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Ethophysiologische Untersuchungen zum Sozialverhalten beim Hausschwein am Beispiel von Mutter-Nachkommen- und sozialen Dominanzbeziehungen

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und unserem Sohn Lennart

gewidmet

Für meine Mutter

Kämpfende Ferkel in einer Sau-Ferkel-Gruppenhaltung (Foto: Birger Puppe, FBN Dummerstorf)

...Es gibt nicht zwei in derselben Gesellschaft lebende Hennen, die nicht genau wissen, wer von ihnen „über" und wer „unter" ihnen ist... Es erwies sich bei den verschiedensten Experimenten, dass die Neigung zur sozialen Gliederung den Hühnern im Blute liegt...

THORLEIF SCHJELDERUPP-EBBE, 1922

…Nichts in der Biologie ist sinnvoll außer im Lichte der Evolution betrachtet…

THEODOSIUS DOBZHANSKY, 1937

…Verhalten ist organismische Interaktion mit der Umwelt auf der Grundlage eines Informationswechsels im Dienst der Fitness…

GÜNTER TEMBROCK, 1988

INHALTSVERZEICHNIS

1 EINLEITUNG

Schweine (Klasse: *Mammalia*, Ordnung: *Artiodactyla*, Familie: *Suidae*) zählen zu den ältesten Haustierrassen der Welt. Schon vor ca. 12000 Jahren brachten Bauern aus dem Nahen Osten die ersten Hausschweine mit nach Europa. Neueren molekularbiologischen Erkenntnissen zufolge aber wurden die Nachkommen dieser Tiere später von europäischen Schweinen verdrängt (LARSON *et al.*, 2007). Diese Tiere entstanden in erster Linie durch die Domestikation des Europäischen Wildschweins *(Sus scrofa)* vor ca. 7000 Jahren und bildeten somit die Grundlage des heute lebenden Hausschweins *(Sus scrofa domestica)* mit seiner Vielzahl von Rassen. Seitdem befinden sich die fast ausschließlich zur kommerziellen Nutzung (vor allem Fleischgewinnung) durch den Menschen gehaltenen Tiere in dessen Abhängigkeit. So gibt es heute in Europa ca. 191 Mio. gehaltene Schweine, in Deutschland sind es 27.1 Mio. (Quelle: Statistisches Bundesamt Deutschland, Stand: 2007) mit einer entsprechenden ökonomischen Bedeutung. Diese immense Anzahl an Schweinen, die unter den vom Menschen gemachten „Regeln“ der Haltung und Zucht leben, bedarf deshalb besonderer Aufmerksamkeit und des Schutzes. Das deutsche Tierschutzgesetz (TierSchG vom 18. Mai 2006, BGBl. I S. 1206) fordert daher ausdrücklich, dass Tiere aus der Verantwortung des Menschen als Mitgeschöpfe anzusehen sind, deren Leben und **Wohlbefinden** zu schützen sind und denen nicht ohne vernünftigen Grund Schmerzen, **Leiden** oder Schäden zugefügt werden dürfen (§1). Die Tierhaltung betreffend müssen Tiere **verhaltensgerecht** untergebracht werden (§2). Auf europäischer Ebene hat die EU 1991 Richtlinien über die Mindestanforderungen für den Schutz von Schweinen erlassen (91/630/EWG), die vor allem seit den 2001 erlassenen Änderungsrichtlinien (2001/88/EG und 2001/93/EG) erhebliche Verbesserungen im Sinne des Tierschutzes bewirkt haben. Diese berücksichtigen ausdrücklich die lange Zeit wenig beachteten sozialen Ansprüche der Schweine, d.h. neben der üblichen Gruppenhaltung von Absetzferkeln und Mastschweinen müssen beispielsweise auch Sauen außerhalb des Abferkelungszeitraumes in Gruppen gehalten werden. Weiterhin sollte die soziale Sau-Ferkel-Beziehung nicht vor 28 Tagen (in Ausnahmefällen 21 Tage) durch das Absetzen der Ferkel beendet werden. In Deutschland wird in Umsetzung dieser EU-Richtlinien die Haltung von Schweinen seit 2006 durch die Zweite Verordnung zur Änderung der Tierschutz-Nutztierhaltungsverordnung (2. TierSchNutztVÄndV) neu geregelt.

In beträchtlichem Maße trägt die Angewandte Ethologie (und hier speziell die Nutztierethologie) als Teilgebiet der Ethologie (allgemein Verhaltensbiologie) dazu bei, wissenschaftliche Erkenntnisse zur Biologie des Verhaltens zu liefern, auf deren Grundlage ethische und gesellschaftliche Normen unseres Verständnisses von Tierschutz sowie – daraus direkt und indirekt abgeleitet – gesetzliche Bestimmungen zur Haltung von Nutztieren entstehen. Allgemein gesehen ist Verhaltensbiologie die Lehre vom Verhalten der Tiere und den biologischen Grundlagen menschlichen Verhaltens. Der Begriff Verhalten lässt sich definieren als organismische Interaktion mit der Umwelt auf der Grundlage eines Informationswechsels im Dienste der Fitness (TEMBROCK, 1992) und hat damit ausdrücklich ein insgesamt eher evolutionär ausgerichtetes Grundkonzept. Der Begriff Fitness als

Ziel des Verhaltens wird somit verstanden als die Eignung eines Individuums (individuelle Eignung) und/oder verwandter Individuen (Gesamt-Eignung) bezüglich des Erfolgs in der Weitergabe seiner genetischen Information an die nächsten Generationen (IMMELMANN, 1982). TINBERGEN (1963) unterscheidet in diesem Zusammenhang vier Fragen, deren Beantwortung bei der Analyse von Verhalten eine besondere Rolle spielt: 1. Verursachung/Kausalität, 2. individuelle Entwicklung (Ontogenese), 3. Nützlichkeit/Anpassungswert (Fitness) und 4. evolutive Entwicklung (Phylogenese). Darauf basierend haben sich zwei Ansätze (Ultimat-Proximat-Dichotomie) in der Analyse der Verhaltensursachen entwickelt (ALCOCK *&* SHERMAN, 1994). Die proximaten Faktoren fragen nach den unmittelbaren bzw. aktuellen Ursachen („Wie") des Verhaltens; die ultimaten Faktoren dagegen nach den mittelbaren, evolutiven, d.h. grundlegenden Ursachen („Warum") des Verhaltens. Grundsätzlich gilt dies auch für die Verhaltensforschung bei Nutztieren (PUPPE, 1995). Aufgrund der speziellen Situation bei Nutztieren – es werden hochentwickelte Wirbeltiere unter eingeschränkten Lebens- und Fortpflanzungsbedingungen gehalten – sind in den letzen Jahren zudem die Befindlichkeiten dieser Tiere in den Fokus wissenschaftlichen Interesses geraten. Unter der (stark anzunehmenden) Voraussetzung, dass Tiere grundsätzlich empfindungsfähig sind und in bestimmtem Maße subjektive Erfahrungen ähnlich denen des Menschen machen können (MANTEUFFEL *&* PUPPE, 1997), ist die Art, wie sie behandelt und gehalten werden, auch von einer ethischen Dimension geprägt (FRASER *et al.*, 1997) und es muss Interesse dafür entwickelt werden, ob Tiere leiden oder sich „wohlbefinden" (THORPE, 1969; BARNARD, 2004). Im Wesentlichen angetrieben durch die Angewandte Ethologie hat das zu konzeptionellen Überlegungen geführt, inwieweit sich emotionale innere Statusformen, wie Leiden und Wohlbefinden bei Tieren, in Konzepte und Modelle der Verhaltensbiologie einordnen lassen (DAWKINS, 1990, 1998; PUPPE, 1996a). So lässt sich die Fitness eines Tieres in Beziehung zu seinem proximaten Bewältigungsverhalten setzen, mit dem Ziel der Aufrechterhaltung der Homöostase. Wohlbefinden ist damit ein Zustand, der etwas über die Fähigkeit eines Tieres aussagt, mit der Umwelt zurechtzukommen (BROOM *&* JOHNSON, 1993) sowie das Ergebnis emotional zu bewerten (PUPPE, 1996a). Daraus ließe sich schlussfolgern, dass Individuen in erster Linie auf homöostatischen Eigenerhalt ihrer Integrität und auf Bewältigungsverhalten zur Sicherung ihrer Reproduktion (BROOM *&* JOHNSON, 1993) sowie auf Deckung ihres Bedarfs und die Vermeidung von Schäden (TSCHANZ, 1985) ausgerichtet sind. BARNARD *&* HURST (1996) weisen allerdings zurecht darauf hin, dass es Situationen gibt, in denen Tiere – in Abwägung zwischen Fitnessmaximierung und physischer Unversehrtheit – Schäden, Tod und damit auch anzunehmende emotionale Beeinträchtigungen in Kauf nehmen, und sie plädieren für eine Hinterfragung der adaptiven Hintergründe und deren adäquate Beachtung bei der Interpretation entsprechender Parameter.

Das Sozialverhalten ist eine Sammelbezeichnung für alle auf einen Artgenossen gerichteten Verhaltensweisen (IMMELMANN, 1982). Dazu gehören in der Verhaltensbiologie neben der Brutpflege (soziale Eltern-Nachkommen-Interaktionen) u.a. auch das Verhalten in der Gruppe (Sozialstruktur). Hausschweine sind soziale Tiere, deren Vorfahren in individualisierten Verbänden, d.h. in Familienstrukturen eng verwandter weiblicher Tiere und ihrer Nachkommen leben, zu denen ausgewachsene männliche Tiere als Einzelgänger nur während der Brunstzeit Zutritt haben (GUNDLACH, 1968; MEYNHARDT, 1978). Infolge des Prozesses der Domestikation von Haustieren erfolgte eine Adaptation gegenüber dem Menschen und den Bedingungen der Gefangenschaft (PRICE, 1984), was bei Schweinen u.a. zu einer Reduktion des Gehirngewichts um ca. 34 % führte (RÖHRS & KRUSKA, 1969). Allerdings sind die domestikationsbedingten Verhaltensänderungen eher quantitativer Natur, d.h. Anpassungen an die vom Menschen geschaffenen ökologischen Bedingungen des Hausstandes. Grundsätzlich neue Verhaltensformen treten dagegen nicht auf (ANDERSEN *et al.*, 2006). Obgleich eine Verwilderung nicht wieder mit einer Zunahme des Hirngewichts einhergeht (RÖHRS & EBINGER, 1999), zeigen zahlreiche Studien an Hausschweinen unter semi-natürlichen Bedingungen, dass arteigenes Verhalten und soziale Organisation erstaunlich wenig verändert sind (z.B. STOLBA & WOOD-GUSH, 1984; NEWBERRY & WOOD-GUSH, 1985, 1986; JENSEN & RECÉN, 1989). Das impliziert, dass der genetische Anteil an der Verhaltenssteuerung entsprechend hoch zu sein scheint und beobachtete Verhaltensänderungen der Tiere eher von der jeweiligen Haltungsumgebung abhängig sind (WECHSLER *et al.*, 1991).

In der vorliegenden kumulativen Habilitationsschrift werden deshalb aus wissenschaftlichen und praktischen Erfordernissen sowie auf der Grundlage von 14 in begutachteten Zeitschriften erschienenen Einzelstudien, zwei eminent wichtige Bereiche des Sozialverhaltens beim intensiv gehaltenen Hausschwein analysiert: die Mutter-Nachkommen-Beziehung (7 Studien) sowie das sozial-agonistische Verhalten und die Dominanzbeziehungen in sozialen Gruppen (7 Studien). Dabei wurde neben der methodisch-analytischen Beschreibung des Sozialverhaltens und seiner Entwicklung besonderen Wert auf die jeweiligen verhaltensphysiologischen Konsequenzen gelegt. Es ist davon auszugehen, dass die Umsetzung der biosozialen Verhaltensansprüche der Tiere – befördert durch die berechtigten Forderungen aus Tierschutz, Ethik und Gesetzgebung – höhere Anforderungen an Haltung und Management von Hausschweinen stellen werden. Daraus wird die allgemeine These abgeleitet, dass die detaillierte Kenntnis der ethologischen Grundlagen sozialen Verhaltens sowie seiner verhaltensphysiologischen Mechanismen und Konsequenzen dazu beitragen kann, sowohl negativen Stress zu minimieren als auch Wohlbefinden und Gesundheit beim Hausschwein zu verbessern. Praktische Implikationen sind in der Vermeidung von Fehlern sowie in Anregungen zur Verbesserung der Tiergerechtheit von Haltungssystemen und Managementmaßnahmen zu sehen. Darüber hinaus soll mit der vorliegenden Habilitationsschrift auch gezeigt werden, dass Hausschweine stärker als bisher eine Rolle als Modelltiere für grundlegende verhaltensphysiologische und psychobiologische Fragestellungen spielen können.

Nach der folgenden kurzen Einführung in die zwei betrachteten Bereiche sozialen Verhaltens beim Hausschwein, werden im Kapitel 2 der vorgelegten Arbeit die Zielstellungen und im Kapitel 3 in knapper Form Hintergrund, Ansatz und Ziel sowie Ergebnisse und Schlussfolgerungen der jeweiligen experimentellen Einzelstudien präsentiert, die dann im Kapitel 4 zusammenfassend diskutiert werden. Nach Zusammenfassung (Kapitel 5) und Literaturverzeichnis (Kapitel 6) sind die zugehörigen vollständigen Originalarbeiten im Kapitel 7 zusammengestellt.

1.1 Sozialkommunikative Mutter-Nachkommen-Beziehungen beim Hausschwein

Grundeinheit der sozialen Organisation beim Europäischen Wildschwein ist die Mutterfamilie mit der Bache und ihren Frischlingen, wobei mehrere Mutterfamilien sich zu größeren Familienverbänden vereinigen (GUNDLACH, 1968). Wichtigstes Element in der sozialen Mutter-Kind-Beziehung ist das Saug-Säugeverhalten. Wild- und Hausschweine sind iteropare und polytoke Säugetiere, d.h. Mütter gebären mehrmals im Laufe ihres Lebens Würfe, die wiederum aus mehreren, ungewöhnlich weit entwickelten Jungen bestehen, die unmittelbar nach der Geburt um den Zugang zur Ressource Milch konkurrieren (PUPPE *et al.*, 2008). Als adaptive Konsequenz hat sich ein charakteristisches und komplexes Muster des Saug-Säugeverhalten herausgebildet (FRASER, 1980; ALGERS, 1993), das nahezu einzigartig bei Säugetieren ist und sowohl aus wissenschaftlich-theoretischen als auch angewandt-praktischen Gründen des genauen Verständnisses bedarf. So können einerseits die Mutter-Nachkommen-Beziehungen beim Hausschwein als ein geeignetes System für grundlegende Untersuchungen von Eltern-Nachkommen-Konflikten *(parent-offspring-conflict),* Geschwister-Konkurrenz *(sibling competition)* sowie des Zusammenhangs zwischen Ressourcenverteilung *(resource allocation)* und Sozialsystem dienen (FRASER *et al.*, 1995; DRAKE *et al.*, 2008), andererseits haben das Überleben und die tiergerechte Aufzucht möglichst vieler gesunder Ferkel eine hohe ethische und ökonomische Bedeutung in Landwirtschaft und Gesellschaft. Es stellt sich zunächst die experimentell zu überprüfende Frage, ob und in welchem Maße das Verhalten von Sau und Ferkeln unter modernen Haltungsbedingungen Voraussagen aus solchen ultimaten Theorien folgt. Bei Säugetieren gibt es einen Konflikt zwischen Eltern und Nachkommen über das Niveau (z.B. Menge an Milch) und die Dauer (z.B. Zeitpunkt des Absetzens) des elterlichen Investments (TRIVERS, 1974; GODFRAY, 1995). So sollte sich die Rate des elterlichen Investments (z.B. Anzahl erfolgreicher Sauakte) mit zunehmendem Alter der Nachkommen verändern (*weaning conflict)*. Nach einem Maximum elterlichen Investments beginnt der allmähliche Absetzprozess, der wiederum mit einem entsprechenden Investmentminimum und dem tatsächlichen Absetzen endet (MARTIN, 1984). Die Beobachtung des Saug-Säugeverhaltens bietet hier einen guten Indikator für die Untersuchung der Milchtransferrate sowie für die Ausprägung des elterlichen Investments (MENDL *&* PAUL, 1989). Insgesamt gesehen sollte die Einbeziehung von Ideen, Thesen und Erkenntnissen der Verhaltensökologie und Soziobiologie eine nützliche Ergänzung der traditionell eher sehr praktisch und leistungsorientiert ausgerichteten Sichtweisen der Nutztierethologie sein (FRASER *et al.*, 1995).

Neugeborene Hausschweinferkel zeigen das typischerweise gut entwickelte Verhalten der frühen Zitzensuche und Milchaufnahme aller Ungulaten (FRASER, 1984), sind aber physiologisch mit wenig Energiereserven ausgerüstet, thermoregulatorisch noch relativ instabil (LE DIVIDICH *&* NOBLET, 1981) und bedürfen deshalb sowie aus Gründen der Entwicklung eines adäquaten Immunschutzes (WILSON, 1974) einer schnellen ersten Kolostrumaufnahme, die durchschnittlich ca. 30-40 min *post natum* erfolgt (BÜNGER, 1985; HOY *et al.*, 1995; TUCHSCHERER *et al.*, 2000). Typisch für Tiere mit einer Fortpflanzungsstrategie, die vergleichsweise hohe Wurfgrößen mit relativ geringem elterlichen Investment verbindet, sind hohe Mortalitätsraten beim Nachwuchs (FRASER, 1990; DRAKE *et al.*, 2008). So sterben immerhin 12-30 % der lebend geborenen Ferkel vor dem Absetzen (ENGLISH *&* MORRISON, 1984) und davon 75-80 % in den ersten 3 Lebenstagen durch in der Regel multifaktorielle Ursachen (BARNETT *et al.*, 2001). Diese hohe Mortalität zu minimieren, ist ein erklärtes Ziel der Tierschutz- und Befindlichkeitsforschung bei Nutztieren (MELLOR *&* STAFFORD, 2004). Verhaltensassoziierte Hauptursachen der frühen Ferkelsterblichkeit liegen klar im Bereich der sozialen Sau-Ferkel-Interaktionen, erstens, durch Mangelernährung infolge von Problemen beim Saugverhalten und der Milchaufnahme, und zweitens, durch Erdrückungsverluste der Ferkel durch die Sau (FRASER, 1990). Dem Prozess der Zitzenwahl und der Entwicklung einer erfolgreichen Milchaufnahme (Saugakte) für alle Ferkel eines Wurfes kommt deshalb in der Entwicklung des sozialen Verhaltens eine besondere Bedeutung zu. Nach teilweise heftigen neonatalen Kämpfen (z.B. HARTSOCK *&* GRAVES, 1976; DE PASSILLÉ *et al.*, 1988; FRASER *&* THOMPSON, 1991) entwickeln Ferkel in ihren ersten Lebenstagen eine charakteristische Zitzen- bzw. Saugordnung (z.B. DONALD, 1937; MCBRIDE, 1963; FRASER & JONES, 1975; JONES-BAADE *et al.*, 1978; HOY *&* MEHLHORN, 1989; HOY *&* PUPPE, 1992; PUPPE *et al.*, 1993), d.h. eine individuell konstante Benutzung einer bestimmten Zitze bei der Milchaufnahme. Eine solche konstante Zitzenordnung kommt auch bei einigen wenigen anderen Säugetierarten vor, wie beispielsweise Hauskatzen (EWER, 1959), Klipp- und Buschschliefern (HOECK, 1977) oder Prairie- (MCGUIRE, 1998) bzw. Kiefernwühlmäusen (MCGUIRE *&* SULLIVAN, 2001) und lässt sich als ein territoriales soziales System infolge der neonatalen Geschwisterkonkurrenz ansehen (HUDSON *&* TRILLMICH, 2008). Dabei werden bei Wildschweinen anscheinend die hinteren Zitzen (FERNÁNDEZ-LLARIO *&* MATEOS-QUESADA, 2005), bei Hausschweinen dagegen eher die kranial gelegenen Zitzen bevorzugt (PUPPE *et al.*, 1993). Es ergibt sich die Frage, inwieweit Platz und Konsistenz der territorialen Saugordnung die weitere soziale Genese der Tiere beeinflussen, z.B. ihre spätere soziale Stellung in der Dominanzhierarchie. Es gibt Hinweise, dass kranial saugende Ferkel später bessere Dominanzplätze erreichen – möglicherweise indirekt vermittelt durch ihr höheres Körpergewicht (SCHEEL *et al.*, 1977; PUPPE *&* Tuchscherer, 1994). Ein weiteres Problem im Kontext der Regulation des intraspezifischen Sozialverhaltens von Mutter und Nachkommen ist die Frage ihrer kommunikativen Beziehungen, z.B. den Mechanismen der wechselseitigen Erkennung. Sauen initiieren und begleiten den Saugakt mit einer stereotypen Folge von Grunzlauten (WHITTEMORE *&* FRASER, 1974; ELLENDORFF *et al.*, 1982; SHILLITO WALSER, 1986). Das Zusammenspiel zwischen Säugegrunzen und Milchejektion ist ein bei Säugetieren in dieser Komplexität einzigartiges System, das Verhalten der Ferkel und die Verteilung von Milch bioakustisch auf adaptive Weise zu synchronisieren (FRASER, 1980). Hier stellt sich die Frage, an welchen inter-individuellen akustischen Merkmalen die Ferkel den Laut an sich bzw. an welchen individuellen Lautmerkmalen sie ihre Muttersau erkennen. Das ist insbesondere bedeutsam, da ferkelführende Sauen auch unter Gruppenbedingungen gehalten werden können, d.h. Ferkel somit die Möglichkeit haben, auch an anderen Sauen als an ihrer eigenen Mutter zu saugen und Milch aufzunehmen (PUPPE *&* TUCHSCHERER, 1995). Akustische Lautmerkmale haben dabei ein hohes biologisches Potential für die Verwandtenerkennung (RENDALL *et al.*, 1996) und können gleichzeitig als ein zeitnaher Indikator für Zustand und Befindlichkeit dienen (MANTEUFFEL *et al.*, 2004).

Spätestens seit den klassisch gewordenen Isolationsversuchen an Rhesusäffchen von Harry Harlow (z.B. HARLOW, 1958; HARLOW *et al.*, 1965) ist bekannt, wie wichtig die soziale Bindung zwischen Mutter und Nachkommen für die Gesundheit und normale Verhaltensentwicklung letzterer ist. Andererseits bieten experimentell induzierte Störungen in diesem Bereich des Sozialverhaltens die Möglichkeit, Mechanismen und Konsequenzen dieser Beziehung modellhaft und gezielt zu untersuchen (LEHMANN & FELDON, 2000). Als besonders bedeutsam sind die generellen Konsequenzen psychosozialen Stresses für Immunität und Gesundheit (HENRY & STEPHENS, 1977; COE, 1993) zu nennen, da hier direkte Verbindungen zwischen Stress und Immunsystem sichtbar werden, die komplexe, regulatorische Antworten in Verhalten und Physiologie nach sich ziehen (DANTZER & KELLY, 1989). Neuere Befunde haben hier zu der These geführt, dass als eine adaptive, neuroimmunologische Reaktion auf psychosozialen Stress (z.B. maternale Separation) auch das zytokinvermittelte Auftreten von Krankheit und/oder Krankheitsverhalten (*sickness behaviour*) verstanden werden kann (HENNESSY *et al.*, 2001; DANTZER, 2005). Das hat insbesondere Implikationen für den Prozess des Absetzens von Ferkeln, da dieser zusätzlich zu Stressoren wie Nahrungs- und Haltungsumstellungen auch mit einer Reihe von psychosozialen Herausforderungen (z.B. die abrupte Trennung sozialer Bindungen zu Mutter und/oder Wurfgeschwistern) verbunden ist (NEWBERRY & SWANSON, 2008; WEARY *et al.*, 2008). So wird beispielweise in der praktischen Schweinehaltung das Absetzalter der Ferkel vorrangig aus ökonomischen Gründen in immer frühere Altersbereiche gelegt und liegt mittlerweile meist zwischen drei und fünf Wochen. Verschärft wird dieser Trend noch bei Produktionsverfahren, wie dem isolierten Frühabsetzen, bei dem die Ferkel teilweise weit vor dem 21. Lebenstag abgesetzt werden und in dessen Folge Probleme in Verhalten und Wohlbefinden bei den Ferkeln beschrieben werden (ROBERT *et al.*, 1999; VON BORELL, 2000). Modellhafte Untersuchungen an isolierten Ferkeln können daher dazu beitragen, das Verständnis ihrer adaptiven Reaktionen im komplexen Wechselspiel zwischen neuroendokrinen Stressreaktionen, immunologischen Antworten und entsprechenden Verhaltensveränderungen zu verbessern und damit Maßnahmen der Verbesserung von Wohlbefinden und Gesundheit der Tiere anzuregen. Gleichzeitig wird in jüngerer Zeit zunehmend postuliert, dass die Untersuchung derartiger psychobiologischer Zusammenhänge von Stress und Immunsystem beim Schwein (LIND *et al.*, 2007) auch grundlegende Implikationen für das Verständnis der Entwicklung psychosomatischer Probleme (z.B. depressive Erkrankungen) beim Menschen hat (DANTZER, 2005).

1.2 Sozial-agonistisches Verhalten und Dominanzstruktur beim Hausschwein

Wie bei anderen sozial lebenden Spezies auch hat sich in Wildschweinrotten eine soziale Rangordnung (Hierarchie) evolutiv herausgebildet, die allerdings spezifisch durch Faktoren, wie Territorialität (Revierbesitz), Erfahrung (Alter) und Körpermasse bestimmt wird (MEYNHARDT, 1978). Eine sozialhierarchische Rangordnung ist das komplexe Ergebnis wechselseitiger, kompetitiver Dominanz-Subdominanzbeziehungen der Individuenpaare (Dyaden) innerhalb einer sozialen Gruppe (PUPPE, 1996b). Zahlreiche Arbeiten zeigen, dass sie auch bei Hausschweingruppen Grundlage ihrer sozialen Organisation ist (RASMUSSEN *et al.*, 1962; MCBRIDE & JAMES, 1964; BEILHARZ & COX, 1967; MEESE & EWBANK, 1973a,b; EWBANK, 1976; PUPPE & TUCHSCHERER, 1994; PUPPE, 1996b). Strukturell lässt sich Dominanz als eine Eigenschaft des Musters agonistischer Interaktionen zweier Individuen definieren, bei dem ein Individuum konsistent als Sieger (dominant), das andere als Verlierer (subdominant) hervorgeht (DREWS, 1993). Dominanz ist somit eine gebräuchliche Bezeichnung für den Zustand der Imbalance oder Asymmetrie von Interaktionen in einer Zweierbeziehung, während der soziale Rang eines Tieres einen Aspekt der hierarchischen Gruppenstruktur darstellt (PUPPE, 1996b). Agonistisches Verhalten wird als Überbegriff aller mit kämpferischen Auseinandersetzungen zwischen Individuen in Zusammenhang stehenden Verhaltensweisen verwendet, umfasst sowohl aggressives als auch defensives Verhalten, wird aber häufig auch als Synonym für rein aggressives Verhalten benutzt (IMMELMANN, 1982). Das agonistische Verhalten beim Hausschwein steht aus verschiedenen Gründen im Zentrum des wissenschaftlichen Interesses in der Nutztierethologie: 1. als soziales Interaktionsverhalten trägt es entscheidend zur Bildung von Gruppenstruktur und Dominanzhierarchie bei (BEILHARZ & COX, 1967; MEESE & EWBANK, 1973a; PUPPE, 1996b); 2. es ist beteiligt am individuellen Zugang zu Ressourcen (MCGLONE, 1986; PUPPE & TUCHSCHERER, 1994); 3. es ist ethologischer Bestandteil ultimater und proximater Auslöse- und Bewältigungsmechanismen einschließlich individueller Charakteristika (HESSING *et al.*, 1993; FORKMAN *et al.*, 1995; ERHARD *et al.*, 1997; BOLHUIS *et al.*, 2005); 4. es hat erhebliche ethologische und physiologische Konsequenzen für das Individuum (MENDL *et al.*, 1992; HESSING *et al.*, 1994a; MORROW-TESCH *et al.*, 1994; HICKS *et al.*, 1998; DE GROOT *et al.*, 2001); und 5. es hat weitreichende praktische Implikationen für Züchtung, Haltung, Management sowie Wohlbefinden, Tierschutz und Ethik (PETHERICK & BLACKSHAW, 1987).

BEUERLE (1975) hat in einer umfangreichen Studie ein Ethogramm des agonistischen Verhaltens beim Wildschwein zusammengestellt, das aggressive Verhaltensinteraktionen, wie beispielsweise Beißen, Schnauzenstöße und Wegschieben, beschreibt. Zum Ethogramm des agonistischen Verhaltens bei neugruppierten Hausschweinen gehört ebenfalls eine Reihe offener aggressiver und submissiver Verhaltenssequenzen (ausführlich beschrieben bei MCGLONE, 1985), bei denen Bisse und Stöße mit 81 % quantitativ am häufigsten vorkommen. Spätere Verlierer unterscheiden sich von Gewinnern in Häufigkeit und Sequenz einzelner Verhaltensweisen und zeigen in deren Folge ein ausgeprägtes Wegdrehen des Körpers (MCGLONE, 1985) oder eine Tendenz zur Flucht weg vom Angreifer (RUSHEN & PAJOR, 1987). JENSEN (1980, 1982) hingegen präsentiert ein Ethogramm sozialer Interaktionsmuster bei etablierten Sauengruppen, das neben als klar aggressiv zu wertenden Verhaltensweisen (z.B. Kopf-Kopf-Stöße, Kopf-Körper-Stöße) auch eine Reihe schwierig zu interpretierender (z.B. Drohen) bzw. neutral (nasale Körperkontakte) bis mild aggressiv (z.B. naso-nasal-Kontakte) zu wertender Verhaltensweisen enthält, die an der Regulation der Sozialordnung beteiligt sind. Es scheint, dass die Einschätzung der Kampffähigkeit des Opponenten (*resource holding potential*, PARKER, 1974) bei Schweinen sich erst im Laufe der Ontogenese bzw. mit zunehmender Kampferfahrung entwickelt (RUSHEN, 1988). Dann spielen offensichtlich Gewichts- bzw. Größenunterschiede (RUSHEN, 1987) und Bekanntheitsgrad (STOOKEY & GONYOU, 1998) eine entscheidende Rolle beim Zustandekommen der agonistischen Interaktionen. Es wird diskutiert, inwieweit der Erfolg im agonistischen Verhalten neben einem höheren sozialen Rang auch die direkte Ressourcenverfügbarkeit und -verteidigung beeinflusst. So kann man bei neu gruppierten Hausschweinen eine kurze „akute“ Periode mit offenen Aggressionen beobachten, die offensichtlich der Klärung der Dominanzverhältnisse dient (PUPPE & TUCHSCHERER, 1994), gefolgt von einer längeren „chronischen“ Periode, die eher als eine intraspezifische Kompetition um Ressourcen, wie z.B. Futter, angesehen wird (FRASER, 1984), so dass temporale und lokale Faktoren beachtet werden müssen (FRASER *et al.*, 1995). Zudem wird die These diskutiert, dass agonistisches Verhalten bei Schweinen in Zusammenhang mit der Intoleranz gegenüber fremden Individuen und ihrer Vertreibung steht (FRASER *et al.*, 1995). Bezüglich der individuellen Ausprägung des aggressiven Verhaltens wird die generelle Existenz zweier Bewältigungsstrategien (*coping style*) mit konsistenter ethologischer und neuroendokriner Charakteristik beschrieben (BENUS *et al.*, 1991; KOOLHAAS *et al.*, 1999): Tiere mit einem (pro)aktiven, hochaggressiven und einem reaktiven, wenig aggressiven Verhaltensmuster. Ob diese bei Hausschweinen experimentell ermittelte (sogenannter „Backtest“) und daraufhin generell postulierte Dichotomisierung der aggressiven Reaktion (HESSING *et al.,* 1993; RUIS *et al.*, 2002; BOLHUIS *et al.*, 2003, 2005) gerechtfertigt ist, wird allerdings aus theoretischen (JENSEN, 1995) und experimentellen Gründen (FORKMAN *et al.*, 1995; JENSEN *et al.*, 1995; D'EATH & BURN, 2002) bezweifelt. Allerdings ist sicher, dass aggressives Verhalten und seine Konsequenzen beim Hausschwein in enger Beziehung zu individuellen Bewältigungsmustern, aber auch sozialen Dominanzbeziehungen stehen (RUIS *et al.*, 2002; BOLHUIS *et al.*, 2005).

In der praktischen Schweinehaltung gibt es eine Reihe von Abschnitten (z.B. Absetzen, Umstallen), die mit einem Wechsel der Haltungsumgebung und dem Neugruppieren bzw. Integrieren neuer Gruppenmitglieder verbunden sind. Folge ist eine erhöhte Anzahl agonistischer Auseinandersetzungen sowie das Neuetablieren der sozialen Rangordnung (PUPPE, 1996b). Daraus ergeben sich einige spezifische Fragen, z.B. inwieweit soziale Faktoren der Ontogenese, wie Verwandtschaft (*relatedness*) oder Bekanntheit (*familiarity*), das agonistische Verhalten beeinflussen und darüber hinaus, wie sich der gleichzeitige Wechsel der Haltungsumwelt darauf auswirkt. Untersuchungen dazu können beitragen, detaillierte wissenschaftliche Kenntnisse über Mechanismen des agonistischen Verhaltens bei Schweinen zu vertiefen, mit deren Hilfe die Art und Weise des Managements dieser sozialen Prozesses im Sinne einer gesetzlich geforderten Verbesserung der Tiergerechtheit verändert werden kann. Ein weiteres Problem besteht in der Art und Weise, wie Dominanzbeziehungen – ausgehend von der strukturellen Dominanzdefinition von DREWS (1993) – methodisch analysiert werden. Obgleich eine Reihe von soziometrischen Parametern entwickelt und nach und nach in die bestehenden Analysemethoden eingeführt wurden (siehe z.B. DE VRIES *et al.*, 1993; DE VRIES, 1998; DE VRIES & APPLEBY, 2000), fehlt bisher insbesondere bei Nutztieren ein konsistenter Ansatz, um Dominanz auf allen Analyseebenen (Dyade, Individuum, Gruppe) zu charakterisieren. Vorteil bei Anwendung eines solchen Verfahrens ist die Möglichkeit, über einheitliche Parameter die speziesspezifische Entwicklung sozialer Dominanzbeziehungen in solchen Haltungssituationen vergleichend zu analysieren, in denen es zu Veränderungen der Sozialstruktur kommt. Zudem könnten verschiedene Nutztierspezies in ihren sozialen Unterschieden und Gemeinsamkeiten verglichen werden – mit direkten Implikationen für ihre Zucht und Haltung.

Es ist weitgehend Konsens, dass mit dem Sozial- und Dominanzverhalten verbundene Merkmale (z.B. sozialer Rang) oder Prozesse (z.B. sozialer Stress) vielfältige, teilweise rückgekoppelte Auswirkungen auf weitere ethologische, neuroendokrinologische oder auch immunphysiologische Regelkreise haben, die wiederum positive oder negative Folgen für Stressbewältigung, Wohlbefinden und Gesundheit haben können (HENRY *&* STEPHENS, 1977; VON HOLST, 1998; SAPOLSKY, 2005; BARTOLOMUCCI, 2007). Dabei hat sich gezeigt, dass die tatsächlichen physiologischen Reaktionen eines Individuums entscheidend von seiner Fähigkeit bzw. Möglichkeit abhängen, die Situation vorherzusagen (*predictability*), zu kontrollieren (*control*), und/oder zu bewältigen (*coping*), aber auch von seinen damit verbundenen emotionalen Erfahrungen (PUPPE, 1996a, 2003) und anderen Randbedingungen der konkreten sozialen Situation. Die Einbeziehung von geeigneten Kosten-Nutzen-Analysen beim Bewältigungsverhalten von Individuen im sozialen Kontext (z.B. bei individuellen Konsequenzen unterschiedlicher Rangpositionen) zeigen zudem, dass die eher „traditionelle" Sicht von linearen Beziehungen zwischen sozialem Rang und Fitness bzw. Wohlbefinden fraglich ist (MENDL *&* DEAG, 1995). So haben beispielsweise neuere Untersuchungen an Pavianen gezeigt, dass erhöhte Glukokortikoidwerte als endokrine Folge der sozialen Stressantwort nicht nur bei Subdominanz, sondern auch als Kosten von Dominanz vorkommen können (CREEL *et al.*, 1996; CREEL, 2001). Andere Untersuchungen an Mäusen belegen, dass die soziale Erfahrung von Subdominanz bzw. eines niedrigen sozialen Ranges immunsuppressive Konsequenzen hat und die Krankheitsfälligkeit fördert (BARNARD *et al.*, 1994, 1996). Die bisherigen Studien an Hausschweinen zeigen, dass soziale Stresserfahrungen im Kontext der Etablierung von Dominanz- und Rangbeziehungen (z.B. sozialer Status), das Verhalten und (neuro)endokrine Reaktionen der Tiere beeinflussen (MENDL *et al.*, 1992; MCGLONE *et al.*, 1993; MORROW-TESCH *et al.*, 1994; HICKS *et al.*, 1998; ZANELLA *et al.*, 1998). Meist scheinen subdominante Tiere erhöhtem Stress ausgesetzt, die Ergebnisse variieren jedoch in Abhängigkeit von Methodik, analysierten Parametern und dem konkreten sozialen Untersuchungskontext. Mehrere Arbeiten lassen darüber hinaus die begründete Annahme zu, dass die Stellung in der Dominanzhierarchie bei Hausschweinen immunphysiologische Konsequenzen (MCGLONE *et al.*, 1993; MORROW-TESCH *et al.*, 1994; HICKS *et al.*, 1998; DE GROOT *et al.*, 2001; SALAK-JOHNSON *&* MCGLONE, 2007; RUDINE *et al.*, 2007) bis hin zur tatsächlichen Krankheitsanfälligkeit (HESSING *et al.*, 1994b) hat. Auch hier ist die These des Auftretens einer stressvermittelten Immunsuppression nicht immer einheitlich belegt (SALAK-JOHNSON *&* MCGLONE, 2007); die Ergebnisse deuten aber insgesamt darauf hin, dass subdominante Tiere hier tatsächlich größere Probleme haben. Schlussfolgernd daraus ergibt sich ein dringender Bedarf an interdisziplinären Studien, die Zusammenhänge zwischen sozialhierarchischem Verhalten und sowohl neuroendokrinen als auch immunphysiologischen Reaktionen bei Hausschweinen zu untersuchen, da hier nachhaltige Effekte auf Gesundheit und Wohlbefinden zu erwarten sind. Dies hat Auswirkungen für Haltung und Management der Tiere sowohl unter intensiven Bedingungen als auch unter den Bedingungen der stetig zunehmenden ökologischen Landwirtschaft.

2 ZIELSTELLUNGEN

Die vorliegende kumulative Habilitationsschrift verfolgt das generelle Ziel, die Entwicklung des Sozialverhaltens beim intensiv gehaltenen Hausschwein unter dem Gesichtspunkt ultimater und proximater Verhaltenssteuerung multidisziplinär zu untersuchen. Es werden wichtige Mechanismen sozialen Verhaltens, Methoden zu seiner Bestimmung und entsprechende ethologische und physiologische Konsequenzen für die Tiere am Beispiel von Mutter-Nachkommen-Beziehungen und sozialen Dominanzbeziehungen auf wissenschaftlicher und praktischer Ebene analysiert. Damit ordnet sich die Arbeit an der Schnittstelle zwischen Grundlagenethologie und Angewandter Ethologie ein. Die Ergebnisse der vorgestellten Studien dieser Habilitationsschrift sollen einerseits der Vertiefung der wissenschaftlichen Zusammenhänge im Bereich des sozialen Verhaltens bei Hausschweinen dienen, andererseits sollen sie durch die gewonnenen Erkenntnisse einen praktischen Beitrag zur möglichen Verbesserung von Wohlbefinden und tiergerechter Haltung leisten. Ausgehend von den allgemeinen Grundsätzen und Zielen der Einleitung (Kapitel 1) sowie den jeweiligen konkreten Zielen und Hypothesen der einzelnen experimentellen Studien (Kapitel 3), verfolgt diese Arbeit zusammengefasst nachfolgende Ziele.

I Verbesserung des bisherigen Verständnisses von verhaltensbiologischen Mechanismen der Sau-Ferkel-Beziehung (Kapitel 3.1).

- Analyse der Entwicklung und Funktion spezifischer Elemente des Saugverhaltens der Ferkel (Saugfrequenz, Saugstabilität) unter soziobiologischen Aspekten und mittels mathematischer Modellfunktionen (Kapitel 3.1.1).
- Aufklärung von akustischen Mechanismen der Muttererkennung durch die Ferkel am Beispiel des Säugegrunzens der Sau (Kapitel 3.1.2).
- Aufklärung psychophysiologischer Konsequenzen gestörter Sau-Ferkel-Beziehungen (maternale Deprivation und soziale Isolation) beim Ferkel (Kapitel 3.1.3).

II Verbesserung des bisherigen Verständnisses von agonistischem Verhalten und Dominanz in sozialen Schweinegruppen (Kapitel 3.2).

- Analyse des Einflusses sozialer (Bekanntheit, Verwandtschaft) und nichtsozialer (Haltung) Umweltfaktoren auf das agonistische Verhalten und seine Konsequenzen nach Absetzen der Ferkel bzw. Neugruppieren in Ferkelgruppen (Kapitel 3.2.1).
- Entwicklung standardisierter soziometrischer Methoden bei der Dominanzstrukturanalyse auf verschiedenen sozialen Ebenen und deren Anwendung bei Schweinen in verschiedenen Haltungsabschnitten (Kapitel 3.2.2).
- Aufklärung der Konsequenzen sozialer Dominanz auf Verhalten, Immun- und Stresssystem bei wachsenden Schweinen (Kapitel 3.2.3).

3 ZUSAMMENFASSENDE DARSTELLUNG DER EINZELSTUDIEN

3.1 Mutter-Nachkommen-Beziehungen

3.1.1 Saugverhalten der Ferkel und Soziobiologie der Sau-Ferkel-Beziehung

Publikationen:

Studie 1

Puppe, B. *&* Tuchscherer, A. (2000): The development of suckling frequency in pigs from birth to weaning of their piglets: a sociobiological approach. *Animal Science* **71**, 273-279.

Studie 2

Puppe, B. *&* Tuchscherer, A. (1999): Developmental and territorial aspects of suckling behaviour in the domestic pig *(Sus scrofa f. domestica)*. *Journal of Zoology* **249**, 307-313.

Studie 3

Puppe, B. (2002): Die Entwicklung der Beziehung zwischen Sau und Ferkel beim Hausschwein – Eine soziobiologische Betrachtung. *Berliner und Münchener Tierärztliche Wochenschrift* **115**, 445-452.

3.1.1.1 Entwicklung der Saughäufigkeit der Ferkel an der Sau

Studie 1 (Kumulative Publikationsliste, Kapitel 7.1)

Puppe, B. *&* Tuchscherer, A. (2000): The development of suckling frequency in pigs from birth to weaning of their piglets: a sociobiological approach. *Animal Science* **71**, 273-279.

Hintergrund, Ansatz und Ziel:

Der Milchtransfer von der Mutter zu den Nachkommen ist einer der wichtigsten Parameter des elterlichen Investments bei Säugetieren (MARTIN, 1984). Gleichzeitig stellt die erfolgreiche Milchaufnahme für die Ferkel die wichtigste nutritive, energetische und immunologische Ressource in den ersten Lebenstagen dar und determiniert entscheidend ihre weitere physische Entwicklung (Fitness). Dabei spielt die Saughäufigkeit bzw. -frequenz eine wichtige Rolle bei der Beeinflussung von Quantität und Qualität der Milchproduktion (ŠPINKA *et al.*, 1997, 1999). Hingegen sollte sich die Dauer des elterlichen Investments mit zunehmendem Alter der Nachkommen verringern (Absetzkonflikt, TRIVERS, 1974). Aus dieser Perspektive beginnt der Absetzprozess unmittelbar nach einem Maximum an elterlichem Investment (z.B. den gewährten Saugakten) und endet bei einem entsprechenden Minimum (siehe Abbildung 1 der Studie). Die Studie wurde an 34 Jungsauen, die mit ihren Würfen in konventionellen Einzelhaltungsbuchten, aber ohne Bewegungseinschränkung gehalten wurden, durchgeführt. Unser Ziel war es, erstens die Entwicklung der täglichen Saughäufigkeit per Video und unterstützenden Direktbeobachtungen während der 35-tägigen Säugezeit zu dokumentieren und dann mit diesen Daten das Saugverhalten als eine Funktion des Absetzprozesses mittels nichtlinearer Regressionsanalyse zu modellieren. Wir wollten zweitens die Beziehungen zwischen Saughäufigkeit und einfach zu erhebenden Merkmalen, welche die Fitness von Nachkommen charakterisieren, analysieren: Anzahl (Wurfgröße) und Qualität (durchschnittliches Ferkelgewicht) der Nachkommen.

Ergebnisse und Schlussfolgerungen:

Der Kurvenlauf erfolgreicher täglicher Saugakte in Beziehung zum Alter der Ferkel charakterisiert Sau-Ferkel-Beziehungen in Übereinstimmung mit den Vorhersagen des evolutionären Modells des Absetzkonflikts (siehe Abbildung 2 der Studie). Das lokale Maximum der Kurve wurde nach 8.5 Tagen erreicht (entspricht 31.4 Saugakten in 24 h) und kann als Beginn des biologischen Absetzprozesses angesehen werden. Ein lokales Minimum wurde in der beobachteten Säugezeit von 35 Tagen jedoch nicht erreicht. Der prozentuale Anteil nichterfolgreicher Saugakte betrug lediglich 5.1 % am Tag 8 und 4.9 % am Tag 35 der Saugperiode. Offensichtlich als Kompensation zur abnehmenden Saughäufigkeit steigerten die Ferkel ihre Beifutteraufnahme deutlich ab der vierten Laktationswoche. Kleine Würfe (4-7 Ferkel) erreichten erst nach 12.4 Tagen ihre maximale Saughäufigkeit und damit ca. 6 Tage später im Vergleich zu großen Würfen (6.2 Tage bei 11-14 Ferkel/Wurf). Sie waren in den durchschnittlichen Individualgewichten (Qualität) tendenziell auch schwerer. Es lässt sich schlussfolgern, dass die Modellierung des Saugverhaltens definierte und vergleichbare Parameter liefert, mit denen sowohl theoretische Fragen von Mutter-Nachkommen-

Beziehungen (z.B. Eltern-Nachkommen-Konflikt, Absetzkonflikt) als auch praktische Probleme der Sau-Ferkel-Beziehungen (Haltungsfragen, Management) untersucht werden können. Dies wird umso wichtiger, je höher der Freiheitsgrad des Verhaltens der Sauen in alternativen Haltungsformen (z.B. in verschiedenen Formen der Gruppenhaltung) wird, die eher den evolvierten Verhaltenserwartungen der Tiere entsprechen.

3.1.1.2 Entwicklung der Saugstabilität der Ferkel an der Sau

Studie 2 (Kumulative Publikationsliste, Kapitel 7.2)

Puppe, B. & Tuchscherer, A. (1999): Developmental and territorial aspects of suckling behaviour in the domestic pig *(Sus scrofa f. domestica)*. *Journal of Zoology* **249**, 307-313.

Hintergrund, Ansatz und Ziel:

Ferkel entwickeln in den ersten Lebenstagen eine hohe lokale Saugstabilität und ordnen sich bei den Saugakten in einer charakteristischen Zitzenordung an (MCBRIDE, 1963). Diese lässt sich eher als eine soziale Territorialordnung denn als eine soziale Hierarchie bezeichnen (PUPPE *et al.*, 1993), scheint aber eine wichtige Determinante für letztere zu sein. Insofern sollten sowohl erreichter Platz in der Saugordnung als auch die Stabilität des Saugens an sich die weitere soziale und physische Ontogenese der Ferkel entscheidend beeinflussen. Die Studie führten wir an insgesamt 327 Ferkeln von 39 Jungsauenwürfen durch. Das Saugverhalten wurde bezüglich der territorialen Konsistenz am Gesäuge der Sau über eine 35-tägige Säugeperiode beobachtet. Mit diesen Daten modellierten wir anschließend die Saugstabilität mittels einer drei-parametrischen Exponentialfunktion. Ziel war es, die Entwicklung der Saugstabilität von Geburt bis Absetzen anhand konkreter mathematischer, gleichzeitig aber verhaltensbiologisch interpretierbarer Parameter zu beschreiben und individuelle Auswirkungen auf die Gewichtsentwicklung und die späteren sozialen Dominanzwerte zu ermitteln.

Ergebnisse und Schlussfolgerungen:

Der Verlauf der Saugstabilität entsprach einer typischen Lernkurve und widerspiegelte somit einen rapide ablaufenden Lernprozess, so dass die Ferkel bereits ab dem vierten Lebenstag Werte um die 95 % Saugstabilität bezüglich der entsprechenden Gesäugeregion erreichten (siehe Abbildung 1 der Studie). Darüber hinaus konnten mittels einzelner mathematischer Funktionskoeffizienten biologische Merkmale des territorialen Saugverhaltens, wie Start- und Endlevel der Stabilität, aber auch die tägliche Saugstabilität ethologisch sinnvoll modelliert werden. Die kranial gelegenen Zitzen wurden präferiert, während Ferkel an den kaudal gelegenen Zitzen geringere Gewichtszunahmen und schlechtere soziale Dominanzwerte nach dem Absetzen aufwiesen. Ferkel mit Präferenz der mittleren Zitzen hatten die geringsten Saugstabilitäten. Saugstabilität und Platz in der Saugordnung sind somit adaptiver Bestandteil des frühen Territorialverhaltens der Ferkel und bilden die Grundlage für die Etablierung ihres späteren Sozialverhaltens und der physischen Entwicklung.

3.1.1.3 Soziobiologische Aspekte der Sau-Ferkel-Beziehung

Studie 3 (Kumulative Publikationsliste, Kapitel 7.3)

Puppe, B. (2002): Die Entwicklung der Beziehung zwischen Sau und Ferkel beim Hausschwein – Eine soziobiologische Betrachtung. *Berliner und Münchener Tierärztliche Wochenschrift* **115**, 445-452.

Hintergrund, Ansatz und Ziel:

Es wurde in den letzten Jahren zunehmend diskutiert, dass die Mutter-Kind-Beziehung bei Hauschweinen nicht nur von aktuell wirkenden proximaten Verhaltensursachen („Wie“ des Verhaltens), sondern – trotz langjähriger Domestikation und künstlicher Selektion – auch von einer Reihe ultimater Verhaltensursachen („Warum“ des Verhaltens) bestimmt wird (Puppe, 1995; FRASER *et al.*, 1995; DRAKE *et al.*, 2008). Das beinhaltet vor allem evolutionäre und soziobiologische Erklärungsmodelle, wie Eltern-Nachkommen-Konflikt, Geschwisterkonkurrenz und Absetzkonflikt, in denen das Sozialverhalten im Sinne einer individuellen Fitnessmaximierung beim Konflikt über die Verteilung von Ressourcen erklärt wird. Hier bildet die Sau-Ferkel-Beziehung mit ihrem komplexen Saug-Säugeverhalten ein ideales System zur Erforschung sowohl ultimater als auch proximater Zusammenhänge mit Implikationen für Theorie und Praxis. Ausgehend von den Ergebnissen und Konsequenzen der Studien 1 (siehe Kapitel 3.1.1.1) und 2 (siehe Kapitel 3.1.1.2) sowie diesbezüglichen Ergebnissen aus der Literatur wird in der Studie ein Überblick gegeben, inwieweit derartige Prinzipien in der Mutter-Kind-Beziehung beim Hausschwein eine Rolle spielen und welche Beziehung zu aktuellen Tendenzen in der Schweinehaltung hergestellt werden können.

Ergebnisse und Schlussfolgerungen:

Die Saug-Säugeinteraktion zwischen Ferkel und Sau ist in vielen Punkten mittels soziobiologischer Erklärungsmodelle interpretierbar, beeinflusst die Ontogenese der Tiere, letztlich auch ihre vom Menschen genutzten Leistungen und erweitert somit das Wissen um soziale Zusammenhänge. Mittels unserer Untersuchungen konnte beispielsweise gezeigt werden, dass die Ferkel als eine Art Gegenstrategie zum mütterlichen Investitionsentzug ihre höchste Saugstabilität dann erreichen, wenn das Maximum der Saughäufigkeit zu fallen beginnt (siehe Abb. 2 der Studie). Es scheint, dass auch die mütterliche Laktationsleistung zeitversetzt durch diese Verhaltensmuster beeinflusst wird (siehe Abbildung 3 der Studie). Darüber hinaus lassen sich ebenso andere wichtige Komponenten in der Haltung, wie Alter der Sauen und Zeitpunkt des Absetzens, mittels soziobiologischer Ideen diskutieren. Die genaue Kenntnis proximater Mechanismen und vor allem ultimater Strategien beim Sozialverhalten wird zunehmend bedeutsamer, je mehr Gesetzgebung und ethisch geprägte Vorstellungen im Tierschutz den Tieren wieder größere Freiräume, ihr „natürliches“ Sozialverhalten auszuleben, ermöglichen. Die Studie leistet hierzu einen wichtigen Beitrag, indem sie entsprechende Ideen aus der Grundlagenforschung der Verhaltensbiologie einführt bzw. vorstellt und Implikationen für die angewandte Forschung beim Schwein zieht.

3.1.2 Sozial-akustische Erkennungsmechanismen in der Sau-Ferkel-Beziehung

Publikationen:

Studie 4

Schön, P.C., Puppe, B., Gromeyko, T. & Manteuffel, G. (1999): Common features and individual differences in nurse grunting of domestic pigs *(Sus scrofa)*: a multi-parametric analysis. *Behaviour* **136**, 49-66.

Studie 5

Puppe, B., Schön, P.C., Tuchscherer, A. & Manteuffel, G. (2003): The influence of domestic piglets' *(Sus scrofa)* age and test experience on the preference for the replayed maternal nursing vocalisation in a modified open-field test. *Acta Ethologica* **5**, 123-129.

3.1.2.1 Individualität und Inter-Individualität in der Säugevokalisation der Sau

Studie 4 (Kumulative Publikationsliste, Kapitel 7.4)

Schön, P.C., Puppe, B., Gromeyko, T. *&* Manteuffel, G. (1999): Common features and individual differences in nurse grunting of domestic pigs *(Sus scrofa)*: a multi-parametric analysis. *Behaviour* **136**, 49-66.

Hintergrund, Ansatz und Ziel:

Soziale Interaktionen zwischen Individuen werden u.a. maßgeblich durch akustische Signale gesteuert. Das Säugegrunzen der Sau ist ein im Grunde genommen einzigartiger Vokalisationstyp bei Säugetieren, da es in repetitiver Weise über mehrere Minuten andauert, den Kontakt zu den Ferkeln beim Saugakt initiiert und diese dann durch die verschiedenen Phasen ihres Saugverhaltens führt und synchronisiert (FRASER, 1980). In einem speziellen, schallgedämmten Akustiklabor, das die Haltung von Grosstieren ermöglicht (SCHÖN *et al.*, 1998), nahmen wir sowohl das mütterliche Säugegrunzen von 5 Sauen als auch das entsprechende Saugverhalten ihrer Ferkel über die Säugeperiode von 5 Wochen per Video auf. Die Laute wurden mittels moderner bioakustischer Verfahren im Zeit- und Frequenzbereich analysiert, über eine Diskriminanzanalyse statistisch ausgewertet und zum Verhalten der Ferkel in Beziehung gesetzt. Ziel war es, potentielle gemeinsame Merkmale und individuelle Unterschiede im Säugegrunzen zu finden, die den Ferkeln die Verhaltenskontext- und Individualerkennung ermöglichen.

Ergebnisse und Schlussfolgerungen:

Die elementare Einheit der Säugevokalisation ist ein kurzer atonaler (< 0.4 s), sich stereotyp wiederholender Grunzlaut, bestehend aus einem Frequenzgemisch mit einem Maximum an Energie im Frequenzbereich unter 1 kHz. Wir fanden eine deutliche Übereinstimmung in der Zeitstruktur des Säugegrunzens zwischen den Sauen und folgerichtig nur einen unwesentlichen Einfluss des Individuums bei den akustischen Parametern des Zeitbereichs. Die Grunzrate verdoppelte sich in typischer Weise ca. 20 s vor dem bevorstehenden Milchfluss (siehe Abbildung 5 der Studie) – ein Zeichen für die Ferkel, ihr Saugverhalten darauf auszurichten. Alle drei gewählten Lautmodelle im Frequenzbereich (multiparametrischer Ansatz, Amplitudenspektrum, Cepstrum) ergaben eine extrem hohe Individualität der Laute, d.h. es war durch die Diskriminanzanalyse eine richtige individuelle Zuordnung der Laute, z.B. zu 96.6 % im Falle der Cepstrumanalyse möglich (siehe Abb. 6 und 7 der Studie). Aus den Ergebnissen schlussfolgern wir, dass der zeitliche Verlauf der Säugevokalisation vergleichsweise invariant ist und ein typisches inter-individuelles Merkmal darstellt, d.h. ein generelles Erkennen des Verhaltenskontextes für alle Ferkel ermöglicht. Die individuelle Erkennung der Muttersau scheint dagegen über die offensichtlich erlernte Mustererkennung der jeweiligen individuellen Frequenzmischung durch ihre Ferkel möglich.

3.1.2.2 Akustische Muttererkennung durch die Ferkel

Studie 5 (Kumulative Publikationsliste, Kapitel 7.5)

Puppe, B., Schön, P.C., Tuchscherer, A. & Manteuffel, G. (2003): The influence of domestic piglets' *(Sus scrofa)* age and test experience on the preference for the replayed maternal nursing vocalisation in a modified open-field test. *Acta Ethologica* **5**, 123-129.

Hintergrund, Ansatz und Ziel:

Die Erkennung der Muttersau im Kontext des Saugverhaltens ist eine überlebenswichtige Notwendigkeit für die Ferkel. Wir konnten in der vorangegangenen Studie zeigen (siehe Studie 4, Kapitel 3.1.2.1), dass akustische Merkmale in der Säugevokalisation potentiell existieren, die den Verhaltenskontext (d.h. einen bevorstehenden Saugakt) anzeigen, aber auch eine individuelle Erkennung ermöglichen. Um die tatsächlichen Reaktionen der Ferkel unter standardisierten Bedingungen zu testen, führten wir in der jetzigen Studie eine Reihe von replay-Experimenten durch und analysierten dabei das open-field- und Wahlverhalten der Ferkel in unserem Akustiklabor für Grosstiere (SCHÖN *et al.*, 1998) in einer eigens dafür entwickelten Testanlage (PUPPE *et al.*, 1999). Wir führten die Studie an insgesamt 135 Saugferkeln in der 1. und 5. Lebenswoche durch. Unser Ziel war es, die Reaktionen von Ferkeln auf die Säugevokalisation der eigenen Muttersau, die Säugevokalisation einer unbekannten Sau, einen künstlich produzierten und dem Frequenzbereich der Säugevokalisation nachempfundenen Laut sowie einer Kontrolle ohne Lauteinspielung zu testen.

Ergebnisse und Schlussfolgerungen:

Alle vorgespielten „echten" Säugevokalisationen initiierten eine generalisierte Annäherungsreaktion der Ferkel in ihrer 1. Lebenswoche. In der Nähe der Soundquelle präferierten die Ferkel signifikant das mütterliche Säugegrunzen gegenüber dem einer fremden Sau und dem künstlichen Laut (siehe Abbildung 2 der Studie). In der 5. Lebenswoche waren die Unterschiede nicht mehr vorhanden und das auch unabhängig von einer früheren Testerfahrung. Zusätzlich verminderte die Vokalisation der eigenen Muttersau die open-field-Aktivität der Ferkel, was wir als starkes Indiz für einen beruhigenden Effekt durch die Mutter werteten. Diese Ergebnisse bestätigen nachhaltig unsere gefundenen Belege für individuelle und inter-individuelle akustische Merkmale in der Säugevokalisation aus der Studie 4 (siehe Kapitel 3.1.2.1). So schlussfolgern wir, dass Ferkel aus dem Distanzfeld offensichtlich die inter-individuelle Bedeutung der Säugevokalisation als solches erkennen können, im Nahfeld dagegen wird die eigene Mutter anhand ihrer individuell-akustischen Merkmale erkannt und bevorzugt. Die fehlenden Reaktionsunterschiede in der 5. Lebenswoche deuten auf die zunehmende Unabhängigkeit der Ferkel von direkter mütterlicher Unterstützung hin. Bezogen auf die landwirtschaftliche Haltung unterstreichen die Befunde, dass Haltungsformen, die auf eine funktionierende soziale Kommunikation der Tiere zielen (z.B. Formen der gemeinsamen Gruppenhaltung von Sauen und Ferkeln), (verhaltens)biologisch grundsätzlich möglich sind und den Fähigkeiten und Bedürfnissen der Tiere entgegenkommen.

3.1.3 Auswirkungen sozialer Deprivation beim Saugferkel

Publikationen:

Studie 6

Kanitz, E., Tuchscherer, M., Puppe, B., Tuchscherer, A. *&* Stabenow, B. (2004): Consequences of repeated early isolation in domestic piglets *(Sus scrofa)* on their behavioural, neuroendocrine, and immunological responses. *Brain, Behavior, and Immunity* **18**, 35-45.

Studie 7

Tuchscherer, M., Kanitz, E., Puppe, B. *&* Tuchscherer, A. (2006): Early social isolation alters behavioural and physiological responses to an endotoxin challenge in piglets. *Hormones and Behavior* **50**, 753-761.

3.1.3.1 Psychoneuroendokrine und immunologische Konsequenzen

Studie 6 (Kumulative Publikationsliste, Kapitel 7.6)

Kanitz, E., Tuchscherer, M., Puppe, B., Tuchscherer, A. & Stabenow, B. (2004): Consequences of repeated early isolation in domestic piglets *(Sus scrofa)* on their behavioural, neuroendocrine, and immunological responses. *Brain, Behavior, and Immunity* **18**, 35-45.

Hintergrund, Ansatz und Ziel:

Für Schweine als ausgesprochen sozial lebende Tierart bedeuten jegliche Störungen sozialer Interaktionen erhebliche Beeinträchtigungen ihrer diesbezüglichen Verhaltensansprüche, so dass vielfältige negative Konsequenzen angenommen werden müssen. Die soziale Deprivation von Saugferkeln, d.h. die totale Isolation von Mutter und Wurfgeschwistern in einer frühen, sensiblen Phase ihrer Ontogenese, stellt daher einen Extremfall sozialer Beeinträchtigung dar. Wir haben sie deshalb als ein geeignetes Modell zur Untersuchung psychisch-vermittelten Stresses gewählt, um zunächst grundlegende Folgen für Verhalten, Stressantwort und Gesundheit zu untersuchen. In dieser Studie isolierten wir 90 Saugferkel aus 9 Würfen vom 3. bis 11. Lebenstag für jeweils 2 h täglich und untersuchten ihr Verhalten im open-field, ihre neuroendokrinologischen Stressreaktionen sowie ihre Immunreaktionen im Vergleich zu 89 nichtisolierten Kontrollferkeln aus 9 Würfen. Die Blutentnahmen zur Analyse der physiologischen Werte erfolgten durch Punktion der *Vena cava cranialis* in Rückenlage der Tiere (Zeitdauer < 30 s). Unser Ziel war es, durch einen komplexen, interdisziplinären Ansatz die vernetzten Mechanismen der beteiligten Systeme anzusprechen und kurz- und langfristige Folgen dieses psychosozialen Stressmodells bei Schweinen zu untersuchen.

Ergebnisse und Schlussfolgerungen:

Wir konnten zeigen, dass eine wiederholte soziale Isolation neonataler Ferkel zu einer verminderten Verhaltensaktivität (Lokomotion, Vokalisation) im open-field Test führte (siehe Abbildung 1 der Studie). Weiterhin wiesen die Tiere erhöhte basale Kortisolwerte und eine verminderte Lymphozytenproliferation *in vitro* gegenüber verschiedenen Mitogenen auf, während die Konzentrationen von Interleukin-1β im Hippokampus erhöht waren. Sechs Wochen nach der Isolation konnten wir erhöhte basale Plasma-ACTH- und Interleukin-1ß-Werte sowie eine erhöhte Bindungskapazität des Glukokortikoidrezeptors im Hippokampus nachweisen. Die Konzentrationen des Kortikotropin-Releasing-Hormons (CRH) im Hypothalamus waren erniedrigt, in der Amygdala als emotionalem Bewertungszentrum (Furcht, Angst) dagegen erhöht. Daraus schlussfolgern wir, dass das verwendete Stressmodell die Verhaltensreaktivität in Richtung depressiv zu wertender Zustände der Tiere beeinflusst und die Aktivität der hypothalamo-hypophysär-adrenalen Achse (HPA-Achse) sowie die neuroimmunologische Regulation des Immunsystems langfristig verändern kann. Das impliziert negative Konsequenzen für Wohlbefinden und Gesundheit der Tiere (z.B. beim Absetzen), wenn sie in der Haltung frühzeitig psychisch vermitteltem Stress ausgesetzt sind. Darüber hinaus belegt dieses Vorgehen, dass Schweine in derartigen Versuchsansätzen als geeignete Modelltiere für die Untersuchungen psychosomatischer Probleme beim Menschen dienen könnten.

3.1.3.2 Reaktionen gegenüber einem Endotoxin-Challenge

Studie 7 (Kumulative Publikationsliste, Kapitel 7.7)

Tuchscherer, M., Kanitz, E., Puppe, B. & Tuchscherer, A. (2006): Early social isolation alters behavioural and physiological responses to an endotoxin challenge in piglets. *Hormones and Behavior* **50**, 753-761.

Hintergrund, Ansatz und Ziel:

Wir konnten in der vorangegangenen Studie zeigen (siehe Studie 6, Kapitel 3.1.3.1), dass frühzeitige und wiederholte psychosoziale Stresserfahrungen nachhaltige Veränderungen in psychophysiologischen Mechanismen der Stressbewältigung von Ferkeln bewirken. Die Frage der jetzigen Studie war deshalb, ob und inwieweit sich bestimmte Verhaltens-, Hormon- und Immunantworten von Ferkeln mit diesem Stressparadigma von ungestressten Kontrolltieren unterscheiden, wenn sie zusätzlich einem Endotoxin-Challenge unterzogen werden. Dazu verwendeten wir Lipopolysaccharid (LPS) aus der Membran gramnegativer Bakterien, das als standardisiertes Modell der Infektion mit einem Krankheitserreger eingesetzt wird, um Infektabwehrreaktionen zu simulieren. Die LPS-Injektion (bzw. Kochsalzlösung als Kontrolle) erfolgte einen Tag nach Ende der Stressbehandlung sowie 6 Wochen danach. Das gesamte Versuchsdesign umfasste 144 Ferkel. Unser Ziel war es, die Auswirkungen sozialen Stresses auf die beteiligten Subsysteme und ihre Interaktionen bei der späteren Krankheitsbewältigung zu untersuchen.

Ergebnisse und Schlussfolgerungen:

Entsprechend unseren Erwartungen induzierte die LPS-Injektion deutliche Anzeichen von Krankheitsverhalten (z.B. Zittern, Erbrechen, Somnolenz) bei allen Ferkeln (siehe Tabelle 1 der Studie) und zog jeweils erhöhte Werte von ACTH, Kortisol und des Tumornekrosefaktors TNFα nach sich. Die zuvor gestressten Tiere zeigten nach LPS-Injektion jedoch anhaltend deutlicheres Krankheitsverhalten und längere Latenzzeiten sowie eine kürzere Dauer beim anschließenden Saugverhalten an der Mutter. Insbesondere das verstärkte Krankheitsverhalten interpretierten wir als Versuch der Tiere, zunächst durch entsprechendes Verhalten die Homöostase wiederherzustellen. Die Konzentrationen an TNFα waren langfristig erniedrigt, die Stresshormonkonzentrationen dagegen nicht beeinträchtigt. Wir konnten aber mittels multipler Regressionsanalysen nachweisen, dass die Korrelationen zwischen Krankheitsverhalten (z.B. Somnolenz bzw. Zittern) und veränderten physiologischen Werten (z.B. Kortisol und TNFα bzw. Kortisol und Kortikosteroidbindungsglobulin CBG) deutlich enger bei zuvor gestressten Tieren waren als bei ungestressten Tieren (siehe Abb. 3 der Studie). Dies spricht für eine generelle Sensitivierung der vernetzten adaptiven Antwort eines Organismus durch frühe psychisch vermittelte soziale Stresserfahrungen und kann zu Problemen bei der späteren Krankheitsbewältigung führen. Insgesamt schlussfolgern wir, dass durch die grundlagenorientierte, interdisziplinäre Untersuchung von psychosozialem Stress das Verständnis der Bewältigung von Stress und Krankheit deutlich verbessert wird. Das trägt dazu bei, entsprechende Haltungs- und Managementverfahren verstärkt dahingehend zu beurteilen, inwieweit Tiere erfolgreich mit ihnen zurechtkommen.

3.2 Agonistisches Verhalten, soziale Hierarchie und Dominanzstruktur

3.2.1 Entwicklung des sozial-agonistischen Verhaltens nach Absetzen der Ferkel

Publikationen:

Studie 8

Puppe, B. (1998): Effects of familiarity and relatedness on agonistic pair relationships in newly mixed domestic pigs. *Applied Animal Behaviour Science* **58**, 233-239.

Studie 9

Puppe, B., Tuchscherer, M. & Tuchscherer, A. (1997): The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. *Livestock Production Science* **48**, 157-164.

3.2.1.1 Effekte von Bekanntheit und Verwandtschaft

Studie 8 (Kumulative Publikationsliste, Kapitel 7.8)

Puppe, B. (1998): Effects of familiarity and relatedness on agonistic pair relationships in newly mixed domestic pigs. *Applied Animal Behaviour Science* **58**, 233-239.

Hintergrund, Ansatz und Ziel:

Das Neugruppieren von Tieren in der Tierhaltung, d.h. das Neuzusammenbringen sich überwiegend unbekannter Tiere, ist eine Quelle sozialen Stresses durch die hierbei initiierten agonistischen Auseinandersetzungen zwischen den Individuen. Innerhalb dieses Funktionskreises des Verhaltens sollten deshalb individuelle Erkennungsmechanismen eine entscheidende Rolle spielen. In dieser Studie wurden daher die agonistischen Auseinandersetzungen an insgesamt 144 Schweinen in 16 neugemischten Gruppen im Alter von 12 Wochen untersucht. Das Ziel war es herauszufinden, ob und inwieweit sich verwandte und vorher bekannte Individuenpaare (124 Dyaden) in ihrem agonistischen Verhalten von sich unbekannten Tieren (452 Dyaden), die entweder verwandt (Geschwister bzw. Halbgeschwister, die unmittelbar nach Geburt getrennt und nicht zusammen gehalten wurden) oder nicht verwandt waren, unterschieden. Zusätzlich wurde der Ort der Auseinandersetzungen (Bucht- oder Trogbereich) protokolliert, um die mögliche Ressource (z.B. sozialer Rangplatz oder Futter) als potentielles Ziel der Kämpfe zu berücksichtigen.

Ergebnisse und Schlussfolgerungen:

Während die Tiere in bekannten als auch in unbekannten Dyaden eine ähnliche Häufigkeit agonistischer Auseinandersetzungen im Trogbereich hatten, waren unbekannte Dyaden signifikant häufiger in Kämpfe im Buchtbereich verwickelt als einander bekannte Tierpaare. Die Verwandtschaft der Tiere hatte offensichtlich keinen Einfluss auf die beobachtete Häufigkeit an agonistischen Auseinandersetzungen. Von insgesamt 5354 beobachteten Kämpfen fanden deutlich mehr im Buchtbereich (3639) als im Trogbereich (1715) statt. Daraus kann geschlussfolgert werden, dass die Motivation für die Kämpfe unterschiedlich ist. Bei unbekannten Tieren scheint es primär um die Klärung der sozialen Dominanzbeziehungen zu gehen, was eine Determination des zukünftigen Zuganges zu Ressourcen einschließt. Eine alternative bzw. ergänzende Erklärung wäre, dass Schweine generell aggressiv auf fremde Subjekte reagieren – möglicherweise als eine besondere Form sozialen Erkundungsverhaltens zur Abschätzung der relativen Kampffähigkeit des Opponenten. Im Gegensatz zu Berichten bei anderen Spezies (z.B. Mäusen) scheint es dagegen keine *a priori* Verwandtenerkennung zu geben; die agonistischen Beziehungen zu anderen Individuen scheinen eher auf erlernter Bekanntschaft zu beruhen. Gruppenzusammenstellungen in der modernen Tierhaltung sollten dies berücksichtigen und/oder den Tieren ausreichend Zeit geben, um ihre sozialen Beziehungen zu klären.

3.2.1.2 Einfluss der sozialen Umwelt und der Haltungsumwelt

Studie 9 (Kumulative Publikationsliste, Kapitel 7.9)

Puppe, B., Tuchscherer, M. *&* Tuchscherer, A. (1997): The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. *Livestock Production Science* **48**, 157-164.

Hintergrund, Ansatz und Ziel:

Das Absetzen der Ferkel von der Mutter ist ein komplexer Prozess, der neben den sozialen (Verlust der Mutter und Wurfgeschwister, Neugruppierung) und nutritiven Veränderungen meist auch eine Veränderung der Umwelt in Form einer neuen Haltungsumwelt einschließt. Das hat nachgewiesenermaßen stressbedingte Konsequenzen für die Tiere und kann in der Praxis zu Krankheiten und Leistungsdepressionen führen. Die vorangegangene Studie (siehe Studie 8, Kapitel 3.2.1.1) hat gezeigt, dass die Bekanntheit mit sozialen Umweltfaktoren (hier: einander bekannte Dyaden in einer Neugruppierungssituation) die sozial-agonistischen Auseinandersetzungen der jeweiligen Tierpaare verringern kann. Dieser Ansatz wurde jetzt auf ganze Gruppen übertragen. An insgesamt 16 Gruppen in einem 2×2-Faktoren-Design wurde untersucht, inwieweit die soziale Umwelt (wurfweises Belassen *vs.* Neugruppieren) und die Haltungsumwelt (Belassen in der gewohnten Haltungsumwelt *vs.* Umstallen) das agonistische Verhalten der Gruppe, das Verhältnis neutrophiler Granulozyten zu Lymphozyten (N/L) als einfach zu messender Parameter für die zelluläre Immunreaktion der Tiere und die Plasma-Glukosewerte der Individuen 1 und 4 Tage nach dem Absetzen beeinflussen (Kontrolle: 1 Tag vor Absetzen). Unser Ziel war es, verhaltensphysiologische Stressreaktionen der Tiere bei diesen verschiedenen Absetzvarianten zu beurteilen.

Ergebnisse und Schlussfolgerungen:

Aus den Ergebnissen wird deutlich, dass das agonistische Verhalten sowohl von der Haltungsumwelt als auch der sozialen Umwelt beeinflusst wurde und die höchsten Werte 4 Tage nach dem Absetzen erreichte. Die Glukose- und N/L-Werte wurden dagegen nur durch die Haltungsumwelt beeinflusst und erreichten ihr höchstes Niveau 1 Tag nach Absetzen. Während die physiologischen Werte eher kurzfristigen, transienten Stress indizieren, lässt sich am Verhalten der Tiere erkennen, dass ihre adaptiven Reaktionen, die neue Situation zu bewältigen, weiter herausgefordert werden, d.h. insbesondere die Notwendigkeit, ihre sozialen Beziehungen neu zu etablieren. Wir schlussfolgern insgesamt, das abgesetzte Ferkel mehr Probleme haben mit der neuen Haltungsumgebung als mit ihnen unbekannten Ferkeln zurechtzukommen. Das sehen wir dadurch begründet, dass in dieser Phase der Ontogenese die soziale Anpassungsfähigkeit vergleichsweise hoch zu sein scheint und ein „physiologisches Stressprogramm“ gegen Individuen gleichen Alters eher unwahrscheinlich ist. Damit wird die evolvierte Fähigkeit von Ferkeln dieses Altersbereichs unterstrichen, sozial miteinander zu interagieren – Grundvoraussetzung für entsprechende Gruppenhaltungen und/oder das Mischen bzw. Neugruppieren von Tieren.

3.2.2 Dominanzstruktur und Soziometrie beim Hausschwein

Publikationen:

Studie 10

Langbein, J. *&* Puppe, B. (2004): Analysing dominance relationships by sociometric methods – a plea for a more standardised and precise approach in farm animals. *Applied Animal Behaviour Science* **87**, 163-182.

Studie 11

Puppe, B., Langbein, J., Bauer, J. *&* Hoy, S. (2008): A comparative view on social hierarchy formation at different stages of pig production using sociometric measures. *Livestock Science* **113**, 155-162.

3.2.2.1 Soziometrische Methoden bei der Dominanzstrukturanalyse

Studie 10 (Kumulative Publikationsliste, Kapitel 7.10)

Langbein, J. & Puppe, B. (2004): Analysing dominance relationships by sociometric methods – a plea for a more standardised and precise approach in farm animals. *Applied Animal Behaviour Science* **87**, 163-182.

Hintergrund, Ansatz und Ziel:

Eines der wichtigsten Konzepte im Sozialverhalten von Tieren ist das der sozialen Dominanz, das gewöhnlich benutzt wird, um das Ergebnis einer Vielzahl kompetitiver Interaktionen zu charakterisieren, in deren Konsequenz eine soziale Hierarchie entsteht. Das Problem ist, dass Dominanz im Grunde ein Synonym für einen multidimensionalen Kontext ist, der nur nachvollziehbar beschreibbar wird, wenn spezies-, definitions- und methodenspezifische Details klar dargelegt werden (PUPPE, 1996b). Dabei ist Dominanz eine gebräuchliche Beschreibung für den Zustand der Imbalance bzw. Asymmetrie in einer Zweierbeziehung (Dyade), während hingegen der soziale Rang eines Tieres einen Aspekt seiner Rollenfunktion in einer hierarchischen Gruppenstruktur darstellt. Dies aufgreifend wollten wir in der Studie einen komplexen Ansatz entwickeln, um – ausgehend von einer strukturellen Dominanzdefinition (DREWS, 1993) – Dominanzbeziehungen auf verschiedenen sozialen Ebenen (Dyade, Gruppe, Individuum) mittels soziometrischer Kenngrößen zu analysieren. Unser Ziel war es, erstens durch eine methodische Vereinheitlichung ein verbessertes und vergleichbares wissenschaftliches Herangehen zu erreichen und zweitens damit die methodische Grundlage zu schaffen, soziale Dominanzbeziehungen bei landwirtschaftlichen Nutztieren umfassend, konsistent und vergleichend untersuchen zu können.

Ergebnisse und Schlussfolgerungen:

Entsprechend unserem Ausgangspunkt haben wir mit der vorliegenden Studie eine methodische Verfahrensweise vorgeschlagen, soziale Dominanzbeziehungen in Tiergruppen schrittweise über die Beobachtung und Auswertung der dyadischen agonistischen Interaktionen durch eine Reihe soziometrischer Kenngrößen – ausgehend von der Dyade, dann für die soziale Gruppe und letztlich auf Ebene des Individuums – strukturell zu analysieren (siehe Tabelle 1 der Studie). Anhand einer Fallstudie bei Hausschweinen und Zwergziegen haben wir darüber hinaus die grundsätzliche Anwendbarkeit aller Methoden beispielhaft dargestellt (siehe Tabellen 1 und 2 der Studie). Wir schlussfolgern, dass die weitverbreitete Verwendung individueller sozialer Rangplätze (und damit auch deren Beziehung zu anderen verhaltensphysiologischen Merkmalen) wissenschaftlich nur dann gerechtfertigt ist, wenn soziometrische Kenngrößen auf Ebene der Dyade (z.B. Asymmetrie) und der Gruppe (z.B. Hierarchiestärke) dies im konkreten Fall erlauben oder wenn sie zumindest für die untersuchte Spezies in vergleichbarer Weise nachgewiesen sind. Somit wird nicht nur ein umfassendes Bild sozialer Dominanzbeziehungen gegeben, sondern auch die Möglichkeit eröffnet, beobachtete Verhaltensunterschiede zwischen Spezies und innerhalb einer Spezies zu erklären (z.B. bei Haltungsunterschieden oder zwischen verschiedenen ontogenetischen Stadien).

3.2.2.2 Soziale Hierarchie in verschiedenen Haltungsabschnitten

Studie 11 (Kumulative Publikationsliste, Kapitel 7.11)

Puppe, B., Langbein, J., Bauer, J. & Hoy, S. (2008): A comparative view on social hierarchy formation at different stages of pig production using sociometric measures. *Livestock Science* **113**, 155-162.

Hintergrund, Ansatz und Ziel:

Das Halten in sozialen Gruppen wird zunehmend aus Tierschutzgründen favorisiert (z.B. Forderung der Europäischen Union nach genereller sozialer Haltung bei Sauen). Wir haben in der vorangegangenen Studie einen komplexen Ansatz zur Analyse sozialer Hierarchien bei Nutztieren entwickelt (siehe Studie 10, Kapitel 3.2.2.1). Soziale Hausschweingruppen werden in den verschiedenen Haltungsabschnitten der modernen Schweineproduktion in der Regel neu gemischt und zusammengestellt. Für die Tiere hat das die Konsequenz vermehrter agonistischer Interaktionen, in deren Folge neue soziale Hierarchien etabliert werden. In der vorliegenden Studie haben wir uns deshalb das Ziel gesetzt, in drei typischen Haltungsabschnitten (Absetzferkel, Mastschweine, reproduktive Sauen) die soziale Hierarchie unmittelbar nach dem Neuzusammenstellen mittels soziometrischer Kenngrößen auf der Ebene der Dyade und der Gruppe umfassend zu charakterisieren. Hierzu beobachteten wir bei insgesamt 57 Gruppen (je 8-10 Tiere) das sozial-agonistische Verhalten der sich jeweils unbekannten Tiere und kalkulierten auf dieser Basis die entsprechenden sozialen Kenngrößen und interpretierten diese methodisch und praktisch.

Ergebnisse und Schlussfolgerungen:

Belegt u.a. durch die entsprechenden Kendall-Indizes (Werte zwischen 0.61 und 0.71) bzw. korrigierten Landau-Indizes (Werte zwischen 0,70 und 0.72) entwickelten die Tiere in allen Haltungsabschnitten eine quasi-lineare Hierarchie. Im Vergleich mit abgesetzten Ferkeln und Mastschweinen benötigten Sauen bei deren Etablierung signifikant weniger agonistische Interaktionen und zeigten Unterschiede bei der sozialen Organisation (mehr unbekannte, aber weniger bidirektionale und signifikant asymmetrische Dyaden, insgesamt höhere Gerichtetheit der Linearität). Wir erklären das dadurch, dass Sauen es im Verlauf der Ontogenese gelernt haben, ihre sozialen Beziehungen effektiv zu regulieren, während die jüngeren Tiere einen Teil der agonistischen Interaktionen auch dazu verwenden müssen, um soziale Erfahrungen zu sammeln. Dies bei den letzteren in adäquater Form durch entsprechend Managementmaßnahmen zu gewährleisten (z.B. soziales Mischen unter kontrollierten Bedingungen), kann die soziale Kompetenz der Tiere erhöhen. Die Charakterisierung des sozialen Verhaltens durch standardisierte soziometrische Kenngrößen sind ein von uns vorgeschlagener, wissenschaftlich fundierter Zugang, der auch in der Tierzucht Berücksichtigung finden sollte (z.B. bei der Selektion von Tieren nicht nur nach Leistungs-, sondern auch nach Verhaltenskriterien). Detaillierte Kenntnisse der sozialen Hierarchiebeziehungen und deren ontogenetische Entwicklung können helfen, sozial bedingten Stress zu vermeiden und somit Haltung, Management und Wohlbefinden der Tiere zu verbessern.

3.2.3 Konsequenzen sozialer Dominanzstrukturen beim Hausschwein

Publikationen:

Studie 12

Tuchscherer, M., Puppe, B., Tuchscherer, A. *&* Kanitz, E. (1998): Effects of social status after mixing on immune, metabolic, and endocrine respones in pigs. *Physiology & Behavior* **64**, 353-360.

Studie 13

Otten, W., Puppe, B., Kanitz, E., Schön, P.C. *&* Stabenow, B. (1999): Effects of dominance and familiarity on behaviour and plasma stress hormones in growing pigs during social confrontation. *Journal of Veterinary Medicine, Series A* **46**, 277-292.

Studie 14

Otten, W., Puppe, B., Kanitz, E., Schön, P.C. & Stabenow, B. (2002): Physiological and behavioral effects of different success during social confrontation in pigs with prior dominance experience. *Physiology & Behavior* **75**, 127-133.

3.2.3.1 Verhaltensimmunologische Effekte von Dominanz

Studie 12 (Kumulative Publikationsliste, Kapitel 7.12)

Tuchscherer, M., Puppe, B., Tuchscherer, A. & Kanitz, E. (1998): Effects of social status after mixing on immune, metabolic, and endocrine responses in pigs. *Physiology & Behavior* **64**, 353-360.

Hintergrund, Ansatz und Ziel:

Wir haben in den vorangegangenen Studien zeigen können, dass das praxisübliche Neugruppieren sich unbekannter Tiere in einer neuen Haltungsumwelt offensichtlich eine soziale Stresssituation darstellt, die mit erheblichen agonistischen Auseinandersetzungen verbunden ist (siehe Studien 8 und 9, Kapitel 3.2.1.1 und 3.2.2.1) und zur Etablierung einer nachweisbaren Dominanzhierarchie führt (siehe Studien 10 und 11, Kapitel 3.2.2.1 und 3.2.2.2). Der letztendlich erreichte soziale Status in der Gruppenhierarchie lässt jedoch den Schluss zu, dass die Tiere unterschiedlich erfolgreich in der Bewältigung dieses sozialen Stresses waren: dominante Tiere waren erfolgreicher, subdominante Tiere dagegen weniger erfolgreich. Ausgehend vom postulierten Zusammenhang zwischen sozialem Bewältigungsverhalten, Stress und Krankheit (siehe HENRY & STEPHENS, 1977; VON HOLST, 1998) war unsere These deshalb, dass der Dominanzstatus psychophysiologisch unterschiedliche Auswirkungen auf das Immunsystem und die Krankheitsanfälligkeit haben sollte. In der vorliegenden Studie sowie auch in den nachfolgenden Studien 13 und 14 (Kapitel 3.2.3.2 und 3.2.3.3) konnten – wie in den vorhergehenden Studien 10 und 11 (siehe Kapitel 3.2.2.1 und 3.2.2.2) hergeleitet – die Dominanzwerte (DV) zwischen +1.00 (absolut dominant) und -1.00 (absolut subdominant) liegen. Wir untersuchten an insgesamt 10 neu zusammengestellten Tiergruppen (9 Schweine je Gruppe, 12 Wochen alt), ob und inwieweit der mittels des Ausgangs der agonistischen Interaktionen kalkulierte Dominanzstatus der Tiere in der Gruppe (dominant: DV > 0.00 *vs.* subdominant: DV ≤ 0.00) ihre zelluläre und humorale Immunantwort beeinflusste. Die Blutentnahmen zur Analyse der physiologischen Werte erfolgte durch Punktion der *Vena cava cranialis* in Rückenlage der Tiere (Zeitdauer < 30 s) 1 Tag vor und 3 Tage nach dem Gruppieren. Ähnlich wie bei Studie 6 (siehe Kapitel 3.1.3.1) wurde die mitogen-induzierte Zellproliferation als ein *in vitro* Index für die zelluläre Immunfunktion verwendet. Darüber hinaus wurden weitere stressrelevante endokrine und stoffwechselphysiologische Parameter analysiert. Hauptziel aber war es, die immunphysiologischen Effekte eines akuten sozialen Stresses im Zusammenhang mit der Etablierung der Dominanzhierarchie aufzuzeigen.

Ergebnisse und Schlussfolgerungen:

Die Immunfunktion neu gruppierter, sich unbekannter Schweine wird durch sozialen Stress beeinflusst. Hochrangige, dominante Tiere reagierten mit einer verstärkten zellvermittelten Immunantwort; niedrigrangige, subdominante Tiere dagegen mit einer Immunsuppression. Dies war am deutlichsten bei der *in vitro* Stimulation von T-Lymphozyten durch das Mitogen Concanavalin A zu erkennen. Beide Effekte verstärkten sich in ihrer jeweiligen Richtung mit zunehmender Anzahl an agonistischen Interaktionen, d.h. mit der wiederholten Erfahrung der Tiere zu gewinnen oder zu verlieren. Auch die humorale Immunantwort (z.B. Immunglobulin G) der dominanten Tiere war stärker verglichen mit den subdominanten Tieren. Weder Stoffwechselmetabolite (z.B. Glukose, Freie Fettsäuren) noch Kortisolkonzentrationen waren hingegen vom Dominanzstatus beeinflusst. Das Neugruppieren selbst verursachte beispielsweise eine Erhöhung der Glukose- und Totalproteinwerte der Tiere und eine Verringerung der Kortisolwerte im Durchschnitt aller Tiere der Gruppe. Wir interpretieren die Resultate dahingehend, dass der psychosoziale Stress durch die dominanten Tiere über eine höhere Situationskontrolle und den positiven Erfolg in den Auseinandersetzungen (sowie den damit verbundenen höheren Sozialstatus) besser bewältigt wird, während subdominante Tiere damit Schwierigkeiten haben. Diese unterschiedliche Stresswahrnehmung beeinflusst – wie vorhergesagt – auch unterschiedlich das Immunsystem der Tiere und damit die potentielle Fähigkeit zur Krankheitsabwehr. Das impliziert vielfältige Beziehungen zwischen psychischer Stresswahrnehmung, Bewältigungsverhalten und Immunsystem, die teilweise endokrin, aber offensichtlich auch über andere Mechanismen vermittelt werden und zudem von Stressart, Stressdauer und weiteren Faktoren abhängen. Auf Nutztiere bezogen heißt das, dass den Tieren in ihrer Haltungsumwelt eine möglichst erfolgreiche Situationskontrolle bzw. Situationsbewältigung ermöglicht werden sollte (Eustress), um dazu beizutragen, ihre Befindlichkeiten und die Gesundheit zu verbessern.

3.2.3.2 Verhaltensendokrinologische Effekte von Dominanz und Bekanntheit

Studie 13 (Kumulative Publikationsliste, Kapitel 7.13)

Otten, W., Puppe, B., Kanitz, E., Schön, P.C. & Stabenow, B. (1999): Effects of dominance and familiarity on behaviour and plasma stress hormones in growing pigs during social confrontation. *Journal of Veterinary Medicine, Series A* **46**, 277-292.

Hintergrund, Ansatz und Ziel:

Wir haben in den vorangegangenen Studien zeigen können, dass Schweine nach dem praxisüblichen Neugruppieren eine Dominanzhierarchie aufbauen (siehe Studie 11, Kapitel 3.2.2.2), ein Vorgang, der in Gruppen einander unbekannter Tiere von besonders häufigen agonistischen Interaktionen begleitet wird (siehe Studie 8, Kapitel 3.2.1.1). Zudem fanden wir, dass der in diesen Auseinandersetzungen erreichte Dominanzstatus als eine Fähigkeit der Tiere verstanden werden kann, diese soziale Stresssituation zu bewältigen und - je nach Erfolg - das Immunsystem unterschiedlich beeinflussen kann (siehe Studie 12, Kapitel 3.2.3.1). Darüber hinaus wird vermutet, dass dahinter bestimmte unterschiedliche Bewältigungsstrategien mit konsistenten ethologischen und physiologischen Mustern stecken könnten, bei denen u.a. agonistisches Verhalten und Dominanz eine entscheidende Rolle spielen (BENUS *et al.*, 1991; HESSING *et al.*, 1993, 1994a). Unsere Frage war deshalb, inwieweit sich ehemals hoch- und niedrigrangige Testtiere nach einer zwei- bis dreiwöchigen Trennungsphase in ihre bekannte Gruppe rückintegrieren oder in eine ihnen unbekannte Gruppe integrieren lassen und mit welchen ethologischen und physiologischen

Konsequenzen dies verbunden ist (vgl. auch OTTEN *et al.*, 1997). Diese experimentelle Herangehensweise simuliert zudem modellhaft Situationen, wie sie auch in der Praxis der landwirtschaftlichen Haltung vorkommen. Um diese Frage zu beantworten, haben wir in der vorliegenden Studie bei 16 neugemischten Tiergruppen (9 Schweine je Gruppe, 12 Wochen alt) über die Kalkulation von insgesamt 8280 direkt beobachteten agonistischen Interaktionen die jeweils ranghöchsten und rangniedrigsten Individuen ermittelt (sozialer Selektionstest). Diese wurden für zwei bis drei Wochen jeweils in Einzelhaltung separiert und mit Dauerkathetern (*Vena jugularis externa*) zur frequenten Blutentnahme versehen. Anschließend brachten wir die ausgewählten Tiere für jeweils 10 h entweder zurück in ihre frühere, bekannte Gruppe oder in eine neue, ihnen unbekannte Gruppe (sozialer Konfrontationstest: modifiziertes „Resident-Intruder-Paradigma“). Dort wurde ihr agonistisches Verhalten sowie weitere Verhaltensmuster, wie Lokomotion, Exploration, Fressen, Liegen *etc.* beobachtet und über insgesamt 10 zeitlich verteilte Blutentnahmen ihre endokrine Stressantwort (Adrenalin, Noradrenalin, Kortisol) untersucht. Die Daten wurden mittels komplexer Varianz- (Repeated Design) und Korrelationsanalysen ausgewertet.

Ergebnisse und Schlussfolgerungen:

Die Rückführung der Testtiere im sozialen Konfrontationstest verursachte heftige agonistische Interaktionen vor allem während der ersten 30 min und dies signifikant häufiger in der unbekannten Gruppe. Während die niedrigrangigen Tiere generell in ihrem subdominanten Status verblieben (mittlerer Dominanzwert DV: -0.95), konnte ca. die Hälfte der vormals hochrangigen Tiere seine dominante Stellung in seiner ehemaligen Gruppe wiedererlangen (DV: +0.09) und war in der unbekannten Gruppe (DV: -0.59) zumindest erfolgreicher als die subdominanten Tiere (DV: -0.94). Die vormals hochrangigen Tiere waren tendenziell häufiger zu Beginn der Fütterungszeiten am Trog; alle anderen Verhaltensweisen wurden dagegen nicht vom Rang und der Bekanntheit beeinflusst. Die Stresshormone erreichten analog zum agonistischen Verhalten ihre höchsten Werte am Beginn des Konfrontationstests mit einem Maximum der Katecholamine bereits nach ca. 5 min und einem verzögerten Anstieg der Kortisolwerte mit Maxima zwischen ca. 20 und 80 min. In den ersten drei Stunden des sozialen Konfrontationstests waren die Katecholaminwerte der vormals hochrangigen Testtiere sowohl in ihrer bekannten als auch in der unbekannten Gruppe signifikant höher als die der vormals niedrigrangigen Tiere. Die Kortisolwerte der vormals hochrangigen Testtiere waren in der unbekannten Gruppe im Vergleich zu den vormals niedrigrangigen Tieren dramatisch erhöht, nicht aber in der bekannten Gruppe. Zum Ende des 10-stündigen Konfrontationstests waren die Unterschiede verschwunden. Die höchsten Korrelationen zwischen dem beobachteten Level an agonistischem Verhalten und gemessenen Adrenalinwerten fanden wir in der ersten Konfrontationsstunde (r=0.69), während die Korrelation zu den gemessenen Kortisolwerten (r=0.56) in der zweiten Konfrontationsstunde signifikant am besten war. Wir schlussfolgern aus den Ergebnissen, dass sowohl das agonistische Verhalten als auch die Aktivierung der beiden physiologischen Stressachsen (sympatho-adrenomedulläres System [SAM] und hypothalamo-hypophysär-adrenales System [HPA]) bei einer sozialen Konfrontation vom früheren Dominanzrang des jeweiligen Tieres und dessen Bekanntheit mit den Opponenten abhängen. Subdominante Tiere scheinen eher weiterhin passiv in derartigen sozialen Konfliktsituationen zu reagieren, d.h. sie verlieren nahezu alle ihrer Kämpfe und können folglich ihren Dominanzstatus kaum verbessern, sind aber auch an weniger agonistischen Interaktionen beteiligt und haben eine veringerte physiologische Stressreaktion. Andere Verhaltensmuster sind wenig beeinträchtigt. Dagegen scheinen dominante Tiere aktiv zu versuchen, ihren hohen Dominanzrang zu halten, was ihnen in der bekannten sozialen Gruppe teilweise, bei unbekannten Gruppen nur unzureichend gelingt. Dies wird in jedem Fall von einer unmittelbaren Aktivierung des SAM-Systems begleitet, führt allerdings nur bei Konfrontation mit einer unbekannten Gruppe (und offensichtlich unzureichender Fähigkeit, den hohen Dominanzrang zu halten) zu einer hohen akuten Kortisolausschüttung und damit zur stressbedingten Aktivierung des HPA-Systems. Die Studie stützt die These, dass verschiedene adaptive Bewältigungsmuster gegenüber sozialem Stress existieren, die aber bei Hausschweinen, in ihrer Gesamtreaktion gesehen, eher für individuelle Variationen und nicht so sehr für konsistente Strategien sprechen. Die Ergebnisse unterstreichen ebenso wie schon Studie 9 (siehe Kapitel 3.2.1.2) die hohe verhaltensphysiologische Anpassungsfähigkeit von Hausschweinen gegenüber sozialen Veränderungen, die eine generelle Voraussetzung für das praxisübliche Neugruppieren ist. Insbesondere aber beim Einführen einzelner Tiere in bestehende Gruppen (z.T. praktiziert in der Sauenhaltung) hängt die Belastung der Individuen sehr stark von der Bekanntheit mit der Gruppe und dem eigenen sozialen Status ab. Vorrangig zu Beginn dieser Prozedur kann es dabei zu Verhaltensproblemen (Aggressionen) und hohen Stressbelastungen kommen, die einer besonderen Beachtung durch das Betreuungspersonal bedürfen.

3.2.3.3 Effekte in Abhängigkeit von früheren Dominanzerfahrungen

Studie 14 (Kumulative Publikationsliste, Kapitel 7.14)

Otten, W., Puppe, B., Kanitz, E., Schön, P.C. & Stabenow, B. (2002): Physiological and behavioral effects of different success during social confrontation in pigs with prior dominance experience. *Physiology & Behavior* **75**, 127-133.

Hintergrund, Ansatz und Ziel:

Wir haben in der vorangegangenen Studie zeigen können, dass vormals dominante Schweine bei einer Rückintegration in ihre bekannte Gruppe nach einer Trennung eine große Varianz bei der Wiedererlangung ihres dominanten Status aufweisen – von absolut dominant bis absolut subdominant (siehe Studie 13, Kapitel 3.2.3.2). Ihre sympatho-adrenomedulläre Stressantwort ist insgesamt stark aktiviert, aber ebenfalls von hoher Varianz gekennzeichnet. Während der „Verliererеffekt" vergleichsweise gut untersucht ist, gibt es zum „Gewinnereffekt" widersprüchliche Ergebnisse. Unsere These lautete deshalb, dass das reaktive Verhalten eines Tieres und die sich daraus ergebenden Konsequenzen in einer Art Rückkopplung ganz offensichtlich stark von seinen eigenen sozialen Erfahrungen geprägt zu sein scheint. Ziel der vorliegenden Studie war es zu untersuchen, welche ethologischen und physiologischen Konsequenzen es für Schweine mit vorhergehender Dominanzerfahrung hat, wenn sie die aktuelle Erfahrung machen müssen, ihren Status zu verlieren. Diese wurden verglichen mit Schweinen, die ihren hohen Dominanzstatus verteidigen konnten, indem sie die erneute Erfahrung des Gewinns von Kämpfen machten. Dazu rekalkulierten wir die Daten der vorangegangenen Studie (siehe Studie 13, Kapitel 3.2.3.2) in der Weise, dass wir nur die 14 dominanten Testtiere bei der Rückkehr in ihre bekannte Gruppe betrachteten und sie entsprechend ihrer neu berechneten Dominanzwerte in erfolgreiche Tiere und nichterfolgreiche Tiere einteilten und ihre jeweiligen ethologischen und endokrinen Ergebnisse analysierten.

Ergebnisse und Schlussfolgerungen:

Die neu kalkulierten Dominanzwerte der vormals dominanten Testtiere bei Rückkehr in ihre bekannte Gruppe ergab eine klare Zweiteilung (siehe Abbildung 1 der Studie): 7 Tiere waren erfolgreich (DV: +0.78), die anderen 7 Tiere dagegen nicht (DV: -0.74). Im Vergleich zu den erfolgreichen Tieren waren die nicht erfolgreichen Tiere zu Beginn der sozialen Konfrontation an mehr Kämpfen beteiligt (die sie dann aber überwiegend verloren), zeigten insgesamt signifikant weniger exploratives sowie lokomotorisches Verhalten, aber erhöhtes Liegeverhalten. Ihre sympatho-adrenomedulläre Stressantwort (Adrenalin, Noradrenalin) war analog zum agonistischen Verhalten ebenfalls zu Beginn der sozialen Konfrontation tendenziell erhöht. Die HPA-Achse war bei allen Tieren aktiviert, aber es gab keine Unterschiede zwischen erfolgreichen und nichterfolgreichen Tieren. Zum Ende der sozialen Konfrontationstests zeigten sich insgesamt keine Unterschiede mehr in den verhaltensphysiologischen Daten erfolgreicher und nichterfolgreicher Tiere. Insgesamt schlussfolgen wir, dass die (neue) Erfahrung von Niederlagen bei ehemals dominanten Tieren ihre aktuellen Verhaltensmuster bei der Situationsbewältigung in Richtung submissives und furchtsames Verhalten ändert und eine stärkere sympatho-adrenomedulläre Stressantwort verursacht. Bei den nicht erfolgreichen Tieren wird im Vergleich zu den erfolgreichen Tieren somit verstärkter emotionaler Disstress induziert. Die gleichlaufende Aktivierung der HPA-Achse, die sich unabhängig vom Erfolg der Tiere einstellte, scheint dagegen eher der Neuartigkeit der sozialen Konfrontationssituation an sich geschuldet. In der praktischen Schweinehaltung sollte deshalb bei der Integration bzw. Reintegration neuer Gruppenmitglieder darauf geachtet werden, dass in der jeweiligen Haltungsumwelt genügend Rückzugs- und Ausweichmöglichkeiten vorhanden sind, damit sie durch entsprechende Verhaltensmuster ihre potentiell problematische Situation der sozialen Neueingliederung in die Dominanzhierarchie bewältigen können. Insbesondere ehemals dominante Tiere scheinen mit einer Neuerfahrung des Verlusts ihres sozialen Status Schwierigkeiten zu haben.

4 ZUSAMMENFASSENDE DISKUSSION UND SCHLUSSFOLGERUNGEN

Die experimentellen Studien der vorliegenden kumulativen Habilitationsschrift behandeln ethologische Mechanismen und verhaltensphysiologische Konsequenzen des Sozialverhaltens beim Hausschwein aus ultimater und proximater Sicht. Dabei werden wissenschaftlich-theoretische und angewandt-praktische Aspekte der Beziehungen von Mutter und Nachkommen sowie entsprechende Probleme der sozialen Dominanzbeziehungen bei Schweinen nach dem Absetzen untersucht. Während im vorangegangenen Kapitel 3 die wichtigsten Ergebnisse und Schlussfolgerungen jeder Untersuchung dargelegt sind, werden die Arbeiten nachfolgend zusammenfassend diskutiert. Darüber hinaus befindet sich eine ausführliche Diskussion jeder Einzelstudie in den jeweiligen Originalarbeiten, die im Kapitel 7 zusammengestellt sind. Ausgangspunkt für die vorgelegte Arbeit ist die These, dass die evolutiv (ultimat) herausgebildeten innerartlichen Sozialbeziehungen das Verhalten und die physiologischen Reaktionen beim Hausschwein unter praktischen Haltungsbedingungen auf proximater Ebene spezifisch beeinflussen. Als komplexe Konsequenz biologischer Antworten der miteinander vernetzten Subsysteme des Organismus werden Wohlbefinden, Gesundheit und Leistung der Tiere in einem stärkeren Maße ontogenetisch determiniert als bisher angenommen. Dies mit entsprechenden verhaltensphysiologischen Methoden und Daten zu belegen, ist übergeordnetes Ziel dieser Arbeit.

4.1 Sozialphysiologische Mechanismen in der Sau-Ferkel-Beziehung

Saugverhalten. Es ist unstrittig, dass die wichtigste soziale Beziehung eines neugeborenen Ferkels die zu seiner Mutter ist, um essentielle Ansprüche an Ernährung, Thermoregulation, Energie- und Immunversorgung sowie an Schutz und sozialer Unterstützung zu gewährleisten (NOWAK *et al.*, 2000; PUPPE *et al.*, 2008). Kürzlich durchgeführte vergleichende Studien bei Hauschweinen und Kreuzungstieren zwischen Haus- und Wildschweinen ergaben, dass die meisten Aspekte maternalen Verhaltens sich während der Domestikation und unter modernen Zucht- und Haltungsbedingungen kaum geändert haben (GUSTAFSSON *et al.*, 1999; ŠPINKA *et al.*, 2000). Um so erstaunlicher ist, dass – obgleich in vielen ethologischen und physiologischen Details vergleichsweise häufig untersucht (siehe FRASER, 1980; ALGERS, 1993) – der Blick auf die Sau-Ferkel-Beziehung aus soziobiologischer bzw. ultimater Sicht und die damit verbundenen theoretischen Vorstellungen (z.B. *resource allocation theory, parent-offspring-conflict theory (weaning conflict)*) relativ neu und noch nicht üblicher Bestandteil des „Theoriegebäudes“ in der Nutztierethologie sind (siehe FRASER *et al.*, 1995; DRAKE *et al.*, 2008). Der komplexe Prozess der Etablierung und Entwicklung des Saugverhaltens der Ferkel an der Sau im Kontext der Milchaufnahme (vgl. Übersichten in GILL & THOMPSON, 1956; FRASER, 1980; ALGERS, 1993) hat hierbei eine entscheidende Bedeutung. In den Studien 1 (siehe Kapitel 3.1.1.1) und 2 (siehe Kapitel 3.1.1.2) wurden deshalb Saughäufigkeit und Saugstabilität als essentielle Parameter des nutritiven Saugverhaltens in ihrer täglichen Entwicklung von Geburt bis Absetzen untersucht und zudem die mütterliche Laktationsleistung empirisch analysiert (siehe Studie 3, Kapitel 3.1.1.3.). Erstmalig haben wir dabei aus den beobachteten Daten Elemente des Saugverhaltens mathematisch modellieren können, so dass definierte und verhaltensbiologisch interpretierbare Funktionsparameter zur Verfügung stehen. Diese können künftig analytisch genutzt werden, um verschiedene Fragen aus dem Problemkreis der Sau-Ferkel-Beziehungen vergleichend beantworten zu können. Wir konnten beispielsweise damit zeigen, dass die Ontogenese der Saughäufigkeit in Übereinstimmung mit Vorhersagen des evolutionären Modells des Absetzkonflikts (MARTIN, 1984) interpretiert werden kann. Das mütterliche Investment verringert sich kontinuierlich nach einem Maximum in der zweiten Lebenswoche der Ferkel (Saughäufigkeit) bzw. – aus physiologischen Gründen leicht zeitversetzt – in der dritten Lebenswoche (Laktationsleistung). Das bestätigt Untersuchungen von JENSEN *&* REDBO (1987) sowie JENSEN *et al.* (1991), die fanden, dass Elemente des mütterlichen Absetzprozesses schon sehr frühzeitig (ab ca. 10 Tagen) beginnen. Als eine Art Gegenstrategie zu diesem mütterlichen Investitionsentzug haben die Ferkel ab diesem Zeitpunkt ihr höchstes Level an territorialer Saugstabilität zur optimalen Ausnutzung der geringer werdenden mütterlichen Ressourcen in einem prägungsartig zu wertenden Lernprozess erreicht (**Abbildung 1**). Diese temporale Koinzidenz unterstützt indirekt die These von BATESON (1994), dass die phänotypischen Auswirkungen der verschiedenen Interessen von Mutter und Nachkommen auf proximater Ebene dynamischer ausbalanciert werden können als eigentlich theoretisch erwartet.

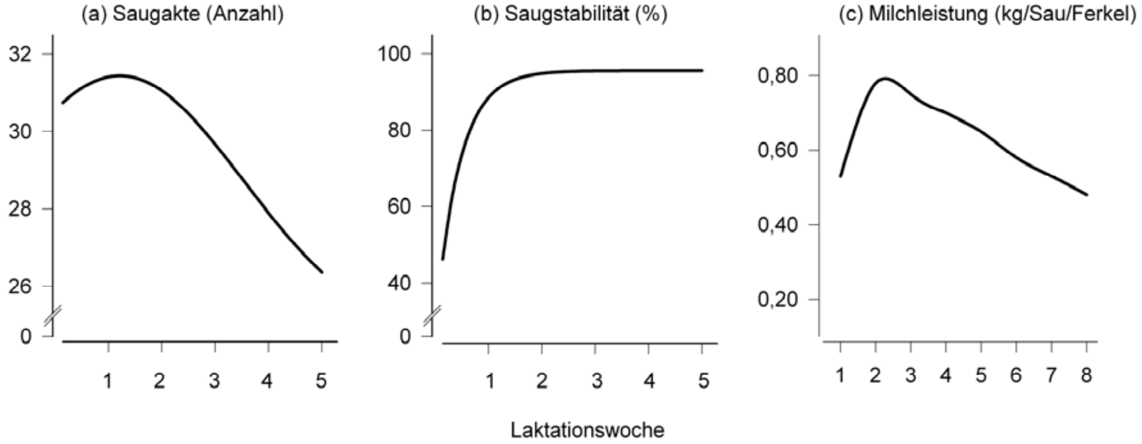

Abbildung 1: Saugverhalten der Ferkel und Milchleistung der Sauen als wichtige Parameter der Mutter-Nachkommen-Beziehung. Vereinfachte Darstellung der Entwicklung der Anzahl von Saugakten mit erfolgreicher Milchaufnahme durch die Ferkel (a), der Entwicklung ihrer Saugstabilität bezogen auf die konstante Präferenz einer bestimmten Zitze (b), sowie der Entwicklung der Milchleistung von Sauen (c). Die Saughäufigkeit erreicht ihr Maximum in der zweiten Lebenswoche der Ferkel und sinkt dann – ebenso wie die mütterlichen Laktationsleistung (Maximum in der dritten Lebenswoche) – kontinuierlich. Gleichzeitig ist das Level der Saugstabilität der Ferkel ab diesem Zeitpunkt am höchsten (PUPPE *et al.*, 2008, verändert).

Wir konnten weiterhin zeigen, dass größere Würfe das Maximum der Saughäufigkeit um ca. 6 Tage vorverlagern. Prinzipiell übereinstimmend mit BØE (1991) kann dies als ein Mechanismus gewertet werden, die individuelle Ressourcenverfügbarkeit bei Bedarf zu erhöhen, was vor allem unter den üblichen praxisrelevanten Bedingungen der Fixierung von Sauen (z.B. Kastenstand) und zunehmenden Wurfgrößen die Gefahr der physiologischen „Überforderung“ der Mütter beinhalten könnte. In der Tat gibt es Hinweise, dass das Niveau des maternalen Investments unter diesen

Haltungsbedingungen überproportional stark zugunsten der Ferkel verschoben wird (FRASER *et al.*, 1995). Auf die von uns postulierten sozialphysiologischen Konsequenzen des Saugverhaltens für die unmittelbare Entwicklung des Sau-Ferkel-Systems weisen auch anderen Studien hin. So konnten ŠPINKA *et al.* (1997, 1999) nachweisen, dass die Saugfrequenz der Ferkel eine wichtige Rolle für die Milcherzeugung hat, indirekt vermittelt durch Insulin- und Prolaktinänderungen infolge der Gesäugemassage. Ebenso ist eine hohe Zitzenkonstanz vorteilhaft für die Ferkel, da so die Gefahr von Verteilungskämpfen und Fehlsaugakten im Rahmen der Geschwisterkonkurrenz verringert werden kann (DE PASSILLÉ *et al.*, 1988). Im Bereich der nichtnutritiven Sau-Ferkel-Beziehungen ist vor allem die Nachmassage des Gesäuges durch die Ferkel nach dem eigentlichen Milchfluss ebenfalls aus ultimater Sicht betrachtet worden (ŠPINKA & ALGERS, 1995; DOSTÁLKOVÁ *et al.*, 2002). Es gibt einige experimentelle Hinweise, dass die Nachmassage den künftigen Milchfluss erhöhen kann (*restaurant hypothesis*: ALGERS & JENSEN, 1985) und dass dieses Verhaltensmuster einige Ähnlichkeiten mit dem Bettelverhalten hat, wie es bei Jungvögeln als ‚ehrliches Signal' *(honest begging)* zur Maximierung der Futterversorgung durch die Eltern vorkommt (JENSEN *et al.*, 1998). Darüber hinaus konnten wir mit unseren Analysen nachweisen, dass territoriale Aspekte des frühen Saugverhaltens der Ferkel (Ort und Stabilität der Zitzenpräferenz) ihre spätere soziale (Platz in der Dominanzhierarchie) und physische (Gewichtszunahme) Ontogenese beeinflussen, wie es bereits von einigen Autoren vermutet wurde (SCHEEL *et al.*, 1977; MATTWEI *et al.*, 1979; ROSILLON-WARNIER & PAQUAY, 1984; HOY & PUPPE, 1992; PUPPE *et al.*, 1993; PUPPE & TUCHSCHERER, 1994; TUCHSCHERER *et al.*, 1994; HOY *et al.*, 1995). Das spricht für ein langfristiges System der sozialen Organisation bei Hausschweinen (**Abbildung 2**) mit einer entsprechenden Adaptation ihres Verhaltens und ihrer physiologischen Reaktionen gegenüber Herausforderungen der Umwelt (siehe auch Kapitel 4.2). Dies sollte bei Gruppenzusammenstellungen in der Haltung beachtet werden.

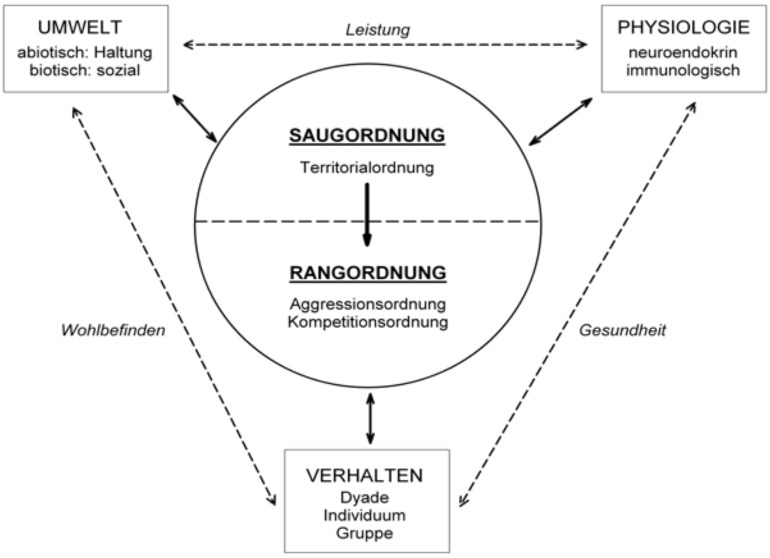

Abbildung 2: Vereinfachtes Schema der sozialen Organisationsstrukturen beim Hausschwein in Relation zu Umwelt, Verhalten und Physiologie. Es wird deutlich, dass die soziale Organisation in vielfältigen Wechselbeziehungen zu ökologischen, ethologischen und physiologischen Faktoren steht und dadurch Wohlbefinden, Gesundheit und Leistung der Tiere netzwerkartig beeinflusst werden (PUPPE, 1996b, verändert).

Sozialakustische Erkennungsmechanismen. Ausgehend vom fein abgestimmten Muster des Saug-Säugeverhaltens demonstrierten wir mittels Lautanalysen (phonetische Analyse: siehe Studie 4, Kapitel 3.1.2.1) und replay-Experimenten (semantische Analyse: siehe Studie 5, Kapitel 3.1.2.2), dass Ferkel ihre Mütter anhand individueller akustischer Merkmale im Säugegrunzen erkennen können. Diese individuellen Unterschiede sind in Übereinstimmung mit einer Studie von BLACKSHAW *et al.* (1996) vor allem im Frequenzbereich zu finden. Umgekehrt scheinen Sauen ihre Ferkel ebenfalls anhand individueller akustischer Merkmale erkennen zu können (ILLMANN *et al.*, 2002). Insbesondere die hochfrequenten Schreie der Ferkel können als kostenintensives, den realen Status des Tieres wiedergebendes Signal *(honest signalling)* an die Mutter interpretiert werden (WEARY & FRASER, 1995; PUPPE *et al.*, 2005). Hingegen ist die Erkennung der eigenen Mutter überlebensnotwendige Voraussetzung für die Ferkel, um ihre Ansprüche an Schutz und Versorgung zu gewährleisten. Damit wird unterstrichen, wie wichtig die ungestörte akustische Kommunikation für die Etablierung und Genese der Mutter-Nachkommen-Beziehung beim Hausschwein ist (ALGERS, 1993; PUPPE *et al.*, 2008). Gleichzeitig werden ältere Befunde bestätigt, die auf die inter-individuelle Bedeutung des Säugegrunzens als ein (aber nicht einziges: RUSHEN *&* FRASER, 1989) Mittel der zeitlichen Synchronisation des Saug-Säugeverhaltens und der Milchejektion hinweisen (WHITTEMORE *&* FRASER, 1974; FRASER, 1980; ELLENDORFF *et al.*, 1982; CASTRÉN *et al.*, 1989; ALGERS *et al.*, 1990; ŠPINKA *et al.*, 2002). Die Ferkel sind somit in der Lage, den Verhaltenskontext an sich zu erkennen und beispielsweise auch andere Sauen als ihre eigene Mutter zu besaugen. Es ist zu postulieren, dass die Bedeutung adäquat funktionierender sozialakustischer Beziehungen in dem Maße steigen wird, in dem alternative Haltungssysteme genutzt werden, die den Tieren mehr Möglichkeiten der Kontrolle ihres Verhaltens geben. Dies sind beispielsweise „Sauen-kontrollierte" Gruppenabferkelsysteme, in denen die Sauen ihre Ferkel nach Bedarf verlassen können, was ihrem natürlichen Verhalten entgegenkommt (AREY *&* SANCHA, 1996; PAJOR *et al.*, 2000). Obgleich auch das Säugen von fremden Ferkeln möglich ist (*cross-, multi-, allo-suckling*) und praktiziert wird (PUPPE *&* TUCHSCHERER, 1995; OLSEN *et al.*, 1998; WATTANAKUL *et al.*, 1998; MALETÍNSKÁ *&* ŠPINKA, 2001; ILLMANN *et al.*, 2007), ist aber gerade hier eine verlässliche Muttererkennung durch die Ferkel biologische Grundlage für das praktische Funktionieren eines solchen Haltungssystems. Dass Mütter auch fremde Nachkommen säugen, ist ein in der Natur weitverbreitetes Phänomen, kommt vor allem bei polytoken Spezies vor und ist in Übereinstimmung mit evolutionären Kooperationsmodellen, die auf Verwandtschaft, Reziprozität oder Mutualismus basieren (PACKER *et al.*, 1992; ROULIN, 2002). Darüber hinaus können auch proximate Mechanismen betroffen sein, die über immunologische (ROULIN *&* HEEB, 1999) oder neuroendokrine (ROULIN, 2003) Konsequenzen für Mutter und/oder Nachkommen die gegenseitigen Beziehungen ausbalancieren können. Insgesamt kommen WECHSLER *&* WEBER (2007) in einer kürzlich erschienenen Übersichtsarbeit zu dem Schluss, dass bei adäquatem Management und Haltung keine Nachteile, aber verbesserte Chancen für die Erhöhung von Wohlbefinden und Tiergerechtheit in alternativen Gruppenhaltungssystemen für ferkelführende Sauen möglich sind. So wurden in einer Serie experimenteller Studien positive Auswirkungen sowohl für Ferkel als auch Sauen beschrieben: verbesserter Futterkonsum der Ferkel nach dem Absetzen (PAJOR *et al.*, 1999), Vermeidung der

Überforderung der Laktationsleistung der Sau (WEARY *et al.*, 1999b; PAJOR *et al.*, 2002), erhöhte Sozialisationserfahrungen für die Ferkel (WEARY *et al.*, 2002) und ein ausgewogeneres mütterliches Investment in die Nachkommen (PITTS *et al.*, 2002). Insgesamt lässt sich daraus interpretieren, dass durch die Beachtung und Einbeziehung der beschriebenen Mechanismen der Mutter-Nachkommen-Beziehungen unter modernen Haltungsbedingungen das Absetzen der Ferkel in Richtung eines mehr graduell ablaufenden Prozesses verbessert werden kann.

Soziale Deprivation. Das – im Vergleich zum eher graduellen und kontinuierlichen Absetzen unter natürlichen oder semi-natürlichen Bedingungen (JENSEN & REDBO, 1987; BØE, 1991; JENSEN & STANGEL, 1992) – abrupte Absetzen der Ferkel von der Sau in der praktischen Tierhaltung ist mit vielfältigen Konsequenzen verbunden, die Leistung, Physiologie, Verhalten, Gesundheit und Ernährung betreffen (vgl. Übersicht in PLUSKE *et al.*, 2003). Es gibt eine Reihe überzeugender Hinweise, dass das Absetzen ein stresshaftes Ereignis für die Ferkel ist (WEARY *et al.*, 2008) und zwar offensichtlich deutlicher ausgeprägt, wenn es vergleichsweise frühzeitig erfolgt (FRASER, 1978; BLECHA *et al.*, 1983; METZ & GONYOU, 1990; DYBKJÆR, 1992; WEARY *et al.*, 1999a; HAY *et al.*, 2001; ORGEUR *et al.*, 2001; KANITZ *et al.*, 2002; MASON *et al.*, 2003; COLSON *et al.*, 2006). In der Nutztierhaltung bisher wenig berücksichtigt sind die psychophysiologischen Konsequenzen des Verlusts sozialer Beziehungen (NEWBERRY & SWANSON, 2008), wie sie beispielsweise durch das frühe Absetzen der Ferkel von der Sau verursacht werden. Es ist allgemein anerkannt, dass ein früher Verlust der sozialen Bindung zur Mutter die spätere Verhaltensentwicklung der Nachkommen sowie ihre Fähigkeit, Krankheit und Stress zu bewältigen, negativ beeinflusst (HOFER, 1996). Hinzu kommen Verhaltensprobleme, die durch qualitativ unzureichende, d.h. inadäquate mütterliche Fürsorge verursacht werden können (LATHAM & MASON, 2008). Hier wird postuliert, dass schon vergleichsweise geringfügige Variationen in der mütterlichen Umgebung adaptive Anpassungen in neuralen und endokrinen Funktionen bei den Nachkommen verursachen, die deren zukünftige Stressreaktivität beeinflussen (MEANEY, 2001; WÜRBEL, 2001; MACRI & WÜRBEL, 2006). Diese Erkenntnisse wurden vorwiegend durch Studien an Labortieren gewonnen, bei Hausschweinen gibt es kaum diesbezügliche Untersuchungen. Die Studien 6 (siehe Kapitel 3.1.3.1) und 7 (siehe Kapitel 3.1.3.2) beschäftigten sich deshalb modellhaft mit ethologischen, neuroendokrinen und immunologischen Konsequenzen psychosozialen Stresses, um zunächst grundlegende Mechanismen der komplexen Reaktionen beim Saugferkel zu verstehen. In Übereinstimmung mit vielen Studien aus der grundlagenorientierten Forschung nutzten wir zunächst das Modell der wiederholten sozialen Isolation von Individuen (HENNESSY, 1986; HALL, 1998). Wir konnten in dieser Komplexität erstmals für isolierte Ferkel zeigen, dass diese psychosoziale Stresssituation zu einer Reihe potentiell negativer Effekte für Stressbewältigung, Wohlbefinden und Gesundheit führte. Bei langfristiger, wiederholter Isolation von Ferkeln verminderte sich ihre unmittelbare Verhaltensreaktivität bei gleichzeitiger Erhöhung basaler Stresshormonwerte und der Suppression von Immunfunktionen. Darüber hinaus veränderten sich nachhaltig auch einige untersuchte stressrelevante Mediatoren dieser Effekte, wie zentrale Stressrezeptor- und Zytokinkonzentrationen in verschiedenen Hirnarealen (z.B. Hypothalamus und Amygdala). Diese Befunde sind in genereller

Übereinstimmung mit verschiedenen Studien, die bei anderen Spezies (z.B. Makaken, Meerschweinchen) beschreiben, dass die Reaktionen von Nachkommen auf soziale Isolation bzw. maternale Separation einer biphasischen Dynamik folgen. Obgleich individuell abhängig von einer Reihe innerer und äußerer Faktoren (BOCCIA *et al.*, 1994), kommt es zunächst zu einer anfänglichen Phase der aktiven Bewältigung und Erregung *(behavioural arousal)*, die mit andauernder Isolation in eine passive Phase mit reduzierter Verhaltensaktivität übergeht. Die in der passiven Phase auftretenden inaktiven Verhaltensmuster können psychobiologisch mit mentalen Zuständen, wie Hoffnungslosigkeit *(despair)* und Depression *(depression),* beschrieben werden (HOFER, 1975; LAUDENSLAGER *et al.*, 1990; HOFER, 1996; HENNESSY *et al.*, 2001; HENNESSY *&* MORRIS, 2005) und können darüber hinaus auch als eine Art stressinduziertes Krankheitsverhalten interpretiert werden (HENNESSY *et al.*, 2001; 2004; HENNESSY *&* MORRIS, 2005). Letzteres wird in erster Linie durch proinflammatorische Zytokine induziert und ist eine adaptive Antwort des Organismus über eine motivationale Reorganisation seiner Prioritäten zur Situations- bzw. Infektbewältigung (HART, 1988; AUBERT, 1999; DANTZER, 2001; JOHNSON, 2002; KONSMAN *et al.*, 2002; KELLEY *et al.*, 2003). Das lässt vermuten, dass eine frühzeitige und/oder andauernde Sensitivierung des Immunsystems durch psychischen Stress stattfindet (DANTZER, 2005), was bei späterer, erneuter Stressdisposition zu Problemen für Gesundheit und Wohlbefinden führen kann. In der Tat fanden wir in unseren Untersuchungen, dass die soziale Isolation der Ferkel ihr mittels Endotoxin-Challenge ausgelöstes Krankheitsverhalten in seiner Wirkung verstärkte (z.B. Grad des Zitterns oder der Somnolenz), mit engen Beziehungen zu damit verbundenen physiologischen Veränderungen von Stresshormonen (z.B. Kortisol) und Zytokinen (z.B. TNFα). Das wird durch weitere eigene Untersuchungen bestätigt, die nachweisen, dass es bei frühisolierten Schweinen zu langfristigen Beeinflussungen von Regulationsmechanismen innerhalb des peripheren und zentralen proinflammatorischen Netzwerks kommt, die insbesondere über TNFα vermittelt werden (TUCHSCHERER *et al.*, 2004). Darüber hinaus deuten die wenigen diesbezüglichen Studien beim Ferkel an, dass soziale Isolation bzw. sogar frühes Absetzen die Genexpression stressrelevanter Marker im sich entwickelnden Gehirn der Tiere verändert (KANITZ *et al.*, 1998; SCHWERIN *et al.*, 2005; POLETTO *et al.*, 2006a,b), was für eine grundlegende Veränderung neuroendokriner Regulationsmechanismen spricht. Das kann zur Beeinträchtigung sensorisch-kognitiver Leistungen führen, wie kürzlich in einer Studie gezeigt wurde. Hier verursachte eine frühe soziale Isolation Defizite in der sozialen Erkennung von Artgenossen (SOUZA & ZANELLA, 2008). Es gibt erste Hinweise, dass die durch eine soziale Isolation verursachten stressbedingten Verhaltensänderungen durch partiellen sozialen Kontakt zu Artgenossen *(social support)* abgemildert werden konnten (HERSKIN *&* JENSEN, 2000). Insofern sind weitere Untersuchungen notwendig (z.B. Effekte kurzzeitiger Isolation, Effekte sozialer Unterstützung), um das komplexe Beziehungsgefüge zwischen Verhalten, Neuroendokrinum und Immunsystem detaillierter zu verstehen. Entsprechende Forschungen am Hausschwein können sowohl in ihren Auswirkungen auf praktische Haltungsbedingungen in der Landwirtschaft beurteilt werden, sind aber auch zunehmend geeignet als Tiermodell für den Menschen (z.B. psychosomatische Erkrankungen).

4.2 Analyse und Konsequenzen sozialer Dominanzstrukturen beim Hausschwein

Sozial-agonistisches Verhalten. Die für die Haltung und das soziale Verhalten von Schweinen kritischste Phase entsteht unter intensiven Haltungsbedingungen nach dem Absetzen von der Sau. Neben dem sozialen Verlust von Mutter und Wurfgeschwistern (siehe auch Kapitel 4.1) müssen die Tiere mit größtenteils unbekannten Tieren interagieren und gleichzeitig in einer neuen Umgebung zurechtkommen (PUPPE *et al.*, 2008). Das führt zu einer sozialen Reorganisation, z.B. von der territorial geprägten Saugordnung zu einer sozialhierarchischen Rangordnung (siehe auch **Abbildung 2**). Die Folge sind erhöhte und zum Teil sehr heftige agonistische Auseinandersetzungen mit der Gefahr der Beeinträchtigung von Gesundheit und Wohlbefinden für die Tiere (PETHERICK *&* BLACKSHAW, 1987; GONYOU, 2001; HELD *&* MENDL, 2001; PUPPE *et al.*, 2008). Deshalb untersuchten wir in den Studien 8 (siehe Kapitel 3.2.1.1) und 9 (siehe Kapitel 3.2.1.2) dieser Arbeit den Einfluss verschiedener Randbedingungen auf die Entwicklung des sozial-agonistischen Verhaltens während des Absetzprozesses. Wir konnten zeigen, dass die Häufigkeit akuter agonistischer Interaktionen der Ferkel nach dem Absetzen durch ihren vorigen Bekanntheits-, nicht aber Verwandtschaftsgrad beeinflusst wurde. Sich vorher unbekannte Ferkelpaare hatten die meisten agonistischen Auseinandersetzungen im Buchtbereich, nicht aber in der Kompetition um Futter. Daraus lässt sich in Übereinstimmung mit einer Studie von STOOKEY *&* GONYOU (1998) schlussfolgern, dass die gegenseitigen Erkennungsmechanismen bei Ferkeln auf erlernter Bekanntschaft und nicht auf genetischer Verwandtschaft beruhen. Obgleich die soziale Erkennung wichtig für die Etablierung und Aufrechterhaltung einer Dominanzhierarchie ist, gibt es über die dazu genutzten sensorischen Informationskanäle erstaunlich wenig gesicherte Kenntnisse (MCLEMAN *et al.*, 2005). Unsere Ergebnisse stützen die These, dass die Motivation für die anfänglichen Kämpfe beim Neugruppieren als eine Form sozialer Exploration betrachtet werden kann, um Informationen über die relative Kampffähigkeit des Opponenten zu erlangen (RUSHEN, 1990). Funktionell sind diese anfänglichen Kämpfe im Kontext der Klärung der sozialen Dominanzbeziehungen (Aggressionsordnung) und der Sicherung des Zugangs zu zukünftigen Ressourcen zu sehen (PUPPE, 1996b). Möglicherweise ist ein Teil der agonistischen Interaktionen auch motiviert durch einen gewissen Grad an Intoleranz gegenüber fremden Individuen (FRASER *et al.*, 1995). Hier sollten künftige Untersuchungen ansetzen, um dies genauer zu klären. Methodisch gilt bei Hausschweinen allerdings zu beachten, dass die über eine Aggressionsordnung gemessene Dominanz nicht notwendigerweise mit der sozialen Dominanz, die auf der Einschätzung des Zugangs zu direkten Ressourcen (z.B. Futter) beruht (Kompetitionsordnung; vgl. SYME, 1974), übereinstimmen muss (MCGLONE, 1986; PUPPE & TUCHSCHERER, 1994), so dass eindeutige Angaben über Art und Weise der Analyse zwingend erforderlich sind. Erste Hinweise zeigen, dass – in Anlehnung an ultimate Erklärungsmodelle aus der Verhaltensökologie – auch die räumliche Verteilung von Ressourcen einen möglichen Einfluss auf das Kampfverhalten neu gruppierter Hausschweine hat (ANDERSEN *et al.*, 2000). Allerdings scheinen unmittelbare Effekte, wie Uniformität zwischen den Tieren (HAYNE *&* GONYOU, 2006) sowie Charakteristika wie Bekanntheit (FRASER, 1974; GIERSING *&* ANDERSSON, 1998; STOOKEY *&* GONYOU, 1998) oder Kampffähigkeit (RUSHEN, 1988), das agonistische Verhalten weitaus höher zu beeinflussen als das Potential der Schweine, ihre Ressourcen zu halten bzw. zu verteidigen (FRASER *et al.*, 1995). Beim Versuch, die Effekte von sozialer Umwelt und Haltungsumwelt bezüglich des agonistischen Verhaltens experimentell zu trennen, konnten wir erstmals demonstrieren, dass physiologische Stresseffekte nur unmittelbar nach dem Absetzen durch beide Arten von Umweltfaktoren verursacht werden. Hingegen führte eine unbekannte Haltungsumgebung lediglich zu einer anhaltenden Erhöhung des agonistischen Verhaltens bis 4 Tage nach dem Absetzen, beeinflusste aber nicht längerfristig die physiologischen Stressparameter. Das spricht einerseits für die hohe Sozialisationsfähigkeit von Ferkeln (NEWBERRY *&* WOOD-GUSH, 1986; PETERSEN *et al.*, 1989), zeigt aber auch, dass der Effekt der unbekannten Umgebung bisher weniger beachtet worden ist. Auch andere Studien zeigen, dass die psychophysiologischen Stresseffekte nach Absetzen und Neugruppieren eher transient sind (MERLOT *et al.*, 2004), während die Effekte auf das Verhalten (z.B. eine generalisierte Furchtsteigerung) anhaltender zu sein scheinen (HAYNE *&* GONYOU, 2003). Insgesamt gesehen kann die Sozialisation einander unbekannter Ferkel vor dem Absetzen das agonistische Verhalten und die damit verbundene Erfahrung von Stress nach dem Absetzen verringern (PLUSKE *&* WILLIAMS, 1996; PITTS *et al.*, 2000; HILLMANN *et al.*, 2003; D'EATH, 2005; HESSEL *et al.*, 2006). Eine verringerte Aggressionsrate im Kontext des Absetzens kann darüber hinaus auch durch eine verhaltensangereicherte Umgebung erreicht werden (O'CONNEL *&* BEATTIE, 1999), insbesondere dann, wenn diese den Ferkeln durch ein Signal als Belohnung „angekündigt", d.h von ihnen „antizipiert" wird (DUDINK *et al.*, 2006).

Dominanzstruktur und Soziometrie. Hausschweine werden nicht nur nach dem Absetzen, sondern auch in weiteren Haltungsabschnitten neu zusammengestellt und sozial gemischt. Das führt in der Regel zu einer Neuetablierung der sozialen Rangordnung mit entsprechenden Dominanz-/Subdominanzverhältnissen (GONYOU, 2001). Das Dominanzkonzept (vgl. ROWELL, 1974; HINDE, 1978; BERNSTEIN, 1981; KAUFMANN, 1983; FRANCIS, 1988; DREWS, 1993; CLUTTON-BROCK *&* PARKER, 1995) ist – beginnend mit der Pionierarbeit von SCHJELDERUPP-EBBE (1922) – trotz einiger Differenzen bezüglich Definition, biologischer Bedeutung und Bestimmungsmethoden, eines der zentralen Konzepte bei der Untersuchung sozialer Organisationsstrukturen. Für die Charakterisierung gibt es mittlerweile eine Reihe verschiedener Parameter und/oder Verfahren auf der Basis der Analyse der agonistischen Interaktionen (vgl. APPLEBY, 1983; DE VRIES *et al.*, 1993; PUPPE & TUCHSCHERER, 1994; DE VRIES, 1995, 1998; DE VRIES *&* APPLEBY, 2000). Diese haben wir zum Teil aufgegriffen, erweitert und als neu zusammengestellte soziometrische Parameter in einem einheitlichen wissenschaftlich-methodischen Ansatz eingeführt, um Dominanz auf verschiedenen Analyseebenen strukturell zu beschreiben (siehe Studie 10, Kapitel 3.2.2.1). Dies ist erstens in klarer Übereinstimmung mit der von Drews (1993) gegebenen Dominanzdefinition und erlaubt dadurch eine strenge Aufstellung und Testung von diesbezüglichen wissenschaftlichen Hypothesen. Zweitens trennen wir, den Überlegungen von CAPITANIO (1991) folgend, die verschiedenen Ebenen der Analyse. Wir schlagen daher vor, bei der methodischen Kalkulation der soziometrischen Parameter und ihrer Ergebnisdarstellung als Ausgangsebene grundsätzlich mit der Dyade zu beginnen (**Abbildung 3**).

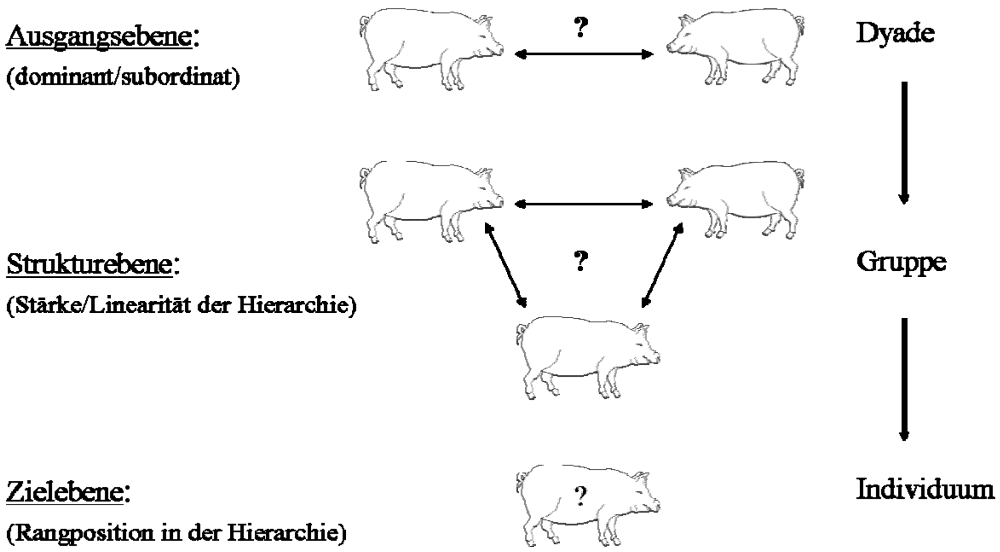

Abbildung 3: Schematische Darstellung der Analyseebenen von Dominanzbeziehungen bei sozial lebenden Tieren am Beispiel des Hausschweins. Die Berechnung von Rangplätzen beim Individuum in einer Dominanzhierarchie ist strenggenommen nur zulässig, wenn die analysierten soziometrischen Parameter auf Ebene der Dyade und der Gruppe dies zulassen (Langbein & Puppe, 2004, verändert).

Auf der Strukturebene folgt die soziale Gruppe, in der die Stärke bzw. Linearität der Rangordnung analysiert wird, bevor als Zielebene die Rangposition des Individuums bestimmt wird. Dieser Logik der Analyse wird in vielen Untersuchungen nicht Rechnung getragen (vgl. Puppe, 1996b; Langbein & Puppe, 2004), was zu fehlerhaften Interpretationen führen kann. Erst wenn sich bei der Majorität aller potentiell möglichen Dyaden einer Gruppe Asymmetrien im Ausgang der agonistischen Interaktionen feststellen lassen, die wiederum zu signifikant strukturierten Dominanzbeziehungen in der sozialen Gruppe führen, hat die strukturelle Kalkulation individueller Rangplätze und – darauf aufbauend – die funktionelle Betrachtung ihrer ethologischen und physiologischen Konsequenzen eine entsprechende Berechtigung. Als weiteren, folgerichtigen Schritt haben wir diesen Ansatz auf die Bedingungen der praktischen Nutztierhaltung übertragen und konnten so das Sozialverhalten von Hausschweinen über die soziometrische Analyse ihrer Dominanzstruktur in verschiedenen Ontogenese- bzw. Haltungsabschnitten erstmals in dieser Komplexität untersuchen (siehe Studie 11, Kapitel 3.2.2.2). Die Quellen und Verwendung sowie Aussagekraft und Validität der verwendeten soziometrischen Parameter haben wir ausführlich in den entsprechenden Originalarbeiten diskutiert (siehe Kapitel 7.10 und 7.11), deshalb soll im Rahmen dieser generellen Diskussion nicht weiter darauf eingegangen werden. Entscheidend ist, dass mit unserem auf Standardisierung und Konsistenz bedachten Ansatz der Analyse und Darstellung sozialer Dominanzstrukturen ein direkter wissenschaftlich-methodischer Vergleich innerhalb und zwischen Spezies auf den Analyseebenen der Dyade, der Gruppe und des Individuums möglich wird. Wir schlagen vor, dass die erhobenen soziometrischen Parameter direkt in die praktische Tierzucht betreffende genetische Kalkulationen integriert werden können (z.B. kommerzielle Zuchtprogramme), um diese durch konkret nutzbare Maßzahlen aus dem Bereich des Sozialverhaltens zu erweitern. Die wenigen Arbeiten, die sich bisher mit genetischer Selektion unter Verwendung des Aggressions- oder Dominanzverhaltens bei Hausschweinen beschäftigt haben (Jonsson & Jørgensen, 1989; Løvendahl *et al.*, 2005), finden hier durchaus heritable Zusammenhänge zu anderen Produktions- (z.B. Tageszunahmen) oder Verhaltensmerkmalen (z.B. Mütterlichkeit). Deshalb haben wir unseren Ansatz der Analyse soziometrischer Dominanzstrukturen bei drei typischen Haltungsabschnitten der modernen Schweinhaltung angewandt, in denen die Tiere neu gemischt und zusammengestellt werden: Absetzferkel, Mastläufer, reproduktive Sauen. So konnten wir erstmals Gemeinsamkeiten und Unterschiede bei der Etablierung der Dominanzstruktur in diesen Phasen herausarbeiten sowie die entsprechenden soziometrischen Parameter liefern. Wir konnten weiterhin methodisch zeigen, dass teilweise hohe korrelative Zusammenhänge zwischen den soziometrischen Parametern auf Ebene der Dyade und der Gruppe bestehen, so dass sich dadurch die Verlässlichkeit der Interpretationen deutlich erhöht. Damit erhärteten wir frühere Studien, die Hausschweinen eine soziale Organisationsstruktur in Richtung einer quasi-linearen sozialen Hierarchie attestieren (BEILHARZ *&* COX, 1967; MEESE *&* EWBANK, 1973a; SAMBRAUS, 1981). Allerdings fanden wir insbesondere bei den Sauengruppen einige Besonderheiten. Trotz weniger beobachteter agonistischer Interaktionen und bidirektionaler bzw. asymmetrischer Paarbeziehungen ist die Stärke bzw. Linearität der Gruppenstruktur ähnlich oder sogar höher als bei Absetzferkel- und Mastschweingruppen. Vergleichbare soziometrische Werte für die Gruppenstruktur der Sauen finden sich auch bei HOY & BAUER (2005). Offensichtlich nutzen Sauen infolge ihrer höheren sozialen Erfahrung auch andere Mechanismen als direkte aggressive Auseinandersetzungen zur Etablierung und Aufrechterhaltung einer sozialen Dominanzstruktur. Dass eine solche Regulation beispielsweise über submissives Verhalten realisiert wird, das zu einer Art „Meideordnung“ *(avoidance order)* bei Sauen führt, wie es JENSEN (1982) sowie JENSEN *&* WOOD-GUSH (1984) vorschlagen, wird anhand unserer Ergebnisse indirekt unterstützt. Daraus lässt sich ableiten, dass Haltungs- und Managementstrategien, die zur Verbesserung der sozialen Fertigkeiten *(social skills)* der Sauen führen (z.B. der Erwerb sozialer Erfahrungen in für Wohlbefinden und Gesundheit vergleichsweise weniger kritischen Perioden als während der Trächtigkeit), vorteilhaft für die Tiere sein können (VAN PUTTEN *&* BURÉ, 1997; AREY *&* EDWARDS, 1998; HOY & BAUER, 2005).

Dominanzstruktur und Konsequenzen. Nachdem wir zeigen konnten, dass Hausschweine nach dem Absetzen und Neugruppieren über offene, agonistische Interaktionen eine nachweisbare Dominanzstruktur mit entsprechenden sozialen Rangplätzen aufbauen (siehe auch **Abbildung 3**), stellt sich natürlich die Frage nach den sozialphysiologischen Konsequenzen für die Tiere. Vergleichweise unstrittig ist, dass diese Situation der sozialen Reorganisation eine Stresserfahrung für die Tiere darstellt, die eine Reihe messbarer verhaltensphysiologischer Adaptationsmechanismen aktiviert (FERNANDEZ *et al.*, 1994; AREY *&* EDWARDS, 1998; DE JONG *et al.,* 1999; DE GROOT *et al.*, 2001; MERLOT *et al.,* 2004; COUTELLIER *et al.,* 2007). Um eine adäquate Beurteilung der komplexen Konsequenzen vornehmen zu können, hat VON BORELL (1995) ein möglichst multidisziplinäres Herangehen vorgeschlagen. Dies aufgreifend haben wir in den Studien 12 (siehe Kapitel 3.2.3.1), 13 (siehe Kapitel 3.2.3.2) und 14 (siehe Kapitel 3.2.3.3) verschiedene Aspekte der immunologischen, endokrinologischen und ethologischen Konsequenzen sozialer Dominanzstrukturen beim Hausschwein untersucht. Wir konnten in Studie 12 (siehe Kapitel 3.2.3.1) zeigen, dass die

Immunfunktion (Lymphozytenproliferation, IgG) der Schweine durch den sozialen Stress des Gruppierens und die damit verbundene soziale Reorganisation beeinflusst wurde. Der soziale Status der Tiere hatte aber keinen Einfluss auf die Kortisolkonzentration im Blutplasma oder andere untersuchte Metabolite des Stoffwechsels. Der zunehmende Erfolg der dominanten Tiere in ihren agonistischen Interaktionen führte jedoch zu einer verbesserten Immunreaktivität, während subdominante Tiere mit einer Immunsuppression reagierten. Dieser immunmodulatorische Effekt der Dominanzstruktur konnte auch in anderen Studien beim Hausschwein nachgewiesen werden (MCGLONE *et al.*, 1993; MORROW-TESCH *et al.*, 1994, SUTHERLAND *et al.*, 2006; RUDINE *et al.*, 2007), obgleich es auch abweichende Berichte gibt, die eine Immunsuppression nach Neugruppieren auch bei dominanten Schweinen beschreiben (DE GROOT *et al.*, 2001). Zudem konnte in ersten Studien gezeigt werden, dass dominante Schweine eine experimentelle Infektion mit einem viralen Krankheitserreger besser bewältigen (z.B. Aujeszkysche Krankheit (HESSING *et al.*, 1994b) und Porzines Reproduktives und Respiratorisches Syndrom (SUTHERLAND *et al.*, 2007)). Enge Beziehungen zwischen Dominanzstatus, Immunität und Krankheitsanfälligkeit gibt es auch in vergleichbaren Studien bei nichtmenschlichen Primaten (COHEN *et al.*, 1997; SCHAPIRO *et al.*, 1998) und Labortieren (BARNARD *et al.*, 1994, 1996; STEFANSKI *&* ENGLER, 1999), teilweise mit widersprüchlichen Ergebnissen zum Einfluss des sozialen Ranges. Nur wenige Arbeiten allerdings zeigen, dass Änderungen im sozialen Status direkte Auswirkungen auf das Immunsystem in Form biologischer Kosten haben (z.B. ZUK *&* JOHNSON, 2000; LINDSTRÖM *et al.*, 2005; HAWLEY, 2006). Die allgemeine biologische Funktion des Immunsystems ist es, entsprechende Verteidigungsmechanismen gegen Infektionen zu liefern und damit zu helfen, die evolutive Fitness eines Tieres zu maximieren (VINEY *et al.*, 2005). Verhaltensökologen sind hierbei vor allem am Zusammenhang zwischen sexueller Signalgebung, sozialem Verhalten (Partnerwahl) und testosteron-vermittelter Immunsuppression als einen Mechanismus der sexuellen Selektion interessiert (*immunocompetence handicap hypothesis:* FOLSTAD *&* KARTER, 1992; ROBERTS *et al.*, 2004). In der Angewandten Ethologie dagegen sind vor allem die Auswirkungen der stressinduzierten Immunsuppression auf Gesundheit, Wohlbefinden und Leistung der Tiere von wissenschaftlichem und praktischem Interesse (MCGLONE, 1990; PUPPE, 2003). Studien an Labor- und Haustieren weisen hier darauf hin, dass sozialer Stress komplexe Änderungen im Hormon-, Neurotransmitter- und autonomen Nervensystem verursacht, die u.a. über zirkulierende Katecholamine und Glukokortikoide das Immunsystem beeinflussen (BOHUS *et al.*, 1993; BLANCHARD *et al.*, 2001; VON BORELL, 2001; SALAK-JOHNSON & MCGLONE, 2007). Folgerichtig wird das hypothalamo-hypophysär-adrenale Stresssystem (HPA-Achse) zur Beurteilung von Stress und Wohlbefinden bei Tieren genutzt (vgl. Übersicht in MORMÉDE *et al.,* 2007). Wenn die biologischen Kosten der Stressbewältigung die Aufrechterhaltung anderer biologischer Funktionen, wie Immunkompetenz, Fortpflanzung *etc.* einschränken, besteht die Gefahr, dass die Tiere Wohlbefinden und Gesundheit beeinträchtigenden „Disstress“ erleiden (MOBERG, 1987, 2000). Adäquate Stressbewältigung kann dagegen auch positive Auswirkungen haben (ZUKIFLI & SIEGL, 1995), was mit dem auf Hans Selye (SELYE, 1976) zurückgehenden Begriff „Eustress“ bezeichnet werden kann (**Abbildung 4**). Das entscheidende Kriterium zur Evaluierung von Stress scheint dabei nicht so sehr die Aufrechterhaltung der Homöostase im Sinne der Beibehaltung unveränderlicher physiologischer Sollwerte zu sein, sondern der als Allostase bezeichnete aktive Prozess der Aufrechterhaltung durch adaptive physiologische Veränderungen (MCEWEN *&* WINGFIELD, 2003; KORTE *et al.*, 2005). Biologische Kosten *(allostatic load)* im Sinne von (Dis)Stress und Beeinträchtigungen des Wohlbefindens (KORTE *et al.*, 2007) entstehen bei Hypo- und/oder Hyperstimulation von Mediatoren der Allostase (z.B. Stresshormone, Immunkompetenz, Neurotransmitter *etc.*). Entscheidend bei der Stressbewältigung ist nicht nur der Stressor selbst (z.B. Art und Dauer der Einwirkung), sondern dessen Kontrollierbarkeit und Vorhersagbarkeit durch ein Individuum (siehe auch **Abbildung 4**) (BOHUS *et al.*, 1993; BASSETT *&* BUCHANAN-SMITH, 2007).

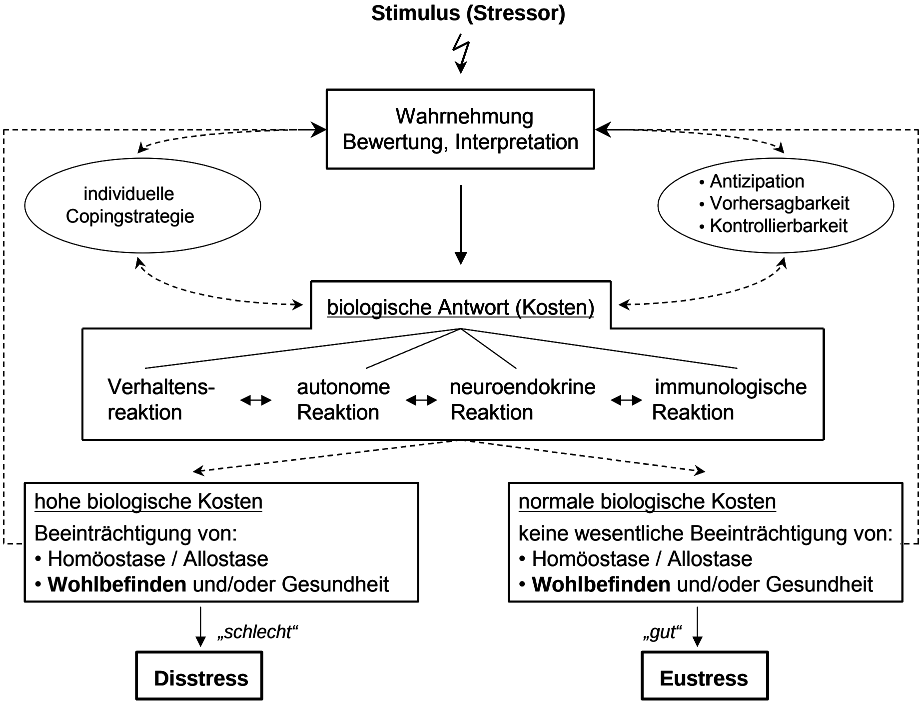

Abbildung 4: Vereinfachtes schematisches Modell der biologischen Stressantwort eines Tieres in Relation zum Wohlbefinden. Die miteinander vernetzten Subsysteme der biologischen Antwort eines Tieres auf einwirkenden und wahrgenommenen Stress hängen sowohl von dessen individueller Bewältigungsstrategie *(coping)* als auch von der Antizipation, Vorhersagbarkeit und Kontrolle der Situation ab. Bei adäquater Reaktion und erfolgreicher Stressbewältigung (Eustress) ist das Wohlbefinden unbeeinträchtigt, möglicherweise sogar verbessert. Dagegen führen Reaktionen, die mit sehr hohen biologischen Kosten bei der Stressbewältigung verbunden sind, zu Disstress mit Problemen bei der Aufrechterhaltung der Homöostase bzw. Allostase und beeinträchtigtem Wohlbefinden (PUPPE, 2003, verändert).

Die Nutztierhaltung betreffend haben wir aus den Ergebnissen der Studie 12 (siehe Kapitel 3.2.3.1) geschlussfolgert, dass es darauf ankommt, Haltung und Management so zu gestalten, dass nicht nur den dominanten Tieren, sondern allen Individuen einer Gruppe die Chance einer möglichst erfolgreichen Stressbewältigung gegeben werden muss, um das biologisch vorhandene Potential von Eustress bezüglich einer „natürlichen“ Verbesserung von Wohlbefinden und Gesundheit (PUPPE, 2003) sowie zur Induktion positiver Emotionen (BOISSY *et al.,* 2007) zu nutzen. Zur praktischen Umsetzung haben wir deshalb als kognitive Umweltbereicherung *(cognitive enrichment)* ein belohnungsorientiertes und die kognitiven Fähigkeiten herausforderndes Fütterungssystem für

Hausschweine entwickelt (ERNST *et al.*, 2005). Erste Ergebnisse zeigen, dass die Tiere tatsächlich mit erfolgreichem Lern- und Bewältigungsverhalten (PUPPE *et al.*, 2007), verbunden mit partiell verbesserter Immunreaktivität und Wundheilung (ERNST *et al.*, 2006), reagierten. Als interessanter Nebeneffekt verbesserten sich – wahrscheinlich infolge der insgesamt erhöhten lokomotorischen Aktivität – auch einige Parameter der Fleischqualität (FIEDLER *et al.*, 2005). Darüber hinaus bietet ein solches System die Möglichkeit, unter sozialen Bedingungen den Einfluss eines positiven Stressparadigmas auf weitere, das Wohlbefinden der Tiere indizierende Parameter zu untersuchen. Hier ergibt sich ein interessanter zukünftiger Forschungsbedarf. So gibt es beispielsweise erste Hinweise, dass endogene Opioide beim Hausschwein vom sozialen Rang der Tiere beeinflusst werden können (ZANELLA *et al.,* 1998). Insgesamt gesehen kann die soziale Bereicherung der Umwelt *(social enrichment)* als ein effektives Mittel der Verbesserung der Haltung angesehen und gezielt eingesetzt werden (SCHAPIRO *et al.*, 1996). Argumente (hauptsächlich aus der biomedizinischen Forschung bei Labortieren kommend), dass Haltungsanreicherungen die Standardisierung und damit die wissenschaftliche Aussagekraft von Experimenten mit Modelltieren gefährden, sind fraglich (WÜRBEL, 2000; 2001; OLSSON *et al.*, 2003) und konnten mittlerweile experimentell bei Mäusen widerlegt werden (WOLFER *et al.,* 2004). Insofern gibt es auch keinen Anlass, Hausschweine, die sich insbesondere für neuroethologische Forschung eignen (siehe LIND *et al.,* 2007), als Modelltiere für die biomedizinische Forschung abzulehnen.

Um den Einfluss vorangegangener sozialer Erfahrungen beim Hausschwein auf ihr späteres Verhalten und endokrine Reaktionen in sozialen Konfliktsituationen genauer analysieren zu können, führten wir in den Studien 13 (siehe Kapitel 3.2.3.2) und 14 (siehe Kapitel 3.2.3.3) gezielte „Resident-Intruder-Experimente“ durch – ein Modell, das mittlerweile auch bei Hausschweinen etabliert wurde, um das Sozialverhalten im Kontext von Aggression oder Dominanz zu untersuchen (ERHARD *&* MENDL, 1997; ERHARD *et al.*, 1997; DE JONG *et al.,* 2000; D'EATH, 2002; D'EATH *&* BURN, 2002; D'EATH *&* PICKUP, 2002). Wir fanden erwartungsgemäß, dass alle reintegrierten Tiere, insbesondere am Anfang der Konfrontationen, deutliche Stressreaktionen aufwiesen, z.B. eine mit dem Anstieg des agonistischen Verhaltens gekoppelte Auslenkung des sympatho-adrenomedullären Stresssystems (SAM-Achse). Dies war besonders ausgeprägt in der unbekannten Gruppe. Allerdings hatten sowohl der soziale Rang des Tieres als auch die Bekanntheit der Gruppe, in die der jeweilige Proband integriert wurde, modifizierenden Einfluss auf das agonistische Verhalten und die endokrinen Reaktionen sowohl der SAM- als auch der HPA-Achse. Ehemals subdominanten Schweinen gelang es in keinem Fall, ihren sozialen Rang zu verbessern; sie waren allerdings auch passiver in den agonistischen Interaktionen und hatten eine insgesamt geringere physiologische Stressantwort. Die ehemals dominanten Tiere dagegen versuchten, ihren hohen sozialen Status zu halten, was in der unbekannten Gruppe nur vereinzelt, in der bekannten Gruppe hingegen immerhin bei der Hälfte der Probanden gelang. Die Erfahrung des Verlusts des sozialen Ranges der nicht erfolgreichen, ehemals dominanten Tiere verursachte dagegen vor allem ihr Gesamtverhalten beeinträchtigenden emotionalen Disstress und aktivierte – offensichtlich zusammen mit der Neuartigkeit der Stresssituation – auch ihr hypothalamo-hypophysär-adrenales Stresssystem. Daraus lässt sich in Übereinstimmung mit Schlussfolgerungen anderer Autoren (MENDL *et al.,* 1992;

DE JONGE *et al.,* 1996; BOLHUIS *et al.,* 2005) schließen, dass soziale Erfahrungen in der Ontogenese (siehe auch **Abbildung 2**) die nachfolgenden Bewältigungsreaktionen gegenüber Herausforderungen beeinflussen (siehe auch **Abbildung 4**) und dass die Tiere auf sich ändernde Umweltbedingungen flexibel reagieren können. Das Vorhandensein konsistenter Bewältigungsstrategien *(coping styles)* (vgl. Übersicht in KOOLHAAS *et al.,* 1999) scheint zwar eine Art Tendenz vorzugeben, sich eher aktiv oder reaktiv zu verhalten (z.B. aggressives Verhalten), das erreichte „Ergebnis“ (z.B. sozialer Rang) ist davon aber kaum abhängig, wie Studien von RUIS *et al.* (2002) und BOLHUIS *et al.* (2005) gleichfalls bestätigen. Das unterstreicht die praktische Relevanz frühzeitiger Sozialisation der Tiere und macht deutlich, dass beim Eingliedern bzw. Wiedereingliedern von Tieren (z.B. Sauenhaltung) deren soziale Vorgeschichte beachtet werden sollte. Eine weitere Möglichkeit ist die Anreicherung der Haltungsumwelt (z.B. mit Stroh), von der insbesondere rangniedere Tiere bezüglich ihres Wohlbefindens (allgemeine Stressreduktion, verringerte Ängstlichkeit) profitieren, wie jüngst gezeigt wurde (O'CONNEL *et al.,* 2004). Neuere Studien haben – ähnlich den Schlussfolgerungen aus unseren Studien – insgesamt gezeigt, dass die Verhaltensplastizität von Nutztieren gegenüber sozialen Umweltbedingungen weitaus größer ist als bisher angenommen (ESTEVEZ *et al.*, 2008). Ob möglicherweise eine erhöhte Gruppengröße durch Verringerung der Aggressionsrate auf der Ebene des Einzelindividuums entsprechende Vorteile bezüglich Adaptationsleistung und Wohlbefinden bringt (ANDERSEN *et al.*, 2004), bedarf entsprechender Kosten-Nutzen-Analysen und weiterer Forschung (TURNER *&* EDWARDS, 2004).

4.3 Fazit und Ausblick

Spätestens seit dem kritischen Blick von Ruth Harrisons Buch *„Animal Machines“* (HARRISON, 1964) auf die intensive Tierproduktion und dem darauf von der britischen Regierung in Auftrag gegebenen Kommissionsbericht unter der Leitung von Roger Brambell (BRAMBELL COMMITTEE, 1965) ist die naturwissenschaftliche Forschung aufgefordert, sich mit den biologischen Grundlagen von Schutz und Befindlichkeiten *(welfare)* bei Haustieren zu beschäftigen (THORPE, 1969). Da auch affektive Zustände Teil evolvierter Anpassungsmechanismen sind und auf proximater Ebene die Motivation der Tiere triggern, ein bestimmtes Verhalten auszuführen oder nicht, können die Maximierung der Fitness und die Optimierung des Wohlbefindens miteinander vernetzte Ziele sein (FRASER & DUNCAN, 1998). Verschiedene Adaptationsprobleme im Kontext der Tier-Umwelt-Interaktion können in der Nutztierhaltung zu Stress, Beeinträchtigungen des Wohlbefindens und ethischen Problemen führen (FRASER *et al.,* 1997). Sozialverhalten, Gruppenstruktur und soziale Organisation einer Tierart sind evolutionär entstandene Charakteristika (vgl. z.B. MCBRIDE, 1976; HENDRICHS, 1978; MENDL *&* HELD, 2001), deren Mechanismen und Konsequenzen unter den Bedingungen moderner Tierproduktion bisher vergleichsweise wenig mit multidisziplinären Ansätzen untersucht wurden. Diese Überlegungen aufgreifend kann insbesondere für den Bereich des Sozialverhaltens beim Hausschwein eine Diskrepanz zwischen den in der Evolution erworbenen Anpassungsmechanismen der Tiere und den üblichen Bedingungen der kommerziellen Haltungsumwelt, auf die sie treffen, postuliert werden (**Abbildung 5**).

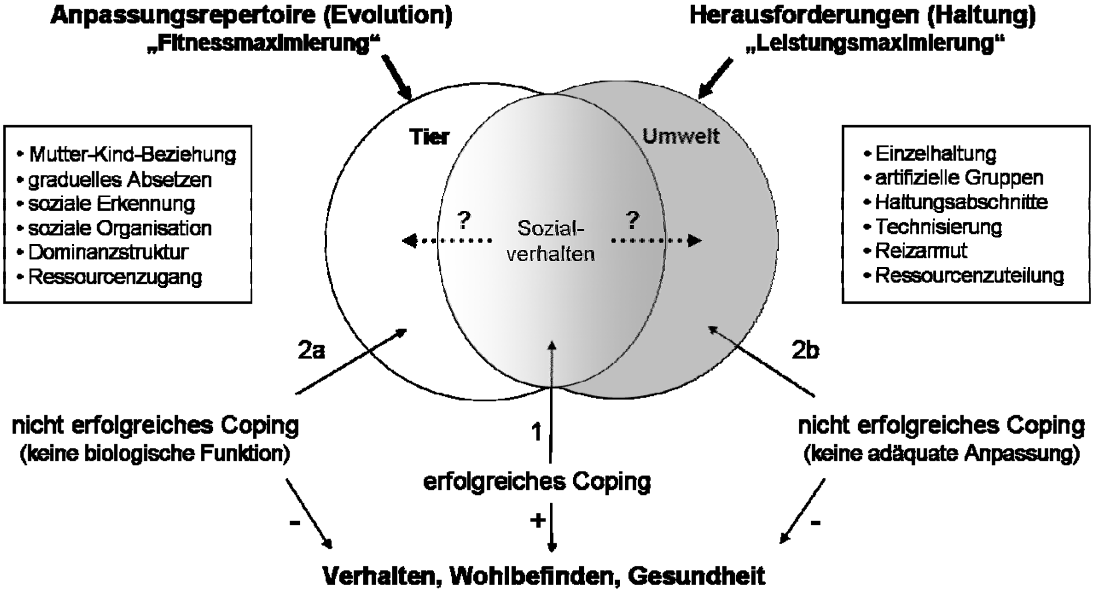

Abbildung 5: Schematisches Modell möglicher Adaptationsprobleme in der Nutztierhaltung, die durch Tier-Umwelt-Diskrepanzen verursacht werden sowie ihre (möglichen) Auswirkungen auf Verhalten, Wohlbefinden und Gesundheit. Probleme in der Anpassungsbewältigung (Coping) mit negativen Konsequenzen im Kontext des Sozialverhaltens können auftreten, wenn die in der Evolution unter dem Gesichtspunkt der Fitnessmaximierung entwickelten Adaptationsmechanismen eines Tieres auf die in erster Linie auf Leistungsmaximierung gerichteten Herausforderungen der Haltungsumwelt treffen: Anpassungen haben keine biologische Funktion mehr (2a) oder es existieren keine entsprechenden Anpassungen gegenüber der aktuellen Haltungsumwelt bzw. sie sind inadäquat (2b). Entsprechen die Umweltherausforderungen dem sozialen Anpassungsbereich der Tiere, ist in der Regel ein erfolgreiches Bewältigungsverhalten mit positiven Auswirkungen möglich (1). Die Frage, wie die Tier-Umwelt-Diskrepanzen durch sinnvolle Verbreiterungen dieses Anpassungsbereichs verringert werden können, ist Teil wissenschaftlicher Fragestellungen und hat praktische Implikationen.

Diesen Hintergrund berücksichtigend zeigen die Studien der vorliegenden Habilitationsschrift exemplarisch, dass eine Verbesserung der Kenntnisse sozialphysiologischer Anpassungsmechanismen beim Hausschwein sowie entsprechende praktische Umsetzungen ultimater und proximater Prinzipien dazu beitragen können, die Diskrepanzen zwischen sozialen „Erwartungen“ der Tiere und realer Haltungsumwelt zu minimieren und auf diesem Wege Verbesserungen im Sinne der Tiere zu erreichen. Das betrifft insbesondere die Kenntnis grundlegender verhaltensphysiologischer Mechanismen der Sau-Ferkel-Beziehung sowie des agonistischen Verhaltens und der Dominanzstruktur in sozialen Schweinegruppen. Dazu ist es hilfreich, den Blickwinkel der Angewandten Ethologie mit Ideen, Theorien und Hypothesen aus Verhaltensökologie und Soziobiologie zu erweitern und mit modernen Methoden der Neuroendokrinologie und Psychoneuroimmunologie zu verbinden. Vorschlag der vorgelegten Arbeit ist deshalb, dass Sozialverhalten der Tiere mit den vorgestellten ethologischen und mathematisch-analytischen Methoden (Modellierung des Saugverhaltens, soziometrische Analyse der Dominanzstruktur) wissenschaftlich zu untersuchen, um mit dieser Hilfe entsprechende verhaltensphysiologische, neuroendokrine und psychoimmunologische Mechanismen und Konsequenzen zu charakterisieren. Unter Einbeziehung geeigneter verhaltenstheoretischer Vorstellungen können damit sowohl weitergehende wissenschaftliche als auch praxisbezogene Schlussfolgerungen abgeleitet werden. Die Verbesserung der Erfüllung sozialer

Verhaltensansprüche der Tiere, wie z.B. die Entwicklung adäquater Beziehungen zu Mutter und Wurfgeschwistern sowie die Möglichkeit, soziale Erfahrungen *(social skills)* in diesbezüglich wichtigen Phasen der Ontogenese zu sammeln, können stress- und verhaltensbedingte Probleme insbesondere in der Saug- und Absetzphase der Ferkel vermeiden helfen. Haltungssysteme, die den sozialen Ansprüchen der Tiere Rechnung tragen (z.B. Gruppenhaltungssysteme für Sauen und Ferkel), sind daher im Grundsatz eher geeignet, Verhalten, Wohlbefinden und Tierschutz zu fördern sowie die ethische Akzeptanz der Nutztierhaltung in der Gesellschaft zu verbessern. Psychosozialer Stress reflektiert die subjektive Antizipation eines Individuums gegenüber Herausforderungen der sozialen Umwelt, die – gerechtfertigt oder nicht – das Empfinden einer möglichen Beeinträchtigung der Kontrolle und Vorhersagbarkeit der Situation und in der Folge eine Kaskade neuroendokriner Adaptationsreaktionen erzeugen (vgl. SAPOLSKY, 2005). Die soziale Stellung (z.B. Dominanzrang) beeinflusst das Ausmass, mit dem Individuen physischen und psychischen Stress empfinden. Hausschweine entwickeln im Verlaufe ihrer Ontogenese soziale Organisationsstrukturen (Saugordnung, Rangordnung), bei denen ihre soziale Stellung bzw. soziale Rolle in komplexen Interaktionen mit Verhalten, Physiologie und Umwelt steht. Davon werden netzwerkartig Wohlbefinden, Gesundheit und Leistung der Tiere beeinflusst, so dass die Kenntnis dieser Zusammenhänge wichtig für die komplexe Beurteilung des Bewältigungsverhaltens der Tiere gegenüber Herausforderungen der Haltungsumwelt ist. Probleme bei der adaptiven Bewältigung, wie sie im Kontext der in der vorliegenden Arbeit beschriebenen sozialen Situationen bei Hausschweinen auftreten können, z.B. bei sozial isolierten oder subdominanten Tieren, haben insbesondere ungünstige Auswirkungen auf deren Immunreaktivität. Managementmaßnahmen, wie Gruppenzusammenstellungen bei der Haltung, können deshalb vom Monitoring dieser Zusammenhänge profitieren. Weiterhin konnte in der Arbeit exemplarisch gezeigt werden, wie der eher abstrakte Begriff des Sozialverhaltens in konkrete soziometrische Parameter gefasst werden kann, die beispielsweise in Zuchtstrategien Verwendung finden können, die neben den bisher üblichen Leistungsparametern auch das Verhalten der Tiere (z.B. agonistisches Verhalten, Dominanzverhalten) berücksichtigen. Darüber hinaus verbessert ein einheitlicher Ansatz zur Bestimmung soziometrischer Kennwerte, die wissenschaftliche und praktische Vergleichbarkeit von Charakteristika sozialen Verhaltens innerhalb und zwischen Rassen und/oder Spezies. Es ist zu postulieren, dass neben seiner „Funktion“ als landwirtschaftliches Nutztier, das Hausschwein als Modelltier für biomedizinische Fragestellungen insbesondere mit psycho- und sozialphysiologischem Hintergrund geeignet ist. Insgesamt bedarf es weiterer Forschungsansätze, die sich mit den sensorischen, sozialkommunikativen, kognitiven und mentalen Fähigkeiten der Tiere beschäftigen, um über adäquate Bereicherungen der Haltungsumwelt, tiergerechtere Managementmaßnahmen und veränderte Zuchtziele das Leben der Tiere unter Menschenobhut zu verbessern. Die Untersuchung und sinnvolle Einbeziehung „natürlichen Verhaltens“ (vgl. Übersicht in LIDFORS *et al.*, 2005; ŠPINKA, 2006) an der Schnittstelle zwischen Tier und Technik in existierenden und künftigen Haltungssystemen ist dabei unerlässlich. Der Bereich sozialen Verhaltens nimmt hierbei eine Schlüsselstellung ein.

5 ZUSAMMENFASSUNG / SUMMARY

Puppe, B. (2008): Ethophysiologische Untersuchungen zum Sozialverhalten beim Hausschwein am Beispiel von Mutter-Nachkommen- und sozialen Dominanzbeziehungen. Habilitationsschrift.

Die vorliegende Arbeit hat das Ziel, die Entwicklung des Sozialverhaltens beim Hausschwein aus dem Blickwinkel ultimater und proximater Verhaltensmechanismen multidisziplinär zu betrachten. Es werden verhaltensbiologische Ansätze der Analyse von Mutter-Nachkommen-Beziehungen und von Dominanzbeziehungen in sozialen Gruppen nach dem Absetzen vorgestellt. Zugleich werden wichtige neuroendokrine und psychoimmunologische Konsequenzen im Kontext dieser beiden Bereiche sozialen Verhaltens präsentiert, die vor allem Wohlbefinden, Stressbewältigung und Gesundheit der Tiere betreffen. Aus den Ergebnissen werden zudem Implikationen für die praktische Tierhaltung gezogen. Die vorliegende Arbeit basiert auf insgesamt vierzehn publizierten Einzelstudien, die vollständig in der kumulativen Publikationsliste der Arbeit vorliegen.

Die Analyse des Saugverhaltens zeigt, dass die Saug-Säugeinteraktion von Ferkel und Sau ein dynamisch ausbalanciertes System zwischen mütterlichem Investment und Ansprüchen der Nachkommen ist, das deutliche Auswirkungen auf die weitere soziale und physische Ontogenese der Ferkel hat. Es werden Möglichkeiten der mathematischen Modellierung von Saughäufigkeit und Saugstabilität vorgeschlagen, die erstens zeigen, dass die Mutter-Nachkommen-Interaktion beim Hausschwein mit ultimaten Erklärungsmustern des Absetzkonflikts beschreibbar ist, und die zweitens einen Ansatz für die verhaltensphysiologische Analyse bei künftigen Fragestellungen bieten. Mittels bioakustischer Lautanalysen und replay-Experimenten wird demonstriert, dass Ferkel sowohl ihre Mütter anhand individueller akustischer Merkmale im Säugegrunzen erkennen können, als auch die inter-individuelle Bedeutung dieses Lautes erfassen. Die Untersuchungen dieser Arbeit zeigen weiterhin, dass die wiederholte soziale Isolation einzelner Ferkel, d.h. deren Trennung von Sau und Wurfgeschwistern, ein geeignetes Deprivationsmodell für sozial lebende Säugetiere, aber auch für den Menschen ist, um gezielt Störungen der Mutter-Nachkommen-Beziehungen in ihren Auswirkungen auf Krankheits- und Stressbewältigung zu untersuchen. Unmittelbare Folgen dieses psychosozialen Stresses für die isolierten Ferkel sind eine verminderte Verhaltensreaktivität, eine Erhöhung basaler Stresshormonwerte und die Suppression von Immunfunktionen. Langfristig verändern sich stressrelevante Mediatoren in zentralen Hirnarealen (z.B. Hypothalamus und Amygdala). Insgesamt kann dies zu einer verschlechterten Bewältigung von Herausforderungen durch die Umwelt beitragen. So wird in der Arbeit auch folgerichtig gezeigt, dass es unter Endotoxin-Challenge zur Beeinträchtigung des Saugverhaltens der zuvor isolierten Ferkel sowie verstärkten Anzeichen von Krankheitsverhalten mit engen Beziehungen zum Stress- und Zytokinsystem kommt. Es lässt sich zusammenfassend schlussfolgern, dass die adäquate Berücksichtigung sozialer Sau-Ferkel-Beziehungen in der Tierhaltung (z.B. durch geeignete Gruppenhaltungssysteme für Sau und Ferkel) Wohlbefinden und Gesundheit der Tiere fördern kann.

Das Absetzen und Neugruppieren der Ferkel ist verbunden mit einer sozialen Reorganisation von der territorialen Saugordnung hin zu einer sozialhierarchischen Dominanzstruktur, die mittels des

Auftretens und des Auganges agonistischer Interaktionen analysiert werden kann. Die soziale Erkennung der Tiere wird durch den Bekanntheitsgrad, nicht aber durch ihre Verwandtschaft bestimmt. Sowohl die Bekanntheit mit der sozialen Umwelt, aber insbesondere auch die mit der Haltungsumwelt beeinflussen hierbei spezifisch das agonistische Verhalten und die Stressantwort der Tiere. Ausgehend von einer strukturellen Dominanzdefinition wird in der Arbeit eine einheitliche und vergleichende methodische Vorgehensweise vorgeschlagen, soziale Dominanzbeziehungen mittels einer Reihe soziometrischer Kennwerte auf Ebene der Dyade, der Gruppe und des Einzeltieres zu analysieren. Unter praxisnahen Bedingungen auf drei typische Haltungsabschnitte angewandt (Absetzferkel, Mastschweine, reproduktive Sauen), werden diese soziometrischen Kennwerte bei jeweils neugemischten, sozialen Gruppen kalkuliert und die sich daraus ergebenden Besonderheiten der jeweiligen Dominanzstruktur interpretiert. Weiterhin wird gezeigt, dass die Immunfunktion einander unbekannter, neu gruppierter Schweine durch sozialen Stress beeinflusst wird. Für die dominanten Tiere führt der zunehmende Erfolg in den und die Kontrolle über die agonistischen Interaktionen zu einer verbesserten Immunreaktivität, während subdominante Tiere in der Folge mit einer Immunsuppression reagieren, die eine potentielle Beeinträchtigung von Gesundheit und Wohlbefinden darstellt. Mittels spezifischer sozialer Konfrontationstests („Resident-Intruder-Experimente“) wird demonstriert, dass sowohl der jeweilige Bekanntheitsgrad der Gruppe als auch die vorhergehenden sozialen Erfahrungen (z.B. der soziale Rangstatus) einen variierenden Einfluss auf Verhalten und endokrine Stressantwort der in diese Gruppen integrierten Tiere haben. Die Möglichkeit, soziale Erfahrungen in entsprechenden Phasen der Ontogenese zu erwerben, ist deshalb essentielle Kompenente einer tiergerechten Haltung und gleichzeitig wichtige Voraussetzung für die spätere Stressbewältigung.

Es lässt sich insgesamt schlussfolgern, dass eine Verbesserung der Kenntnisse sozialphysiologischer Anpassungsmechanismen beim Hausschwein sowie entsprechende praktische Umsetzungen ultimater und proximater Verhaltensprinzipien dazu beitragen können, die Diskrepanzen zwischen sozialen „Erwartungen“ der Tiere und realer Haltungsumwelt zu minimieren und auf diesem Wege Verbesserungen im Sinne der Tiere zu erreichen.

Schlüsselwörter: Sozialverhalten, Mutter-Nachkommen-Beziehungen, Saugverhalten, Agonistisches Verhalten, Soziale Dominanz, Stress, Wohlbefinden, Immunreaktivität, Gesundheit, Hausschwein

SUMMARY

Puppe, B. (2008): Ethological and physiological investigations on social behaviour in domestic pigs examplified with mother–offspring relationships and social dominance. Habilitation Thesis

The aim of the present work is to examine interdisciplinary the development of social behaviour caused by ultimate and proximate factors in domestic pigs. Behavioural approaches of analysing mother–offspring relationships and dominance relationships within social groups after weaning are discussed. Moreover, important neuroendocrine and psychoimmunological consequences for both types of social behaviour are presented in relation to animal welfare, coping with stress and health. In addition, implications for animal husbandry are concluded from the results. The present work is based on 14 published studies which are completely given in the cumulative list of publications.

The analysis of suckling behaviour shows that the nursing–suckling interaction of a sow and their piglets is a dynamically balanced system between maternal investment and the requirements of the offspring. It has considerable consequences for the further social and physical ontogeny of piglets. Mathematical modelling approaches of suckling frequency and suckling stability are suggested which, firstly, show that the mother–offspring relationship in domestic pigs can be described by ultimate causes of behaviour and, secondly, offer a methodological approach for behavioural and physiological studies in the future. By applying bioacoustical analysis of nursing vocalisation and corresponding replay experiments it is demonstrated that piglets are able to both recognise their mothers' voice on the basis of individual call features and comprehend the inter-individual meaning of this vocalisation. Furthermore, a repeated social isolation of piglets, i.e. the social separation from mother and littermates, is a useful model of deprivation not only for socially living mammals, but also for humans to investigate specific disturbances in the mother-offspring relationships and possible consequences for coping with stress and sickness. Decreased behavioural reactivity, elevated level of stress hormones and immune suppression of isolated piglets are found as direct consequences of this psychosocial stress. Long–term consequences are measurable alterations of stress-specific mediators in different brain areas (e.g. hypothalamus and amygdala) indicating an impaired coping with environmental challenges. For example, the present work shows that isolated piglets react to an endotoxin–challenge with impaired suckling behaviour and enhanced sickness behaviour, both strongly coupled with alterations in the stress and cytokine system. It is concluded that an adequate consideration of social sow–piglet relationships in animal husbandry (e.g. by proper group-housing systems for sows and piglets) can improve animal welfare and health.

Weaning and mixing of piglets leads to a social reorganisation from the territorial suckling order towards a hierarchical dominance structure which can be determined by the outcome of agonistic interactions. The social recognition of animals is caused by familiarity rather than by relatedness. Familiarity with both the social environment and especially the housing environment affects the agonistic behaviour and the stress response of piglets. Based on a structural definition of dominance a standardised and comparative approach is suggested to analyse dominance relationships by sociometric methods at dyadic level, group level and individual level. By applying this approach in three typical stages of pig production sociometric measures are calculated in newly mixed groups (weaning piglets, growing pigs, reproductive sows) and the resulting characteristics of the respective dominance structure are interpreted. Furthermore, the present work shows that social stress also affects the immune function of unfamiliar pigs in newly mixed groups. While dominant pigs react with an enhanced immune function caused by increasing success in and better control of the agonistic interactions, subdominant pigs suffer from immune suppression which may result in impaired health and welfare. By using specific social confrontation tests (resident–intruder experiments) it is demonstrated that behaviour and endocrine stress response of individuals to be integrated in a social group vary with their familiarity with the group as well as their previous social experience (e.g. social rank). Therefore, animal–friendly husbandry should offer possibilities to acquire social experiences during ontogeny as an important skill for coping with stress.

In conclusion, improving knowledge on behavioural and physiological mechanisms of social adaptation as well as an appropriate consideration of ultimate and proximate causes of behaviour in animal husbandry may contribute to diminish discrepancies between social “expectations” of the animals and real housing conditions. Thus, a substantial improvement of farm animal welfare can be achieved.

Keywords: Social Behaviour, Mother–Offspring Relationships, Suckling Behaviour, Agonistic Behaviour, Social Dominance, Stress, Welfare, Immune Response, Health, Domestic Pig

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(*) Die im Literaturverzeichnis (Kapitel 6) aufgeführten Zitationen des Verfassers dieser Habilitationsschrift (Erst- und/oder Koautor) sind in keinem Fall identisch mit den Studien der kumulativen Publikationsliste dieser Arbeit im Kapitel 7

7 KUMULATIVE PUBLIKATIONSLISTE

Originalarbeiten zum Kapitel 3.1.1

Saugverhalten der Ferkel und Soziobiologie der Sau-Ferkel-Beziehung

Publikationen:

7.1 Studie 1

Puppe, B. *&* Tuchscherer, A. (2000): The development of suckling frequency in pigs from birth to weaning of their piglets: a sociobiological approach. *Animal Science* **71**, 273-279.

7.2 Studie 2

Puppe, B. *&* Tuchscherer, A. (1999): Developmental and territorial aspects of suckling behaviour in the domestic pig *(Sus scrofa f. domestica)*. *Journal of Zoology* **249**, 307-313.

7.3 Studie 3

Puppe, B. (2002): Die Entwicklung der Beziehung zwischen Sau und Ferkel beim Hausschwein – Eine soziobiologische Betrachtung. *Berliner und Münchener Tierärztliche Wochenschrift* **115**, 445-452.

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The development of suckling frequency in pigs from birth to weaning of their piglets: a sociobiological approach

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Abstract

The suckling frequency in 34 first-lactation sows and their litters in conventional farrowing pens was observed during a 35-day lactation period from birth to weaning. In order to quantify the ontogenetic development of this behaviour a non-linear regression function was used as a model. The maximum (MAX) of the curve was determined at day 8·5 (31·4 sucklings per 24-h period) and was considered as the biological beginning of the weaning process. The occurrence of MAX was shifted towards an earlier time by about 6 days earlier from small (4 to 7 piglets per litter) to large litters (11 to 14 piglets per litter). However, no significant influence of the litter size on the development of the daily suckling frequency and the average individual piglet weight was found. Nevertheless, there was a tendency for an inverse relationship between the litter size and the piglet weight during lactation and, further, for an increased daily suckling frequency during early lactation and a lower frequency during late lactation in larger litters. As a compensation for the decreasing suckling frequency piglets markedly increased their creep food consumption in the last week of the suckling period (day 28 to 35). The results indicate that the suckling behaviour in domestic pigs reflects sow-piglet relationships which are consistent with a theoretically predicted model of weaning conflict. Hence, it is suggested that modelling the suckling behaviour using well defined and comparable measures may be a suitable approach for the evaluation of the weaning process both in terms of sociobiology (e.g. weaning conflict, parent-offspring conflict) and of farm animal science (e.g. performance, housing conditions).

Keywords: *litter size, piglets, sows, suckling, weaning.*

Introduction

Milk transfer from mother to offspring is a useful measure of parental investment in mammals (see Martin, 1984). The nursing and sucking behaviour is important in behavioural studies of the parent-offspring interaction as an indirect measure of milk transfer (Mendl and Paul, 1989). Martin (1984) suggests that lactation constitutes the major component of parental investment for many mammals. Hence, the relation between the rate of parental investment (e.g. the suckle bout frequency) and offspring age can be drawn in a curve in which weaning may be viewed as the phase of parental care where the rate of parental investment drops most sharply (Figure 1). If so, then weaning is the period bounded by the two principal points of inflexion in the curve (Martin, 1984). To test a simple practical application of this hypothesis, in the present study we used the development of the observed daily suckling frequency of domestic pigs. The interests of parents (in this case the mother) and the offspring for the duration of parental investment ('weaning conflict') as a function of investment through the period of parental care (Trivers, 1974) should produce a curve which might be used to evaluate existing housing conditions regarding the state of sow-piglet relationships, their welfare and production efficiency. For example, consistent with parent-offspring conflict theory, Pajor *et al.* (1994) have demonstrated that piglets in confined pens and sows in 'get-away' pens can bias milk production in their respective favours.

It is well known that the domestic pig has a complex nursing and sucking behaviour which in many ways is closely related to the milk ejection (Fraser, 1980). Consequently, Špinka *et al.* (1997 and 1999) have presented evidence that the nursing frequency plays

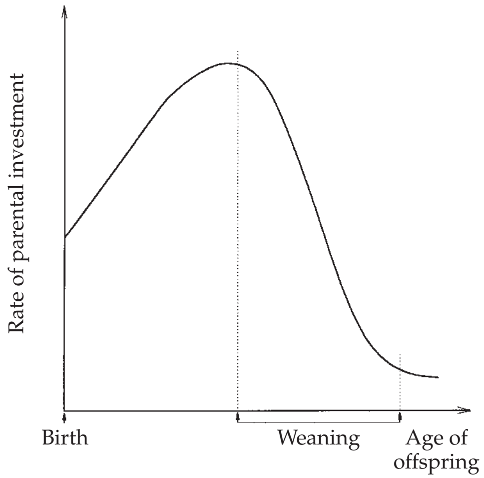

Figure 1 General relation between the rate of parental investment, measured in units of parental resources invested per time unit, and age of offspring (redrawn after Martin, 1984).

a crucial rôle in adjusting the milk output. When the intervals between nursings are shortened and hence the number of nursings per day increases, milk production rises significantly.

Numerous studies on nursing and sucking behaviour in pigs have shown that the absolute number of daily sucklings in a 24-h period varied considerably between the studies and the population used, depending on, for example, housing condition, breed, individual sow or observed week of lactation (e.g. Barber *et al.*, 1965; Hernandez *et al.*, 1987; Jensen, 1988; Jensen and Recén, 1989; Bøe, 1991 and 1993; Wattanakul *et al.*, 1997; Herskin *et al.*, 1999). Mostly, a number of around 24 daily sucklings (or an average frequency of around one suckling per hour) were reported to occur during the first 1 to 2 weeks. However, little information is given on the development of suckling frequency from birth until weaning to evaluate relative changes occurring within the whole period. In pigs, artificial selection has apparently led to increased litter sizes. On the other hand, pigs have evolved a system of brood reduction by mechanisms of high neonatal competition and mortality among new-born piglets (Fraser *et al.*, 1995). Hence, it seems an open question as to if and how the suckling frequency as a possible feature of parental investment is affected by differences in litter size normally occurring in domestic pigs under commercial farm animal conditions. As a first hint, Auldist *et al.* (1998) have shown recently that although there is a linear and positive relationship between milk yield and litter size during a 28-day lactation, only milk composition in late lactation and only suckling interval during early lactation were affected by the litter size.

Therefore, the objectives of this study were as follows: (1) to investigate the development of daily suckling frequency in first-lactation sows over a 35-day lactation period and to calculate it as a function of the weaning process; (2) to analyse relationships between the suckling rate (which might be regarded as the level of parental care given) and two parameters which are thought to characterize the fitness of the offspring: the different number (litter size) and quality (average piglet weight) of piglets in a litter.

Material and methods

Animals and experimental procedure

The daily frequency of the suckling behaviour was observed in 34 first-lactation German Landrace sows and their litters during a 5-week period from birth until weaning. The sows were farrowed individually and allowed to move freely in a conventional farrowing pen (4·5 m^2) with a concrete floor and little straw. Although the sows could not leave their piglets this system allowed both the sow and the piglets some freedom of movement, and hence the sows could control the suckling rate to some degree. Sows were given food individually to appetite twice daily. Supplementary solid food ('creep food') was offered as commercial pellets from day 10 until weaning from an *ad libitum* hopper. All animals had the opportunity of *ad libitum* access to water. The canine teeth of the piglets were clipped at birth.

Suckling behaviour observations

All 34 sows and their litters were video-taped for 24 h daily over the whole 5-week observation period by a time lapse video-recorder (Mitsubishi HS-S5600). Every successful suckling bout occurring daily in a 24-h interval was noted. The particular behaviour of sow and piglets showing all the distinct phases of a whole suckling episode as described by Fraser (1980) and which led to a visible milk ingestion as recently described also by Schön *et al.* (1999), Herskin *et al.* (1999) and Puppe and Tuchscherer (1999) was defined as a single suckling bout. Additionally, in order to evaluate the correctness of the results obtained by the video-technique (the milk intake of piglets was sometimes difficult to recognize on video), eight of the 34 video-recorded sows were directly observed at the expected time of the highest daily suckling frequency (day 8) and at the end of the suckling period (day 35).

Measures of weight and creep food consumption

The piglets were weighed nine times during the suckling period: four times in the 1st week after birth, twice in the 2nd week, and thereafter at the end of each following week up to the weaning day. In order to compare litters with different numbers of piglets, an 'average piglet' of each litter was defined, dividing litter weight at a certain time by the corresponding litter size. Litter creep food consumption was determined by daily weighing of the creep food before feeding and re-weighing of the remainder after the intake. The resulting difference was used as a measure for the creep food consumption.

Statistical and graphical analysis

All statistical analyses were performed using various procedures of the Statistical Analysis Systems Intsitute (SAS, 1989). The development of the daily suckling frequency during the period of lactation (first 35 days of life) was modelled by the non-linear regression function

$$\text{suckling frequency} = \begin{cases} b_0 + b_1.e^{-(b_2.\text{day}-b_3)^2}; & \text{day} \le 35 \\ 0 & ; \text{otherwise} \end{cases} \quad (1)$$

and the regression parameters were estimated with the help of the SAS procedure NLIN using the Marquardt method. The maximum (MAX) of the regression function (1)

$$\text{suckling frequency}_{\text{MAX}}(\text{day}_{\text{M}}) = b_0 + b_1$$
$$\text{reached at day}_{\text{M}} = b_3/b_2 \quad (2)$$

can be considered as the biological beginning of the weaning process when the parental care starts to clearly decrease (see Martin, 1984). Repeated-measures ANOVA (procedure GLM) were used to analyse the ontogenetic development of the suckling frequency and the piglets' weight as influenced by litter size (three previously defined litter size classes: small, medium, large) as the between-subject factor and day as the within-subject factor.

Additionally, the non-linear regression function (1) was extended by the effect of litter size (LS) and its interaction with day in order to uncover the influence on the development of suckling frequency in a three-dimensional non-linear response surface (see equation (3) below) with six parameters estimable by the SAS procedure NLIN.

$$\text{suckling frequency} =$$
$$= \begin{cases} b_0 + b_1.e^{-(b_2.\text{day}-b_3)^2} + \ldots \\ \ldots + b_4.\text{LS} + b_5.\text{LS.day}; \; \text{day} \le 35 \\ 0 \qquad\qquad\qquad ; \text{otherwise} \end{cases} \quad (3)$$

Results

Observation techniques

Of all directly observed successful suckling episodes (280 at day 8 and 234 at day 35), 95·0% at day 8 and 95·3% at day 35 could also be detected by the observer using the video-technique. Because results from the two were adequately similar, only data obtained by the video-technique were used in the following analyses. Moreover, the direct observation of the eight sows showed that 15 additional suckling episodes which occurred at day 8 and 12 episodes at day 35 were unsuccessful, i.e. without a visible milk intake by the piglet. Taking all suckling attempts together, this means a rate of unsuccessful suckling of only 5·1% at day 8 and 4·9% at day 35.

Development of daily suckling and creep food consumption

The development of the daily suckling frequency can well be described by an exponential regression function (Figure 2). The suckling bouts increased in the first days after birth, and after reaching MAX (day 8·5 with 31·4 sucklings) they slowly decreased up to the weaning day. Over the whole observation period of 35 days the daily suckling frequency was generally higher than 24 successful attempts per day. However, the creep food consumption of the piglets (Figure 3) was relatively low in the first 3 weeks but a considerable increase was then found in the last week before weaning (days 28 to 35). The food intake

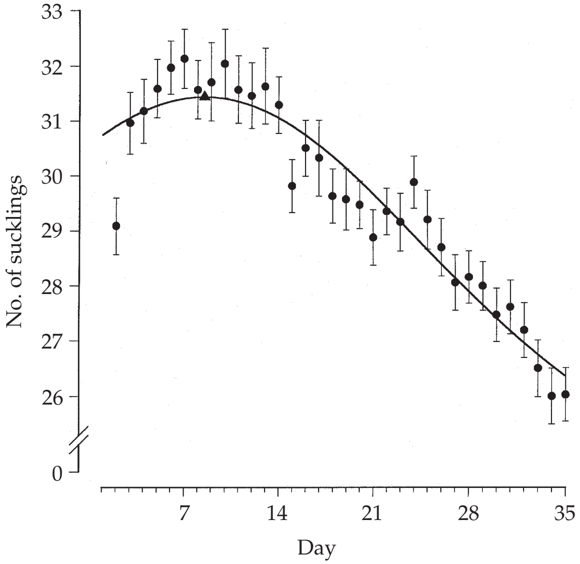

Figure 2 The development of the daily suckling frequency over a 35-day lactation period: mean number of suckling bouts per day (means ± s.e.), the estimated non-linear regression function (equation 1) (———) and the estimated maximum (▲) at day 8·5 (all regression coefficients are significantly different from 0; $P = 0{\cdot}05$).

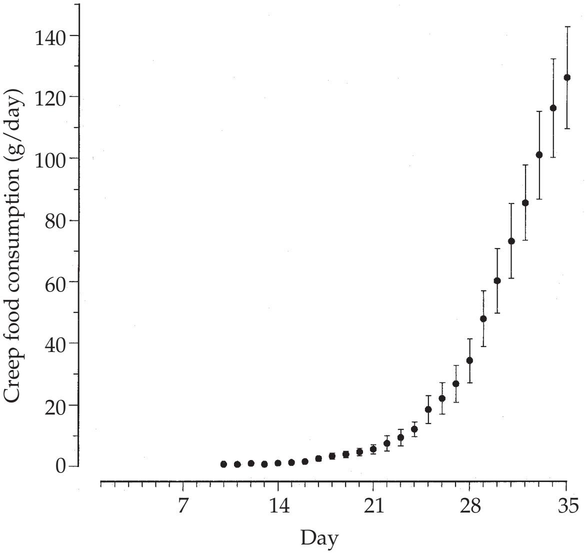

Figure 3 Creep food consumption of an 'average piglet' per litter: means ± s.e. (creep food was provided from day 10 until weaning).

varied greatly among litters (daily CV ranged from 72·2 to 212·0) and was not affected by litter size ($P > 0·05$). At the 3rd week of lactation the suckling frequency of the litters was negatively correlated with the creep food consumption (Pearson's $R = -0·397$ at day 20, $R = -0·391$ at day 24, and $R = -0·342$ at day 26; at each case $P < 0·05$).

Table 1 *Maximum points (equation 2) estimated by a non-linear regression function (equation 1): maxima are given for three different litter size classes and for all litters*

	Small 4-7 piglets	Medium 8-10 piglets	Large 11-14 piglets	All litters†
No. of litters	8	19	7	34
Day (x)	12·4	8·5	6·2	8·5
Suckling bouts (y)	31·4	31·2	32·3	31·4

† Corresponds with ▲ in Figure 2.

Relationships between daily suckling frequency, 'average piglet' weight and litter size

Comparing the three litter size classes, the calculated MAX was shifted towards an earlier time by about 6 days from the small to the large litter size class (Table 1). However, the corresponding suckling bouts at this point increased only for approximately one successful suckling attempt. Figure 4 and 5 summarize the complex relationships between the development of daily suckling frequency and 'average piglet' weight and the litter size. As expected, the data showed very clearly that the suckling frequency ($F_{33,264} = 4·91$; $P < 0·001$) and the weight ($F_{8,144} = 511·73$; $P < 0·001$) depend on the time from birth to weaning. However, an effect of litter size could not be found ($P > 0·05$) on either suckling frequency or weight. As well, no direct correlation occurred at any day between suckling frequency and 'average piglet' weight in the litters (Pearson's R: $P > 0·05$). Nevertheless, some tendencies shown in the three-dimensional surface figures should be noted. First, there was a slight tendency that the smaller the litter size the lower was the suckling frequency in the first days after birth but the higher in the last days of the suckling period (Figure 4). It also seems that with decreasing litter size the points of highest suckling frequency gradually shifted towards later days in the suckling period (Figure 4, see also Table 1). Second, piglets living in smaller litters tended to be slightly heavier than piglets in larger litters (Figure 5).

Discussion

It is well known that the suckling behaviour of pigs and their piglets is of great importance for their subsequent physical and social development of the latter (Puppe and Tuchscherer, 1999). Jensen and Recén (1989) have suggested that suckling can be regarded as a special case of piglets' foraging strategy affected by the sows' behaviour. The present study is an approach to interpret suckling behaviour from a more sociobiological and fundamental perspective. This view also includes the relationships between the suckling behaviour and parameters which are probably more interesting in commercial farming: litter size and piglet weight development.

It should be noted that the interpretations of the present study are based on a relatively simple

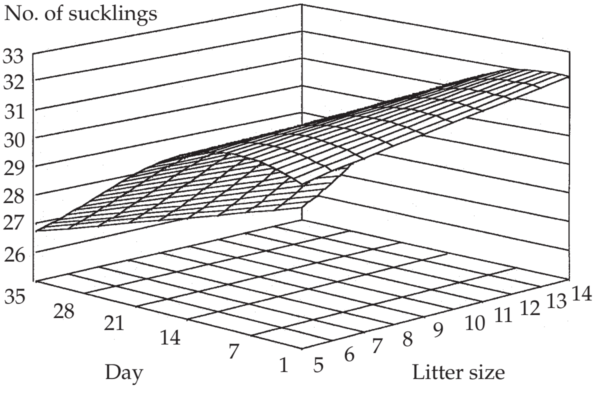

Figure 4 The development of the daily suckling frequency over a 35-day lactation period related to litter size: estimated non-linear response surface (equation 3).

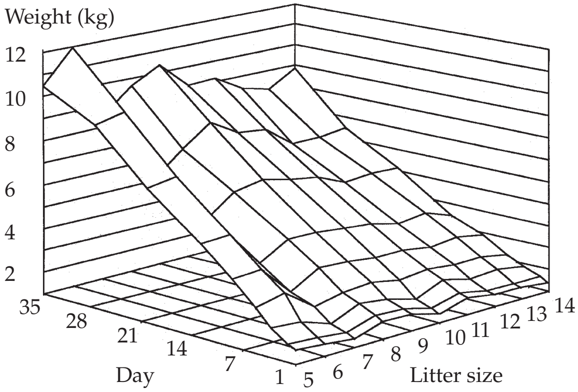

Figure 5 Weight development of an 'average piglet' per litter over a 35-day lactation period related to litter size: means.

behavioural measure, the observed daily suckling frequency. It is known that some features of suckling behaviour may not always be a reliable indicator of the actual milk transfer and hence, the maternal investment (Cameron, 1998). For example, in domestic horses the time spent suckling does not adequately predict the milk intake (Cameron *et al.*, 1999). In the case of the domestic pig, however, some evidences exist that the milk yield is under significant behavioural control (Fraser, 1980; Špinka *et al.*, 1997). For example, the spontaneous changes in the nursing frequency both between day 1 and 2 and day 2 and 3 were positively correlated with the changes in milk output (Spearman's $R = 0{\cdot}64$ and 0·77, respectively) (Špinka *et al.*, 1997).

In contrast to some other studies (Whatson and Bertram, 1980; Castrén *et al.*, 1989; Illmann and Madlafousek, 1995) the incidence of observed unsuccessful sucklings with values around 5% was comparatively low, showed no tendency to change over time, and was therefore in accordance with the findings of Bøe (1991). We can only speculate on possible causes for this finding. Most hypotheses on non-nutritive nursings are based on the view that they are of a functional nature, increasing the tactile stimuli input to the udder and thus the milk production by increasing the local blood flow in the udder and the release of galactopoietic and gastrointestinal hormones (Algers, 1993). However, Illmann *et al.* (1998) have found that the additional massage provided during non-nutritive nursings did not induce higher milk output during subsequent milk ejection. We suppose that from a (socio)biological perspective the incidence of unsuccessful sucklings might be as small as possible because it is a costly behaviour (e.g. time, energy). A 'well functioned' sow-piglet relationship may therefore be characterized by a high incidence of successful and a low incidence of unsuccessful sucklings.

It is clear that the performance of behavioural patterns within the mother-young relationship can be modified by the experimental environment. In the present study the sows had only limited opportunities to deny the piglet's access to the udder. Additional weaning strategies of the sow (e.g. 'early leavers' or 'late leavers') as described in sow-controlled housing systems (Pajor *et al.*, 1999) may influence the absolute values of maternal care given (e.g. also the suckling rate), but in principle it seems unlikely that the process of weaning as predicted by Martin (1984) is completely altered. Hence we suggest, that the suckling frequency, as used in the present study, at least reflects relative changes in the parental investment during the suckling period in a given environment.

The main result of this study is that the ontogenetic development of the suckling frequency can be well modelled by an non-linear (exponential) regression function (see Figure 2) which is consistent with a curve that is theoretically predicted from weaning conflict theory (see Figure 1). According to that model the suckling frequency reaches a maximum at the beginning of the 2nd week (MAX at day 8·5). After Martin (1984) this maximum labels the beginning of the weaning process when the maternal care starts to decrease. A similar time point was found by Bøe (1991) who has shown that from the 2nd week after birth the number of sucklings per 24-h period, the proportion of sucklings initiated by the sow and the time the sows spent with the piglets decreased gradually while the proportion of sucklings terminated by the sow and initiated by the piglets increased. As a further important result of the present study, the beginning of the weaning process (defined by MAX) of the large litters started about 6 days earlier compared with the small litters. This is in agreement with the findings of Bøe (1991) and seems reasonable as large litters have a higher demand on maternal investment and thus are more costly for the sow. As well, larger litters should produce higher suckling stimuli for the sow and, in that way, may reach an earlier MAX.

In the present study, the model is primarily limited to the development of the suckling frequency within the 35-day period of observation. Of course, weaning at day 35 is more a 'technological' point made by humans than the actual biological weaning time. With the present study the question cannot be answered when the weaning process is finally terminated, i.e. when the second point of inflexion is reached according to the definition of Martin (1984). Furthermore, a precise definition of such a point seems to be open. For instance, Jensen and Recén (1989) observed that free-ranging domestic pigs had an average weaning time of 17·2 weeks. Because of the observation that when no suckling had been observed for two consecutive days the udder of the sow was clearly degenerated within 2 to 3 days afterwards, the authors used the appearance of the mothers udder to determine the weaning time with an accuracy of 1 week.

Similar to the findings of Pajor *et al.* (1991) we have demonstrated that creep food consumption began to markedly increase in the week before weaning. Although our method only permits between-litter comparisons the negative correlations between suckling frequency and creep food intake in the 3rd week indicate that the piglets were forced to compensate for the decrease of suckling and therefore the failure of milk intake.

Although we could not find significant effects, the observed tendencies in the present study were that the larger the litters the lower were the piglets' weights during lactation and the higher was the daily suckling frequency during early lactation but the lower it was during late lactation. Both tendencies are in agreement with results found by Auldist *et al.* (1998). A possible explanation is that the sow is interested in earlier weaning of large litters (and reducing the time until her next litter) because the 'quality' of piglets might be lower. Further studies with more consideration given to the energetic expenditure of lactation should shed more light on problems of the cost:benefit ratio in the domestic pig. For instance, there are studies in house mice, *Mus musculus* (König *et al.,* 1988), pigs, *Sus scrofa* (Tuchscherer *et al.*, 1994), rats, *Rattus norvegicus* (Künkele and Kenagy, 1997) and guinea pigs, *Cavia porcellus* (Künkele and Trillmich, 1997) which show that energetic measures are very good at accounting for the physiological mechanisms underlying the behavioural relationships between mother and infants. Especially circumstances in which parents invest more in either the quality or the quantity of their offspring requires experimental data. Nevertheless, modelling the suckling behaviour may be a useful approach to describe the development of parental investment with the help of well defined and comparable measures, as demonstrated in the present study. It is obvious that weaning in pigs is a gradual and prolonged process with a measurable within-litter variation (Jensen, 1995). Hence, the well defined point MAX describes the mother-offspring relation in a better way than looking for a very clear or abrupt change in the real suckling behaviour. Beyond it, defined points based on simple behavioural observations can help to clarify problems raised from a theoretical and practical point of view. For instance, Jensen and Recén (1989) found that older sows invest more in their current young so that weaning occurred later in later parities because the mother's chance of survival decreases with age. This idea, however, was questioned by the results of Bøe (1991). More practically, if the age of the mother and/or the litter size have an influence on various aspects of suckling behaviour this should be considered in fostering programmes. The latter was demonstrated by Winfield *et al.* (1974) for the teat preference of piglets. Housings systems for sows with suckling piglets enabling both to control their nursing and suckling behaviour seems to be advantageous for a biologically based development of mother-offspring relationships. From a biological point of view, a fixed weaning date is therefore not very appropriate because potentially different developmental states of the offspring are ignored.

In conclusion, we suggest that modelling the suckling behaviour with well defined and comparable measures may be a useful approach for evaluating the weaning process both in terms of sociobiology (e.g. weaning conflict, parent-offspring conflict) and of farm animal science (e.g. performance, housing conditions).

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Developmental and territorial aspects of suckling behaviour in the domestic pig (*Sus scrofa f. domestica*)

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Abstract

The suckling behaviour of 327 piglets from 39 litters of primiparous domestic sows *Sus scrofa f. domestica* was analysed regarding the development of territorial suckling consistency during a 5-week suckling period. Additionally, the consistent use of a certain teat pair (1–7) during the whole suckling period was related to weight gain within the suckling period and to social dominance after weaning and mixing of the piglets. The piglets increased their suckling stability consistent with a three-parametric exponential model delivering a time-dependency that is typical for learning curves and which may be a useful methodological approach for studies on behavioural ontogeny. Thus, the development of suckling stability can be regarded as a continuous learning process. After extremely rapid learning within the first 4 days of life a moderate increase of suckling consistency with final stabilization occurred within the second week. After this time, the piglets maintained a high suckling consistency of about 95% up to the end of the observed suckling period. The anterior teats were preferred, whereas piglets suckling at the posterior teats showed a tendency to have lower weight gains and poorer dominance values. However, the lowest suckling stability (consistency) was found with piglets occupying the middle teats. The study shows that individual differences (e.g. weight gain, position in a social hierarchy) have their roots in early ontogenetic characteristics of the neonates (e.g. suckling behaviour, physical strength). The results are discussed with regard to findings and interpretations of behavioural ontogeny in pigs.

Key words: domestic pig, suckling consistency, weight gain, dominance, ontogeny, learning curve

INTRODUCTION

The nursing and suckling behaviour of domestic pigs *Sus scrofa f. domestica* has been frequently investigated ethologically and physiologically (for reviews see Fraser, 1980; Hurnik, 1985; Algers, 1993). A sow gives birth to a large number of precocial young which begin to compete for nutritional resources immediately after they are born. Fraser & Thompson (1991) suggested that the intense neonatal competition among piglets may be an adaptive means of achieving early brood reduction and may thus form part of a strategy to maximize reproduction. The resulting 'teat order' (McBride, 1963) can be regarded as a territorial type of social system rather than a dominance hierarchy (Mattwei, Derenbach & Steinhauf, 1979; De Passillé & Rushen, 1989; Puppe, Tuchscherer, Hoy *et al.*, 1993). The development of a stable teat order is progressive (Van Loen & Molenaar, 1967; Wesley, 1967; Hemsworth, Winfield & Mullaney, 1976; Jones-Baade, Schumacher & Sambraus, 1978; Mattwei *et al.*, 1979) and can have a great influence on the physical and behavioural development of piglets (Gill & Thompson, 1956; Fraser & Jones, 1975; Scheel, Graves & Sherritt, 1977; Hoy & Puppe, 1992; Puppe, Tuchscherer, Hoy *et al.*, 1993). Additionally, observations on the suckling behaviour of piglets provide information on how the behaviour of the young is adapted to maternal investment (cf. Fraser , Kramer *et al.*, 1995) or to housing and social conditions (Whatson & Bertram, 1980; Newberry & Wood-Gush, 1985; Jensen, Stangel, & Algers, 1991). In highly social mammals, the relationship between behavioural development and social organization is particularly interesting (for some examples in a wild member of the *Artiodactyla*, the collared peccary *Tajassu tajacu*; see Byers & Bekoff, 1981; Byers, 1983; Babbitt & Packard, 1990).

Some authors have suggested that a high level of the

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so-called teat fidelity, i.e. a high territorial stability of suckling behaviour, is advantageous to the ontogenetic development of domestic piglets because it reduces teat disputes and missed nursings (De Passillé, Rushen & Hartsock, 1988) or it may ensure better places in the later rank order (Puppe & Tuchscherer, 1994). In particular, piglets suckling at the anterior part of the udder seem to have a better weight development until weaning (Fraser & Jones, 1975; Hoy & Puppe, 1992).

The first aim of the present study was to analyse the suckling stability (consistency) from birth to weaning in litters of first-lactating (primiparous) domestic sows. A more general estimation of the development should be possible with the help of these data formalized as a learning curve. The second aim was to show if or how territorial effects of the teat region influence the development and what may be the consequences for the surviving piglets with respect to physical (weight gain) and social (dominance value after weaning and new mixing) parameters.

MATERIALS AND METHODS

Animals, housing and experimental procedure

A total of 327 piglets from 39 litters of primiparous German landrace sows *Sus scrofa f. domestica* were investigated under standard farming conditions. The mean littersize was 8.4 piglets per sow with a range from 6 to 13 piglets. Previously, the sows were selected for physiologically and morphologically intact teats, i.e. we used only sows with 14 lactating teats (7 teat pairs). Each sow and her litter were kept in a single pen (4.5 m^2) and were observed for the whole suckling period, i.e. from the piglets' birth to the 35th day of age (weaning day). The sows were fed twice a day and the piglets were given additional food from the 10th day after birth. All animals had *ad libitum* access to water. The piglets were individually tagged and they were weighed 2 times weekly during the suckling period; the average daily weight gain of every piglet was calculated.

The offspring were weaned at an age of 5 weeks by the removal of the mother. After 7 weeks without observation, 126 piglets were randomly selected and newly mixed in 14 groups of 9 pigs each, at an age of 12 weeks. Afterwards, the agonistic behaviour of all animals was continuously observed for 3 days (10 h daily), and an interaction matrix (wins *vs* defeats) of all group members was generated. The animals were kept on concrete floor cages (6.5 m^2) with little straw. The animals were given *ad libitum* access to water, and they were fed twice a day for 2 h each (animal to feeding place ratio 2.25:1 with sufficient food for all animals). The trough was located along one side of the pen and was closed with a grating between the feeding times. The area up to 1 m right in front of the feeder was regarded as a 'trough area', the remaining place was called the 'pen area' (Puppe, 1998).

Suckling behaviour observations

Five morning suckling bouts in each litter were observed daily within the first 10 days of age and twice a week after this period. The particular behaviour of sow and piglets showing all distinct phases of a whole suckling episode as described by Fraser (1980) was defined as a single suckling bout. Altogether, we recorded between 80 and 90 suckling bouts for each piglet. Within a suckling episode the milk ingestion can be well recognized because the grunting rate of each sow strongly increases before the milk ejection occurs and the milk flow is coincident with rhythmic and rapid mouth movements of the piglets and a visible and audible swallowing of the milk (see Schön *et al.*, 1999). Hence, we defined the behaviour of the young as suckling (or nutritive sucking) that leads to the successful procurement of milk from the mother's teat, i.e. it results in the ingestion of milk (for more details on suckling behaviour definition, see also Ellendorff, Forsling & Poulain, 1982; Hall, Hudson & Brake, 1988; Rushen & Fraser, 1989). Additionally, we registered the suckling piglet and the used teat number (1–14) of every suckling bout within each litter. Thus we could define two additional 'territorial levels' of the sows' udder, the corresponding number of teat pair (1–7) and the type of used udder region (anterior, middle, posterior). The anterior/posterior region included the two cranial/caudal teat pairs, whereas the middle region included the three remaining teat pairs at the middle of the udder (Hoy & Puppe, 1992; Puppe, Tuchscherer, Hoy *et al.*, 1993).

The daily suckling bouts of each piglet in a litter were successively labelled whether or not a territorial change regarding teat, teat pair or udder region occurred (yes = 1, no = 0). Afterwards, the daily suckling stability (consistency of suckling position) was calculated by the number of changes made by the piglets in relation to all the changes that were possible, i.e. the observed number of suckling bouts minus 1 (Puppe, Tuchscherer, Hoy *et al.*, 1993; Puppe & Tuchscherer, 1995). The obtained stability coefficient ranged from 0 (100% stability) to 1 (0% stability).

As a result of these calculations, each piglet was allocated a 'preferred teat pair', i.e. a teat pair that was used most by the piglet in all observations during the whole suckling period.

Dominance value calculation

The agonistic interactions of dyads after mixing were observed in the trough and the pen area. An agonistic interaction was defined as a fight with an overt bodily attack or a displacement event with physical contact of 2 individuals for more than 2 s and intervening periods of at least 8 s, while the fight either was interrupted or the pigs showed other behaviours (Puppe, 1998). Each end of a fight was immediately evaluated by the observer regarding the outcome. An individual dominance value (DV) for each pig was calculated by the number of observed wins and defeats of a pig in relation to all decisive fights of the animal with other group members (Puppe & Tuchscherer, 1994; Otten *et al.*, 1997; Tuchscherer *et al.*, 1998). DV was used as a relative measure which could vary between −1.00 (no wins) to +1.00 (no defeats).

Statistical analysis and estimation of suckling stability development as a learning curve

All data were analysed using various procedures of the SAS-system (SAS, 1989). To evaluate the increase of the mean suckling stability, the observed differences between days following each other were tested against 0 by *t*-test. For a general estimation, the suckling stability was modelled by a 3-parametric exponential 'learning function' (1), where the regressand y stands for the trait suckling stability at udder region, teat pair or teat, and the regressor x for the day of observation. For $x \rightarrow \infty$ the function tends towards the parameter a which can be interpreted as the final status of the learning process, whereas the starting level of the learning process is represented by the parameter c of $f(x)$ at time $x = 0$. The first derivation (2) describes the slope of $f(x)$ at any time x and can be interpreted as the 'learning progress' at time x; the parameter $b = f'(0)$ is the slope of $f(x)$ at time $x = 0$.

$$y = f(x) = a - (a - c)^{*}\mathrm{e}^{\frac{-b^{*}x}{a-c}} \qquad (1)$$

$$y' = f'(x) = \frac{\mathrm{df}(x)}{\mathrm{d}x} = b^{*}\mathrm{e}^{\frac{-b^{*}x}{a-c}} \qquad (2)$$

Least squares estimates of the parameters *a, b, c* were fitted by the SAS procedure PROC NLIN using the Marquardt method. Moreover, observed differences in the individual suckling stability, the daily weight gain or the dominance values between teat pairs were tested by the Wilcoxon 2-sample test within a non-parametric ANOVA (PROC NPR1WAY).

RESULTS

Development of litter suckling stability within the suckling period

The suckling stability of the observed litters increased significantly ($P \leq 0.05$) each day up to day 4 after birth for all defined 'territorial levels' of the udder (Fig. 1a), e.g. the mean stability at the same teat pair increased from 53.8 ± 2.9% at day 1 to 86.2 ± 2.8% at day 4. After this period, the daily stability further increased (but not significantly) up to the end of the second week with values around 95%. From the beginning of the third week, the suckling stability hardly showed any changes.

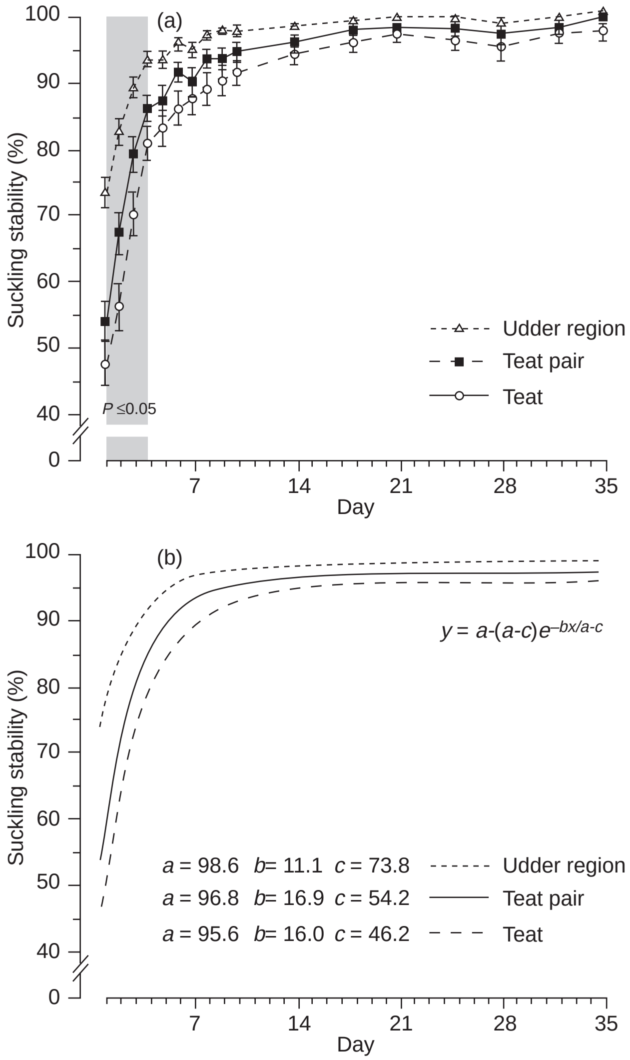

Fig. 1. Development of litter suckling stability for different 'territorial levels' of the sows' udder (udder region, teat pair, single teat) within the observed suckling period: (a) means ± SE (the grey shadowed area represents a daily significant increase with $P \leq 0.05$); (b) calculated three-parametric exponential learning curve.

The course can clearly be described by fitting data with a three-parametric exponential regression model (Fig. 1b). After a dramatic increase during the first days, the graph slowly tends to reach a plateau. The calculated use of a particular udder region was stable ($a = 98.6\%$) from approximately the 7th day after birth ($b_{(7)} < 0.5\%$). The same holds for the preferred teat pair after 9 days ($b_{(9)} < 0.5\%$, $a = 96.8\%$) and to the single teat after 12 days ($b_{(12)} < 0.5\%$, $a = 95.6\%$). The modelled starting level of stability (parameter c) differed between 73.8% for the udder region, 54.2% for the teat pair, and 46.2% for the single teat. This might be an *a priori* preference for an udder region in some piglets or a very rapid initial process that 'imprints' individuals for a certain region.

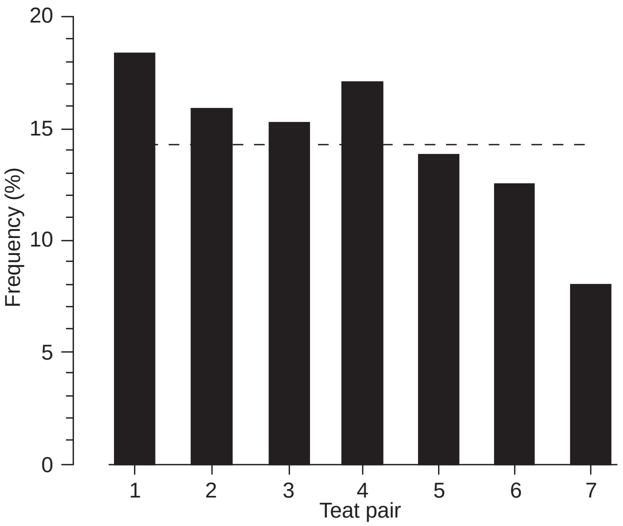

Fig. 2. Distribution of piglets at the udder within the suckling period. The dotted line represents the uniform distribution (14.3%) of piglets at the seven teat pairs.

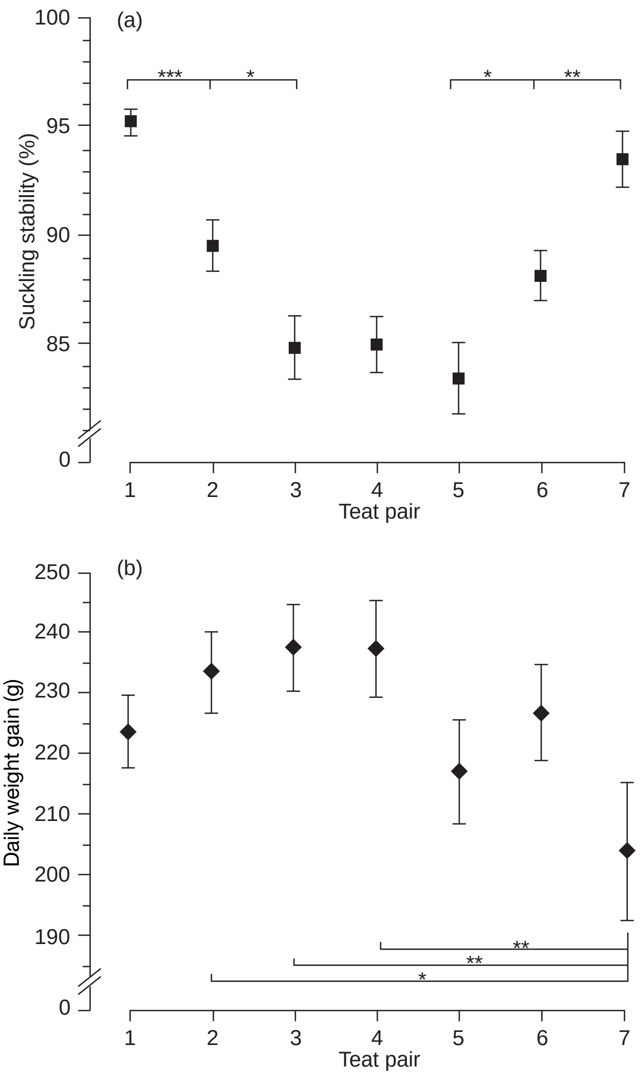

Fig. 3. Relationship between the teat pair use ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$) and (a) the individual suckling stability (means ± SE), (b) the individual daily weight gain (means ± SE).

Relationships between teat pair use and other parameters

Figure 2 shows the distribution of piglets at the udder. The posterior teat pairs were clearly used on fewer occasions by the piglets than the anterior or middle teats (estimated skewness = 0.21).

Piglets that preferred the middle teats suckled with significantly lower stability than piglets preferring the anterior or posterior udder region (Fig. 3a). However, the daily weight gain of piglets suckling posterior was lower than that of the other piglets. When splitting the udder into seven teat pairs this finding was significant for piglets at teat pair 7 compared with piglets at teat pair 2, 3 and 4 (Fig. 3b). In addition, those piglets which were able to use only the posterior udder region (teat pair 6 and 7) within the suckling period showed a tendency to reach lower individual dominance values after weaning and mixing (Fig. 4). Partly, this tendency was significant (trough area: teat pair 6 and 7 *vs* teat pair 4).

DISCUSSION

Development of suckling stability

At birth, the first activity of a piglet is to search for a teat and to suckle. Within approx. 45 min this activity leads the majority of piglets to the udder and to the first ingestion of colostrum (Hoy *et al.*, 1997). The present study demonstrates that piglets subsequently develop suckling preference for a distinct teat within the observed suckling period of 5 weeks. We suggest that the development of this behaviour can follow a learning curve with a three-parametric exponential function. The three parameters of the function can be used for a detailed estimation of the starting (parameter *c*) and final (parameter *a*) level of the suckling stability, and for the modelling of the learning progress on every day (parameter *b*). Because of the use of well-defined parameters, this approach may be very advantageous, especially for studies with an ontogenetic or comparative background.

Despite a different starting level, during the first 4 days after birth the piglets significantly increase their suckling stability at all observed ‘territorial levels’. It seems that the rewarding properties of neonatal suckling (Nowak *et al.*, 1997) lead to the establishment of a preferential relationship with a particular teat. This can be interpreted as an adaptive and rapid learning process to survive these critical days. A high suckling stability helps to ensure that piglets suckle frequently with less competition, a factor that has been shown to be related to survival (Hartsock, Graves & Baumgardt, 1977). Over 50% of the losses of liveborn piglets occur in the first 2–3 days of life (for a review, see English & Morrison, 1984).

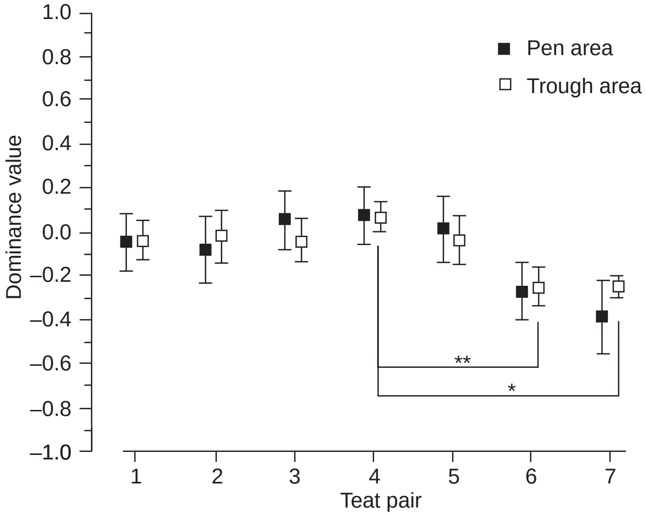

Fig. 4. Relationship between the teat pair use and the individual dominance values after regrouping and new mixing in the trough and the pen area (means ± SE, $^*P \leq 0.05$, $^{**}P \leq 0.01$).

After a period of a further but moderate increase of suckling stability within the second week, the piglets maintain a high suckling consistency at all 'territorial levels' of more than 95%. In another study in litters of primiparous sows we investigated the relation between the daily suckling frequency and the offspring age. The suckling frequency increased in the first days after birth, and after reaching a maximum value in the second week (on average at day 8.7 with 31.5 sucklings per day) it slowly decreased up to the end of the observation period at day 35. Such a maximum point can be considered as the biological beginning of the weaning process when the parental care starts to decrease (see Martin, 1984). Reaching the highest suckling stability exactly at this time may be a behavioural adaptation for the surviving piglets to compensate for the subsequent decrease of maternal investment by a strict teat fidelity. Thus the disruptive sibling competition causing milk inhibition may be avoided (Fraser, 1980).

It is clear that the suckling stability for a single teat must be lower than the use of a certain udder region with several teats. Surprisingly, the calculated differences are relatively small or hardly exist. This means that the single teat is the real object of preference of the piglet's efforts to obtain milk. Some territorial changes may occur when a sow frequently changes her nursing position (Puppe, Tuchscherer, Hoy *et al.*, 1993), when the litter is very large (Hemsworth *et al.*, 1976), under inappropriate housing conditions (Jones-Baade *et al.*, 1978), or if the mother–young interaction (e.g. the acoustic communication) is disturbed for various reasons (cf. Algers, 1993). It must be noted that we only included piglets that reached the weaning day, i.e. all other piglets were excluded from the analysis.

Although there is some research on the postnatal behavioural ontogeny in the European wild boar *Sus scrofa* L. (e.g. Gundlach, 1968), little information is given on the actual process of suckling order stabilization. It seems that some characteristics of suckling in the wild boar (including the development of individual teat preferences) are indistinguishable from those described in the domestic pig (Horrell, 1997). Hence, it can be assumed that the nursing-suckling interaction both in wild and domestic pigs is a relatively fixed behavioural pattern.

Teat pair use, physical and social development

We are in agreement with several other authors that the anterior teats are more often used by the piglets (Rosillon-Warnier & Paquay, 1984; Newberry & Wood-Gush, 1985; De Passillé *et al.*, 1988). Besides some behavioural explanations related to piglets' postnatal orientation (cf. Orihuela & Solano, 1995), a high initial milk yield from the anterior teats and a positive feedback with even a higher yield when the anterior teats are stimulated may explain this (Fraser, 1984). In agreement with this, many authors found that piglets at the anterior teats gain more weight during the suckling period (for a review, see Hoy & Puppe, 1992). However, our results show that only piglets suckling at the end of the udder (teat pair 7) had a significantly lower weight gain, whereas the piglets at the other teat pairs showed no differences. It must be noted that in our study primiparous sows were investigated. It may be that the weight gain advantage of the piglets suckling at the anterior teats becomes more apparent with increasing parity number of the sow (Dyck *et al.*, 1987; Fraser, Thompson & Rushen, 1992; Kornblum, Molnar & Guenther, 1993). Then, the offspring may benefit more from the behaviour of the previous litter(s) of a sow (e.g. a high rate of udder massage), and from an increasing investment of the older mothers. If we take the body weight gain as a 'currency' for measuring fitness in domestic animals, behaviour-production relationships should be investigated at different stages of lactation for a better evaluation of the interplay between ultimate and proximate causation of functional questions (cf. Spinka & Algers, 1995).

The teat use of piglets suckling at the middle of the udder (teat pair 3, 4 and 5) was least consistent, as previously reported by various other authors (Donald, 1937; Fraser, 1975; Fraser & Thompson, 1986; De Passillé *et al.*, 1988; Puppe, Tuchscherer, Hoy *et al.*, 1993). A higher amount of fighting and more missed milk ejections in piglets occupying the middle teats may be responsible for this fact (Fraser & Thompson, 1986).

When unfamiliar pigs are mixed, vigorous fighting occurs (Puppe, Tuchscherer & Tuchscherer, 1997; Puppe, 1998) which was thought to lead to the establishment of a dominance hierarchy (Meese & Ewbank, 1973). In the present study we found a tendency in piglets occupying the posterior teat pairs of having lower dominance values after weaning and mixing than piglets occupying the anterior or middle teat pairs. This tendency was significant only in the trough area (teat pairs 6 and 7 *vs* teat pair 4). As other authors have suspected (Scheel *et al*., 1977), the teat order can form a certain basis of the later social dominance hierarchy in domestic pigs, but the relationships are not very marked (Rosillon-Warnier & Paquay, 1984). The main reasons for this may be physical developmental differences rather than the actual behavioural and social ontogeny of the piglets. Further, Algers, Jensen & Steinwall (1990) suggest that the strategy of agonistic behaviour among piglets is a function of the variation in productivity of the sows' teats, i.e. piglets occupying teats with a poor quality (mostly posterior) show less agonistic and more submissive behaviour after weaning and regrouping.

In conclusion, the present study describes elements of the early social and physical development of domestic piglets. They increase their suckling stability following a three-parametric exponential learning curve which can be used for modelling the starting and final level as well as the daily stability.

The physical and social development of the offspring are partly influenced by territorial aspects of their early suckling behaviour, i.e. how and where the majority of suckling bouts at the mothers' udder occurred. Because domestic pigs are characterized by a slight overproduction of young, their suckling behaviour regarding territoriality, consistency and ontogeny may play an increasing role for the understanding of sibling competition and fitness. The ontogeny of suckling consistency may have been developed as a beneficial behavioural adaptation of piglets securing their needs in the parent–offspring conflict.

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Die Entwicklung der Beziehung zwischen Sau und Ferkel beim Hausschwein – Eine soziobiologische Betrachtung

The development of the relationship between sow and piglet in the domestic pig – A sociobiological approach

Birger Puppe

Zusammenfassung: Das Saug- und Säugeverhalten beim Hausschwein ist eine komplexe soziale Verhaltensinteraktion, in deren Sequenz die Milchübergabe von der Sau an die Ferkel erfolgt. In zunehmenden Maße können auch für Nutztiere ultimate (funktionale) Erklärungsmodelle aus der Grundlagenethologie und/oder Soziobiologie für die Interpretation derartiger sozialer Verhaltensmuster herangezogen werden. Die vorliegende Arbeit gibt daher eine Kurzübersicht zu verhaltensphysiologischen Aspekten des Saugverhaltens beim Hausschwein und erörtert danach die Entwicklung und Konsequenzen von Elementen des Saugverhaltens und der mütterlichen Laktationsleistung unter dem Blickwinkel eines möglichen Eltern-Nachkommen- bzw. Absetzkonflikts. Es kann geschlußfolgert werden, daß – in Ergänzung zu proximaten (kausalen) Erklärungsmodellen – auch die Ontogenese beim Hausschwein unter Berücksichtigung derartiger Prinzipien interpretiert werden kann. In tiergerechten Haltungssystemen, die den Tieren einen höheren Freiheitsgrad bezüglich ihres Verhaltens (z. B. ihr Investment) gewähren, werden ultimate Verhaltensursachen stärker berücksichtigt werden müssen. Insbesondere die über proximate Mechanismen erfolgende Umsetzung ultimater Strategien scheint noch nicht vollständig verstanden und bedarf gezielter Forschung. Trotzdem kann postuliert werden, daß sich nachhaltige Verbesserungen für Wohlbefinden, Gesundheit und Produktivität nur unter Kenntnis und Integration von sowohl ultimaten als auch proximaten Verhaltensursachen erreichen lassen.

Schlüsselwörter: *Sus scrofa,* Hausschwein, Saug-/Säugeverhalten, Ontogenese, Absetzkonflikt, Soziobiologie.

Summary: The nursing and suckling behaviour of the domestic pig is a complex social behavioural interaction aimed to ensure the milk flow from the sow to the piglets. Ultimately caused theoretical frameworks from basic ethology and/or sociobiology are increasingly used to explain such social behavioural patterns. The present paper gives a short survey of ethophysiological aspects of the nursing and suckling behaviour in domestic pigs and, thereafter, discusses the development and consequences of some parts of the suckling behaviour and the maternal lactation performance in terms of a possible parent-offspring and weaning conflict. It is concluded that – additional to proximately caused mechanisms – the ontogeny of domestic pigs can be interpreted with such principles. In housing systems allowing the animals more control over their behaviour (e.g. their investment) ultimate causes should be more considered. Especially the pathways on which the ultimate strategies become effective via proximate mechanisms do not seem to be understood in total and require specific research. However, it is suggested that effective improvements of welfare, health and productivity can only be reached with the knowledge and integration of both ultimate and proximate causes of behaviour.

Keywords: *Sus scrofa,* domestic pig, nursing and suckling behaviour, ontogeny, weaning conflict, sociobiology.

Fragestellung der vorliegenden Arbeit

In den letzten Jahrzehnten haben sich eine Reihe klassischer ethologischer Vorstellungen zugunsten soziobiologischer Ansätze verändert (vgl. Wickler, 1987). Das betrifft auch und insbesondere das soziale Brutpflegeverhalten. Brutpflege verlangt elterliche Investitionen, es kostet Zeit und Energie, bringt Risiken und/oder senkt die Lebenserwartung des Pflegenden. Andererseits erhöht sie die Überlebens- und Fortpflanzungschancen der Nachkommen und damit die Fitness der Individuen über die möglichst erfolgreiche Weitergabe des eigenen Genpools. Es ist unter anderem das Verdienst der Soziobiologie, erstens, derartige Kosten-Nutzen-Überlegungen sowohl inhaltlich als auch sprachlich in die Betrachtungen zur Biologie sozial lebender Individuen eingeführt zu haben (vgl. Voland, 1993), und zweitens, darauf hingewiesen zu haben, daß Eltern vor einem ernsthaften Optimierungsproblem stehen. So wird beispielsweise postuliert, daß es einen Konflikt zwischen Eltern und Nachkommen über Dauer und Menge des elterlichen Investments gibt (Trivers, 1974). Sowohl die Eltern als auch die Nachkommen verfügen über potentielle Möglichkeiten dieses zu manipulieren. Während die jeweils aktuellen Nachkommen dazu tendieren, die Eltern in Richtung einer Maximierung der elterlichen Fürsorge zu veranlassen, müssen die Eltern die Balance zwischen dem Investment in die aktuellen und die künftigen Nachkommen finden, die ihnen, zumindest theoretisch, den größten reproduktiven Erfolg sichert (Fraser et al., 1995). Letzteres bedeutet, daß das Verhalten von Individuen evolutionär darauf ausgerichtet

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worden ist, eine maximale Weitergabe eigener oder verwandter Gene zu erreichen. Es gibt etliche Hinweise darauf, daß solche Prinzipien auch bei Nutztieren wirken (Übersicht in Fraser et al., 1995) und somit deren Verhalten und letztlich auch deren spezifische Leistungen, die für den Menschen von wirtschaftlichem Interesse sind, beeinflussen. Um so erstaunlicher ist die Tatsache, daß die Nutztierethologie erst in den letzten Jahren begonnen hat, zunehmend und ernsthaft solche Denkweisen in ihre Untersuchungen einzubeziehen. So hat der Begriff des Elterninvestments auch Eingang gefunden in den Sprachgebrauch der Nutztierethologie (vgl. Hurnik et al., 1995).

Als ein wichtiger Indikator elterlichen Investments wird das Saug- und Säugeverhalten angesehen (Martin, 1984; Mendl und Paul, 1989). Das Saug- bzw. Säugeverhalten beim Hausschwein *(Sus scrofa)* ist eine komplexe Verhaltensinteraktion zwischen Mutter und Nachkommen, in deren Sequenz die Milchübergabe von der Sau an die Ferkel erfolgt. Obwohl als Phänomen bereits relativ frühzeitig in der wissenschaftlichen Literatur beschrieben (z. B. Donald, 1937; Barber et al., 1955; Gill und Thomson, 1956; McBride, 1963) war die nachfolgende Forschung in erster Linie am Zusammenhang der beobachteten Verhaltenssequenzen mit verschiedenen Leistungs- und/oder Produktionsmerkmalen interessiert (vgl. Fraser, 1980). Später folgten dann verhaltensphysiologische Untersuchungen (vgl. Ellendorff et al., 1982; Algers, 1993; Špinka et al., 1999, Uvnäs-Moberg, 2001), deren Anliegen unter anderem darin bestand, die beobachteten Verhaltensabläufe während des Saugaktes mit zeitnah erhobenen physiologischen (z. B. Druckverhältnisse im Gesäuge) und/oder hormonellen Daten (z. B. Oxytocinfreisetzung) in Korrelation zu bringen und somit auf proximater (unmittelbarer bzw. kausaler) Ebene („Wie“) Erklärungsmuster für das Saug-/Säugeverhalten zu finden. Diese physiologischen Zusammenhänge der Laktation sind mittlerweile gut bekannt und haben in entsprechenden Lehrbüchern Eingang gefunden (vgl. Porzig und Sambraus, 1991; Whittemore, 1993).

Eine ganz andere Ebene von Erklärungsmodellen beschäftigt sich dagegen mit den evolutionär entwickelten Gründen („Warum“) für ein Verhalten – den sogenannten ultimaten (mittelbaren bzw. funktionalen) Faktoren (Alcock und Sherman, 1994; Dewsbury, 1999; bei Nutztieren: vgl. Wechsler, 1992; Puppe, 1995). Neben einer von Tinbergen (1963) vorgeschlagenen Herangehensweise, die generell fordert, sich mit den Ursachen, dem Fitnesswert, der Ontogenese und der Evolution von Verhalten zu befassen, hat vor allem die Soziobiologie, eine Disziplin innerhalb der Verhaltenswissenschaften, die sich mit dem adaptiven Wert sozialer Verhaltensweisen und ihrem Einfluß auf die Fitneß (d. h. der Weitergabe des eigenen Genpools) von Individuen beschäftigt (Wilson, 1975), entscheidende theoretische Anstöße gegeben. So sind folgerichtig in jüngerer Zeit eine Reihe von Arbeiten erschienen, die versuchen, über die klassischen verhaltensbiologischen und ethophysiologischen Erklärungen hinaus, soziobiologische Ansätze, wie sie in der Grundlagenethologie seit längerem bei der Interpretation von Verhaltensweisen verwendet werden, in die Forschung bei Hausschweinen einzubeziehen (vgl. Fraser und Thompson, 1991; Fraser et al., 1995; Jensen et al., 1998; Puppe und Tuchscherer, 1999, 2000; Pajor et al., 2000). Man kann zwar das Saugverhalten beispielsweise als eine frühe Form der Futteraufnahme betrachten, aber es besteht weitgehend Konsens darüber, daß eine Reihe weiterer nichtnutritiver Faktoren mit diesem Verhalten verbunden sind und somit die Ontogenese der Nachkommen mitbestimmen (vgl. Hall und Williams, 1983).

Ziel der vorliegenden Arbeit ist es, mögliche prä- und postnatale Mutter-Nachkommen-Konflikte beim Hauschwein vorzustellen. Schwerpunkt liegt dabei auf der Erörterung der postnatalen Saug-Säuge-Interaktion zwischen Sau und Ferkel und der mütterlichen Laktationsleistung unter dem Blickwinkel eines soziobiologischen Absetzkonflikts. Es wird postuliert, daß der Verlauf sowohl von Saugverhalten als auch Laktation als ein Resultat evolvierter sozialer Verhaltensmechanismen von Sau und Ferkeln angesehen werden kann. Beide können somit als potentielle Parameter für das mütterliche Investment verwendet werden. Kenntnisse über solche Zusammenhänge können helfen, Design und Beurteilung von Haltungsumwelten zu verbessern.

Das Saugverhalten beim Hausschwein – eine Kurzübersicht

Fast unmittelbar nach der Geburt beginnen die Ferkel mit ihren ersten Saugversuchen und die überlebenden Tiere nehmen meist innerhalb der ersten 30–45 min die für die weitere Entwicklung so wichtige Kolostralmilch auf (Hoy et al., 1995; Tuchscherer et al., 2000). Eine Übersicht zur kausalen Analyse der Verhaltenssteuerung des gesamten Säugeaktes gibt Fraser (1980). Proximate Faktoren für den Beginn des Saugverhaltens bei den Ferkeln sind wahrscheinlich am ehesten in angeborenen Appetenzen zu suchen, wie z. B. in auslösenden Orientierungsreizen, die über verschiedene sensorische Kanäle (Akustik, Geruch, Tastsinn, Wärme) wahrgenommen werden und zusätzlich im Motivationsaufbau durch Hunger (vgl. Porzig und Sambraus, 1991). Die Säugebereitschaft der Sau dagegen scheint auf proximater Ebene vor allem unter erheblicher neurohormoneller Konrolle zu stehen (vgl. Whittemore, 1993). Insbesondere das Oxytocin spielt eine vielfältige Rolle bei der Steuerung des Verhaltens und solchen physiologischer Prozessen, die im Zusammenhang mit der Laktation stehen (Übersicht in Uvnäs-Moberg et al., 2001).

Bis zum Aufbau und zur Stabilisierung einer sogenannten Zitzen- oder auch Saugordnung (Donald, 1937; McBride, 1963) zeigt sich eine durch teilweise heftige Kämpfe begleitete Geschwisterkonkurrenz um die Ressource Zitze bzw. Milch (Hartsock et al., 1977; Fraser und Thompson, 1991). Zusammen mit Krankheiten und Erdrückungsverlusten durch die Sau ergibt sich so eine hohe Verlustrate in den ersten 2–3 Tagen (Englisch und Morrison, 1984). Neben Geburtsmasse, allgemein-körperlicher und physiologischer Reife haben insbesondere Verhaltensweisen wie die Fähigkeit der Ferkel zur frühen Orientierung und Milchaufnahme (Tuchscherer et al., 2000) oder auch die frühe kompetitive Durchsetzungsfähigkeit (de Passillé et al., 1988) entscheidenden Anteil an den Überlebenschancen der Jungtiere. Das hat zu der Hypothese geführt, daß Schweine evolutiv an eine Strategie der leichten Überproduktion von Nachkommen adaptiert sind, in der eine vergleichsweise hohe neonatale Nachkommenmortalität infolge Geschwisterkonkurrenz als eine Art Optimierungsfaktor innerhalb des Wurfes durchaus eingerechnet ist (vgl. Fraser, 1991; Fraser et al., 1995).

Die externe Organisation des Saugverhaltens über die gesamte Säugezeit ist in erster Linie durch soziale sowie temporale und lokale Faktoren bestimmt. Während die sozialen Faktoren die Interaktion der Wurfgeschwister untereinander und zur Mutter beinhalten, lassen sich die beiden letzteren Faktoren beispielsweise durch die Häufigkeit (und/oder Dauer) des Saugens pro Tag sowie durch den präferierten Ort am Gesäuge und die Konsistenz (Stabilität) seiner Benutzung beschreiben. Für die tägliche Saughäufigkeit werden meist um die 24 Saugakte je 24 h innerhalb der ersten 1–2 Wochen angegeben (vgl. Puppe und Tuchscherer, 2000). Eine Reihe von Untersuchungen kommt zu dem Schluß, daß Ferkel die kranial gelegenen Zitzen präferieren – verbunden mit einer besseren Gewichtsentwicklung und einer besseren sozialen Stellung (Übersichten in Scheel et al., 1977; Rosillon-Warnier und Paquay, 1984; Puppe und Tuchscherer, 1999), d. h. *de facto* einer Erhöhung ihrer individuellen Fitneß. Insofern läßt sich das Saugverhalten auch als eine wichtige Determinante für die weitere Ontogenese der Tiere verstehen.

Beispiele für Mutter-Nachkommen-Konflikte beim Hauschwein

Pränatale Konflikte

Wickler und Seibt (1983) haben die These aufgestellt, daß Mütter iteroparer (mehrere Aufzuchten) und polytoker (mehrere Junge per Aufzucht) Spezies eine Aufzucht von Nachkommen mit abnormal geringer Wurfgröße unter dem Gesichtspunkt eigener Fitneßerwägungen mittels unter evolutionärem Druck entstandenen physiologischen Mechanismen „zurückweisen" können. Aus Gründen hoher biologischer Kosten sollte ein solcher Mutter-Nachkommen Konflikt schon sehr frühzeitig durch die Mutter „gelöst" werden, z. B. durch einen präpartalen, intra-uterinen Infantizid (Wickler, 1986). So haben Polge et al. (1966) gezeigt, daß Hauschweinsauen, die weniger als 5 Embryos angelegt haben, die Trächtigkeit mittels hormoneller Gegenaktivierungen nicht weiter führen. Der Vorteil ist offensichtlich; eine nächste, erfolgreichere Aufzucht und somit eine Fitneßerhöhung wird früher möglich.

Postnatale Konflikte

Der weitaus größte Teil der Untersuchungen aber konzentriert sich auf den postnatalen Zeitraum. Martin (1984) postuliert in Interpretation des Triverschen Eltern-Nachkommen-Konflikts, daß die Laktation bei Säugetieren eine Hauptkomponente des elterlichen Investments darstellt. Zwischen dem Grad der elterlichen Investition (z. B. aufgewendete Zeit und/oder Energie) und dem Alter der Nachkommen ergibt sich eine Kurve, die das Absetzen der Nachkommen definiert (Abb. 1). Der eigentliche Absetzprozeß beginnt ab dem Punkt, an dem das elterliche Investment nach einem Maximum deutlich zu sinken beginnt und endet am zeitlich folgenden Minimum. Insofern ist eine solche Kurve als Ergebnis der Höhe des elterlichen Pflegeaufwandes im Konflikt zu den Pflegeansprüchen der Jungen zu verstehen. Dieser Interessenskonflikt wird in der Terminologie der Soziobiologie auch als Absetzkonflikt („weaning conflict") bezeichnet. Bei Übertragung dieses theoretischen Ansatzes auf das unter kommerziellen Bedingungen gehaltene Hausschwein ist zunächst zu klären, ob es Elemente des mütterliche Investments gibt, die einer solchen Kurve folgen, und wenn ja, ist zu fragen, welche Konsequenzen sich daraus ergeben.

Saug-/Säugeverhalten

Modellierung der Saughäufigkeit. Durch mathematische Modellierung lassen sich sowohl Einsichten in die zugrunde liegenden Mechanismen als auch die Konsequenzen biologischer Prozesse gewinnen. Das ist auch bei Elementen des Saugverhaltens des Hausschweins möglich, beispielsweise bei der Ontogenese der Saughäufigkeit (Abb. 2, oben; Puppe und Tuchscherer, 2000). Gleichzeitig liefern die Parameter der verwendeten nichtlinearen Regressionsfunktion definierte und biologisch interpretierbare Größen dieses komplexen sozialen Verhaltens. Es ist klar zu erkennen, daß die Entwicklung der Anzahl der erfolgreichen Saugakte (mit Milchaufnahme der Ferkel) einer theoretischen Verlaufsvorhersage des Eltern-Nachkommen-Konflikts (vgl. Abb. 1) entspricht (Puppe und Tuchscherer, 2000). Nach anfänglichem Anstieg des mütterlichen Investments mit einem berechneten Maximum bei 8,5 Tagen und im Mittel 31,4 gewährten täglichen Saugakten fiel das Investment im Verlaufe der weiteren Säugeperiode. Soziobiologisch gesehen kann das Maximum (MAX) als der biologische Beginn des Absetzprozesses interpretiert werden, da ab hier das mütterliche Investment deutlich zu sinken beginnt (Martin, 1984; Puppe und Tuchscherer, 2000). Das Ende dieses Prozesses wäre durch ein Minimum charakterisiert, das im vorliegenden Beispiel aber (noch) nicht erreicht wurde. Darüber hinaus konnte mittels dieses Modells gezeigt werden, daß größere Würfe (11–14 Ferkel) einen deutlich früheren Beginn des Absetzens im Vergleich zu kleineren Würfen (4–7 Ferkel) aufwiesen (6,2 vs. 12,4 Lebenstage) – möglicherweise ein Effekt des insgesamt höheren Saugstimulus. Da Ferkel aus größeren Würfen die in der Regel kleineren Individualgewichte aufweisen, erhebt sich die Frage nach den Umständen, unter denen Sauen entweder in die Qualität oder die Quantität ihrer Nachkommen investieren. So hat Mendl (1994) vorgeschlagen, daß eine mögliche Alternativstrategie von Müttern kleinerer Säugetierwürfe darin bestehen könnte, ihr Investment zu erhöhen, um auf diesem Wege die Qualität (und damit die Fitneß) ihrer Nachkommen zu erhöhen. Allerdings bedarf es hier weiterer Untersuchungen und Daten, um die Umstände zu klären, unter denen eine solche Strategie eine adaptive Alternative zum meist postulierten Abbruch oder gar Infantizid werden kann.

Modellierung der Saugstabilität. Die Entwicklung der Saugstabilität der Ferkel an einer bestimmten Zitze ließ sich mit

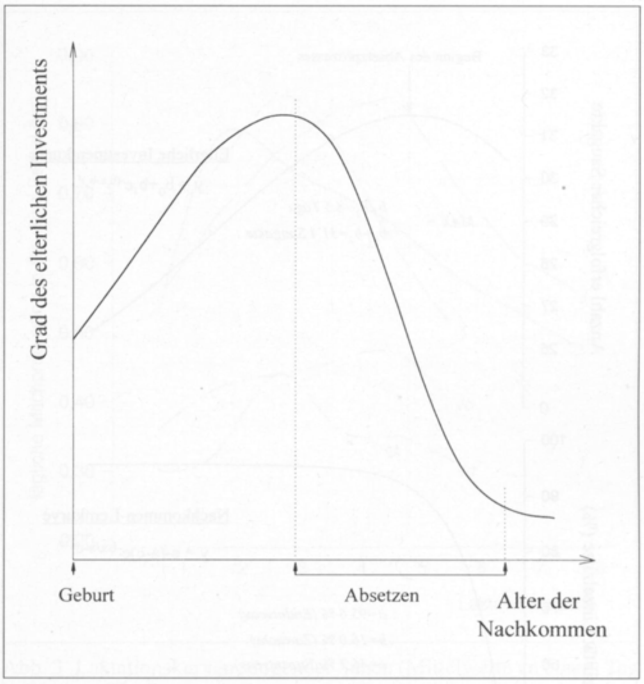

Abb. 1 Schema der Beziehung zwischen Grad des elterlichen Investments und dem Alter der Nachkommen. Modifiziert nach Martin (1984) und verändert entnommen aus Puppe und Tuchscherer (2000). Weitere Erklärungen im Text.

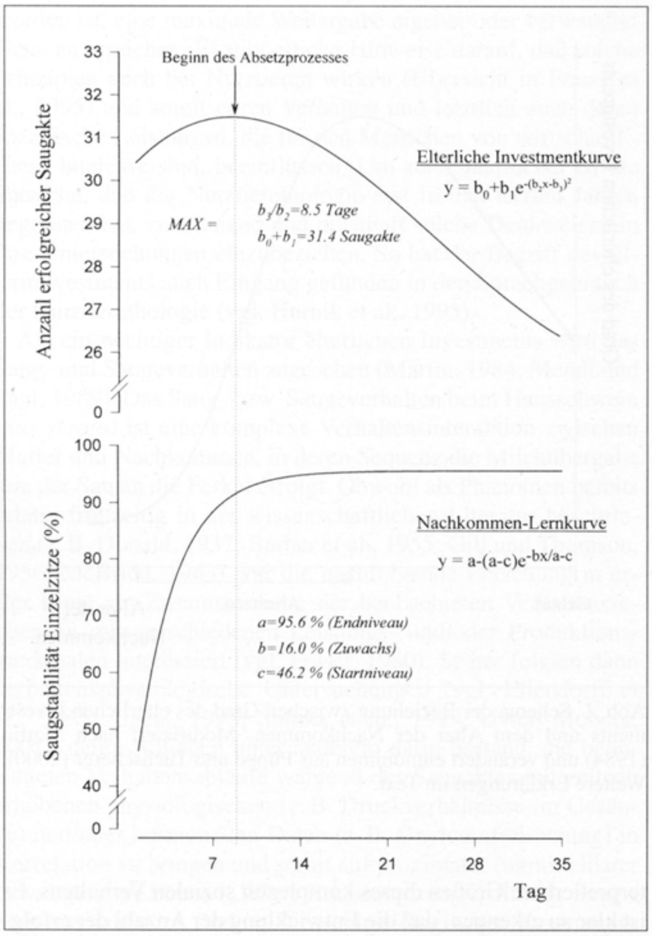

Abb. 2 Modellierung der Entwicklung von täglicher erfolgreicher Saughäufigkeit (oben, 34 Würfe) und Saugstabilität (unten, 39 Würfe) bei Hausschweinen (Jungsauen) im Verlaufe einer 35tägigen Säugezeit. Modifiziert und zusammengefaßt nach Puppe und Tuchscherer (1999, 2000). Es sind die dabei verwendeten Gleichungssysteme sowie wichtige berechnete Parameter angegeben. Die Würfe (Sau und Ferkel) wurden jeweils einzeln in Laufbuchten gehalten, die Sauen waren nicht fixiert. Weitere Erklärungen im Text.

einer 3-parametrigen Exponentialfunktion modellieren und widerspiegelt somit den Verlauf einer typischen Lernkurve (Abb. 2, unten; Puppe und Tuchscherer, 1999). Als definierte und nutzbare Parameter der Kurve können das Start- (hier: 46,2 %) und erreichte Endniveau (hier: 95,6 %) sowie der Zuwachs in der Saugstabilität zu einem bestimmten Zeitpunkt (hier: 16,0 %) gelten. Auffallend war der rapide, durchaus prägungsähnliche Anstieg der Saugstabilität in den ersten 4 Lebenstagen. Als eine Art Gegenstrategie der Ferkel zum mütterlichen Investmentsentzug kann interpretiert werden, daß die maximale Saugstabilität in etwa zu dem Zeitpunkt erreicht wurde (Abb. 2, gepunktete Linie), als der soziobiologisch definierte Absetzprozess begann. Danach bleibt die Saugstabilität der Ferkel innerhalb des Untersuchungszeitraumes auf einem hohen Niveau konstant. Ein deutlicher Abfall der Stabilität kann erst zum Ende des Absetzprozesses erwartet werden, der hier nicht erreicht wurde. Es konnte weiterhin gezeigt werden, daß die Stabilität des Saugens der Ferkel auch in Bezug zur Präferenz eines bestimmten Zitzenpaares bzw. der präferierten Gesäugeregion (z. B. kranial oder kaudal) Endwerte von über 90 % erreichte (Puppe und Tuchscherer, 1999). Das läßt darauf schließen, daß tatsächlich die Einzelzitze das „Zielobjekt“ der lokalen Präferenz ist, während beispielsweise die Gesäugeregion eher als Orientierungshilfe für die Tiere dienen könnte.

Gesäugemassage. Es konnte gezeigt werden, daß die Dauer und Intensität der Gesäugemassage der Ferkel vor und nach dem Saugakt die Milchergiebigkeit der entsprechenden Zitze beeinflußt (Algers und Jensen, 1991). Dies wird ganz offensichtlich sowohl über die Hormone Oxytocin und Prolaction als auch über eine Reihe anderer aktiver Substanzen (z. B. Somatostatin, Glucagon u. a.) vermittelt (Algers et al., 1991). Insbesondere die Nachmassage kann somit zur Manipulation der Muttersau bezüglich der Gewährung von Ressourcen (Milch) genutzt werden – ein Verhalten, das gewisse Ähnlichkeiten zum „Betteln“ der Jungtiere bei Vögeln hat (Jensen et al., 1998). Die typische Grunzvokalisation der Sau während des Sauaktes hingegen scheint als ein Signal zu dienen, das die Ferkel durch den Saugakt führt (Schön et al., 1999). So steigt ca. 20 sec vor dem eigentlichen Milchfluß die Grunzrate der Sau rapide an – ein Zeichen für die Ferkel, jetzt unbedingt an der Zitze zu sein und möglichst rasch und viel Milch in der begrenzten Zeit des Milchflusses aufzunehmen. Dabei spielen offensichtlich Mechanismen der Verwandtenerkennung („kin recognition“) eine wichtige Rolle, denn Ferkel präferieren deutlich die Vokalisation der eigenen Mutter (vgl. Puppe et al., 1999). Insgesamt aber scheint nicht ein Mechanismus des *a priori* Wissens der Verwandschaft entwickelt zu sein, sondern eher ein Lernprozeß entsprechender Reize (z. B. Geruch, Vokalisation), anhand derer die Tiere beispielsweise bekannte und unbekannte Tiere unterscheiden (vgl. Puppe, 1998).

Fehlsaugakte. Es kommt vor, daß ein Teil der Saugakte ohne Milchaufnahme durch die Ferkel endet. Diese nichtnutritiven Saugakte können bis zu 30 % betragen (Illmann et al., 1999). Neben verschiedenen Erklärungen, welche die Ursache im wesentlichen in „Interaktionsproblemen“ zwischen Sau und Ferkel im Verlaufe des Saugaktes sehen, kann dies auch als ein manipulatives Mittel der Sau verstanden werden, das Investment in den gegenwärtigen Nachwuchs zu begrenzen (Illmann et al., 1999). Die Sauen scheinen dazu zu tendieren, ihr Investment unter dem von den Jungen geforderten Level zu halten (vgl. Fraser et al., 1995). Andere Ergebnisse scheinen widersprüchlich. Während Barber et al. (1955) beobachteten, daß die Sauen vor allem in der späten Laktationsperiode (5.–8. Woche) entweder mit Fehlsaugakten oder sogar einer Totalverweigerung auf die Versuche der Ferkel Milch zu erhalten, reagierten, terminierten Whatson und Bertram (1980) den Höhepunkt an Fehlsaugakten zwischen der 2. und 3. Lebenswoche *post partum* Puppe und Tuchscherer (2000) fanden keinen Unterschied bei den Fehlsaugakten am Anfang und am Ende der Säugezeit. Es ist offensichtlich, daß eine Reihe weiterer Mechanismen für die „Entstehung“ von Fehlsaugakten verantwortlich ist (vgl. hierzu Fraser, 1977), so daß nur über eine Vereinheitlichung von Fragestellung und Untersuchungsmethodik ein Erkenntnisgewinn bezüglich deren Rolle als Mittel des elterlichen Investments möglich scheint.

Mütterliche Laktationsleistung

Während das Saugverhalten in Form gewährter Saugakte eher als ein indirektes Maß mütterlicher Investition angesehen werden kann (Mendl und Paul, 1989), läßt sich als ein direktes Äquivalent des energetischen Investments in die Nachkommen die mütterliche Laktationsleistung heranziehen. Letztere wird beim Hausschwein überwiegend dadurch bestimmt, daß die Ferkel jeweils vor und nach dem Saugakt gewogen werden

(„weigh-suckle-weigh"-[WSM]-Methode) – aus der Differenz ergibt sich die aufgenommene Menge Milch. Nachteile dieser Methode liegen in den durch das ständige Wägen verursachten Störungen der sozialen Interaktionen von Sau und Ferkeln (z. B. des Säugeintervalls), im Potenzieren von leichten Wägefehlern bei den Ferkeln und in dem nicht wirklich exakt zu bemessenden Gewichtsverlusten beispielsweise durch Defäkationen u. ä., die eine Unterschätzung der tatsächlichen Milchleistung verursachen können (vgl. den Hartog et al., 1984; Pettigrew et al., 1985). Als allerdings aufwendigere Alternative kann hier die Markierung mit Isotopen (D_2O) eingesetzt werden („isotope-dilution"-[ID]-Methode), die eine offensichtlich genauere Schätzung der Milchleistung erlaubt (Pettigrew et al., 1985).

Abbildung 3 zeigt die auf die tägliche Milchmenge pro Sau und Ferkel abgeglichenen Laktationskurven verschiedener Studien. Erstens, obgleich im Niveau sehr unterschiedlich, ist der prinzipielle Kurvenverlauf über die Säugezeit ähnlich dem in Abbildung 1 dargestellten Verlauf. Zweitens, es existiert ein lokales Maximum der Milchproduktion, welches – je nach Studie – zwischen der zweiten und fünften Laktationswoche liegt (vgl. hierzu auch Hartman et al., 1984). Betrachtet man ein solches Maximum als Zeitpunkt der höchsten Investition in die Nachkommen, ergibt sich eine Differenz von ungefähr einer bzw. möglicherweise zwei Wochen zwischen den verschiedenen Investitionsmaxima – zum einen ein Maximum in der zweiten Woche (gemessen durch das Saugverhalten: vgl. Abb. 2) und zum anderen um die dritte Woche herum (bestimmt durch die mütterliche Laktationsleistung: vgl. Abb. 3). Dabei ist zu beachten, daß die dargestellten Laktationskurven von älteren Studien stammen (hier waren die Originaldaten für die weitere Berechnung bzw. Standardisierung zugänglich). Heutige moderne Zuchtlinien scheinen u. a. durch den darauf liegenden Selektionsdruck insgesamt mehr Milch zu produzieren (Sinclair et al., 1999), so daß es nicht unwahrscheinlich ist, daß auch das Laktationsmaximum etwas früher erreicht wird. So geben beispielsweise Whittemore (1993) sowie Beyer und Jentsch (1994) das Laktationsmaximum mit ungefähr drei Wochen an. Interpretiert man das im Sinn eines möglichen Absetzkonflikts, könnte dies bedeuten, daß auf proximater Ebene verschiedene ethologische und physiologische Mechanismen zeitversetzt „agieren" können. Das erscheint grundsätzlich auch sinnvoll, da aus etlichen Studien bekannt ist, daß die Milchproduktion bei Hausschweinen unter erheblicher Kontrolle des Verhaltens der Tiere steht (Fraser, 1980). So sind beispielsweise die spontanen Änderungen in der Saugfrequenz in den ersten Lebenstagen nach der Geburt signifikant positiv mit den Änderungen in der Milchmenge korreliert (Špinka et al., 1997). Andere Verhaltensweisen (z. B. eine sinkende Anzahl der durch die Sau initiierten Saugakte) zeigen, daß die Mütter relativ frühzeitig beginnen den Absetzprozeß einzuleiten (vgl. Jensen und Recén, 1989; Bøe, 1991) – möglicherweise bevor bisher bekannte physiologische Regelmechanismen dies überhaupt erkennen lassen.

Schlußfolgernd aus Untersuchungen an Hausmäusen *(Mus musculus)* scheint sich anzudeuten, daß es in erster Linie die Mütter sind, die mittels der Steuerung ihrer Laktationsleistung den Absetzkonflikt und damit auch den Zeitpunkt des Absetzens bestimmen (vgl. König und Markl, 1987; König et al., 1988). Dabei wird das mütterliche Pflegeverhalten (einschließlich der Aufrechterhaltung der Laktation) durch ein komplexes Zusammenwirken verschiedener, von den Nachkommen ausgehender Stimuli (einschließlich ihres Verhaltens), die ihrerseits eine Reihe neurophysiologischer Prozesse in der Mutter auslösen, beeinflußt (vgl. Übersicht in Stern, 1997). Letztere bilden die proximaten Realisierungsvarianten mittels derer die ultimaten, evolutionär entwickelten Mechanismen wirken können.

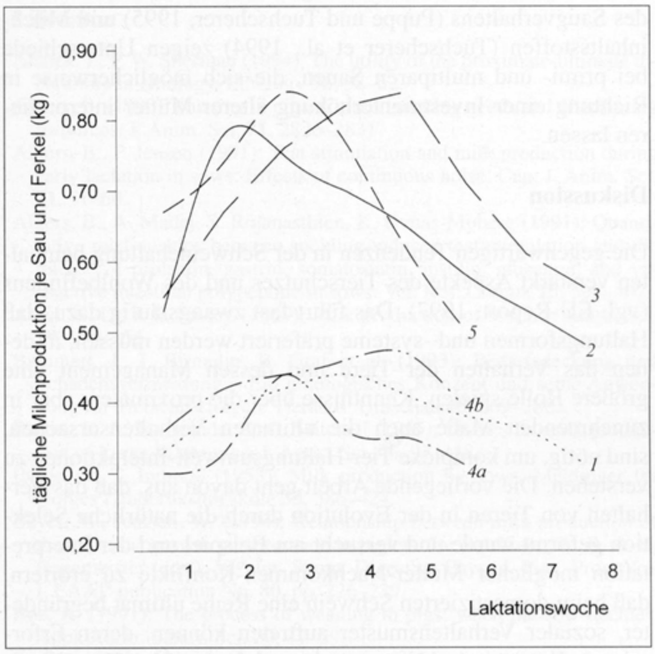

Abb. 3 Laktationskurven säugender Sauen (Mittelwerte an einem Tag je Woche). Die Daten wurden verschiedenen, älteren Originalarbeiten entnommen und – wenn nötig – auf die tägliche Milchmenge (kg) je Sau und Ferkel umgerechnet. Die Bestimmung der Milchmenge in den Originalarbeiten war grundsätzlich durch die „weigh-suckle-weigh"-Methode erfolgt. Weitere Erklärungen im Text. 1 – Hempel (1928): 22 Sauen, veredelte Landrasse; 2 – Niwa et al. (1951): 9 Sauen, Middle White; 3 – Berge und Indrebø (1953): 11,3 Sauen, Norvegian Landrace; 4 – Allen und Lasley (1960): a – 19 Sauen, Duroc, b – 14 Sauen, Landrace; 5 – Hartman et al. (1962): 6 Sauen, Hampshire und Duroc.

Alter der Mütter

Folgt man der Logik des Mutter-Nachkommen-Konflikts, sollte das Alter der Mütter Auswirkungen auf ihre Investmentmotivation haben; ältere Mütter sollten mehr in die Nachkommen investieren, da ihre Chancen auf weitere Nachkommen immer geringer werden (Trivers, 1974). Bisherige Untersuchungen am Schwein haben dazu widersprüchliche Ergebnisse gezeigt. Während Jensen und Recén (1989) fanden, daß Sauen älterer Würfe diese später absetzten, d. h. *de facto* mehr investierten, konnten Untersuchungen von Bøe (1991, 1993) dies nicht bestätigen. Kürzlich durchgeführte Studien (Gustafsson et al., 1999; Špinka et al., 2000) haben gezeigt, daß die zentralen Muster mütterlichen Verhaltens beim Schwein durch den Domestikationsprozeß offensichtlich wenig verändert worden sind. Allerdings betrafen die aufgetretenen wenigen Veränderungen speziell Verhaltensweisen (z. B. Säugeverhalten), die mit dem mütterlichen Investment zu tun hatten. Insofern scheint die von Gustafsson et al. (1999) aufgestellte These nicht ungerechtfertigt, daß domestizierte Sauen aufgrund ihrer züchterisch bedingten Adaptation an menschlichen Schutz und Versorgung weniger von einem Investment in ihre künftige Nachkommen profitieren als das beispielsweise bei Wildschweinen der Fall ist und deshalb ein vergleichsweise hohes Investment in die aktuellen Nachkommen betreiben. Hier müssen gezielte Untersuchungen ansetzen, um dies zu klären. Einige erste Studien bezüglich des Saugverhaltens (Puppe und Tuchscherer, 1995) und Milchinhaltsstoffen (Tuchscherer et al., 1994) zeigen Unterschiede bei primi- und multiparen Sauen, die sich möglicherweise in Richtung einer Investmenterhöhung älterer Mütter interpretieren lassen.

Diskussion

Die gegenwärtigen Tendenzen in der Schweinehaltung beinhalten verstärkt Aspekte des Tierschutzes und des Wohlbefindens (vgl. EU-Report, 1997). Das führt fast zwangsläufig dazu, daß Haltungsformen und -systeme präferiert werden müssen, in denen das Verhalten der Tiere und dessen Management eine größere Rolle spielen. Kenntnisse über die proximaten, aber in zunehmenden Maße auch die ultimaten Verhaltensursachen, sind nötig, um komplexe Tier-Haltungsumwelt-Interaktionen zu verstehen. Die vorliegende Arbeit geht davon aus, daß das Verhalten von Tieren in der Evolution durch die natürliche Selektion geformt wurde und versucht am Beispiel und der Interpretation möglicher Mutter-Nachkommen-Konflikte zu erörtern, daß beim domestizierten Schwein eine Reihe ultimat begründeter, sozialer Verhaltensmuster auftreten können, deren Erforschung, Kenntnis und Nutzung eine solche künftige Beurteilung – neben den bisherigen Ansätzen dazu – verbessern könnte. So schlägt beispielsweise das besonders im deutschen Sprachraum verbreitete Bedarfsdeckungs- und Schadensvermeidungskonzept vor, funktionale (ultimate) Betrachtungsweisen des Verhaltens wie den Bedarf eines Tieres und dessen Deckung zum Zwecke von Selbstaufbau und Selbsterhaltung (Bammert et al., 1993) sowie Fortpflanzung (Tschanz et al., 1997) als essentielle ethologische Grundlage bei der Beurteilung von Tierschutzfragen heranzuziehen. Dazu gehört auch die Gewährleistung einer arttypischen Fürsorge für die Nachkommen (Tschanz et al., 1997). In der vorliegenden Arbeit werden deshalb einige verhaltens- und soziobiologische Grundlagen der Mutter-Kind-Beziehung beim Hausschwein erörtert.

Zeitpunkt des Absetzens

Es ist unstrittig, daß die intensive Nutztierhaltung in der Regel ein Kompromiß zwischen den wirtschaftlichen Interessen des Menschen als Tierhalter und den biologischen Ansprüchen der Tiere ist. Das gilt auch und insbesondere für die Wahl des geeigneten Absetzzeitpunktes. Aus menschlicher Sicht werden hier zwei Prinzipien durchgesetzt, deren Eignung im Grunde wenig mit den in der vorliegenden Arbeit skizzierten biologischen Grundlagen zu tun hat, nämlich zum einen „absolut abrupt" und zum anderen „möglichst früh" in der Ontogenese der Nachkommen. Biologisch evolvierte Prinzipien wären dagegen als eher „langsam" bzw. „graduell" zu bezeichnen sowie zu einem Zeitpunkt stattfindend, an dem die Nachkommen neben der ernährungsseitigen Selbständigkeit auch eine weitestgehende soziale Unabhängigkeit von der Mutterbindung erreicht haben. Oder anders ausgedrückt, das Absetzen ist ein biologischer Prozeß, der in Verlauf der Evolution über wechselseitige Kosten-Nutzen-Erwägungen zwischen Eltern und Nachkommen ausbalanciert worden ist. Während der langsame Milchentzug bei Wildschweinen in einem Alter von etwa 3–5 Monaten erfolgt und sie ihre ersten „echten" Rangkämpfe in der Rotte erst mit ca. 10 Monaten bestreiten müssen (Meynhardt, 1978), setzen seminatürlich gehaltene Hausschweine ihre Nachkommen bei ungefähr 13 (vgl. Wechsler et al., 1991) bis ca. 17 Wochen (Jensen und Récen, 1989) ab, was damit im starken Kontrast zu der abrupten Trennung von Sau und Ferkeln in der kommerziellen Schweineproduktion steht, die meist im Zeitraum von etwa 3–5 Wochen durchgeführt wird. Produktionsverfahren wie das Isolierte Frühabsetzen (SEW, Absetzen <21 Tage, meist zwischen dem 12. und 16. Lebenstag, Übersicht bei von Borell, 2000) verschärfen diesen Trend noch. Ein solches extremes Frühabsetzen erscheint zunächst insofern problematisch, da in dieser Zeit noch ein relativ hohes Investment der Mutter vorhanden ist (siehe Abb. 2), was auf ein starkes Bindungsniveau der Mutter-Kind-Beziehung schließen läßt. Ferkel scheinen zudem auf solch abrupte Unterbrechungen ihrer Kontakte zur Mutter mit vermehrten und anhaltenden Verhaltensproblemen (z. B. gegenseitiges Besaugen) zu reagieren (vgl. von Borell, 2000). Darüber hinaus ist bekannt, daß auch die mit dem „normalen" Absetzen unter Produktionsbedingungen (z. B. in der 5. Woche) verbundenen Änderungen der sozialen und Haltungsumwelt zu vielfältigen Reaktionen der Ferkel im Bereich des Verhaltens sowie metabolischer und immunologischer Parameter (Puppe et al., 1997), bis hin zu Verschiebungen hirnspezifischer Rezeptoren (Kanitz et al., 1998) führen können. Die Sauen dagegen reagieren auf das Absetzen der Ferkel beispielsweise mit einer starken Zunahme ihrer Lautäußerungen (Sambraus und Baier, 2000), was ebenfalls auf ein noch vorhandenes, starkes Bindungsniveau hinweist.

Zwei bekannte Alternativen scheinen hingegen eher dem biologisch evolvierten Verlauf des Absetzprozesses zu entsprechen. Eine Möglichkeit, dem individuell unterschiedlichen Entwicklungzustand der Tiere eines Wurfes Rechnung zu tragen, könnte in einem gesplitteten Absetzen („split weaning") bestehen (Pluske und Williams, 1996), das schwerere Tiere etwas eher, leichtere Tiere etwas später absetzt. Letztere profitierten durch die dadurch mögliche höhere Milchaufnahme in ihrem Wachstum im Vergleich mit einer normal abgesetzten Kontrollgruppe (Pluske und Williams, 1996). Auch die Reproduktionsleistung der Sauen scheint davon zu profitieren (Vesseur et al., 1997). Weit konsequenter noch ist der Ansatz des Stolba-Familienstalls für Hausschweine (vgl. Wechsler et al., 1991). Hier werden trotz produktionsorientierter Ziele die Ferkel nicht abgesetzt, sondern von der Muttersau natürlich entwöhnt.

Gruppenhaltung und mütterliches Investment

Viele kommerzielle Haltungssysteme für ferkelführende Sauen (z. B. Einzelbuchten mit Kastenständen) verlagern den Mutter-Nachkommen-Konflikt stark zugunsten der Nachkommen einfach dadurch, daß die Mütter in ihrer Handlungsfreiheit stark eingeschränkt sind. Es kann postuliert werden, daß alternative Haltungssysteme, in denen den Sauen eine größere Kontrolle über das Niveau ihres elterlichen Investments gewährt wird (z. B. Laufbuchten oder besser noch Gruppenhaltungssysteme), sich ein „natürlicheres" Verhältnis einstellen wird, das unter Kenntnis der Zusammenhänge und der richtigen Managementmaßnahmen sowohl den Sauen als auch den Ferkeln Vorteile bieten kann (vgl. Fraser et al., 1995). So sind beispielsweise Ferkel von Müttern, die aktiv ihre Abferkelbucht verlassen können, gezwungen, eher Beifutter aufzunehmen (Pajor et al., 1999), was sie auf das abrupte Absetzen besser vorbereitet (Weary et al., 1999). Die Sauen wiederum verlieren während der Laktation weniger an Gewicht, und tendieren dazu, wieder eher oder in besserer Kondition in den Östrus zu kommen; insgesamt scheint der Absetzstreß geringer (Pajor et al., 1994, 1999, 2000). Allerdings variieren die Sauen ungewöhnlich stark in ihrer mütterlichen Fürsorge (Pajor et al., 2000). Eine mögliche Erklärung hierfür scheint darin zu liegen, daß Domestikation und künstliche Selektion bisher wenig Druck in Richtung mütterlicher Fürsorge entwickelt haben (Price, 1984), sondern eher Leistungsmerkmale in den Vordergrund gestellt haben. Es gibt einige Hinweise darauf, daß Haltungssysteme, in denen die Tiere mehr Entscheidungsfreiheit und Kontrolle über ihr Verhalten haben, Vorteile für deren Wohlbefinden und Stressbewältigung bringen (vgl. Fraser et al., 1995; Pajor et al., 2000).

Schlußfolgerungen

Die vorliegende Arbeit macht deutlich, daß neben proximaten Faktoren vor allem auch in der Evolution entstandene ultimate Mechanismen helfen, soziales Verhalten bei domestizierten Schweinen zu erklären und somit ihre diesbezüglichen Verhaltensbedürfnisse zu verstehen. Funktionale Erklärungsmodelle aus dem Bereich des elterlichen Investments und des Absetzkonflikts können hierzu herangezogen werden. Bestehende proximate Erklärungsmodelle (z. B. physiologische Mechanismen der Milchproduktion) sollen damit keineswegs außer Kraft, sondern in ihrer Kausalität ergänzt werden. Sowohl das Saugverhalten als auch die Laktationsleistung variieren mit dem Alter der Ferkel in einer charakteristischen, mittels ultimater Erklärungsmodelle vorhersagbaren Weise. Es läßt sich schlußfolgern, daß bestimmte Elemente des Absetzprozesses intensiv gehaltener Hausschweine zwar schon relativ frühzeitig (d. h. in der zweiten Lebenswoche der Ferkel) beginnen, daß dieser dann aber einen langsamen Verlauf nimmt, in dem wahrscheinlich weitere Elemente dieses Prozesses einsetzen. Über das Ende des Prozesses, zeitliche Abhängigkeiten und deren Bedeutung ist dagegen bei intensiv gehaltenen Hausschweinen wenig bekannt. Als meßbarer Beginn des Absetzprozesses können Parameter gelten, die einen Investitionsaufwand der Mütter an Zeit, Energie oder anderen Ressourcen definieren und hier ein Maximum erreichen. Die in der Regel in der kommerziellen Produktion angestrebte (und züchterisch umgesetzte) Maximierung der Reproduktionsrate sollte daher unter den immer wichtiger werdenden Aspekten von Wohlbefinden und tiergerechter Haltung die evolutiv entwickelten Strategien, an die das soziale Verhalten der betreffenden Tierart adaptiert ist, in Betracht ziehen. Untersuchungen, ob und wie solche Strategien unter den Bedingungen der Domestikation und der kommerziellen Aufzucht und Haltung wirken, scheinen daher notwendig, um nicht nur das „Wie", sondern auch das „Warum" von Verhaltensweisen verstehen zu können. Besonderes Augenmerk sollte darauf gerichtet werden, über welche proximaten Mechanismen mögliche ultimate Strategien bei Nutztieren „umgesetzt" werden. Nur durch die Einbeziehung solcher grundlegenden Kenntnisse lassen sich nachhaltige Verbesserungen für Wohlbefinden, Gesundheit und Produktivität im Sinne von Mensch und Tier erreichen, insbesondere wenn man unterstellt, daß künftige Haltungssysteme den Tieren einen größeren Freiheitsgrad des Verhaltens gewähren sollen.

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Originalarbeiten zum Kapitel 3.1.2

Sozial-akustische Erkennungsmechanismen in der Sau-Ferkel-Beziehung

Publikationen:

7.4 Studie 4

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7.5 Studie 5

Puppe, B., Schön, P.C., Tuchscherer, A. & Manteuffel, G. (2003): The influence of domestic piglets' *(Sus scrofa)* age and test experience on the preference for the replayed maternal nursing vocalisation in a modified open-field test. *Acta Ethologica* **5**, 123-129.

COMMON FEATURES AND INDIVIDUAL DIFFERENCES IN NURSE GRUNTING OF DOMESTIC PIGS (SUS SCROFA): A MULTI-PARAMETRIC ANALYSIS

by

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Summary

The nursing vocalisation of domestic pigs was investigated with respect to common features and individual differences. The sow's repeated grunting during nursing can be regarded as a contact call and a signal of the mother to start and synchronise the suckling behaviour of the piglets. Analyses in the time domain revealed the gross structure of the call, whereas in the frequency domain the fine structure of single grunts was investigated. Three parameter groups with parameter vectors extracted from single grunts centred around the maximum of the grunting rate were used for classification of frequency patterns. The parameter vectors were compared by a discriminant function analysis. The main results are: (1) a strong correlation in the time course of the nurse grunting between the sows; (2) an individual frequency pattern of the single grunts as demonstrated by the discriminant analysis with erroneous discriminations of less than 1.6% if an optimum set of features of the amplitude spectrum was included. Thus, it can be concluded that a common structure of the time course of the nurse grunting is the typical inter-individual characteristic of the vocalisation. Individual differences between sows occurred in the absolute grunting rate, however. Major individual differences were found in the frequency pattern of the single grunts and were expressed over the whole frequency range of a grunt. Hence, based on the parameter groups

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'whole amplitude spectrum' and 'cepstrum', individual sows could well be distinguished whereas this was not the case using only single features of the amplitude spectra.

Taken together, the results show that there is a species-typical pattern in the nurse grunting that could be recognised by all piglets, and an individual label that could be used to discriminate the own mother from other nursing sows.

Keywords: vocalisation, bioacoustics, domestic pigs, nurse grunting, inter-individual classification.

Introduction

Acoustic utterances play an important role for communication in many animals. Sounds are transferred very quickly to distant places and are relatively insensitive to intervening objects. As a result, they are ideal carriers for signals that are sent in order to alarm others, to keep in contact with remote members of the group, or to attract conspecifics over large distances. It is assumed that, during evolution, the meaning which was attributed by the sender was tuned to that attributed by the receiver if a vocalisation serves intra-specific communication that alters the behaviour of the group members (Hauser, 1997). This requires a clear specification of the produced sounds that distinguishes them from vocalisations with different meanings. As a result, any meaningful communicative vocalisation must have a certain unique phonetic form. For intra-specific communication this form must have inter-individually invariant elements that are able to label its meaning by this characteristic syntax and its frequency structure. Other formal characteristics could carry information on the individual which was sending.

The aim of the present study was to analyse a type of vocalisation with rather clear meaning in domestic pigs: the nurse grunting. In the sow, this vocalisation is elicited and controlled by the secretion of the hormone oxytocin, and it accompanies the various stages of nursing behaviour (Ellendorf *et al.*, 1982; Algers *et al.*, 1990). The piglets, as receivers, respond with an immediate approach to the sow's teats. A formal phonetic analysis should clarify which elements of the grunting are able to carry its specific meaning by its invariance across individuals. Further we looked for syntactic elements with intra-individual invariance which differed between the sows so that they may deliver non-ambiguous information on the individual.

The general vocal abilities of pigs with some suggestions for their biological meanings were described by various authors (Grauvogl, 1958; Kiley, 1972; Klingholz & Meynhard, 1979; Klingholz *et al.*, 1979). Behavioural audiogram studies have shown that the frequency range of hearing in pigs ranges from 42 Hz to 40.5 kHz, displaying best sensitivities from 250 Hz to 16 kHz (Heffner & Heffner, 1990). However, the animals were found to be unable to localise pure tones above 4 kHz. This is indicated by the fact that their ability to use binaural intensity cues is greatly restricted if not completely absent (Heffner & Heffner, 1989).

The complex nursing and suckling behaviour of domestic pigs consists of several distinct phases. Each phase is characterised by different sow and piglet behaviours and a characteristic pattern of sow grunting (Fraser, 1980). The sows' repeated grunting calls during nursing are a comparatively simple and well structured vocalisation, which can be regarded as a contact call (Shillito Walser, 1986; Castren *et al.*, 1989; Blackshaw *et al.*, 1996). Temporal variations in the vocalisation of the sow seem to act as signals that affect and synchronise the behaviour of the piglets (Jensen & Algers, 1984, 1985).

Following the latest developments in human speech recognition or in technical acoustics (Schukat-Talamazzini, 1995; Hoffmann & Westendorf, 1997) sound analysis in animal communication should generate procedures that allow the numerical comparison of individual frequency patterns in a similar way. Because of the sometimes complex and variable structure of mammalian calls as well as variations in the absolute amplitudes of the recorded vocalisations due to recording conditions, the computer aided signal processing employing frequency analyses and mathematical statistical methods (multi-parametric approach) is most suitable for vocalisation analyses (Hammerschmidt & Todt, 1995; Riede, 1997; Schrader & Hammerschmidt, 1997; Schön *et al.*, 1998).

Despite existing data on nurse grunting of domestic sows (Shillito Walser, 1986; Blackshaw *et al.*, 1996) there is no detailed numerical description of the formal parameters of this vocalisation which may carry the inter-individual meaning. In addition, it is an open question which particular elements may be used by the piglets to distinguish their own mother from other sows. Therefore, the aim of this study was to investigate the vocal patterns of the nurse grunting in domestic sows and to discern auditory features that could form the basis of the piglets' ability to discriminate individual sows. To this end, we used tools which included statistical procedures in order to reveal the time-dependent course of the nurse grunting and the inter-individual differences of call parameters in the time and frequency domain.

Materials and methods

Animals and general procedure

Five German Landrace sows (*Sus scrofa*) and their litters were used in this study during the nursing period of five weeks. Each sow and its piglets were brought into a special acoustic room which allowed the housing of the animals as well as acoustical and behavioural recordings in a noise reduced environment (Schön *et al.*, 1998). From each sow, five successful nursing episodes were investigated. Because of the strong correlation between the grunting calls of the sow and the suckling behaviour of its piglets, the calls and the behaviour of a whole nursing episode were recorded with a video camera (Canon, UC8Hi) with a separate microphone (Sennheiser, MKE 46). Additionally, the milk flow during each nursing episode was observed. The start of the milk flow is coincident with the rhythmic and rapid mouth movements of the piglets and a visible and audible swallowing of the milk (Gill & Thompson, 1956; Whittemore & Fraser, 1974; Fraser, 1980; German *et al.*, 1997). Accordingly, termination of the milk flow is characterised by the first visible signs of the end of this behavioural sequence when the piglets start to display rather slow mouth movements followed by nosing the udder, trying to occupy other teats or to drive out other piglets.

Both recorded signals (the video signal and the audio signal) were fed to the analysis computer and displayed online on the computer screen. The analysis tools for data acquisition and data pre-processing under LabVIEW® (Schön *et al.*, 1998) allowed the precise separation of sow and piglet vocalisations.

Parameter extraction and data pre-processing

1. Extraction of parameters from the time signal

In the first step, the nurse grunting was digitised on the computer for a period ranging from 2 minutes prior to the half time of the milk flow episode until 1 minute after this time. The calls and the behaviour during this period were displayed online on a computer screen and stored as synchronised data sets.

Figure 1 shows an example of the time signal of a recorded nurse grunting. The sampling rate of all digitised calls was 16 kHz.

The time signal was divided into 10-s-intervals starting with −1 and 0 in relation to the middle of the milk flow (intervals before and after the milk flow). For each interval, we determined the number of grunts, the average duration of grunts, and the average interval between the grunts.

In the next step, the time courses of the grunting rate and the grunt duration were synchronised to the minimum of intervals between the grunts to eliminate subjective influences

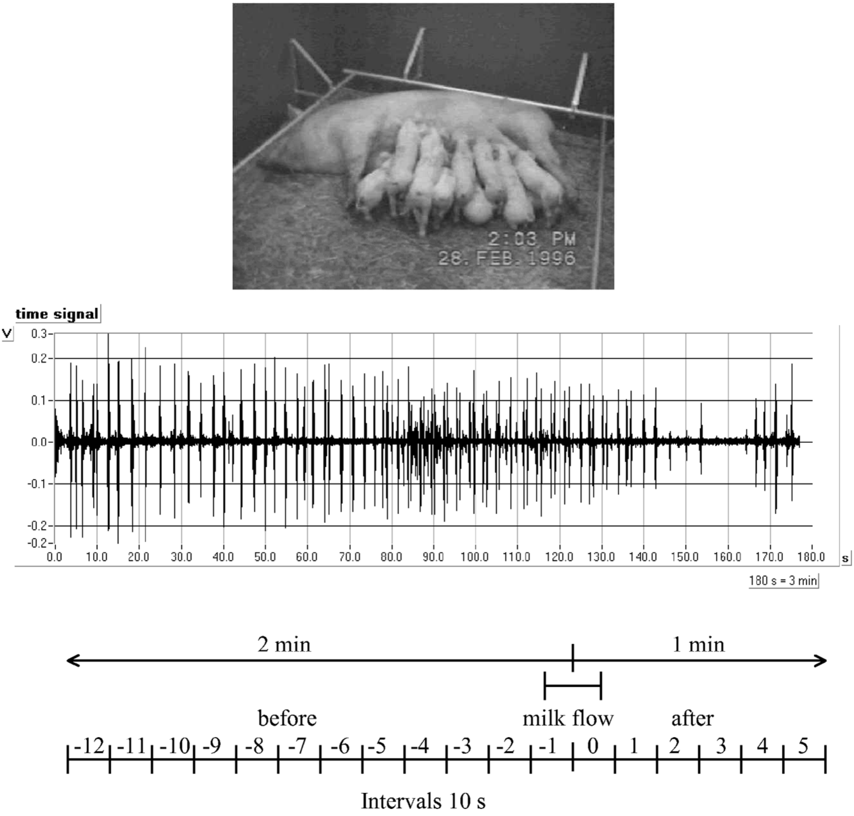


Fig. 1. Example of the recorded time signal of the nurse grunting and the behaviour of the sow-piglet unit. The division of the time axis (intervals) used for the extraction of parameters from the time signal is shown below.

of the observation of the milk flow. This means that the curves were shifted along the abscissa so that the intervals with the minimum grunting rates were assembled in the same 10-s-intervals (*e.g.* in the interval −3, see Fig. 5). The synchronisation was used both for the parameter extraction from the time domain and the parameter extraction from the frequency domain.

2. Extraction of parameters from the frequency domain

Figure 2 shows the time signal of an interval of nurse grunting with the respective sonagram. Because there is no clear structure in the sonagrams of the grunts, a fundamental frequency and harmonic frequencies cannot be confidently distinguished. The fundamental period in a periodic signal is the smallest value T_0 that satisfies the relation $f(t+T_0) = f(t)$ for all values of t. In a voice signal T_0 is the time between two cyclic (recurring) events. The inverse of the fundamental period, the fundamental frequency, appears in the amplitude spectrum as the first remarkable frequency (F_0). This first frequency (F1) varies considerably in the grunting

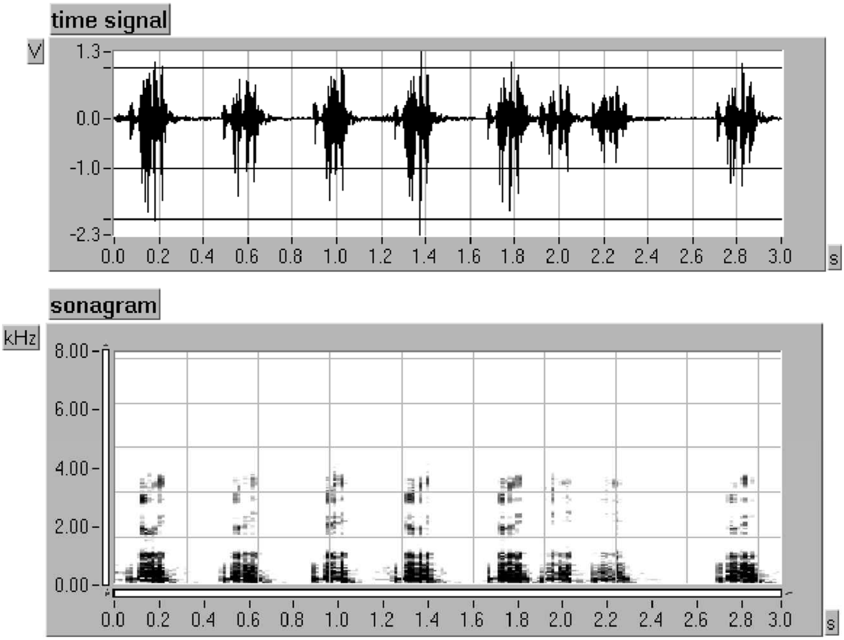


Fig. 2. Time signal and sonagram of a 3-s-interval of the nurse grunting.

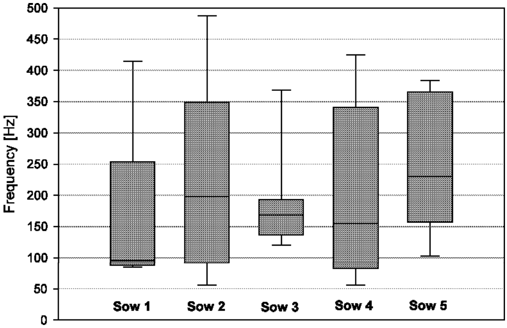


Fig. 3. Range of frequency (F1) of the FRQ-parameter group. The box plot shows the median, 10th, 25th, 75th, and 90th percentiles as vertical boxes from 5 replications for each sow.

calls of the investigated sows. The variation coefficient ranged from 49.0 (Sow 3) to 78.9% (Sow 4) (Fig. 3). Thus, the grunting resembles more a noisy than a tonal sound. Each single grunt has a constant pattern over time which means that a dynamic frequency modulation fails to occur. Therefore a parameter extraction from the entire frequency domain of a single grunt could be performed (Fig. 4).

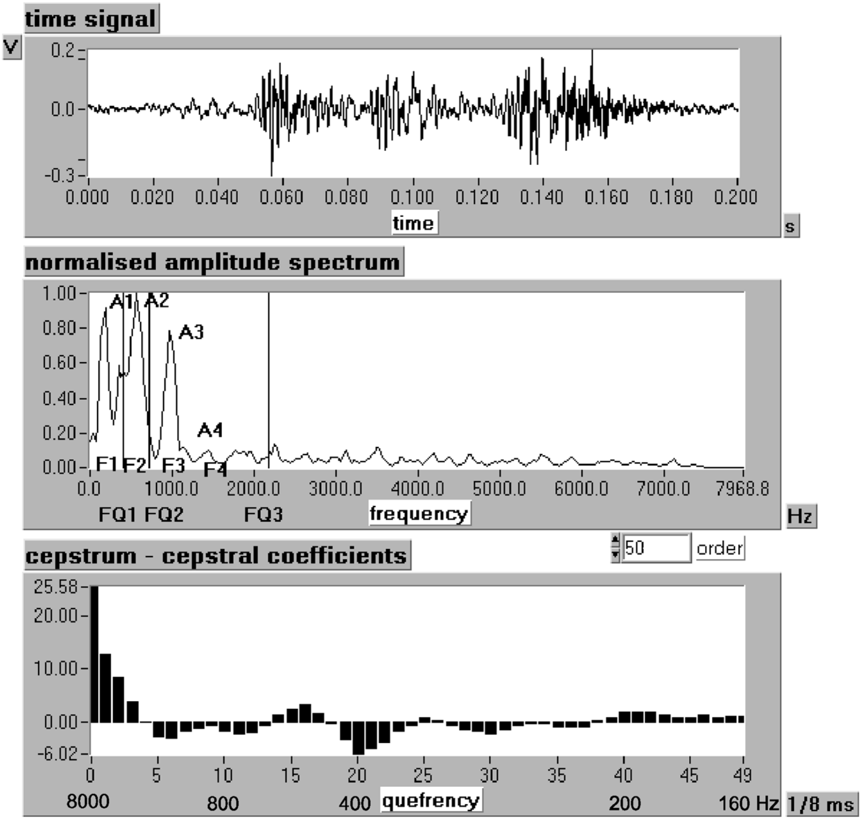


Fig. 4. Time signal, amplitude spectrum and cepstral coefficients c_q, $q = 0, \ldots, 49$ (order = 50) from a single grunt (sampling rate: 16 kHz).

For the extraction of the time and frequency parameters we used the minimum interval between the single nurse grunts as a synchronisation point. Twenty single grunts before and ten single grunts after this point were chosen for analysis. This means that a time interval of approximately 45 s was covered. Altogether 721 grunts from the five sows investigated were included in the analysis (sow 1: 130 grunts, 2: 145, 3: 150, 4: 146, 5: 150). For every single grunt a normalised amplitude spectrum and a cepstrum with its coefficients to an order of 50 was produced. The amplitude spectrum was extracted using a short time FFT (Fast Fourier Transformation) with an FFT-size (window) of 512 points (32 ms), a Hamming windowing function, an FFT-overlap of 60% (19.2 ms), a superposition of the single spectra (the values of the amplitude frequency of all FFT-windows were averaged), and a normalisation of the accrued sum spectrum to one. From the single grunts we extracted 3 parameter groups as shown in Fig. 4. The first parameter group was calculated from the first ten frequencies exceeding a threshold of 0.05 of the normalised amplitude spectrum (F1, . . . , F10) and the respective amplitudes (A1, . . . , A10), the frequency quartiles (FQ1, FQ2, FQ3) and the sum of energy of the normalised spectrum (ES). In the following this parameter vector will be named FRQ. The threshold of 0.05 corresponds to the noise in the acoustic room. The first 10 frequencies were extracted by a peak detection algorithm (fits a quadratic polynomial to sequential groups of data, for each peak the quadratic fit was tested against the threshold level). The frequency quartiles were received by dividing the area below the plot of the amplitude spectrum in four equal parts (quartiles). The points of the intersections with the x-axis are the frequency quartiles. The sum of energy corresponds to the whole area below the plot of the normalised amplitude spectrum.

The second parameter group (SPEC) was extracted from the data points of the whole amplitude spectrum. With an FFT-size of 512, 256 features from each amplitude spectrum are gained.

The third parameter group (CEP) was built from the cepstrum. For the cepstrum, the amplitude spectrum is logarithmed followed by a second Fourier-transformation. The coefficients of this transformation are the coefficients of the cepstrum. This approach is based on the source filter model of vocal production. The goal is to separate periodic components of the signal (*i.e.* the fundamental frequency and its harmonics) from non-periodic components (Noll, 1964; Nossair & Zahorian, 1991; Owren & Linker, 1995). The cepstrum roughly separates both influences with the lowest coefficients essentially reflecting properties of non-periodic components and the higher coefficients reflecting the properties of the periodic components (fundamental frequency and its harmonics). In the present work the cepstrum is used exclusively to get a further model of the call with fewer parameters for the classification analysis. Classification analyses were carried out on the basis of 50 coefficients which was enough to cover the range necessary for an optimum result of classification. These 50 coefficients correspond to a frequency range from 160 Hz (C50 = 6.25 ms) to 8000 Hz (C1 = 0.125 ms).

The data pre-processing and the extraction of the parameters were carried out with custom applications developed with the graphical programming language LabVIEW® (Finneran & Hastings, 1997; Schön *et al.*, 1998).

Statistical methods

The goal of the statistical analysis was to obtain a detailed numerical description of the individual and species-specific formal parameters of the vocalisation.

1. Parameters from the time signal

The influence of animals (sows) and replications on the time course was calculated by the repeated measurement analysis of variance using the GLM procedure of SAS® (SAS Institute Inc., 1989), where the factor ‘time’ was the repeated factor and the factors ‘sow’ and ‘replication’ were the class variables. Using the repeated measurement analysis of variance, only data sets without missing values are suitable for analysis. Because of missing values at the beginning or at the end due to different durations of nursing behaviour, only the time interval between −6 to 2 (see Fig. 1) was available for this analysis. These intervals represent the number of single grunts of each sow (sow 1: 235 grunts, 2: 205, 3: 293, 4: 242, 5: 222).

2. Parameters from the frequency domain
The classification of parameter vectors was calculated with the SAS procedure DISCRIM. The discriminant function analysis by the k-nearest-neighbour method with k = 2 was used, and the probability of misclassification (*i.e.* assigning the parameter vector to the wrong class) was estimated. The data set that the procedure DISCRIM uses to derive the discriminant criterion is the training or calibration data set. We used all parameter vectors for the training data set. The estimation of the error rate was carried out by crossvalidation. Using a non-parametric method that means that each parameter vector of the classification model (formed by the training data set) is excluded stepwise, a new classification model is built without the excluded parameter vector, and the excluded parameter vector is tested concerning the class membership (Sumpf *et al.*, 1998).

The option *backward* in the SAS-procedure *stepdisc* reduces the number of features of the parameter vectors by a stepwise discriminant analysis starting with the whole parameter vector. Insignificant features are stepwise eliminated from the parameter vector (Stepwise Discriminant Analysis Backward = SDAB). Features are removed according to the criterion of the level of significance of the F-test from a covariance analysis ($p = 0.05$).

Results

1. Analysis in the time domain

Figure 5 shows that all individual curves of the time course of the sows' nurse grunting are very similar, whereas the absolute values are different. The most prominent feature of the gross structure of nurse grunting in the time domain is a sharp increase in the grunting rate 19.2 ± 1.5 s before the milk ejection occurs (Table 1). This is reflected by the opposite course of the parameters 'interval between grunts' with a minimum and 'number of

TABLE 1. *Delay between minimum of grunting intervals and the start of the milk flow, and the duration of milk flow*

Animal	Delay [s]	Duration of milk flow [s]
Sow 1	25.0 ± 3.7 (16.8-37.0)	16.6 ± 0.8 (14.0-19.0)
Sow 2	19.1 ± 4.7 (7.5-34.8)	18.2 ± 2.7 (10.0-26.0)
Sow 3	19.2 ± 0.7 (16.4-20.7)	16.0 ± 1.2 (12.0-19.0)
Sow 4	13.1 ± 2.6 (3.6-17.9)	19.8 ± 3.9 (9.0-33.0)
Sow 5	19.6 ± 1.6 (16.1-25.4)	15.8 ± 1.5 (13.0-21.0)
Mean ± SE (Range)	19.2 ± 1.5 (3.6-37.0)	17.3 ± 1.0 (9.0-33.0)

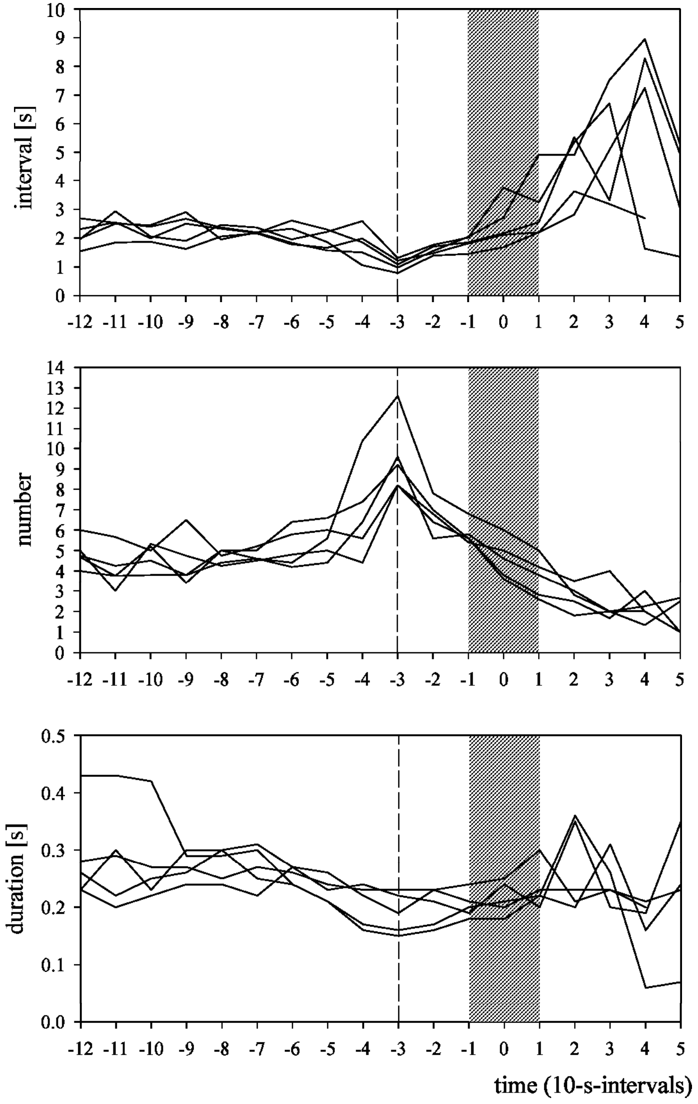


Fig. 5. Average interval between grunts, number of grunts and average duration of grunts as a function of time for the 5 investigated sows (as a mean from 5 replications). The dotted line corresponds to the minimum intervals between the single grunts and the shaded area labels to the observed duration of milk flow.

grunts per time unit' with a maximum at this point (Fig. 5). However, the 'duration of single grunts' exhibits no conspicuous changes over the nurse grunting episode. The average duration of the subsequent milk flow was 17.3 ± 1.0 s (Table 1).

The results from the repeated measurement analysis of variance investigating the influence of individuals (sows) and replications show that the repeated factor 'time' (defined in 10-s-intervals and representing the x-axes of Fig. 5 as the course of grunts during a nursing episode) affected significantly all three y-parameters of Fig. 5 (interval: $F_{7,112} = 8.58, p = 0.0001$; number: $F_{7,112} = 38.16, p = 0.0001$; duration: $F_{7,112} = 4.97, p = 0.0001$). This influence emphasises that a similar time course of the individual nurse grunting seems to be present. No influence of the factor 'replication' for any parameter was found. This means that there is a nearly constant level of the parameters in the time domain over the five replications. A weak influence of the factor 'sow' was only found for the parameter 'number of grunts' ($F_{4,16} = 2.81, p = 0.0608$). Hence, the absolute number of grunts during a nursing episode seems to be an individual characteristic. In contrast, the time course showing a pronounced maximum rate before the milk ejection is a common structure in all animals.

2. *Analysis in the frequency domain*

The classification results assigning the single grunts around the time of the highest grunting rate to each one of the five sows are shown in Fig. 6. Whereas the use of parameters from the FRQ-group produces a high misclassification result (46.3%), SPEC- and CEP-parameters generate very low misclassifications (3.8 and 3.4%, respectively). It is striking that the inclusion of the two time signal features ('grunt duration' and 'interval between grunts') did not lead to a significant reduction of misclassification. In the case of the FRQ-parameter group, the improvement amounted to at most 1.5%, and in the SPEC-parameter group the classification even worsened by 0.6%.

However, in all cases the stepwise reduction of the parameter vectors (SDAB) resulted in a remarkable improvement of the classification, *i.e.* the precision of the individual discrimination of the sows' vocalisations. The effect was most pronounced in the SPEC-parameter group, where a reduction from 256 initial features to only 116 features resulted in an improvement of misclassification from 3.8 to 1.5% (Fig. 6). The "optimum classification" results were reached with the cepstrum (CEP-parameter group) where a

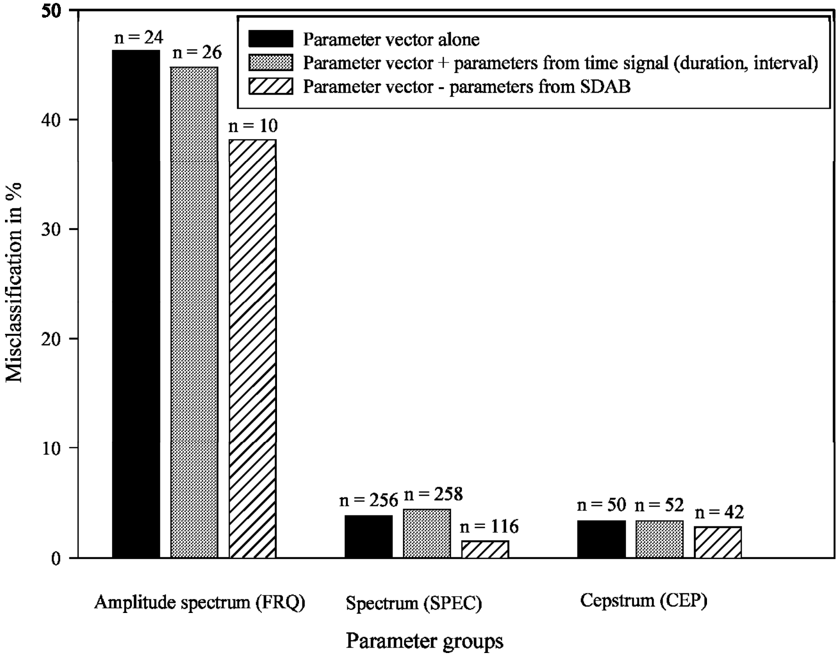
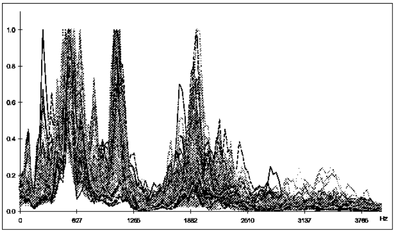


Fig. 6. Results of the three chosen parameter groups (FRQ, SPEC, CEP) using the discriminant analysis function of SAS (*n* is the number of features of the used parameter vectors).

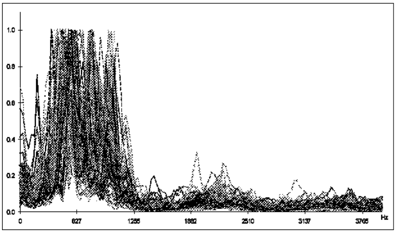
misclassification of only 3.4% was achieved with the 50 coefficients initially used, *i.e.* 96.6% of the nurse gruntings were assigned to the right sow. After the parameter reduction the remaining 42 features led to a misclassification of 2.8% (Fig. 6).

As Fig. 6 shows there were only weak results regarding an individual classification based on features from the FRQ-group. This is due to the strong variance in frequencies within the sows (Fig. 3). Hence, it is not possible to reliably identify a certain frequency as the fundamental frequency and others as its harmonics (see method section). A successful classification is possible, however, with the parameter group which includes the complex of features of the ‘amplitude spectrum’ (SPEC) and the ‘cepstrum’ (CEP). Figure 7 summarises the amplitude spectra of each tested sow superimposed for 90 single grunts. Although there is clearly a common structure in the grunts of each sow considerable differences between the individuals are recognisable. Similar results were found for the CEP-parameter group.

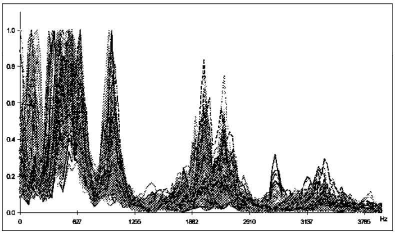
Sow 1:



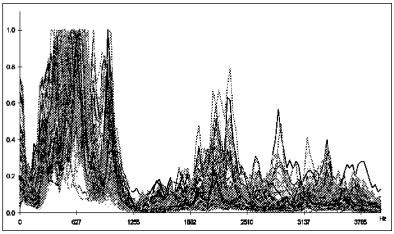
Sow 2:



Sow 3:



Sow 4:



Sow 5:

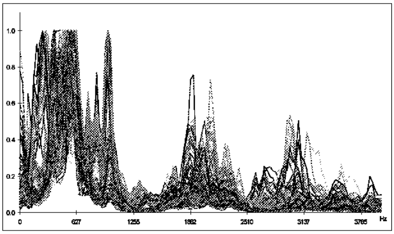


Fig. 7. Structure of the amplitude spectra of the five sows tested superimposed for 90 single grunts of each individual.

Discussion

The results of this study investigating the time and the frequency structure of nurse grunting in domestic pigs contribute new knowledge to the understanding of pig vocalisation. This concerns especially the elements of vocalisation which are able to represent the invariant formal elements that carry the specific meaning, and the elements which are individual and may be used for kin recognition, *e.g.* the recognition of the own mother by the piglets. Additionally, the study supplies some new methodological tools to analyse animal vocalisation when a multi-parametric approach is used.

Common characteristics of nurse grunting: the time course of grunting

In contrast to many other calls in pigs (see Kiley, 1972) the nursing vocalisation of the sow is specific for a certain naturally occurring situation. The present study demonstrates that a common characteristic of nurse grunting of all sows lies in the time course of the grunting rate. The pure time course of the sows' nursing grunts is a comparatively well investigated phenomenon. Concurrent with other experimental studies (Whittemore & Fraser, 1974; Ellendorff *et al.*, 1982; Blackshaw *et al.*, 1996), it seems that the typical form which labels the vocalisation 'nurse grunting' is the rhythmic and repeated occurrence of short grunts with an increasing rate. This rate has its maximum value shortly (about 20 s) before the milk ejection occurs. The high grunting rate signals to the piglets that availability of milk has to be expected in a few seconds. This is an extremely important signal for the piglets since the sow's milk ejection is short, lasting only some seconds. This means that all piglets may use the temporal vocalisation patterns of the sow as a trigger of their suckling behaviour. The pattern is linked to the probable time of milk ejection and is relatively independent from individual features (*e.g.* the absolute number of grunts). Taken together with other characteristics of pigs' nursing and suckling behaviour (*e.g.* teat preference), the communicative function may be interpreted as a beneficial adaptation mechanism in mammals feeding a great number of precocious littermates to ensure a largely undisturbed milk ingestion for all youngs (see Fraser, 1980).

Individual properties of nurse grunting: the frequency pattern of the grunts

In comparison to the evaluation of common characteristics of the nursing vocalisation with regard to a more sophisticated analysis turned out to be necessary to fix the individual particularities of the nurse grunting enabling a piglet to recognise its own mother. The parameter group FRQ which was extracted from single features of the normalised amplitude spectrum delivered weak classification results, *i.e.* the sows could hardly be distinguished on the basis of these parameters. In contrast to that, the parameter group which used all points of the 'amplitude spectrum' (SPEC) delivered very good classification results as well as the parameter group 'cepstrum' (CEP). The latter delivered good classification results with a fewer number of features. This means that the individuality of a sow seems to be expressed in a complex pattern of frequencies in a single grunt rather than in single frequency bands. The biological significance of single features of the parameter vector has to be tested in behavioural experiments, where these elements can be changed artificially, and the recognition of the sow by the piglets then indicates the used information carried by the particular parameters.

In an earlier study, Blackshaw *et al.* (1996) used a relatively simple approach to distinguish sows on the basis of only five variables measured from the grunts shown in the sonagram image (maximum and minimum frequency, the frequency of the greatest power, duration of and interval between the grunts). However, our more detailed multi-parametric analysis provides more precise and partly different results. First, we generally had some difficulties to surely determine such characteristic frequencies in the nurse grunting from the sonagram (see method section). Second, in contrast to Blackshaw *et al.* (1996) the discriminant function analysis was not used to compare simply pairs of sows. For our analysis a five class model was used where each class of the model represented one sow in relation to five, *i.e.* the level of the classification task was higher. Third, the results from the FRQ-group which includes parameters similar to the parameters used by Blackshaw *et al.* (1996) clearly demonstrate the weakness of this model to classify the sows, *i.e.* the parameters vary too much to consistently differentiate between the sows. Fourth, despite the general similarity in the time course of the nursing vocalisation of different sows, the individuality in the rapid grunts around milk ejection can be best revealed by analysing the whole spectrum or the cepstrum.

The missing of surely determinable fundamental frequencies and their harmonics could mean that nurse grunting is rather a noisy than a tonal vocalisation. The cepstrum roughly separates the influences of periodic and non-periodic components, where the lowest coefficients essentially reflect the properties of the non-periodic components and the higher coefficients the properties of the periodic components (fundamental frequency and its harmonics). As most of the higher cepstrum coefficients proved to be insignificant for a successful classification of the sows this is a further hint that fundamental frequencies and their harmonics play a negligible role as formal elements of individuality within the context of nurse grunting. Instead, this vocalisation is more or less a noisy sound which, nevertheless, is typical for a particular animal.

The results show that the specific call ‘nurse grunting’ has its inter-individual cues in the time domain (temporal course) whereas individual cues are encoded in the frequency domain. However, in other pig calls (*e.g.* ‘grunts’, ‘squeals’ and ‘screams’) inter-individual and individual cues could well be encoded in different patterns of the frequency spectrum, since alarm calls and stress calls are characterised by high pitched ‘screams’. These seem to be an inter-individual cue.

General conclusions

The elementary unit of the nursing vocalisation in sows is the grunt which is mainly an atonal, noisy sound composed of various frequencies with the maximum of energy in the lower frequency range below 1 kHz. Its typical duration is shorter than 0.4 s.

The species-specific meaning of the nurse grunting serving arousal and approach of the piglets to their mother’s teats is carried by a typical, inter-individual syntax. Its main formal element is the rate of grunting starting with about 5 grunts per 10 s. Approximately 40 to 50 s prior to milk ejection the rate starts to increase reaching a double rate roughly twenty seconds prior to milk ejection. Although these values may vary to some degree between individuals the time course of the grunting rate is a fixed pattern and displayed by all sows.

The individuality of a sow is expressed by the composition of the grunts’ frequencies. Caused by the noise-like characteristic of the grunts the individual differences can best be discovered by a multi-parametric analysis.

The present results demonstrate that piglets may be able to recognise and predict the readiness to nurse of an arbitrary sow. This ability can explain the phenomenon of cross-suckling in group housed sow/piglet communities. Should the piglets be able to perform complex frequency discriminations of the grunts they will also be enabled to distinguish their own mother from other sows in order to avoid too high competition for the teats (Puppe & Tuchscherer, 1995). Thus, despite the fact of domestication, the acoustic communication of nursing pigs displays all properties which can be expected in social animals typically living in larger groups of individuals.

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ORIGINAL ARTICLE

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The influence of domestic piglets' (*Sus scrofa*) age and test experience on the preference for the replayed maternal nursing vocalisation in a modified open-field test

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Abstract The nursing vocalisation of lactating sows is an important part of the complex nursing and suckling behaviour of domestic pigs (*Sus scrofa*). It helps to lead the young through the several phases of a nursing episode. A total of 135 suckling piglets were investigated regarding their reactions to different vocalisations and sounds successively replayed in combined choice/open-field experiments. Two piglet samples were used to determine the impact of age (sample 1: weeks 1 and 5, *n*=93) and previous test experience of naive older piglets (sample 2: week 5, *n*=42) on their behavioural responses. In the 1st week after birth, the nursing vocalisation of sows released an initial generalised approach and contact response in the young, indicating high motivation to gain nutritional or social support. Close to the sound source, the piglets then strongly preferred the nursing vocalisation produced by their own mothers compared to that produced by another, unrelated and unfamiliar sow, or an artificial sound composed of similar frequencies as the nurse grunting, or a control without sound. However, at the end of the suckling period (5-week-old piglets) no clear differences in the reactions to the sounds could be identified. Moreover, the lack of a clear response at week 5 seems largely independent of any earlier test experience of the piglets. As well in the 1st as in the 5th week the piglets showed a decrease in open-field activity when sow grunting was present, indicating that the replay of the nursing vocalisation might have some calming effect.

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Keywords *Sus scrofa* · Nurse grunting · Sound discrimination · Replay · Open field

Introduction

The domestic pig (*Sus scrofa*) displays a complex nursing and suckling behaviour, consisting of several distinct phases of suckling by the piglets and a characteristic pattern of grunting by the sow (reviewed by Fraser 1980). The nursing vocalisations of the sow have been suggested as a way of attracting piglets to the udder (McBride 1963; Fraser 1980; Lewis and Hurnik 1986; Rohde Parfet and Gonyou 1991) and appear to be necessary for the synchronised behaviour during suckling (Algers and Jensen 1985). These vocalisations consist of relatively stereotyped and repeated grunts, take some minutes (normally 2–3 min), and lead the piglets through the several phases of a successful nursing episode (Whittemore and Fraser 1974; Fraser 1980; Algers and Jensen 1985). The elementary unit is the single grunt that is mainly an atonal, noisy sound composed of various frequencies with the maximum of energy in the lower frequency range below 1 kHz (Schön et al. 1999). Whereas the time course of the grunting rate is a relatively fixed pattern indicating the oncoming milk flow (Ellendorff et al. 1982), individual differences are expressed by the composition of the grunt frequencies (Schön et al. 1999). It has been shown that such differences have the potential to be used as possible auditory cues for kin recognition (e.g. Rendall et al. 1996). Pigs are social animals naturally living in family groups where several mothers related to each other simultaneously give birth to multiple young (Gundlach 1968). Hence, for the offspring the recognition of their own mother is an important task. Indeed, there is some evidence that very young piglets (<2 days old) are able to discriminate between their own mother and an alien sow by auditory cues (Shillito Walser 1986a, 1986b; Horrell and Hodgson 1992). This ability can be regarded as a learning process; it seems to increase over time and was experimentally investigated up to 2 weeks after birth. However, there are no data for later development until weaning time in natural or commercial housing.

It is well known that a number of mammalian species have established forms of co-operative care of young including non-offspring nursing in communal nesting systems (reviewed in Packer et al. 1992; König 1997). To improve commercial housing systems for farm animals, allowing more naturally evolved behaviour, communal nursing and rearing systems were "reintroduced" (Puppe and Tuchscherer 1995; Wechsler and Brodmann 1996). This may include some spatial dispersion of sows and piglets that may impair tactile, olfactory, or visual contact. Under such circumstances, social communication and mutual recognition can be maintained by vocalisations. Because it covers distances, acoustic communication can maintain social behaviours, including suckling and nursing behaviour. It can be assumed that the offspring would prefer kin vocalisation, but similar vocalisations of non-kin group members should be able to release some behavioural responses, too. Hence, it was the aim of the present study to analyse the response of domestic piglets to different replayed sounds in a modified open-field/choice arena. An artificially produced sound as well as an example of the typical nursing vocalisation of an alien sow were replayed and each was compared to the replayed nursing vocalisation of the piglets' own mothers at the beginning (week 1) and the end (week 5) of the suckling period (piglet sample 1). Additionally, the piglets' open-field and choice behaviour without any replayed sound was used as a control. To evaluate a possible effect of test experience of the older piglets in the first piglet sample we repeated the investigations at week 5 with naive animals without test experience (piglet sample 2).

Auditory stimuli have the advantage that they can be easily and repeatedly tested by replay from a tape. The animals' responses to playbacks might be used to test vocal recognition and the ability to discriminate the calls and, respectively, the senders behind the calls (Rendall et al. 1996). Studies of choice (for an early example in pigs, see Yerkes and Coburn 1915) and open-field behaviour (for early examples in pigs, see Beilharz and Cox 1967 or Fraser 1974) are widely used to investigate particular behavioural aspects. Whereas measures that refer to the choice behaviour of an animal give information about what the animal prefers, and how strongly it prefers it, open-field behaviours may depend on various motivations, which may result in some difficulties of interpretation (see De Passillé et al. 1995). Therefore, in the present study we used two simple measures, locomotor activity and vocalisation, that have been proven as general signs of excitement in pigs (Kiley 1972; Fraser 1974). Combining all the methods mentioned above (replay, open-field, choice behaviour) we have developed an automatic monitoring system for medium-sized animals, such as pigs (Puppe et al. 1999).

Methods

Animals, sounds, and general procedure

The study was conducted in the experimental pig unit of the Research Institute for the Biology of Farm Animals in Dummerstorf, Germany. A total of 135 (70 male, 65 female) piglets (*Sus scrofa*) from 19 primiparous German Landrace sows (previously standardised to 6–8 piglets per sow) were used in two piglet samples to analyse their open-field activity and choice behaviour during four different replay situations (three sounds and one control). Whereas one piglet sample (n=93) was tested both at the beginning and the end of a 5-week suckling period, a second piglet sample (n=42) was only tested at their 5th week of life. The piglets of a litter were kept together with their mother in a conventional farrowing pen.

At the 1st day after birth each sow and her piglets were brought into an acoustically dampened chamber (for technical details, see Schön et al. 1998) that allowed housing as well as recording (microphone Sennheiser MKE 46, DAT recorder Sony DCT-790) of the typical maternal vocalisation (nurse grunting, Schön et al. 1999) during a suckling episode (Fig. 1, top). The recordings were later used for replaying the maternal vocalisation (MV). For the replay of the nursing vocalisation of an alien sow (AV) we used a recording that was made in an earlier experiment (Schön et al. 1999) under the same conditions to ensure that all piglets were unrelated and unfamiliar to this animal. In general, the part of nurse grunting 20 s prior to milk flow was used in the replay of MV and AV, and it was presented repeatedly throughout the 5-min replay period. The third sound example was an artificially produced sound (AS) consisting of a modulated sine tone (Fig. 1, bottom) taking into consideration results of earlier studies (Blackshaw et al. 1996; Schön et al. 1999) that revealed that the nurse grunting's maximum of energy lies in the lower frequency range below 1 kHz. It is known that hearing in pigs ranges from 42 Hz to 40.5 kHz with a region of best sensitivity from 250 Hz to 16 kHz (Heffner and Heffner 1990), and that pigs have a well-developed ability to localise low-frequency tones (Heffner and Heffner 1989). Because there is also evidence that pigs habituate in their response to uniform sounds (Talling et al. 1998), the sine tone was additionally modulated both in frequency and amplitude (for details, see Fig. 1). Thus, in the frequency, but not in the temporal domain, the artificial sound was similar to the nurse grunting. The objective was to ensure that piglets were able to hear the sound and to investigate if they might be attracted by the presence of any sound. All sounds were digitally adapted under a sound analysis system based on LabVIEW (Schön et al. 1998) and were displayed with an intensity of approximately 60 dB.

For reasons of experimental availability, the piglets of the first sample could only be investigated in four time-shifted trials (trial 1: five litters; trial 2: three litters; trial 3: three litters; trial 4: two litters). The second piglet sample was observed in one trial consisting of six litters. Both in the 1st week (day 4, piglet sample 1) and the 5th week (day 32, piglet samples 1 and 2) after birth the open-field behaviour of all piglets was individually observed without sound presentation as a control (CO). In the next 3 days, the three different sounds were daily presented to each piglet (one sound class per day) using a semi-random order of MV, AV, and AS for each litter.

Open-field activity and choice behaviour

A modified open field (for details, see Puppe et al. 1999) was used to test the behavioural response and the choice behaviour of the piglets. Each piglet was individually tested. The open field was divided into 12 imaginary neutral segments, 63×67 cm. Additionally, two identical choice segments were separated at the front, each of which could be reached by a piglet through a 30-cm opening. A loudspeaker with a nearly linear frequency transfer function displayed the sounds behind one of the choice segments (i.e. the sound segment), and the other choice segment served as the re-

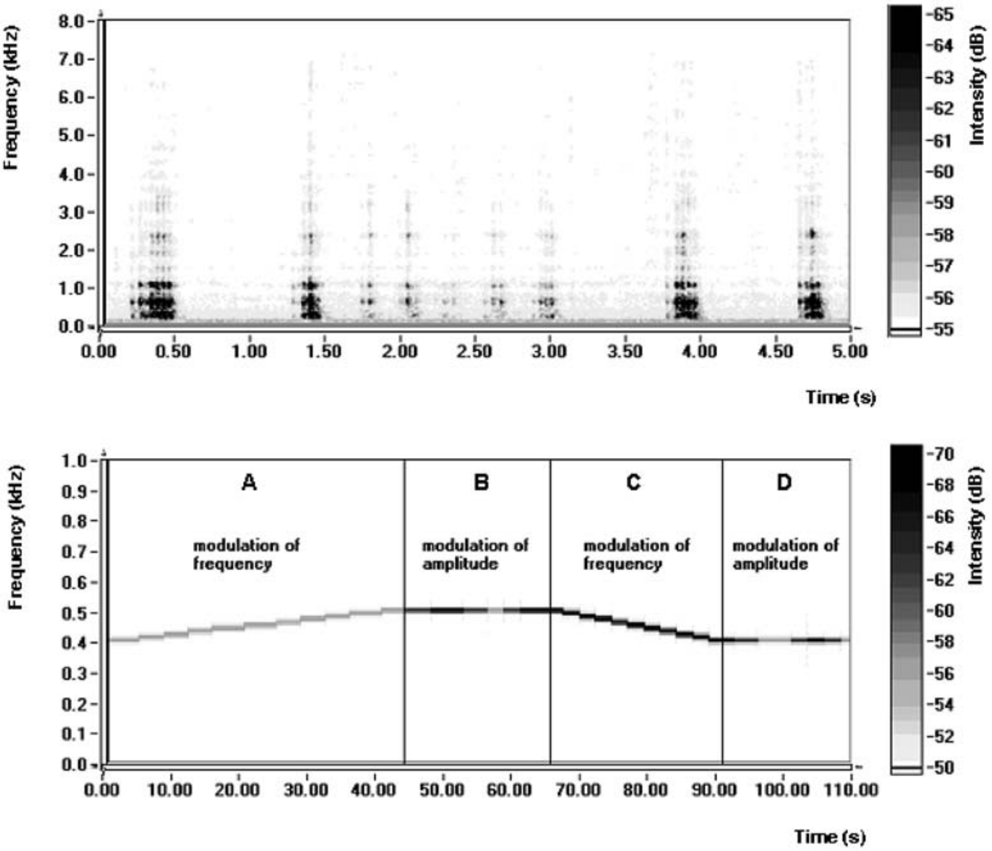

Fig. 1 Sonograms of the replayed sound examples. All sounds were presented repeatedly throughout a 5-min replay period (for further explanations see text). *Top*: Example of the nurse grunting of domestic sows used as either maternal vocalisation (MV) or alien sow vocalisation (AV) recorded with a sampling rate of 16 kHz and a bandwidth from 20 Hz to 16 kHz (FFT order 512 data points, Hamming-window overlap 90%). *Bottom*: A sine tone modulated both in frequency and amplitude used as artificial sound (AS): *A* increasing frequency from 400 to 500 Hz (10-Hz steps), *B* increasing/decreasing/increasing amplitude of 55, 60, 65, 60, 55, 60, and 65 dB (7 steps), *C* decreasing frequency from 500 to 400 Hz (10-Hz steps), *D* decreasing/increasing/decreasing amplitude of 65, 60, 55, 60, 65, 60, and 55 dB (7 steps)

spective “localisation control”. To avoid any side-dependent effects both segments were changed randomly in their function.

After a short habituation period of 5 min, each of the sound examples was presented for 5 min. The piglet’s behaviour was observed by an automatic monitoring system based on passive infrared motion detectors (PIDs) that were positioned over each segment of the open field. The PIDs were combined with a computer-aided on-line data analysis that detected activity whenever an animal moved from segment to segment. This monitoring system (previously described in detail; see Puppe et al. 1999) allowed a reliable registration of some behavioural patterns—the choice behaviour regarding the sound segment (percentage and latency of first choice, number of times the segment was entered and mean duration of stay) and the open-field activity (number of entered segments) regarding all segments. Whereas the percentage and latency of first choice was considered to measure the piglets’ voice recognition at a distance, the number of times the sound segment was entered and the mean duration of stay was thought to indicate their discrimination ability close to the sound source. Additionally, the overall incidence of each piglet’s vocalisation was counted by an observer who was not visible to the animal.

Statistical methods

All statistical analyses were done with the SAS System for Windows (SAS Institute 1999, release 8.01). Because the gender of the piglets had no influence (P>0.05) on the piglets’ behavioural responses in the present study, this variable was removed from further analyses. The following parameters were used as class variables in the evaluation model: the replayed sound type analysed as MV versus AV, AS, and CO (df=1), respectively; the investigated age of the piglets (week 1 or 5, df=1); and the effect of the biological mother (piglet sample 2: 6 sows, df=5) nested in the first piglet sample (13 sows) within the respective trial (four trials, df=9). Because the piglets’ behavioural responses to be analysed as dependent variables differed regarding their “data type”, three different procedures from the SAS System were required.

First, the CATMOD procedure in the SAS/STAT software was used to analyse the categorical data by “analysis of variance”. Here, the term analysis of variance is used in a general sense to denote the analysis of response functions and the partitioning of variation among these functions into various sources. The response functions were mean scores because the dependent variable (percentage of first choice of the sound segment) is scaled ordinally (1: right choice; 0: false choice). Hypotheses about contrasts of the parameters were tested with the generalised Wald (1943) statistic (χ^2 values).

Second, continuous data (latency of first choice of the sound segment, duration of stay in the sound segment) were analysed by the MIXED procedure in the SAS/STAT software. This procedure has the flexibility of modelling not only the means of the data (as in the standard linear model) but their variances and covariances as well. We used restricted maximum likelihood estimates of variance parameters, which are generally preferred to the ANOVA estimates (Searle 1988; Harville 1988). Hypothesis tests were calculated for the significance of each of the effects in the model (F- or t-values).

Third, parameters describing counted data (number of times the sound segment was entered, activity, vocalisation) were analysed with the GENMOD procedure of the SAS/STAT software on the basis of a statistical model formulated as a generalised linear model by the selection of the log link function and the Poisson response probability distribution. The GENMOD procedure fits generalised linear models, as defined by Nelder and Wedderburn (1972). The class of generalised linear models is an extension of traditional linear models that allows the mean of a population to depend on a linear predictor through a nonlinear link function and allows the response probability distribution to be any member of an exponential family of distributions. A GENMOD procedure

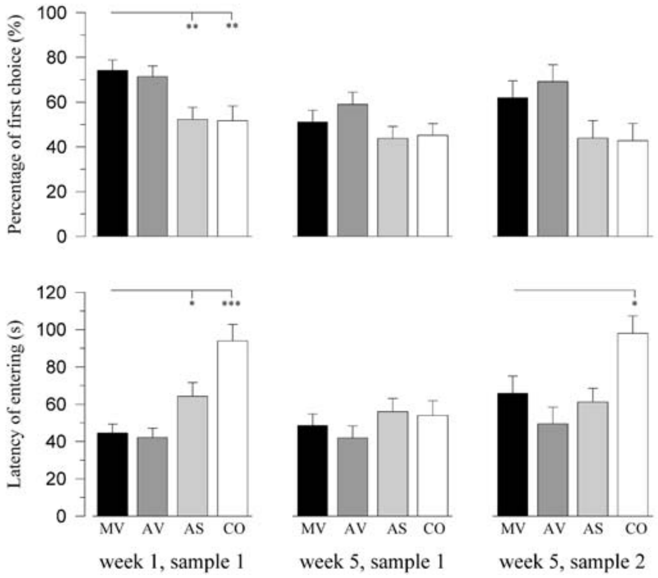

Fig. 2 First choice and latency of entering the sound segment at piglets' 1st (sample 1) and 5th week (samples 1 and 2) during the replay of the maternal vocalisation (*MV*), an alien sow vocalisation (*AV*), an artificial sound (*AS*), and the control without any sound (*CO*). Data are given as means±SE. Significant differences to MV (procedures CATMOD and MIXED, respectively) are indicated by *asterisks*: *P<0.05, **P<0.01, or ***P<0.001

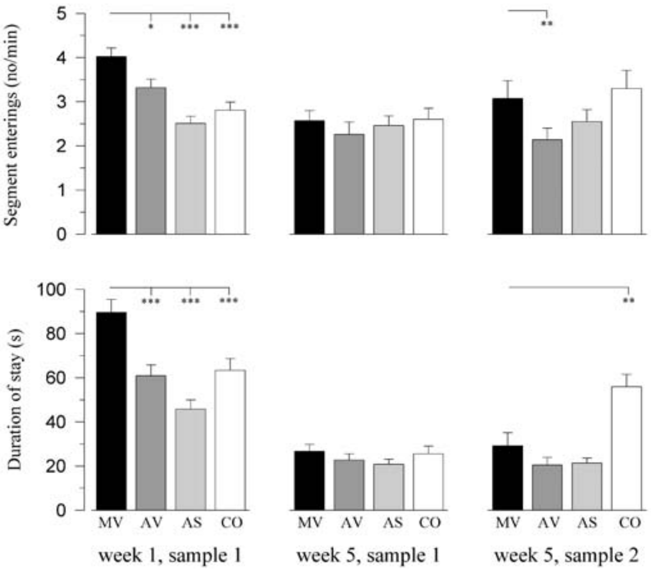

Fig. 3 Number of times the sound segment was entered and duration of stay at piglets' 1st (sample 1) and 5th week (samples 1 and 2) during the replay of the MV, AV, AS, and CO. Data are given as means±SE. Significant differences to MV (procedures GENMOD and MIXED, respectively) are indicated by *asterisks*: *P<0.05, **P<0.01, or ***P<0.001

type-III analysis was done, consisting of specifying a model and computing likelihood ratio statistics for type-III contrasts for each term in the model (χ^2 values). If the test values used indicate first-kind risks of $P \leq 0.05$ the results are considered to be significant.

Results

The respective nested effect of sow and trial (piglet sample 1) or sow (piglet sample 2) showed no significant influence (P>0.05), except for the activity of the piglets in both samples (χ^2=82.4 and χ^2=23.3, respectively; P<0.001), indicating that only the general activity level, but not the choice behaviour or the vocalisation rate, were affected by a genetic (i.e. mother) or social (i.e. litter) background. Hence, the following results are presented in a summarised manner, showing the mean values for each sound class and observation week in both piglet samples.

Choice behaviour

Figures 2 and 3 summarise the observed parameters of the piglets' choice behaviour regarding the sound segment and indicate significant differences between the MV and the other two sounds and the control. The percentage and latency of first choice towards MV were significantly different only from the AS and the CO and not from the AV (Fig. 2). However, close to the sound source, the number of times the sound segment was entered and the duration of stay at the piglets' own mother's voice were significantly different from the AV, the AS, and the soundless CO (Fig. 3), indicating piglets had some recognition of their own mother's voice. In contrast, at week 5 no significantly different discrimination between the MV and the other sounds or the control could be found in the piglets of the first sample. Generally, a significant overall effect of the observed week (not additionally indicated in the figures) was found in the piglets' latency of first entering with P<0.05 (F=6.4) and for the other parameters with P<0.001: the level of the piglets' reactions at week 5 was generally lower compared to week 1.

The piglets that were only investigated at week 5 (piglet sample 2) showed only few significant differences in their choice behaviour (predominantly MV vs CO) and a definite preference for the maternal vocalisation could not be verified. Only the MV was significantly preferred in the number of times that sound segment was entered compared to the AV (Fig. 3). When compared to week 1 of the piglet sample 1, an overall effect of the observed week was only detected for the duration of stay (F=37.4, P<0.001). Similar to the piglets of the first sample the piglets of the second sample had a lower response level regarding this parameter. For the other parameters, however, an overall effect of the week was not found.

Activity and vocalisation

The piglets' open-field activity and the incidence of vocalisations are presented in Table 1. For both piglet

Table 1 Frequency of the piglets' behavioural patterns (locomotor activity and vocalisation) observed in the open field at week 1 (piglet sample 1) and week 5 (piglet samples 1 and 2) during the replay of the maternal vocalisation (*MV*), an alien sow vocalisation (*AV*), an artificial sound (*AS*), or the control without any sound (*CO*). Data are given as means with the respective mean coefficients of variation (*CV*) in parentheses. Significant differences to MV (GENMOD procedure) are indicated by asterisks: $^{*}P<0.05$, $^{**}P<0.01$, or $^{***}P<0.001$

Frequency (no./min)	Week/Sample	MV	AV	AS	CO	(Mean CV)
Locomotor activity						
Segment entered	1/1	9.0	9.5	9.7	10.3**	(38.6)
	5/1	10.9	10.2	12.5**	12.4**	(33.3)
	5/2	8.9	9.3	11.6***	10.9**	(33.8)
Vocalisation						
Sound segment	1/1	1.4	1.0*	0.7***	1.1	(63.5)
	5/1	0.6	0.5	0.6	0.7	(88.8)
	5/2	0.8	0.5	0.6	1.1	(85.5)
Control segment	1/1	0.7	0.7	0.9*	1.2***	(97.3)
	5/1	0.6	0.5	0.7	0.8	(85.5)
	5/2	0.5	0.3	0.9	1.0	(98.8)
Neutral segment[a]	1/1	0.5	0.6	0.5	0.5	(33.2)
	5/1	0.8	0.7	0.7	0.7	(23.7)
	5/2	0.9	0.9	0.8	0.8	(24.1)

[a] Given as an average score of one segment

samples, the investigated week had a significant ($P<0.01$) overall effect (not indicated in the table) on the piglets' reaction to all parameters presented here, except for the activity of the sample 2 piglets in the 5th week ($\chi^2=3.3$, $P=0.07$): the piglets' activity and their vocalisation in an average neutral segment at week 5 were generally higher, whereas the vocalisation in the sound or control segment was lower compared to week 1. The absolutely highest vocalisation rate was detected when the piglets of the first sample entered the sound segment during the MV at week 1 (1.4 vocalisations per min).

In detail, the locomotor activity of both piglet samples during MV was significantly lower compared to the control without sound. At week 5 the activity during the AS was also significantly higher than the activity during the MV. Whereas the piglets of the first sample reacted partly differently in their vocalisation rate when confronted with the different sounds at week 1, no differences were found for either piglet sample at week 5. It is especially remarkable that the MV at week 1 provoked significantly more piglet vocalisations in the sound segment (MV vs AV and AS) and less in the control segment (MV vs AS and CO). Additionally, the highest variations of the behavioural response were found with the vocalisation in the two choice segments (sound and control segment).

Discussion

It is well known that pigs have a considerable repertoire of vocalisations uttered in several situations (for reviews, see Kiley 1972; Klingholz et al. 1979). The nurse grunting of lactating sows before and during a suckling event regulates social and nutritional relationships between mother and offspring. As indicated in the Introduction (see relevant references cited therein), very early recognition of the mother's voice could be expected. It seems that piglets learn the specific characteristics of maternal vocalisation either in utero or in the first hours after birth (Shillito Walser 1986a). Whereas no experimental studies exist for the former hypothesis, the latter seems very likely for at least two reasons. First, there are individual differences in the composition of the grunt frequencies (Schön et al. 1999) that may potentially be used by the piglets as cues for an individual recognition of their mother. Second, the first 4 days of life are characterised by an extremely rapid learning process imprinting the individuals for a specific teat (Puppe and Tuchscherer 1999). This is accompanied by the nurse grunts of the mother during suckling and is rewarded by the milk intake. Indeed, there is some evidence that piglets improve their recognition ability in the first hours after birth (see Shillito Walser 1986b). For example, Horrell and Hodgson (1992) have shown that piglets at the age of 12 h had difficulties differentiating between the vocalisation of their own mother and that of an unrelated sow, whereas piglets at the age of 36 h or older were well able to do so.

Our results revealed that the discrimination of their own mothers and alien sows by the piglets at week 1 failed in two choice parameters (percentage of first choice and latency of entering the sound segment) but was successful in two other parameters (number of times the field was entered and duration of stay in the sound segment). However, for all parameters, the control without sound and the artificially produced sound were significantly less attractive than grunting. This may suggest that 1-week-old piglets react in a distance-dependent way in their response to the early nursing vocalisation. It seems that, regardless of the individual source, the nurse grunting is able to release a generalised approach response in the young. The piglets may use it as acoustic information of a distant female indicating her readiness to nurse as shown by the first choice equality between the piglets' own and an alien mother. Close to the sound source, the piglets seem to discriminate more critically and prefer the maternal nurse grunting. This seems reasonable because it is of vital interest for the piglets not to fail a suckling event. Predominantly the mother provides for her offspring, but in some cases (e.g. death of the mother, signs of disorder or problems with milk production, etc.) alien sows (under natural conditions mostly related with the mother) may adopt piglets. It is known that pigs have well-developed mechanisms of establishing a communal nursing system with synchronised nursing behaviour (Wechsler and Brodmann 1996) including a temporary or permanent switch of some or all individuals from their own mother to other lactating females (Puppe and Tuchscherer 1995). In such cases, exclusive imprinting on the vocalisation of their own mother would be rather disadvantageous. The present findings on the behavioural response of piglets to the replays of nursing vocalisation support very well the conclusions of our previous study (Schön et al. 1999) on the kind of auditory cue that encodes the call information. This study was based on a computer-aided multi-parametric analysis of a great number of acoustic features of the nurse grunting. Whereas an inter-individual meaning indicating an upcoming suckling episode may be encoded both by the occurrence of the call itself and its temporal vocalisation patterns, the individual composition of grunts seems to be the basis of maternal recognition by the piglets. Moreover, there is some evidence that piglets are better at discriminating sows than sows are able to distinguish their own piglets from others (see Horrell and Hodgson 1992).

Surprisingly, both the older piglets experienced with the test situation (piglet sample 1) and the piglets without such experience (piglet sample 2) showed no clear sound discrimination at the end of the suckling period (week 5), except for the difference of entering the sound segment with maternal and alien sow grunting whereas the naive piglets preferred their mother's voice. To a certain degree, this finding is in contrast to some other studies (Lewis and Hurnik 1986; Horrell and Hodgson 1992) reporting a clear preference for the vocalisation of their own mother also by older piglets (up to 14 days old). An obvious explanation would be that the older and experienced piglets of sample 1 have possibly recognised the artificial test situation where the sound stimulus was not rewarded. However, the coeval but inexperienced piglets of sample 2 also showed no clear preference, except to some degree when they were close to the sound source and more frequently entered the mother's vocalisation segment.

Moreover, the older naive piglets' overall choice activity (except the duration of stay at the sound segment) was similar to that of the 1-week-old piglets of sample 1. Although the nursing frequency of 5-week-old piglets is very high (Puppe and Tuchscherer 2000) it seems that the piglets' motivation always to follow their mother might have changed at this age. Apparently, during the weaning process the maternal investment gradually decreases and the young acquire nutritional and social independence from the mother (Martin 1984). At the beginning of the suckling period ungulate young are completely dependent on milk for nutrition, followed by a period of combined solid and milk intake. For pigs, the solid intake increases markedly at about 3–4 weeks of age (Puppe and Tuchscherer 2000). It is therefore plausible that piglets then cease suckling as a possible strategy to optimise their total foraging behaviour (see Jensen and Recén 1989).

Related to the present study we therefore speculate that piglets at the end of the suckling period (week 5) may be able to discriminate the sounds, but they do not show this behaviour because they are more interested in foraging and exploring the new environment in the test situation. This view has been supported by the increased vocalisation released in the neutral segments of the open field at week 5, the simultaneous decreased vocalisation rate in the sound and control segments, and the mostly higher activity scores. Altogether, this might be interpreted as an attempt at active exploration.

Alternatively, the increased vocalisation at week 5 may also be interpreted in terms of increasing discomfort by the disruption of the mother–young relationship (Hennessy et al. 1995). This may explain the higher rate of entering the sound segment replaying the maternal vocalisation, possibly indicating the piglets' search for their mother. At week 1 the piglets responded to maternal grunts with an increasing rate of vocalisation directly in the sound segment. This may serve as an attempt to communicate with the sow, and this view is supported by experiments of Weary et al. (1997), who found that isolated piglets doubled their call rate in response to playback of sow calls. Generally, the replay of the nursing vocalisation seems to have a slight calming effect on the tested piglets (as indicated by the decreased activity) compared to the replays of the artificial sound or the control.

In conclusion, the kind of replayed sounds affects both the choice and the open-field behaviour of suckling domestic piglets in their very early postnatal life. The nursing vocalisation of sows by itself released an initial generalised approach and contact response in the young, indicating their high motivation to gain nutritional or social support. In the absence of visual and olfactory cues but close to the sound source, the maternal vocalisation was clearly preferred to any other sound including an alien sow vocalisation. However, 5-week-old piglets showed no clear discrimination response, and this was largely independent from their experience with the presented test situation. This might be due to a change in the motivation of these piglets reflecting their increased independence of direct maternal support.

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7.7 Studie 7

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Consequences of repeated early isolation in domestic piglets (*Sus scrofa*) on their behavioural, neuroendocrine, and immunological responses

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Abstract

Stress in the form of intermittent maternal deprivation and social isolation during early postnatal life in rats and monkeys produces persistent changes in physiology and behaviour. In farm animals physiological consequences of disrupting mother–infant interactions with respect to health and animal welfare are relatively unknown. Therefore, the aim of the present study was to investigate the behavioural, neuroendocrine and immunological consequences of a 2 h daily social isolation from day 3 to day 11 of age in domestic piglets as well as potential long-term effects on the brain–endocrine–immune regulation. Repeated social isolation resulted in significantly decreased open-field activity (locomotion, vocalization) during the isolation period, increased basal cortisol concentrations and decreased lymphocyte proliferation in response to concanavalin A and pokeweed mitogen one day after the isolation. There was also a significant increase of interleukin-1β (IL-1β) concentration in hippocampus in isolated piglets compared to controls at this time. Six weeks after isolation significant enhanced basal ACTH concentrations as well as higher IL-1β content and glucocorticoid receptor (GR) binding in hippocampus were found. These endocrine and immune responses were associated with decreased CRH levels in the hypothalamus and increased CRH content in the amygdala. The present data indicate that early social isolation in pigs may cause changes in behavioural, neuroendocrine, and immune regulation and produce long-term effects not only on the activity of the hypothalamic–pituitary–adrenal (HPA) system, but also on the immune–brain circuitry with possible negative consequences in health and welfare of commercial pigs. Using the pig as a suitable animal model, the finding of this study may also have some implications for the etiology of anxiety and depression in humans.

Keywords: Early-life stress; Behaviour; Immunity; HPA response; Limbic system; Pigs

1. Introduction

Psychosocial stress in form of intermittent maternal separation and social isolation during early postnatal life has been repeatedly shown to induce profound and irreversible alterations in neuroendocrine and behavioural mechanisms of adaptation. In the human, early-life experience may modulate stress–responses and coping, with long-term implications for emotional health and cognitive function (Heim et al., 1997). In rats, daily handling during a critical neonatal period leads to a significant and permanent modulation of key molecules that govern hormonal secretion in response to stress (Meaney et al., 1996).

As shown in many studies, the hypothalamic–pituitary–adrenal (HPA) axis is the neuroendocrine system that is subjected to programming by early-life events (Liu et al., 1997; Meaney et al., 1994). In addition to its purely endocrine role, the HPA axis has bidirectional communication with the immune system, and during development signals from one system impinge on the functional development of the other (Hall and O'Grady, 1989; Stein and Miller, 1993). Therefore, immune reactivity may also

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be modified by alterations of the postnatal environment by affecting the brain–endocrine–immune connections. Stressful stimuli may also influence both peripheral immune function and brain cytokine expression (Dantzer and Mormede, 1995; Plata-Salaman et al., 1999).

In commercial pig production, many management practices like housing conditions, handling by humans and disruption of social contacts may influence feed intake, growth, behaviour, stress susceptibility, and health of the animals (Minton, 1994; Von Borell, 1995; Weiler et al., 1998). An environment in which stressors are minimized would likely be favourable for efficient production and for helping ensure the welfare of this species. However, the challenge for those interested in maintaining the welfare of pigs lies in identifying biologically meaningful determinants of "stress." Although domestic pigs are socially living animals, as the majority of farm animals, basic physiological effects of early isolation from mother and/or siblings are only scantily investigated. Only one study based on behavioural observations in early isolated piglets has been concluded that social isolation is a stressful event compared with a group-housed control (Herskin and Jensen, 2000). On the other hand it is well known that some handling procedures in the commercial pig production (Ruis et al., 2001) and also some experimental housing (Schrader and Ladewig, 1999) require individual housing (isolation) of the concerned animals. Based on findings in primates (Boccia et al., 1997; Lyons et al., 2000; Smith and French, 1997) and rodents (Levine, 2001; Makino et al., 1999; McCormick et al., 1998) young piglets would be expected to respond with considerable psychophysiological alterations to a social deprivation paradigm. Hence, the present multidisciplinary approach was designed to investigate the influence of a repeated social isolation in very young piglets on important behavioural, neuroendocrine, and immunological measures of their stress response. Besides improved knowledge of developmental adaptation in another species, there are considerable economic reasons to prevent possibly negative consequences of early social isolation for welfare and health in the commercially used pig. Therefore, the objectives of the current work were to analyse the effects of repeated social isolation during early neonatal life in pigs on behaviour, HPA function and immune reactivity and to examine whether these neonatal life events have long-term consequences on the brain–endocrine–immune regulation.

2. Materials and methods

2.1. Animals

Piglets were taken from 18 litters (German Landrace), bred, and raised in the experimental pig unit of our institute. After birth litters were culled to 10 animals, usually 5 males and 5 females. The animals were used in 9 trials and in each trial 2 litters were randomly assigned to a control (C) and isolation (IS) litter with totalling 89 control and 90 isolation piglets. During the suckling period sows and their piglets were housed in a loose farrowing pen ($6\,m^2$) with a warm water heated lying area for the piglets, and with unrestricted access to food and water. The piglets were weaned at 28 days of age by removal of the sow, mixed from 2 litters, and placed in weaning pens (stocking density $0.45\,m^2$ per pig) in an environmentally controlled room (16L:8D, with room temperature of 28 °C in the first days after weaning and continuous decrease to 22 °C up to an age of 8 weeks). Piglets were offered a commercially pelleted diet from an automatic feeder. Food and water were provided ad libitum.

2.2. Experimental design

On days 3–11 of age, all piglets were isolated from mother and siblings for 2 h daily (7:00–9:00 h). The experimental animals were transported (<60 s) in a carrying cage from the farrowing pen to the test room located in the same experimental station. Here each piglet was placed alone into an opaque plastic box ($0.60 \times 0.40 \times 0.32$ m) with sawdust on the floor and adequate air passage. The isolated piglets were kept under the same temperature and humidity as used in the farrowing pen. After isolation each piglet was returned to its mother. The control piglets and mother were left undisturbed in the farrowing pen during this time.

To investigate the short-term and long-term effects of isolation on the immune and endocrine system blood samples were taken one day after the end of the isolation period (day 12) and 45 days after isolation (day 56) in a supine position by anterior vena cava puncture (the whole procedure lasted approximately 30 s). The day 56 was chosen because it is characterized by habituation to the weaning challenge (day 28) and low social conflicts within the groups.

Blood samples for plasma extraction were collected in ice-cooled polypropylene tubes containing EDTA solution, placed on ice, and subsequently centrifuged at 2000*g* for 15 min at 4 °C. Another part of blood samples was allowed to clot overnight at 4 °C and centrifuged at 1000*g* for 15 min at 4 °C. Plasma and serum were then stored at −20 °C until analysis.

At the same age of 12 and 56 days four piglets (two male and two female piglets) from each control and isolated litter were euthanized by an injection of T61 (Intervet, Germany). The brains were quickly removed and placed on ice, and the hippocampus, amygdala and hypothalamus were dissected, frozen in liquid nitrogen, and stored at −80 °C.

All experimental procedures were performed between 08:00 a.m. and 11:00 a.m.. The procedures involving use and treatments of the animals were approved by the Committee on Animal Care and Use of the Agricultural Ministry of Mecklenburg–Vorpommern, Germany.

2.3. Body weights

Body weights of all piglets were continuously recorded and the daily weight gains were calculated from birth until day 28 and 56 of age, respectively.

2.4. Behaviour

An open-field (2.20 × 2.20 m) was used to test the individual behavioural responses of each piglet 1 day before as well as 3 and 7 days after the start of the daily 2-h isolation period. As methodically described earlier by Puppe et al. (1999), the behaviour of the tested animals was continuously recorded (focal sampling) during a 10-min session using The Observer® (Noldus Information Technology, 1995). In the present study, the following main behavioural patterns were subsequently analysed as duration per session: inactivity (motionless standing or sitting), locomotion (all forms of locomotor or exploratory activity), elimination (urination or defecation), and attempts to escape (attempts to leave the open-field, e.g., the piglets contacted the wall and/or sprang against the wall). Additionally, it was noted when each behaviour was accompanied by any kind of vocalization; either grunts (low-pitched calls) or screams (high-pitched calls).

2.5. Immune parameters

Mitogen-induced lymphocyte proliferation was used as an in vitro index of cellular immune function performed as previously described (Tuchscherer et al., 1998). T cell-specific mitogen, concanavalin A (25 μg/ml; ConA), B-cell-specific mitogen, lipopolysaccharid (100 μg/ml; LPS), and T cell-dependent B cell mitogen, pokeweed mitogen (12.5 μg/ml; PWM), were used in the lymphocyte proliferation assay (all mitogens from Sigma–Aldrich, St. Louis, USA). The specified concentrations of mitogens were shown to be an optimum by dose titration experiments. Peripheral blood mononuclear cells (5×10^6) were incubated in 96-well flat-bottomed cell culture plates 72 h in a 5% CO_2 humidified incubator at 37 °C. The following cell proliferation assay is based on the reduction of tetrazolium salt into a blue formazan product by mitochondrial dehydrogenase of viable cells (commercial kit, Boehringer 1465007, Mannheim, Germany). After 72 h incubation, cultures were pulsed with 20 μl of the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) labeling reagent (final concentration 0.5 mg/ml) for 4 h at 37 °C, and solubilized to dissolve the dark blue crystals overnight. The optical density (OD) was measured by a microplate reader (Dynatech, Denkendorf, Germany) at a test wavelength of 550 nm and a reference wavelength of 690 nm. The results are expressed as the proliferation index (PI) on MTT assay calculated according to the formula for results of triplicate assays:

$$\mathrm{PI} = \frac{\text{OD (550/690 nm) of stimulated cells}}{\text{OD (550/690 nm) of non-stimulated cells}}.$$

Total serum IgG concentrations were determined using a modified indirect competitive ELISA based on the method described by Morrow-Tesch et al. (1994). Briefly, polystyrene microtitre plates were coated with 25 μl rabbit anti-pig IgG diluted in 50 mM bicarbonate buffer, pH 9.6, at 4 °C overnight. Plates were than washed three times with PBS containing 0.05% Tween 20. Diluted serum samples (1:30,000 in PBS–Tween) or pig IgG standards (1.08–0.142 μg/ml) were added to the plates (50 μl/well). After incubation at 37 °C for 60 min, plates were washed with PBS–Tween and 50 μl of a solution of rabbit anti-pig IgG conjugated to horseradish peroxidase was added. Plates were incubated at 37 °C for 60 min, washed, and 100 μl of substrate (10 ml of 0.1 M citric acid buffer, pH 4.9; 4 mg *o*-phenylendiamine, and 4 μl of 30% H_2O_2) was added to the wells. Plates were incubated for 20 min at room temperature in the dark and than colour development was stopped by adding 100 μl of 2 M H_2SO_4. Absorbance was measured with a microplate reader (Dynatech, Denkendorf, Germany) at a test wavelength of 490 nm and a reference wavelength of 690 nm. All reagents were obtained from Sigma–Aldrich (St. Louis, USA). The intra- and inter-assay variation coefficients were 4.1 and 7.7%, respectively.

2.6. Hormone analyses

ACTH-like immunoreactivity was analysed in duplicate in 200 μl plasma using a highly sensitive and specific sandwich-immunoradiometric assay according to the instructions of the manufacturer (Nichols Institute Diagnostics, San Juan Capistrano, USA). The assay has been also validated for animal specimens (Nichols Institute Diagnostics, Geneva, Switzerland) and has no cross-reactivities with α-MSH, β-MSH, β-endorphin, or β-LPH (Zahradnik et al., 1989). Additionally, in our laboratory the assay was validated for quantifying ACTH concentrations in pigs. Briefly, plasma samples from CRH-challenged pigs were serially diluted and assayed. The displacement curves generated from these samples were found to parallel the human standard curve provided in the assay kit. The ACTH assay incorporates a monoclonal and polyclonal antibody binding specifically to the N- and C-terminal regions of ACTH, thereby detecting only the intact ACTH molecule. The intra- and inter-assay coefficients of variation were 3.2 and 7.8%, respectively and the assay sensitivity was 1.6 pg/ml.

Plasma cortisol concentrations were measured in duplicates using a commercially available ^{125}I-RIA kit (DRG International, USA) according to the manufacturer's guidelines. The antibody for this assay was highly specific with less than 5% cross-reactivity with any potentially competing plasma steroids (DRG International, USA). The assay was validated for use with porcine plasma. Serial dilutions of two porcine plasma pools (>200 and <20 ng/ml cortisol) with the provided diluent demonstrated parallelism to the standard curve. Recovery was conducted by addition of four cortisol standards (0, 5, 100, and 600 ng/ml) to porcine plasma in a 25 μl reaction volume. Recovery levels ranged from 85 to 100%. Sensitivity of the assay was 3 ng/ml, and intra- and inter-assay coefficients of variation were 5.3 and 9.8%, respectively.

2.7. Corticosteroid binding globulin (CBG)

Plasma samples were assayed for CBG using a modified binding assay previously described by Kanitz et al. (2002). Twenty-five μl blood plasma were incubated with 0.78 nM unlabeled cortisol (Hydrocortisone, Merck, Darmstadt, Germany) and 25 pM [^{3}H]cortisol (specific radioactivity 68 Ci/mmol, Amersham–Pharmacia Biotech, Freiburg, Germany). This volume was adjusted to 0.5 ml with 0.01 M sodium dihydrogen phosphate buffer, pH 7.4. For determination of nonspecific binding, a 100-fold excess of unlabeled cortisol was added. After incubation for 15 min in a 45 °C water bath, the samples were chilled for 15 min at 0 °C. Unbound cortisol was removed by mixing with 0.5 ml of an ice-cold dextran-coated charcoal solution followed by 20 min incubation at 4 °C and centrifugation at 1000*g* for 10 min. Aliquot of supernatant fraction (500 μl) were taken for scintillation counting (LKB Wallac, Turku, Finland). The intra- and inter-assay coefficients of variation were 7.8 and 9.1%, respectively.

2.8. Quantification of IL-1β

IL-1β content was assayed in hippocampus and hypothalamus. Frozen tissue samples were weighed and placed in homogenization buffer (4 °C) at a ratio of 100 mg tissue/ml of buffer. Buffer contained a protease-inhibitor cocktail including 1 mM phenylmethyl sulfonylfluoride (PMSF), 1 μg/ml pepstatin A, 1 μg/ml aprotinin, and 1 μg/ml leupeptin in phosphate-buffered saline solution, pH 7.2, containing 0.05% sodium azide and 0.5% Triton X-100. Samples were homogenized using a Teflon-glass homogenizer and subjected to one freeze–thaw cycle, and incubated at 4 °C for 60 min. The final homogenate was centrifuged at 120,000*g* for 20 min (Centrikon T-1080, Kontron Instruments, Watford, UK) to precipitate cellular organelles. Tissue supernatants were analysed for IL-1β concentrations using a commercial pig ELISA (Biosource, Nivelles, Belgium) in duplicate at 1:5 dilution. The ELISA was run according to the manufacturer's instructions. The limit of detection for this assay was 15 pg/ml. Intra- and inter-assay coefficients of variation were 4.9 and 9.6%, respectively. Data are expressed as pg of IL-1β per mg of tissue protein.

2.9. Quantification of CRH

CRH concentrations were determined in the amygdala and hypothalamus. CRH was extracted from frozen tissues with 1 ml of 0.5 N HCl containing 1 mg ascorbic acid and 0.01% Triton X-100 by using a Teflon-glass homogenizer. The extract was transferred to a microcentrifuge tube, placed in a shaking bath at 70 °C for 10 min, and sonicated at 37 °C for 10 min. The samples were then centrifuged at 10,000*g* for 15 min. The supernatant was lyophilized in a Speed-Vac evaporator (Jouan GmbH, Unterhaching, Germany) and resuspended in 250 μl RIA buffer (50 mM phosphate buffer, 12 mM EDTA, 0.1% Triton X-100, and 0.02 mg/ml aprotonin). CRH-like immunoreactivity was quantitated in the tissue extracts, using commercially available ^{125}I-RIA kit (PhoenixPeptide, Belmont, CA) according to the manufacturer's instructions. The RIA kit was validated before use with porcine tissue extracts. Dose–response curves for brain tissue extracts, and increasing concentrations of CRH added to the tissue extracts were parallel to the standard curve. The cross-reactivity test, provided by Phoenix Pharmaceuticals, indicated 0% cross-reactivity with ACTH, LH-RH, [Arg8]-Vasopressin, Urocortin, PACAP-38, and BNP-45. The assay sensitivity was approximately 7 pg/ml. The intra- and inter-assay variabilities for CRH were 8.5 and 11.8%, respectively. Values for CRH are expressed as pg of CRH per mg of tissue protein.

2.10. Glucocorticoid receptor (GR) binding

Hippocampal tissue was homogenized in ice-cold buffer containing 10 mM Tris base, 12.5 mM EDTA, 10 mM sodium molybdate, 0.25 mM saccharose, and 1 mM dithiothreitol (pH adjusted to 7.5) using a Teflon-glass homogenizer. The homogenates were centrifuged at 0–4 °C for 60 min at 120,000*g* in a Centrikon T-1080 ultracentrifuge (Kontron Instruments, Watford, UK). The supernatant cytosol fractions were decanted and used immediately. The GR binding was assessed by incubating 100 μl cytosol with 5 μl aliquots of [^{3}H] dexamethasone (specific activity 43 Ci/mmol; Amersham–Pharmacia Biotech, Freiburg, Germany) over a concentration range of 0.2–24 nM previously described by Kanitz et al. (1998). Binding equilibrium was reached after 24 h at 2 °C. This has been shown to be sufficient for maximal exchange, and binding remains stable over this period (Kalimi and Hubbard, 1983). Although the exchange is probably not complete, it was assumed to be equivalent in all experimental groups. Non-specific binding was determined with a parallel incubation that contained 500-fold excess of RU 28362 (kindly donated by Roussel Uclaf, Romainville, France), which binds selectively to the glucocorticoid receptor. In the presence of unlabeled RU 28362 the remaining [^{3}H]dexamethasone activity mainly represented the binding to MR, while GR could be calculated by subtraction of MR from total [^{3}H]dexamethasone binding. The separation of bound and free [^{3}H]dexamethasone was performed by precipitation with dextran-coated charcoal. After addition of 500 μl of an ice-cold charcoal suspension the samples were mixed for 15 min at 4 °C. Immediately afterwards, they were centrifuged at 4 °C at 1500g for 10 min. An aliquot of the supernatant was aspirated into a scintillation vial, 5 ml scintillation liquid was added, and the radioactivity was counted in a spectral liquid scintillation counter (LKB Wallac, Turku, Finland) at an efficiency of 50%. The apparent maximum binding capacity ($B_{\max}$) of [^{3}H]dexamethasone and dissociation constant (K_D) were evaluated from Scatchard plots (Scatchard, 1949) generated for each animal individually.

The protein concentrations in all tissues were determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

2.11. Data analysis

All statistical analysis were done with the SAS System for Windows (Version 8.02, Statistical Analysis Systems Institute, Cary, NC) by an analysis of variance (ANOVA) using the GLM procedure. Independent variables (fixed classification variables) for all measures of the present study were the treatment of the piglets (isolation vs. control), age (i.e., the respective test days), the interaction age × treatment, gender, and the random nested effect of the sow within the treatment. The data analysis differed concerning the use of the variable age. For the immune, endocrine, and brain tissue parameters different piglets of both treatment groups were tested either at the day 12 of age or at the day 56 at age. However, the behavioural measurements were always taken on the same animals at three test days related to the start of isolation (1 day before, 3 and 7 days after). Hence, the variable age in the ANOVA-model was now used as a repeated factor.

Basically, the results are presented as least-squares means (LS-means) and standard errors (SE) computed for each effect in the model. Additionally, all pairwise LS-means differences for the fixed effects in the ANOVA-model at the respective test days were tested (t test).

3. Results

3.1. Behaviour

The results of the open-field behaviour are shown in Fig. 1. As expected, neither in the behaviour nor in the vocalization differences occurred between the control and the later isolated piglets one day before the isolation started. However, the process of repeated social isolation altered some behavioural reactions of the piglets during the isolation treatment. All behavioural patterns shown in Fig. 1 significantly changed over the test days ($F(2,194) = 43.10$ (inactivity), $= 55.92$ (locomotion), $= 17.92$ (elimination), $= 28.76$ (vocalization), all p values $< .001$). Both inactivity ($F(1,97) = 7.68$, $p < .01$) and locomotion ($F(1,97) = 10.05$, $p < .01$) were significantly affected by the treatment of the piglets (isolation vs. control) as overall main effect. Whereas isolated piglets displayed more inactivity compared to the control piglets at day 3 ($t = 3.10$, $p < .01$) and at day 7 ($t = 2.33$, $p < .05$) after the start of isolation, the locomotion of the isolated piglets was lower at both days ($t = 3.99$, $p < .001$ and $t = 1.99$, $p < .05$). Although elimination and vocalization were not affected by the overall treatment, the isolated piglets showed less elimination at day 7 ($t = 2.10$, $p < .05$) and vocalised less at day 3 ($t = 2.12$, $p < .05$). The total vocalization basically consisting of grunts and screams are displayed, the percentage of screams of isolation treated piglets was less than 3%. However, the escape behaviour (fewer than 6% of all behaviours after isolation) was not affected by treatment (data not shown).

3.2. Immune changes

Statistical analysis revealed a significant overall main effect of treatment (isolation vs. control) on the lymphocyte proliferation in response to mitogen ConA ($F(1,142) = 3.87$, $p < .05$) and PWM ($F(1,126) = 5.71$, $p < .05$), but isolation had no effect on the lymphocyte proliferation in response to B-cell mitogen LPS ($F(1,105) = .23$, $p = .63$). As shown in Fig. 2, isolated piglets displayed a significantly reduced in vitro lymphocyte proliferation in response to ConA ($t = 2.96$, $p < .05$) and PWM ($t = 3.69$, $p < .01$) one day after the isolation period (day 12) compared with control piglets. However, at day 56 of age (45 days after the end of the isolation treatment) there was no effect of treatment on mitogen-induced lymphocyte proliferation. The factor age had a significant influence on the mitogen-induced lymphocyte proliferation to PWM ($F(1,126) = 22.68$, $p < .001$) and LPS ($F(1,105) = 9.26$, $p < .01$) whereas it

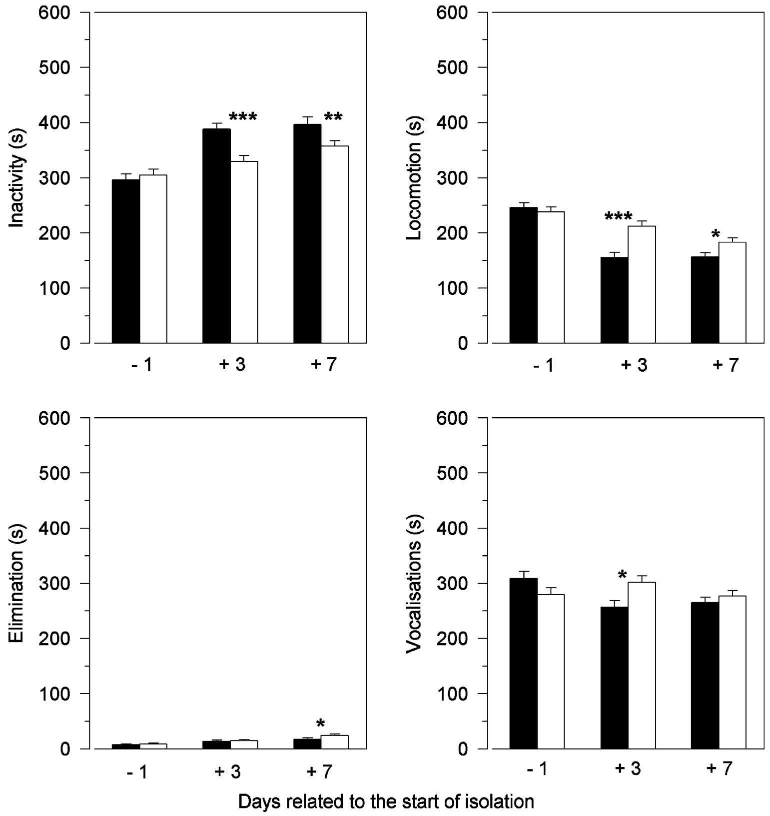

Fig. 1. Open-field behaviour of isolated (filled bars) and control (open bars) piglets before (−1 day) and after (+3 and +7 days) the start of isolation at their third day of life. Significant differences between isolated and control piglets are indicated by asterisks: $^*p < .05$, $^{**}p < .01$, and $^{***}p < .001$.

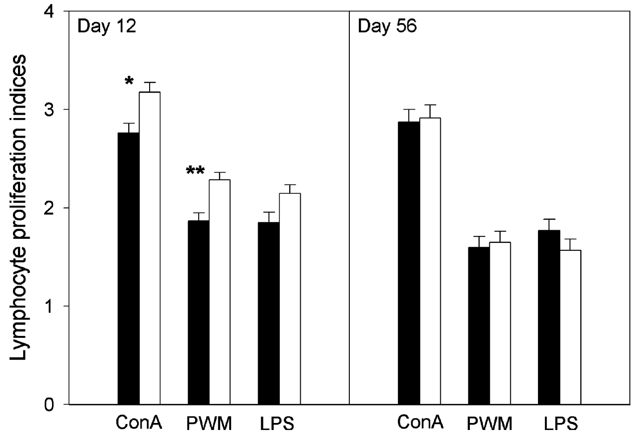

Fig. 2. Lymphocyte proliferation indices in response to T-cell and B-cell mitogens of isolated (filled bars) and control (open bars) piglets at days 12 and 56 of age. Significant differences are indicated by asterisks: $^*p < .05$ and $^{**}p < .01$.

did not affect the proliferation to T-cell mitogen ConA ($F(1,142) = .43$, $p = .51$).

Serum IgG concentrations decreased significantly with age of the piglets ($F(1,152) = 94.64$, $p < .001$), but they were not significantly influenced by the treatment at day 12 (isolation: 15.98 ± 0.77 g/L; control: 14.66 ± 0.46 g/L; $t = 2.02$, $p = .18$) or day 56 of age (isolation: 11.04 ± 0.56 g/L; control: 9.55 ± 0.56 g/L; $t = 1.88$, $p = .24$).

3.3. Endocrine changes

A significant influence of isolation was found on plasma concentrations of ACTH ($F(1,114) = 10.49$, $p < .01$) and cortisol ($F(1,145) = 19.62$, $p < .001$). There was also an effect of age on the stress hormone levels (ACTH: $F(1,114) = 7.25$, $p < .01$; cortisol: $F(1,145) = 4.56$, $p < .05$). The repeated isolation of neonatal piglets caused a significant increase of cortisol concentrations at day 12 of age ($t = 4.58$, $p < .001$) and a significant increase of ACTH levels ($t = 2.76$, $p < .05$) at day 56 (Fig. 3).

Plasma CBG concentration increased significantly with age of the piglets ($F(1,147) = 164.27, p < .001$) but there was no treatment effect on CBG concentration neither at day 12 nor day 56 of age (data not shown).

3.4. Brain parameters

GR binding in the hippocampus was significantly affected by the factor treatment ($F(1,44) = 12.09$. $p < .01$). The values for the maximum binding capacity $B_{\max}$ were significantly higher in isolated piglets

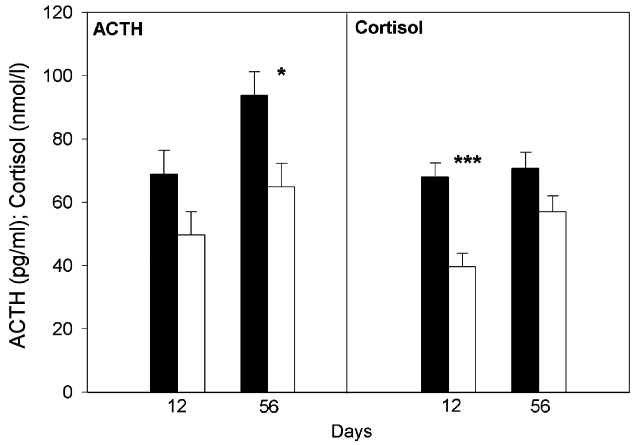

Fig. 3. Basal plasma ACTH and cortisol concentrations in isolated (filled bars) and control (open bars) piglets at days 12 and 56 of age. Significant differences are indicated by asterisks: $^*p < .05$ and $^{***}p < .001$.

compared with control piglets at day 56 ($t = 2.93$, $p < .05$; Fig. 4).

The hippocampal IL-1β concentration followed a similar pattern as that for the GR binding. There is a main effect of treatment ($F(1,109) = 30.90$, $p < .001$) on the IL-1β content in the hippocampus. As shown in Fig. 5, hippocampal IL-1β concentration was significantly higher in isolated piglets both at day 12 ($t = 3.29$, $p < .01$) and day 56 ($t = 4.58$, $p < .001$). Similarly, the IL-1β concentration in the hypothalamus was significantly affected by the factor treatment ($F(1,92) = 12.13$, $p < .001$). Here, IL-1β concentration in isolated piglets only tended to be higher at both tested days (day 12: $t = 2.53$, $p = .06$; day 56: $t = 2.38$, $p = .08$; Fig. 5).

Additionally, CRH concentrations were measured in specific brain areas like hypothalamus and amygdala. CRH concentration in the hypothalamus did not differ between isolated and control piglets at day 12, but there was a significant reduction in CRH content of isolated piglets at day 56 ($t = 2.91$, $p < .05$; Fig. 5). As a main effect of treatment CRH concentration in the amygdala was slightly enhanced in isolated piglets ($F(1,97) = 3.06$, $p = .08$). In contrast to the hypothalamic changes, CRH concentration in the amygdala tended to be higher in isolated piglets at day 56 ($t = 2.14$, $p = .10$; Fig. 5).

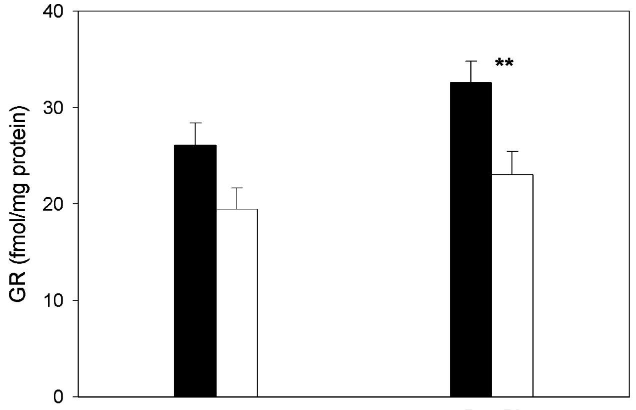

Fig. 4. Hippocampal glucocorticoid receptor (GR) binding in isolated (filled bars) and control (open bars) piglets at days 12 and 56 of age. Significant differences are indicated by asterisks: $^{**}p < .01$.

A significant influence of age was found on hypothalamic IL-1β ($F(1,92) = 17.22$, $p < .001$) and CRH concentrations ($F(1,119) = 6.07$, $p < .05$), but there was no effect of age for the other examined parameters in hippocampus and amygdala.

3.5. Daily weight gain

The daily weight gain was both affected by treatment ($F(1,152) = 5.78$, $p < .05$) and age ($F(1,152) = 121.55$, $p < .001$). At day 12 isolated piglets showed a significantly lower daily weight gain compared to the control piglets (218.43 g/d vs. 244.55 g/d; $t = 2.57$, $p = .05$), but there was no treatment effect at day 56 of age (isolation: 313.35 g/d; control: 325.75 g/d; $t = 1.01$, $p = .74$).

It should be noted that statistical analysis revealed no significant influence of gender on investigated parameters in this study.

4. Discussion

Repeated daily social isolation in pigs during the first days of life produced short- and long-term effects in the offspring. These effects consisted of: (i) decreased behavioural activity in the open-field; (ii) higher basal ACTH and cortisol levels; (iii) suppressed immune function; (iv) increase in cytosolic GR binding in the hippocampus; (v) higher central IL-1β concentrations, and (vi) a different CRH activation in hypothalamus and amygdala.

Maternal separation is known to produce an array of biological effects in mammals (Coe et al., 1985). Whereas physiological responses to a brief separation are adaptive for the survival of the organism, prolonged or repeated episodes of separation could produce negative effects on long-term development. Our results show that social isolation was perceived as a stressful condition by neonatal pigs. This was indicated by physiological changes, which were considered indicative for higher state of stress, such as an increase of basal cortisol (Harbuz and Lightman, 1992; Minton, 1994) and a decrease in immune responsiveness (Kanitz et al., 2002; Tuchscherer et al., 1998), and by alterations of behavioural responses in the open-field test (Jensen et al., 1997; Ruis et al., 2001).

It is well known from studies in rodents and monkeys that their isolated infants show a biphasic response during maternal separation. After an initial period of behavioural activation, they become progressively le-

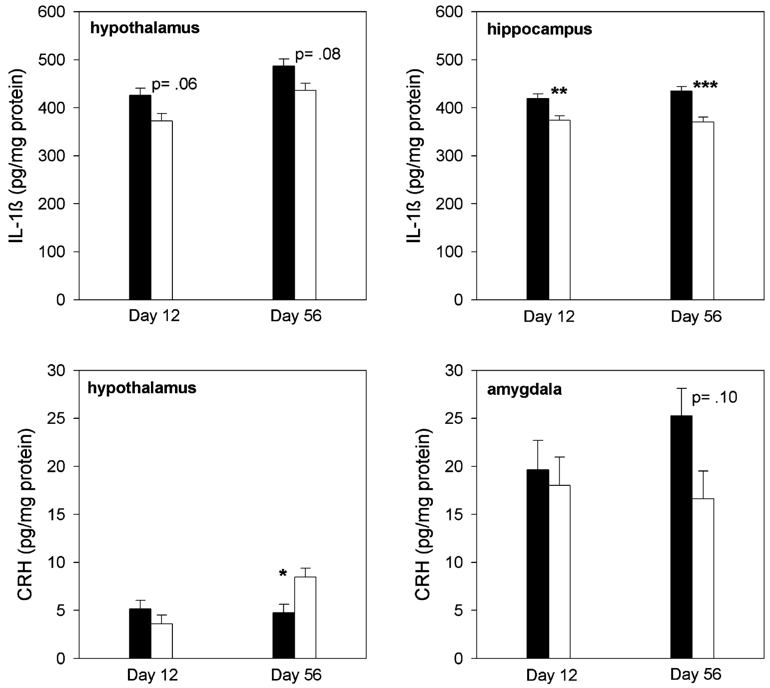

Fig. 5. Interleukin-1β (IL-1β) and corticotropin-releasing-factor (CRF) content in different brain areas of isolated (filled bars) and control (open bars) piglets at days 12 and 56 of age. Significant differences are indicated by asterisks: *$p < .05$, **$p < .01$, and ***$p < .001$.

thargic and show rather passive patterns of behaviour (Hennessy et al., 2001; Hofer, 1996). Regardless of the passive stage is interpreted in terms of mental "despair" (Hofer, 1996) or of stress-induced sickness behaviour (Hennessy et al., 2001), it is often considered as an animal model for human depression. Behaviour of pigs in an open-field test has been examined in a variety of studies (Fraser, 1974; Lathrop and Friend, 1986, 1987; Thodberg et al., 1999; Von Borell and Ladewig, 1992) and has been correlated with emotional reactivity (Herskin and Jensen, 2000; Puppe et al., 1999). In the present study, the open-field behaviour of the isolated piglets was characterised by a significant decrease of locomotion, a simultaneously increased inactivity, and by partly decreased reactivity patterns like eliminative behaviour and vocalization compared to controls. According to the passive stage of the described temporal response in laboratory animals and similar to the results of Herskin and Jensen (2000) in newly weaned piglets these behavioural reactions may be interpreted as decreased emotional reactivity or even as first signs of depression.

This open-field behaviour was also accompanied by an impaired cellular immunocompetence. Repeated social isolation in the first days of life caused a significant decrease in immune reactivity at day 12, as assessed by T-cell mitogenesis without affecting B-lymphocyte proliferation induced by LPS. Consistent with earlier findings, psychosocial events seems to affect primarily the T-lymphocytes, and less the B-lymphocytes (Dominguez-Gerpe and Lefkovits, 1996; Kanitz et al., 2002; Neveu et al., 1994; Tuchscherer et al., 1998). Some studies described long-term effects of postnatal stress on immunity in monkeys (Coe et al., 1989; Gordon et al., 1992; Lubach et al., 1995). The present study in pigs did not reveal similar results. There was no effect of isolation on lymphocyte proliferation at day 56 of age regardless of increased HPA activity. This finding may include an age-dependent effect, because it was documented that neonatal lymphocytes are more sensitive to glucocorticoid inhibition than are those from older pigs (Yang and Schultz, 1986). Stress is also known to have an effect on humoral immunity (Griffin, 1989). In our experiment the IgG concentrations the main class of immunoglobulins in pigs were not significantly influenced by social isolation at both test days. Whereas at day 12 of age the IgG concentration is dependent on absorption of intact antibodies from ingested colostrums, at day 56 of age the IgG content reflects the de novo synthesis by piglets.

Numerous experiments have demonstrated that maternal separation and/or isolation procedures are critical for the regulation of the rat pup's HPA axis and that these stressors produce persistent changes in physiology and behaviour depending on the nature of the stressor and the duration of the separation period (Levine, 2001; Levine et al., 1992; Matthews et al., 1996). However, it was also shown that these effects can diminished by social support (e.g., maternal care) or environmental enrichment (Francis et al., 2002; Liu et al., 1997; Lyons et al., 2000). With respect to the HPA regulation it was shown, that a brief maternal separation (handling) in rats increases GR expression in the hippocampus and frontal cortex, while a protracted maternal separation is associated with decreased GR levels in the hypothalamus, hippocampus, and frontal cortex (Bhatnagar et al., 1995; Meaney et al., 1996; Plotsky and Meaney, 1993). These varieties in GR levels were associated with altered glucocorticoid negative feedback sensitivity (Meaney et al., 1996) and with differences in resting-state levels of mRNAs encoding for CRH and CRH immunoreactivity in the hypothalamus (Francis et al., 1996; Plotsky and Meaney, 1993). In the present study on pigs neonatal treatment did not result in the same changes in HPA function as in rats. However, also in the pig the repeated social isolation caused long-term effects on the neuroendocrine system. Isolated pigs had a higher number of GRs in the hippocampus, lower CRH concentration in the hypothalamus and greater plasma ACTH concentrations at day 56 of life. Weaver et al. (2000) also found permanent alterations in HPA function in pigs in response to neonatal handling in a manner dissimilar to that detected in the rat. These species differences may be due to different developmental stage of the HPA axis at birth, a failure of a stress hyporesponsive period in neonatal pigs (Kanitz et al., 1999; Otten et al., 2001) compared to rats, and thus, to a different temporal susceptibility of various components of the HPA system to environmental regulation.

Interestingly, isolation stress also induced in tendency changes of CRH concentration in limbic areas. In contrast to the reduction of CRH in the hypothalamus the concentration in the amygdala was elevated at day 56. Although both hypothalamic and amygdala-derived CRH are thought to interact in the activation of brainstem arousal centres such as the locus coeruleus (Gray, 1991), they do not necessarily respond in a similar fashion to the same stimuli. It has previously been reported that while sustained glucocorticoid administration inhibits the hypothalamic CRH system, it causes a subtle but significant increase in the levels of CRH in the amygdala (Makino et al., 1994; Watts, 1996). A similar dissociation between levels of CRH mRNA in the amygdala and in the hypothalamus was also described by Albeck et al. (1997) and Makino et al. (1999) after psychological stress. In contrast to the study of Makino et al. (1999) in which a psychological stressor failed to elevate any components of the HPA axis, our results indicated both an activation of the HPA system and the limbic system. The response of the amygdala CRH system to the isolation stress in the present study supports the hypothesis provided by Herman and Cullinan (1997) that limbic stress pathways are sensitive to stressors involving higher-order sensory processing, and may refer to the predominantly psychological component of the used stressor.

In addition to the neuroendocrine activation, it was found that social isolation of neonatal pigs also influenced the immune system by affecting the IL-1β concentrations in different brain regions. To our best knowledge it is the first report about changes of IL-1β protein level in different brain regions in pigs. IL-1β is one of the key mediators of immunological and pathological responses to infection, antigenic challenge, and stress (Maier and Watkins, 1998; O'Neill, 1997; Pugh et al., 1999). The present finding that social isolation in pigs increases central IL-1β protein levels is also consistent with studies by other researchers who have shown that stressful events may influence brain cytokine expression. For instance, it was reported that immobilization stress in rats increased IL-1β mRNA expression in the hypothalamus, although no changes occurred in other brain regions (Minami et al., 1991). Inescapable tail shock, on the other hand, increased IL-1β protein levels in various brain regions, including the hypothalamus and hippocampus (Nguyen et al., 1998). IL-1β appears to play a intermediate role in these stress responses because central IL-1β administration produces endocrine, neurochemical, and behavioural changes similar to those produced by stressors (Shintani et al., 1995). Central injection of IL-1 receptor antagonist also significantly inhibits both the brain monoamine and pituitary adrenal response to immobilization stress (Shintani et al., 1995) and the potentiation of fear conditioning and escape learning failure following inescapable tail shock (Maier and Watkins, 1995). It is important to note that stressors like immobilization (which involves forcefully preventing any movement by the rat) and inescapable tail shocks have a physical component. Both of these stressors leave open the possibility for injury induced IL-1β activity. In contrast, the stressor used in the present experiment was purely psychological and had no physical components. In addition, the alteration in brain IL-1β level supported the hypothesis that IL-1β might be an important neural mediator of stress effects.

In conclusion, the present results demonstrate that social isolation of neonatal pigs may cause long-term effects on the activity of the HPA axis and the brain–endocrine–immune system. Although the data do not allow to clarify causal relationships between neuroendocrine and immune regulation in isolated pigs, they indicate that the used psychological stressor seems able to join the same immune–brain circuitry that mediates the innate immune host defence against pathogens. The present results on the impact of social stress during the neonatal period are not only important for the assessment of possible negative consequences in health and welfare of commercially used pigs but might also have implications for the etiology of anxiety and depression in humans.

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Early social isolation alters behavioral and physiological responses to an endotoxin challenge in piglets

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Abstract

Psychosocial stress in the form of maternal deprivation and social isolation during early postnatal life induces persistent alterations in behavioral and physiological mechanisms of adaptation. One consequence may be an increased susceptibility to diseases in later life. Therefore, the aim of the present study was to investigate in domestic piglets the effects of a repeated social isolation (2 h daily from day 3 to day 11 of age) on behavioral, endocrine and immune responses to an endotoxin challenge with lipopolysaccharide (LPS) 1 day or 45 days after the isolation period. Peripheral LPS administration caused serious sickness behavior (somnolence, shivering, vomiting) and provoked profound increases in circulating tumor necrosis factor-α (TNF-α), ACTH and cortisol concentrations. The prior social isolation treatment enhanced signs of sickness and impaired suckling behavior. Early isolated piglets responded to LPS by an increase of shivering on day 12 and by increased vomiting on day 56 compared to controls. Further, there were considerable delays and reductions of time isolated piglets spent suckling on day 12. The repeated isolation stressor diminished TNF-α increases after LPS, whereas stress hormone levels were not significantly affected by isolation treatment. Finally, stronger relationships between signs of sickness and physiological measures were revealed in early isolated piglets. The duration of somnolence in isolated piglets was related to changes of cortisol and TNF-α concentrations, and the highest impact on duration of shivering was found for changes in cortisol and corticosteroid binding globulin levels. The present results suggest a sustained adaptive sensitization of coping with infection by social stress experience during early development in piglets.

Keywords: Social isolation; Lipopolysaccharide; Behavior; Sickness; Hypothalamic–pituitary–adrenal axis; Immunity

Introduction

Recent concepts of sickness behavior in humans and animals emphasize its adaptive value and suggest that the sometimes dramatical behavioral changes after infection are a manifestation of a central motivational state that reorganizes the organism's priorities in order to cope with infectious pathogens (e.g., reviewed by Hart, 1988; Dantzer, 2001; Johnson, 2002; Kelley et al., 2003). Sickness behavior appears to be mediated by peripherally and centrally produced proinflammatory cytokines and activated neural pathways which are involved in the interplay between immune and central nervous system (Dantzer et al., 1998; Kelley et al., 2003; Dantzer, 2004). Because the activation of the brain cytokine system is also associated with human depression, it is thought that behavioral responses developed in sick individuals may be related to behavioral states occurring during depression (Dantzer, 2001; De La Garza, 2005).

Recently, it has been suggested that stress induced by maternal separation may also cause sickness behavior which may share common symptoms and underlying mechanisms to responses after infection (Hennessy et al., 2001). In fact, there is growing evidence supporting the assertion that psychosocial stress in early life may affect the later susceptibility to diseases and the process of recovery (Heim et al., 1997; Huot et al., 2001; Heim et al., 2002; Milde et al., 2004). For instance, it was shown that maternal deprivation of rat pups influenced the development and course of experimental

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inflammatory autoimmune diseases in later adulthood as indicated by modified macrophage activity and differences in clinical symptoms (Laban et al., 1995; Teunis et al., 2002). Also in domestic pigs early social isolation is a stressful event and produces sustained changes in behavioral, neuroendocrine and immune regulation (Herskin and Jensen, 2000; Kanitz et al., 2004).

A valuable tool for studying the impact of social stress on the responsiveness of the immune-neuroendocrine system is the presentation of an immune challenge. The endotoxin lipopolysaccharide (LPS), derived from the cell walls of gram-negative bacteria and often used as an experimental immune stimulus, is known to induce an acute phase response (APR) with distinct signs of sickness. The APR is part of a general homeostatic reaction and therefore serves as the first line of defence of the organism against infection (Hart, 1988; Baumann and Gauldie, 1994; Johnson et al., 1996). The physiological responses of this defence mechanism include activation of peripheral and central cytokine production, activation of the hypothalamic–pituitary–adrenal (HPA) axis by triggering the corticotropin-releasing hormone (CRH) release in the hypothalamus and a complex array of behaviors (Dantzer, 2001; Larson and Dunn, 2001). Sickness is characterized by non-specific clinical and behavioral symptoms such as anorexia, fever, somnolence, lethargy, vomiting, diarrhea, decreased food and water intake and decreased general activity (Hart, 1988; Maier and Wakins, 1998; Dantzer, 2001). Numerous studies have demonstrated that domestic pigs respond to an LPS challenge by serious sickness behavior, activation of HPA axis and elaboration of systemic proinflammatory cytokines (Warren et al., 1997; Wright et al., 2000; Kanitz et al., 2002; Sakumoto et al., 2003). Therefore, LPS challenge is useful model in pigs to study the relationship between behavioral and physiological responses to stress and immune stimuli.

Previously, we have shown that social isolation of neonatal pigs may cause long-term effects on proinflammatory regulation at the periphery and in the brain following immune challenge, with particular importance of tumor necrosis factor-α (TNF-α) in mediating these interactions (Tuchscherer et al., 2004). Besides the neuroendocrine response to infectious or inflammatory stimuli, the behavioral response is an important component of the mechanisms for maintaining homeostasis and promoting recovery (Hart, 1988; Johnson et al., 1996). Nevertheless, the study of behavioral pattern in sick animals has not received much attention. Thus, information about possible effects of social stress in early life on signs of sickness and sickness behavior in response to infection or inflammation is scarce.

The objective of the present study was to investigate if and how early psychosocial stress experience by domestic piglets can modulate coping mechanisms against a later risk of infection. For this purpose, we examined the effects of repeated social isolation from days 3–11 (2 h daily) on behavioral, endocrine and immunological responses to an acute endotoxin challenge (LPS) on days 12 and 56. Because of possible similarities between stress-induced and infection-induced coping mechanism we predicted that early stress experiences may sensitize responses to LPS in the previously stressed individuals. Revealing these relationships may also be helpful to develop treatments reducing suffering in diseased animals (Gregory, 1998), and are thus important for improving their welfare.

Materials and methods

Animals and housing conditions

Piglets from 18 German Landrace litters, bred and raised in the experimental pig unit of our institute, were randomly allocated to an isolation treatment (nine litters) or to non-isolated controls (nine litters). After birth the litter size was standardized to 10 piglets. Each piglet could be recognized by a tattoo number in the ear and a number painted on the back. During the suckling period sows and their piglets were housed in a loose farrowing pen (6 m^2) with a warm-water-heated lying area for the piglets at room temperature of 28±1°C. The piglets were weaned at 28 days of age by removal of the sow, mixed from 2 litters and placed in weaning pens under controlled temperature and lighting conditions (16L:8D, with a room temperature of 28±1°C in the first days after weaning and a continuous decrease to 22±1°C up to an age of 8 weeks). Piglets were offered a commercially pelleted diet from an automatic feeder. Food and water were provided ad libitum.

Experimental design and behavioral measures

Repeated social isolation of piglets was carried out as previously described by Kanitz et al. (2004). On days 3–11 of age, all piglets of an experimental litter were isolated from their mother and siblings in a separate test room for 2 h daily. Here, each piglet was placed alone into an opaque plastic box (60×40×32 cm) with sawdust on the floor, adequate air passage and temperature control (28±1°C). Piglets of the control litters remained undisturbed in the farrowing pen during this time. To investigate the short-term and long-term effects of isolation on behavioral and physiological responses to an endotoxin challenge LPS was applied either 1 day after the end of the isolation period (day 12) or 45 days after isolation (day 56). Day 56 was chosen because it is characterized by habituation to the weaning process (separation from the mother on day 28) and the efficiency of the immune system is comparable to adults (Gaskins, 1998).

From the nine isolated litters and the nine non-isolated control litters, eight piglets per litter were randomly assigned to receive an intraperitoneal (i.p.) injection of either LPS (two piglets) or saline (two piglets) on day 12, or to receive an i.p. injection of either LPS (two piglets) or saline (two piglets) on day 56. Thus, 8 groups with 18 animals each were formed. LPS from *Escherichia coli* (serotype 0111:B4, Sigma Chemical Company, St. Louis, MO, USA) was administered at a dose of 100 μg/kg of body weight dissolved in 2 ml sterile saline. Previous studies have shown that this dose of LPS activates the acute phase response without being lethal (Kanitz et al., 2002). The i.p. injection of sterile saline was volume-equivalent. Body weight of each pig was measured immediately before the injection.

After LPS or saline injection, the behavior of experimental piglets was observed for the subsequent 3 h in their home pen to determine sickness symptoms: (1) shivering (piglets lay separately on the floor or stood and displayed rapid, synchronous muscle contractions, frequently accompanied by piloerection and slow respiration), (2) somnolence (piglets lay separately in a drowsy state with both eyes closed) (3) vomiting and (4) locomotor activity (piglets displayed actual movement from place to place). For each piglet, the behavioral state was directly recorded by scan sampling every minute (Altmann, 1974). In addition, at 12 days of age the suckling behavior of piglets leading to successful intake of milk was recorded (the time piglets spent suckling and massaging the udder; Fraser, 1980). From these data, duration and latency of all behaviors were calculated for the observed 3-h period. All behavioral observations were carried out by trained observers blind to the treatment of piglets. Preobservations of this simple and well-defined behavioral pattern revealed a high intra-observer consistency of more than 95%.

Blood samples were taken while piglets were in a supine position by *anterior vena cava* puncture (the whole procedure lasted approximately 30 s) immediately before LPS or saline injection (0 h, baseline for all measures), 1 h later for analysis of TNF-α and 3 h later for analyses of ACTH, cortisol, CBG and IgG. These time points were identified with the strongest amplitude of peripheral TNF-α and hormone levels in responses to LPS in piglets (Kanitz et al., 2002). EDTA blood samples were centrifuged at 2000×*g* for 15 min at 4°C to separate plasma. Whole blood samples were allowed to clot for 4 h at room temperature and centrifuged at 1000×*g* for 15 min at 4°C to obtain serum. Plasma and serum were then stored at −20°C until analysis.

The procedures referring to animal treatments were approved by the Committee on Animal Care and Use of the Agricultural Department of Mecklenburg-Vorpommern, Germany.

Hormone analyses

Plasma ACTH concentrations were determined by an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, USA). The intra- and inter-assay coefficients of variation were 3.2% and 7.8%, respectively. The assay sensitivity was 1.6 pg/ml.

Plasma cortisol levels were obtained using a commercially available radioimmunoassay kit (DRG International, Inc., USA) with a highly specific cortisol antibody and a detection threshold of 0.3 μg/100 ml. The intra- and inter-assay coefficients of variation were 5.3% and 9.8%, respectively.

Assay for corticosteroid binding globulin (CBG)

Plasma samples were examined for CBG using a modified binding assay previously described by Kanitz et al. (2002). Briefly, 25 μl blood plasma was incubated with 0.78 nM unlabeled cortisol (Hydrocortisone, Merck, Darmstadt, Germany) and 25 pM ^{3}H-cortisol (specific radioactivity 68 Ci/mmol, Amersham Pharmacia Biotech, Freiburg, Germany). Non-specific binding was determined in parallel using a 100-fold excess of cold cortisol. The separation of bound and free ^{3}H-cortisol was performed by precipitation with dextran-coated charcoal at 4°C and subsequent centrifugation at 1000×*g* for 10 min. The intra- and inter-assay coefficients of variation were 7.8% and 9.1%, respectively.

Assay for TNF-α and IgG

TNF-α concentrations were analyzed in plasma samples using commercially available pig ELISA kits (Biosource, Nivelles, Belgium) according to the manufacturer's instruction. The sensitivity of the assay was 6 pg/ml and intra- and inter-assay coefficients of variation were 6.4% and 9.0%, respectively.

Total serum IgG concentrations were determined with a modified indirect competitive ELISA based on the method described by Morrow-Tesch et al. (1994) using a rabbit anti-pig IgG antibody (Sigma-Aldrich, St. Louis, USA). The intra- and inter-assay variation coefficients were 4.1% and 7.7%, respectively.

Statistical analysis

Statistical analysis was performed using the SAS System for Windows (SAS Institute Inc., 2004). Initially, an analysis of variance (ANOVA) was done using the GLM procedure for the behavioral measurements and physiological responses to LPS or saline. The individual physiological responses were calculated by subtracting the baseline before injection (0 h) from the measures 1 h after injection (TNF-α) or 3 h after injection (ACTH, cortisol, CBG and IgG). The ANOVA model for physiological and behavioral data (except for suckling behavior) included the fixed classification variables social treatment (isolation and control), injection (LPS and Saline), age (test days 12 and 56) and the random variable sow nested within social treatment. The model for suckling behavior is equal to the model above, but without an age effect. A classification variable sex was not included in the model because sex had no significant effect ($P > 0.10$) for all traits investigated. The main effects and their appropriate interactions were tested (ANOVA *F*-tests). Additionally, least squares means (LS means) and standard errors (SE) were computed for each effect in the model and all pairwise differences of these LS means were tested by a post hoc test using the Tukey–Kramer procedure.

Relationships between the signs of sickness behavior and physiological measures were calculated for LPS-induced piglets by a multiple linear regression analyses using PROC REG. Initially, the best model was fitted by stepwise

Table 1
Behavior after LPS or saline injection and signs of sickness after LPS injection in isolated and control piglets on days 12 and 56

	Day 12				Day 56			
	LPS		Saline		LPS		Saline	
	Isolated	Control	Isolated	Control	Isolated	Control	Isolated	Control
Locomotor activity								
Duration (min/1 h *)	15.1±2.1	10.4±2.1	12.6±2.1^{a}	16.9±2.2	18.2±2.1	15.2±2.1	24.3±2.1^{a}	22.1±2.1
Duration (min/3 h)	16.9±2.7	12.3±2.7^{A}	28.0±2.8^{a}	34.2±2.8^{A,b}	19.4±2.7^{B}	16.2±2.7^{C}	40.9±2.8^{a,B}	46.1±2.8^{b,C}
Suckling behavior								
Duration (min/3 h)	8.0±1.0^{A}	9.5±1.0^{B}	13.6±1.0^{A}	16.2±1.0^{B}	–	–	–	–
Latency (min)	44.2±2.9^{a,A}	31.8±2.9^{a,b}	21.3±3.0^{A}	17.3±3.0^{b}	–	–	–	–
Somnolence								
Duration (min/3 h)	59.4±5.8^{a}	73.3±5.8	0.0±0.0	0.0±0.0	80.5±5.8^{a}	84.4±5.8	0.0±0.0	0.0±0.0
Latency (min)	70.1±5.8	65.5±5.4	0.0±0.0	0.0±0.0	62.1±5.2	50.0±5.1	0.0±0.0	0.0±0.0
Shivering								
Duration (min/3 h)	47.7±4.9^{A}	19.6±4.9^{A,a}	0.0±0.0	0.0±0.0	53.0±5.0	49.7±5.0^{a}	0.0±0.0	0.0±0.0
Latency (min)	60.4±3.4	55.6±3.5^{A}	0.0±0.0	0.0±0.0	35.1±3.1	34.4±3.1^{A}	0.0±0.0	0.0±0.0
Vomiting								
Duration (min/3 h)	0.8±0.3^{B}	1.0±0.3	0.0±0.0	0.0±0.0	3.4±0.3^{A,B}	1.5±0.3^{A}	0.0±0.0	0.0±0.0
Latency (min)	78.1±10.3	94.6±12.0^{a}	0.0±0.0	0.0±0.0	49.6±5.3	50.6±6.7^{a}	0.0±0.0	0.0±0.0

Data are expressed as LS-means±SE ($n = 18$ per group).
Significant differences are indicated by equal superscripts within a row (equal small letters: $P < 0.05$; equal capital letters: $P < 0.01$).
* First third of the 3-h observation period.

model selection in PROC REG, i.e., those physiological variables with the highest impact on signs of sickness behavior were selected.

Effects and differences were considered significant if $P \leq 0.05$.

Results

Effects of social isolation and LPS on behavior and signs of sickness

LS means and standard errors of behavioral responses and signs of sickness during the 3-h period after LPS or saline injection in isolated and control piglets on days 12 and 56 are presented in Table 1.

ANOVA indicated that all behavioral parameters and signs of sickness were significantly influenced by injection (LPS vs. saline; all P values < 0.001) and age (day 12 vs. day 56; at least $P < 0.05$). Whereas the locomotor activity was not significantly affected by social treatment ($F(1,116) = 0.19$, $P = 0.66$), ANOVA revealed a significant main effect of social treatment on duration $F(1,50) = 3.96$, $P < 0.05$) and latency of suckling behavior ($F(1,50) = 7.63$, $P < 0.01$). On day 56 feed intake was not observed.

As expected, only piglets with LPS injections developed signs of sickness, whereas saline-injected piglets did not exhibit any sickness responses (shown as 0±0 in Table 1).

There was no significant influence of social treatment neither on duration of somnolence ($F(1,116) = 0.60$, $P = 0.44$) nor on latencies for displaying a somnolent state ($F(1,49) = 2.43$, $P = 0.13$). However, ANOVA revealed a significant main effect of social treatment on the duration of shivering ($F(1,116) = 3.89$, $P < 0.05$) and vomiting ($F(1,116) = 3.74$, $P < 0.05$). The latencies to shivering ($F(1,47) = 0.73$, $P = 0.39$) and to vomiting ($F(1,19) = 0.93$, $P = 0.35$) were not significantly affected by social treatment.

Statistical analysis revealed a significant social treatment × injection interaction effect on locomotor activity ($F(1,116) = 5.80$, $P < 0.05$). All other tested interactions were non-significant. Isolated piglets showed a higher locomotor activity after LPS and a lower activity after saline compared to LPS or saline injected control piglets (3-h observation period). But post hoc test (Tukey–Kramer) failed to reach significance between different social treated piglets in both LPS ($t = 1.98$, $P = 0.20$) and saline ($t = 2.73$, $P = 0.12$) induced animals.

Remarkably, the locomotor activity in LPS-injected piglets occurred mainly in the first third of the 3-h observation period on day 12 (isolated: 89%, control: 85%) and day 56 (isolated: 94%, control: 94%), whereas locomotor activity in saline-injected piglets was clearly lower during the first hour after injection on day 12 (isolated: 45%, control: 49%) and day 56 (isolated: 59%, control: 48%) (see Table 1).

Pairwise comparisons (Tukey–Kramer procedure) of the LS means of LPS injected animals (Table 1) indicated that the latency to suckling was significantly longer in isolated piglets compared to controls ($t = 3.01$, $P < 0.05$). Furthermore, the duration of shivering in isolated piglets was significantly increased compared to control animals on day 12 ($t = 3.94$, $P < 0.01$), whereas vomiting in isolated piglets reached significant higher values on day 56 ($t = 4.36$, $P < 0.01$).

Effects of social isolation and LPS on endocrine and immune changes

Fig. 1 shows the LS means and standard errors of changes in plasma ACTH (Fig. 1A), cortisol (Fig. 1B) and CBG (Fig. 1C) concentrations of both social treatment groups on days 12 and 56. ANOVA indicated that LPS induced significant changes in all investigated endocrine measures compared to

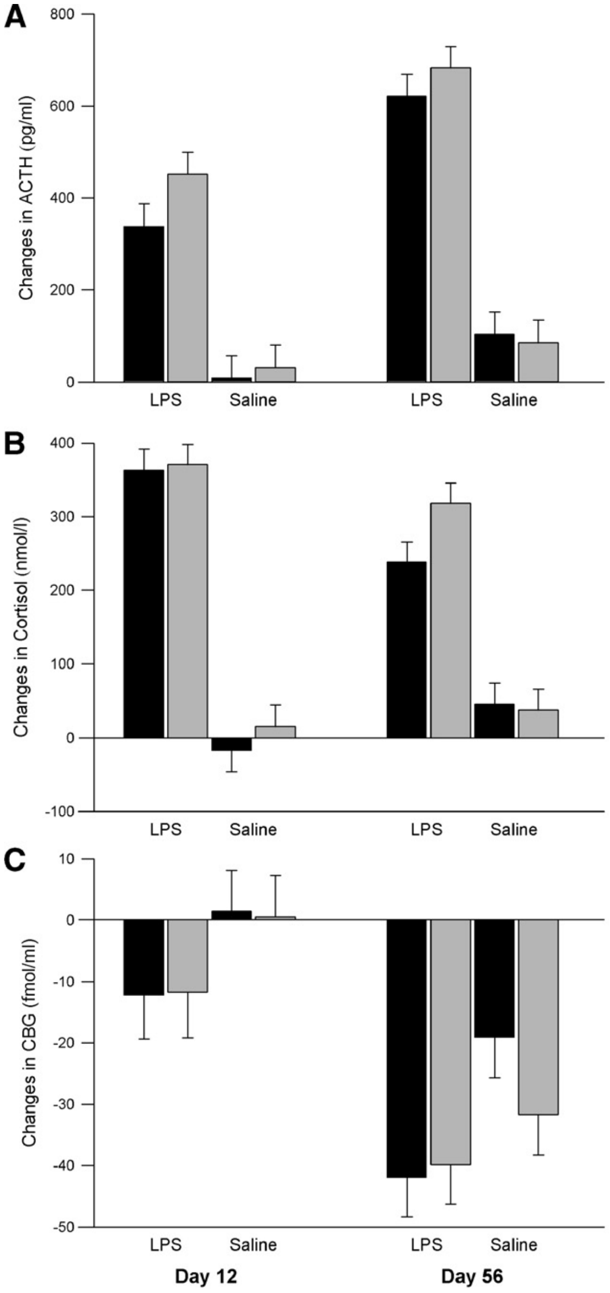

Fig. 1. Temporal changes in plasma ACTH (A), cortisol (B) and CBG (C) concentrations in isolated (black bars) and control (grey bars) piglets on days 12 and 56 (LS means±SE; before and 3 h after LPS or saline injection).

saline (at least $P<0.01$). However, there was no significant effect of social treatment on stress hormone increases (ACTH: $F(1,110)=1.66$, $P=0.20$; cortisol: $F(1,114)=1.84$, $P=0.18$) and CBG decreases ($F(1,108)=0.33$, $P=0.56$). Changes of ACTH ($F(1,110)=23.47$, $P<0.001$) and CBG ($F(1,108)=33.58$, $P<0.001$) were mainly affected by the factor age of piglets. In contrast, increases of cortisol were not influenced by age ($F(1,114)=1.28$, $P=0.26$).

ANOVA revealed significant main effects of injection ($F(1,64)=59.22$, $P<0.001$) and age ($F(1,64)=35.59$, $P<0.001$) on enhancement of plasma TNF-α concentration 1 h after LPS. Considering only LPS-induced piglets, ANOVA indicated a main effect of social treatment ($F(1,44)=8.22$, $P<0.01$) on increases of TNF-α levels. The Tukey–Kramer procedure showed that the TNF-α increase was significantly lower in socially isolated piglets compared to control animals on day 56 ($t=3.64$, $P<0.01$, Fig. 2A).

Statistical analysis revealed significant main effects of injection ($F(1,116)=3.93$, $P<0.05$) and social treatment ($F(1,116)=5.85$, $P<0.05$) on changes of IgG concentrations, but no age effect ($F(1,116)=0.01$, $P=0.9$). On day 12 the decrease of IgG concentration in LPS-induced isolated piglets tended to be higher compared with controls, but failed to reach statistical significance (Tukey–Kramer: $t=2.78$, $P=0.10$, Fig. 2B).

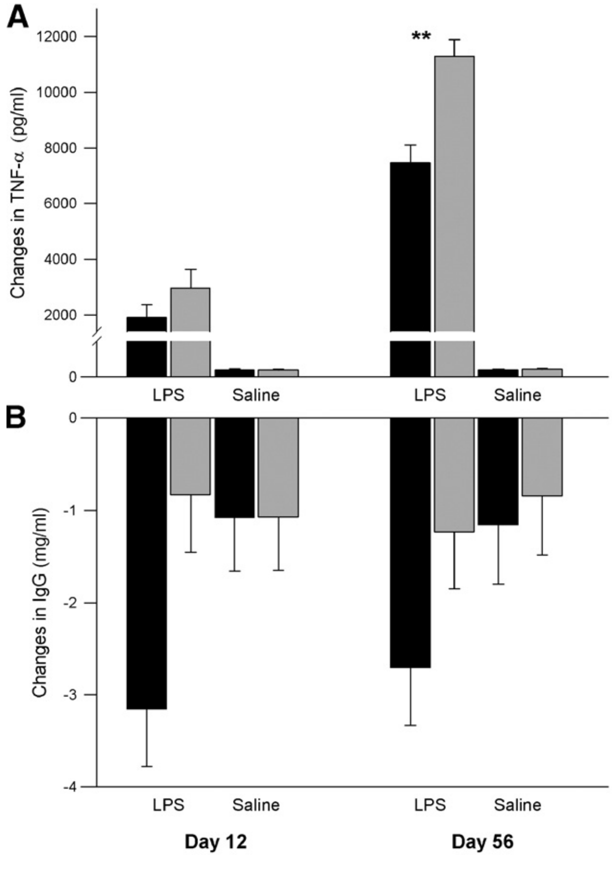

Fig. 2. Temporal changes in plasma TNF-α (A) and IgG (B) concentrations in isolated (black bars) and control (grey bars) piglets on days 12 and 56 (LS means ± SE; before and 1 h (TNF-α) or 3 h (IgG) after LPS or saline injection; significant difference: $^{**}P<0.01$).

Relationship between signs of sickness and physiological measures in LPS-induced piglets

The regression analyses showed that the duration of somnolence in isolated piglets was interrelated to changes of plasma TNF-α and cortisol concentrations (Fig. 3A). Higher duration of somnolence was related to higher increases of TNF-α and simultaneously lower cortisol increases ($R^2=0.40$, $P<0.001$). In control piglets these relations were absent ($R^2=0.03$, $P=0.59$). The highest impact on duration of shivering in isolated piglets was calculated for changes of both cortisol and CBG concentrations (Fig. 3B). Here, longer shivering was related to higher increases of cortisol and lower decreases of CBG levels ($R^2=0.22$, $P<0.05$). In contrast, changes of cortisol and CBG in control piglets did not significantly affect shivering ($R^2=0.01$, $P=0.85$).

Discussion

The results of the present study demonstrate that the process of repeated early social isolation can affect behavioral and physiological responses to an acute endotoxin challenge in piglets. The prior social isolation impaired suckling behavior, enhanced signs of sickness, decreased serum IgG content and diminished cytokine response to LPS.

The behavioral and physiological responses following infection or immune challenge are important mechanisms for maintaining homeostasis and promoting recovery (Johnson et al., 1996). A commonly used methodology for modeling bacterial infection is peripheral administration of endotoxin LPS (Tilders et al., 1994), also inducing behavioral symptoms of sickness (Dantzer et al., 1998).

In the present study, pigs were injected i.p. with a dose of 100 μg LPS/kg body weight which caused serious sickness behavior, characterized by somnolence, shivering and vomiting as well as reduced locomotor activity and suckling behavior. As expected, LPS provoked a profound increase in circulating TNF-α concentrations and an enhancement of plasma ACTH and cortisol levels. These findings confirm that LPS administration in pigs evokes a multitude of sickness symptoms (Johnson and von Borell, 1994; Kanitz et al., 2002), activation of HPA axis (Warren et al., 1997; Wright et al., 2000) and synthesis and release of proinflammatory cytokines (Warren et al., 1997; Matteri et al., 1998; Sakumoto et al., 2003; Tuchscherer et al., 2004). Furthermore, the acute challenge with LPS in our experiment resulted in a decrease of plasma CBG levels and reduction of IgG contents within 3 h after application. These data are comparable to previous studies showing decreased CBG and IgG levels following inflammation or septic shock (Shanks et al., 1999; Beishuizen et al., 2001; Kanitz et al., 2002). However, in the present study, the sickness and physiological responses to an acute LPS administration were found to be dependent on age. Older piglets displayed longer duration of somnolence, shivering and vomiting and responded with greater increases of ACTH and TNF-α concentrations as well as greater decreases of CBG. This

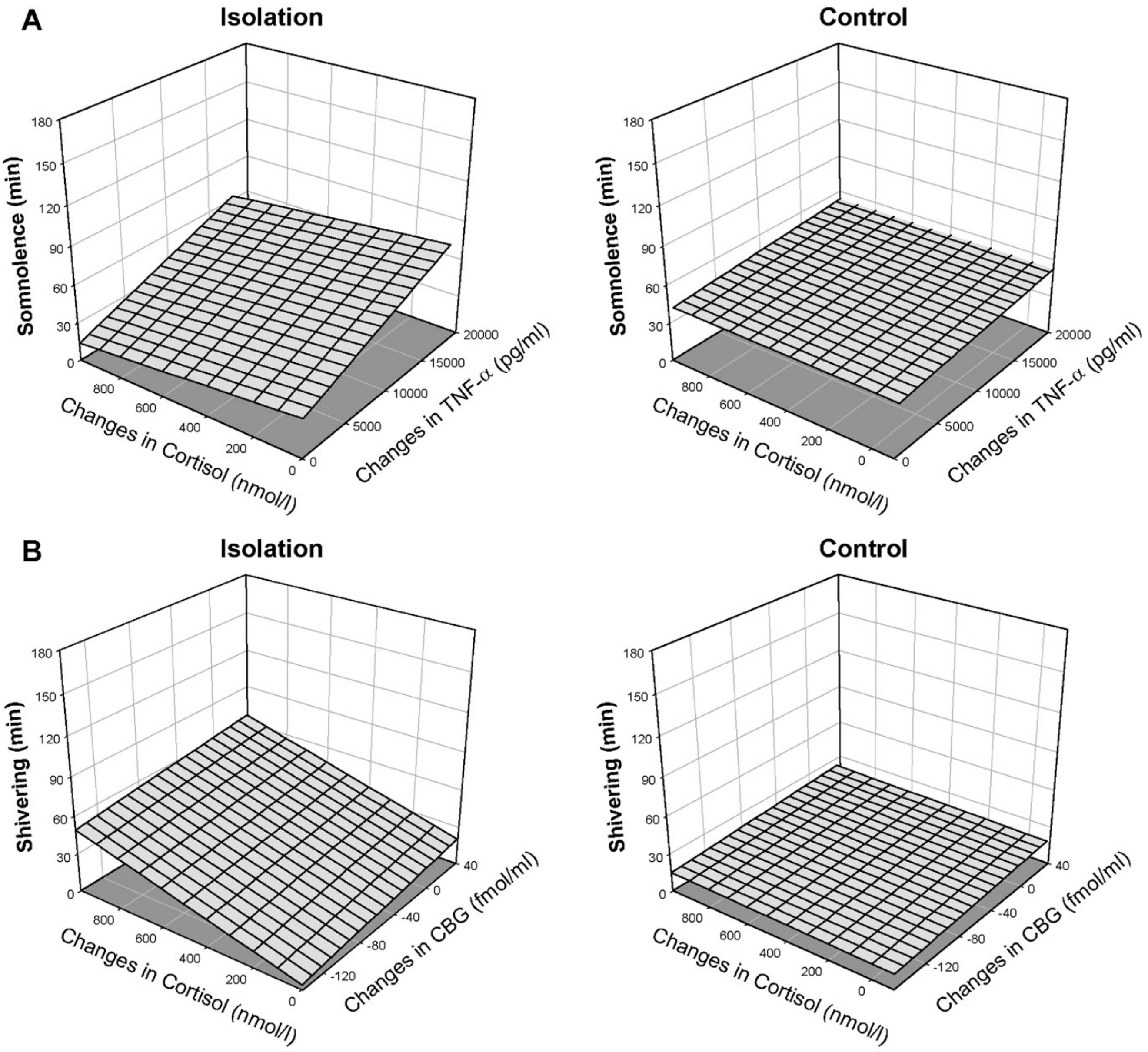

Fig. 3. Relationship between duration of somnolence and changes of plasma cortisol and TNF-α concentrations (A) in isolated and control piglets within 3 h after LPS (regression response surface: somnolence (isolation) = 29.827 + 0.003*ΔTNF-α − 0.017*Δcortisol; somnolence (control) = 39.072 + 0.001*ΔTNF-α + 0.001*Δcortisol) and relationship between duration of shivering and changes of plasma cortisol and CBG concentrations (B) in isolated and control piglets within 3 h after LPS (regression response surface: shivering (isolation) = 17.048 + 0.096*ΔCBG + 0.046*Δcortisol; shivering (control) = 19.504 + 0.048*ΔCBG + 0.003*Δcortisol).

fact may be due to different developmental stages of the neuroendocrine and immune function in piglets at 12 days of age compared with piglets at 56 days of age (Becker and Misfeldt, 1993; Schwager and Schulze, 1997; Matteri et al., 1998). Although in pigs all components of the native and acquired immune systems are functional at birth, they are generally less efficient than in the adult (Salmon, 1984; Hammerberg et al., 1989). To date, only very few data are available on proinflammatory and behavioral responses to an LPS challenge in neonatal pigs (Klir et al., 1997; Matteri et al., 1998; Kanitz et al., 2002). In pigs, the monocytes and macrophages which primarily produce proinflammatory cytokines appeared to be fully functional from time of birth (Schwager and Schulze, 1997), and recently it has been shown that cytokine production of T lymphocytes in pigs was moderately consistent measured at 2, 5 and 8 weeks of age (De Groot et al., 2005). Unlike lymphocytes, our results indicate that a dramatic increase in LPS-induced TNF-α production of myeloid cells occurs in pigs between 12 and 56 days of age.

In the present study, we found an influence of prior social isolation on LPS-induced behavioral and physiological responses. Early isolated piglets responded to LPS by a significant increase of shivering on day 12 and by increased vomiting on day 56 compared to non-isolated controls. This higher intensity of sickness symptoms could indicate the severity of illness (Johnson and von Borell, 1994; Klir et al., 1997). Certain reflexes and more complex behavior, such as shivering, piloerection and taking a hunched posture apparently serve a thermogenic function (Hennessy et al., 2001). As recently shown, rats and birds developed a short-term hypothermia (2 h) in response to endotoxin (Fraifeld and Kaplanski, 1998; Owen-Ashley et al., 2006). Therefore, the increased shivering in isolated piglets in this study may be a sign of a mild hypothermia.

Further, the time isolated piglets spent in locomotor activity was slightly increased in response to LPS, whereas activity after saline was decreased. Generally, reduced activity, exploration and social interactions, and increased sleepiness are considered as a strategy of energy conservation in order to fight against infection (Hart, 1988; Aubert, 1999). The increased locomotor activity of isolated piglets in the present study was unexpected and could denote alterations in behavioral responses to an immune challenge provoked by early stress experience. However, similar findings in pigs were reported by Sakumoto et al. (2003). An intravenous treatment with LPS resulted in an increased behavioral standing rate and a decrease in lying rate. In our study, the locomotor activity in LPS-injected piglets occurred mainly within the first hour following injection, indicating that the decrease in locomotion may take some time to develop as a non-specific sickness symptom. In addition, from studies in rodents and monkeys, it is known that their infants show a biphasic behavioral response to maternal separation. After an initial period of intense vocalization and locomotor activity, the passive behavioral patterns of the second stage of separation represent stress-induced sickness behavior (Hofer, 1996; Hennessy et al., 2001; Hennessy and Morris, 2005).

LPS is a potent inhibitor of feed-motivated behavior in pigs (Johnson and von Borell, 1994; Sakumoto et al., 2003). The prior isolation treatment in our study impaired the suckling behavior of piglets at 12 days of age. The delay and reduction in time early isolated piglets spent suckling and massaging the udder may reflect a stronger disturbance of feed-motivated behavior (Johnson and von Borell, 1994).

In addition, the LPS-associated increases in activity of the HPA axis and the decreases of CBG concentration did not differ between social isolated and non-isolated piglets. The secretion of glucocorticoids is part of a negative feedback loop that regulates the immune system to prevent it from overreacting (Johnson et al., 1996; Warren et al., 1997; Kanitz et al., 2002). It is well known from studies in rodents that exposure to stressors alters the subsequent responsiveness of a variety of systems and therefore may represent an important factor in the modulation of adaptational reactions to an immune challenge (Quan et al., 2001; Carobrez et al., 2002; Johnson et al., 2002). Although, numerous studies in rodents have demonstrated that repeated periods of maternal separation in early life enhances HPA responses to different stressors (Plotsky and Meaney, 1993; Liu et al., 2000; Avitsur et al., 2006), recently it was shown in rats and also in pigs, that social stress experience did not necessarily affect the activity of the HPA axis after LPS injection (Carobrez et al., 2002; Tuchscherer et al., 2004). The present results of stress hormone changes are consistent with this finding. Obviously, the responsiveness of the HPA axis to stressors later in life may differ depending on developmental stage of the HPA axis during separation (Meaney et al., 1985; Kattesh et al., 1990) and on the specific nature of the stimulus following the maternal separation procedure.

However, earlier investigations in animal separation models suggest that it is more likely that alterations of the HPA axis activity occur at the higher organizational level as indicated by long-term effects on glucocorticoid receptor and corticotropin-releasing hormone regulation in the brain after social stress (Ladd et al., 2000; Buwalda et al., 2001; Kanitz et al., 2004). Furthermore, with respect to the HPA axis it is also accepted that decreases of CBG serve to increase levels of biologically available cortisol in an effort to combat immune challenge (Savu et al., 1981; Beishuizen et al., 2001) or stress (Fleshner et al., 1995; Tinnikov, 1999; Heo et al., 2005). Therefore, independent of the function of corticosteroids the consideration of CBG is crucial to understanding the control of the behavioral and physiological responses to stress (Breuner and Orchinik, 2002).

The behavioral and physiological effects of LPS have been attributed to the release of proinflammatory cytokines (Kelley et al., 1994; Matteri et al., 1998). Previous studies suggest that TNF-α is an important component of the acute phase response to infection and affects sickness behavior in pigs (Warren et al., 1997; Abraham et al., 1998; Balaji et al., 2002; Sakumoto et al., 2003). In our study, the increases of circulating TNF-α concentrations 1 h after LPS administration were diminished by the prior social isolation.

Contrary to our result, a recent study using separation model in mouse pups showed increased lung cytokine responses to an influenza viral infection of adults neonatally stressed by repeated separation (Avitsur et al., 2006). It seems that repeated social stress in early life may cause a dysregulation in proinflammatory responses to an immune stimulus later in life. Therefore, alteration in the secretion of these cytokines can be disadvantageous for host's recovery from infectious or immune challenge.

There is growing interest in the relationship between behavior and physiological parameters to characterize different adaptive capacity (Barnard et al., 1998). In the present study, the relationships between signs of sickness and physiological measures were stronger in early isolated piglets compared to controls. Interestingly, although LPS-induced cortisol increases were not significantly affected by social treatment, the calculation of relationship between changes of cortisol and TNF-α concentrations was found with the highest impact on duration of somnolence in isolated piglets. The increase in plasma cortisol was linked with a reduction of LPS-induced somnolence, whereas a rise in TNF-α was associated with increased somnolence. As shown in rabbits, TNF-α induces dose-dependent increases in slow-wave sleep (Krueger et al., 2003). Thus, our results in pigs confirm the important role of TNF-α in modulating of somnolence. Furthermore, there was a clear relationship between changes in plasma cortisol and CBG concentrations and displaying shivering in early isolated piglets. Higher increases of cortisol and smaller decreases of CBG were related to enhanced shivering reflecting a pronounced relation of biologically active cortisol on this energy costly sickness sign. In addition to cytokines glucocorticoids represent another class of key molecules in the regulation of sickness behavior (Dantzer, 2004). The present results demonstrate that glucocorticoids may differently affect distinct signs of sickness.

Recently, it has been suggested that social stress in form of maternal separation may also induce sickness behavior as well as aspects of the acute phase response (Hennessy et al., 2001). Moreover, there are some indications that the brain cytokine system can become sensitized in response to environmental stressors (Tilders and Schmidt, 1999) or to psychosocial stress during early development (Kanitz et al., 2004). Therefore, the early stress experience of piglets in our study may have altered their responses to an acute LPS challenge by a certain sensitization of the immune and endocrine system. This stress-induced sensitization may be expressed by the stronger relationship between behavioral and physiological responses to the immune challenge.

In summary, the present results indicate that social isolation of neonatal pigs may persistently alter sickness behavior as well as behavioral and physiological responses to an acute endotoxin challenge. Furthermore, the findings emphasize the important role of behavioral responses to an immune stimulus for maintaining homeostasis. Taking into consideration similarities between stress-induced and infection-induced mechanisms of coping, our results suggest a brain-mediated sensitization of adaptive responses by early isolation treatment. Thus, the primary characterization of relationship between behavior and physiological measures has important pathophysiological implications and may helpful to improve our understanding of sickness behavior and animal welfare.

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APPLIED ANIMAL
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Effects of familiarity and relatedness on agonistic pair relationships in newly mixed domestic pigs

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Abstract

The effects of familiarity and relatedness on agonistic pair relationships (dyads) in different pen regions (pen area or trough area) were studied in 16 groups of newly mixed domestic pigs of similar weight (9 pigs per group) at an age of 12 weeks. The agonistic interactions (AI) within 124 familiar (related) and 452 unfamiliar (related and unrelated) dyads were continuously recorded for 3 days (10 h daily) after mixing. Whereas pigs, both in dyads familiar and unfamiliar to each other, showed the same frequency of AI in the trough area, unfamiliar dyads exhibited significantly more AI in the pen area than familiar dyads. The relatedness of unfamiliar dyads had apparently no influence on AI. It is discussed that, besides establishing a dominance hierarchy, pigs react aggressively on strange subjects. Furthermore, the results are briefly discussed with reference to dominance and resource usage in pigs. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Pig agonistic behaviour; Relatedness; Familiarity; Pair relationships; Dominance

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1. Introduction

One source of social stress in pigs is fighting that occurs when strange conspecifics are mixed for the first time, and this overt aggression may have vast undesirable consequences (e.g., reviewed by Petherick and Blackshaw, 1987). Fighting behaviour between two strange animals is partly motivated by uncertainty about their relative

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fighting ability (Enquist and Leimar, 1983; Rushen, 1990). This uncertainty should decrease when the weight is very dissimilar (Rushen, 1987; Rushen and Pajor, 1987) or, obviously more important, when the dyad members are acquainted to each other (Fraser, 1974).

In the past, aggressive behaviour of pigs has often been interpreted in terms of dominance hierarchies (e.g., Ewbank, 1976). As dominance is an expression of the relationship between two individuals, the minimal unit to which the concept applies is the dyad (Capitanio, 1991). It is well known that domestic pigs are able to establish a dominance hierarchy (e.g., Beilharz and Cox, 1967; Meese and Ewbank, 1973) based on bi-directional agonistic interactions (Puppe and Tuchscherer, 1993, 1994). Although the common practice in pig husbandry generates groups consisting of dyads familiar as well as unfamiliar to each other, it seems, however, that most studies of aggression and dominance mainly have dealt only with the individual or the group level of dominance (Puppe, 1996).

In the last years evidence from both theoretical and empirical studies has accumulated showing that the useful application of principles from behavioural biology and sociobiology (e.g., resource defence theory) could help in explaining conflict and cooperation (see Fraser et al., 1995). The authors conclude that aggression involved in establishing social dominance is more likely to be influenced by manipulating traits of the competing animals (e.g., competitive ability, familiarity) rather than by the ability to defend resources. Therefore, it should be expected that in a group of domestic pigs the effect of familiarity on agonistic behaviour is stronger in fights immediately related to the clarification of social relationships than in fights related to the usage of food around the trough.

Additionally, under farming conditions, domestic pigs within a social group can be confronted with conspecifics which are characterised not only by a different degree of familiarity but also of relatedness. Under such circumstances the question arises whether or not pigs are able to recognise related companions (e.g., half-siblings) without any prior experience of them, and to react with a decreasing degree of agonistic interactions (for such an example in mice, see Kareem and Barnard, 1982). Hence, the purpose of this study was to investigate the behaviour of dyads of newly mixed pigs as a crucial level of a social organisation, to elucidate the effects of familiarity (familiar or unfamiliar) and, in a preliminary study, of relatedness (unfamiliar related or unrelated) on agonistic pair relationships in different pen regions.

2. Material and methods

2.1. Animals and experimental procedure

The study was carried out using 16 newly mixed groups, each consisting of nine 12 week old German Landrace pigs (34.7 kg, SD $= \pm 3.3$ kg, $n = 144$; within-group-SD $= \pm 2.4$ kg, $N = 16$). Two groups were simultaneously observed (one observer per group) for three days in eight successive trials (2×8). The two observers were randomly assigned to the groups.

The groups were put together by random selection from a pool of 48 littermate groups (6 groups per trial). The litters were weaned at an age of 5 weeks by the removal of the mother. The lightest and heaviest pigs were sorted out, leaving 4 pigs with weights as similar as possible in each group. This design was chosen to create the optimum environment for overt aggression, since wide variations in weight have been shown to reduce the amount of time pigs spend fighting (Rushen, 1987; Francis et al., 1996).

Using 144 animals in 16 groups, 576 pair relationships are possible (36 per group). Of these, 124 pairs (21.5%) were familiar and 452 pairs (78.5%) were unfamiliar to each other. All opponents of the familiar dyads were related to each other through their parents (brothers and sisters). The unfamiliar dyad members were related through their father (half-siblings) and/or their grandparents (55.8%), or they were unrelated to each other (44.2%). The groups were kept on concrete floor (6.5 m^2) with little straw. There were limited feeding times from 0900 to 1100 and 1500 to 1700 h (4 h daily), during which the animals had the only access to the trough (animal to feeding place ratio 2.25:1 with sufficient food for all pigs). Animals were given ad libitum access to water. The trough was located along one side of the pen and was closed with a grating between the feeding times. The area up to 1 m right in front of the feeder was regarded as ‘trough area’, the remaining place was called ‘pen area’.

2.2. Agonistic behaviour

Immediately after mixing, the number of overt agonistic interactions of all dyads ($N = 576$) in each group was recorded by direct observation for 3 days (10 h daily from 0800 to 1800 h, continuous event sampling). A dyad agonistic interaction (AI) was defined as a fight or a displacement event with physical contact of two individuals for more than 2 s and intervening periods of at least 8 s, while the fight either was interrupted or the pigs showed other behaviours. Similar to other investigations fights occurred as overt bodily attacks like ‘head to head knocks’ and ‘head to body knocks’, ‘parallel/inverse parallel pressings’, ‘bitings’, and ‘displacements’ displayed by the piglets in the pen area or in the trough area (for more details on agonistic behaviour in pigs, see Jensen, 1982; McGlone, 1985; Rushen and Pajor, 1987; Rushen, 1988).

2.3. Statistical analysis

Analyses of data were performed by using the SAS system (SAS, 1989). Differences of AI regarding familiarity (familiar, unfamiliar) or relatedness (related, unrelated) were tested by Nonparametric Anova (Wilcoxon 2-Sample Test). Prior to this, AI-data for the pen and trough area were corrected by eliminating the effects of trial (8) and the nested effects of group in each trial (2).

3. Results

Altogether, 5354 AI were observed after mixing, 3639 AI in the pen area and 1715 AI in the trough area. At least one fight was observed in the majority of all unfamiliar

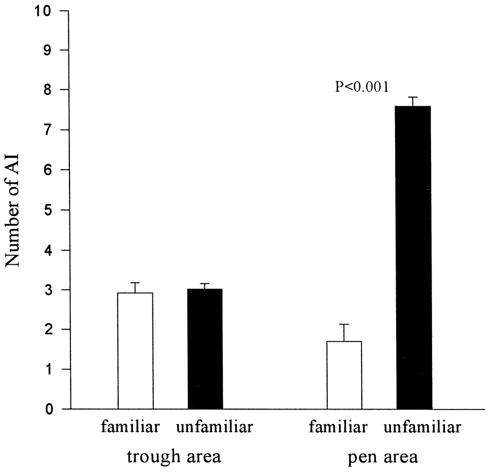

Fig. 1. Number of dyad agonistic agonistic interactions (AI) in the trough area and in the pen area of familiar and unfamiliar pairs (lsmeans + SE).

and familiar dyads in the trough area (80.6% and 80.5%, respectively). In the pen area, however, significantly more unfamiliar dyads displayed at least one fight (96.2% vs. 68.5%, $\chi^2 = 85.0$, $p < 0.001$).

Whereas no differences in the number of AI were found between familiar and unfamiliar dyads in the trough area, pigs of unfamiliar dyads fought significantly more in the pen area ($z = 12.6$, $p < 0.001$) than pigs in familiar dyads (Fig. 1).

In any case ($p > 0.05$) the relatedness (related vs. unrelated dyads) in unfamiliar dyads did not influence the number of observed AI (Fig. 2).

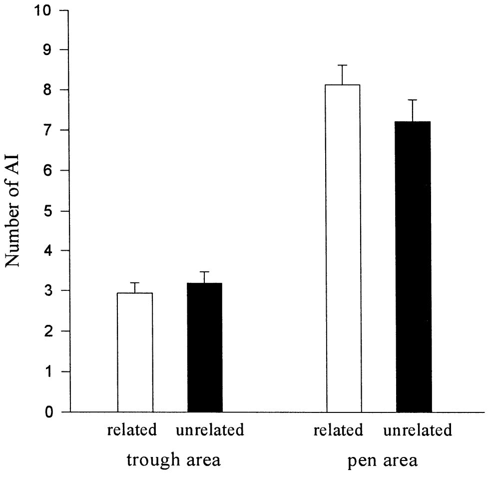

Fig. 2. Number of unfamiliar dyad agonistic agonistic interactions (AI) in the trough area and in the pen area of related and unrelated pairs (lsmeans + SE).

4. Discussion

Although social pair relationships can be described by several behavioural measures studies that quantify and qualify pair relationships, especially the dyad-level dominance in pigs, are almost missing. The present work presents an attempt to analyse pair relationships based on the level of agonistic interactions in newly mixed groups of domestic pigs in dependence on the familiarity or relatedness of the dyad members and on the observed pen region where the fights occurred.

The results show different reactions of the pigs if we regard the area where the encounters occurred. Pigs in unfamiliar dyads fight significantly more in the pen area than dyad members familiar to each other. It is well known that pigs unfamiliar to each other show more aggression than familiar ones (Arey and Franklin, 1995; Ekkel et al., 1997; Puppe et al., 1997; Otten et al., 1997). A possible and common explanation is that unfamiliar pigs fight to establish a dominance hierarchy, and this hierarchy is then used to regulate the pigs' access to resources. Both the position in a hierarchy (Schnebel and Griswold, 1983) and the access to food (McGlone, 1986) may be viewed as resources which are limited and defendable. Hence, the increased agonistic interactions of unfamiliar dyads in the pen area observed in our study could possibly be used by the pigs to clarify agonistic dominance relationships, and subsequently, to regulate future relationships including the control of resources.

However, an alternative explanation is that agonistic interactions may be triggered by unfamiliarity alone and the real goal could be to drive away a stranger. Other recent studies have demonstrated that elevated levels of fighting in unfamiliar mixed pigs can be found some days (Puppe et al., 1997) or even many weeks (Ekkel et al., 1997) after mixing. The work by Stolba and Wood-Gush (1984) has shown that in an outdoor environment only familiar pigs tended to form subgroups, whereas the unfamiliar pigs maintained separate sleeping areas for over 6 months. Generally, the strongest social relationships were found in littermates (Newberry and Wood-Gush, 1986; Petersen et al., 1989). These findings and the data of agonistic behaviour in the present study support the hypothesis that, besides establishing a dominance hierarchy, pigs react aggressively on strange subjects. The motivation behind this fighting may lie in the uncertainty about relative fighting abilities of the unfamiliar animals as a special form of curiosity and social exploration behaviour (see Rushen, 1990).

The present data also show that unfamiliarity causes no differences in the number of agonistic interactions for food competition. This may be explained by the fact that the motivation for fighting in the trough area is very different from that in the pen area. The possession of the limited feeding place including food intake and holding that position seems to be more important than to react on strangers or to establish a dominance hierarchy. For example, subordinate pigs initiate a considerable amount of aggression against dominant pigs to defend their position at the feeding trough (Baxter, 1989).

Only in the unfamiliar dyads, the experimental design in the present study allowed a differentiation between 'related' and 'unrelated' individuals. However, the agonistic behaviour in these dyads was unaffected by relatedness. Similarly, Appleby (1983) found that the frequency of agonistic interaction in red steer stags was the same between related and unrelated animals, whereas the degree of relatedness in mice leads to different results in social interactions (Kareem and Barnard, 1982) or fitness-related scores (König, 1994).

How individuals classify their conspecifics as kin can be achieved in a number of ways (e.g., reviewed by Hepper, 1986). The present results give rise to the supposition that there is no a priori knowledge on kin in growing pigs unfamiliar to each other (e.g., half-siblings or distantly related animals). A learning process associating conspecific cues seems to be present, enabling the pigs to discriminate other animals during agonistic interactions, i.e., pigs may use familiarity with conspecifics as a guide to their probable degree of relatedness (see Bekoff, 1981). Since pigs have a highly developed olfactory system the use of olfactory cues is not unlikely. Accordingly, chemical signals (e.g., androstenone) are able to modulate the aggressive behaviour and the development of dominance relationships in pigs (McGlone, 1990).

In summary, the present study supports evidence that, in pigs, familiarity affects only the agonistic pair relationships in the pen area and not in the through area. There was no indication that the relatedness of unfamiliar dyads has an influence on the number of agonistic interactions.

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LIVESTOCK
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The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs

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Abstract

We investigated the effect of different weaning methods the on group agonistic behaviour, and the two physiological parameters neutrophil/lymphocyte (N/L) ratio and individual plasma glucose level in 16 groups of pigs (10 animals/group). Measures were taken 1–2 days before, 0–1 days, and 3–4 days after weaning. Different degrees of environmental familiarity were induced by placing weaned piglets either into unfamiliar enclosures (UE) or leaving them in their familiar housing environment (FE). Half of these treatment groups consisted of littermates (LM), the other groups were built up from piglets of different litters (DL). Hence, four treatment groups were produced in a 2 × 2 factorial design, each consisting of 4 groups (10 pigs/group). The piglets reacted differently with respect to the time of the behavioural and physiological stress response after weaning. Whereas most changes in agonistic behaviour occurred at day 4 after weaning (day −1 vs. day 4), the two physiological parameters particularly changed at day 1 after weaning (day −1 vs. day 1). Agonistic behaviour increased significantly over time, and this effect was significantly affected by housing condition (UE or FE) and social environment (LM or DL). At four days after weaning, the N/L ratio declined after an initial increase one day after weaning to a level which was similar to the level before weaning. Nevertheless, the N/L ratio changed significantly with time and was affected by housing condition. One day after weaning only the piglets which were brought to an unfamiliar housing environment showed an increase of plasma glucose level. No overall effect of time could be observed. In summary, newly weaned piglets appear to have more problems coping with an unfamiliar housing environment than coping with unfamiliar piglets. Whilst the physiological effects are very transient, the behavioural adaptation (e.g. agonistic behaviour) continues to rise to day 4. The present study indicates that behavioural and physiological measures that can be gained easily reflect the animals' ability to cope with environmental conditions as well as social disturbances. It is concluded that simultaneous effects of social and non-social environmental changes, though regularly met in animal husbandry, are still poorly understood and need further research in order to shed more light upon the behavioural and physiological events during these processes. © 1997 Elsevier Science B.V.

Keywords: Pig; Agonistic behaviour; Weaning; Housing; N/L ratio; Glucose; Stress

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1. Introduction

Weaning in mammals is a complex process that involves a number of behavioural, physiological, nutritional, environmental and social changes for the offspring. A genetical influence on the weaning process and a measurable within-litter variation of free-ranging domestic pigs was described by Jensen (1995a). Under free-ranging conditions, piglets are able to peacefully make social bonds with other piglets when they have the opportunity to early contact piglets of the same age from other litters (Petersen et al., 1989). However, under practical farming conditions most of the piglets are weaned by the removal of the mother and mixing with unfamiliar animals in a new housing environment. Postweaning mixing of pigs unfamiliar to each other leads to vigorous fighting and social stress (Friend et al., 1983; Algers et al., 1990; Puppe and Tuchscherer, 1993, 1994). The level of agonistic and aggressive behaviour can be modified by some social (e.g. group composition) and non-social (e.g. pen size, environmental enrichment) factors (see Ewbank and Bryant, 1972; Schaefer et al., 1990; Moore et al., 1994). After regrouping, pigs appear to experience social stress sufficient to cause a subsequent reduction in weight gain performance (Stookey and Gonyou, 1994).

The aim of this study was to investigate the effect of different weaning methods of piglets on agonistic behaviour, energy demand, and immunophysiological response to increasing stress. It has been shown that in pigs exposed to stress, changes of the blood neutrophil/lymphocyte (N/L) ratio reflect short-term elevations of the plasma cortisol level, because there is a tendency for neutrophils (N) to increase while lymphocytes (L) decrease (Widowski et al., 1989; Moore et al., 1994; Wallgren et al., 1994). This suggests that in controlled situations an increasing N/L ratio can reliably be correlated to increasing stress in pigs. Other authors found that the stress response in pigs is parallelled by elevated levels of plasma glucose (Barnett et al., 1983; Fernandez et al., 1994). Hence, changes in relative leukocyte counts, particularly the N/L ratio, may be a simple, useful, and easily gained physiological stress indicator for pigs (Widowski et al., 1989; Metz and Gonyou, 1990; Moore et al., 1994).

The weaning methods used in the present study varied both in housing condition (i.e. piglets remained in their familiar pen or were placed into an unfamiliar pen) and in social condition (i.e. piglets remained in littermate groups or were mixed with unfamiliar individuals). These conditions reflect variation in common management practices, and it is anticipated that they create different levels of stress responses that are measurable by behavioural and physiological parameters as used in this study.

2. Material and methods

2.1. *Experimental procedure*

The study was carried out using 16 groups of German Landrace pigs consisting of 160 female and castrated male piglets from 16 litters of multiparous sows. Immediately after farrowing the litters were equalized to 10 piglets per farrowing pen (slatted floor, 3.6 m^2). Weaning was performed when the piglets were 42 days (±1 day) of age, their average weaning weight was 11.6 ± 2.1 kg (mean ± SD).

Different degrees of environmental familiarity were induced by placing weaned piglets either into unfamiliar enclosures (UE) or leaving them in their familiar housing environment (FE). The FE-groups remained in the farrowing pens, whilst the UE-groups were placed into flat-decks. The main housing conditions were similar. Half of these treatment groups consisted of littermates (LM), the other groups were built up from piglets of different litters (DL), i.e. half of the piglets of each housing group were unfamiliar to each other. Hence, four treatment groups resulted in a 2 × 2 factorial design, each consisting of 4 groups (10 pigs/group). The farrowing pens after weaning were extended to the same size as the flat-decks (5.1 m^2). The average female/male ratio of the groups was 53.8% to 46.2%. All groups were fed ad libitum at 08:00 and 14:00 (4 places per enclosure) and were kept on slatted floor without straw. Water was supplied ad libitum.

2.2. *Agonistic behaviour*

Agonistic interactions were recorded by direct observation one to two days before weaning (day

−1), at the day of weaning or one day after weaning (day 1), and three to four days after weaning (day 4). The groups were observed daily from 08:00 to 16:00 (continuous sampling). Agonistic behaviour was defined as dyadic encounter during which a piglet ostensibly attacked another piglet. Similar to other investigations (e.g. Jensen, 1982; McGlone, 1985; Rushen and Pajor, 1987), fights were defined as overt bodily attacks like 'head to head knocks' and 'head to body knocks', 'parallel/inverse parallel pressings' and 'bitings' displayed by the piglets within the pen area. Additionally, 'replacements' were recorded as agonistic interactions when one piglet replaced another at the trough area. When winner and loser could definitely be recognized, fights were recorded as 'decisive'. Although it is difficult to clearly detect submissive behaviour patterns in pigs (Meese and Ewbank, 1973), we interpreted turning away from an attack and entering an asymmetric parallel configuration (Rushen and Pajor, 1987) as a defensive movement, indicating a loser. For each group, an aggression score was calculated expressed as the group mean value of all piglets' aggressive behaviour bouts.

2.3. N/L ratio and glucose

To avoid additional stress, the randomly sorted piglets were individually brought in a separated room immediately in the neighbourhood of the housing environment. Blood samples were taken from each pig in a supine position by anterior vena cava puncture one day prior to weaning (day −1), one day after weaning (day 1), and four days after weaning (day 4) at 8:00 h (immediately prior to feeding). The whole procedure needed no longer than 30 s including 20 s for the blood sample taking.

Relative leukocyte counts (lymphocytes, monocytes and basophils, eosinophils, neutrophils) were made by microscopic examination of the smears stained using the May–Grünwald–Giemsa method. The N/L ratio was determined from counts of at least 200 leukocytes in total. Plasma glucose (mmol/l) was determined by the glucose-oxidase-peroxidase method (Merckotest® No. 14365).

2.4. Statistics

Analyses of data were performed by using the SAS system (SAS, 1989), except for data in Table 1, where only percentage measures of agonistic behaviour are given. Repeated measures analysis of variance was performed by GLM procedure testing the within-subjects effect of time (day −1, day 1, day 4), and the interaction of time with between-subject factors housing condition (FE, UE), social environment (LM, DL), and their interaction. If significant, contrasts were tested between day −1 and day 1, and day −1 and day 4, respectively. Results were regarded to be significant at $P \leq 0.05$ (NS = not significant: $P > 0.05$).

3. Results

3.1. Agonistic behaviour

Fig. 1 summarizes all group aggression scores before and after weaning involving an increase of aggressive behaviour after weaning in groups which were placed into an unfamiliar housing condition. For all groups ($n = 16$), the mean aggression scores increased from 2.15 ± 0.17 encounters per animal and hour before weaning (day −1) to 2.87 ± 0.28 (day 1) and 3.58 ± 0.41 (day 4) after weaning (mean ± SEM).

The percentage of recognized wins or defeats of

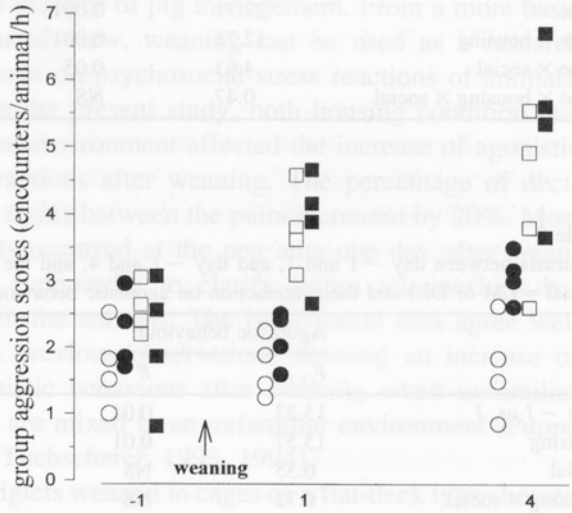

Fig. 1. Group aggression scores (encounters/animal/hour) of pigs around weaning: FE–LM (○), FE–DL (●) UE–LM (□), and UE–DL (■). Each treatment consists of 4 groups (10 pigs/group) each ($n = 4$).

Table 1
Percentage measures of agonistic behaviour in pigs around weaning (16 groups, 10 pigs/group)

Agonistic behaviour	Weaning		
	Day − 1 (%)	Day 1 (%)	Day 4 (%)
Percentage of aggressions in the pen area (pen and trough area = 100%)	50.0	80.1	69.5
Percentage of clearly recognized wins or defeats (all aggressions = 100%)	62.9	75.9	84.2

all aggressions increased from 62.9% before weaning to 84.2% at day 4 after weaning (Table 1). Whereas before weaning (day − 1) aggression was equal in the pen and trough area (50% each), immediately after weaning (day 1) most aggression occurred in the pen area as dyadic fights (80.1%), mainly to establish social relationships among the piglets.

Agonistic behaviour increased significantly over time (Tables 2 and 3), and this effect was significantly affected by housing condition and social environment (Table 2). The tested contrasts show (Table 3) that weaning process increased agonistic behaviour between day − 1 and 1 as well as between day − 1 and day 4. One day after weaning (day − 1 vs. 1) only the housing condition affected agonistic behaviour, whereas at day 4 after weaning (day − 1 vs. 4) both housing condition and social environment affected agonistic behaviour.

3.2. *N/L ratio*

In contrast to the aggression scores, the N/L ratio of all piglets declined after an initial increase one day after weaning to a level at day 4 after weaning

Table 2
Effect of time and interactions with housing condition (housing = FE or UE) and social environment (social = LM or DL) on agonistic behaviour, N/L ratio, and plasma glucose level around weaning

	Agonistic behaviour		N/L ratio		Glucose	
	$F_{(2.24)}$	P	$F_{(2.270)}$	P	$F_{(2.246)}$	P
Time	22.71	0.001	16.59	0.001	1.60	NS
Time × housing	12.71	0.001	4.19	0.05	9.74	0.001
Time × social	4.63	0.05	0.12	NS	0.73	NS
Time × housing × social	0.47	NS	0.54	NS	0.81	NS

Table 3
Contrasts between day − 1 and 1, and day − 1 and 4, and the effect of housing condition (housing = FE or UE) and social environment (social = LM or DL) and their interaction on agonistic behaviour, N/L ratio, and plasma glucose level

	Agonistic behaviour		N/L ratio		Glucose	
	$F_{(1.12)}$	P	$F_{(1.135)}$	P	$F_{(1.123)}$	P
Day − 1 vs. 1	13.23	0.01	17.72	0.001	0.04	NS
Housing	15.57	0.01	5.72	0.05	13.87	0.01
Social	0.35	NS	0.02	NS	0.06	NS
Housing × social	0.72	NS	0.68	NS	0.02	NS
Day − 1 vs. 4	75.49	0.001	0.04	NS	2.66	NS
Housing	38.73	0.001	2.58	NS	0.01	NS
Social	13.67	0.01	0.47	NS	1.70	NS
Housing × social	1.30	NS	1.18	NS	1.34	NS

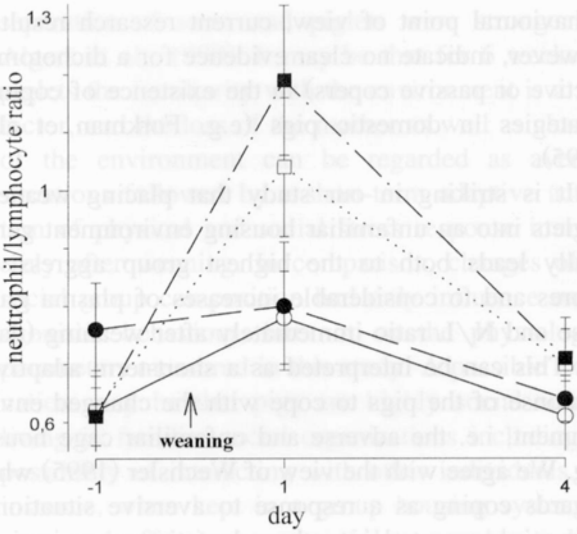

Fig. 2. Neutrophil/lymphocyte ratio of pigs around weaning: FE–LM: $n = 39$ (○), FE–DL: $n = 37$ (●), UE–LM: $n = 40$ (□), UE–DL: $n = 37$ (■). Each treatment consists of 4 groups. Data are means ± SEM (bars).

which was similar to the level before weaning (Fig. 2). The N/L ratio changed significantly with time (Table 2), and this effect depended significantly on housing condition. The contrasts show (Table 3) that the weaning process increased the N/L ratio only between day −1 and day 1, and at this time a significant influence of housing condition was found. No contrast effect between day −1 and 4 was found.

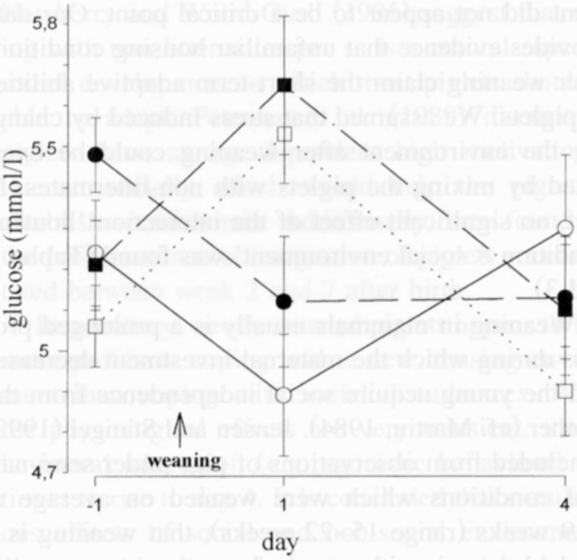

Fig. 3. Blood plasma glucose (mmol/l) of pigs around weaning: FE–LM: $n = 30$ (○), FE–DL: $n = 37$ (●), UE–LM: $n = 30$ (□), UE–DL: $n = 37$ (■). Each treatment consists of 4 groups. Data are means ± SEM (bars).

3.3. *Glucose*

One day after weaning the piglets which were removed into an unfamiliar housing environment showed an increase of plasma glucose level, whereas the other animals responded with a decrease (Fig. 3). Four days after weaning no differences compared to the time before weaning were observed. Hence, no overall effect of time was found (Tables 2 and 3). Merely, the interaction 'time × housing condition' (Table 2) and the contrast effect of housing condition between day −1 and day 1 (Table 3) were significant.

4. Discussion

Management methods are important sources of environmental variation which can effect social behaviour (Broom, 1993). By means of four different treatment groups of pigs we simulated an increasing lack of familiarity in housing condition (UE or FE) and social environment (LM or DL) and, thus, of the controlability and predictability of the environmental situation. These treatments reflect variation in common practice of pig management. From a more basic point of view, weaning can be used as a research concept on psychosocial stress reactions of animals.

In the present study, both housing condition and social environment affected the increase of agonistic interactions after weaning. The percentage of decisive fights between the pairs increased by 20%. Most fights occurred at the pen area one day after weaning, supposedly to clarify future relationships between the animals. The behavioural data agree well with previous observations showing an increase of agonistic behaviour after weaning when unfamiliar pigs are mixed in an unfamiliar environment (Puppe and Tuchscherer, 1993, 1994).

Piglets weaned in cages of a flat-deck type showed elevated cortisol levels compared with piglets kept in the maternity pen (Dantzer and Mormède, 1981; Marx and Haecker, 1981). This can be interpreted as a stress response of the animals on the very poor environmental condition of the flat-decks. A number of authors presented experimental evidence that stress in pigs can also induce immunological reactions (Blecha et al., 1985; McGlone et al., 1993; Moore et al., 1994; Morrow-Tesch et al., 1994; Hessing et al., 1994). Most of these authors emphasized the role of N/L ratio as a useful and simple stress measure. Wallgren et al. (1994) proved that the treatment with ACTH induced high concentrations of cortisol in the blood plasma of pigs. Simultaneously, the number of lymphocytes (L) decreased while the neutrophil number (N) increased. Blecha et al. (1983) pointed out that weaning stress in pigs younger than 5 weeks causes physiological changes detrimental to cellular immunity reactivity. Our study provides evidence that it also may be the case for 6 weeks old piglets, but the effect seems to be transient.

In a recent study, Fernandez et al. (1994) observed a positive relationship between aggressive behaviour and plasma glucose levels in domestic pigs. Barnett et al. (1983) found elevated levels of plasma glucose as a chronic stress response of gilts in an unpleasant handling treatment. It has been suggested that activation of the sympathic nervous system in response to behavioural stress plays an important role in the control of glucose availability during aggressive behaviour (cf. Fernandez et al., 1994). In agreement with these findings, in our study the N/L ratio as well as the plasma glucose level of piglets substantially increased with increasing stress, particularly induced by the unfamiliarity of the housing condition at one day after weaning. We also showed that four days after weaning the physiological measures approached the levels prior to weaning, but the aggressive scores further increased. From this inconsistency we conclude that the activation of behavioural and physiological mechanisms may be time-variant. After a short physical adaptation response immediately after weaning (day 1), the pigs more and more begin to clarify the social relationships (day 1 and day 4) by using agonistic interactions. As indicated by aggression results the pigs are still stressed, although the physiological measures in this study show no further indication. This might also be explained by the effect that not all pigs responded in a uniform manner. Socially related coping strategies of the animals or simple individual variations are discussed as possible reasons (for a review, see Jensen, 1995b). From a behavioural point of view, current research results, however, indicate no clear evidence for a dichotomy (active or passive copers) in the existence of coping strategies in domestic pigs (e.g. Forkman et al., 1995).

It is striking in our study that placing weaned piglets into an unfamiliar housing environment generally leads both to the highest group aggressive scores and to considerable increases of plasma glucose and N/L ratio immediately after weaning (day 1). This can be interpreted as a short-term adaptive response of the pigs to cope with the changed environment, i.e. the adverse and unfamiliar cage housing. We agree with the view of Wechsler (1995) who regards coping as a response to aversive situations being incorporated into the adaptative organisation of animals and having been shaped by evolution. This implies that pigs will often have difficulties in successfully coping with intensive housing systems.

Surprisingly, the pigs' response to the 'housing effect' was overtly significant for all parameters, but the 'social effect' was only significant for the agonistic behaviour and not significant for the physiological parameters. However, Metz and Gonyou (1990) found that weaning at an age of 2 weeks resulted in a significant increase of the N/L ratio compared with weaning at 4 weeks, but they also found that behavioural familiarity with the environment did not appear to be a critical point. Our data provides evidence that unfamiliar housing conditions after weaning claim the short-term adaptive abilities of piglets. We assumed that stress induced by changing the environment after weaning could be exerbated by mixing the piglets with non-littermates. In fact, no significant effect of the interaction 'housing condition × social environment' was found (Tables 2 and 3).

Weaning in mammals usually is a prolonged process during which the maternal investment decreases and the young acquire social independence from the mother (cf. Martin, 1984). Jensen and Stangel (1992) concluded from observations of pigs under semi-natural conditions which were weaned on average at 18.9 weeks (range 15–22 weeks), that weaning is a gradual process without any dramatic changes to the piglets in respect to activity budget or social relationships. In contrast, under practical husbandry conditions (weaning between 4–7 weeks) the effects of separation of sows and piglets were stronger (cf. Algers et al., 1990). It may be that for 6 weeks old piglets the familiarity with the environment is a vital factor, and the loss of the mother as well as changes of the environment can be regarded as aversive situations followed by a short-term adaptive activation of physical and social stress response immediately after weaning. In comparison, changes of the social group composition had only influence on the agonistic behaviour, but not on the physiological parameters measured in this study. A possible explanation may be that pigs are highly social animals living in familiar social organizations including the possibility of interacting with other individuals. For instance, when kept in a group housing system for sows and offspring, young piglets are able to build up a multi-suckling system with the opportunity of social interactions with strange animals (Puppe and Tuchscherer, 1995). We interpret the present findings such that young piglets primarily use behavioural mechanisms (e.g. agonistic interactions) to establish a social organization or clarify social relationships. An evolved physiological stress program against other individuals of the same age appears not very likely. This is in agreement with conclusions of Newberry and Wood-Gush (1986) and Petersen et al. (1989). From observations of piglets in a semi-natural environment between birth and 13 weeks of age Newberry and Wood-Gush (1986) suggest that mixing with non-littermates per se is a natural aspect of social development to which most piglets should be able to adapt. Petersen et al. (1989) discuss that piglets have an age-dependent high motivation to interact socially which would help the piglets to establish social bonds. Additionally, they found that the process of social integration of piglets was facilitated between week 2 and 7 after birth.

In summary, newly weaned piglets appear to have more problems coping with a new housing environment than coping with social disturbances. Whilst the physiological effects are very transient, the behavioural adaptation (e.g. agonistic behaviour) continues to rise to day 4. It is concluded that simultaneous effects of social and non-social environmental changes, though regularly met in animal husbandry, are still poorly understood and need further research (cf. Lawrence et al., 1991) to shed more light upon the behavioural and physiological events during these processes.

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7.11 Studie 11

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Analysing dominance relationships by sociometric methods—a plea for a more standardised and precise approach in farm animals

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Abstract

Social dominance is a multidimensional phenomenon occurring in all gregarious farm animals and finds its reflection in a dominance hierarchy. Hence, numerous studies have tried to analyse dominance relationships as well as to correlate outcoming results (mostly individual ranks) with other behavioural and/or physiological features of the animals. Although the concept of dominance, once established, has been developed continuously and several sociometric measures were cumulatively introduced, a consistent analysing approach has not been achieved, especially in farm animals. Thus, considerable inconsistencies in the used methodology may impair obtained results and interpretations. The present paper is a plea for a more standardised and complex approach when analysing dominance relationships, not only in farm animals. First, derived from a structural definition of dominance, we suggest in detail the preferably consistent use of appropriate sociometric measures at all social levels of analysis: the dyad as the starting level, the group as the highest level, and the individual as the basic level. Second, we applied this procedures in a case study to analyse social dominance in a group of dwarf goats ($n = 12$) and pigs ($n = 10$), respectively, to comparatively demonstrate benefits and problems of such an approach in two different farm animal species.

It is concluded that the use of individual ranks is actually only reasonable when fundamental sociometric measures both at the dyadic level (e.g. percentage of dyads which have a significant asymmetric outcome) and at the group level (e.g. the strength of hierarchy) are successfully tested by statistical methods as also presented in this paper. The calculated sociometric measures deliver not only a more comprehensive "picture" of the social relationships within a group as simple ranks do, but also indicate possible reasons of differences in the behavioural development. For instance, whereas the dwarf goats maintained a quasi-linear dominance hierarchy over time with a high rate of overt agonistic behaviour, pigs after the establishment of their hierarchy showed a reduced agonistic behaviour which

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makes it questionable to calculate reliable sociometric measures. These species-dependent variations may be primarily caused by different kinds of the fighting behaviour in goats (i.e. ritualised, low costs) and pigs (i.e. more seriously, high costs).

Overall, a more consistent and standardised approach of analysing social dominance in (farm) animals may improve the scientific value of single studies and makes it easier to compare various studies within a species and between species.

Keywords: Dominance; Dyads; Social hierarchy; Sociometric measures; Pig; Dwarf goat

1. Introduction

In recent years a rising number of studies in different species of gregarious farm animals have dealt with the analysis of social dominance related to various aspects of animal behaviour, physiology and/or performance:

- pig (e.g. Mendl et al., 1992; Puppe and Tuchscherer, 1994; Marchant et al., 1995; Puppe, 1996; Drickamer et al., 1999; Otten et al., 2002);
- cattle (e.g. Reinhardt and Reinhardt, 1975; Kondo and Hurnik, 1990; Hasegawa et al., 1997; Plusquellec et al., 2001);
- goat (e.g. Orgeur et al., 1990; Patón et al., 1995; Keil and Sambraus, 1996; Barroso et al., 2000);
- horse (e.g. Clutton-Brock et al., 1976; Keiper and Sambraus, 1986; Ellard and Crowell-Davis, 1989; van Dierendonck et al., 1995);
- poultry (e.g. Rushen, 1982; Nol et al., 1996; Pagel and Dawkins, 1997).

Nevertheless, the results from various studies differ considerably. Whereas, for example, some authors found a significant correlation between a certain physical attribute and social rank (Bouissou, 1972; Lee and Craig, 1981; Lott and Galland, 1987; Ellard and Crowell-Davis, 1989; Greenberg-Cohen et al., 1994; von Holst et al., 2002), other authors have not found such relationship (Sambraus and Osterkorn, 1974; Eccles and Shackleton, 1986; Rutberg, 1986; Arnold and Grassia, 1982; Cassinello, 1995). Considering coherence between social rank and the level of stress hormones, Zanella et al. (1998) found highest levels of plasma cortisol in middle-ranking pigs when studying group housed sows. In contrast, Tuchscherer et al. (1998) as well as de Groot et al. (2001) did not find any relationship between rank and cortisol level in pigs. On the contrary, Hasegawa et al. (1997) revealed a high level of cortisol for high-ranking individuals in cattle. When focusing on production traits, Patón et al. (1995) accounted for a direct correlation between social rank and milk production, whereas results in studies done by Lee et al. (1982) and Barroso et al. (2000) indicated middle-ranking animals as the most productive.

Independent from real-existing biological varieties, a possible reason for these frequently observed deviating results may lie in the fact that there is no consistent approach throughout all these studies how to calculate the various attributes of social dominance and how to interpret each of them. Used parameters differ at all levels of social relationship: dyadic level, group level and individual level. Moreover, quite often some indices are calculated without fulfilling basic requirements which are demanded for correct interpretation. Overall, considerable differences or deficiencies in the used methodology may impair obtained results or conclusions and complicate the comparability within a species and, especially, between various species. Therefore, the aim of the present paper is two-fold. First, derived from the definition of dominance and existing sociometric measures at different levels, we suggest a more uniform analysis schedule which seems to be necessary for a standardised and precise analysis of social hierarchy in farm animals. Second, based on a case study in two different species (dwarf goats and pigs), we apply this procedure to demonstrate benefits and problems of this approach.

1.1. *The structural definition of dominance and the levels of analysis*

Over the last four decades a variety of theoretical papers on the concept of dominance have been published (Rowell, 1966; Syme, 1974; Bernstein, 1981; Kaufmann, 1983; Dunbar, 1988). Drews (1993) tried to give a synopsis of this still ongoing debate. Based on the original definition of dominance given by Schjelderup-Ebbe (1922) he has formulated a structural approach of dominance. Structural dominance definitions describe the pattern of an observable type or set of interactions without referring to their function. Drews (1993) summarised the essence of this dominance concept as follows: “Dominance is an attribute of the pattern of repeated, agonistic interactions between two individuals, characterised by a consistent outcome in favour of the same dyad members . . . ”. Hence, dominance initially is a dyad-level phenomenon (Capitanio, 1991), and the dyad should be the starting level of every analysis (Puppe, 1996). Whereas the next higher level is the social group, at a minimum consisting of three individuals, calculations of indices for single individuals are valid only when measures at the two other levels indicate the existence of a certain kind of dominance hierarchy. Nevertheless, the individual level is mostly the actual focus of studies on dominance (e.g. the social rank of an individual) and often the only level considered.

Adapted from this general concept of dominance a plethora of meaningful sociometric parameters has been developed to evaluate dominance at all levels of analysis (e.g. Landau, 1951; Kendall, 1962; Appleby, 1983; Boyd and Silk, 1983; Jackson and Winnegrad, 1988; de Vries et al., 1993; Puppe and Tuchscherer, 1994; de Vries, 1995; de Vries and Appleby, 2000). The majority of all these procedures use a binomial approach, that is, in each dyadic agonistic (aggressive) interaction an individual is scored as either winner or loser (Brown, 1963). However, many studies on dominance simply calculated the ranks, i.e. the social position of an individual in relation to all other group members, e.g. based on the ratio of wins and defeats. This makes it impossible to judge the dominance rank of an individual with regard to the other sociometric levels (e.g. the strength or stability of dyadic relationships or dominance hierarchy) and may lead to some misinterpretation of the results.

1.2. *Collecting valid data on dominance–subordinate relationships*

Before starting an investigation on dominance relationships in a group of animals, a suitable set of behavioural actions associated with this phenomenon has to be identified. Following Drews (1993) all forms of agonistic behaviour clearly refer to dominance. However, chosen behaviour should be, as far as possible, unambiguous and easy to observe in every context. Moreover, it should be described comprehensibly. It is still under discussion if predominantly overt agonistic interactions like fights or related actions with physical contact, displacements or chase should be only considered and if behaviours like threats, gestures of fear or anticipatory avoiding are applicable as well when studying social relationships (Lehner, 1996). To our mind, focusing on overt agonistic interactions between dyads of individuals should be preferred because they normally deliver clear and unambiguous results between various observers. Additionally, to be sure about the outcome of an agonistic encounter not only aggressive behaviour displayed by one individual should be considered but ditto clear submissive behaviour displayed as a reaction by the other combatant. As submissive behaviour patterns, for example, Tuchscherer et al. (1998) defined any signs of displacement, turning the body or at least the head away from the other individual and any kind of fleeing.

Collecting data on dominant–subordinate relationships can generally be conducted by one of the two following methods. First, continuous sampling of all occurring agonistic interactions by observing a group in their normal home pen over a certain period of time. This observation can be done directly or by video if the group is not too large and the individuals are well distinguishable. Second, in the case a stable dominance order is already established (i.e. animals stayed together already for some time) and direct agonistic interactions are only rarely observable a more suitable method is to match each individual with every other individual in an equal number of times in an adequate competition situation forcing the animals to show agonistic interactions. For instance, an appropriate procedure to do so is the “food competition test”. In this test two individuals were given a small amount of food in a trough or feeder offering space for only one animal (Ellard and Crowell-Davis, 1989; Hessing et al., 1994).

The first method is thought to be more time consuming, however, it delivers valid data for calculating a more general ‘aggressive order’ (McGlone, 1986) independent of a special social situation (two animals isolated from the group) or a special context (food competition). The second method indicates dominance a priori only in the special context of a used resource (e.g. food) and delivers a ‘competitive order’ (Syme, 1974). It should be noted that both methods may differ in their results concerning dominance (McGlone, 1986; Ellard and Crowell-Davis, 1989).

2. A plea for a comprehensive analysis schedule by sociometric methods

Based on the above mentioned problems with regard to dominance we suggest to bear in mind the different levels of social relationships and the appropriate parameters as shown in Table 1 when analysing dominance relationships in farm animals. At least, such an approach should involve some meaningful sociometric measures at all levels of analysis: the dyadic level, the group level, and the individual level. Measures at the dyadic level are the prerequisite to evaluate subsequent calculations at the group level as well as at the individual level. Applying such a comprehensive approach, we expect a more standardised and precise “picture” of the social hierarchy in a group of animals irrespective whether it was studied for its own or in correlation to other biological measures.

Table 1
Sociometric indices describing various aspects of social dominance at dyadic level, group level and individual level

Parameter	Description	Statistical test	Literature
Dyadic level			
Unknown dyads	Dyads with no interactions		
One-way dyads	Dyads with wins only for one individual (strictly unidirectional)		
Two-way dyads	Dyads with wins for both individuals (bidirectional)		
Tied dyads	Dyads with the same number of wins for both animals		
Circular triads	Intransitivities in dominance		de Vries (1995)
Significant dyads	Dyads which have a significant asymmetric outcome	Binomial test	Lohse et al. (1982)
Group level			
Strength of hierarchy			
Landau index, *h*	Hierarchy strength index based on dominated animals		Landau (1951), Appleby (1983)
Kendall index, *K*	Hierarchy strength index based on circular triads	Chi-square test	Kendall (1962), Appleby (1983)
Improved Landau index, h'	Hierarchy strength index corrected for tied and unknown dyads	Randomisation test	de Vries (1995)
Directional consistency index, DCI	Ratio of agonistic interaction in the main direction to reversed agonistic interactions		van Hooff and Wensing (1987)
Stability of hierarchy			
Rank correlation coefficient	Index of hierarchy stability over time	Spearman's rank correlation	
Matrix correlation index	Index of hierarchy stability over time	Permutation test	de Vries (1993)
Individual level			
Dominance index, DI_{dom}	Ratio of animals which are dominated by an individual in relation to all animals with which it has interacted		Lamprecht (1986)
Dominance index, DI_{AI}	$\sum$ number of wins minus defeats to the number of all interactions		Bowen and Brooks (1978)
Rank number	Placing each individual into an ordinal rank according to the DI		
Agonistic index, AGI	Number of agonistic interactions per individual and time unit		Puppe and Tuchscherer (1994)
Aggressive index, ARI	Number of initiated agonistic interactions per individual and time unit		Barroso et al. (2000)

2.1. Proven sociometric measures at different levels of analysis

2.1.1. Dyadic level

Collected data on the outcome of agonistic interactions between pairs of individuals, based on a clear winner/loser criterion, should be entered into a dyadic interaction matrix (see Appendix A) (Lehner, 1996). Every individual is listed on both axes. Animals on one axis are labelled as winners and animals on the other axis as losers. This matrix is the basis for evaluating the dominance status of individuals within every dyadic relationship, calculating the dominance rank of single individuals within a group and analysing the strength of linearity of a dominance hierarchy as well its stability over time. The maximum number of possible dyads in a group ($n =$ number of individuals) can be easily calculated as

$$\mathrm{dyad}_{\max} = \tfrac{1}{2}n(n-1) \tag{1}$$

In a first step, based on the matrix, we can calculate a number of simple parameters like the amount of coverage of the collected behaviour over all dyadic relationships (dyad$_{\max}$ minus unknown dyads). The dominance structure can be revealed only if the chosen behaviour occurs in most relationships. A first impression of the level of unidirectionality within a matrix is given by the number of one-way dyads in which the behaviour is shown by one actor only. Tied dyads, however, are characterised by an equal number of wins in a given dyad for both actors.

The majority of all theoretical definitions consider dominance primarily as an attribute of dyadic agonistic relationships and not of single individuals (Drews, 1993; de Vries, 1998). An animal is called dominant over another individual when it wins significantly more than it loses within an observed number of encounters with this individual. It has been claimed in many theoretical papers on dominance that the consistency of the outcome has to be tested for significance (e.g. by binomial sign test) before further calculations are done (Boyd and Silk, 1983; Lundberg, 1987; Lehner, 1996). Surprisingly, we are aware of only a few studies on dominance in farm animals which used empirical measurements (Hunter et al., 1988; Araba and Crowell-Davis, 1994; Côté, 2000) or objective statistical methods to test dyadic agonistic relationships for asymmetry of the outcome of wins and defeats (Puppe and Tuchscherer, 1994; Martin and Beaugrand, 1997). In contrast, some mathematical procedures and derived computer programs for calculating sociometric parameters (e.g. MatMan Version 1.0 for Windows (MatMan, 1998); Java Applets "HierarchyMatrixApplet" Version 2.1 (Java Applets, 2001)) which found broad acceptance consider dyads as clarified whenever an animal wins only one agonistic encounter more than it loses (Appleby, 1983; de Vries, 1995, 1998). In this case, the required significance in the dyad asymmetry according to the definition of dominance (Drews, 1993) is not fulfilled and this may lead to some misinterpretations. As far as we know, up to now there is no agreement or even a discussion if and to what extent dyadic relationships of a given matrix should be tested as significantly asymmetric before including it for further analysis. However, we suggest that it is at least fundamental to offer the number of dyads that are proved as significant in order that proximate calculations of sociometric parameters can provide a serious picture of the group structure. This is needed in particular for social matrices with a high number of two-way relationships, i.e. high level of bidirectionality.

2.1.2. Group level

In a next step, based on dyadic relationships between single individuals, an index for the strength (linearity) of the dominance hierarchy of a group should be calculated. A frequently used procedure of dealing with data on dominance is to calculate only individual ranks based on dominants and subordinates or wins and defeats, respectively, and assign group members according to their dominance index (Schein and Fohrman, 1955; Lott, 1979; Zanella et al., 1998). However, this feigns the overall impression of linearity even for non-linear hierarchies by obscuring irregular relationships like non-linear (circular) triads (Beilharz and Mylrea, 1963; Boyd and Silk, 1983). Dominance hierarchies can generally be divided into three types: strictly linear, non-linear but significantly different from chance (quasi-linear) and non-linear. A linear dominance order is completely transitive, meaning when A is dominant over B and B is dominant over C than A is dominant over C as well. However, this kind of linearity is not common especially for larger groups of animals, neither in primate nor in non-primate species (de Waal and Luttrell, 1989; Koenig, 2000; Greenberg-Cohen et al., 1994). Only 15% of 25 species of birds and mammals surveyed by Jackson and Winnegrad (1988) showed strictly linear hierarchies. Appleby (1983) and more recently de Vries (1995, 1998) developed statistical test procedures, based on the work of Kendall (1962) and Landau (1951), appropriate to test significance of linearity in dominance hierarchies. Both indices differ depending on their sensitivity in case of unknown relationships (no interactions between two individuals) or tied relationships (equal number of wins for both animals within a dyad). The Kendell index (K) is derived from the concept of “circular triads” and calculates values between 0 and 1. The calculation of circular triads is described in detail by de Vries (1995). The Landau index (h) refers to the concept of the “number of dominated animals” and ranges from 0 to 1 as well. A value of 1 means the dominance hierarchy is totally transitive. Both indices are identical for most dominance matrices. However, as de Vries (1995) stated, the value of K is slightly smaller than that of h when the number of individuals in a group is even due to the fact, that the maximum number of circular triads is constrained under such circumstances. Moreover, the calculation of circular triads is disabled when there are tied dyads present in the matrix. To overcome these problems de Vries (1995) developed a method for testing the significance of linearity for dominance dyads some of which are unknown and some of which are tied (h' corrected for unknown relationships). Moreover, he developed a computer program for the analysis of sociometric matrices called MatMan, which implemented the test statistic for K as well as h' (de Vries et al., 1993).

Another index for the strength of the dominance hierarchy of a group of individuals is the directional consistency index (DCI) (van Hooff and Wensing, 1987). The DCI calculates cross all dyads, reflects the frequency with which a behaviour (e.g. wins in agonistic encounters) occurred in the more frequent direction relative to the total number of this behaviour, i.e. the level of unidirectionality. It ranges from 0 (completely equal exchange) to 1 (completely unidirectional). Compared to K, h or h', the DCI accounts much more for the asymmetry of the outcome of dyadic relationships. As an example, the DCI amounts 0.09 for a number of dyadic relationships with a ratio of wins to defeats of six to five and 0.71 for a number of dyads with a relation of six to one, whereas K, h and h' are 1 for both matrices. However, the DCI has disadvantages, too, as it is insensitive towards unknown relationships, and a test statistic is missing until now.

Whereas the linearity indicates the strength of a dominance hierarchy at a fixed point in time it is important as well to know about the stability of group structure over longer periods. Dominance is a time-related variable which generally remains dynamic (Reinhardt and Reinhardt, 1975; Hall, 1986). Especially when investigated in young animals, ontogeny alone guarantees that physical features vary and resulting ranks can may change in parallel (Bernstein, 1981; Puppe and Tuchscherer, 1994). In addition, stable groups over longer periods of time are rather uncommon in animal husbandry because of frequent regrouping, partly or total. To evaluate hierarchy stability a number of studies simply calculated the dominance index (DI) for all group members at two different times and correlated the DI or the deduced ordinal ranks. Another method is described by de Vries (1993). It assessed the rowwise correlation between two matrices, based on the weighted sum of correlations between all pairs of corresponding rows. This procedure was evaluated suitable especially for square matrices, whereas rows and columns correspond to the same individuals as dyadic agonistic interaction matrices. In combination with a permutation test as an adequate test statistic this procedure is also implemented in the MatMan program (de Vries et al., 1993).

2.1.3. *Individual level*

For dominance hierarchies which are linear or quasi-linear (non-random) it is reasonable to calculate individual DIs which provide a measure of how dominant an individual is with regard to other group members. Sometimes it seems that almost every researcher working on animal dominance developed his own DI (for a review, see Lundberg (1987), Lehner (1996), de Vries (1998)). In principle, three different methods can be distinguished:

(1) The DI can be calculated from any kind of data concerning the number of observed agonistic interactions and their outcome ($\mathrm{DI}_{\mathrm{AI}}$). For instance, it can be the ratio of wins minus defeats to all decisive fights (Bowen and Brooks, 1978):

$$\mathrm{DI}_{\mathrm{AI}} = \sum \frac{\text{wins} - \text{defeats}}{\text{wins} + \text{defeats}} \tag{2}$$

In this case, this index ranges from -1 (absolutely submissive) to $+1$ (absolutely dominant). Such an approach may be especially useful when only few unknown relationships occur and nearly all of the animals of a group are involved in a similar number of agonistic interactions (e.g. in newly mixed groups). However, results can be confusing in the way that an individual which is dominant over only few group members but achieved a high number of successful agonistic interactions can get a higher DI than an individual which is dominant over many group members but achieved only a lower number of successful interactions.

(2) A second group of methods tried to reorganise the matrix such that a numerical criterion calculated over the whole matrix is minimised or maximised (Slater, 1961; Boyd and Silk, 1983; Jameson et al., 1999; de Vries and Appleby, 2000). In the case of the I&IS method developed by de Vries (1998) the matrix is reorganised in a way that the number of inconsistencies under the diagonal of the matrix and the strength of those inconsistencies is minimised. However, these methods only deliver ordinal ranks for single individuals thus masking dominance differences between adjacent ranks.

(3) In a further group of methods, the DI is somehow related to the number of group members which are dominated by an individual ($\mathrm{DI}_{\mathrm{dom}}$). For instance, this can be calculated by the ratio of the number of animals which are dominated by an individual in relation to all animals with which it has interacted (Lamprecht, 1986):

$$\mathrm{DI}_{\mathrm{dom}} = \frac{\text{subordinates}}{\text{subordinates} + \text{dominants}} \times 100\% \qquad (3)$$

Under certain circumstances this index seems more reliable to us compared to the type of indices mentioned above because it considers the performance of an individual in all contested dyads instead of focusing only on its absolute number of agonistic interactions (Côté, 1999), and it delivers cardinal equivalents to ranks offering detailed information about the distances between adjacent ordinal ranks. In our case, this index ranges between 0 (absolutely submissive) and 100 (absolutely dominant).

A further meaningful parameter to score the level of agonistic interactions is the agonistic index (AGI) which is simply the number of all agonistic interactions (AI) an individual is involved in per time unit (e.g. per hour) (Puppe and Tuchscherer, 1994; Schrader, 2002). The AGI can be used as an individual parameter as well as on group level when it gives information about the level of agonistic interactions of the whole group under varying study conditions (e.g. space availability stocking rate, feeding regime) (Puppe et al., 1997). Furthermore, some authors calculated an aggressive index (ARI) on the basis of initiated AI by an individual per time unit (Araba and Crowell-Davis, 1994; D’Eath, 2002). Individual rank is often thought to be somehow related to the level of aggressive behaviour an animal exhibits or the number of agonistic interactions it is involved in (Reinhardt et al., 1987; Orgeur et al., 1990; Barroso et al., 2000), and even in stable groups high levels of agonistic interactions can occur (Fournier and Festa-Bianchet, 1995). The AGI as well the ARI are of interest when evaluating dominance relationships at a given point of time as well when comparing stability of dominance hierarchy over a period of time.

3. Case study: dwarf goats and pigs

In order to demonstrate benefits and problems of the suggested approach we analysed the agonistic behaviour of two different farm animal species (pigs and dwarf goats) under typical farming conditions as a case study. Thereafter, the results are discussed both in their biological meaning and especially with regard to the methodological evaluation of the used sociometric measures.

3.1. Animals and observations

Both a group of dwarf goats (*Capra aegagrus* f. *hircus*) and pigs (*Sus scrofa* f. *domestica*) were observed in two consecutive periods, first, directly after weaning and regrouping at an age of about 7 weeks (period 1), and second, at an age of about 14 weeks (period 2). Overt agonistic interactions were analysed using the all-occurrences sampling method (Lehner, 1996). Animals were observed for 6 h per day over a period of 4–6 days. The total time of observation amounted 24 h as a minimum and 36 h as a maximum during each period.

After weaning, a group of twelve unacquainted goats were kept in a pen (4 m × 3 m) with straw as litter, hay and water ad libitum and a wooden pyramid with three levels to allow goats to realise climbing behaviour (period 1). For experimental reasons goats were relocated to another pen with the same features in period 2. Concentrate was offered during defined times throughout the day, ad libitum during period 1 and at an amount of 400 g per day per animal in period 2, respectively.

Ten unacquainted piglets were weaned in a strawless flat-deck (1.3 m × 2.3 m) with standard food (four places) and water offered ad libitum (period 1). For period 2 the pigs were relocated to a greater pen (1.6 m × 3.6 m) under the same conditions. However, group size was only nine in period 2 because one pig died at the end of period 1.

For both species the occurrence and the outcome of all AI were observed by species-experienced observers. An AI was defined as a fight or a displacement with physical contact initiated by one individual and featuring aggressive behavioural elements, followed by any form of submissive behaviour performed by the opponent. In pigs, the following aggressive behaviour patterns were recorded as related to dominance: “head to head knocks”, “head to body knocks”, “parallel/inverse pressings”, “bitings” and “physical displacements” (for detailed description, see Jensen (1982), McGlone (1985), Rushen (1988), Puppe (1998)). In dwarf goats, the following aggressive behaviour patterns were recorded as related to dominance: “butts”, “front clash”, “rear clash”, “head wrestling”, “agonistic chase” and “threats” (for detailed description, see Shank (1972)). In both the species the loser was defined as the animal which first stopped fighting, turned away from an attack, tried to flee or was displaced from a location. Data on AI were collected regardless of the accompanying behavioural context (e.g. social or food competition), and were only assessed when just two individuals were involved.

3.2. Calculation of sociometric measures and statistical analysis

Data on wins and defeats were transformed into a winner–loser matrix with rows labelled as wins and columns labelled as defeats (see Appendix A). Based on these matrices we calculated numerous sociometric measures at all three levels of social relationship following Table 1. At first we calculated the number of possible dyads and the total number of AI. According to the total observation time and the number of individuals we determined the mean AGI for each species and observation period. Differences between observation periods with regard to the AGI were tested using the paired *t*-test (SAS, 1999).

In order to characterise processes on the dyadic level we computed the percentage of various types of dyadic relationships (unknown dyads, one-way dyads, two-way dyads, tied dyads and circular triads) using the MatMan program. In a next step we calculated the percentage of dyads with a significant asymmetric outcome using the sign test (Dixon and Mood, 1946). At least five agonistic encounters with a strictly unidirectional outcome were required to reach significance ($P < 0.05$) (for other ratio of wins to defeats in bidirectional dyads, see Table 9 in Lohse et al. (1982)).

To evaluate the strength of dominance hierarchy at the group level we computed h' (number of randomisations: 10 000), K and the DCI as implemented in MatMan. As a consequence of our approach which considers the dyad as the basis of dominance we repeated the calculation of h' and DCI considering only those dyads which were tested to have an significant asymmetric outcome. The stability of hierarchy over time was estimated by means of the rowwise matrix correlation procedure offered by MatMan. We calculated Spearman's rho averaged over all pairs of corresponding rows. To test the matrix correlation for significance we used the R_r statistic (number of permutations: 10 000) which is described as more powerful in the case that tied relationships occur (Hemelrijk, 1990).

At the individual level, we calculated the AGI, $\mathrm{DI}_{\mathrm{dom}}$ and the deduced ordinal rank of each individual.

3.3. Results

Table 2 summarises results for sociometric measures at the dyadic level and the group level for goats and pigs separately for both observation periods. The 66 possible dyads in the dwarf goats exhibited a total number of 938 AI in period 1 and 782 AI in period 2. In the overwhelming majority of all AI the initiator and the winner of an AI were identical, even though it was extremely difficult to record it. The mean AGI of the group only showed a tendency to decline from period 1 to period 2 (4.34 versus 3.62, $t = 1.91$, $\mathrm{d.f.} = 11$, $P < 0.081$). In pigs, the number of AI were much lower already in period 1, even though the number of all possible dyads were only slightly smaller. Moreover, AGI declined dramatically from period 1 to period 2 (1.64 versus 0.44, $t = 6.55$, $\mathrm{d.f.} = 8$, $P < 0.001$).

In goats the number of unknown relationships was extremely low in both observation periods ($\leq$1.5%). In pigs, however, this measure increased from merely 4.4% in period 1 to 36.1% in period 2. In both species, the percentage of one-way relationships increased from period 1 to period 2, whereas the percentage of two-way relationships decreased. In pigs

Table 2
Sociometric indices at dyadic level and group level of a male dwarf goat group ($n = 12$) and a pig group ($n = 10$) for two observation periods (for further details, see text)

	Dwarf goats		Pigs	
	Period 1	Period 2	Period 1	Period 2
Number of dyads	66	66	45	36
Agonistic interactions (AI)	938	782	263	47
Agonistic index for the group (AGI ± S.D.)	4.34 ± 1.31	3.62 ± 1.46	1.64 ± 0.60	0.44 ± 0.22
Unknown dyads (%)	0.0	1.5	4.4	36.1
One-way dyads (%)	27.3	42.4	42.2	50.0
Two-way dyads (%)	72.7	56.1	53.3	13.9
Tied dyads (%)	7.6	4.6	13.3	0.0
Circular triads (%)	24.3	20.7	51.8	71.7
Significant dyads (%)	53.0	63.6	35.0	2.8
Landau index, h'	0.762 ($P < 0.001$)	0.801 ($P < 0.001$)	0.510 ($P < 0.05$)	0.392 (n.s.)
Kendall index, K	0.762 ($P < 0.001$)	0.793 ($P < 0.001$)	0.481 ($P < 0.05$)	0.283 (n.s.)
DCI	0.676	0.783	0.681	0.787
Landau index, h'_1 (significant dyads)	0.552 ($P < 0.05$)	0.724 ($P < 0.001$)	0.333 (n.s.)	–
DCI_1 (significant dyads)	0.88	0.87	0.86	–

the percentage of one-way relationships was much higher already in period 1 compared to dwarf goats. The proportion of circular triads was below 25% in both periods in goats, but twice as much already in period 1 in pigs. Finally, the percentage of dyads with a significant asymmetric outcome increased from 53.0% in period 1 to 63.6% in period 2 in dwarf goats. However, agonistic relationships in pigs were characterised by a much lower level of significant dyads in both periods.

The improved Landau index h' indicates that dwarf goats established a quasi-linear hierarchy significantly different from purely random relationships in both observation periods. The quite low DCI was caused by the persistent high percentage of two-way dyads. h' in goats is still significant in both periods even when only significant dyads were considered. Here, the higher values of the DCI (0.87–0.88) mirrored a more stable amount of unidirectionality of the hierarchy when including only clarified dyads. The measures of strength of hierarchy in pigs (h', DCI) indicate only in period 1 a quite “moderate” quasi-linear hierarchy but less pronounced than in goats. In period 2, h' was not significant while the DCI still referred to a certain level of unidirectionality. h' in pigs is no longer significant in period 1 considering only the significant dyads. Nevertheless, the DCI is similar to the values in dwarf goats. Due to the low level of significant dyads in period 2 (2.8%) we did not calculate h' and DCI including only significant relationships.

For dwarf goats the rowwise matrix correlation between both observation periods revealed an averaged Spearman’s rho of 0.602 ($P < 0.001$) indicating a certain level of stability of the rank order over a period of 6 weeks. Correlation between ranks calculated using the Spearman’s rank order correlation was significant as well ($R_s = 0.71$, $P < 0.01$). For pigs we did not calculate the matrix correlation because of the high number of unknown dyads combined with low level of AI in period 2.

Individual AGI, DI_{dom} and ordinal ranks for goats and pigs are given in Table 3. In dwarf goats slight changes of individual ranks from period 1 to period 2 indicate a certain level

Table 3
Sociometric indices at individual level of a male dwarf goat group ($n = 12$) and a pig group ($n = 10$) for two observation periods (for further details, see text)

Animal	Dwarf goats						Pigs					
	Period 1			Period 2			Period 1			Period 2		
	AGI	DI_{dom}	Rank	AGI	DI_{dom}	Rank	AGI	DI_{dom}	Rank	AGI	DI_{dom}	Rank
1	2.8	77.8	4	2.9	50.0	5	1.4	37.5	7	0.3	83.3	1
2	4.1	80.0	3	3.4	36.4	9	1.8	55.6	4	0.4	50.0	4
3	5.4	40.0	7	3.7	70.0	3	2.0	75.0	2	0.7	40.0	6
4	6.1	18.2	10	3.2	50.0	6	2.3	100.0	1	0.6	80.0	2
5	4.8	90.0	1	6.9	90.9	2	1.8	71.4	3	0.4	80.0	3
6	5.2	0.0	12	5.1	0.0	12	1.4	28.6	8	0.1	25.0	8
7	3.4	72.7	5	2.2	44.4	7	2.7	50.0	5	0.3	33.3	7
8	2.0	33.3	9	1.9	44.4	8	0.6	50.0	6	0.3	50.0	5
9	4.1	55.6	6	2.8	70.0	4	0.9	12.5	9	–[a]	–[a]	–[a]
10	4.8	81.8	2	5.1	100.0	1	1.5	0.0	10	0.8	14.3	9
11	3.3	40.0	8	2.3	36.4	10						
12	6.2	18.2	11	4.0	9.1	11						

[a] Pig number 9 died after period 1.

of social dynamics of the hierarchy especially in the middle of the dominance order. We found a quadratic relationship between the $\mathrm{DI}_{\mathrm{dom}}$ and the AI only in observation period 2 ($R^2 = 0.72$, $P = 0.003$). High-ranking and low-ranking dwarf goats were involved in a higher number of agonistic interactions than middle-ranking individuals. Moreover, the four highest-ranking goats had as much AI with the four lowest-ranking individuals as among themselves ($\mathrm{AI}_{\mathrm{Rank\ 1–4\ versus\ 9–12}} = 16.2$ versus $\mathrm{AI}_{\mathrm{Rank\ 1–4}} = 19.0$; n.s.). In pigs we did not found any relationship between $\mathrm{DI}_{\mathrm{dom}}$ and the AI in none of the both observation periods.

4. Discussion

4.1. Agonistic behaviour

Results concerning sociometric parameters at dyadic level, group level and individual level reveal general differences concerning the observability and usefulness of overt agonistic interactions in dwarf goats and pigs. A high AGI in dwarf goats in both observed periods confirmed observations of Eccles and Shackleton (1986), Thompson (1993), Fournier and Festa-Bianchet (1995), Barroso et al. (2000) and Côté and Festa-Bianchet (2001) all of whom found a high level of AI in free-ranging and captive horned gregarious ungulates even when groups are staying together for longer periods of time. On the contrary, in pigs the AGI was much lower already in period 1 and nearly absent in period 2. This fits studies of Meese and Ewbank (1973), McGlone (1986), and Arey and Franklin (1995) who observed a sharp decrease of agonistic interactions in piglets within a few days following grouping. The drastic reduction of overt aggression in pigs is predominantly caused by increasing familiarity of the group members rather than by their actual genetic relatedness (Puppe, 1998; Stookey and Gonyou, 1998). However, the constantly high level of overt agonistic behaviour in dwarf goats interferes with a widely accepted assumption on the general function of social hierarchies reducing agonistic interactions in group living animals (Keiper, 1986; Drews, 1993; Lindberg, 2001). Gonyou (2003) argued that the high degree of relatedness among individuals in natural family groups and scattered availability of resources at various times of the year causes the decrease of aggression over time in the gregarious pig. However, goats are a gregarious species as well, living in female-dominated groups of related individuals. Therefore, we assume that the observed species-dependent variations in the level of agonistic interactions are primarily caused by a different kind of fighting behaviour in pigs and goats. Whereas pigs use their lower tusks as weapons in social conflicts and often try to bite and heavily injure their counterpart (Frädrich, 1974; Beuerle, 1975; Gonyou, 2001), goats and other horned Bovidae fight in a highly ritualised form where damages or serious injuries are really seldom (Shank, 1972; Barrette, 1986; Festa-Bianchet, 1991). Therefore, the costs of fighting in goats appear to be lower, and it is not disadvantageous for them to maintain agonistic interactions at a high level even in stable hierarchies (Clutton-Brock et al., 1979; Drews, 1993). Because of potentially higher costs of fighting pigs, however, are forced to reduce this behaviour drastically once the hierarchy is established and, thenceforward, prefer distance threats, vocalisations or active avoidance behaviour to sustain rank order (Frädrich, 1974; Beuerle, 1975). Hence, it is not surprising that some studies have preferred to use these behavioural patterns of submission and avoidance when studying social relationships, especially in well-established groups of animals (Jensen, 1982; Hunter et al., 1988; Araba and Crowell-Davis, 1994; van Dierendonck et al., 1995; Bradshaw et al., 2000). But some problems should be noted when using such behavioural patterns. First, the use of behavioural signs of avoidance and submission demands an extremely precise and reliable recognition of often subtle behavioural features which may increase the level of subjectivity and decrease comparability between different studies (Beilharz and Mylrea, 1963; Beilharz and Zeeb, 1982; Jensen, 1980). Moreover, avoidance or submission are observable only when sufficient space is available for subordinates to show adequate behaviour already over a distance or to avoid anticipatory dominants (Jensen, 1982). Secondly, analysing such behaviour patterns is probably driven by different motivations resulting in a 'subordination hierarchy' or 'avoidance order' often contrary to the dominance order (Rowell, 1974; Jensen, 1982). Third, submissive behaviour especially in captivity is strongly related to the hormonal status (Rowell, 1974) and both may be rather incorporated in a general stress response than in the formation of a dominance hierarchy.

A further common approach to overcome the problem of a lack of overt agonistic interactions in stable groups is to force animals to show overt agonistic behaviour, e.g. when valuable resources are restricted. In contrast to the 'aggressive order' described above such measurements result in a 'competitive order' which should be validated in terms of its measurement of priority of access as well as its generality (Syme, 1974). Being aware of the criticism that results from paired contests do not necessarily deliver a reliable image compared to real dominance relationships within the whole group (Stricklin et al., 1985; Craig, 1986; Craig and Ramos, 1986) we suggest short-term restricted resource availability to encourage agonistic encounters but observed in the entire group and within their normal home pen (Rushen, 1984; Craig, 1986; Lindberg, 2001).

4.2. *Sociometric measures*

The percentage of one-way dyads, two-way dyads and ties illuminates different aspects on the quality of the collected data with regard to the directionality of dyadic relationships. The higher amount of dyads of the first type indicates a lower level of bidirectionality over all dyads in pigs compared to dwarf goats already immediately after grouping (van Hooff and Wensing, 1987; Koenig, 2000). This fits the general assumption that pigs solve rank problems within a few days after grouping and thereafter establish a stable dominance order (Meese and Ewbank, 1973; McGlone, 1986). However, the definition of a dyad as unidirectional or bidirectional alone does not specify unambiguously the level of asymmetry of its outcome from a statistical point of view. The percentage of statistically significant dyads is much lower in pigs in period 1 compared to dwarf goats. An important reason for this discrepancy is probably the much lower number of agonistic interactions observable in pigs in the same observation time as in goats. For our understanding, significant dyads are most important to evaluate the overall quality of collected data on social relationships as they assess objectively the temporal consistency of the outcome of dyadic interactions. Moreover, this parameter answers the general claim formulated in various theoretical papers to judge the strength of single dyads at first (Boyd and Silk, 1983; Drews, 1993), and is thus necessary to evaluate the explanatory power of further sociometric measures calculated at the group level as well at the individual level. This is all the more important since following sociometric procedures often do not consider the strength of single dyads of the matrix. For instance, broadly used indices to evaluate the strength of hierarchy of a group like the Kendall index or the corrected Landau index handle a number of dyads with a ratio of wins to defeats of six to five (n.s., sign rank test) as clarified (i.e. as consistent in the outcome) in the same way as for a number of really asymmetric dyads with a ratio of wins to defeats of six to one ($P < 0.01$, sign rank test).

In this study, results concerning the corrected Landau index reveal the formation of a quasi-linear dominance order more transitive than it would be expected by chance within a few days after grouping of previously unacquainted individuals both in dwarf goats and pigs. Similar results were obtained in a number of investigations concerning wild, feral and domestic goats (Addison and Baker, 1982; Fournier and Festa-Bianchet, 1995; Côté, 2000; Barroso et al., 2000). The much weaker hierarchy in pigs compared to goats was caused by two different factors: the number of tied dyads and the number of circular triads both double as high in pigs as in dwarf goats. Both parameters are likely to be influenced by the much lower number of agonistic interactions observed in pigs. Searching literature on social dominance in pigs we found many studies which have postulated a nearly linear hierarchy and, consequently, have calculated individual ranks (Beilharz and Cox, 1967; Meese and Ewbank, 1973). But it was also mentioned that spontaneous changes of rank can occur quite often (Meese and Ewbank, 1972). Surprisingly with regard to pigs, we do not know any published investigation which really analysed the strength of the hierarchy using sociometric matrices as presented in this paper. Based on our results it is questionable if the hierarchy of a large group of pigs under commercial housing conditions is as strong as formerly assumed. However, because of the smaller sample size (number of agonistic interactions) compared to goats, we cannot definitely decide whether social relationships were indeed rather intransitive in pigs or if the lower degree of linearity might be influenced by methodological effects. Several authors have postulated a rather stable and more transitive hierarchy for small groups but more triangular relationships, consequently resulting in a more intransitive and complex hierarchy for larger groups (Gonyou et al., 1992; Moore et al., 1996; Gonyou and Stricklin, 1998). It seems that the broad uniformity of pig groups in commercial production systems (e.g. weight, strain) exacerbates the recognition of reliable signs indicating dominance (Ewbank, 1976; Rushen, 1988).

If we accept the theoretical approach that dominance refers to dyads and a dyad is clarified only if there is a clear winner and a loser in the last consequence we have to test each single dyad for significant asymmetric outcome and afterwards should calculate sociometric parameters including clarified dyads in the matrix only. When analysing h' including only the significant dyads we still found a significant quasi-linear hierarchy in goats, but not in pigs anymore. Whereas this very puristic approach is hard to realise in free-ranging animals, however, it has been already successfully applied in hens under stall housing conditions (Martin and Beaugrand, 1997).

Both dyadic parameters and group parameters indicate an increase of the stability of pair relationships as well of the strength of hierarchy in period 2 in dwarf goats. Nevertheless, the percentage of two-way relationships was still more than 50% in period 2 meaning that the majority of all subordinates were still winning several fights. Stewart and Scott (1947) and Ross and Scott (1949) found only a low number of pairs of goats to have strictly unidirectional relationships. Probably not all observed fights were solely related to agonistic behaviour. Shank (1972) described two different forms of agonistic interactions in feral goats. “Dominance fights” are highly ritualised and primarily related to the establishment of a rank order, whereas so-called “sparring fights” most probably have its function in developing fighting abilities and reaffirming the existent rank order.

Several authors postulated highly stable dominance hierarchy over time in groups of wild, feral and domestic ungulates (Rutberg, 1983; Keiper and Sambraus, 1986; Hass, 1991; Fournier and Festa-Bianchet, 1995; Barroso et al., 2000; Côté, 2000). In general, similar results were found for dwarf goats in this study. However, slight changes in the rank positions of single individuals occurred especially in the middle of the hierarchy still indicating a certain level of social dynamics. Probably, this flexibility was related to balanced age, weight and size of the individuals as well as its young age. Various authors assumed more flexible hierarchies in young animals compared to highly stable hierarchies in adult ones (Schloeth, 1961; Reinhardt and Reinhardt, 1975; Bouissou, 1985).

In the present case study, it was impossible to statistically test the stability of hierarchy over time in pigs because of the low number of overt AI, and resulting, the high number of unknown dyads in period 2. Although there are only few studies which have actually correlated the rank order of pigs at two different times, it was found that dominance relations in pigs are often situation-dependent, and thus, relatively unstable (Meese and Ewbank, 1972). Puppe and Tuchscherer (1994) have shown that the rank order in pigs had a certain short-term stability, but is extremely unstable in the long term.

The significant curvilinear relationship between the DI_{dom} and the individual AGI as well as the distribution of AI between goats with different ranks indicate that high-ranking goats have interacted with rank neighbours as much as with the low-ranking individuals. The latter is in contrary to results in some free ranging ungulates where dominant animals do not interact very often with low-ranking individuals (Thouless and Guinness, 1986; Thouless, 1990; Côté, 2000). However, in those studies subordinates had the possibility to avoid encounters with dominants. In an investigation with flocks of White Leghorn pullets reared in small pens. Lee and Craig (1981) found a similar curvilinear relationship between AGI and rank as we did. We assume different motivations in high-ranking goats when interacting with adjacent rank neighbours or with low-ranking individuals. Whereas the first is important for rank conservation or to climb hierarchy, with regard to the winner-winning theory (Jackson, 1991; Chase et al., 2002), interactions with low-ranking individuals encourage self-confidence of the dominants.

5. Conclusions

Because dominance is a multi-dimensional phenomenon it is necessary to characterise dominance at different levels of analysis (dyad, group, individual) as well as with well-defined and preferably standardised sociometric methods delivering a comprehensive “picture” of the social hierarchy of the observed species. Therefore, a suggestion of useful sociometric measures (see Table 1) and their application is given in the present paper. Actually, the use of individually calculated ranks for correlation with other animal features (e.g. stress level, performance, other behaviours) is only allowed when the interpretation of the sociometric measures both at the dyad level and at the group level reveals no serious limitation. Additional measures which are only indirectly related to the rank order but describe some further aspects of agonistic behaviour (e.g. the kind or level of agonistic behaviour of a whole group) may be helpful when it is difficult to monitor an exact dominance hierarchy or when it is sufficient to describe more general relationships between agonistic behaviour and physiological features.

Whenever we study social relationships in a group of animals we have to bear in mind that "for an organisation at any given level, its mechanism lies at the level below and its purpose at the level above" (Capitanio, 1991). Whereas the dominance status of an individual refers to a dyadic relationship the dominance rank characterises its position within a group. Following the structural definition of dominance by Drews (1993) the dyad is the starting level when analysing any kind of dominance relationships. Testing the outcome of all possible dyads over the whole group is the very basis to evaluate any other sociometric parameter both at the lower level (e.g. individual rank) and at the higher level (e.g. strength and stability of group hierarchy). Thereby, it is not enough to assume an animal as dominant in a dyadic relationship because it simply has won more often in a number of encounters with the same opponent or judging the outcome using various empirical indices. An animal is considered dominant over another individual by definition only if it wins significantly more than it loses. Other pair-related parameters like the number of unknown dyads are useful to evaluate a certain behaviour as an essential feature of the majority of all dyads and therefore suitable to study dominance (coverage). Although clarified dyadic relationships are the main prerequisite to calculate dominance hierarchy they do not necessarily deliver explicit information about its character. It is therefore important to determine the strength of the hierarchy and to test it for statistical significance before we have a reason to analyse individual dominance ranks. With regard to individual dominance ranks we suggest to prefer procedures which deliver cardinal equivalents of ordinal ranks reflecting the more realistic "dominance distance" between single individuals, whereas ordinal measurements may distort the relationship between dominance and related variables. Overall, a more uniform and standardised approach of social dominance in farm animals may improve the scientific value of a single study and makes it easier to compare various studies within a species and between different species.

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Appendix A

Agonistic interaction matrices of a group of dwarf goats and a group of pigs analysed in this study: (a) dwarf goats—period 1; (b) dwarf goats—period 2; (c) pigs—period 1; (d) pigs—period 2.

(a)

loser

		1	2	3	4	5	6	7	8	9	10	11	12
winner	1		6	5	5	3	9	6	2	6	7	4	11
	2	7		9	14	17	18	5	8	5	2	6	21
	3	5	5		17	2	38	4	4	4	1	6	45
	4	2	1	2		2	24	3		8		8	20
	5	5	5	12	9		22	8	1	7	12	17	34
	6	1											2
	7	4	6	6	25	2	8		3	7	4	7	9
	8	2	1	3	9	1	4	2		1	1	2	3
	9	4	5	7	18	3	21	5	10		1	3	22
	10	2	5	12	45	8	18	5	4	7		8	25
	11	3	2	8	9	1	12	4	1	3	5		8
	12						9		9	1		3	

(b)

loser

		1	2	3	4	5	6	7	8	9	10	11	12
winner	1		4	3	9	3	6		1	6	2	5	12
	2	5		2	2	3	23	4	2	2		2	14
	3	3	3		6	1	32	5	6	1		7	12
	4	6	10	1		5	4	2	1	2	1	6	8
	5	12	14	12	24		29	13	13	24	7	16	44
	6								1				
	7	1	2		1	3	19			1		3	12
	8	3	3	3	1		19						3
	9	3	8	6	3	1	12	1	4		3	8	4
	10	18	17	23	23	24	18	9	5	12		5	14
	11	2	4	3	1		12	2	1		1		6
	12			5			7	1	1				

(c)

loser

		1	2	3	4	5	6	7	8	9	10
winner	1			2	2		5	1		3	4
	2	7		1		2	7		5	13	4
	3	5	15			1	3	1	6	2	7
	4	3	10	12		15	6	4	4	7	1
	5		12	5	4			4	7	4	
	6		5	1	1	2		1		3	3
	7		1	1	1		1		1	1	2
	8	9	3	1	1	3	5	1		8	1
	9	4							1		1
	10	1	1		1		3			1	

(d)

loser

		1	2	3	4	5	6	7	8	10
winner	1		5	2			1	1		3
	2	1					1	1		3
	3	1	2							2
	4			1			1		2	1
	5	1	2	2				1		
	6									3
	7				1					2
	8							1		
	10		1			3	1	1		

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A comparative view on social hierarchy formation at different stages of pig production using sociometric measures

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Abstract

A standardised and comprehensive approach to describe dominance relationships in gregarious farm animals quantitatively was recently developed, incorporating a combination of appropriate sociometric measures. The present study applied this approach to a comparative analysis of the social hierarchies within 57 groups of domestic pigs at different age/production stages with a total of 496 animals. Unacquainted pigs were grouped to three age categories which correspond to the typical production stages: weaned pigs (PIG28, 12 groups), growing pigs (PIG80, 16 groups), and reproductive sows (SOW, 29 groups). Based on observed agonistic interactions, sociometric values were calculated both at the dyadic and at the group level and may be considered as preliminary reference values for further studies. As indicated by the respective values of the Kendall index (PIG28: 0.66, tested as significant in 69.0% of the observed groups; PIG80: 0.71, 87.5%; SOW: 0.61, 69.0%), and the improved Landau index (PIG28: 0.70, 75.0%; PIG80: 0.72, 93.7%; SOW: 0.71, 72.4%), a social organisation towards a quasi-linear social hierarchy was predominantly developed throughout all age/production categories. However, compared to weaned and growing pigs, sows were characterised by significant differences concerning establishment (fewer agonistic interactions) and kind (more unknown dyads, fewer two-way and significant dyads, higher directional consistency index) of their social hierarchy. It seems that sows have effectively adapted their agonistic behaviour towards pen-mates to regulate social dominance relationships, whereas younger pigs frequently display agonistic interactions also to gain additional experience on social cues (e.g. the fighting ability of an opponent). Hence, it is concluded that the effective experience of socialisation during sensitive periods may increase the social skills of pigs which in turn can improve their welfare and health, e.g. by adjusted aggressive behaviour. The consideration of comparable and standardised sociometric measures in livestock breeding may help to improve husbandry conditions.

Keywords: Pig; Dominance; Sociometric measures; Social hierarchy; Ontogeny

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1. Introduction

Evolutionary advantages could be gained by living in groups and, as a consequence, this may have lead to the development of social interactions and relationships among members of the group (Mendl and Held, 2001). The social structure of domestic pigs, *Sus scrofa*, is based on a dominance hierarchy which is established after vigorous fighting when unacquainted pigs are brought together (Meese and Ewbank, 1973; Puppe and Tuchscherer, 1994). Amongst newly acquainted pigs

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mutual recognition during aggression seems to be related to an acquired familiarity (Puppe, 1998; Stookey and Gonyou, 1998), although the mechanisms underpinning individual discrimination remain rather unclear (McLeman et al., 2005). The decrease in aggression after mixing may be caused by an increase of simple habituation or, more obviously, by an increase of the clarity of mutual dominance relationships (Rushen, 1988; Jensen and Yngvesson, 1998). The repeated regrouping which is common in pig production, however, means that new hierarchies have to be established frequently. It is well known that high rates of aggression are involved in this process, which in turn may cause serious problems in animal welfare and performance (e.g. Stookey and Gonyou, 1994; Puppe and Tuchscherer, 1994; Puppe et al., 1997; Gonyou, 2001).

Social housing of sows is required by EC directive 2001/88/EC (EU, 2001). To adequately consider the process of social hierarchy formation respective knowledge of the animals' current and previous behaviour is necessary. However, the possible effects of regrouping on sociometric measures describing the hierarchy structure itself have not been systematically studied. Recently, we have suggested a comprehensive approach to analyse dominance relationships in animals by the consistent use of appropriate sociometric measures at different levels of analysis (Langbein and Puppe, 2004). This approach is important for two specific reasons. Firstly, the use of individually calculated ranks is only reasonable when sociometric measures at the dyadic level and the group level provide an adequate justification for their calculation. This would possibly improve the scientific value when correlating social rank with other biological parameters. Secondly, the establishment and implementation of a consistent and standardised approach for analysing social dominance would facilitate intra-and inter-species comparisons.

Therefore, it was the aim of this paper to investigate the social hierarchy formation in groups of domestic pigs (*Sus scrofa*) at different ages, utilising the suggested approach. For this study the three ages which correspond to the three typical stages of pig production were considered as these usually involve the mixing and regrouping of some or all animals: weaned pigs, growing pigs and reproductive sows. As this paper was the first study in which the same sociometric measures were consistently taken over a large number of social groups, a further goal of the present study was to investigate possible correlations that may exist between the sociometric measures of the different groups which were observed.

2. Materials and methods

2.1. Animals and housing

The present study is based on data of agonistic interactions of 57 newly-mixed groups, consisting of 8–10 pigs of the German Landrace per group, a total of 496 pigs, which were observed during the first 2 to 3 days after regrouping and mixing. The observed groups fell into three different age categories which corresponded to the typical stages of pig production: weaned pigs, growing pigs and reproductive sows. The housing, feeding and management conditions were also comparable with those used in commercial production. All members of these newly constituted groups were unfamiliar to each other and had similar weights. Animals were fed with commercial feed pellets. The respective standard rations could feed quasi ad libitum meaning that the animals had continuous access to feed but the feeders were filled manually twice a day, i.e. in the morning and in the afternoon. Water was available ad libitum through nipple drinker. The key data of all groups under observation are summarised in Table 1.

Firstly, 12 groups of castrated male piglets were observed immediately after weaning and mixing (Ernst et al., 2005) on day 28 of age (PIG28). Feed was supplied by a commercial feeding dispenser with four

Table 1
Key data of pig groups in three age/production categories during the observation periods

	PIG28	PIG80	SOW
Observed groups (No.)	12	16	29
Group size (animals/group)	10	9	8
Weight/animal (kg) (mean ± SD)	8.5 ± 0.8	34.6 ± 2.1	229.7 ± 18.9
Floor space/animal (m^2)	0.44	0.71	2.50
Observation period (h)	24 (3 d × 8 h)	30 (3 d × 10 h)	48 (2 d × 24 h)
Feeding (feed supply)[a]	ad lib (twice/d)	ad lib (twice/d)	ad lib (twice/d)
Animal-feeding-place-ratio	2.50:1	2.25:1	4.00:1
Housing	Partly slatted floor with little straw	Slatted floor with little straw	Concrete floor with little straw

[a] All animal groups could feed their respective standard rations quasi ad libitum on the basis of two daily feeding times in the morning and in the afternoon where the feeder were filled manually.

feeding places. Secondly, 16 groups of growing pigs with an average ratio of 54.2% females to 45.8% castrated males (Otten et al., 1999), were observed after regrouping on day 80 of age (PIG80). Feed was given in a feeder with four feeding places. The third age category observed consisted of 29 groups of multiparous, reproductive sows (SOW) which were grouped for the first time, as described by Hoy and Bauer (2005) in so-called stimulation pens. Previously, the sows were weaned from individual farrowing crates after an average nursing period of 25.5 days. Just as the weaning and growing pigs, the sows were unfamiliar to each other and were not housed together during previous production periods. Their parity number ranged from 2 to 9 and they were fed from a tube feeder with two feeding places.

2.2. Behavioural observations, sociometric and statistical analysis

The present study was based on the observation of agonistic interactions (AI) within dyads of pigs and the subsequent analysis of the dominance relationships between all group members. As shown in Table 1 all groups were observed during the first 2 to 3 days after regrouping for a total time varying between 24 and 48 h. The occurrence and outcome of all AI within the observation period were observed directly (PIG28 and PIG80; see Otten et al., 1999) or by video (SOW; see Hoy and Bauer, 2005). The AI were recorded by experienced observers with the characteristics of an AI clearly defined. As described in numerous previous studies, an AI was defined as an overt fight or a displacement with physical contact initiated by one individual and featuring aggressive behavioural elements typical for pigs, followed by any form of submissive behaviour performed by the opponent (Puppe, 1998; Tuchscherer et al., 1998; Otten et al., 1997, 1999; Langbein and Puppe, 2004; Hoy and Bauer, 2005).

The gathered data were transformed into a winner–loser matrix with the rows labelled as wins and the columns as defeats. Sociometric measures used to describe dominance relationships at dyadic and group level, as shown in Table 2, were calculated based on these matrices and strictly followed the approach used by Langbein and Puppe (2004). As all of these sociometric measures were clearly defined and previously discussed by Langbein and Puppe (2004), only a short description shall be given here.

Firstly, the sociometric values were analysed at a dyadic level, i.e. the percentages of unknown or tied dyads (dyads with no observed interactions or with the same number of wins for both individuals), one-way dyads (uni-directional relationships with wins only for one individual) or two-way dyads (bi-directional relationships with wins for both individuals), and the percentages of circular triads (intransivities, e.g. A is dominant over B, B is dominant over C, but C is dominant over A) within any given group. All calculations were done using MatMan (version 1.0 for Windows, Noldus Information Technology, Wageningen, The Netherlands) — a commonly used software program for the analysis of sociometric matrices (de Vries et al., 1993). In addition, the percentage of significant dyads was calculated as the percentage of dyads with a significant asymmetric outcome (e.g. 5 wins vs. 0 defeats or 6 wins vs. 1 defeat, etc.) explicitly tested by a sign test (Puppe and Tuchscherer, 1994; Langbein and Puppe, 2004).

Secondly, various sociometric measures were analysed at a group level. The group agonistic index (AGI) was the overall agonistic level of a group expressed as the number of AI per individual and hour (Puppe and Tuchscherer, 1994; Puppe et al., 1997). Subsequently, sociometric measures describing various aspects of the strength or linearity of the social hierarchy were calculated using the respective indices provided by

Table 2
Sociometric measures (mean±SE) of pig groups in three age/production categories

	PIG28	PIG80	SOW
Dyadic level			
Unknown dyads (%)	10.00±2.25 a	2.60±0.82 a	22.29±2.23 b
One-way dyads (%)	60.56±4.11 a	35.07±2.18 b	68.35±2.36 a
Two-way dyads (%)	29.44±3.55 a	62.33±2.61 a	9.36±1.32 b
Tied dyads (%)	4.63±1.08	5.38±1.15	2.95±0.59
Circular triads (Number)	13.56±2.73	8.67±0.92	7.73±0.68
Significant dyads (%)	37.59±4.78 a	53.64±3.58 a	22.66±1.71 b
Group level			
Group agonistic index, AGI	2.01±0.18 a	2.46±0.21 a	0.45±0.02 b
Kendall index, *K*	0.66±0.07	0.71±0.03	0.61±0.03
Significant groups (%)	75.0	87.5	69.0
Improved Landau index, *h′*	0.70±0.06	0.72±0.03	0.71±0.03
Significant groups (%)	75.0	93.7	72.4
Directional consistency index, DCI	0.78±0.03 a	0.71±0.02 a	0.91±0.01 b

Significant differences (at least $P<0.05$) between the age/production categories are indicated by different letters.

MatMan (see Appendix), i.e. the Kendall index (K, see Appleby, 1983), the improved Landau index (h', see de Vries, 1995), and the directional consistency index (DCI, see van Hooff and Wensing, 1987). Whereas K expresses the degree of linearity in a hierarchy based on the concept of 'circular triads', h' refers to the concept of 'number of animals each individual dominated' corrected for tied and unknown dyads (de Vries, 1995). The DCI, in addition, reflects the frequency with which wins occurred in the more frequent direction relative to the total number of agonistic interactions. To determine the statistical significance of linearity for each observed group the χ^2 procedure (K) and a two-step randomisation process using 10000 randomisations (h') were performed. All these indices range from 0 (complete absence of linearity and equal exchange, respectively) to 1 (strictly linear hierarchy and complete uni-directionality, respectively).

The overall effect of age/production category (PIG28, PIG 80, SOW) on single sociometric measures was estimated with a nonparametric ANOVA (Kruskal–Wallis Test) using the statistical software package InStat (version 3.06 for Windows, GraphPad Software Inc., San Diego, CA, USA). In case of significant effects, subsequent Dunn's post-hoc tests (Siegel and Castellan, 1988) were applied to test pairwise differences between age/production categories. To detect possible correlations between different sociometric measures within each age category Spearman's rank correlation procedure was performed.

3. Results

3.1. Sociometric measures

Means of all calculated sociometric measures for the observed pig groups of all three age/production categories are shown in Table 2. At the dyadic level, age/production category significantly affected the percentage of unknown dyads ($\mathrm{KW}=32.2$, $P<0.001$), one-way dyads ($\mathrm{KW}=32.3$, $P<0.001$), two-way dyads ($\mathrm{KW}=43.7$, $P<0.001$) and significant dyads ($\mathrm{KW}=29.7$, $P<0.001$). However, tied dyads and circular triads were unaffected by age/production category. Post-hoc tests revealed that the SOW groups had a higher percentage of unknown dyads and, correspondingly, a lower number of two-way dyads and significant dyads compared to the PIG28 and PIG80 groups (see Table 2). The only difference between weaned and growing pigs was that the PIG80 groups had fewer one-way dyads when compared to the PIG28 and SOW groups.

At the group level, age/production category significantly affected the AGI ($\mathrm{KW}=42.6$, $P<0.001$) and the DCI ($\mathrm{KW}=31.9$, $P<0.001$), but not h' and K. Compared to the PIG28 and PIG80 groups the SOW groups had the lowest AGI and the highest DCI (see Table 2). Table 2 also shows that h' and K reached values between 0.61 and 0.72 in all groups of the observed age/production categories indicating the establishment of a social organisation towards a quasi-linear social hierarchy throughout all the age/production categories. This was supported by the fact that both linearity measures were tested as significant in the majority of all groups (between 69% and 75% of all observed groups for K, between 72.4% and 93.7% of all groups for h', Table 2). Here, the highest values were reached by the PIG80 groups.

3.2. Correlations between sociometric measures

Altogether nine correlations were found between those sociometric measures analysed in this study which were tested as significant (at least $P<0.05$) in each of the age/production categories (Table 3). As one could expect, with increasing AGI the percentage of unknown dyads decreased and the percentage of significant dyads increased. This entails that unknown dyads were negatively correlated with significant dyads.

Table 3
Spearman correlation coefficients between sociometric measures tested as significant (AGI = Group agonistic index, DCI = Directional consistency index)

Measure 1	Measure 2	PIG28	PIG80	SOW
AGI	Unknown dyads	−0.765**	−0.890***	−0.735***
AGI	Significant dyads	+0.766**	+0.854***	+0.844***
Unknown dyads	Significant dyads	−0.912***	−0.741**	−0.663***
One-way dyads	Two-way dyads	−0.813**	−0.985***	−0.385*
One-way dyads	DCI	+0.925***	+0.524*	+0.596***
Two-way dyads	DCI	−0.625*	−0.523*	−0.820***
Circular triads	Kendall index, K	−0.998***	−0.997***	−0.998***
Circular triads	Landau index, h'	−0.993***	−0.998***	−0.977***
Landau index, h'	Kendall index, K	+0.993***	+0.998***	+0.977***

The table shows only those relationships which were calculated as significant in each of the age/production categories (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

With increased number of one-way dyads the percentage of two-way dyads decreased and the DCI increased indicating that all these measures reliably characterise the degree of directionality within the dyads. Consequentially, there was an inverse correlation between the two-way dyads and the DCI. Furthermore, an increase in the number of circular triads reduced the values of h' and K, the latter being positively correlated. This indicates a high mutual reliability of the calculated measures used to analyse the occurrence and strength of a social hierarchy.

4. Discussion

The present study is, to the best of our knowledge, the first systematic attempt to calculate well-defined sociometric measures during the formation of a social hierarchy in farm animals at different age/production stages in a consistent, comparative and comprehensive manner. The approach recently suggested by Langbein and Puppe (2004) was strictly applied to a relatively large number of domestic pig groups which were newly grouped at different stages of their ontogeny corresponding to typical stages of production.

4.1. Formation of a social hierarchy using sociometric measures

In all observed age/production stages the majority of groups was strongly motivated to establish a social hierarchy with a quasi-linear formation as indicated by the calculated values of h' and K, respectively. This corroborates previous studies (e.g. Beilharz and Cox, 1967; Meese and Ewbank, 1973) which surmised a social organisation of this type, however, on the basis of empirical reasons. Slight deviations between h' and K values appeared depending on the level of unknown dyads in a given matrix. As de Vries (1995) outlined in his critical review of the Kendall index, values of linearity of a hierarchy calculated by this index are systematically too low when unknown dyads occur. Indeed, the greatest deviations between K and h' occurred in the SOW groups of the present study, where also the highest percentages of unknown dyads were found.

However, the present study clearly shows that differences in important sociometric measures depend on the age/production stage. Despite the facts that the sows were observed over a longer period (48 h) and their animal-feeding-place-ratio was more unfavourable 4:1, a lower level of overt aggressive interactions between sows was noted compared to the two other age/production categories observed only for 24 (PIG28) or 30 h (PIG80) and with an animal-feeding-place-ratio of 2:1. This, as well as the higher amount of unknown dyads and the lower amount of significant dyadic relationships, indicated that sows rely on overt agonistic interactions likely to a lesser extent than younger pigs during the formation of a social hierarchy. This is supported by the study of Hoy and Bauer (2005), who found that the directionality of the hierarchy in sows (measured by the DCI) is unaffected by a high or low level of agonistic interactions. Therefore, it is reasonable to assume that sows have developed additional behavioural mechanisms to regulate their social relationships. It seems that, in the course of their ontogeny, sows have gained some social experience in establishing and maintaining a hierarchy without extensive use of overt aggressive behaviour. This result indirectly supports the suggestions of Jensen (1982) which indicate that groups of sows regulate internal aggressions trough an “avoidance order”. Moreover, the higher amount of unidirectional dyads and resulting higher DCI in sow groups indicated that adult, experienced pigs can better judge the individual fighting ability of an opponent compared to younger individuals. This could be realised, for instance, on the basis of certain morphological cues (Rushen, 1990). In contrast, weaned and growing pigs displayed no significant differences in their sociometric characteristics except for a lower level of one-way dyads in the growing pigs. The high bidirectional fighting between young pigs, however, appears to be at least partly motivated by the uncertainty concerning the relative fighting abilities and can be considered as a form of social exploration (Rushen, 1988, 1990). Additionally, when agonistic behaviour is viewed in terms of biological costs, it is reasonable to assume that the costs of fighting (e.g. serious injuries) in sows are higher than in younger pigs because of their higher physical potential. Indeed, it has been reported that fighting between sows is a serious welfare problem in pig production with detrimental effects on reproductive parameters (Arey and Edwards, 1998).

It is commonly assumed that competition over resources may affect the level of animal aggression and dominance. Housing and feeding conditions in the three observed age/production categories of the present study differed slightly so that influences on agonistic behaviour due to differences in age or competition can not be excluded. However, previous studies have shown that pigs fight predominantly after mixing in the pen area and not in the trough area (Puppe and Tuchscherer, 1994; Puppe, 1998). This fights result in a social hierarchy based on an aggressive order which is not related directly to a competitive order or resource usage (McGlone, 1986). Further studies are necessary to evaluate possible effects of resource competition on measures of social hierarchy.

Any explanation as to why there were only minor differences in the sociometric values of the studied pigs of either 4 or 12 weeks of age can only be speculative. Jensen (1994) also found that age (week 1, 5 and 9) did not influence the incidence of fighting with strangers in test situations. However, age determined the duration and course of the fighting. The constant social interactions (including the agonistic behaviour) between individuals during the early weeks of a pigs life may be thus interpreted to be the normal way in which pigs gain social experience and become socialised. Consequentially, it is reported that any social experience (e.g. by social mixing under controlled conditions) gained during these and other sensitive periods may not only increase social skills in young pigs (Pitts et al., 2000; D'Eath, 2004) but also in older animals (Van Putten and Buré, 1997; Hoy and Bauer, 2005).

4.2. Correlations between sociometric measures

As an important methodological result, significant correlations between several sociometric measurements were detected throughout all age/production categories (see Table 3). The highly "logical" pattern of mutual relationships shown by the results increases the reliability and consistency of the calculated sociometric values and may serve as an internal validation. A high correlation could be verified between h' and K values which both can be regarded as different measures of linearity for a dominance hierarchy. Furthermore, both showed a strong negative correlation with the number of circular triads exhibited. As both indices are based on different concepts (h': number of dominated animals, K: number of circular triads, see de Vries, 1995) and circular triads indicate intransitivities in the dominance relationships, all these correlations support the conclusion that a quasi–linear hierarchy was predominantly developed throughout all observed pig groups. Surprisingly, no consistent correlation was found between either h' and DCI nor between K and DCI in any age group. Although in several studies the DCI was used as an indicator of linearity within hierarchies (Koenig, 2000; French and Smith, 2005; Roden et al., 2005) and was quite high in the SOW groups it should rather be interpreted as an index of directionality in the dyadic interactions. Correlation between two-way dyads and the DCI but not between two-way dyads and h' or K support this theory. The AGI was inversely correlated to the number of unknown dyads and positively correlated to the number of significant dyads, but did not show any correlation with the measure of hierarchy strength. This suggests that at least a part of the agonistic behaviour which occurred after the grouping of unacquainted pigs was motivated by reasons other than purely establishing a dominance hierarchy. Indeed, it has been discussed that piglets may aggressively react also to the strangeness of a subject, maybe to drive away a stranger (Puppe, 1998). It must be noted that the present study deliberately used data obtained from pig groups under "productive conditions", therefore, the observed three age/production periods partly differed in their housing conditions. That means that a variation in certain housing conditions (e.g. feeding) may also affect characteristics of the agonistic behaviour. Nevertheless, the calculated sociometric measures firstly provide a comprehensive "picture" of the social hierarchy of pigs within different stages of production. Furthermore, the calculated data may serve as preliminary reference values for similar investigations in pigs or in other species.

4.3. Conclusions

Commercial used pig groups develop a social hierarchy after grouping at all different stages of production. However, compared to weaning and growing pigs, sow groups are characterised by important differences concerning establishment (e.g. fewer agonistic interactions) of their social hierarchy and the form that it took (e.g. lower level of bi-directionality). It seems that sows have effectively adapted their agonistic behaviour towards pen-mates to regulate social dominance relationships, whereas younger pigs seem to perform agonistic interactions frequently to gain additional experience on social cues (e.g. the fighting ability of an opponent). It can be indirectly concluded that the effective experience of socialisation during sensitive periods may increase the social skills of pigs resulting in improved welfare and health, e.g. by adjusting aggressive behaviour. Further studies under experimentally varying conditions using the methods described should be made to evaluate other factors influencing the sociometric measures of hierarchy formation. Nevertheless, the consideration of comparable and standardised sociometric measures in livestock breeding can uncover processes of social hierarchy formation, and thus, may help to improve husbandry conditions.

Appendix A

Calculation of indices used for estimation of linearity of a social hierarchy

(1) Kendalls coefficient of linearity K (Appleby, 1983):

$$K = 1 - \frac{24d}{N^3 - N} \qquad \text{for odd values of } N$$

$$K = 1 - \frac{24d}{N^3 - 4N} \qquad \text{for even values of } N$$

where d is the number of circular triads, and N is the group size.

(2) Improved Landau index h' (de Vries, 1995):

$$h' = h + \frac{6}{N^3 - N}(\text{number of unknown dyads})$$

where

$$h = \frac{12}{N^3 - N}\sum_{i=1}^{N}\left(S_i - \frac{1}{2}(N-1)\right)^2$$

and N is the group size, and S the number of individuals dominated by individual i.

(3) Directional consistency index DCI (van Hooff and Wensing, 1987):

$$\text{DCI} = \frac{H - L}{H + L}$$

where H is the total number of interactions whose outcome is in the most frequent direction within each dyad, and L is the number of interactions occurring in the less frequent direction.

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Effects of Social Status After Mixing on Immune, Metabolic, and Endocrine Responses in Pigs

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TUCHSCHERER, M., B. PUPPE, A. TUCHSCHERER AND E. KANITZ. *Effects of social status after mixing on immune, metabolic, and endocrine responses in pigs.* PHYSIOL BEHAV **64**(3) 353–360, 1998.—The effects of social rank on immune, metabolic, and endocrine responses were studied in 10 newly mixed groups of German Landrace pigs (9 individuals each) at an age of 12 weeks. Immediately after mixing, the agonistic interactions (AI) of all group members were continuously recorded over 3 days (10 h daily). An individual dominance value (DV) was calculated by the number of wins minus defeats in relation to all decisive fights (DV ≤ 0, subordinate; DV > 0, dominant). Blood samples were taken 24 h before and 3 days after mixing. The data showed that the social status had a significant effect on lymphocyte proliferation in responses to different mitogens: socially dominant pigs had higher proliferative response than subordinate pigs. In addition, during the observation period the lymphocyte activation by mitogens increased in the dominant animals and decreased in the subordinate animals with increasing number of agonistic interactions. The rise in total serum IgG concentration 3 days after mixing was higher in dominant pigs compared with subordinates. The dominance status did not significantly affect plasma metabolic levels nor cortisol concentrations. However, mixing appeared to increase glucose and total protein values and to decrease alkaline phosphatase and cortisol levels in both, dominant and subordinate pigs. In conclusion, mitogen induced cell proliferation seems to be a valuable marker for acute social stress in pigs. © 1998 Elsevier Science Inc.

Pig Social stress Agonistic behavior Dominance Immunity Metabolites Cortisol

MIXING animals that are unfamiliar to each other is common in livestock production systems. In pig production, this is accompanied by increased agonistic behavior and may result in social stress (24,32,61). It is well known that stress modifies the secretion of various hormones which have different effects on the immune system (for review, see 7,25,52). Whereas the "classic view" states that stress causes a suppression of the effectiveness of the immune system (37), recent studies suggest a more differentiated influence of social stressors on various aspects of the immunologic defense mechanisms (10,26). Chronic stress in socially isolated animals was associated with the suppression of the immune function (15). In contrast, acute psychological stressors were found to enhance some immune responses. For example, short restraint stress enhanced the primary serum antibody response to sheep red blood cells in rats (49) and social reorganization in Rhesus monkeys resulted in significantly higher lymphocyte proliferation of high-ranking animals (12). A lack in immune reactivity and higher disease susceptibility was described in lower ranking pigs compared with dominants (31,47,56).

The aim of the present study was to investigate the short-term effects of the social status of unfamiliar, newly mixed domestic pigs on their physiological responses in this acute stress situation. To this end, we analyzed a complex of behavioral, immunologic, metabolic, and hormonal measures in order to characterize the interactions between behavior, immune system, and neuroendocrinum in a social stress paradigm frequently occurring in pig husbandry.

METHODS

Animals and General Procedure

Ten groups of pigs at an age of 12 weeks (German Landrace) were studied immediately after new mixing of nine unfamiliar pigs each. The groups were randomly mixed using only clinically healthy pigs ($n_{\max} = 90$) with approximately same weights (33.5 ± 3.2 kg, mean ± SD). This design was chosen to create the optimum environment for overt aggression, since wide variations of weights were shown to reduce the amount of time unfamiliar pigs spend fighting (60). The groups were kept on concrete floor (6.5 m^2) with little straw. There were limited feeding times from 0900 to 1100 and 1500 to 1700 hours (4 h daily), during which the animals had access to the trough (animal to feeding place ratio 2.25:1). Animals were given ad libitum access to water. The mean female to castrated male ratio was 56.7% to 43.3%.

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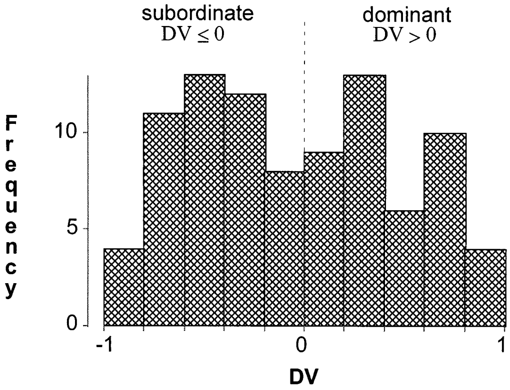


FIG. 1. Frequency plot of the dominance values (DV) of all observed pigs ($n = 90$).

Agonistic Behavior

Immediately after mixing, the number of overt agonistic interactions in each group was recorded by direct observation for 3 days (10 h daily from 0800 to 1800 h, continuous event sampling). An agonistic interaction (AI) was defined as a fight or a displacement event with physical contact of two individuals for more than 2 s and intervening periods of at least 8 s, while the fight either was interrupted or the pigs showed other behaviors. Fights occurred as overt bodily attacks like "head-to-head knocks" and "head to body knocks," "parallel/inverse pressings," "bitings," and "displacements" (35,45,62). Each end of a "decisive" fight was immediately evaluated by the observer regarding the possible outcome. The loser was defined as a pig which first stopped fighting, turned away from an attack and tried to flee or was displaced from a location (e.g., the trough). A typical movement was that the loser positioned itself in an asymmetric parallel configuration (61), whereas the pursuer (winner) turned around the loser and bit it. Matrices were generated for each group, and an individual dominance value (DV) was calculated for each animal by the numbers of wins minus defeats in relation to all decisive fights with the other group members over the whole observation period (53,55,56). DV was used as a relative measure which could vary between –1.00 (no wins) to +1.00 (no defeats). The pigs were divided into two categories with respect to the distribution of their DV (Fig. 1). Thus, animals with indexes greater than 0 were relatively successful as they won more than half of their fights. In the following they are labeled as dominant. In contrast, pigs with an index equal or smaller than 0 were considered to be subordinate.

Blood Sampling

Blood samples were taken from each pig in a supine position by anterior vena cava puncture in a separated room 24 h before mixing (baseline data), and the day after the 3-day behavioral observation period at 0800 hours (1 h before feeding). Fixation and venipuncture required less than 1 min per pig. One part of heparinized blood samples was centrifuged for 15 min at 2000 × *g* to separate plasma. Plasma samples were stored frozen at –20°C in polypropylene vials until they were assayed for glucose and cortisol levels. Another part was directly used in in vitro tests. Remaining blood samples were allowed to clot overnight at 4°C, centrifuged for 15 min at 1000 × *g* and serum was stored at –20°C until it could be analyzed.

Physiological Measures

Only one of the immunologic parameters taken in this study (lymphocyte proliferation index against the mitogen concanavalin A) was analyzed in all pigs of the ten observed groups ($n_{\max} = 90$). All other physiological measures were studied in all members of four randomly selected groups ($n = 36$).

In vitro cell-mediated immunity. Mitogen-induced lymphocyte proliferation was used as an in vitro index of cellular immune function, as lymphocyte stimulation in the presence of mitogens is recommended for testing the capacity of cell mediated immunity (6,34). Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by density gradient centrifugation. Briefly, 5.0 mL of blood, diluted with an equal volume of Roswell Park Memorial Institute (RPMI) 1640 culture medium were carefully layered on 4 mL of Histopaque 1077. After centrifugation (20 min at 400 × *g*), lymphocytes were collected from the interface and washed twice in RPMI-1640 medium (15 min at 100 × *g*). If erythrocytes remained after centrifugation, a Tris buffer (0.16 M NH4Cl; 0.17 M Tris) was used for lysing. Cell concentration was determined using a hemocytometer and was adjusted to 5×10^6 cells/mL of RPMI-1640 medium supplemented with sodium bicarbonate (25 mM), Hepes buffer (25 mM), L-glutamine (2 mM), 2-mercaptoethanol (0.05 mM), gentamicin solution (50 μg/mL) and 10% fetal bovine serum. Viability of cells was determined by trypan blue dye exclusion (>95%). All reagents were obtained from Sigma.

Two T cell-specific mitogens, concanavalin A (25 μg/mL; ConA) and phytohemagglutinin (25 μg/mL; PHA), and one T cell-dependent B cell mitogen, pokeweed mitogen (12.5 μg/mL; PWM), were used in the lymphocyte proliferation assay (all mitogens from Sigma). These concentrations of mitogens were shown to be optimum by dose titration experiments. Isolated lymphocytes (100 μL) suspended in complete medium were pipetted in triplicate into 96-well flat-bottomed cell culture plates (Nunc) followed by 100 μL of the appropriate mitogen or complete medium (controls). The plates were incubated for 72 h in a 5% CO_2 humidified incubator at 37°C. The following cell proliferation assay is based on the reduction of tetrazolium salt into a blue formazan product by mitochondrial dehydrogenase of viable cells (commercial kit, Boehringer 1465007). After 72 h of incubation, cultures were pulsed with 20 μL of the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) labeling reagent (final concentration 0.5 mg/mL) for 4 h at 37°C, and solubilised to dissolve the dark blue crystals overnight. The optical density (O.D.) was measured by a microplate reader (Dynatech) at a test wavelength of 550 nm and a reference wavelength of 690 nm. The results are expressed as the proliferation index (PI) on MTT assay calculated according to the formula for results of triplicate assays:

$$\mathrm{PI} = \frac{\text{O.D. (550/690nm) of stimulated cells}}{\text{O.D. (550/690nm) of non-stimulated cells}}$$

Humoral immunity. An enzyme-linked immunosorbent assay (ELISA) was used to determine total porcine immunoglobulin G (IgG) (g/L). The assay was an indirect, competitive ELISA previously described by Morrow-Tesch et al. (51) using rabbit anti-pig IgG antibody (Sigma Chemical). Serum levels of the humoral immunocomponents complement protein C3c, α_2-macroglobulin and immunoglobulin IgM (g/L) were monitored by nephelometrical analysis (Behring 100, Marburg, Germany) using rabbit antisera to human blood immunoproteins with public reaction to the porcine immunoproteins (43).

Metabolites. Plasma glucose (mmol/L) was determined by the glucose-oxidase-peroxidase method using a microtiter plate reader

(2). Total protein content (g/L) was determined by biuret-method (Merckotest 3327). Triglycerides (mmol/L) and alkaline phosphatase enzyme activities (U/L) were determined using commercially available kits (LT Lehmann, TR 100, AP 020; Berlin, Germany). Free fatty acids (FFA) levels (mmol/L) were analyzed by colorimetric micro-determination (19).

Cortisol. Plasma concentrations of cortisol were measured in ethanol-extracted samples using a single antibody radioimmunoassay technique with a specific antiserum raised in rabbits and tritiated cortisol, as described by Otten et al. (53). The cross-reactivity with any potentially competing steroids was $< 5\%$. The sensitivity of the cortisol assay was 3.1 nmol/L at 90% B/Bo and the intra- and interassay coefficients of variation were 4.8 and 9.4%, respectively.

Statistical Analysis

Data were analyzed using SAS-software (63). All variables were checked for normal distribution using the Kolmogorov test. Physiological measures were approximately normal distributed. Consequently, the general linear model procedure could be applied to test differences in the levels of social status (dominant versus subordinate pigs), the effects of sex (castrated male versus female) and group number in immunologic, metabolic and endocrine parameters. The individual physiological responses to the stressor "mixing" were calculated by subtracting the baseline (1 day before mixing) from the poststressor measures (3 days after mixing) and were analyzed by the paired *t*-test. Differences were considered significant if $p \leq 0.05$. Relations between changes in physiological responses and behavioral measures of newly mixed pigs were estimated and tested using a random model multiple quasi linear regression analysis. The model included the effects of individual DV, number of AI and interactions DV × AI.

RESULTS

In our study a mean number of 69.99 AI per animal was recorded during the observation period (2.33 AI per observation hour). Nevertheless, no health problems and no serious wounds/injuries were observed throughout the study. In addition, there were no significant differences between females and castrated males and no significant group effects in any of the behavioral and physiological measures.

Immunological Responses

The different lymphocyte proliferation indices in response to ConA, PHA, and PWM of dominant ($DV > 0$) and subordinate ($DV \leq 0$) pigs 1 day before and 3 days after mixing are shown in Fig. 2. After the establishment of a social rank order lymphocyte responses to T cell mitogens ConA ($p = 0.001$) and PHA ($p = 0.01$) as well as to B cell mitogen PWM ($p = 0.05$) were significantly higher in dominant pigs than in subordinate, whereas lymphocyte responses in blood samples of the same individuals taken before mixing did not differ significantly. No significant differences were found between dominant and subordinate pigs in humoral measures: IgG, IgM, C3c, and α_2-macroglobulin.

The ConA- and PHA-stimulated lymphocyte proliferation indices between 1 day before and 3 days after mixing increased in the dominant pigs and decreased in the subordinates. Both social classes differed significantly in these parameters (Table 1). However, the changes in B cell proliferative responses to PWM were not significantly different between dominant and subordinate pigs. The rise in total serum IgG concentration 3 days after mixing was significantly higher in dominant pigs compared to subordinates ($p = 0.05$). Changes in the other humoral immune measures were not significantly correlated with the social status of the pigs.

When both, the number of AI and the individual DV were included in the analysis, an increasing number of AI in the more dominant individuals resulted in an enhanced change in proliferation indices of ConA ($R^2 = 0.40$, $p = 0.0001$), PHA ($R^2 = 0.46$, $p = 0.0001$) or PWM ($R^2 = 0.49$, $p = 0.001$) stimulated lymphocytes whereas in the more subordinate pigs the change of proliferation indices decreased with an increasing number of AI (see Fig. 3 for ConA stimulated lymphocytes). The multiple regression analysis showed that the complement protein C3c change depended on DV, AI and the interaction DV × AI ($R^2 = 0.20$, $p = 0.05$), as well (Fig. 4). DV, AI and DV × AI did not effect serum concentration changes of IgG, IgM, and α_2-macroglobulin.

Metabolic and Endocrine Responses

There were no statistically significant differences in cortisol concentrations between dominant and subordinate pigs 3 days after mixing (Fig. 2). Remarkably, the cortisol concentration in blood samples taken before mixing were significantly lower in dominant individuals compared to subordinates (97.5 ± 31.1 vs. 123.1 ± 37.4 nmol/L; $p = 0.05$).

Whereas the levels of metabolites (glucose, total protein, FFA, triglyceride) and alkaline phosphatase enzyme activities did not differ significantly between dominant and subordinate pigs, mixing led to increased glucose and total protein values and decreased alkaline phosphatase and cortisol levels in both pig groups (Table 2). There was a slight, nonsignificant increase of the mean glucose value of subordinate pigs after the establishment of social hierarchy compared to dominant pigs, whereas dominants showed some greater rise in total protein level. In contrast, the decrease of alkaline phosphatase enzyme activities was significantly higher in subordinate animals compared to dominant ones ($p = 0.05$). No significant differences were obtained between dominant and subordinate animals with respect to changes in triglycerides and FFA (Table 2). The decrease in plasma cortisol concentration was stronger in subordinate than in dominant pigs during the observation period, but because of the small sample size and the high individual variation this difference was not significant. It should be noted that neither metabolic variables nor cortisol concentration was significantly influenced by individual DV, number of AI or interaction effect DV × AI.

DISCUSSION

Social Status and Immunological Responses

The stress response may be considered as a defense mechanism against environmental challenges that animals use to maintain homeostasis (10), in particular the immune system serves the defense of the organism against bacterial and viral infections (59). It is therefore surprising that much evidence suggests that various stressors are able to suppress some types of immune functions (17,25).

Mixing pigs with unacquainted penmates is a stressful event which may lead to a higher susceptibility to diseases (32,47,56). The paper by Morrow-Tesch and coworkers (51) described interaction between heat stress and social status in their effects on the pig's immune system. However, little is known about the effects of social stressors on immune functions in pigs alone.

In order to shed some light on this dependence we examined the effects of the establishment of social rank order in newly mixed groups of domestic pigs on the physiological responses giving special emphasis to the immune reactions. Lymphocyte function was assessed for the ability to proliferate as induced by T cell

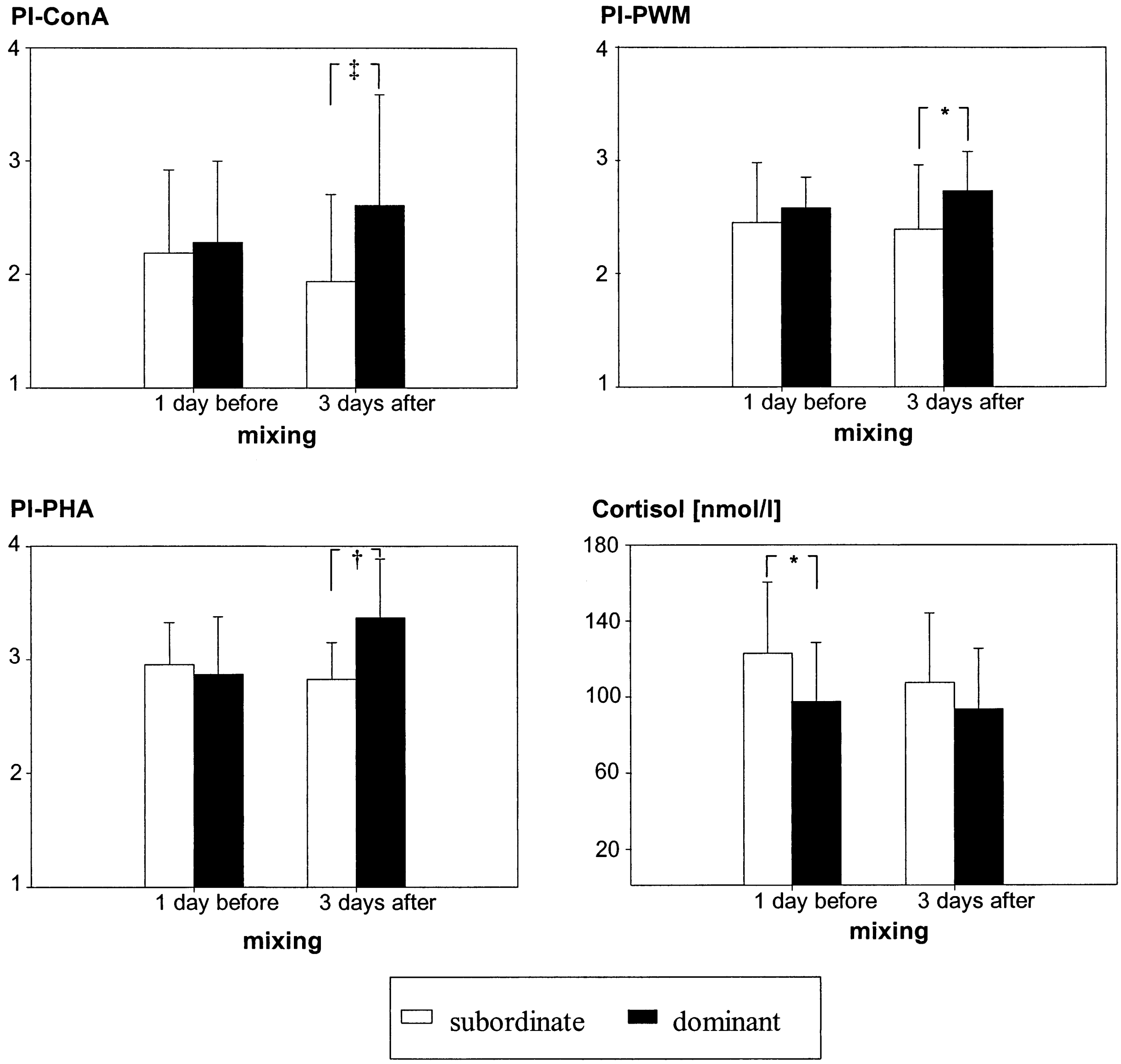


FIG. 2. Proliferation indices in response to ConA, PHA and PWM and concentration of cortisol (means and standard deviations, $*p = 0.05$, $\dagger p = 0.01$, $\ddagger p = 0.001$) of dominant ($DV > 0$) and subordinate ($DV \leq 0$) pigs 1 day before mixing and 3 days after mixing.

mitogens, ConA and PHA, as well as T cell-dependent B cell mitogen, PWM. Our results indicate that the proliferative responses of peripheral blood lymphocytes to these mitogens were significantly affected by the social status of the pigs; however, the ConA stimulated proliferation after social mixing had the strongest association with dominance values. The proliferation indices increased in high ranking individuals and decreased in low ranking animals while the later dominance status was not correlated with the baseline measures of immunity before mixing.

The differences may result from different strategies of coping with stressful events giving rise to different individual immune reactivity (12,33,42). Obviously, newly mixed pigs with similar weights are highly motivated to fight for a dominant position (46,60). Individual behavioral differences may also be important for understanding the complex social relations among animals living in social groups (30). Studies in rodents revealed that an individual behavioral strategy determines the acquired position in a social structure (39). Hessing et al. (30) demonstrated a persistent stability of individual behavioral characteristics. Controllability and/or predictability of stressors are major factors that determine coping (10,57). It seems very likely that agonistic interactions between the animals are predictable and can be controlled only by dominant pigs, while for subordinates it is an aversive situation. Consequently, a successfully coping individual might exhibit the immune pattern of a short-term stressor, whereas a subordinate "helpless" individual might exhibit the pattern of a chronic stressor (12).

Moreover, the fundamental genetics as well as the influence of prenatal and early postnatal experience may play a significant role (33,48,54).

When looking at the interaction effects between DV and AI we found that proliferation indices especially increased in more dom-

TABLE 1

CHANGES IN CELLULAR AND HUMORAL IMMUNE RESPONSES BETWEEN ONE DAY BEFORE MIXING AND AFTER THE ESTABLISHMENT OF A SOCIAL RANK ORDER IN SUBORDINATE AND DOMINANT PIGS

immune responses (poststressor measures-baseline)	**subordinate DV≤0**			**dominant DV>0**		
	n	mean	SD	n	mean	SD
PI-ConA	52	**−0.25‡**	0.44	38	**+0.33‡**	0.71
PI-PHA	21	**−0.12†**	0.22	15	**+0.50†**	0.41
PI-PWM	21	**−0.06**	0.47	15	**+0.15**	0.29
IgG [g/l]	21	**+0.73***	2.61	15	**+2.26***	1.61
IgM [g/l]	21	**+0.04**	0.15	15	**+0.07**	0.42
C3c [g/l]	21	**+0.02**	0.04	15	**+0.03**	0.02
α_2-macroglobulin [g/l]	21	**+0.03**	0.09	15	**+0.02**	0.17

* p=0.05; † p=0.01; ‡ p=0.0001 (significances subordinates vs. dominants)

inant animals if the number of AI was higher. Accordingly, we observed a decrease of proliferation indices in more subordinate pigs with increasing AI. Thus, it seems that repeated wins (i.e., dominant pigs with many AIs) are accompanied by an enhanced cellular immunocompetence, whereas a high number of defeats (i.e., subordinate pigs with many AIs) results in immunosuppression.

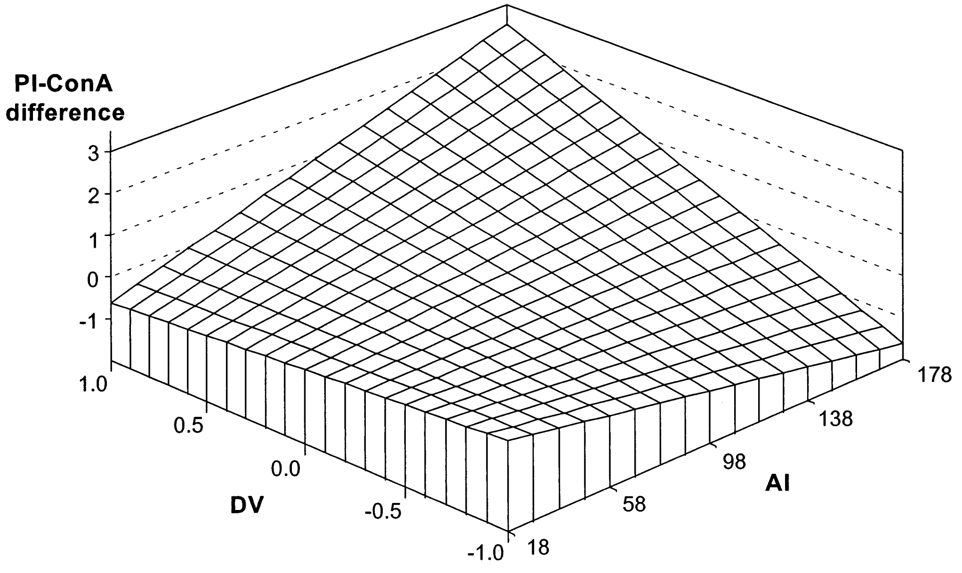
Lymphocytes are the key cells controlling the immune response. It is assumed that greater proliferation in vitro indicates a more effective cell function (14,59). Hessing et al. (31) found that the specific lymphocyte proliferation stimulated by Aujesky disease virus was higher in dominant pigs than in low ranking ones. Therefore, stressed pigs of low social status with suppressed cell-mediated immunity may be more susceptible to pathogens (47). Such a different proliferation response of high and low ranking animals was also observed in studies of nonhuman primates involving group formation stress (12), social separation stress (40) or stress by restricted resources (9), in rats and mice after aggressive interactions (28,58), and in house mice kept in different social and physical environments (3).

The biologic origins of the differences of cellular immune functions in dominant and subordinate pigs remains an important question. Perhaps, the dominance status influences immune parameters in pigs only transiently during times of acute stress, such as mixing unfamiliar animals. The nonstressed pigs in the study of McGlone et al. (47) showed no relationship between social status and natural killer cell activity; whereas among stressed pigs, dominant pigs had higher natural killer cytotoxicity than pigs of intermediate or submissive status, which may be considered as a survival and reproduction advantage over subordinate animals (64).

The fact that psychosocial events affect primarily the proliferation to ConA and PHA, but less to PWM-stimulation may reflect differences in cell populations stimulated by the mitogens. ConA and PHA are known to stimulate T cell division, whereas PWM has an effect on B lymphocytes, reflecting that T lymphocytes seem to be more affected by stress than B lymphocytes (18,66). A study of purified T and B cell subpopulations after stress will probably yield more conclusive data.

Social stress of mixing may influence humoral immune reactivity (22,31). However in the present study, only little changes were noted for humoral immune measures of both pig groups, dominant and subordinate. The higher increase of IgG concentration in dominant pigs was mainly effected by the number of AI.

Complement, a multimolecular system of plasma proteins, pro-

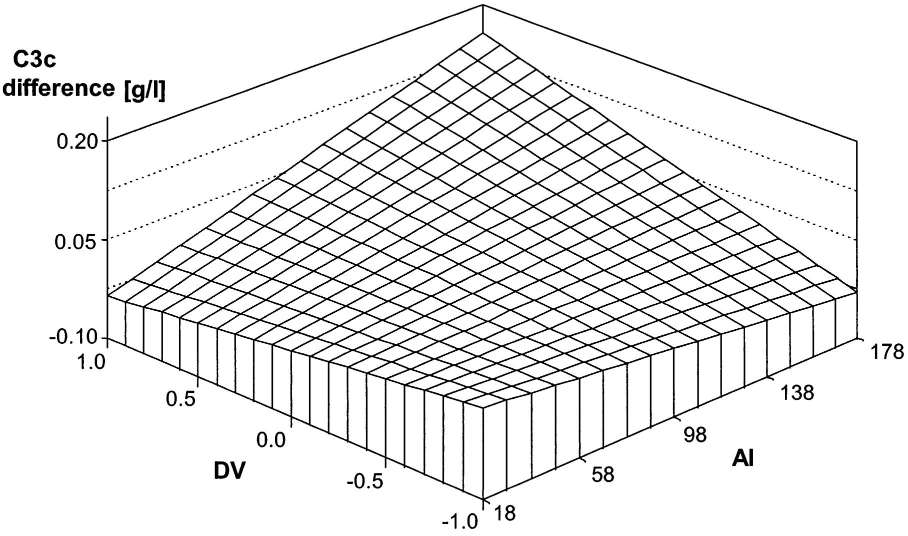


regression response surface:

$$\text{PI-ConA difference} = -0.3503 - 0.6253 \cdot DV + 0.0045 \cdot AI + 0.0152 \cdot DV \cdot AI$$

($R^2 = 0.4$)

FIG. 3. ConA stimulated proliferation index change (PI-ConA difference) between 1 day before mixing and after the establishment of a social rank order in relation to the individual DV and the number of AI.



regression response surface:

C3c difference = -0.0084 - 0.0501*DV + 0.0004*AI + 0.0008*DV*AI

($R^2 = 0.2$)

FIG. 4. C3c change (C3c difference) between 1 day before mixing and after the establishment of a social rank order in relation to the individual DV and the number of AI.

vides the organism with a first line of nonspecific humoral defense against infections before the immune response is fully activated. However, only few studies indicate that the activity of the complement system can be modulated by psychological stress (13,44,65). In the present study no significant differences between C3c values in dominant and subordinate pigs were found, but the interaction effects DV × AI were significantly correlated with levels of C3c component. These findings are supported by results in male guinea pigs (65). These authors found also no significant relationship between complement activity and dominance status, whereas the frequencies of definite agonistic behavioral patterns were significantly correlated with the complement activity.

Multiple communicative pathways among the nervous, endocrine, and immune systems facilitate physiological immunoregulation (7,16,38). Relationships between behavior and immune changes during social stressor are mediated via the central nervous system by circulating hormonal and neurochemical factors and/or by direct sympathetic innervation of lymphoid tissue (41).

Most of the contradictory results described in literature can be explained by the fact that the response of the immune system to stress depends on several factors, such as the intensity, the duration, and the frequency of the stressor, and also on the immune parameters analyzed. The above results suggest that cellular immunity is more susceptible to social stress than the humoral responses.

Since the establishment of a dominance hierarchy was accomplished with absence of "large" wounding, we suppose that psychosocial stress experienced by the animals was responsible for the immunologic changes observed.

TABLE 2

CHANGES IN METABOLIC AND HORMONAL RESPONSES BETWEEN ONE DAY BEFORE MIXING AND AFTER THE ESTABLISHMENT OF A SOCIAL RANK ORDER IN SUBORDINATE AND DOMINANT PIGS

metabolic and hormonal responses (poststressor measures-baseline)	**subordinate DV≤0** n	mean	SD	**dominant DV>0** n	mean	SD
glucose [mmol/l]	21	**+0.32**	0.98	15	**+0.14**	0.57
total protein [g/l]	21	**+0.44**	3.28	15	**+2.32**	7.68
alkaline phosphatase [U/l]	21	**−66***	58	15	**−30***	25
free fatty acids [mmol/l]	21	**+0.006**	0.05	15	**+0.004**	0.02
triglycerides [mmol/l]	21	**+0.06**	0.13	15	**+0.10**	0.15
cortisol [nmol/l]	21	**−15.6**	17.3	15	**−3.9**	21.9

* p=0.05 (significance subordinates vs. dominants)

Social Status and Metabolic and Endocrine Responses

Most stressors are known to cause increase in blood glucose levels mediated through the hyperglycemic effects of epinephrine, glucagon, and the glucocorticoids. Warriss and Brown (67) and Fernandez et al. (21) found positive relationships between plasma glucose level and aggressive behavior or dyadic encounters in domestic pigs. Nevertheless, mixing had no long-term effects on energy metabolism in pigs (29,57). The present results showing slight increases in glucose and FFA levels may reflect the mobilization of body energy sources in response to agonistic interactions.

The decrease of alkaline phosphatase activity after establishment of rank order in newly mixed unfamiliar pigs is consistent with previous studies in lambs given restraint and isolation stress (1,50) or in beef steers that were exposed to feed deprivation and transportation stress (23). Admittedly, the physiological significance of decreased alkaline phosphatase enzyme activities associated with widely differing types of stresses is not clear. Other metabolites measured in this study showed no significant relationships with social hierarchy.

The plasma concentration of cortisol has been widely used to reflect the effects of different stressors on immune function. Present baseline plasma cortisol levels are comparable to those generally obtained in pigs from European breeds blood sampled by *vena* puncture (4,31,66). In pigs, the hypothalamic-hypophysal-adrenal axis responds rapidly and sensitively to acute physical stress (4,53) and returns to prestress levels within one hour (36). Cortisol, which is the major adrenocortical hormone in the pig, is known to be immunosuppressive at pharmacological levels (68). Numerous studies have demonstrated negative effects of various stressors on the lymphocyte proliferative function in pigs mediated by cortisol (11,20,66). On the other hand, the adult pig lymphocyte is highly resistant to the cytolytic as well as the inhibitory effects of steroids (69).

The lack of significant relationship between dominance rank and cortisol values in the present study agree with observations in Rhesus Macaques (27). Moreover, no significant differences due to varying degrees of mobility were found in the profile of cortisol concentrations in pigs (5). Also, Blecha et al. (8) reported an absence of correlation between high cortisol concentrations and decreased lymphocyte blastogenic responses in regrouped pigs. Data from the current experiment indicate that plasma concentrations of cortisol measured 3 days after mixing cannot account for the stress-associated reduction in lymphocyte blastogenic function of subordinate pigs.

In summary, the present results indicate that the immune function of newly mixed groups of unfamiliar pigs is differentially affected by social stress, depending on the dominance value, number of fights, and the immune parameter measured. Stress has been shown to be both immunosuppressive and immunostimulatory. A high-rank position is correlated with enhanced and a low-rank position with suppressed cell-mediated immune reactions. Both effects are enhanced by an increased number of AI. Apparently, the T cell function seems to be mainly influenced by social stress. The data from the current experiment also suggest that the controllability and/or predictability of stressors may be a critical factor in modulating immune functioning. Therefore, these findings support the demands to improve health and welfare in pigs by the creation of new housing and management systems, which offer animals improved coping abilities to achieve physical and psychic control of (social) environment.

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Effects of Dominance and Familiarity on Behaviour and Plasma Stress Hormones in Growing Pigs During Social Confrontation

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With 4 figures and 2 tables

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Summary

Behavioural patterns and plasma adrenaline, noradrenaline and cortisol responses were studied in domestic pigs with different dominance status during 10-h social confrontation tests with a familiar and an unfamiliar group. Eight trials were carried out, where in each trial two groups of nine growing pigs (12 weeks old) were randomly formed. The pigs with the highest (HR) and lowest (LR) rank from each group were selected as test animals, provided with surgically implanted catheters and kept in single housing for 2 to 3 weeks. After this period, each test animal was introduced once into the familiar and once into the unfamiliar group for 10 h. Introduction of the test animals into the groups caused frequent agonistic interactions during the first 30 min and significantly more agonistic interactions during the confrontation with the unfamiliar group. The agonistic behaviour was accompanied by a rapid increase of plasma catecholamines and cortisol. HR pigs showed significantly higher plasma catecholamine concentrations and more agonistic interactions during the first 30 min compared with the LR pigs. During confrontation with the unfamiliar group, HR pigs experienced more defeats and showed a higher increase of plasma cortisol levels than during the confrontation with the familiar group. No influences of rank or familiarity were found on the other behavioural patterns examined. The results show that agonistic behaviour and activation of the sympatho-adrenomedullary and the hypothalamic-pituitary-adrenal system in pigs during a social confrontation test are determined by the former dominance rank of the animals and the familiarity of the group.

Introduction

Under group housing conditions, domestic pigs establish a social dominance order, which is the result of agonistic interactions between the animals (Meese and Ewbank, 1973). The individual animal within the dominance hierarchy must learn to react in an adequate manner to the behaviour of other animals of the group. Therefore, the dominance order is important for stability within the group, which lowers the aggression level, reduces the risk of injury and regulates access to resources. Mixing of unfamiliar pigs, which is a common procedure in pig production, causes disturbances of the social structure within the group. Increased aggression and fighting are a result of mixing animals and can lead to reduced growth performance, increased injuries, strong neuroendocrine changes and even death (e.g. McGlone et al., 1981; McGlone and Curtis, 1985; Warris and Brown, 1985; Fernandez et al., 1994a; Stookey and Gonyou, 1994; Barnett et al., 1996; Puppe et al., 1997). Activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adrenomedullary (SAM) system during agonistic interactions has been reported in various species such as mice (Ely and Henry, 1978; Benus et

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al., 1987), rats (Sgoifo et al., 1996), guinea pigs (Sachser, 1987) and tree shrews (Von Holst, 1985). In these species, there is evidence that the dominance status has a distinct effect on the behavioural and physiological response to a social stress situation, resulting in different coping strategies depending on dominance status (Benus et al., 1991). The existence of different coping strategies in pigs has been a matter of debate (Mendl and Deag, 1995). Recent studies showed no clear evidence for coping strategies in pigs because of poor intersituation consistency and a lack of bimodal distribution (Forkman et al., 1995; Jensen, 1995; Spoolder et al., 1996). There is still a need to clarify the mechanisms of response variations shown by pigs when they are confronted with a social stress situation. Furthermore, little information is available about the effects of dominance status combined with familiarity in social stress situations on agonistic interactions and the activation of the HPA and SAM axis in pigs. Recently, we showed that in pigs social confrontation with a group causes different, but inconsistent, behavioural and physiological stress reactions (Otten et al., 1997).

The present study was designed to investigate behavioural reactions and plasma stress hormone responses of pigs during a social confrontation test. For this purpose, we addressed two questions. (i) When a test animal is confronted with a group, does the familiarity influence its behaviour and the stress hormone reaction during the test situation? (ii) Does the former dominance rank of the test animal determine behavioural and endocrine changes during the confrontation test? To answer these questions, we used a multidisciplinary approach to evaluate simultaneously the relationships between behavioural patterns and plasma stress hormone changes during a 10-h encounter.

Materials and Methods

Animals and housing

Eight trials were carried out. In each trial, pigs were randomly separated into two groups, each consisting of nine young growing pigs derived from six different litters (144 animals in total, 77 female and 67 castrated male animals). In each group, a maximum of two animals came from the same litter. Male animals were castrated at an age of 2 weeks. At the time of mixing, the pigs were ≈ 12 weeks old (80.4 ± 0.5 days; all values are given as mean ± SE). The mean female to castrated male ratio was 54.2 % to 45.8 %. The animals of each group were kept in a pen (4.0 m × 1.6 m) with a concrete floor and little straw. The feeder was placed in the middle of the long side of the pen. Animals were fed a standard diet, and the feed was supplied at 0900 and 1500 h with free access to the trough from 0900 to 1100 and from 1500 to 1700 h (animal to feeding place ratio 2.25 : 1). Water was supplied *ad libitum*.

Selection test

In the 16 selection groups, a total of 8280 overt agonistic interactions were continuously recorded for 3 days immediately after mixing (10 h daily from 0800 to 1800 h). An agonistic interaction was defined as a fight or a displacement event with physical contact of two individuals for more than 2 s and intervening periods of at least 8 s, while the fight either was interrupted or the pigs showed other behaviours (Puppe, 1998). Fights occurred as overt bodily attacks like ‘head to head knocks’, ‘head to body knocks’, ‘parallel/inverse pressings’, ‘bitings’ and ‘physical displacements’ (Jensen, 1982; McGlone, 1985; Rushen, 1988). The end of a ‘decisive’ fight was immediately evaluated by the observer regarding the possible outcome. The loser was defined as the pig which first stopped fighting, turned away from an attack and tried to flee or was displaced from a location (Tuchscherer et al., 1998). A typical movement was that the loser positioned itself in an asymmetric parallel configuration (Rushen and Pajor, 1987), whereas the pursuer (winner) turned around the loser and bit it. Matrices were generated for each group, and an individual dominance value (DV) was calculated for each animal by the number of wins minus defeats in relation to all decisive fights with the other group members over the whole observation period (Puppe and Tuchscherer, 1994). Hence, the dominance value, which could vary between – 1.00 (no wins) to + 1.00 (no defeats), was used as a relative measure indicating the mean fighting success of an individual in agonistic behaviour related to its group mates. Although a large number of measures of dominance exist (for a review, see Lundberg, 1987), such an approach may be especially useful when all or nearly all of the animals of a group are involved in a relatively high number of agonistic interactions (e.g. after new mixing), and when correlations between social stress behaviour and physiological coping mechanisms are investigated (e.g. Tuchscherer et al., 1998).

From each group, the animal with the highest (HR) and the animal with the lowest (LR) dominance value were removed and kept as test animals in a single housing pen, while the two groups remained in their pens. When the subsequent surgical procedure in the test animals was successful, an additional animal with a middle dominance value ($DV = -0.04 \pm 0.06$) was removed from the group. In two cases the first implantation procedure was not successful, and the test animal was replaced by an animal with a similar dominance value. In this case the animal with the middle rank remained in the group in order to keep a constant group size.

For the selected test animals, an average of 73.4 ± 5.3 agonistic interactions with 95.0 % of the other eight animals of the group were observed (mean: 7.6 group mates, range: 6–8), providing the basis for calculation of their mean individual dominance values. The dominance values differed significantly between the selected HR animals and LR animals ($+0.73 \pm 0.04$ vs. -0.66 ± 0.04, $z = 4.39$, $P < 0.01$, Wilcoxon Rank Sum Test), but no differences were found between the body weights of the HR and LR animals (33.9 ± 1.7 kg vs. 31.5 ± 1.7 kg, $t = 1.01$, $P > 0.05$).

Surgical procedure for vein catheters

After an isolation period of 1 week, a catheter was placed into the external jugular vein of the test animals. General anaesthesia was achieved by two intravenous injections of saline containing 200 mg Xylazin and 2 g Ketamin in total. For protection of the catheter outlets, the test animals were equipped with neck straps. The length of the catheter outlets was ≈ 50 cm, which enabled blood sampling without restraining the animal. In the subsequent recovery period (7–11 days) the test animals were habituated to frequent handling by simulating the manipulations for blood sampling three times a day. All procedures involving use and treatments of the animals were approved by the local committee on animal care and use.

Social confrontation test

After the recovery period, each test animal was confronted once with its origin (familiar) group and once with the other (unfamiliar) group, each group now consisting of the six remaining pigs. Half of the test animals were first confronted with the familiar and then with the unfamiliar group, and half the other way round. The average time interval between the two confrontation tests was 6.0 ± 0.3 days. During the confrontation test, all pigs in the group were equipped with neck straps, in order to achieve comparable conditions for all animals. The housing conditions in the test pen were the same as described for the selection test. The average body weight of the HR test animals at the time of the confrontation tests was 54.6 ± 2.4 kg and for the LR test animals 49.4 ± 2.3 kg ($t = 1.59$, $P > 0.05$). Each social contact test started at 0800 h in order to avoid circadian influences, and lasted 10 h.

Behavioural measurements

For the calculation of the new individual dominance values of the test animals, the agonistic interactions of the group dyads were recorded by continuous sampling as described for the selection test. Additionally, to analyse the whole behaviour of the test animals, the agonistic behaviour and other behavioural patterns were measured by instantaneous sampling at a 30-s interval (Table 1). For evaluation, the behavioural data were summarized at 30-min intervals and presented as a percentage of all observations.

Blood sampling and hormone analyses

Twenty-five blood samples were obtained from each test animal before and during the confrontation test on a fixed time schedule. Three samples were taken 30, 15 and 5 min before the confrontation test. During the 10-h test period, blood samples were taken every hour (10 samples). Additional samples were taken 5, 10, 20 and 40 min after introducing the animal into the group and after the beginning of the two feeding times. For blood sampling, a handler who was familiar to all the animals entered the pen, and the samples were taken from the free-moving test animal. The blood samples were collected in ice-cooled polypropylene tubes containing EDTA solution, placed on ice, and subsequently centrifuged at 2000 *g* for 15 min at 4°C. Plasma was then stored at -80°C until analysis.

Plasma concentrations of adrenaline and noradrenaline were analysed in duplicate using high-pressure liquid chromatography with electrochemical detection. The catecholamines were prior extracted from the plasma samples by absorption on aluminium oxide. Inter- and intra-assay CVs were tested on mid-range

Table 1. Behavioural patterns of the test animals measured during the social confrontation test

Behaviour	Definition
Agonistic interactions	A fight or a displacement event with physical contact with the other group members (for further explanation, see text)
Locomotion	Subsequent locomotive behaviour without physical contact with the other group members
Exploration	All forms of manipulative behaviour with physical contact with the other group members (except agonistic interactions), directed towards the technical pen equipment (except trough or watering-place) or towards the straw
Standing/sitting	Standing or sitting alone without physical contact with the other group members
Lying	All forms of lying behaviour with or without physical contact with other group members
Eating/drinking	Staying at the trough or the watering-place with or without the intake of food or water
Waiting	Staying (waiting) at the trough or the watering-place, but behind the other animals eating or drinking

plasma samples. The intra- and inter-assay CVs for adrenaline were 11.5 % and 12.5 %, respectively. For noradrenaline, the intra-assay CV was 2.1 %, and the inter-assay CV was 1.9 %.

Plasma concentrations of cortisol were measured in duplicate in ethanol-extracted samples using a single antibody radioimmunoassay technique. The intra- and inter-assay CVs were 4.8 % and 9.4 %, respectively. The hormone analysis procedures used in this study were previously described in detail (Otten et al., 1997).

Statistical analysis

Data were analysed by a repeated measurement analysis of variance using the GLM procedure of SAS® (Statistical Analysis System Institute, 1989). The statistical model for all data included the repeated factors *time* (number of levels depending on the sample schedule of each parameter) and *group* (familiar, unfamiliar). The class variables included in the model were *rank* (highest and lowest ranking), *trial* (1–8) and *sex* (female, castrated male). In addition, all interactions between the repeated factors and the class variables were tested. Differences within repeated factors were also tested for significance with Student's *t*-test.

For the calculation of Pearson's correlation coefficients, data of all time-dependent variables were pooled in three time periods: the first, the second and the remaining 8 hours of the confrontation test. Before correlation analysis, a logarithmic transformation of plasma catecholamine concentrations was performed.

Missing values due to blocked catheters or ill animals reduced the data sets for statistical analysis. Hence, data from 27 animals (five female and eight castrated male HR animals, six female and eight castrated male LR animals) were available for analysis.

Results

Behavioural patterns and dominance values

During the 10-h period of social confrontation, the test animals were involved in 66.5 ± 7.7 agonistic interactions with 91.7 % of the other six animals of the group (mean: 5.5 group mates, range: 4–6). The mean dominance values achieved in the confrontation test were +0.09 ± 0.23 for the HR animals and −0.95 ± 0.02 for the LR animals in the familiar group ($P < 0.01$), and −0.59 ± 0.14 for the HR animals and −0.94 ± 0.03 for the LR animals in the unfamiliar group ($P < 0.01$). The confrontation of the test animals with the familiar and the unfamiliar groups resulted in a decrease of the mean dominance values of these animals compared with the selection test. The decrease of the dominance values in the familiar group did not differ significantly between the HR (0.74 ± 0.17) and the LR (0.28 ± 0.04) animals. However, the decrease of dominance values was significantly greater for the HR (1.37 ± 0.12) than for the LR (0.33 ± 0.05) animals in the unfamiliar group ($P < 0.01$). The HR animals showed greater variation of dominance value in both groups because seven HR animals in the familiar group and one HR animal in the unfamiliar group succeeded in having more wins than defeats in their agonistic interactions (Fig. 1).

The effect of time was significant for the frequency of agonistic interactions ($P < 0.001$). The highest frequency of agonistic interactions between the test animals and animals from the group was found during the first 30 min of the social confrontation test, but the frequency decreased rapidly thereafter (Fig. 2). Regarding the 10-h testing period, significant influences on agonistic interactions were also found for the effects of group ($P < 0.05$), group × time ($P < 0.05$) and rank × time ($P < 0.01$). During the first 30 min, HR animals introduced into the unfamiliar group were involved in more agonistic interactions than LR animals ($P < 0.05$), whereas in all following 30-min periods the frequency of agonistic interactions was not different between the HR and LR animals.

It follows from the experimental design that the behaviour of staying at the trough or at the watering place was almost exclusively shown during the two feeding times. Although there were no significant influences of the other effects measured, HR animals showed a tendency for the most frequent eating or drinking behaviour during the first 30 min of each feeding time, whereas the highest frequency of eating and drinking for the LR animals occurred during the second 30-min period of the feeding times (Fig. 2).

The time-related changes of the other behavioural patterns are summarised in Fig. 3. The effect of time was significant for all behaviours examined ($P < 0.001$). Locomotion, standing/sitting and exploration were highest during the first 30 min after introduction into the groups and decreased during the first 3 h. Conversely, the frequency of lying was lowest at the

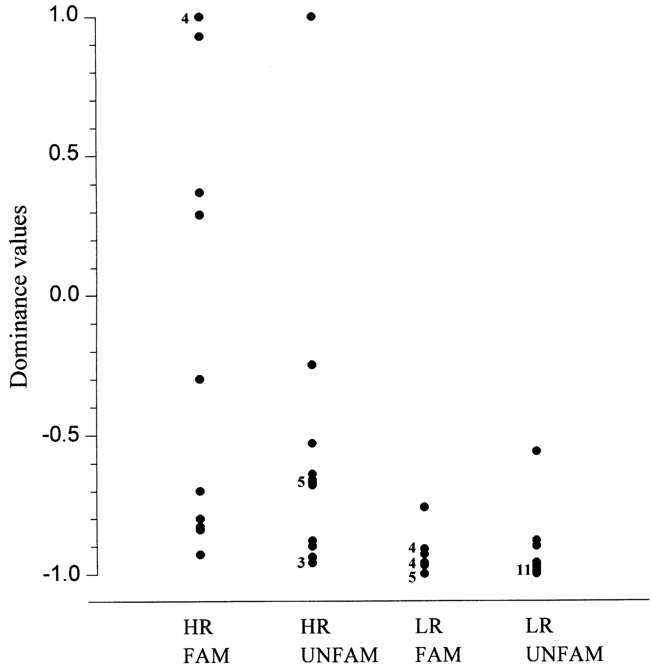


Fig. 1. Dominance values of test animals, classified in the selection test as high (HR) and low ranking (LR), during the confrontation tests with a familiar (FAM) and an unfamiliar (UNFAM) group (HR animals: $n = 13$, LR animals: $n = 14$). If the same dot equals several animals, the number of animals is given.

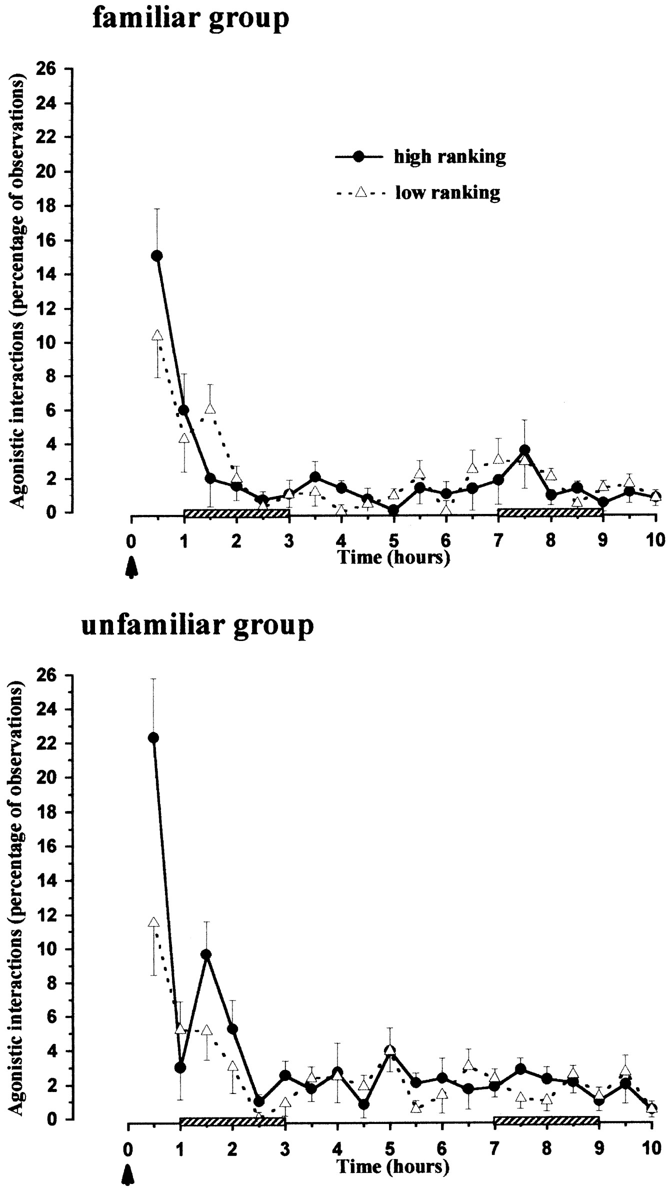


Fig. 2. Agonistic interactions and eating/drinking behaviour of high and low ranking pigs during the confrontation test with a familiar and an unfamiliar group. Results are presented as least-square means ± standard error. The arrow indicates the beginning of the confrontation. Feeding times are indicated by hatched bars.

beginning of the test and highest between and after the two feeding times, where lying was also the behaviour with the highest frequency of all behavioural patterns (Fig. 3). No significant influences of rank, group, sex and related interactions were found for these behavioural patterns.

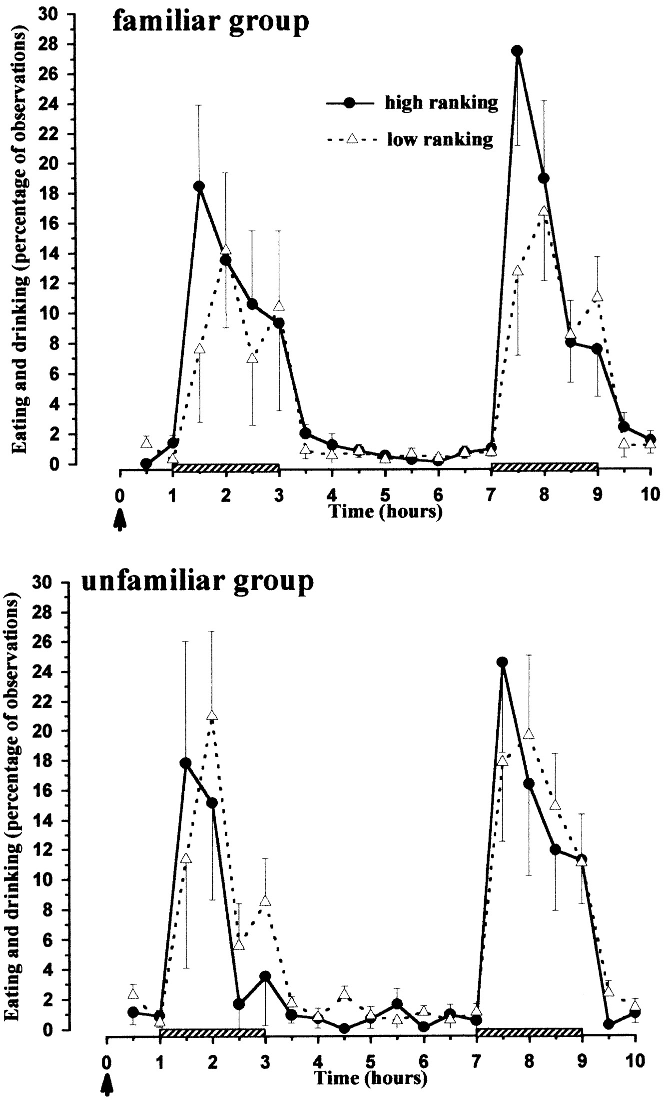


Fig. 2.—*continued*

Stress hormones

The agonistic behaviour at the beginning of the social confrontation test was accompanied by a rapid increase of plasma adrenaline, noradrenaline and cortisol concentrations (Fig. 4). The highest adrenaline and noradrenaline concentrations were measured 5 min after the beginning of the confrontation test. In the next 2 h, the plasma concentrations of adrenaline and noradrenaline decreased approximately to the levels found immediately before the test. The more frequent agonistic behaviour of the HR animals at the beginning of the confrontation test (see Fig. 2) was accompanied by higher plasma adrenaline and noradrenaline concentrations compared with the LR animals (Fig. 4). Up to 3 h after the beginning of the test, the plasma catecholamine concentrations of the HR animals remained on a higher level compared with

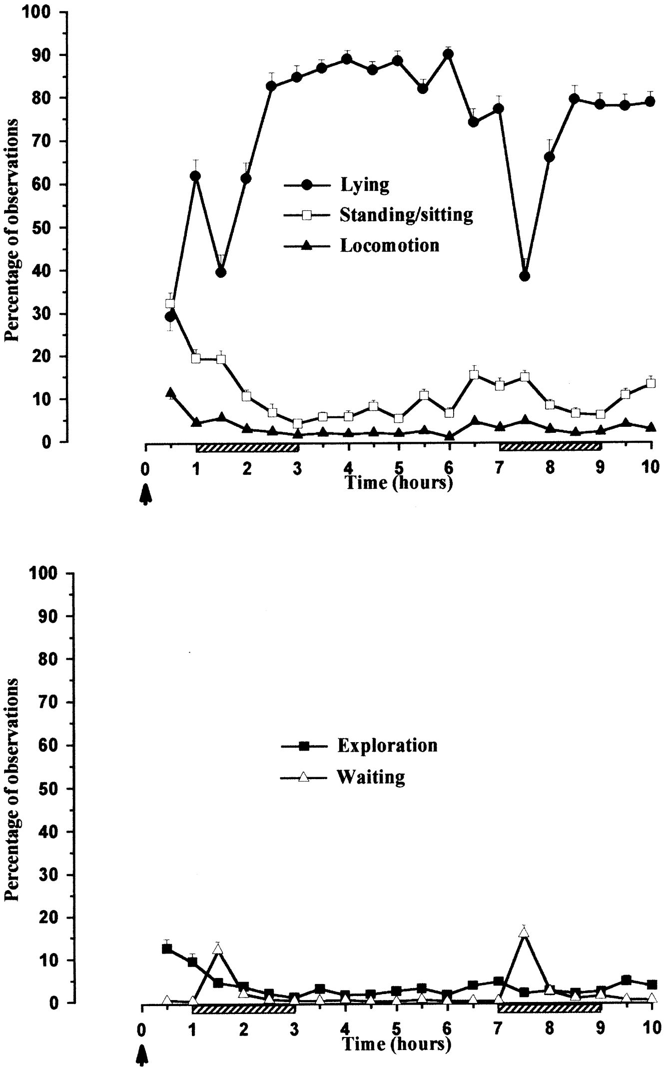


Fig. 3. Time-related changes in lying, standing and sitting, locomotion, waiting and exploration behaviours. Because no significant differences for rank and group effects were found, the data were pooled for all test animals and treatments. Results are presented as means ± standard error.

the LR animals in both groups (rank, $P < 0.05$; rank × time, $P < 0.01$ for adrenaline and noradrenaline).

In general, the confrontation of a test animal with one of the groups caused an increase of the plasma cortisol levels within the first 2 h (Fig. 4). The cortisol reaction of the LR animals was similar in the two groups and showed an approximately 2-fold increase compared with the values immediately before the confrontation. These animals had maximum cortisol concentrations after 40 min in the familiar group with a second maximum after 7 h during the second feeding time. In the unfamiliar group, the cortisol levels of the LR animals reached a maximum after 20 min. In contrast, the HR animals showed only a slight increase when confronted with the familiar group, with maximums after 20 min and within the two feeding times. However, a dramatic increase of the cortisol concentrations was noted for these animals after confrontation with the unfamiliar group. Compared with the values immediately before the confrontation, the cortisol concentrations showed an approximately 5-fold increase with a maximum after 80 min. The differences between HR and LR animals diminished and disappeared after a test duration of 7 h (rank × time, $P < 0.01$; rank × group × time, $P < 0.01$). The influence of sex on the plasma stress hormone values was not significant.

Correlation between behaviour and stress hormones

The significant Pearson correlation coefficients between agonistic behaviour and locomotion as an expression of physical activity and plasma levels of stress hormones are shown in Table 2. Before correlation analysis, data were pooled in three time periods: the first hour of the confrontation test covering the maximum of agonistic behaviour, locomotion and plasma catecholamine levels, the second hour covering the maximum of plasma cortisol concentrations, and the remaining 8 h with few agonistic interactions and little locomotion. The highest correlation coefficient was found between plasma levels of adrenaline and noradrenaline during the first hour of the confrontation test ($r = 0.80$, $P < 0.001$). The increased agonistic behaviour during the first hour was best correlated with plasma levels of adrenaline ($r = 0.69$, $P < 0.001$) and less pronounced with plasma levels of noradrenaline ($r = 0.49$, $P < 0.01$) and cortisol ($r = 0.41$, $P < 0.05$) during the first hour. Due to the delayed increase of the plasma cortisol levels, a better correlation was found between the frequency of agonistic interactions during the first hour and the cortisol levels of the second hour ($r = 0.56$, $P < 0.01$). The locomotion after the second hour was positively correlated with the noradrenaline levels during and after the second hour ($r = 0.42$, $P < 0.05$). Significant relations also existed between the plasma levels of noradrenaline and cortisol ($r = 0.56$, $P < 0.01$), and adrenaline and cortisol ($r = 0.57$, $P < 0.01$) during the first hour as well as between adrenaline and cortisol levels after the second hour ($r = 0.62$, $P < 0.001$).

Discussion

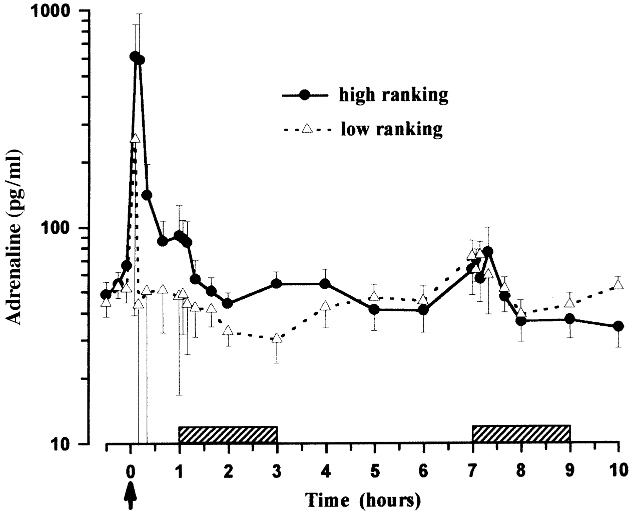
The present study was designed to investigate how behavioural reactions and plasma stress hormones of pigs during a social confrontation test are influenced by their familiarity with the group and how these changes are furthermore determined by former dominance rank. In our experiment, we found that the incidence of agonistic interactions and the activation of the HPA and SAM axis during the social confrontation test depend on the familiarity of the group and the former rank of the test animals, although the test animals were kept under single housing conditions for 2 to 3 weeks before the social confrontation tests.

Behaviour and dominance

The confrontation with the familiar or the unfamiliar group caused very frequent agonistic interactions between the test animal and animals of the group during the first 30 min of the test. In the unfamiliar group, the test animals fought significantly more than in the familiar group. A reason for aggressive behaviour between animals is the establishment of a dominance hierarchy which regulates the access to resources (McGlone, 1986). Moreover, it has been suggested that, besides establishing a dominance hierarchy, agonistic behaviour may be triggered by unfamiliarity alone and the real goal could be to drive away a stranger (Puppe, 1998). It is well known that, after mixing, unfamiliar pigs show more aggressive behaviour than familiar ones (Arey and Franklin, 1995; Ekkel et al., 1997). In our experiment, the test animals were kept separated from the groups in single housing for 2 to 3 weeks between the selection test and the confrontation tests. Despite this separation period, these animals fought significantly more in the unfamiliar than in the familiar group, which provides evidence that pigs are able to recognize former group mates, even after a separation of approximately 3 weeks.

Furthermore, test animals which had previously been highest ranking were involved in

familiar group



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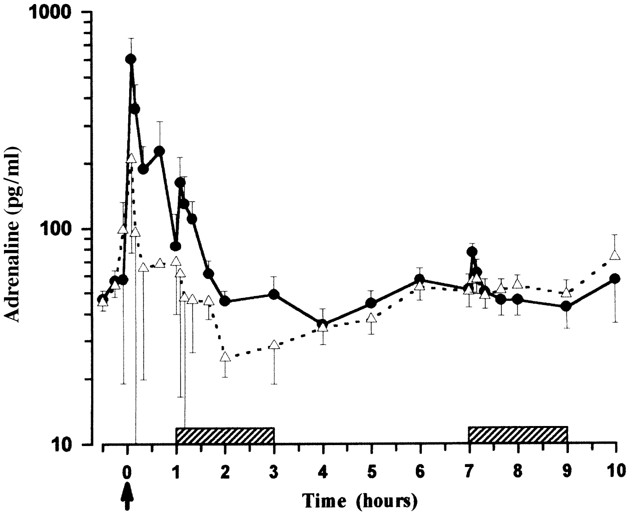
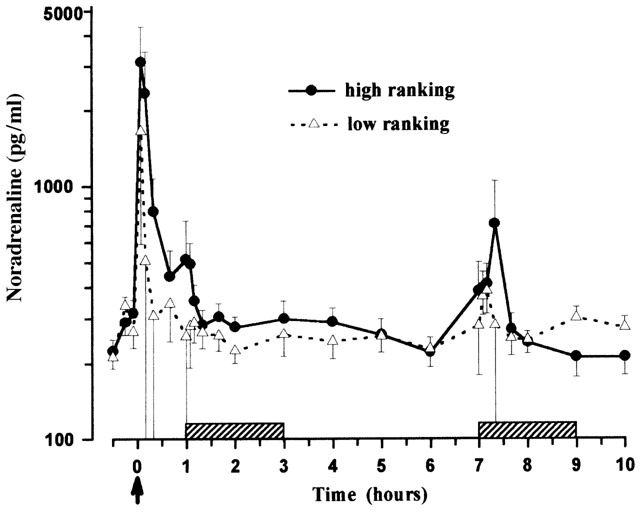


Fig. 4. Plasma levels of adrenaline, noradrenaline and cortisol of high and low ranking pigs before and during the confrontation test with a familiar and an unfamiliar group. Results are presented as least-square means ± standard error. Log-scales are used for the presentation of adrenaline and noradrenaline values.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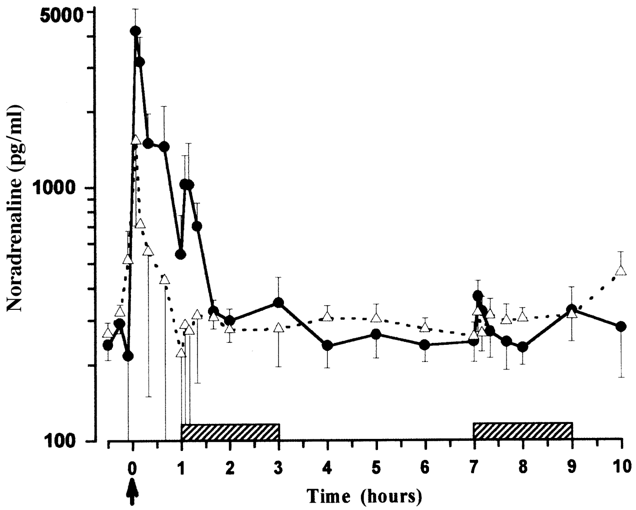
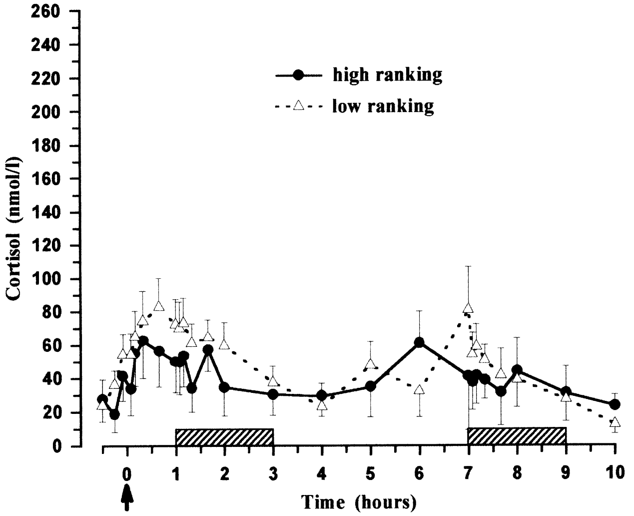

unfamiliar group

Fig. 4.—*continued*

more agonistic interactions during the first 30 min of the test than animals which had previously been lowest ranking. These findings are comparable to results from a study in which young growing pigs classified as aggressive and non-aggressive at the age of 1–2 weeks showed a consistency of their behavioural patterns in later social tests at the age of 10 and 15 weeks (Hessing et al., 1993). Although we could not determine whether more agonistic interactions familiar group



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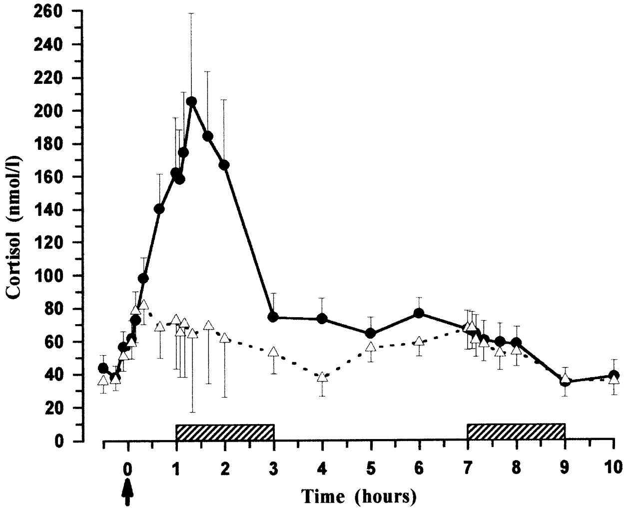

Fig. 4.—*continued*

were initiated by the test animal or by animals of the group, the HR animals made more effort to defend their former rank position at the beginning of the test. As a result, the HR animals succeeded in winning more of the agonistic interactions, and the mean dominance values during the confrontation tests were still higher compared with the LR animals. The motivation for aggressive behaviour between newly mixed animals may be high individual aggressiveness

Table 2. Significant Pearson correlation ($P < 0.05$) between agonistic interactions (AI), locomotion (LO) and plasma levels of adrenaline (A), noradrenaline (NA) and cortisol (C) during the confrontation test. For correlation analysis the data were pooled for the first (1), the second (2) and the remaining 8 h (3) of the confrontation test

		AI			LO			A			NA			C		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
AI	1			0.59				0.69			0.49			0.41	0.56	
	2			0.55												
	3															
LO	1					0.48										
	2						0.40									
	3											0.42	0.42			
A	1								0.70	0.52	0.80			0.57	0.44	0.41
	2									0.79						0.51
	3															0.62
NA	1											0.56		0.56		
	2												0.67			
	3															
C	1														0.63	0.70
	2															
	3															

(Erhard and Mendl, 1997) or uncertainty about relative fighting abilities, which can also be affected by the body weight of the animals (Rushen, 1988, 1990; Puppe, 1998). Because body weight did not differ between HR and LR animals, the difference in agonistic behaviour may be caused by prior experiences during agonistic encounters. Prior experiences of winning are likely to increase the propensity to behave aggressively and experiences of defeat to decrease it (Rushen, 1988; Mendl and Deag, 1995). Internal (rank as prior experience) and external (familiarity of the group) factors were of great importance for the modulation of agonistic behaviour of the test animals in our study. All other behavioural patterns, however, were influenced neither by the former dominance rank nor by the familiarity of the group in the conflict situation. Compared with the LR animals, the HR animals seemed to be favoured in terms of access to resources (eating/drinking), but this tendency was weak.

Behaviour and stress hormones

We observed that the high release of adrenaline and noradrenaline at the beginning of the confrontation test was closely related to the occurrence of agonistic interactions. A high correlation between agonistic behaviour and catecholamines was only found during the first hour of the test, whereas after the first feeding time only the locomotion of the animals as an expression of physical activity was positively correlated with the noradrenaline levels. The stronger activation of the SAM axis and the more agonistic behaviour of the HR animals after introduction into the groups also show the close relation between these parameters. There are very few data available about the influence of social stress on plasma catecholamine concentrations in pigs. In a previous study, a linear correlation between plasma levels of catecholamines and aggressive behaviour was found during dyadic encounters in pigs. However, no differences were found between dominants and subordinates (Fernandez et al., 1994b). In rats, the occurrence of active coping behaviour and aggression is characterized by a higher SAM reactivity (Fokkema et al., 1988; Sgoifo et al., 1996). However, adrenomedullary stimulation with an increased release of adrenaline can also occur during emotional distress situations and fear (Kågedal and Goldstein, 1988). The elevated catecholamine levels of HR animals after the initiating fighting period may be partly explained by an increased distress of these animals, which generally experienced more defeats, shown by a strong decrease of their dominance values.

It is generally known that social stress situations, e.g. mixing animals, or novelty can cause an activation of the HPA axis in pigs (Fernandez et al., 1994b; Hessing et al., 1994; Mormède et al., 1994; Bradshaw et al., 1996). The results of our study also showed an increase of plasma cortisol levels associated with enhanced agonistic behaviour after introduction of the test animals into the groups. However, during the first hour of confrontation, the adrenocortical response was less related to the frequency of agonistic interactions than the sympatho-adrenomedullary reaction. The adrenocortical reaction of the LR animals was similar during confrontation in the two groups, whereas HR animals showed lower cortisol levels after being introduced into the familiar group, but a very pronounced increase after confrontation with the unfamiliar group, indicating a strong activation of the HPA axis. This finding does not support the assumption that pigs classified as dominant/subdominant or aggressive/non-aggressive show consistent adrenocortical responses (Hessing et al., 1993). Other studies revealed differences in plasma cortisol between dominant and subordinate pigs only for baseline levels or under poor environmental conditions (Fernandez et al., 1994a; De Jonge et al., 1996). In another study, gilts were categorized as 'High Success', 'Low Success' and 'No Success' animals according to their relative success in agonistic interactions (Mendl et al., 1992). The 'No Success' pigs were involved in the fewest agonistic interactions and used aggression least often. The 'Low Success' pigs used more aggression during agonistic interactions, but also experienced the highest level of aggression and the greatest number of displacements from other animals. These pigs also had the highest baseline cortisol levels and the highest cortisol response to an ACTH challenge. The authors hypothesized that the repeated experience of defeat in agonistic interactions lead to elevations in SAM activity and longer-term HPA responses to stress in the 'Low Success' pigs (Mendl et al., 1992; Marchant et al., 1995). In relation to our results, it seems therefore possible that the elevated catecholamine levels and the strong increase of the HPA activity found in HR animals are not only caused by increased agonistic behaviour but also by the experience of more defeats in the unfamiliar group. The LR animals in our study reflected more the reaction shown by 'No Success' pigs. They had already experienced higher levels of defeat prior to the test situation (i.e. during the selection test) and possibly reacted with an avoidance strategy.

The present results indicate that during a confrontation test pigs show different agonistic behaviour and plasma stress hormone concentrations which are determined by the former dominance rank of the test animals and the familiarity of the group. No influences of rank or familiarity were found on the other behavioural patterns examined. In general, the frequent agonistic behaviour at the beginning of the test was accompanied by a HPA and SAM activation. The extent of the behavioural and endocrine responses, however, may be influenced by success and prior experiences during agonistic interactions. Further studies should consider the effects of early experiences of animals on the development of individual coping patterns.

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Physiological and behavioral effects of different success during social confrontation in pigs with prior dominance experience

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Abstract

The impact of a 10-h social confrontation on behavior, plasma adrenaline, noradrenaline and cortisol was studied in 14 domestic pigs (eight castrated males and six females) with prior dominance experience. Prior to the experiments, animal groups, each consisting of nine growing pigs (12 weeks old) from different litters, were composed randomly. After 5 days, the pig with the highest rank from each group was removed, provided with a jugular vein catheter and kept in single housing for 2–3 weeks. After this period, each test animal was returned into its familiar group for a 10-h social confrontation. The reintroduction of the test animals into the familiar groups caused frequent agonistic interactions during the first 30 min. Seven animals succeeded to win most of their encounters during the confrontation test and were classified as High Success (HS) animals, whereas seven other animals lost most of their encounters and were classified as Low Success (LS) animals. The reintroduction of the test animals into the groups provoked also marked changes in behavioral and physiological measures. The frequent fighting behavior during the first 30 min was accompanied by a rapid increase of plasma catecholamines and a delayed increase of cortisol. Immediately after introduction, LS pigs tended to show higher plasma adrenaline and noradrenaline concentrations than HS pigs. There was also a tendency for a sustained increase of noradrenaline/adrenaline ratios in HS pigs, whereas the ratios of LS pigs remained nearly unchanged. No significant differences between both groups were found in cortisol levels and in the frequency of agonistic interactions. However, LS animals showed less locomotion, more lying and spent less time exploring the pen or other animals. These results show that during a social confrontation the experience of defeats for formerly high-ranking pigs is accompanied by increased submissive or passive behavior and a higher sympathoneural and adrenomedullary reaction, which may indicate more emotional distress and fear compared to successful animals.

Keywords: Pig; Social stress; Agonistic behavior; Dominance; Coping; Catecholamines; Cortisol

1. Introduction

It is well known that domestic pigs establish a social hierarchy of dominant and submissive animals, which is the result of agonistic interactions between the individuals [11]. Frequent regrouping and mixing of the animals is a common practice in livestock production systems and causes the need for reestablishment of the social structure. This may result in social stress and welfare or production problems. Several studies have shown that some behavioral and physiological consequences of social stress in dominant or submissive pigs are differently expressed [3,14,15,26]. Generally, there is evidence that dominant animals have the advantage of being able to cope with stressful situations compared to their submissive conspecifics [27]. For instance, for dominants, social interactions are more predictable and can be controlled [26]. Furthermore, prior experiences of winning are likely to increase the propensity to behave aggressively and experiences of defeat to decrease it [12,20]. However, acute social defeat may have a long-lasting negative impact on the loser animal, including an increasing loss of social control [10,19]. Although the ‘loser effect’, in which an individual losing one encounter is likely to lose the next, is relatively well understood, studies of the potential ‘winner effect’ have produced mixed results [1]. Considerable evidence demonstrates that the agonistic role of an individual is apparently determined by its own social experience [2,22].

In a previous study of our group, it could be shown that the agonistic behavior and the activation of the sympatho–

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adrenomedullary (SAM) and the hypothalamic–pituitary–adrenal (HPA) system in pigs during a social confrontation test are determined by the former dominance rank of the animals (high vs. low ranking) and the familiarity of the group (familiar vs. unfamiliar) [14,15]. This was true although the test animals were socially separated for 2–3 weeks before the social confrontation test was done. One surprising 'secondary result' of the study mentioned above is of special interest for the concern of the present paper: Whereas the low-ranking pigs in no case could improve their rank in the later social confrontation test, the high-ranking pigs showed a great variation in their later obtained dominance values from absolute dominance to absolute submissiveness. These animals also showed a higher variation and an increased activation of the SAM and HPA systems compared to the low-ranking animals. The question arises which behavioral and physiological consequences the loss of winning might have in animals with prior dominance experience compared to winners who keep winning. Hence, the present study deals with the effects of different success in agonistic encounters on behavioral reactions and plasma stress hormone responses of formerly high-ranking pigs. As a test situation, the social confrontation with the familiar group was used after a separation period of 2–3 weeks. In the present study, the data of 14 high-ranking pigs as classified in the study of Otten et al. [15] were recalculated and analyzed with the aim to elucidate the behavioral and physiological consequences for winners when they are able to continue to win or, alternatively, when they predominantly begin to lose.

2. Methods

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Agricultural Department of Mecklenburg-West Pomerania, Germany.

2.1. Animals and housing

Eighteen experimental groups were randomly composed, each consisting of nine young growing pigs (German Landrace). In each group, animals were derived from six different litters with a maximum of two animals from the same litter. At the time of mixing, the pigs were approximately 12 weeks old (80.4 ± 2.6 days). The animals were kept on a concrete floor (6.5 m^2 per group) with little straw. Feed was supplied at 09:00 and 15:00 h, and the animals had free access to the trough from 09:00 to 11:00 h and from 15:00 to 17:00 h (animal/feeding place ratio = 2.25:1). Water was supplied ad libitum.

2.2. Selection of dominant animals

As described previously, agonistic behavior of all animals was recorded by direct observation for 3 days immediately after mixing (10 h daily from 08:00 to 18:00 h, continuous event sampling) [14,15]. Agonistic interactions were defined as fights or displacements of two animals for more than 2 s and intervening periods of at least 8 s [16]. The animal, which first stopped fighting, turned away from an attack, tried to flee or was displaced was claimed to be the loser, whereas the other animal was defined as the winner [26]. Individual dominance values were calculated for each animal by the number of wins minus defeats in relation to all decisive fights with its group mates over the whole observation period. The individual dominance values, ranging from -1.00 (no wins) to $+1.00$ (no defeats), were used as a relative measure for the dominance of an animal within the group, indicating the mean fighting success related to its group mates. The animal with the highest dominance value in each group was considered to be the highest ranking animal. These animals were removed from the groups 5 days after mixing and kept in single housing pens. Two other animals, one with the lowest and one with a middle dominance value, were also removed from each group at the same time for other investigations, whereas the remaining six animals stayed in their pens. The mean female/castrated male ratio in these groups was 54.2%:45.8%.

2.3. Surgery

After an isolation period of 1 week, the test animals underwent surgery under general anesthesia with two intravenous injections of 200-mg Xylazin and 2-g Ketamin in total and a catheter was inserted in the external jugular vein. For protection of the catheter outlets, the test animals were equipped with neck straps. In the following recovery period (7–11 days), the test animals were habituated to frequent handling by simulating the manipulations for blood sampling three times a day, including rinsing of the catheters.

2.4. Social confrontation test

After the recovery and handling period, each test animal was confronted with its familiar group of six animals in a test pen (4.0 × 1.6 m) with a concrete floor and little straw. Each confrontation test lasted 10 h from 08:00 to 18:00 h with feeding times from 09:00 to 11:00 h and from 15:00 to 17:00 h (animal/feeding place ratio = 1.75:1). During the confrontation test, all pigs in the group were equipped with neck straps in order to achieve comparable conditions for all animals.

2.5. Behavioral measurements and classification of the animals

Agonistic interactions between the test animal and group members were recorded by continuous sampling as described above. A new individual dominance value was calculated for the test animals during the social confrontation test as described at the selection of the test animals. Due

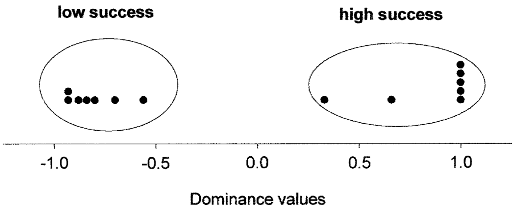

Fig. 1. Dominance values of HS ($n=7$) and LS ($n=7$) pigs during the social confrontation test with familiar groups.

to infections during the experimental period, four animals could not be used in the confrontation test. From the remaining 14 pigs, seven animals (five castrated males and two females) with new dominance values greater than 0 were classified as High Success (HS) animals and seven animals (three castrated males and four females) with dominance values lower than 0 were classified as Low Success (LS) animals (Fig. 1). The average body weight of the HS test animals at the time before the experimental groups were composed was 35.5 ± 2.8 kg (LSM ± S.E.) and for the LS test animals 32.7 ± 2.7 kg (at the time of surgery 44.6 ± 2.8 and 40.8 ± 2.8 kg and at the time of the confrontation tests 56.1 ± 3.7 and 51.5 ± 3.5 kg). No significant differences in body weight between HS and LS animals were found at all ages examined.

In addition, the following behavioral patterns of the test animals were recorded in 30-s interval sampling: agonistic interactions, exploration of the pen or other animals, locomotion, lying, standing or sitting, eating or drinking, waiting at the trough or watering place. For evaluation, the behavioral data were summarized in 30-min intervals and presented as the percentage of each behavioral pattern on all observations (for more details, see Otten et al. [15]).

2.6. Blood sampling and hormone analyses

Twenty-five blood samples were obtained from each test animal before and during the confrontation test on a fixed time schedule. Three samples were taken 30, 15 and 5 min before the confrontation test. During the 10-h test period, blood samples were taken every full hour (10 samples). Additional samples were taken 5, 10, 20 and 40 min after introducing the animal into the group and after the beginning of the two feeding times. Blood samples were taken with ice-cooled polypropylene sampling tubes containing EDTA (Kabevette, Kabe Labortechnik, Nürnbrecht-Elsenroth, Germany), immediately placed on ice and subsequently centrifuged at 2000 × *g* for 15 min at 4 °C. Plasma was then stored at − 80 °C until analysis.

Plasma concentrations of adrenaline and noradrenaline were analyzed by high-pressure liquid chromatography with electrochemical detection after extraction from the plasma samples by absorption on aluminum oxide [14]. Intra- and interassay coefficients of variation, tested on midrange samples, were 11.5% and 12.5% for adrenaline and 2.1% and 1.9% for noradrenaline. Plasma concentrations of cortisol were analyzed in ethanol-extracted samples using a single-antibody radioimmunoassay technique [9]. The intra- and interassay coefficients of variation were 4.8% and 9.4%.

2.7. Statistical analysis

Data were analyzed by a repeated-measurement analysis of variance using the GLM procedure of SAS [24]. The statistical model for the behavioral and physiological data included the repeated factor *time* (number of levels depending on the sample schedule of each parameter) and the class variables *dominance value* (HS and LS) and *gender* (female, castrated male). In addition, *F*-tests were performed on all interactions between the repeated factor and the class variables, and differences within repeated factors were tested with Student's *t* test.

3. Results

3.1. Dominance values and behavior

After introduction of the test animals into their familiar groups, agonistic interactions between the intruding animal and the group mates were observed. Compared with the previous selection test, the new confrontation resulted in a decrease of the mean dominance values of the test animals (0.03 ± 0.16 vs. 0.71 ± 0.16; $P<.01$). The seven HS animals, which succeeded to win most or all of their agonistic interactions, had a mean dominance value of 0.78 ± 0.11 compared to − 0.74 ± 0.11 for the LS animals ($P<.001$; Fig. 1).

The highest frequency of agonistic interactions between the intruding animals and group members was observed during the first 30 min of the confrontation test. During this period, LS pigs tended to be involved in more agonistic interactions than HS pigs (Fig. 2). No significant effects of *dominance value* or *gender* were found on the level or time course of the agonistic interactions.

A significant influence of *dominance value* was found on the frequency of exploration behavior [$F(1,10)=13.70$,

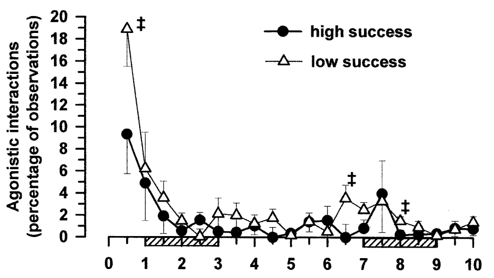

Fig. 2. Agonistic interactions of HS ($n=7$) and LS ($n=7$) pigs during the social confrontation test. Animals were introduced into their familiar groups at time = 0. Feeding times are indicated by hatched bars. Values are least-square means ± S.E. Differences tend to be different with $P<.10$ (‡).

$P<.01$] and locomotion [$F(1,11)=4.95$, $P<.05$] and a significant influence of the interaction *dominance value × time* was found on the frequency of exploration behavior [$F(19,190)=4.29$, $P<.001$], locomotion [$F(19,209)=1.71$, $P<.05$] and lying [$F(19,209)=1.88$, $P<.05$]. HS pigs spent more time exploring the pen or other animals during the first 2 h and showed more locomotion during the first hour and before the two feeding times than the LS pigs (Fig. 3). In contrast, lying behavior of LS pigs was significantly increased during the first 30 min of the confrontation test. After this period, lying was the behavior with the highest proportion of all behavioral patterns in all animals (Fig. 3). No significant effects of *gender* or related interactions were found on any of the behavioral patterns.

3.2. Stress hormones

A significant influence of the interaction *dominance value × time* was found on the plasma concentrations of adrenaline [$F(24,264)=1.94$, $P<.01$] and noradrenaline [$F(24,264)=1.86$, $P<.01$]. Immediately after starting the confrontation test, LS pigs tended to have higher adrenaline and noradrenaline concentrations than HS pigs (Fig. 4). During the first 2 h, the plasma concentrations of adrenaline and noradrenaline decreased approximately to the levels found immediately before the test. Slight increases of catecholamine concentrations were noted at the beginning of each feeding time. After the frequent encounters of the first 30 min and the encounters at the beginning of the two feeding times, there was a tendency for a sustained increase of noradrenaline/adrenaline ratios in HS pigs, whereas the ratios of LS pigs remained nearly unchanged (Fig. 4).

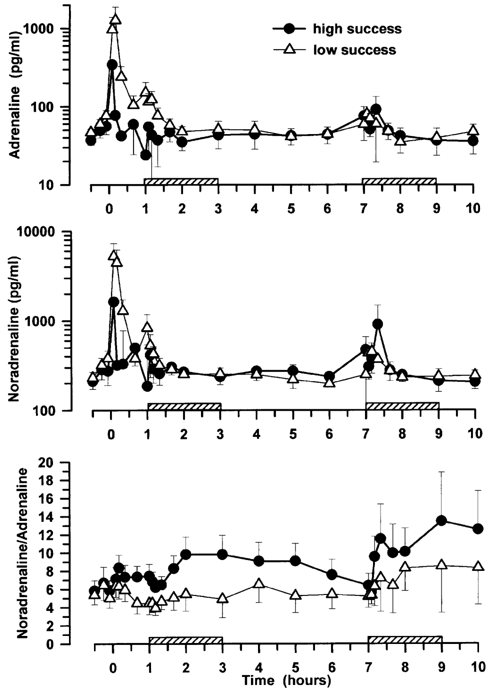

Fig. 4. Plasma levels of adrenaline, noradrenaline and noradrenaline/adrenaline ratio of HS ($n=7$) and LS ($n=7$) pigs during the social confrontation test. Log scales are used for the presentation of adrenaline and noradrenaline values. Animals were introduced into their familiar groups at time = 0. Feeding times are indicated by hatched bars. Values are least-square means ± S.E.

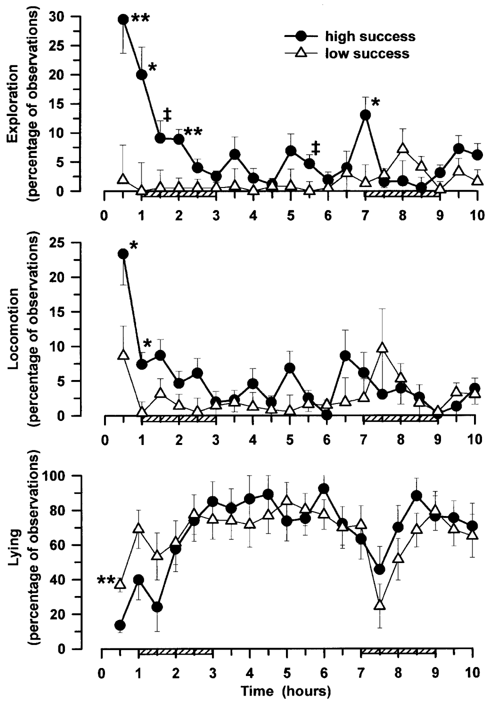

Fig. 3. Exploration, locomotion and lying of HS ($n=7$) and LS ($n=7$) pigs during the social confrontation test. Animals were introduced into their familiar groups at time = 0. Feeding times are indicated by hatched bars. Values are least-square means ± S.E. Differences tend to be different with $P<.10$ (‡) or are significantly different with $P<.05$ (*) or $P<.01$ (**).

In general, the test situation caused an increase of the plasma cortisol levels in the intruding animals within the first 2 h (Fig. 5). Cortisol concentrations of the LS animals

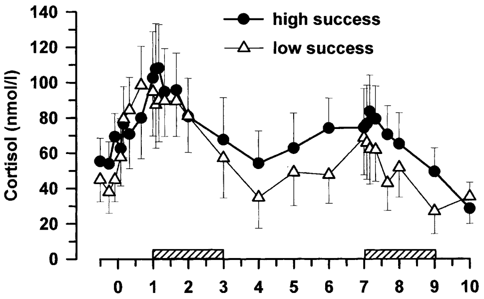

Fig. 5. Plasma levels of cortisol of HS ($n=7$) and LS ($n=7$) pigs during the social confrontation test. Animals were introduced into their familiar groups at time = 0. Feeding times are indicated by hatched bars. Values are least-square means ± S.E.

reached a maximum after 40 min, whereas HS animals reached maximum cortisol concentrations not until 10 min after starting the first feeding time. No significant differences were found between HS and LS animals. However, LS pigs showed a tendency for lower cortisol levels after the first feeding time (Fig. 5).

The effect of *time* was found to be significant with $P < .01$ for all behavioral and physiological parameters examined.

4. Discussion

In the present study, we investigated behavioral and physiological consequences of success during social confrontation in pigs with prior dominance experience. We found that the formerly high-ranking pigs, which lost most or all of their encounters after reintroduction into their familiar groups (LS animals), tended to have more agonistic interactions with pen mates immediately after reintroduction, showed less exploration and locomotion but more lying during the first hours of the confrontation test than the HS animals. Immediately after the beginning of the confrontation, the LS animals also tended to show higher plasma catecholamine concentrations, indicating a higher SAM activation at this time.

4.1. Behavior

In the related study of our group, it was shown that agonistic behavior and the activation of the SAM and HPA systems in pigs during a social confrontation test are determined by the former dominance of the animals (high vs. low ranking) and the familiarity of the group (familiar vs. unfamiliar) [14,15]. The frequency of agonistic behavior during the confrontation test strongly depended on the former dominance rank of the animals with high-ranking pigs showing more agonistic interactions during the first 30 min after introduction into the groups than low-ranking pigs. Test animals had also more agonistic interactions in the unfamiliar than in the familiar group. No effects of the former rank or the familiarity of the confrontation group were found on the other behavioral patterns examined such as locomotion, exploration and lying [15]. The present results, however, revealed significant differences between HS and LS pigs in exploration, locomotion and lying behavior but only a tendency for differences in the frequency of agonistic behavior during the first 30 min. Taken together, the results of both studies indicate that the agonistic behavior of the intruding animal in the confrontation test is determined by its former dominance rank and by the familiarity of the group, whereas behavioral patterns typical for submissive or passive behavior seem to be predominantly affected by the new experience of defeats during this test situation. However, the occurrence of submissive or passive behavior in LS pigs seems to be different from the behavior of losers who kept losing, because in the previous study, no differences in nonagonistic behavior were found between low-ranking pigs losing their agonistic encounters during the confrontation test and high-ranking pigs [15].

The question arises why some of the formerly dominant animals keep winning while the other animals start losing their encounters in the same test situation. Some authors suggest that the subordinate animals play a major role in maintaining the dominance relationships within the group [7,18]. Normally, when familiar pigs meet again, the fights are shorter and involve more defensive behavior by the loser than fights of unfamiliar pigs. This behavior of the loser may result from a change in its assessment of its own winning chance [21]. In the present study, all members of the group were previously subordinated to the intruding animal, which should be interested in keeping its former rank position. It is clear that the first encounters during the confrontation test determine the chance of the former winner to keep winning [6] and that the behavior of the group mates is of decisive importance for this chance. The agonistic interactions during the first 30 min show that the pigs later classified as LS tended to be involved in more fights than the HS pigs. We suppose that during the frequent fights of the later LS animals in this period, exhaustion and signs of weakness are sufficient for the subordinates to reevaluate their chance of winning and to raise the assessment of their own fighting. This may explain why LS animals were behaviorally less active while at the same time they were more involved in agonistic interactions than HS animals. Consequently, the concerned test animals begin to lose, whereas the others are able to assert themselves.

4.2. Catecholamines

The frequent agonistic behavior after reintroduction of the test animals into their groups was accompanied by a strong increase of plasma catecholamines, indicating an increased activation of the SAM system. Immediately after starting the confrontation test, LS pigs showed higher plasma adrenaline and noradrenaline concentrations than HS pigs. After the frequent encounters of the first 30 min, LS pigs tended to have lower noradrenaline/adrenaline ratios compared to the HS animals, indicating a higher adrenomedullary stimulation following the occurrence of submissive behavior at the beginning of the test.

Activation of the sympathetic nervous system and the release of catecholamines from the adrenal medulla was found in relation to different demanding behaviors such as exercise and aggressive encounters [3] and with the occurrence of an aggressive/active coping strategy in rats [4,23]. Adrenomedullary stimulation with an increased release of adrenaline, resulting in lower noradrenaline/adrenaline ratios, is primarily caused by emotional arousal and fear, whereas sympathoneural activity with an increase of plasma noradrenaline levels is predominantly affected by conditions involving physical exercise [8,23,25,28]. In a study with rats in a resident–intruder situation, noradrenaline/adrenaline ratios decreased in defeated intruders with the passing of time in association with the exhibition of clear passive/submissive patterns of behavior [23]. The observed responses in the present study, less exploration and locomotion and more lying together with increased adrenomedullary stimulation, strongly indicate more alertness, fear and uncertainty of the LS animals after the new experience of frequent defeats.

4.3. Cortisol

In general, the social stress situation caused an increase of the plasma cortisol levels in the intruding animals within the first hour and at the beginning of the two feeding times. Activation of the HPA axis is known to be caused by social stress and/or novelty in pigs [3,5,15]. In a study with gilts, it was shown that the success in agonistic interactions determines the stimulation of the HPA axis in a different manner. A high level of aggression associated with low success in agonistic interactions caused the highest basal cortisol levels and a higher adrenal responsiveness to ACTH compared to animals with high or no success in agonistic encounters [13]. Our previous results also showed that formerly high-ranking pigs, which experienced defeats during a confrontation with an unfamiliar group, reacted with a strong increase of plasma cortisol levels compared to low-ranking animals [15]. One might assume that the new experience of frequent defeats may be responsible for the elevated HPA activity. However, the present data revealed no significant differences in plasma concentrations of cortisol between HS and LS pigs. It seems therefore possible that not only the success but also the novelty of the confrontation situation and the familiarity of the group determine the stimulation of the HPA axis in formerly dominant pigs. This is supported by ethophysiological findings on the effect of different weaning methods in pigs [17]. In this study, the authors conclude that piglets appear to have considerable problems in coping with a new environmental situation (e.g. an unfamiliar housing environment).

In conclusion, the results of this study show that during a social confrontation with a familiar group, formerly high-ranking pigs show different coping patterns depending on the success in agonistic encounters. The new experience of defeats is accompanied with submissive or passive behavior, i.e. reduced exploration and locomotion and more lying, and a higher sympathoneural and adrenomedullary stimulation, which also indicates more emotional distress and fear compared to successful animals. Including the results of our previous study, the activation of the HPA axis in formerly dominant pigs seems to be more determined by the novelty of the confrontation situation and the familiarity of the group than by the experience of defeats during social confrontation.

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8 ANHANG

8.1 Abbildungsverzeichnis

8.2 Abkürzungsverzeichnis

ACTH	Adrenocorticotropic hormone (Adrenokortikotropes Hormon)
bzw.	beziehungsweise
CRH	Corticotropin-releasing hormone (Kortikotropin-freisetzendes Hormon)
d.h.	das heißt
DV	dominance value (Dominanzwert)
etc.	*et cetera* (und so weiter)
EU	Europäische Union
h	Stunde(n)
HPA	hypothalamo-hypophysär-adrenal
IgG	Immunglobulin G
kHz	Kilohertz
LPS	Lipopolysaccharid
min	Minute(n)
Mio.	Million(en)
N/L	neutrophils-to-lymphocytes ratio (Verhältnis neutrophiler Granulozyten zu Lymphozyten)
r	Korrelationskoeffizient nach Pearson
s	Sekunde(n)
SAM	sympatho-adrenomedullär
TNFα	Tumornekrosefaktor α
vgl.	vergleiche
vs.	*versus* (contra)
z.B.	zum Beispiel

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8.4 Eidesstattliche Erklärung

Ich erkläre hiermit, dass ich ein Verfahren zum Erlangen der Habilitation bisher an keiner wissenschaftlichen Einrichtung beantragt habe.

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Dummerstorf, April 2008

Birger Puppe