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**Quantitative genetic analyses for dairy cow
behavior traits and traits reflecting
human-animal-technic interactions**

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List of abbreviations

AD	avoidance distance
AMF	average milk flow
AMS	automatic milking systems
CA	calving age
DUR	milking duration of each visit in min
DIMcl	days in milk (DIM) in classes
EBV	estimated breeding values
EC	electrical conductivity
EC_fl	electrical conductivity of the front left quarter
EC_fr	electrical conductivity of the front right quarter
EC_rl	electrical conductivity of the rear left quarter
EC_rr	electrical conductivity of the rear right quarter
ECM	energy corrected milk
FG	farm effect combined with performance class in the herd
FP	fat percentage
FPR	fat to protein ratio
GLMM	generalized linear mixed models
HAR	human animal relationship
Huthcl	days in milk in classes according to Huth (1995)
INT	interval between two consecutive milkings
INTcl	interval in classes
IQR	inter quarter ratio
KO	knocking off of the milking device as a binary trait; threshold \geq one udder quarter
LN	lactation number
MA	milkability
MS	milking speed
MT	milking temperament
MY	milk yield
MY_fl	milk yield of the front left quarter
MY_fr	milk yield of the front right quarter

List of abbreviations

MY_rl	milk yield of the rear left quarter
MY_rr	milk yield of the rear right quarter
MY_total	total milk yield per day
NR90	non-return rate 90 days after insemination
n.s.	not significant
OBV	original breeding values
PCA	principal component analysis
PC1	first principal component
PP	protein percentage
QBA	qualitative behavior assessment
QBA_PC1	cows individual scores from the 1 st principal component analysis
QTL	quantitative trait loci
RB	behavior during release from restrain
RBV	relative breeding values
RZE	complex conformation
RZG	total merit index
RZM	complex milk production
RZN	functional herd life
RZR	complex female fertility
RZS	complex udder health
SCC	somatic cell count
SCS	somatic cell score
S.E.	standard error
STEM	structural equation models
ToD	time of day
TTI	tolerance to tactile interaction
VIS3	visits per cow and day as a binary trait; threshold ≥ 3 visits
VIS4	visits per cow and day as a binary trait; threshold ≥ 4 visits
YS	year and season

SUMMARY

The aim of the present study was to carry out a quantitative genetic analysis for traits reflecting dairy cow behavior, as well as for traits reflecting animal-technic and human-animal interactions. Furthermore, on the basis of records from various databases, three different scientific studies were carried out (see **Chapters 3, 4 and 5**). These studies are based on the main objective of the thesis, but were developed as separate research studies with the purpose of publication in multiple international scientific journals.

Functional traits reflecting animal behavior and temperament, in combination with stress indicators, are becoming increasingly important in dairy cattle breeding. Thus, in the first research study (**Chapter 3**), (co)variance components were estimated on the basis of already recorded traits from routine performance testing and voluntary cow traffic. The focus of the analysis was on average milk flow (AMF), since this trait partly reflects the human-animal-relationship in the milking parlor and also stress responses at a hormonal level. Measurements were available for AMF in kilograms of milk per minute of milking time for 629,161 Holstein cows from the calving years 1990 to 2008. For the genetic-statistical analysis, alternative modeling based on two different approaches was used. The first approach was based on the threshold method, while the second expanded the statistical model with the incorporation of production and functional traits such as fertility, health and behavior traits using structural equation models (STEM). STEM models have the potential to infer relationships and feedback situations among traits by reflecting a more physiological background. The heritabilities for the AMF trait were, for all models, in a moderate range (0.29 - 0.38). Due to the moderate heritabilities, we propose to systematically integrate the AMF trait with a high weighting value into total breeding values for dairy cattle and to specific select towards AMF. In general, from an animal breeding perspective, due to antagonistic relationships between AMF and milk content (AMF and Fat % $rg = (-0.18) - (-0.73)$; AMF and Protein % $rg = (-0.21) - (-0.85)$), as well as between AMF and udder health (AMF and SCS $rg = 0.17 - 0.43$), no trait maximization, but a trait optimization with an intermediate optimum for this trait would be desirable.

Regarding the statistical models, substantially smaller standard deviations of posterior estimates for structural equation coefficients compared with regression coefficients recommend recursive model applications.

In a second scientific study (**Chapter 4**), the impact of the milking technique on the individual animal and the reaction of the animal on the technique were investigated. With the use of objectively recorded and longitudinal data from automatic milking systems (AMS), auxiliary traits that reflect animal behavior in the milking system were defined, free from subjective impressions of classifiers. Data was available for a period of 30 days and included 70,700 observations (visits to the AMS) from 922 Holstein cows kept on three German farms equipped with the DeLaval company's "FeedFirst" AMS System. AMS traits used as behavior indicators include: AMS visits per cow per day, defined as two binary traits, with thresholds for i) more than three visits per day (VIS3) and ii) more than four visits per day (VIS4). The VIS3 and VIS4 traits were defined as binary traits to emphasize the "extra" voluntary component, i.e., the additional AMS visit beyond the typical herd average. Further temperament traits were the knocking off of the milking device (KO) from at least one udder quarter, also defined as a binary trait, milking duration of each visit in the AMS in minutes (DUR), the average milk flow (AMF) and the interval (INT), defined as the time span between two consecutive AMS visits. Since milk yield is an important factor influencing these traits, univariate models with and without milk yield as a covariate were compared. A second AMS dataset included measurements for total milk yield per day (MY_total) and electrical conductivity (EC) of milk, also available on an udder quarter basis. The heritability for AMF ($h^2 = 0.25$) was moderate, indicating possibilities for AMS milkability improvements via breeding. Heritabilities estimated with univariate models, with and without milk yield as a covariate, were comparable, with slightly larger heritabilities for some traits in models with milk yield as a covariate ($h^2 = 0.17$ for VIS3, $h^2 = 0.08$ for VIS4 and $h^2 = 0.18$ for INT). This suggests a behavior or temperament background behind these traits, rather than milk yield being the only motivator for, e.g., AMS visits. Heritabilities for EC varied between 0.37 and 0.46, depending on the udder quarter; this information at an udder quarter level could be useful for precision dairy farming. In terms of genetic relationships, an increase of AMF, a decrease in DUR and a decrease of INT may contribute to desired behavior and AMS efficiency, without

losing genetic gain for production. Hence, due to unfavorable correlations with health indicator traits, these traits should be considered as traits with an intermediate optimum. Based on these results, there is an apparent possibility to breed cows for AMS systems based on AMS data, though it is imperative to have further validation based on larger datasets.

In a preceding study, four behavioral traits intended to assess dairy cow's responsiveness towards humans were identified as repeatable in terms of inter- and intra-observer reliability (Ebinghaus et al., 2016). In **Chapter 5**, genetic (co)variance components were estimated for these traits. Data from 1,761 cows were used to analyze the trait tolerance to tactile interaction (TTI), from 1,766 cows for the behavior during release from restraint in the feeding gate (RB), and from 1,880 cows for the avoidance distances towards an unfamiliar person at the feeding place (AD). Furthermore, the Qualitative Behavior Assessment (QBA) was conducted on 565 cows. For further analysis, the cows' individual scores of the first principal component (PC1) which explained almost 70 % of the variation (QBA_PC1) characterized by descriptors relating to relaxation/attraction/trust on the negative and descriptors relating to fear/distress/aversiveness on the positive end were used. Estimated heritabilities and their corresponding standard errors for behavior traits reflecting human animal interactions were 0.27 ± 0.06 for the AD, 0.04 ± 0.05 for TTI, 0.11 ± 0.06 for RB and 0.13 ± 0.17 for QBA_PC1. Estimated breeding value (EBV) correlations (r_{EBV}) for the most influential sires among the behavioral measures AD, TTI, RB (restriction: sires with at least 5 daughters), and QBA_PC1 (no restriction) were moderately positive, indicating a relationship to one another and that they are partly influenced by a similar genetic component. The positive EBV correlation between AD and SCS reinforced the concept that breeding towards less fearful animals would have a positive impact on the animals' health. Moreover, sires with high EBV for longevity traits tend to have daughters with lower avoidance distances and less fearful behavior. The EBV correlation between the composite functional herd life (RZN) and AD was -0.28.

Although behavior traits depend, to a large extent, on the environment and management factors, the genetic component seems to play an important role. These results support the idea of including behavior traits that reflect the human-animal-relationship into breeding strategies.

A general discussion about the results and the applied statistical models from the previous chapters are given in **Chapter 6**. Additionally, some concerns and outlooks regarding behavior traits and traits reflecting human-animal-interactions and their inclusion in animal breeding strategies are discussed.

ZUSAMMENFASSUNG

Das Ziel der Arbeit bestand darin, quantitativ-genetische Analysen durchzuführen. Diese Analysen bezogen sich auf Merkmale, die das Milchkuhverhalten sowie die Tier-Technik und Mensch-Tier Interaktionen widerspiegeln. In der vorliegenden Arbeit wurden auf Grundlage von verschiedenen Datenbanken drei verschiedene wissenschaftliche Studien durchgeführt (siehe **Kapitel 3, 4 und 5**). Diese Kapitel basieren auf dem Gesamtziel der Arbeit, wurden aber als separate Forschungsstudien mit dem Ziel der Veröffentlichung in internationalen wissenschaftlichen Fachzeitschriften konzipiert.

Funktionale Merkmale, die das Tierverhalten und das Temperament in Verbindung mit Stressindikatoren widerspiegeln, werden in der Milchviehzucht immer wichtiger. Daher wurden in **Kapitel 3** (Ko)varianzkomponenten auf Basis bereits erfasster Merkmale der routinemäßigen Leistungsprüfung und des freiwilligen Kuhverkehrs geschätzt. Der Fokus der Analysen richtete sich auf das Merkmal durchschnittliches Minutengemelk (DMG), da dies z. T. direkt die Mensch-Tier-Beziehung im Melkstand und Stressreaktionen auf hormonaler Ebene reflektiert. Zur Verfügung standen Messungen von DMG in Kilogramm pro Minute Melkzeit von 629.161 Holstein Kühen aus den Kalbejahren 1990 bis 2008. Für die genetisch-statistische Analyse wurden alternative Modellierungen verwendet, die auf zwei unterschiedlichen Ansätzen basieren. Der erste Ansatz basiert auf der Schwellenwertmethode. Im zweiten Ansatz wurde durch eine Erweiterung der statistischen Modellierungen der Einbezug von produktions- und funktionalen Merkmalen (Fruchtbarkeits-, Gesundheits-, und Verhaltensmerkmale), unter Anwendung der Strukturgleichungsmodelle (STEM) - Theorie, betrachtet. STEM Modelle haben das Potenzial Beziehungen und Feedback-Situationen zwischen den Merkmalen abzuleiten, da diesen ein physiologischer Hintergrund zu Grunde liegt. Die Erblichkeiten lagen für alle Modelle im moderaten Bereich (0,29 - 0,38). Aufgrund der moderaten Erblichkeiten wird vorgeschlagen, das Merkmal DMG in die Gesamtzuchtwerte beim Milchrind systematisch mit hohem Gewicht zu integrieren und zielgerichtet auf DMG zu selektieren. Generell ist aus tierzüchterischer Sicht, aufgrund antagonistischer Beziehungen zwischen DMG und

Gehaltsmerkmalen der Milch (DMG und Fett % $r_g = (-0,18) - (-0,73)$; DMG und Eiweiß % $r_g = (-0,21) - (-0,85)$) sowie zwischen DMG und Merkmalen der Eutergesundheit (DMG und somatischer Zellzahl $r_g = 0,17 - 0,43$), keine Merkmalsmaximierung, sondern eine Merkmalsoptimierung im Sinne eines intermediären Optimums anzustreben. In Bezug auf die statistischen Modelle empfehlen wesentlich kleinere Standardabweichungen der posterior(i) Schätzungen für Strukturgleichungskoeffizienten, im Vergleich zu Regressionskoeffizienten, die Anwendung von rekursiven Modellen.

In einer zweiten wissenschaftlichen Studie (**Kapitel 4**) wurde die Auswirkung der Melktechnik auf das einzelne Tier und die Reaktion der Tiere auf die Technik untersucht. Mit Hilfe von objektiv gemessenen longitudinalen Daten aus automatischen Melksystemen (AMS) wurden Hilfsmerkmale definiert, die das Verhalten der Tiere frei von subjektiven Eindrücken von Klassifikatoren, widerspiegeln. Die Daten waren aus einem dreißigtägigen Zeitraum verfügbar und beinhalteten 70.700 Beobachtungen (AMS Besuche) von 922 Holstein Kühen, die in deutschen Betrieben gehalten wurden und mit dem AMS-System „FeedFirst“ von DeLaval ausgestattet waren. Die Merkmale, die als Verhaltensindikatoren benutzt wurden waren: AMS-Besuche pro Kuh und Tag definiert als binäres Merkmal mit den Schwellen i) mehr als drei Besuche pro Tag (VIS3) und ii) mehr als vier Besuche pro Tag (VIS4). Diese Merkmale wurden als binäre Merkmale definiert, um die „extra“ freiwillige Komponente hervorzuheben. Darunter fielen zusätzliche AMS-Besuche, die über den typischen Herdendurchschnitt hinausgingen. Weitere Temperamentindikatoren waren das Abschlagen des Melkgeschirrs (KO) von mindestens einem Euterviertel, auch als binäres Merkmal definiert, die Melkdauer jedes AMS Besuchs in Minuten (DUR), das durchschnittliche Minutengemelk (DMG) und das Intervall (INT) definiert als die Zeitspanne zwischen zwei aufeinander folgende AMS Besuchen. Da die Milchleistung ein wichtiger Faktor ist, die diese Merkmale beeinflussen kann, wurden univariate Modelle mit und ohne Milch kg als kovariable verglichen. Ein zweiter Datensatz enthielt Messungen für die Gesamtmilchleistung pro Tag (MY_total) und die elektrische Leitfähigkeit (EC) (genutzt als Gesundheitsindikator), die auch auf Euterviertelbasis verfügbar war. Die Erbllichkeit für DMG ($h^2 = 0,25$) war moderat, was für die Möglichkeit einer Verbesserung der

Melkbarkeit im AMS durch Zucht spricht. Heritabilitäten, die mit univariaten Modellen mit und ohne Milch kg als Kovariable geschätzt wurden, waren mit etwas höheren Heritabilitäten für einige Merkmale, die im Modell auf Milch kg korrigiert wurden ($h^2 = 0,17$ für VIS3; $h^2 = 0,08$ für VIS4 und $h^2 = 0,18$ für INT), vergleichbar. Diese Ergebnisse bestätigen die Hypothese, dass nicht nur die Milchleistung als Motivator hinter diesen Merkmalen steckt (z.B. AMS-Besuche), sondern auch eine Verhaltenskomponente. Erblichkeiten für EC variierten je nach Euterviertel zwischen 0,37 und 0,46 und lagen somit im moderaten bis hohen Bereich. Verschiedene EC Erblichkeiten für verschiedene Euterviertel ermöglichen euterviertelspezifische Zuchtstrategien und könnten z.B. in „precision dairy farming“ genutzt werden. In Bezug auf die genetischen Beziehungen könnte eine Zunahme von DMG bei einer gleichzeitigen Abnahme der DUR ($r_g = -0,88$) zum gewünschten Verhalten und zur AMS-Effizienz beitragen, ohne den genetischen Gewinn für die Produktion zu verlieren (r_{EBV} zwischen DMG und MY_total = 0,40; und zwischen DUR und MY_total = 0,25). Auch hier sollte aufgrund von ungünstigen Korrelationen mit Gesundheitsindikatoren keine Merkmalsmaximierung sondern eine Merkmalsoptimierung angestrebt werden. Die Zunahme von automatisch objektiv aufgezeichneten AMS Merkmalen mit einem moderaten genetischen Hintergrund rechtfertigt die Abwandlung von Milchvieh-Zuchtzielen in Richtung einer höheren Gewichtung von Verhaltensmerkmalen, insbesondere bei der Entwicklung spezifischer Roboterindizes. Die Selektion in Richtung eines verbesserten Verhaltens und Temperaments wird gleichzeitig die Milchproduktivität verbessern. Dennoch schlagen wir weitere Untersuchungen mit einem größeren Datensatz vor, um die Ergebnisse der vorliegenden Pilotstudie zu validieren.

In einer vorhergehenden Studie wurden vier Verhaltensmerkmale, die Rückschlüsse auf die Reaktivität der Tiere gegenüber dem Menschen zulassen, hinsichtlich der Inter- und Intra-Observer-Reliabilität als wiederholbar identifiziert (Ebinghaus et al., 2016). Daher wurden in **Kapitel 5** genetische (Ko)varianzkomponenten für diese Merkmale geschätzt. Daten von 1.761 Kühen wurden verwendet, um das Merkmal Berührungstoleranz (TTI) zu analysieren. Dabei wurde von 1.766 Kühen für das Merkmal Verhalten beim Freilassen aus der Fixierung im Fressgitter (RB) und von 1.880 Kühen für das Merkmal Ausweichdistanz gegenüber einer unbekannten Person am

Fressgitter (AD) berücksichtigt. Darüber hinaus wurde eine qualitative Verhaltensbeurteilung (QBA) bei 565 Kühen durchgeführt. Für die weitere Analyse wurden die individuellen Faktorenwerte der ersten Komponenten aus einer Hauptkomponenten-Analyse (PC1), die fast 70 % der Variation ausmachten (QBA_PC1), durch Deskriptoren in Bezug auf Entspannung/ Anziehung/ Vertrauen am negativen und Deskriptoren in Bezug auf Angst/ Notlage/ Aversivität am positiven Ende verwendet. Geschätzte Heritabilitäten und ihre entsprechenden Standardfehler für Verhaltensmerkmale, die Mensch-Tier-Interaktionen widerspiegeln, betrugen $0,27 \pm 0,06$ für die AD, $0,04 \pm 0,05$ für TTI, $0,11 \pm 0,06$ für RB und $0,13 \pm 0,17$ für QBA_PC1. Die geschätzten Zuchtwertkorrelationen (r_{EBV}) für die einflussreichsten Vererber zwischen den Verhaltensmerkmalen AD, TTI, RB (Einschränkung: Vererber mit mindestens 5 Töchtern) und QBA_PC1 (keine Einschränkung) waren moderat positiv, was auf eine Beziehung zwischen diesen Merkmalen schließen lässt. Außerdem werden diese Merkmale von einer ähnlichen genetischen Komponente beeinflusst. Die positive EBV-Korrelation zwischen AD und SCS bestärkt das Konzept, dass die Züchtung in Richtung weniger ängstlicher Tiere einen positiven Einfluss auf die Gesundheit der Tiere haben kann. Außerdem haben Bullen mit einem hohen EBV für die Nutzungsdauer tendenziell Töchter mit einer geringeren Ausweichdistanz und weniger angstvollem Verhalten. Die EBV-Korrelation zwischen dem Relativzuchtwert Nutzungsdauer (RZN) und AD betrug -0,28. Obwohl Verhaltensmerkmale in hohem Maße von den Umwelt- und Managementfaktoren abhängen, scheint die genetische Komponente eine wichtige Rolle zu spielen. Diese Ergebnisse unterstützen die Idee Verhaltensmerkmale, die die Beziehung zwischen Mensch und Tier widerspiegeln, in Zuchtstrategien zu integrieren.

Eine allgemeine Diskussion über die Ergebnisse und die angewandten statistischen Modelle aus den vorangegangenen Kapiteln wird in **Kapitel 6** gegeben. Außerdem werden einige Bedenken und Ausblicke in Bezug auf Verhaltensmerkmale und Merkmale, die Mensch-Tier-Interaktionen widerspiegeln, und deren mögliche Einbeziehung in Tierzuchtstrategien, diskutiert.

CHAPTER 1

General introduction

Historically, changes in the demand for livestock products have been driven by human population growth, income growth and urbanization. The production response in various livestock systems has been associated with science and technology, as well as an increase in herd numbers (Thornton, 2010). Dairy cattle breeding in Germany, for example, has been influenced in the last years by several of these aspects, implementation of new technologies (e.g., automatic milking systems (Breitschuh, 2010)) and increasing herd sizes (Barkema et al., 2015).

Domestication and the use of conventional livestock breeding techniques have been largely responsible for the increased yield of livestock products that has been observed over recent decades (Leakey et al., 2009). Selection towards high production, has been heavily criticized for the following reasons: i) the increase in milk yields have been accompanied by a negative impact on functional traits, e.g., animals facing increased frequency of health problems (Ingvarsen et al., 2003) and diminished reproduction abilities (Bell and Roberts, 2007), and ii) reduced breeding capacity, decreased longevity and modification of normal behavior (Lopez et al., 2004; Oltenacu and Algers, 2005). Furthermore, these aspects are indicative of an overall decline in the welfare of dairy cows (Oltenacu and Algers, 2005). According to Groen et al. (1997), functional traits are those animal traits that increase farming efficiency not by leading to a higher production output, but rather by reducing costs. In addition to these economic reasons, concerns for animal welfare, as well as consumer demand, advocate for the consideration of functional traits in breeding goals (Simianer and König, 2002). This includes, for example, traits such as udder health, milking speed or average milk flow (AMF), behavior traits and traits reflecting dairy cows' responsiveness towards humans. In order to thoroughly investigate some of these issues and to encourage breeding efforts to focus on new functional traits, the LOEWE Project ('Landes-**O**ffensive zur Entwicklung **W**issenschaftlich-ökonomischer **E**xzellenz') regarding human-animal-society was founded. It is a project funded by the German state of Hessen. The project's focus is the interdisciplinary investigation of human-animal relationships throughout history and in society. This is not just a question of adequately accounting for the presence of animals in society and of understanding the coexistence of humans and animals. In fact, forms of the "creation" of animals, whether through animal breeding, animal husbandry, animal ethology, animal

research or presentation are investigated through Human-Animal-Interactions (Speitkamp, 2016). The current thesis is part of a subproject within the LOEWE initiative that focuses on the human-animal relationship in dairy farming: Effects of humans, husbandry and selection. Of interest for this investigation is the use of new traits as indicator behavior traits from an ethological perspective complemented with an emphasis on understanding the genetics from an animal breeding point of view. The use of new technologies such as automatic milking systems (AMS) to objectively record traits that reflect behavior (animal-technic interaction), along with the use of reliable and validated traits that reflect dairy cows' responsiveness towards humans will play an important role in the research.

Therefore, the objective of the current study was to carry out quantitative genetic analyses for traits reflecting dairy cow behavior, as well as for traits reflecting animal-technic and human-animal interactions, along with their relationships to traits from conventional performance testing and to evaluate the inclusion of these traits in breeding programs. In order to meet this objective, the focus of each chapter was:

In **Chapter 3** alternative statistical models to infer genetic (co)variance components of AMF were applied. Alternative modeling for the analysis was based on two different approaches: First, a threshold methodology was employed by treating AMF as a binary trait, in accordance with previous findings where AMF was described by bimodal or multimodal curves (distribution of daughter records within sires). In a second step, extensions of statistical modeling were then used to consider the inclusion of production and functional traits, combined with the application of the STEM theory. In this context, (co)variance components and structural equation coefficients were compared with results from standard mixed models, as well as with results from standard mixed models accounting for the effect of the response trait through the incorporation of a linear regression.

In **Chapter 4** alternative functional traits generated by the AMS reflecting animal behavior and health were identified. Afterwards, genetic relationships among those novel traits with production traits like milk yield were estimated. Genetic parameters for milk yield and electrical conductivity for different udder quarters were also estimated.

Heritabilities and relative breeding values were estimated for previously validated behavioral measurements that reflect the human-animal interaction in **Chapter 5**. Furthermore, correlations for estimated breeding values (EBV) were calculated among the behavior measurements, as well as between these traits and production traits from two different points in time (before and after the behavioral measurement). Finally, a calculation was made for the correlations between important relative breeding values included in the overall breeding value in Germany and these behavioral traits.

The importance of the findings regarding behavior and the human-animal relationship are discussed extensively in **Chapter 6**. The implementation of a selection index towards behavior and the use of new DNA technologies such as genetic markers are also discussed in this section.

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CHAPTER 2

Literature overview

Behavior traits in dairy cattle breeding

Dairy cow behavior in the context of breeding schemes is a complex trait category that includes temperament, curiosity, aggressiveness and cow-calf-associations. Temperament traits such as fearfulness or aggressiveness are important to consider as they affect how animals respond to their respective husbandry systems and handling conditions on the farm (Haskell et al., 2014). Feeding behavior (Durunna et al., 2011) or voluntary visits to automatic systems (König et al., 2006) (Figure 1) are also relevant traits in quantitative genetic studies, with mostly moderate heritabilities (ratio of additive genetic variation to total phenotypic variation). Typically, estimates of heritability are necessary for establishing the degree to which the traits of interest are under genetic control, and hence the scope for changing them via selection. Milking behavior, which is considered in official breeding evaluation for dairy cows, reflects the temperament of cows during milking. It is based on subjective assessment by the farmer and, to a large extent, on the milking speed or average milk flow (AMF). The AMF is partly reflective of the behavior of dairy cows because cows' agonistic behavior can cause physiological changes (i.e., the oxytocin hormone level and the noradrenalin concentrations are affected (Kondo and Hurnik, 1988)), thus reducing milk flow in the ongoing lactation. Heritabilities for milking temperament, as well as for several other behavioral traits have been estimated in previous research studies (Table 1).

Less work has focused on the genetic correlations between temperament and other traits in dairy cattle. Research suggests that animals demonstrating calm temperaments have better yields (Drugociu et al., 1977; Lawstuen et al., 1988; Breuer et al., 2000) and faster milking speeds (Lawstuen et al., 1988; Sewalem et al., 2011). There is a positive relationship between temperament and survival in the herd, in that calmer cows are less likely to be culled (Haile-Mariam et al., 2004; Sewalem et al., 2010). There is also evidence of positive effects on health, specifically regarding better resistance to mastitis, lower udder edema and better general health from animals with calmer temperaments (Lawstuen et al., 1988). There are, however, conflicting reports on the relationship between temperament and somatic cell count (Fulwider et al., 2008; Sewalem et al., 2011).

Table 1. Heritabilities for behavioral traits in Holstein dairy cows, according to literature from 2000 to 2011

References	Trait	h^2	No. of animals
Schrooten et al., 2000	milking temperament (1-9)	0.15	656 (bulls)
Pryce et al., 2000	milking temperament (1-9)	0.07	44,672
Sewalem et al., 2002	milking temperament	0.08	656,839
Hiendleder et al., 2003	milking temperament (1-9)	0.07	16 (grandsires)
Rensing and Ruten, 2005	milking speed	0.10	382,500
König et al., 2006	milking frequency (DIM 1)	0.16	1,216
	milking frequency (DIM 2)	0.19	1,112
	milking frequency (DIM 3)	0.22	1,004
Lassen and Mark, 2008	milking speed: freestalls	0.29	19,347
	milking speed: tiestalls	0.35	10,843
Sewalem et al., 2011	milking temperament (1-5)	0.13	1,940,092
	milking speed	0.14	1,620,175

The implementation of breeding programs aimed at improving functional traits, such as behavior, remains challenging, despite the evidence that such traits can considerably impact production.

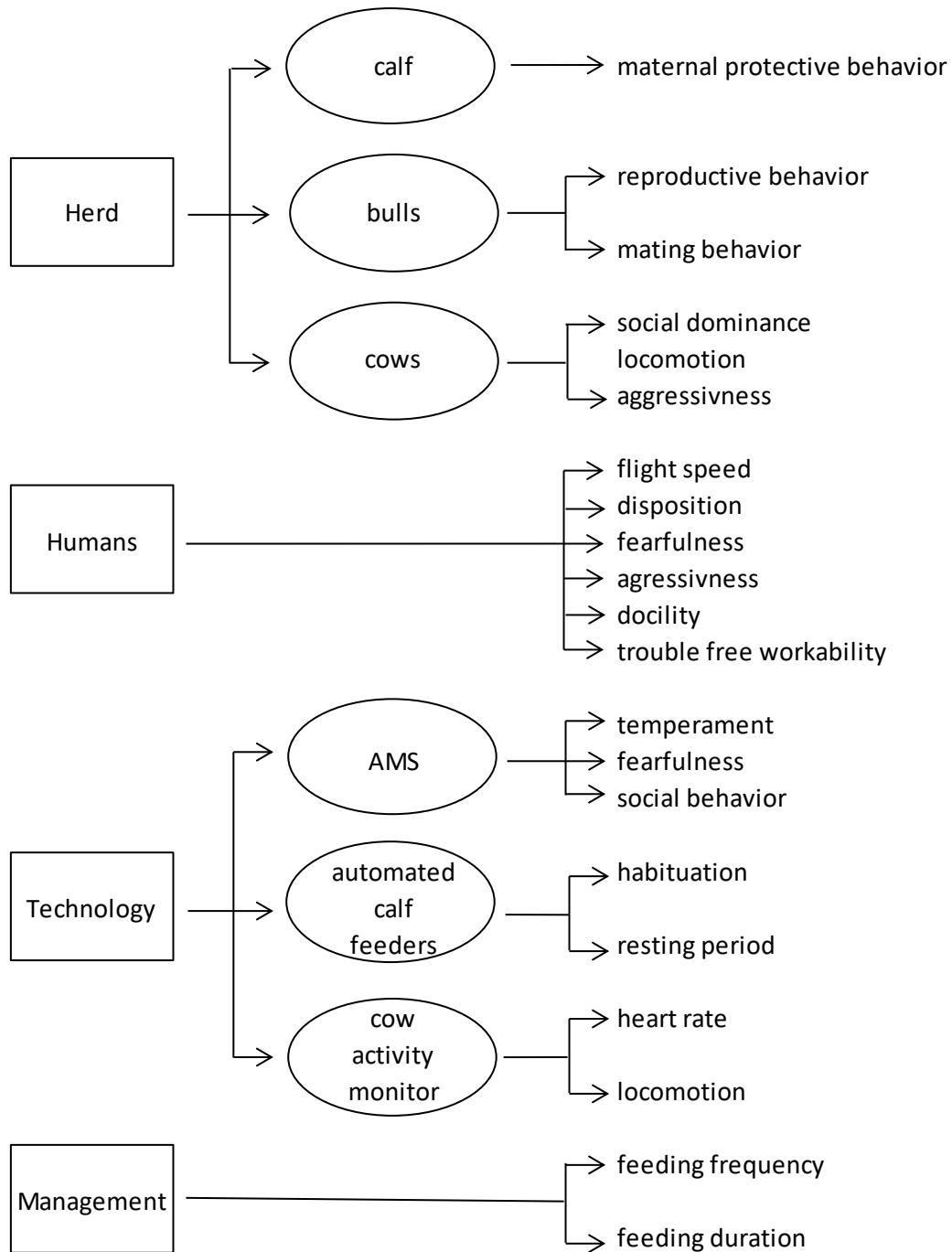


Figure 1. Components of dairy cattle behavior measured on different levels: herd, humans, technology and management

Challenge of functional traits, especially of behavior traits

In the conventional breeding scheme, it is often costly and time consuming to collect sufficient numbers of daughter records for a wide range of functional traits in order to obtain daughter-based sire predictions with sufficient accuracy. It is also important to take into account that many functional traits have a low degree of heritability and are therefore difficult and/or expensive to measure. Furthermore, many functional traits are only expressed late in life (e.g., stayability (Ducrocq et al., 1988)) making it very difficult to include them in the breeding program. Due to the high relevance of functional traits and current concerns about animal welfare, it may be appropriate to consider integrating new phenotypic traits related to behavior, health, fertility and human-animal related traits into modern dairy cattle breeding programs. In order to implement these new traits into a routine breeding value estimation, it is important to ensure that recording efforts do not disturb the working routine on a dairy farm and do not negatively affect the temper of the cows (Kramer et al., 2013). It is also necessary to keep in mind that evaluation of behavioral traits, as previously stated by Adamczyk et al. (2013), is difficult due to problems with handlers' varying definitions and subjectivity of measurements.

Two main components play an important role in the observation and characterization of behavior: Time and objectivity. These components often contribute to evaluation differences among observers. Alternatively, the use of new technologies could be employed to more objectively assess animals' behavior.

Use of new technologies for behavior recording

The utilization of new technologies, e.g., automatic milking systems, in Germany is evolving. The number of farms with so-called "milking robots", or AMS, has increased from 100 farms in 1997 to nearly 4,500 in 2013 (Eckl, 2001; Breitschuh, 2010) (Figure 2). AMS are equipped with extensive data collection technology that provide automatic, repeated measures and objective phenotypic data recording. The technique is used for better control of animal health, as well as for the control of estrus. Milk temperature, milk content, milk quantity and, in some instances, body weight are recorded at each milking. AMS allows the cow to independently select milking times,

number of milking visits, feeding and lying times. The cows have relatively a free choice to decide when and how many times they want to visit the AMS, thus allowing them to act according to their natural behavior. These data would allow for the derivation of behavioral indicator traits. However, information regarding aspects of reliability and validity is insufficient with respect to the use of average milk flow and new behavior trait indicators delivered by automatic milking systems. In this case, the interdisciplinary work between ethology and animal breeding is of extreme importance. Ethologist can provide with reliable traits regarding behavior and traits reflecting dairy cows' responsiveness towards humans.

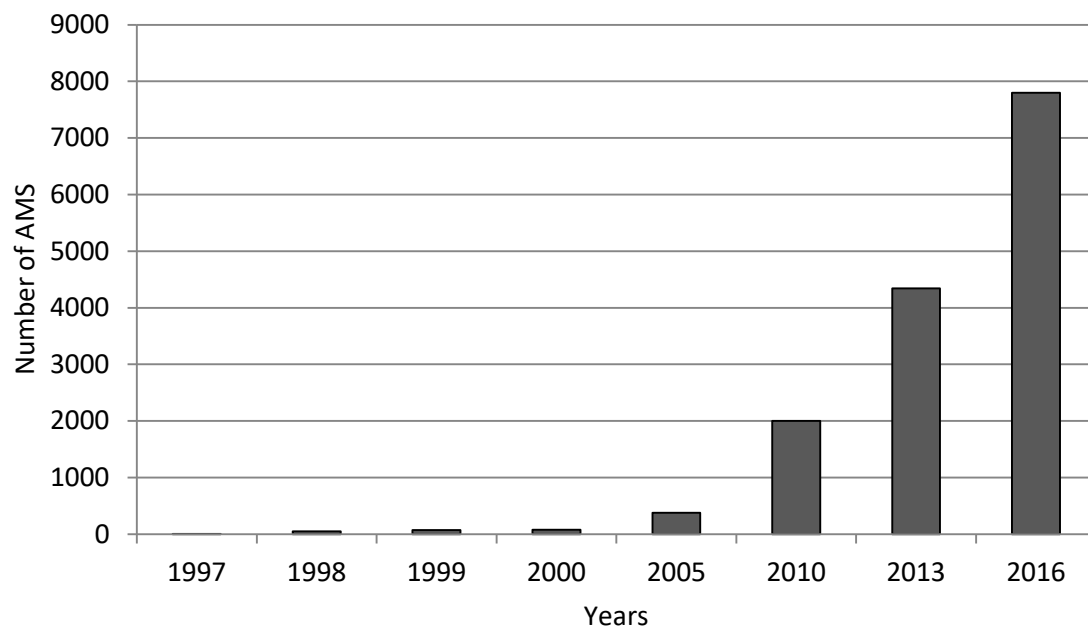


Figure 2. Farms with automatic milking systems in Germany (Eckl, 2001; Breitschuh, 2010; ADR, 2013; Deutscher Bauernverband, 2016)

The human-animal-relationship

As part of the domestication process, animals adapt to dealing with human interaction, as well as to surviving in a captive environment through genetic changes that occur over generations (Price, 1984). In this regard, human-livestock interaction has been of substantial interest over the past 30 years. Further, these interactions may result in behavioral and physiological changes in the animal, with considerable consequences on the animal's performance and overall welfare (Hemsworth et al., 1993). Such consequences could affect not only the animals, but also handlers to the extent that

job-related characteristics, such as job satisfaction, motivation and commitment may be affected (Hemsworth and Coleman, 2011). Early research on human-livestock relationships was initially conducted due to its implications for farm animal productivity, since fear responses to humans were shown to reduce productivity as a result of stress. However, ever-increasing interest in animal welfare and a better appreciation for the role of handlers in determining animal performance and welfare has led to a substantial amount of research being conducted on human-livestock relationships since the 1990s (e.g., ease of handling of cattle (Boivin et al., 1992); fear of humans in sows (Hemsworth et al., 1990); flocking instinct in sheep (Hutson, 1993)). The majority of research in this area has been done on a phenotypic level, though some has focused on the genotypic level (Hemsworth et al., 1990). Further development of research at the genotypic level is still possible, especially for traits reflecting human-animal relationships in dairy cattle.

Breeding program - selection index -

Animal behavior and traits reflecting dairy cows' responsiveness towards humans are an important component of animal welfare. The unfavorable genetic relationship between milk production and welfare indicators suggests that the most-effective route to mitigating welfare regression, or even to improving welfare, is by developing and adopting a selection index in which welfare-related traits are included and weighted appropriately (Oltenacu and Broom, 2010). With such an index, the genetic progress for any of the considered traits is smaller than when selection is for a single trait, but overall economic response is greater (Oltenacu and Algers, 2005). This may justify placing selection pressure on behavior itself in order to achieve more significant genetic progress in animal welfare. The challenge in building such an index, is the lack of complete information regarding heritabilities for behavior traits and traits reflecting dairy cows' responsiveness towards humans. Also genetic and phenotypic correlations of these traits with all the parameters that could be used in the selection index must be estimated.

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CHAPTER 3

Alternative strategies for genetic analyses of milk flow in dairy cattle

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ABSTRACT

Measurement for average milk flow (AMF) in kilograms of milk per minute of milking time from 629,161 Holstein cows from calving years 1990 to 2008 were used to estimate genetic covariance components using a variety of statistical models. For bivariate linear threshold model applications, Gaussian-distributed AMF (linear sire model) was categorized into 2 distinct classes (threshold sire model) by setting arbitrary thresholds for extremely slow and extremely fast milking cows. In different bivariate runs with the 2 traits, Gaussian AMF and binary AMF, within a Bayesian framework, thresholds for the binary trait were 1.2, 1.6, 2.6, 2.8 kg/min. Posterior heritabilities for AMF from the linear and the threshold models in all runs were in a narrow range and close to 0.26, and the posterior genetic correlation between AMF, defined as either a Gaussian or binary trait, was 0.99. A data subset was used to infer genetic and phenotypic relationships between AMF with test-day traits milk yield, fat percentage, protein percentage, somatic cell score (SCS), fat-to-protein ratio, and energy-corrected milk using recursive linear sire models, and multiple trait linear sire models accounting for the effect of a trait 1 on a trait 2, and of trait 2 on trait 3, via linear regressions. The time-lagged 3-trait system focused on the first test-day trait after calving (trait 1), on AMF (trait 2), and on the test-day trait (trait 3) after the AMF measurement. Posterior means for heritabilities for AMF from linear and recursive linear models used for the reduced data set ranged between 0.29 and 0.38, and were slightly higher than heritabilities from the threshold models applied to the full data set. Genetic correlations from the recursive linear models and the linear model were similar for identical trait combinations including AMF and test-day traits 1 and 3. The largest difference was found for the genetic correlation between AMF and fat percentage from the first test day (i.e., -0.31 from the recursive linear model vs. -0.26 from the linear model). Genetic correlations from the linear model, including an additional regression coefficient between AMF and SCS and between AMF and fat-to-protein ratio recorded after the AMF measurement data. Structural equation coefficients from the recursive linear model and corresponding regression coefficients from the linear model with additional regression, both depicting associations on the phenotypic scales, were quite similar. From a physiological perspective, all models

confirmed the antagonistic relationship between SCS with AMF on genetic and phenotypic scales. A pronounced recursive relationship was also noted between productivity (milk yield and energy-corrected milk) and AMF, suggesting further research using physiological parameters as indicators for cow stress response (e.g., level of hormones) should be conducted.

Keywords: milk flow, genetic parameters, recursive model, threshold model

INTRODUCTION

Functional traits reflecting dairy cow behavior are of increasing importance (König et al., 2007). The importance of behavior addresses an economic component, but also animal welfare issues from a consumer's perspective (Kramer et al., 2013). Nevertheless, dairy cattle behavior is a complex trait category that includes temperament, curiosity, aggressiveness, or cow-calf associations. Also, feeding behavior (Durunna et al., 2011) or voluntary entries into automatic milking systems (König et al., 2006) were traits of interest in quantitative-genetic studies, which mostly identifies moderate heritabilities. Ethologists are interested in human-dairy cow relationships by using different stimuli or specific test procedures (Rousing and Waiblinger, 2004). In this context, preliminary work has been done in beef cattle (e.g., by assessing temperament scores or visual flight-speed scores when the animal is jumping out of the crush; Hoppe et al., 2010). Handling of dairy cows (i.e., the effect of humans) in a positive or negative way can either increase or decrease productivity and fertility (Hemsworth et al., 2000; Waiblinger et al., 2002). Jensen and Anderson (2005) discussed the importance of behavior with regard to adaptation of animals to their social and physical environments.

However, a substantial lack of official recording schemes exist for behavior or temperament traits in dairy cattle. Traits used in official genetic evaluations partly included temperament of cows in the parlor during milking based on subjectively appraisals of procedures, and to a large extent milking speed usually defined as

average milk flow (**AMF**; Rensing and Ruten, 2005). Also, AMF reflects dairy cattle behavior because agonistic behavior of cows caused physiological changes (i.e., affecting the oxytocin hormone level and norepinephrine concentrations; Kondo and Hurnik, 1988), and in ongoing lactation reduced milk flow. The genetic correlation between temperament and AMF (Sewalem et al., 2011) also indicates that a cow entering the parlor voluntarily has a calm temperament and a better milk flow, whereas a more nervous cow has a longer milking time. Concentrations of cortisol and endogenous opioids in plasma were higher, and oxytocin concentrations were lower for cows milked in unfamiliar surroundings (Bruckmaier et al., 1993; Bruckmaier et al., 1997). In such cases where release of oxytocin is impaired, milk ejection is inhibited, thus causing a production loss and an increased risk for a mammary infection (Bruckmaier, 2005). Milk flow can be recorded on 2 different scales. The first one is a subjective scoring, mostly done by farmers on a scale from 1 to 5 or from 1 to 9. Subjectively scored milk flow is a trait with low to moderate heritability (Rupp and Boichard, 1999; Rensing and Ruten, 2005). The second scale is an objective measurement in kilograms per minute (during the complete milking event or only in the main milking phase). When objectively measured, AMF is a trait with moderate to high heritability (Lassen and Mark, 2008).

An increase in AMF is associated with a desired decrease in milking and labor time. However, AMF should be considered as a trait with an intermediate optimum, because extremely fast milking cows have higher incidences of clinical mastitis and an increased somatic cell count (**SCC**) (Zwald et al., 2005). Complex trait associations between AMF and other traits of dairy cattle breeding goals were inferred in numerous previous studies by applying standard linear mixed model theory (e.g., Sewalem et al., 2011; Berry et al., 2013). Linear mixed models are suitable for Gaussian-distributed traits, and they do not take into account recursive relationships among traits. Structural equation models (**STEM**), on the other hand, as originally developed for biology (Haldane and Priestley 1905), have the potential to infer relationships and feedback situations among traits by reflecting a more physiological background. Structural equation models are models with recursive or with simultaneous effects, and were applied in previous studies to health and fertility traits (e.g., Sorensen and Varona, 2006; López de Maturana et al., 2007). When applying STEM, associations between

traits are reflected by solutions for so-called structural equation coefficients. In a time-lagged 3-trait system, high milk yield (**MY**) may increase AMF, and AMF could affect levels of MY in the ongoing lactation. Karacaören et al. (2006) and Samoré et al. (2010) found a positive genetic correlation between MY and AMF, but they did not investigate the recursive relationship between the 2 traits. More obvious are causalities between udder health and AMF (Luttinen and Juga, 1997): clinical or subclinical mastitis (extremely high SCC) extends the milking process but, in turn, antagonistic relationships between AMF and SCC were identified.

The aim of the present study was to apply alternative statistical models to infer genetic (co)variance components of AMF. Alternative modeling was based on 2 different approaches. First, we used threshold methodology by treating AMF as a binary trait, based on previous findings that AMF was described by bimodal or multimodal curves (distribution of daughter records within sires). In a second step, extensions of statistical modeling considered the inclusion of production and functional traits, combined with the application of STEM theory. In this context, (co)variance components and structural equation coefficients were compared with results from standard mixed models and with results from standard mixed models accounting for the effect of the response trait by incorporation a linear regression.

MATERIALS AND METHODS

Threshold Models Versus Linear Models

Data and Trait Definitions. Measurements for AMF (in kg/min) recorded with a stopwatch were available for 629,161 first-parity Holstein cows located in the regions of the federal states of Hessian and Lower Saxony, Germany. Official measurements for AMF were from the early period of lactation and mostly recorded at the second official test date of a cow. Calvings spanned the period from January 1990 to December 2008 and included cows from 24,459 different herds. Cows were daughters of 26,475 sires with, on average, 24 daughters per sire (minimum = 1 daughters per sire; maximum = 35,632 daughters per sire). The pedigree file traced back to founder animals born in 1940 included 2,229,849 animals.

Table 1. Description of the applied statistical models for genetic analyses of average milk flow (AMF)

Statistical model	Model description	No. of observ.	Traits ¹
Model [1]	Bivariate linear-threshold sire model	629,161	AMF as Gaussian and as binary trait
Model [2]	Recursive linear sire model	7,924	Trait_1, AMF, Trait_3
Model [3]	Linear multiple trait sire model	7,924	Trait_1, AMF, Trait_3
Model [4]	Linear multiple trait sire model with linear regressions	7,924	Trait_1, AMF, Trait_3

¹Trait_1 = test-day traits before the measurement date for average milk flow;

Trait_3 = test-day traits after the measurement date for average milk flow.

To treat AMF as a binary trait, 4 different binary traits were generated grouping the cows according to 4 different thresholds: 1.2, 1.6, 2.6, 2.8 kg/min. Cows with values above the threshold received a score of 1, and otherwise 0. For the 4 different thresholds, 95.44, 81.29, 21.62, and 14.39 % of the cows, respectively, received a score of 1.

Statistical Modeling. The variety of applied statistical models is described in Table 1. Bivariate linear-threshold sire models were applied to the combinations of all binary AMF traits with Gaussian-distributed AMF in 4 consecutive runs for the 4 different defined thresholds. The bivariate linear-threshold sire model (equation [1]) for the joint analysis of observed values y for Gaussian AMF (index 1) and of the underlying liability π when defining AMF as a binary trait (index 2) was

$$\begin{aligned} y_1 &= X\beta_1 + Z_s s_1 + Z_h h_1 + e_1 ; \\ \pi_2 &= X\beta_2 + Z_s s_2 + Z_h h_2 + e_2 , \end{aligned} \quad [1]$$

where y and π = vectors of observed AMF and unobserved liabilities for AMF, respectively; β = vector of systematic effects; s = vector of sire-additive genetic effects; h = vector of herd-test-day effects; e = vector of residual effects; and X , Z_s , and Z_h = the associated incidence matrices. Vector β included the fixed effects of milking frequency, age at first calving, and days in milk (**DIM**). For the random sire additive genetic and herd-test-day effects, multivariate normal prior distributions were assumed:

$$\begin{aligned} s &\sim N(0, \mathbf{G}_0 \otimes \mathbf{A}), \text{ and} \\ h &\sim N(0, \mathbf{H}_0 \otimes \mathbf{I}), \end{aligned}$$

where $\mathbf{G}_0 = \begin{bmatrix} \sigma_{s1}^2 & \sigma_{s1s2} \\ \sigma_{s1s2} & \sigma_{s2}^2 \end{bmatrix}$ is the co (variance) matrix between sire transmitting abilities, with σ_{s1}^2 and σ_{s2}^2 denoting sire variances for both traits, and σ_{s1s2} is the covariance for sire effects between trait 1 and trait 2, and \mathbf{A} is the additive relationship matrix between sires; $\mathbf{H}_0 = \begin{bmatrix} \sigma_{h1}^2 & \sigma_{h1h2} \\ \sigma_{h1h2} & \sigma_{h2}^2 \end{bmatrix}$ is the (co)variance matrix between herd-test day effects, with σ_{h1}^2 and σ_{h2}^2 denoting herd-test day variances for both traits, and σ_{h1h2} the covariance for her-test day effects between trait 1 and trait 2, and \mathbf{I} is an identity matrix. Residuals were also assumed to be correlated, and the following distribution was assumed:

$$e \sim N(0, \mathbf{R}_0 \otimes \mathbf{I}),$$

where $\mathbf{R}_0 = \begin{bmatrix} \sigma_{e1}^2 & \sigma_{e1e2} \\ \sigma_{e1e2} & 1 \end{bmatrix}$ with σ_{e1}^2 denoting the residual variance for trait 1 and σ_{e1e2} the residual covariance between trait 1 and trait 2. Consequently, the residual correlation between 1 binary trait and 1 Gaussian trait is $\sigma_{e1e2}/\sigma_{e2}$.

Recursive Models Versus Linear Multiple Trait Models

Data and Trait Definitions. A subset of 7,924 cows was used to explore relationships between AMF with test-day production traits and test-day traits reflecting energy efficiency. This subset included cows from similar types of herringbone parlor milking systems to illustrate identical human-animal interactions before and during the milking routine, and excluding data from automatic milking systems (limited contact with humans) and tiestall barns (direct contact between cows and humans). Furthermore, in the selected herds, we implemented recording schemes for further traits reflecting agonistic cow behavior as defined by Waiblinger et al. (2006). Test-day production traits included MY, fat percentage (**FP**), protein percentage (**PP**), somatic cell score (**SCS**), and energy efficiency indicators on a test-day basis [fat-to-protein ratio (**FPR**) and energy-corrected milk (**ECM**)]. Energy-corrected milk determines the amount of energy in the milk standardized to 3.5 % fat and 3.2 % protein, and was calculated using the formula of the GfE (2001):

$$\text{ECM} = \text{MY} \times (0.38 \times \text{FP} + 0.21 \times \text{PP} + 1.05)/3.28$$

According to Neuenschwander et al. (2005), ECM is an indicator for energy needed for milk production, and also an indicator for metabolic stress of the cow. Test-day SCC was transformed to test-day SCS by applying the formula by Ali and Shook (1980):

$$\text{SCS} = \log_2(\text{SCC}/100,000) + 3$$

Descriptive statistical parameters for all traits are summarized in Table 2.

Table 2. Descriptive statistics for the test-day records (MY=milk yield, FP=fat percentage, PP=protein percentage, FPR=fat-to-protein ratio before (_1) and after (_3) the measurement date for average milk flow (AMF), including number of observations (N), means (X), SD, and minimal (Min.) and maximal (Max.) values

Trait	Statistical parameter				
	N	X	SD	Min.	Max.
AMF ¹ (kg/min)	629,161	2.14	0.61	0.26	6.00
AMF ² (kg/min)	7,924	2.18	0.66	0.39	5.82
MY_1 (kg)	7,924	29.37	5.95	3.20	59.60
MY_3 (kg)	7,924	28.11	5.98	3.00	50.70
FP_1 (%)	7,924	3.85	0.65	1.64	8.55
FP_3 (%)	7,924	3.90	0.68	1.66	7.19
PP_1 (%)	7,924	3.26	0.29	2.17	4.50
PP_3 (%)	7,924	3.41	0.28	2.45	4.48
SCS_1	7,924	2.31	1.56	0.01	9.64
SCS_3	7,924	2.44	1.59	0.01	9.64
FPR_1	7,924	1.19	0.20	0.49	3.09
FPR_3	7,924	1.15	0.18	0.47	2.24
ECM_1 (kg)	7,924	18.42	3.33	2.04	39.72
ECM_3 (kg)	7,924	18.02	3.31	2.27	35.00

¹AMF from the whole data set as used for model [1].

²AMF from the reduced data set as used for models [2], [3], and [4].

Statistical Modeling. Structural equation models applications focused on traits recorded in the early stage of lactation. Trait definition depicted a biological system including AMF and the same 2 test-day traits. Trait 1 (e.g., MY_1) referred to the test-day record before the measurement date for AMF, and trait 3 (e.g., MY_3) was the official test-day record of the respective test-day trait after AMF recording. Hence, AMF was trait 2 in the biological system. Such time-lagged recursive systems, where trait 1 affects AMF and AMF itself affects trait 3 without considering reciprocal causalities, were introduced by König et al. (2008) and Rehbein et al. (2013) for disease traits (trait 2). In matrix notation, the recursive linear model (equation [2]) was defined as

$$\begin{pmatrix} \Lambda y_1 \\ \Lambda y_2 \\ \dots \\ \Lambda y_n \end{pmatrix} = X\beta + Z_h h + Z_s s + e, \quad [2]$$

where $i = 1, 2, \dots, n$, denoting the n cows having records for the 3 traits. Here, \mathbf{y}_i is a vector including observations for the 3 traits. Systematic and random effects, and associated incidence matrices, were defined in a similar way to equation [1]. A special feature of the recursive model is matrix $\mathbf{\Lambda}$ containing the structural equation coefficients λ . Coefficients λ_{ij} describe alterations of trait i with respect to changes by one unit of trait j . Here, λ_{21} is the alteration in AMF with respect to test-day trait 1. The rate of change in test-day trait 3 with respect to the change of 1 kg/min in AMF is depicted by λ_{32} . Hence, matrix $\mathbf{\Lambda}$ was:

$$\mathbf{\Lambda} = \begin{bmatrix} 1 & 0 & 0 \\ -\lambda_{12} & 1 & 0 \\ 0 & -\lambda_{32} & 0 \end{bmatrix}.$$

Conditional and prior distributions of the data, parameters, and effects correspond to the study by Heringstad et al. (2009). Standard multiple-trait linear sire models simultaneously included the 3 traits (e.g., MY_1, AMF, and MY_3). Hence, the general form of the multiple trait linear sire model (equation [3]) was:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_s\mathbf{s} + \mathbf{Z}_h\mathbf{h} + \mathbf{e}; \quad [3]$$

with vectors, matrices, and effects as specified for the Gaussian trait in equation [1].

The general form of the multiple trait linear sire model (equation [4]; not shown) was achieved by including trait 1 as a linear regression coefficient in the model for trait 2 and including trait 2 as a linear regression coefficient in the model for trait 3. The intention of equation [4] was to depict a recursive system by using the framework and possibilities of standard mixed models. Hence, we focused on a comparison of solutions for regression coefficients (equation [4]) with corresponding structural equation coefficients (equation [2]).

Data analyses were conducted in a Bayesian framework via Markov chain Monte Carlo sampling and using the SIR-Bayes package (Wu et al., 2008). For equation [4], the software package DMU (Madsen and Jensen 2000) was used because SIR-Bayes software does not allow modeling of regressions (only classification variables). Settings and algorithms for both software packages were identical; 10,000 iterations were defined for the burn-in period, and 100,000 iterations after burn-in were used to infer posterior genetic parameters, regression coefficients, and structural equation coefficients. The decision for the length of burn-in rounds and for the total number of

iterations based on convergence diagnostics [algorithms by Raftery and Lewis (1992) as implemented in the BOA R-package] and on visual inspections of trace plots. For the recursive models, we inspected the posterior samples of λ_{ij} , and for all other models main evaluation criterion was the covariance for sire effects. Parameter estimates from the STEM cannot be directly compared with corresponding parameter estimates from standard mixed models. For final comparisons, the transformation of STEM parameters followed the procedure as described by Gianola and Sorensen (2004).

RESULTS AND DISCUSSION

Threshold Models Versus Linear Models

Distributions for Milking Speed. The idea to transform almost Gaussian data into binary traits was explained and applied by Pashmi et al. (2009). In their study, a threshold for SCC was defined to classify SCC records into binary subclinical mastitis (0 = healthy, 1 = sick). This approach might be suitable for such traits where a broader range of outcomes has an identical biological or physiological value. In the case of SCC, veterinarians do not distinguish, for example, between 80,000 and 85,000 somatic cells for healthy cows; on the upper SCC scale, they do not distinguish, for example, between 350,000 and 355,000 somatic cells for affected cows (Caraviello et al., 2005). Similar differentiations apply to AMF; farmers and breeding organizations accept a broad range of intermediate milk flow, but they select against extremely slow and against extremely fast milkers (Sewalem et al., 2010). Also, statistical concerns favor the applications of threshold models for a categorically defined AMF trait. The pattern of phenotypic daughter records for AMF relatively often follow a bimodal structure instead of an optimal symmetric Gaussian distribution, showing 1 pronounced peak. Bagnato et al. (2003) addressed a similar topic by studying variations of parameters which describe milk flow curves. In a preliminary study focusing on 4 influential Holstein sires, König et al. (2011) identified 1 sire with a bimodal daughter distribution for AMF (i.e., one maximum at 2.2 kg/min, and another maximum at 2.6 kg/min). In the present study, we also analyzed daughter distributions for AMF within sires or within groups of sires by creating subsets of sires according to estimated breeding

values of sires or according to their number of daughters. For all sire groups (Figure 1) and also for specific influential sires (Figure 2), daughter records for AMF seemed to be Gaussian distributed. Only for the subset of sires with lowest breeding values for AMF, we found tendencies for bimodal distributions.

Genetic Parameters. Theoretically, linear model applications require a Gaussian data distribution and normally distributed residuals, variance homogeneity, additivity of systematic effects, and independency between variance and mean. In spite of obvious Gaussian distributed curves (Figure 1 and 2), the data were not normally distributed [utilization of the Kolmogorov-Smirnov test and applying the procedure PROC univariate in SAS (ver. 9.2; SAS Institute Inc., Cary, NC) for the whole data set including 629,161 observations], but heritabilities for different data distributions and analyzed with different models were very similar. Posterior heritabilities for AMF from the bivariate threshold-linear sire equation [1] are depicted in Figure 3 for binary AMF, and in Figure 4 for Gaussian AMF. Different subfigures display heritabilities from the same bivariate run (e.g., Figure 3a and 3b for the run using the 1.2 kg/min threshold for the binary trait). Heritabilities on both scales on the observable scale (Figure 3a) and on the underlying liability scale (Figure 3b) were 0.26. Identical heritabilities for linear and binary AMF were also found for the remaining threshold definitions (Figure 3b vs. 4b, Figure 3c vs. 4c, Figure 3d vs. 4d). Ilahi and Kadarmideen (2004) mentioned a loss of information with associated decreasing heritabilities when transforming AMF from a continuous scale into a few discrete classes. Generally, posterior means for heritabilities of AMF were in a narrow range close to 0.26, along with small posterior standard deviation. Curves for posterior distributions of heritabilities were symmetric with identical shape and within a small range (heritabilities from the different iterations ranged between 0.22 and 0.30). Sire variances were slightly smaller from the linear model for the Gaussian data, but simultaneously smaller residual variances were the reason for almost identical heritabilities from equations [1] and [2].

Nevertheless, a simulation study (König, 1999) clearly showed the importance of heritabilities when ranking sires in top lists according to their genetic evaluations.

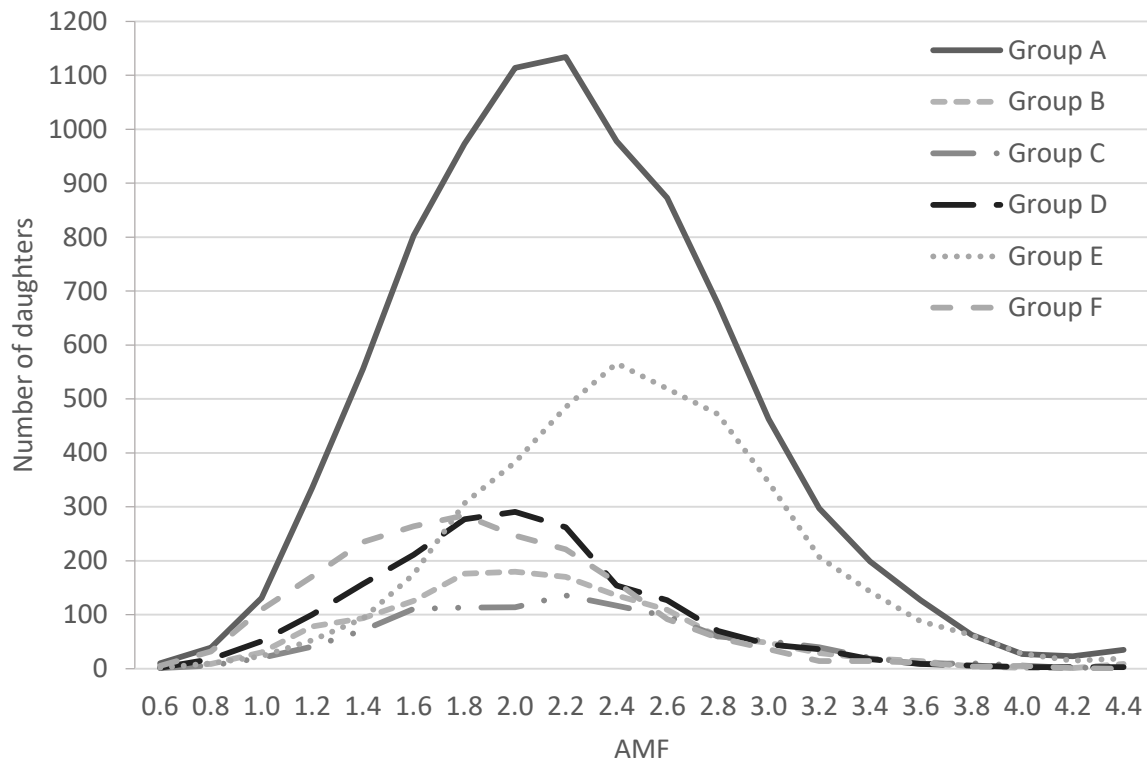


Figure 1. Daughter distributions for average milk flow (AMF) for bulls with the highest (group A) and lowest (group B) total breeding value, highest (group C) and lowest (group D) relative breeding value for production, and highest (group E) and lowest (group F) relative breeding value for AMF

Classically, previous quantitative genetics studies compared heritabilities for binary traits from linear model applications with results for binary traits from threshold models (e.g., Huang and Shanks, 1995). The advantages of threshold over linear models have already been shown with simulated data (Meijering and Gianola, 1985; Hoeschele, 1988). However, variable results were reported when using field data. Similar performance of threshold and linear models were indicated the studies by Weller et al. (1988), Renand et al. (1990), and Matos et al. (1997).

Theoretically, comparison of heritabilities estimated on both scales with an underlying liability and observable for a 0 and 1 trait suggests a heritability transformation according to Dempster and Lerner (1950). This transformation equation indicates higher heritability estimates on the underlying liability scale. In the present study, we

applied linear models to AMF defined as a Gaussian trait, and threshold models to AMF defined as a binary trait.

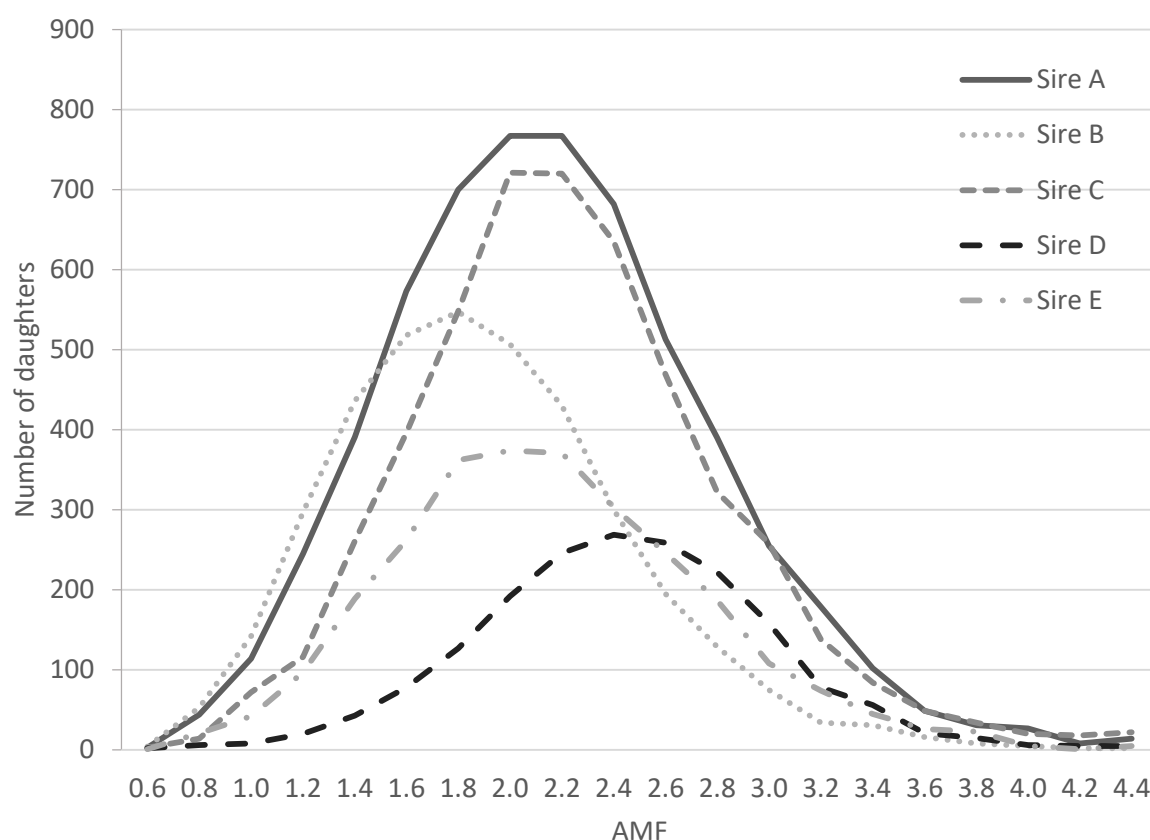


Figure 2. Daughter distributions for average milk flow (AMF) for daughters of the 5 bulls with the highest number of daughters (sire A with 5,854 daughters, sire B with 3,731 daughters, sire C with 4,900 daughters, sire D with 1,816 daughters, and sire E with 2,742 daughters)

Nevertheless, we also used the Dempster-Lerner equation to transform heritabilities from the threshold model ($h^2 = 0.26$ for all class definitions) into the observed scale. Transformed heritabilities were 0.06 for the 1.2 kg/min threshold, 0.12 for the 1.6 kg/min threshold, 0.13 for the 2.6 kg/min threshold, and 0.11 for the 2.8 kg/min threshold. Those results are in agreement with previous studies focusing on genetic analysis of binary data with different models (e.g., Weller and Ron, 1992): differences in transformed heritabilities increased with increasing deviations of observations allocated either to the 0 or to the 1 response.

Posterior genetic correlations between AMF defined as a Gaussian trait (linear model) and AMF defined as a binary trait (threshold model) were larger than 0.99 (figure 5). Minor differences were found for the different threshold definitions: creation of extreme categories [thresholds of 1.2 kg/min (Figure 5a) and 2.8 kg/min (Figure 5d)] resulted in marginally lower genetic correlations compared with bivariate runs using the thresholds of 1.6 (Figure 5b) or 2.6 kg/min (Figure 5c). Posterior density distributions for genetic correlations were centered to the mean with a pronounced maximum symmetric in shape, and characterized by an extremely small range for estimates from different iteration rounds. Such a high genetic correlation indicates an identical ranking of sires according to their breeding values irrespective the trait definition and the statistical modeling. This might be due to the large amount of data, allowing an optimal allocation of records to different subcells.

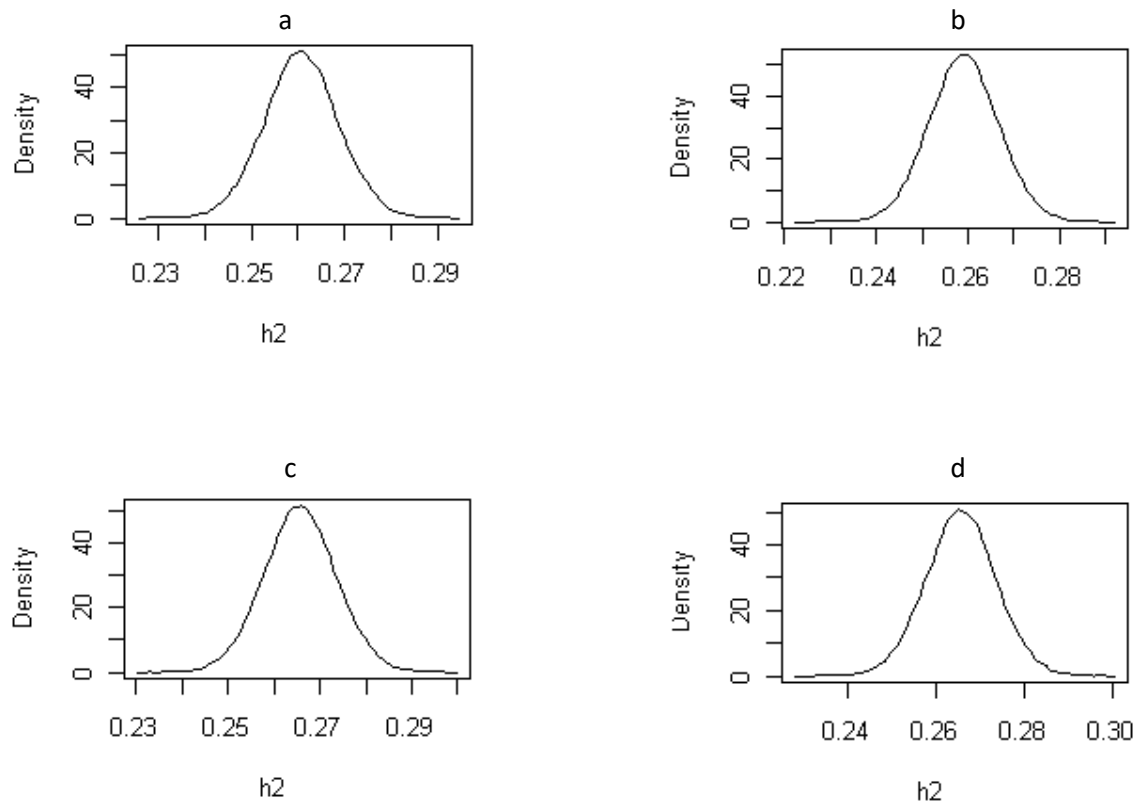


Figure 3. Heritabilities for average milk flow (AMF) defined as a binary trait from bivariate models with thresholds of 1.2 (a), 1.6 (b), 2.6 (c), and 2.8 kg/min (d)

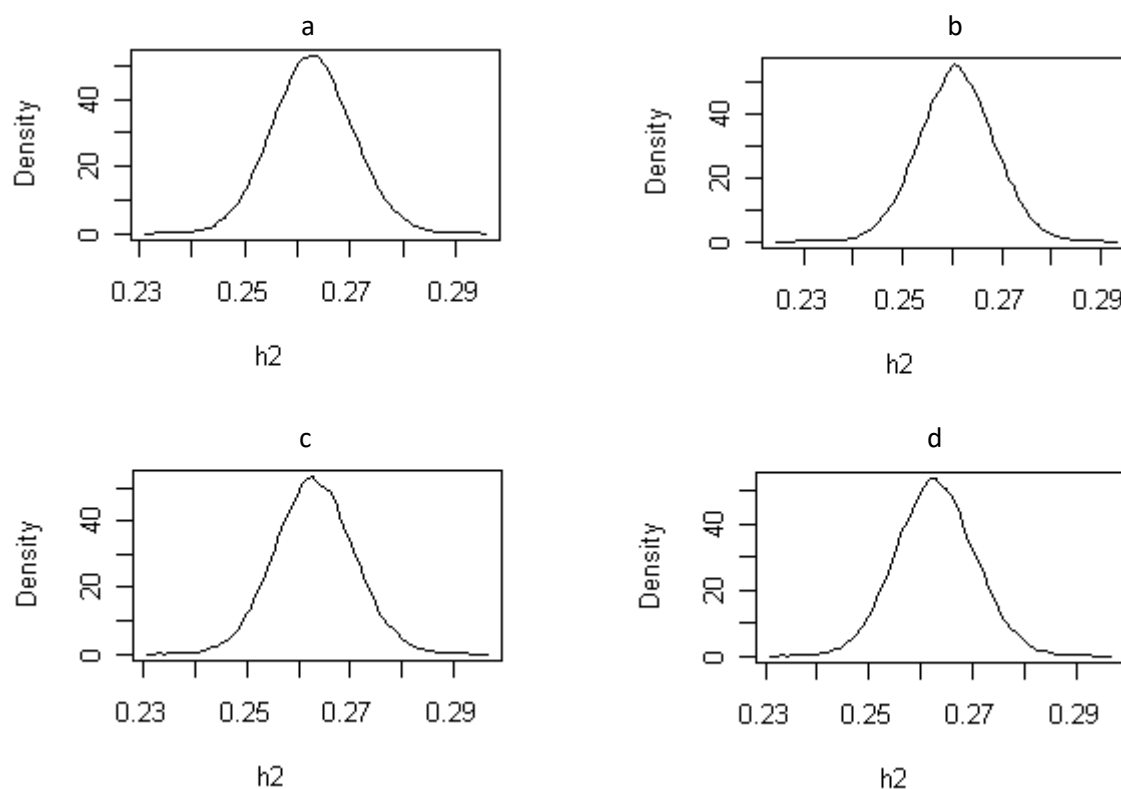


Figure 4. Heritabilities for average milk flow (AMF) as defined as a Gaussian-distributed trait from bivariate models with thresholds of 1.2 (a), 1.6 (b), 2.6 (c), and 2.8 kg/min (d)

Recursive Models Versus Linear Multiple Trait Models

Genetic Parameters. Table 3 provides posterior means of heritabilities for AMF from equations [2], [3], and [4]. The lowest variation of heritabilities (0.29 - 0.30) from different runs with different AMF test-day trait combinations resulted from the recursive equation [2], and highest variations were found for the linear equation [4], including an additional linear regression coefficient (0.29 - 0.38). Moderate heritabilities for AMF, in the range from 0.29 to 0.38, and the inclusion of AMF into overall breeding goals for dairy cattle are a basis to implement efficient selection strategies on AMF. Heritabilities for AMF from the reduced data set for AMF were slightly higher compared with the estimates from the whole data set (equation [1]). Schierenbeck et al. (2011) summarized advantages when basing genetic evaluations on a subset of preselected herds: harmonization in data recording in more or less standardized environments, resulting in larger additive-genetic variances and heritabilities.

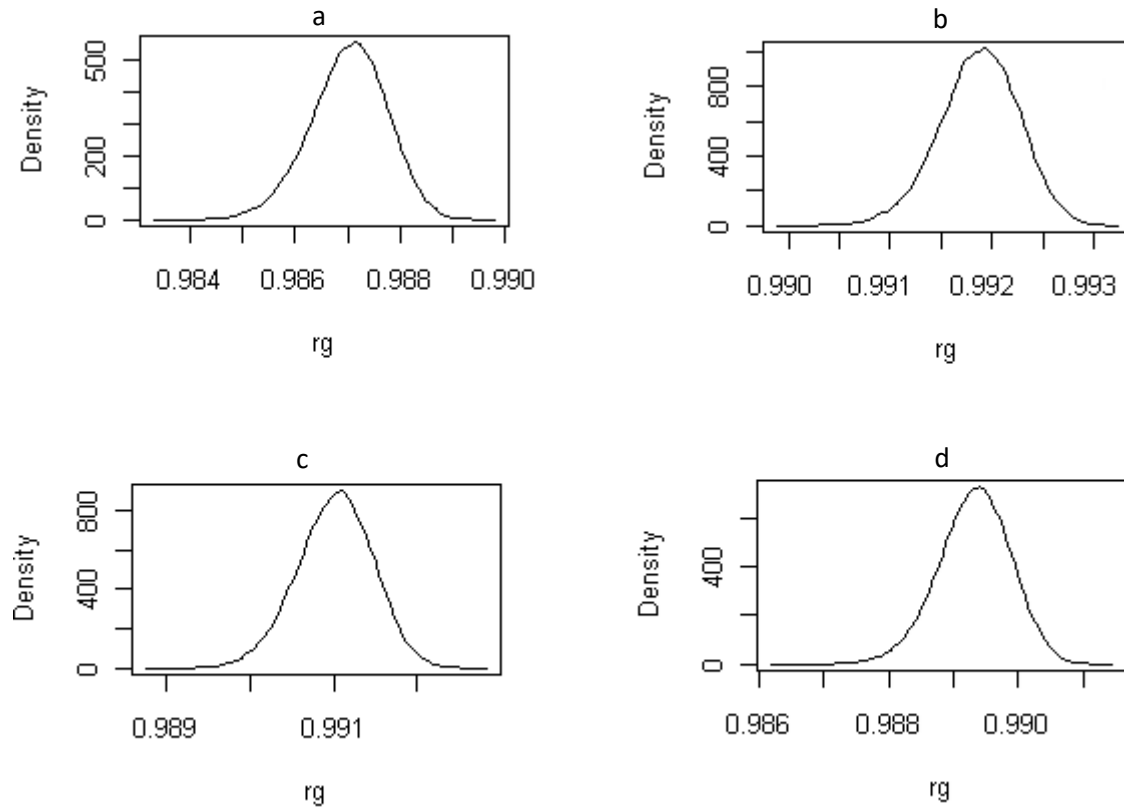


Figure 5. Genetic correlations (r_g) between Gaussian-distributed average milk flow (AMF) and AMF defined as a binary trait from bivariate models with thresholds of 1.2 (a), 1.6 (b), 2.6 (c), and 2.8 kg/min (d)

In recent years, breeders associations have discussed different strategies on how to include milkability traits in selection criteria (Gray et al., 2011). The overall breeding goal for Holsteins in Germany does not explicitly consider AMF; likewise, in the total merit index for the Fleckvieh breed, AMF is only included with a relative economic weight of 3 % (Dodenhoff and Emmerling, 2009). Data for AMF are either objective measurements in kilograms per minute, subjectively assigned scores for slow- and fast-milking cows reflecting impressions of farmers or a combination of both. The present study confirms quite high heritabilities for objective measurements of AMF, suggesting continued implementation of a population-wide recording system instead of using farmer surveys. Moderate to high heritabilities were also found in previous studies by Santos and Bagnato (1998; $h^2 = 0.33$) or by Ilahi and Kadarmideen (2004; $h^2 = 0.44$) in Brown Swiss cows.

Table 3. Posterior heritability estimates for test-day traits (MY= milk yield, FP= fat percentage, PP= protein percentage, FPR= fat-to-protein ratio before (_1) and after (_3) the measurement date for average milk flow (AMF) from 3 different sire models (posterior SD in parentheses)

Trait	Statistical model		
	Recursive linear	Linear	Linear + regression
	Model [2]	Model [3]	Model [4]
AMF ¹ (kg/min)	0.29-0.30 (0.04)	0.34-0.36 (0.04)	0.29-0.38 (0.04)
MY_1 (kg)	0.28 (0.04)	0.30 (0.04)	0.30 (0.04)
MY_3 (kg)	0.26 (0.04)	0.28 (0.04)	0.32 (0.04)
FP_1 (%)	0.31 (0.04)	0.38 (0.05)	0.38 (0.05)
FP_3 (%)	0.36 (0.04)	0.42 (0.05)	0.43 (0.05)
PP_1 (%)	0.30 (0.04)	0.44 (0.04)	0.44 (0.05)
PP_3 (%)	0.38 (0.04)	0.48 (0.05)	0.42 (0.05)
SCS_1	0.08 (0.02)	0.09 (0.02)	0.09 (0.02)
SCS_3	0.07 (0.02)	0.07 (0.02)	0.06 (0.02)
FPR_1	0.30 (0.04)	0.50 (0.05)	0.50 (0.05)
FPR_3	0.31 (0.04)	0.44 (0.04)	0.39 (0.05)
ECM_1 (kg)	0.25 (0.03)	0.25 (0.04)	0.25 (0.04)
ECM_3 (kg)	0.22 (0.03)	0.24 (0.04)	0.25 (0.04)

¹Range of heritabilities for AMS from all runs for the different trait combinations.

Because of the strong association between AMF of cows and labor time in modern dairy cattle farming systems (Devir et al., 1993), AMF has increasing relevance on profitability. Given a routine supply of records and an efficient dissemination of superior animals, both components (i.e., high heritabilities and economic values) would facilitate the implementation of efficient selection strategies for AMF.

Quite similar heritabilities across statistical models and test days were found for MY and ECM (Table 3). As expected, highest heritabilities were found for FP and PP. For all models, we observed higher heritabilities for FP and PP from test days after the AMF measurement. The FP directly after calving was influenced by mobilizations of body fat depots. In fact, such a compensation of negative energy balance, via body fat mobilization especially, was observed in high-yielding Holstein cows (Collard et al., 2000) and was more pronounced in herds characterized by suboptimal feeding strategies. A high agreement in heritabilities for same traits across models was found for SCS_1 (0.08 - 0.09) and for SCS_3 (0.06 - 0.07). Major heritabilities fluctuations

showed FPR_1 with 0.30 (recursive equation [2]) versus 0.50 (multiple-trait linear equations [3] and [4]), and FP_1 (0.31 from equation [2] and 0.38 from equations [3] and [4]). Again, both traits are associated with energy deficiency directly after calving. Parameter estimates from a STEM being developed to depict physiological mechanisms explicitly differed from standard mixed models.

Genetic correlations between AMF with all test-day traits are displayed in Table 4. Interestingly, genetic correlations from the recursive equation [2] and the linear equation [3] were quite similar. The largest difference when comparing both models was found for the genetic correlation between AMF with FP_1 (-0.31 vs. -0.26, respectively). However, partly substantial differences were found for estimates from equation [4], especially for the genetic correlation between AMF with SCS_3 and between AMF with FPR_3. Here, application of the linear model plus the modeling of an additional regression from trait 1 on trait 2 (AMF), and from trait 2 on trait 3, changed the sign of the correlation. However, divergent estimates from equation [4] were associated with highest posterior standard deviation. Generally, MY and AMF were positively correlated on the genetic scale, indicating that a high production level is associated with a better milk flow. Similar results with genetic correlations between 0.31 and 0.41 were found by Karacaören et al. (2006). König et al. (2006) studied milking behavior of cows in automatic milking systems and observed that higher frequencies of voluntary entries into the milking system and faster milking processes were found for cows in early lactation stage with highest test-day MY.

Genetic correlations between MY and AMF and between percentage traits (PP and FP) and AMF differed in sign. Opposite results for MY and percentage traits reflect the natural genetic antagonism between productivity and protein or fat content as proven in several previous studies (e.g. Chauhan and Hayes, 1991). Equations [2], [3], and [4] confirmed the genetic antagonistic relationship between SCS_1 and AMF, with correlations ranging between 0.17 and 0.35. Moore et al. (1983) discussed the consequences of selection due to the antagonistic relationships; they suggested selection toward faster AMF because of the low heritability for SCS (0.03 in their study) and because selection response in SCS is small. Genetic correlations between AMF with FPR_1 and FPR_2 were small and close to zero. Hence, we identified no

associations on the genetic scale between milk flow and an energy efficiency trait. Bergk and Swalve (2011) analyzed relationships between FPR and other functional traits and found an intermediate optimum for FPR. Relationships for traits characterized by intermediate optima with other traits are difficult to infer via linear regressions or correlations.

Table 4. Posterior genetic correlation estimates between test-day traits (MY= milk yield, FP= fat percentage, PP= protein percentage, FPR= fat-to-protein ratio before (_1) and after (_3) the measurement date for average milk flow (AMF) with AMF from 3 different sire models (posterior SD in parentheses)

Trait	Statistical model		
	Recursive linear	Linear	Linear + regression
	Model [2]	Model [3]	Model [4]
MY_1 (kg)	0.38 (0.08)	0.41 (0.08)	0.36 (0.11)
MY_3 (kg)	0.36 (0.08)	0.36 (0.09)	0.73 (0.07)
FP_1 (%)	-0.18 (0.09)	-0.17 (0.09)	-0.22 (0.11)
FP_3 (%)	-0.29 (0.08)	-0.30 (0.08)	-0.73 (0.06)
PP_1 (%)	-0.31 (0.08)	-0.26 (0.08)	-0.21 (0.09)
PP_3 (%)	-0.26 (0.08)	-0.26 (0.08)	-0.85 (0.03)
SCS_1	0.35 (0.14)	0.31 (0.13)	0.17 (0.14)
SCS_3	0.43 (0.13)	0.42 (0.12)	-0.19 (0.26)
FPR_1	-0.05 (0.09)	-0.04 (0.08)	-0.07 (0.10)
FPR_3	-0.18 (0.08)	-0.17 (0.08)	0.21 (0.27)
ECM_1 (kg)	0.29 (0.09)	0.32 (0.10)	0.18 (0.14)
ECM_3 (kg)	0.19 (0.09)	0.18 (0.10)	0.46 (0.17)

Structural Equation and Random Regression Coefficients. Structural equation coefficients λ from the recursive equation [2] and corresponding linear regression coefficients b from equation [4] for identical trait combinations and pathways are displayed in Tables 5 and 6. Effects on the first pathway (λ_{21} and b_{21}) describe the change in AMF with respect to trait_1 (Table 5), and changes of trait_3 with respect to the change of 1 kg/min in AMF are depicted via λ_{32} and b_{32} in Table 6. On the first pathway, structural equation coefficients λ_{21} and linear regression coefficients b_{21} were quite similar for the effects of MY_1, FP_1, PP_1, SCS_1, and ECM_1 on AMF. Moderate differences, that is, $\lambda_{21} = 0$ and $b_{21} = 0.22$, were found for FPR_1. A value $\lambda_{21} = 0$ is the general assumption of a standard linear mixed model. Interestingly, genetic

parameters on this specific pathway (heritabilities for FPR_1, AMF, and genetic correlations between both traits) were almost identical for equations [2] and [4]. Partly moderate differences for same trait combinations were found when comparing λ_{32} and b_{32} on the second pathway. Effects were always in the same direction, but linear regressions depict a stronger effect of AMF on MY_3 and of AMF on ECM_3 than corresponding structural equation coefficients from the recursive system. However, posterior standard deviations for regressions coefficients for effects of AMF on MY_3 and ECM_3 were substantially larger than standard deviations for remaining traits. Generally, standard deviations were larger for regression coefficients compared with structural equation coefficients for same trait combinations and pathways, and supporting recursive model applications.

Table 5. Posterior estimates for structural equation coefficients λ_{21} ¹ from the recursive model [2] and for regression coefficients b_{21} ² from the multiple trait linear model [4] (posterior SD of estimates in parentheses)

Trait_1 ³	Model parameter	
	λ_{21} ¹ (model [2])	b_{21} ² (model [4])
MY_1 (kg)	0.01 (0.00)	0.01 (0.01)
FP_1 (%)	0.01 (0.01)	0.07 (0.09)
PP_1 (%)	-0.13 (0.03)	-0.14 (0.13)
SCS_1	0.14 (0.01)	0.16 (0.08)
FPR_1	0.00 (0.04)	0.22 (0.29)
ECM_1 (kg)	0.01 (0.00)	0.04 (0.03)

¹ λ_{21} = rate of change in average milk flow (in kg/min per unit of trait_1) with respect to trait_1.

² b_{21} = rate of change in average milk flow (in kg/min per unit of trait_1) with respect to trait_1.

³Trait_1 = test-day traits before the measurement data for average milk flow; MY = milk yield, FP = fat percentage, PP = protein percentage, SCS = somatic cell score, FPR = fat-to-protein ratio.

Table 6. Posterior estimates for structural equation coefficients λ_{32}^1 from the recursive model [2] and for regression coefficients b_{32}^2 from the multiple trait linear model [4] (posterior SD of estimates in parentheses)

Trait_3 ³	Model parameter	
	λ_{32}^1 (model [2])	b_{32}^2 (model [4])
MY_3 (kg)	-0.93 (0.10)	-4.69 (3.65)
FP_3 (%)	0.29 (0.01)	0.89 (0.18)
PP_3 (%)	0.43 (0.01)	0.72 (0.13)
SCS_3	0.78 (0.01)	0.79 (0.28)
FPR_3	-0.03 (0.00)	-0.09 (0.14)
ECM_3 (kg)	-0.68 (0.05)	-1.09 (1.79)

¹ λ_{32} = rate of change in trait_3 with respect to the change of 1 kg/min in average milk flow.

² b_{32} = rate of change in trait_3 with respect to the change of 1 kg/min in average milk flow.

³Trait_3 = test-day traits after the measurement data for average milk flow; MY = milk yield, FP = fat percentage, PP = protein percentage, SCS = somatic cell score, FPR = fat-to-protein ratio.

The present study revealed almost identical genetic and phenotypic associations between AMF with test-day traits before and after the AMF measurement for recursive applications (equation [2]), and for linear models accounting for time lagged relationships via linear regressions (equation [4]). Rehbein et al. (2013) gave an overview for recursive model applications in animal breeding and pointed out the superiority of STEM over linear models when exploring mutual relationships, especially among productivity and functional traits. Application of STEM requires substantial extensions of standard mixed model theory by premultiplication of equation systems with matrix \mathbf{A} , and availability of specific software packages [e.g., SirBayes programs by Wu et al. (2008)]. Simplification of equation systems can be realized in a time-lagged multiple-trait approach via the modeling of an additional regression (i.e., defining trait 1 not only as a trait, but also as an effect on trait 2). Valente et al. (2013) emphasized the advantage of STEM to infer mutual relationships among traits, but they also raised the concern of whether STEM are proper for the improvement of multiple trait selection. In contrast, multiple-trait equations [3] and [4] have a direct focus on overall additive-genetic effects and on genetic values of animals. Generally, should a statistical model used for genetic evaluations of a specific trait account for the effect of related traits? This question is of increasing importance in animal breeding, because animal

breeding continuously expands trait recording, which offers possibilities to correct for the health status of cows (Sun et al., 2010). Bohmanova et al. (2006) predicted breeding values for MY with random regression models by accounting for the fertility effects days open and pregnancy state. In another study, Dechow et al. (2001) found substantially different genetic parameters for BCS when adjusting for milk yield in the statistical model.

Physiological mutual relationships between AMF and SCS_3 reflected by structural equation and regression coefficients confirm the genetic antagonism between both traits. Increased AMF is the result of decreased tension of the teat sphincter, and a relatively weak sphincter provides less resistance to infection of the udder by specific major pathogens causing clinical mastitis (Boettcher et al., 1998). The first pathway only shows minor effects of SCS_1 on AMF ($\lambda_{21} = 0.14$, $b_{21} = 0.16$). Those statistical parameters close to zero reflect the biological or physiological expectation, because, to our knowledge, no physiological evidence exists for the effect of SCS early in lactation on milk flow. We also found a negative effect of increasing AMF on MY_3: increasing AMF contributes to impaired udder health, with detrimental effects on milk production in the ongoing lactation (Rehbein et al., 2013). Due to the antagonistic relationship between MY and FP and PP, both content traits increased simultaneously (PP: $\lambda_{32} = 0.43$; FP: $\lambda_{32} = 0.29$). Relationships between MY and AMF and between ECM and AMF were of identical magnitude on all scales and for all statistical parameters: genetic correlations, structural equation coefficients, and regression coefficients. For MY and ECM, negative coefficients λ_{32} and b_{32} indicate a substantial milk yield decline and associated undesired lactation persistency for fast-milking cows. High productivity with a balanced energy status contributed to an increase in AMF, but fast-milking cows reacted with impaired lactation persistency. The influence of milking frequency on lactation persistency was already shown by Hickson et al. (2006). Understanding those relationships between AMF and milking frequency with persistency and peaks of milk production requires ongoing studies on physiological or biological scales. Karacaören et al. (2006) applied random regression models and found alterations of genetic relationships between energy indicator traits such as BW with AMF during lactation.

Solutions for structural equation coefficients λ_{12} and λ_{23} on the phenotypic scale support the idea of AMF as a trait with an intermediate optimum. Cows with the lowest response to stress situations are cows with a quick milk flow that is independent of environmental nuisance and robust against abnormal human behavior in the milking parlor. When selecting cows with large AMF, physiological background suggests impaired udder health. Structural equation models have the potential to infer genetic and phenotypic relationships by considering a more physiological background, and estimates from the present study confirm physiological and practical expectations. However, a more detailed understanding of dairy cow behavior is possible via the implementation of testing schemes to record novel behavior or temperament phenotypes. Such testing schemes focusing on human-animal interactions (e.g., Waiblinger et al., 2006; Breuer et al., 2000) have been partly implemented in participating herds.

CONCLUSIONS

Threshold models, recursive linear models, linear multiple-trait models, and linear multiple-trait models accounting for the effect of a trait A on trait B via linear regressions revealed moderate heritabilities for AMF in a narrow range close to 0.30. Almost identical heritabilities from linear models for assumed Gaussian-distributed AMF and from threshold models for binary AMF recommend continuation of linear model applications in official genetic evaluations. Due to antagonistic genetic relationships with udder health, AMF should be considered as a trait with intermediate optimum.

Antagonistic relationships between AMF and SCS were also confirmed on the phenotypic scale via structural equation coefficients and via linear regression coefficients. The present study revealed almost identical genetic and phenotypic associations between AMF with relationships via linear regressions (equation [4]). Substantially smaller standard deviations of posterior estimates for structural equation coefficients compared with regression coefficients recommend recursive model applications. Furthermore, recursive models also inferred time-lagged relationships

between productivity and AMF and between energy indicators ECM and FPR with AMF.

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CHAPTER 4

Genetic parameters for longitudinal behavior and health indicator traits generated in automatic milking systems

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ABSTRACT

Genetic (co)variance components were estimated for alternative functional traits generated by automatic milking systems (AMS), and reflecting dairy cow behavior and health. Data recording spanned a period of 30 days and included 70,700 observations (visits to the AMS) from 922 Holstein cows kept in three German farms. The three selected farms used the same type of AMS and specific selection gates allowing “natural cow behavior on a voluntary basis”. AMS traits used as behavior indicator traits were: AMS visits per cow and day as binary trait, with a threshold for equal or greater than three visits (VIS3) and equal or greater than four visits (VIS4), knocking off the milking device with a threshold of at least one udder quarter also as a binary trait (KO), milking duration of each AMS visit in min (DUR), average milk flow in kg/min (AMF) and the interval between two consecutive milkings (INT). Electrical conductivity (EC) of milk from each udder quarter and in total was used as a health indicator trait. For genetic analyses, in univariate and bivariate models, linear and generalized linear mixed models (GLMM) with a logit link function were applied to Gaussian distributed and binary traits, respectively. The heritability was 0.08 ± 0.03 for VIS3, 0.05 ± 0.05 for VIS4, 0.03 ± 0.03 for KO, 0.19 ± 0.07 for DUR, 0.25 ± 0.07 for AMF and 0.07 ± 0.03 for INT. Heritabilities for EC varied between 0.37 ± 0.08 and 0.46 ± 0.09 , depending on the udder quarter. On the genetic scale, an increased number of AMS visits (VIS3 and VIS4) was associated with an increase of KO ($r_g = 0.24$ and $r_g = 0.55$, respectively). From a genetic perspective, high milk yielding cows visited the AMS more often ($r_g = 0.49$ for VIS3 and $r_g = 0.80$ for VIS4), had a faster AMF ($r_g = 0.40$), and shorter INT ($r_g = -0.51$). When considering these traits as behavior indicator traits, selection of cows with desired temperament simultaneously increases milk yield. An increase of automatically and objectively recorded AMS traits with moderate heritabilities justifies modifications of dairy cattle breeding goals towards higher emphasis on behavioral traits, especially when developing specific robot indices. Nevertheless, ongoing research in this regard with a larger data is suggested, in order to validate the results from the present pilot study.

Keywords: behavior, automatic milking systems, visit, duration, interval, average milk flow.

INTRODUCTION

Over the past century, dairy cattle breeding has strongly focused on the improvement of production efficiency to satisfy growing consumer markets that demanded animal products at a low cost level (Oltenacu and Algers, 2005). More recently, the importance of functional traits such as fertility, longevity, and conformation has gradually increased in dairy cattle breeding (Miglior et al., 2005). Today, the economic competitiveness of the dairy industry is directly related to public acceptance of breeding techniques (Oltenacu and Algers, 2005). Increasing consumer demands towards animal welfare are forcing dairy breeders to further integrate trait categories that reflect animal health and behavior. Genetic improvements in animal behavior or temperament might contribute to correlated response in other functional traits. Temperament in response to human handlers (docility) has been used as a criterion for genetic selection in beef cattle (Le Neindre et al., 1995). In several countries, subjectively scored dairy cattle temperament is included in breeding indices (Pryce et al., 2000). However, due to challenges in time-efficient and objective trait recording, assumed low heritabilities as well as difficult economic evaluations, only a few studies addressed dairy cattle temperament from a quantitative-genetic or breeding goal perspective (Gutiérrez-Gil et al., 2008; Jensen et al., 2008; Haskell et al., 2014).

Accelerated installation of novel automated technical systems (e.g., automated calf feeders, cow activity monitors, and automatic milking systems (**AMS**)) contributes to a growing amount of currently unused “big data”, including phenotypes for health and behavior traits (Barkema et al., 2015). So far, regarding dairy cows’ behavior, behavior traits used in official genetic evaluations include the temperament in the milking parlor based on a subjective farmer scoring system (Sewalem et al., 2002). Novel technologies, like AMS, allow for automatic, repeated measures and objective phenotypic data recording. Behavior phenotypes can be subjectively scored, or can be objectively measured with technical devices, e.g., the number of escapes, flight times,

or AMS visits (Gutiérrez-Gil et al., 2008; Café et al., 2011; König et al 2006). Milking and cow traffic records from AMS might also be suitable indicators for the development of novel behavior and milking efficiency traits (i. e., milking duration of each AMS visit in min (**DUR**) and average milk flow (**AMF**)). Due to their already existing importance in general as well as special breeding goals for AMS herds, traits like temperament and milking efficiency are turning into the main focus (Vosman et al., 2014). Regarding animal health, electrical conductivity on an udder quarter basis might be a valuable predictor for clinical mastitis (Fernando et al., 1982). To sum up, moderate to high heritabilities of objectively recorded behavior and health traits offer an interesting and currently unused potential to improve and enhance already implemented genetic evaluations for milkability (i.e., average milk flow), milking efficiency and udder health traits. Utilization of traits directly generated from the AMS could help to improve the German Holstein AMS breeding index “RZRobot”. Till now, the RZRobot is based only on the following indicator traits from official recording schemes: Milking speed, somatic cell count, feet and legs, udder component score, rear teat placement and teat length (DHV, 2014).

The aims of the present study were (1) to estimate genetic parameters for dairy cow behavior and health indicator traits generated in AMS, (2) to infer genetic relationships among those novel traits with production traits such as total milk yield per day, and (3) to estimate genetic parameters for milk yield and electrical conductivity from different udder quarters.

MATERIALS AND METHODS

Data. Data recording spanned a period of 30 days and included 70,700 AMS observations (= individual visits into the AMS) from 922 Holstein cows kept in three German farms. The requirement for farm selection was the utilization of the same AMS system: “FeedFirst” (DeLaval GmbH, Glinde, Germany) with a selective guided cow traffic, and a similar herd size (~ 300 cows). Selective guided cow traffic combines the advantages of both, free and guided cow traffic, allowing the cows to show their “natural” behavior (Umstätter, 2002; Harms and Wendl, 2004). Hence, for the additional AMS visit beyond the typical herd average, it was assumed to be the cow’s own decision to enter the milking system. In “FeedFirst” systems, cows have free access to the feeding area by one way gates from the lying area. However, in order to reach the lying area from the feeding area, the cows have to pass a selection gate that directs the cows to the milking station or the lying area depending on the milking permission. If the time since last milking is long enough and a milking permission has been established, the cow is directed to the waiting area in front of the milking unit. If the cow has no milking permission, she is directed back to the lying area. With pre-selection, daily fetch rates are, on average, 1 to 5 percent of the herd (Harms and Wendl, 2004). Pedigree data were traced back to 1940, including 22,714 animals, and a total of 297 different bulls with daughter records. The most influential sire had 37 daughters, and the average no. of daughters per sire was 2.99.

Two different data sets were merged. The first dataset included traits recorded in close intervals, leading to a longitudinal data structure for novel behavior (temperament) and health indicator traits (Table 1). The AMS traits used as behavior indicators were: AMS visits per cow and day defined as two binary traits, with thresholds for i) more than three visits per day (**VIS3**) and ii) more than four visits per day (**VIS4**). If a cow had values equal or above the threshold, she received the score “1”, otherwise a “0” was assigned. Hence, VIS3 and VIS4 were defined as binary traits to emphasize the “extra” voluntary component, i.e., the additional AMS visit beyond the typical herd average. Usually, in selective guided cow traffic systems, only cows that voluntarily visited the AMS one time per day are fetched and forced by the farmer to visit the AMS at least

twice a day (Ketelaar-de Lauwere et al., 1998). Since fetching is time-consuming, we argue that farmers do not force the cows to visit the AMS more than twice a day, and that cows visiting the AMS at least three times a day (VIS3) do that on a voluntary basis due to food motivation or udder pressure (Nixon et al., 2009). Cows visiting the AMS more than four times a day (VIS4) may have other motivations than the primary motivation of being rewarded with food, such as curiosity. Further temperament traits were the knock off of the milking device (**KO**) from at least one udder quarter, also defined as a binary trait, milking duration of each visit in the AMS in min (**DUR**) and average milk flow in kg/min (**AMF**). DUR and AMF primarily reflect the same aspects of behavior and are also influenced by udder pressure and udder morphology. The traits VIS3 and VIS4 are affected by some common aspects, i.e. food motivation, udder pressure, but also temperament. KO due to stepping and kicking potentially reflects different important aspects of behavior, i.e., discomfort, nervousness, and fear (Rousing et al., 2004). The interval (**INT**), defined as the time span between two consecutive AMS visits, was used as a behavior and social dominance indicator (Table 1). A second AMS dataset included measurements for total milk yield per day (**MY_total**), and electrical conductivity (**EC**) of milk. Data for milk yield and EC were also available on an udder quarter basis: Milk yield for the front left quarter (**MY_fl**), for the front right quarter (**MY_fr**), for the rear left quarter (**MY_rl**) and for the rear right quarter (**MY_rr**). Abbreviations for EC for each quarter were **EC_fl**, **EC_fr**, **EC_rl** and **EC_rr**. Electrical milk conductivity was used as a health trait indicator reflecting the udder health status of individual udder quarters.

Statistical analysis

Impact of environmental and animal related effects on behavior traits. Analyses of variance for the behavior indicator traits INT and DUR, and the estimation of least squares means within levels of fixed effects were performed using the software package PROC MIXED in SAS (Version 9.2; SAS Institute Inc., Cary, NC). The applied linear model was:

$$y = X\beta + Zu + e, \quad [1]$$

Table 1. Definition of the traits generated in the automatic milking system and an explanation for their use as behavior and health trait indicators

Trait	Distribution	Definition	Indicator	Explanation
AMF (kg/min)	Normal	Average milk flow	Stress, Temperament	Under stress conditions more cortisol is excreted hampering oxytocin release, leading to reduced milk flow
DUR (min)	Normal	Milking time during a visit in the AMS	Stress, Temperament	Milk let down from stressed cows is slower, leading to increased DUR
VIS3	Binary	If more than three visits a day then score = 1, otherwise score = 0	Temperament Food motivation?	Cows with desirable temperament, visit the AMS more often
VIS4	Binary	If more than four visits a day then score = 1, otherwise score = 0	Temperament, Curiosity	Cows with desirable temperament, visit the AMS more often
KO	Binary	If the milking device is knocked off at at least one quarter then score = 1, otherwise score = 0	General discomfort, Temperament	More nervous cows, more kickings Bad temperament, more knocked offs
INT (h)	Normal	Interval between two consecutive visits	Social dominance, Temperament	Shorter intervals imply more visits to the AMS (higher dominance), indicating free choices to access the robot and also the feeding and resting area
EC (mS/cm)	Normal	Electrical conductivity	Health	Pathological changes of udder tissue produce a change in the electrical conductivity

where \mathbf{y} was the vector of observations, $\boldsymbol{\beta}$ was the vector of fixed effects including lactation numbers from 1 to 5, the day when the cow entered the AMS, days in milk in classes according to Huth (1995) (< 14 d = 1, 14 d - 77 d = 2, 77 d - 140 d = 3, 140 d - 231 d = 4, > 231 d = 5), time of day classes (**ToD**) when the cow visited the AMS (22 - 4 = 1, 4 - 10 = 2, 10 - 16 = 3, 16 - 22 = 4), and explicitly for DUR specific INT classes (< 8 h = 1, 8 h - 10 h = 2, > 10 h = 3), \mathbf{X} was the corresponding incidence matrix for fixed effects, \mathbf{u} was the vector of random cow effects for repeated measurements with the corresponding incidence matrix \mathbf{Z} , and \mathbf{e} was the vector for random residual effects.

Estimation of genetic parameters. In this regard, model I was extended by including the pedigree based on genetic relationship matrix. Variance components and heritabilities for behavior, health and production traits were estimated via single trait animal models using the AI-REML procedure, as implemented in the DMU software package (Madsen and Jensen, 2000).

For the Gaussian distributed traits MY_total, MY_fl, MY_fr, MY_rl, MY_rr, EC_fl, EC_fr, EC_rl, EC_rr AMF, INT and DUR, a linear animal repeatability model was applied:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{W}\mathbf{p} + \mathbf{e}, \quad [\text{II}]$$

where \mathbf{y} was the vector of observations, $\boldsymbol{\beta}$ was the vector of fixed effects with the corresponding incidence matrix \mathbf{X} , \mathbf{u} was the vector of random genetic effects with the corresponding incidence matrix \mathbf{Z} , \mathbf{p} was the vector of random permanent environmental effects with the corresponding incidence matrix \mathbf{W} , and \mathbf{e} was the vector of random residual effects. Traits and their corresponding effects as considered in model I and II are given in Table 2.

Repeatabilities for the longitudinal traits were calculated as follows:

$$W = \frac{(\sigma_a^2 + \sigma_{pe}^2)}{(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)}$$

where σ_a^2 was the additive genetic variance, σ_{pe}^2 was the permanent environmental variance, and σ_e^2 was the residual variance.

Table 2. Significance of fixed effects and of covariates for the different traits

Traits ¹	Effects ²						Covariate ³
	Robot	LN	Date	INTcl	DIMcl	ToD	CA
MY_rr, _rl, _fr, _fl	***	n.s.	***	***	***	***	**
EC_rr, _rl, _fr, _fl	***	n.s.	***	***	***	***	*
AMF	***	n.s.	***	***	***	***	n.s.
MY_total	***	n.s.	***		***	***	***
EC	***	***	***	***	***	***	***
INT	***	*	***		***	***	**
DUR	***	**	***	***	***	***	**
VIS3	***	***	***		***		***
VIS4	***	n.s.	***		***		***
KO	***	**	*	*	***	***	***

* P < 0.05 significant, ** P < 0.01 very significant, *** P < 0.001 highly significant, n.s.: not significant

¹ MY_fl, _fr, _rl, _rr: milk yield at a quarter basis: front left, front right, rear left and rear right

EC_fl, _fr, _rl, _rr: electrical conductivity at a quarter basis: front left, front right, rear left and rear right

AMF: average milk flow

MY_total: total milk yield per day

EC: electrical conductivity from all four quarters

INT: interval between two consecutive milkings

DUR: milking time during a visit in the milking robot

VIS3: at least three visits to the milking robot per day

VIS4: at least four visits to the milking robot per day

KO: knock off of the milking device from at least one udder quarter

² Robot: the AMS the cow used. Consecutively numbered across herds

LN: lactation number 1 - 5

Date: day the cow entered the AMS

INTcl: interval in classes; < 8 h = 1, 8 h - 10 h = 2, > 10 h = 3

DIMcl: DIM in classes; < 14 = 1, 14 - 77 = 2, 77 - 140 = 3, 140 - 231 = 4, > 231 = 5

ToD: time of day when the cow visited the AMS in classes; 22 - 4 = 1, 4 - 10 = 2, 10 - 16 = 3, 16 - 22 = 4

³ CA: calving age in month = (calving date - birth date)/30.4375

For the binary traits VIS3, VIS4 and KO, a generalized linear mixed model (**GLMM**) with a logit link function was applied:

$$\eta = X\beta + Zu + Wp \quad [III]$$

where $\boldsymbol{\eta}$ was the vector of logits, $\boldsymbol{\beta}$ was the vector of fixed effects with the corresponding incidence matrix \mathbf{X} , \mathbf{u} was the vector of random genetic effects with the corresponding incidence matrix \mathbf{Z} , \mathbf{p} was the vector of random permanent environmental effects with the corresponding incidence matrix \mathbf{W} . The binary traits along with their effects are given in Table 2.

Heritabilities were calculated using the variance of the logit link function. This implies a correction of the residual variance by factor $\pi^2/3$ (Southey et al., 2003).

The covariance structure of random effects for models II and III was:

$$\text{var} \begin{bmatrix} \mathbf{u} \\ \mathbf{p} \\ \mathbf{e} \end{bmatrix} = \begin{pmatrix} \mathbf{G} \otimes \mathbf{A}_U & 0 & 0 \\ 0 & \mathbf{R} \otimes \mathbf{I}_P & 0 \\ 0 & 0 & \mathbf{R} \otimes \mathbf{I}_R \end{pmatrix}$$

where \mathbf{G} was the variance-covariance matrix for genetic effects, \mathbf{A}_u was an additive genetic relationship matrix for u animals in the whole pedigree, \mathbf{P} was the variance-covariance matrix for permanent environmental effects, \mathbf{I} was an identity matrix for n cows and r observations, \mathbf{R} was the residual variance-covariance matrix, and \otimes denotes the Kronecker product.

Because milk yield is an important factor influencing AMF, INT, DUR, VIS3, VIS4 and KO, univariate models with and without milk yield as a covariate were compared.

Furthermore, bivariate animal models were applied to all trait combinations, in order to estimate genetic correlations. Bivariate GLMM (model III) were applied to binary trait combinations (e.g., VIS3 with KO), bivariate GLMM - linear models (model III and II) for the combination of one binary with one Gaussian trait (e.g., VIS3 with MY), and linear - linear models (model II) for Gaussian - Gaussian trait combinations including EC, AMF, MY_total, INT and DUR.

In some bivariate runs, SE for genetic correlations were quite large. However, SE for heritabilities from single trait animal models were in an acceptable range. Hence, in addition, estimated breeding values (**EBV**) from single trait animal models were correlated, and EBV correlations were compared with genetic correlations. For this purpose, EBV from the most influential sires with at least 5 daughters were considered (47 sires).

RESULTS AND DISCUSSION

Descriptive statistics. Descriptive statistics for all traits used in this study after data editing are given in Table 3. The degree of individual variation in milking frequencies reflect the variation in milking intervals from 2 h to 18 h. Some cows entered the AMS as soon as the minimum “milk allowing interval” has passed, whereas other cows only are milked when they are fetched by the stockperson after 14 h. A large variation in the range from 1 to 15 minutes (average of 6.3 minutes) was also observed for DUR. A fraction of 52 % of all cows visited the AMS more than 3 times per day (VIS3), but only 10 % of all cows visited the AMS more than 4 times per day (VIS4).

The mean of the udder health indicator EC ranged from 4.92 mS/cm to 4.96 mS/cm on an udder quarter level. Norberg et al. (2004) used an inter-quarter ratio (IQR) for EC and found out that IQR performed better than the absolute conductivity level, in order to distinguish between clinically and subclinically infected cows. In contrast, in early studies, Linzell and Peaker (1975) and Fernando et al. (1982) stated that absolute conductivity and IQR had similar accuracies for the detection of mastitis. In the present study, the analysis focused on the absolute EC, as generated by the AMS, instead of defining inter-quarter ratios.

Impact of environmental and animal related effects on behavior traits. Table 2 shows the significance level of each effect for the traits DUR and INT (results from model I). DUR differed significantly ($P < 0.05$) for all ToD classes (Figure 1a), apart from the comparison DUR in ToD 1 and DUR in ToD 3. Cows spent the most time in the milking box between 4 and 10 (= ToD 2). Regarding INT, all ToD classes differed significantly ($P < 0.05$) from each other (Figure 1b). The longest interval was found between 10 and 16 (Figure 1b). This is the time of the day when cows usually eat or lie (Krohn and Munksgaard, 1993; DeVries and Keyserlingk, 2005). The shortest milking interval was found between 4 and 10. Cows determine their activity rhythm according to environmental factors such as light. They are more active during daytime because of the circadian rhythm – the light change between day and night – which acts as a natural timer (Zeeb und Bammert, 1985). Hopster et al. (2002) demonstrated that low-

ranking cows are forced by social competition to visit the AMS at times that are not preferred, particularly during the midnight hours, leading to irregular or long intervals between milkings.

Table 3. Descriptive statistics for all traits and effects

Traits/Effects ¹	Statistical parameters			
	Mean	SD	Min.	Max.
MY_total (kg)	31.88	11.07	1.70	88.42
AMF (kg/min)	2.05	0.64	0.11	4.00
MY_fl(kg)	2.90	1.09	0.21	6.80
MY_rl (kg)	3.76	1.41	0.61	8.40
MY_fr (kg)	3.01	1.17	0.21	7.40
MY_rr (kg)	3.67	1.44	0.21	8.60
EC (mS/cm)	4.92	0.37	3.78	6.06
EC_fl (mS/cm)	4.95	0.42	3.76	6.30
EC_rl (mS/cm)	4.95	0.40	3.91	6.30
EC_fr (mS/cm)	4.96	0.41	3.91	6.30
EC_rr (mS/cm)	4.95	0.41	3.91	6.30
DUR (min)	6.27	1.84	1.02	15.00
INT (h)	9.16	2.65	2.05	18.00
VIS3	0.52	0.50	0	1
VIS4	0.10	0.29	0	1
KO	0.08	0.28	0	1
DIM(days)	164.56	90.04	5.00	364.00
CA (month)	37.16	10.04	20.47	71.23

¹ MY_total: total milk yield per day

AMF: average milk flow

MY_fl, _fr, _rl, _rr: milk yield at a quarter basis: front left, front right, rear left and rear right

EC: electrical conductivity from all four quarters

EC_fl, _fr, _rl, _rr: electrical conductivity at a quarter basis: front left, front right, rear left and rear right

DUR: milking time during a visit in the milking robot

INT: interval between two consecutive milkings

VIS3: at least three visits to the milking robot per day

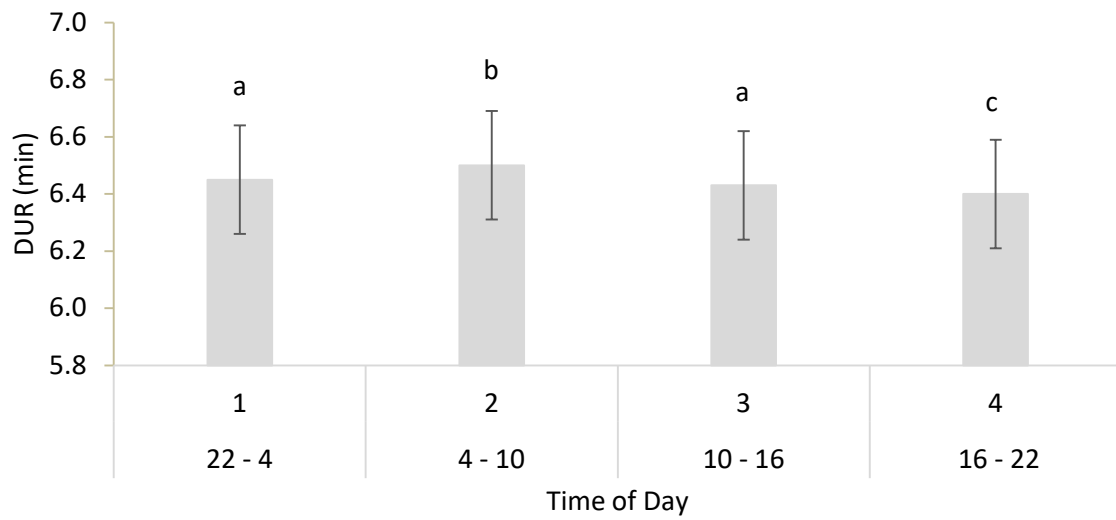
VIS4: at least four visits to the milking robot per day

KO: knock off of the milking device from at least one udder quarter

DIM: days in milk

CA: calving age

A



B

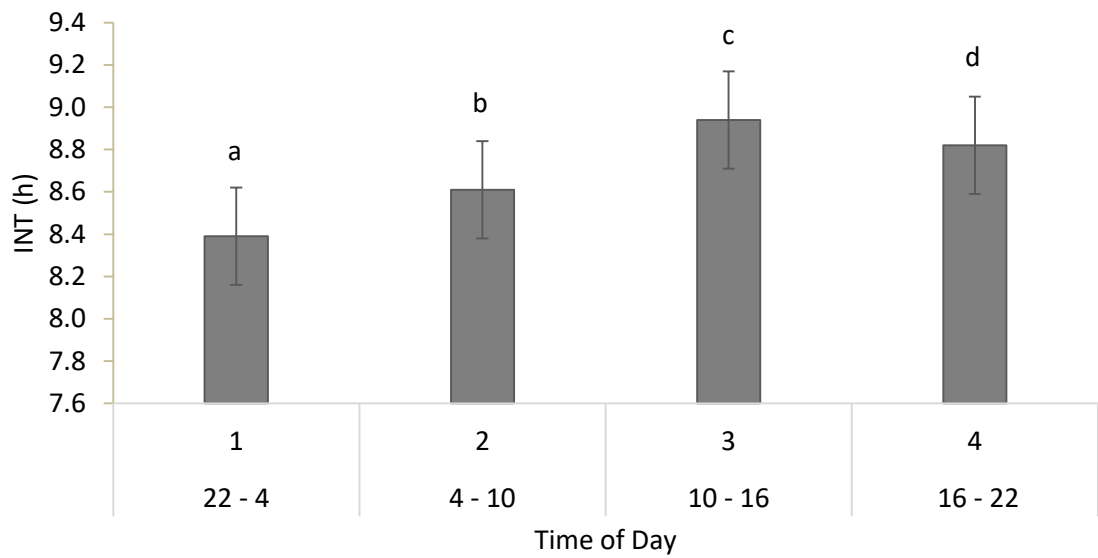


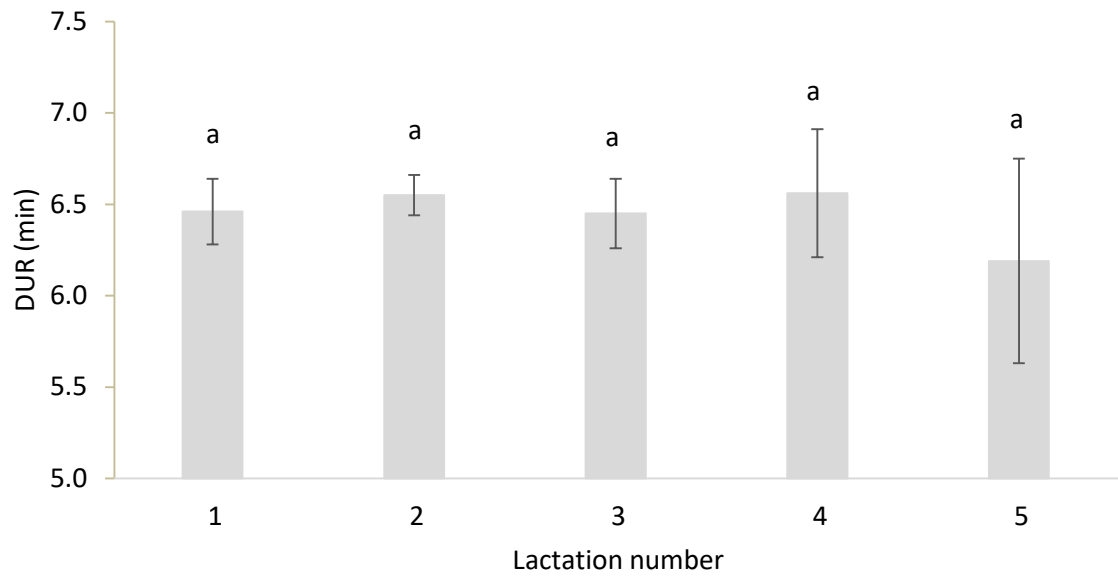
Figure 1. Least squares means for (A) DUR (milking time during a visit in the AMS) (\pm SE) and (B) INT (interval between two consecutive visits to the AMS) (\pm SE) in dependency of time of the day in classes. Significant differences of least squares means (< 0.05) are indicated with different letters above the bars

There was no significant impact ($P > 0.05$) of the effect lactation number (LN) on DUR (Figure 2a), meaning that cows with different LN spent on average the same time in the AMS. With regard to the LN effect, least squares means for INT significantly differed ($P < 0.05$) (Figure 2b). Longer INT for first lactation cows were probably due to fearfulness and lower ranking, i.e., they were not allowed to visit the AMS in close intervals due to the social herd structure (Ketelaar-de Lauwere, 1996). The INT increase in the fifth lactation (Figure 2b) could be due to reduced mobility of older cows (Alban, 1995). Prescott et al. (1998) found that low-yielding cows did not significantly increase their level of voluntary AMS visits when changing feeding strategies, which indicates further motivations to visit the AMS, for example curiosity.

Genetic parameters

Variance components and heritabilities for behavior traits. The heritability was 0.25 for AMF, 0.19 for DUR, 0.07 for INT, 0.03 for KO, 0.08 for VIS3 and 0.05 for VIS4 (Table 4). König et al. (2006) estimated a heritability for milking frequency in the range from 0.16 and 0.27, depending on the statistical modelling. Reported heritabilities for INT were in a range from 0.09 to 0.26 (Carlström et al., 2013). The heritability for INT found in the present study was slightly lower (0.07). Also the heritability for AMF (0.25) was slightly lower compared to estimates based on conventional milking technique data (Santos et al., 2015), but still moderate with relatively small standard errors. The moderate heritabilities for the behavior trait indicators DUR and AMF indicate the possibilities for AMS milkability improvements via breeding. Heritabilities estimated with univariate models with and without milk yield as a covariate were comparable, with slightly larger heritabilities for some traits in models with milk yield as a covariate (0.17 for VIS3, 0.08 for VIS4 and 0.18 for INT). Heritabilities and additive genetic variance show that beside environmental components such as food, fetching and machine calibration (e.g. restriction of the INT) there is a genetic component behind the traits. Research showed that there is a possibility of automatically record behavior traits (König et al., 2006; Schwartzkopf-Genswein et al., 2012). The characterization of the traits in the context of animal welfare imply further studies including temperament and behavior tests as validated in animal ethology (e.g., Ebinghaus et al., 2017).

A



B

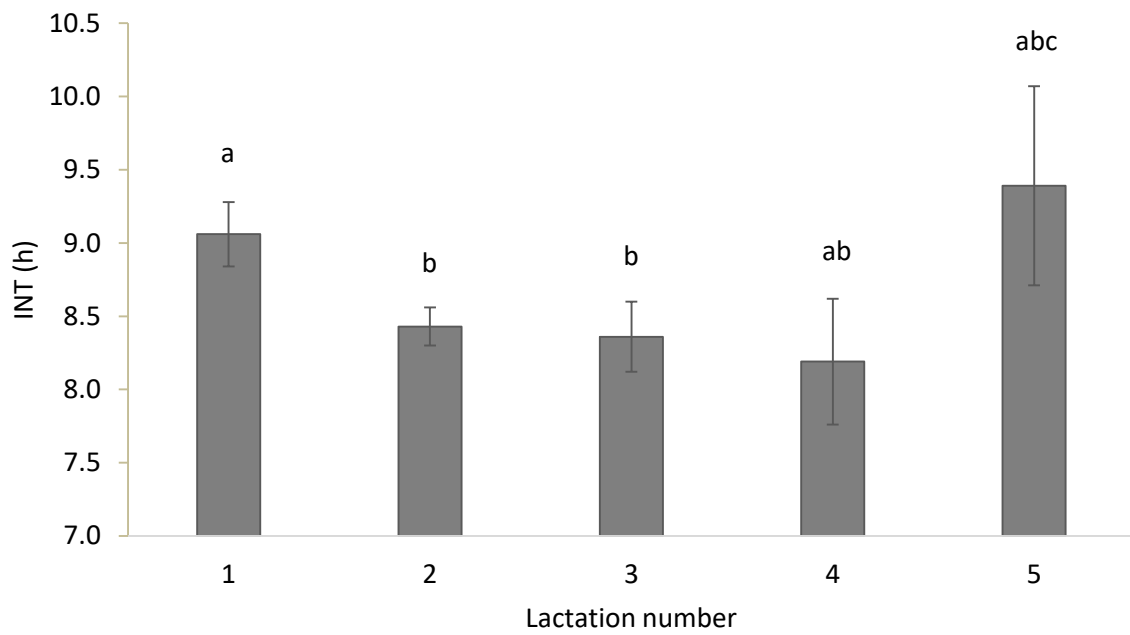


Figure 2. Least squares means for (A) DUR (milking time during a visit in the AMS) (\pm SE) and (B) INT (interval between two consecutive visits to the AMS) (\pm SE) in dependency of lactation numbers. Significant differences of least squares means (< 0.05) are indicated with different letters above the bars

Table 4. Heritabilities (h^2) with their corresponding standard errors (SE), additive genetic variances (σ_a^2), permanent environmental variances (σ_{pe}^2), residual variances (σ_e^2) and repeatabilities (w^2) for all traits

Traits ¹	σ_a^2	σ_{pe}^2	σ_e^2	$h^2 \pm SE$	w^2
AMF (kg/min)	0.09	0.13	0.13	0.25 ± 0.07	0.63
DUR (min)	0.57	1.28	1.23	0.19 ± 0.07	0.60
INT (hour)	0.42	1.44	4.15	0.07 ± 0.03	0.31
KO	0.17	1.44	3.29	0.03 ± 0.03	0.33
VIS3	0.38	1.11	3.29	0.08 ± 0.03	0.31
VIS4	0.30	2.01	3.29	0.05 ± 0.05	0.41
MY_total (kg)	1.62	3.49	4.13	0.18 ± 0.06	0.55
MY_fl (kg)	0.16	0.47	0.23	0.19 ± 0.06	0.73
MY_fr (kg)	0.04	0.55	0.27	0.05 ± 0.06	0.69
MY_rl (kg)	0.15	0.70	0.47	0.11 ± 0.06	0.64
MY_rr (kg)	0.10	0.72	0.49	0.08 ± 0.05	0.63
EC (mS/cm)	0.07	0.03	0.03	0.53 ± 0.09	0.77
EC_fl (mS/cm)	0.07	0.04	0.04	0.46 ± 0.09	0.73
EC_fr (mS/cm)	0.07	0.05	0.04	0.44 ± 0.09	0.75
EC_rl (mS/cm)	0.05	0.05	0.04	0.37 ± 0.08	0.71
EC_rr (mS/cm)	0.06	0.05	0.04	0.38 ± 0.09	0.73

¹ See Table 3

Variance components and heritabilities for udder quarter specific productivity and udder health traits. Heritabilities for EC from different udder quarters varied between 0.37 and 0.46 (Table 4). For udder health indicators, Juozaitienė et al. (2015) estimated a heritability of 0.51 for EC, reflecting heritabilities for corresponding traits from our study. The moderate to large heritabilities suggest the inclusion of EC into udder health indices. From such a perspective, additional value is generated when treating each quarter separately, in order to identify pathological changes (Umstätter, 2002). The benefit would be in the use of information for monitoring and managing animal health (Kramer et al., 2013).

The heritability for milk yield per day (MY_total) was 0.18. For the different udder quarters, heritabilities ranged from 0.05 for MY_fr to 0.19 for MY_fl (Table 4). Tančin et al. (2006) suggested productivity improvements via consideration of specific udder quarter information. Also in the present study, identical traits from different udder quarters seem to have a different genetic background in terms of genetic (co)variance

components, suggesting udder quarter specific selection strategies. Information at an udder quarter level can be used in precision dairy farming. Eastwood et al. (2004) defined precision dairy farming as “the use of information technologies for assessment of fine-scale animal and physical resource variability aimed at improved management strategies for optimizing economic, social, and environmental farm performance.” In this regard, electrical devices used in precision dairy farming analyzed different milk characteristics and produced indicators based on temperature, conductivity, milk quantity, production balance across quarters or milk flow (Boichard and Brochard, 2012).

Correlations among behavior indicator traits. Genetic correlations (r_g) as well as EBV correlations (r_{EBV}) between AMF and DUR were negative (-0.88 and -0.63, respectively (Table 5)), indicating that fast milking cows, i.e. cows with a good temperament, spent less time milking in the milking robot. Hence, genetic associations between AMF and DUR confirm farmers’ observations at a phenotypic scale. Both temperament indicators milking time and milk flow are economically important, because they determine AMS capacity and efficiency. Explanations for the strong genetic relationship between AMF and DUR with acceptable standard errors can be as follows: The combative behavior of cows causes physiological changes (i.e., affecting the oxytocin hormone level and norepinephrine concentrations (Kondo and Hurnik (1988)), and in ongoing lactation, reduced milk flow. Szentléleki et al. (2015) reported a significantly higher milking speed if cows were calm in the pre-milking process compared to nervous cows. Another explanation is due to the fact that cows with faster AMF need less time to be milked, and therefore spend less time in the milking box.

The genetic correlation between DUR and KO was also negative ($r_g = -0.25$), with less knock offs of the milking device being associated with longer milk ejection. A hypothesis would be that nervous or fearful cows show more knock offs, leading to a shorter milk ejection because of negative impact on oxytocin release. As outlined by Rousing et al. (2004), kicking and stepping during milking resulted in knock off of the milking device, incomplete milking, and reduced milk yield. Stressed animals require

not only more handling during milking (Rushen et al., 1999) but they may kick off the milking device during milking. Fearfulness in cows can result in slower milk let down and milk retention (Munksgaard et al., 2001). EBV correlations between DUR and KO were close to zero. EBV correlations only based on EBV from influential sires, but due to EBV accuracies lower than 1, r_{EBV} are “less extreme”, i.e., an underestimation of genetic correlations (Calo et al., 1973).

Low positive correlations were estimated between VIS3 and DUR ($r_g = 0.28$, $r_{EBV} = 0.11$), and between VIS4 and DUR ($r_g = 0.37$, $r_{EBV} = 0.12$), indicating that an increase of visiting frequencies was associated with a longer milking duration per visit. Extremely long DUR may reflect an inhibited milk flow, being supported on the genetic scale with $r_g = -0.88$ between DUR and AMF. The genetic correlation between AMF and INT was positive, but close to zero ($r_g = 0.14$, $r_{EBV} = 0.02$), and estimated with quite large standard errors. Accordingly, Hogeveen et al. (2001) found that a longer milking interval was associated with an increase in the milk flow rate (on a phenotypic level), independent from the milk performance level. An increase in milking speed (e.g., AMF) lead to a reduction of total milking time per cow, with positive impact on AMS capacity and AMS efficiency (Gäde et al., 2007).

INT and KO were negatively correlated ($r_g = -0.19$, $r_{EBV} = -0.13$), meaning that shorter intervals were genetically associated with more knock offs. This might be associated with udder deformations, resulting in improper attachment of the milking device. Also, the correlation between DUR and INT was slightly negative ($r_g = -0.15$, $r_{EBV} = -0.09$), indicating that shorter intervals were associated with a longer milking duration. Milking duration depends not only on the amount of milk, but also on the temperament of the cow. Kaihilahti et al. (2006) stated that 19 % of the cows kicked off the teat cups during milking, with impact on milk duration and milking time independent from the milk yield level. In most cases, EBV correlations were in agreement with genetic correlations.

Table 5. Genetic correlations among behavior indicator traits (above the diagonal with standard errors in brackets) and corresponding correlations among breeding values from bulls with at least 5 daughters (below the diagonal)

Trait ¹	AMF (kg/min)	DUR (min)	INT(hour)	KO	VIS3	VIS4
AMF (kg/min)		-0.88 (0.08)	0.14 (0.23)	0.17 (0.40)	-0.24 (0.23)	-0.20 (0.34)
DUR (min)	-0.63		-0.15 (0.28)	-0.25 (0.47)	0.28 (0.27)	0.37 (0.38)
INT (hour)	0.02	-0.09		-0.19 (0.47)	-0.62 (0.19)	-0.88 (0.22)
KO	-0.02	0.06	-0.13		0.24 (0.47)	0.55 (0.79)
VIS3	-0.00	0.11	-0.96	0.13		0.89 (0.14)
VIS4	-0.00	0.12	-0.86	0.21	0.84	

¹ See Table 1

Correlations between behavior and production traits. As indicated in Table 6, MY_{total} was positively correlated with DUR ($r_g = 0.97$; $r_{EBV} = 0.25$), with VIS3 ($r_g = 0.49$; $r_{EBV} = 0.52$) and with VIS4 ($r_g = 0.80$; $r_{EBV} = 0.47$). These findings suggest that sires with high EBV for milk yield, tend to have daughters with longer milking duration, but also daughters that come easily or voluntary into the milking box. These findings were also reported by König et al. (2006) when defining milking frequencies as a behavior indicator trait. In their models, milk yield was used as covariate in statistical models, showing that milk yield was not the only force determining voluntary visits. Kazlauckas et al. (2005) compared two groups of mice (with high and low exploration of an object) and concluded that although both groups were driven by food motivation, high exploratory mice showed increased locomotion. Results from such research, as conducted in other species, support the hypothesis that there are additional motivations to visit the AMS apart from the offered food. In accordance with Nixon et al. (2009), selection for increased milk production implies an increase of milking frequencies. Associations between calm temperament and high milk production were shown by Lawstuen et al., (1988) and Breuer et al. (2000). Also across species, temperament and behavior was favorably correlated with productivity. For example in pigs, selection for calm temperament simultaneously improved carcass and meat quality traits (Reverter et al., 2003). Nevertheless, the definition of temperament differed, complicating the comparison of correlation coefficients from different

studies. Accordingly, Hoppe et al. (2010) found that selection towards improved temperament in beef cattle simultaneously improved production traits. In the present study, milk yield was positively correlated with AMF ($r_g = 0.40$; $r_{EBV} = 0.47$), indicating that higher productivity was associated with a faster milk flow, supporting previous results from conventional milking systems (Santos et al., 2015). Selection towards desired temperament had a positive effect on the number of visits, and on the duration in the milking box, as well as on milk production and milk efficiency (i. e., AMF).

Table 6. Genetic correlations (r_g) and breeding value correlations (r_{EBV}) considering bulls with at least 5 daughters between milk yield and behavior indicator traits

Traits ¹	MY_total (kg)	
	r_g	r_{EBV}
AMF (kg/min)	0.40 (0.19)	0.47
DUR (min)	0.87 (0.35)	0.25
INT (hour)	-0.51 (0.23)	-0.52
KO	0.21 (0.42)	0.05
VIS3	0.49 (0.23)	0.52
VIS4	0.80 (0.30)	0.47

¹ See Table 1

MY_total: total milk yield per day from AMS

The correlation between MY_total and KO ($r_g = 0.21$; $r_{EBV} = 0.05$) was moderate positive, indicating antagonistic genetic relationships from a breeding perspective. Knocking off the milking device has different reasons, e.g., udder morphology, mastitis or the temperament of the cow. More aggressive cows produce more knock offs of the milking device due to the agitation in the milking box. The behavior indicator INT was negatively correlated with MY_total ($r_g = -0.51$; $r_{EBV} = -0.52$). Shorter milking intervals, indicating a good individual behavior or temperament, were also associated with higher milk production per cow and hour (Hogeveen et al., 2001). Extended milking intervals cause practical problems in AMS, and so far, direct selection tools are missing.

Nevertheless, some genetic correlation standard errors were quite large, encouraging further analyses in this regard.

Correlations between behavior and health traits. Genetic correlations among all behavioral traits and EC were positive, but also close to zero (Table 7). The strongest positive genetic correlation was found between INT and EC (0.19), indicating that longer milking intervals had a negative impact on animal health, or that animals having health problems have longer milking intervals. The number of AMS visits (VIS3) was positively correlated with EC ($r_g = 0.15$). The risk of bacterial invasion into the mammary gland increased with the number of milkings per day (Hogeveen et al., 2001). The genetic correlation between AMF and EC was low, but again positive ($r_g = 0.11$), confirming the unfavorable relationships between temperament and health traits (Santos et al., 2015).

Table 7. Genetic correlations (r_g) with posterior standard deviations (in brackets) and breeding value correlations (r_{EBV}) considering bulls with at least 5 daughters between behavior indicator traits and electrical conductivity of the udder (EC)

Traits ¹	EC (mS/cm)	
	r_g	r_{EBV}
AMF (kg/min)	0.11 (0.23)	0.05
DUR (min)	0.18 (0.22)	-0.06
INT (hour)	0.19 (0.37)	-0.03
KO	0.16 (0.24)	0.04
VIS3	0.15 (0.21)	-0.01
VIS4	0.08 (0.23)	0.11

¹ See Table

Increased AMF is also due to decreased tension of the teat sphincter. A weak sphincter decreased resistance to infection of the udder by specific major pathogens, causing clinical mastitis (Boettcher et al., 1998). Hence, from an animal breeding perspective, AMF should be treated as a trait with an intermediate optimum, as already suggested by Santos et al. (2015). Generally weak genetic correlations suggest that breeding on behavior indicator traits does not impair health indicator traits. Regarding the correlations between AMS behavior indicator traits with EC, all EBV correlations were close to zero, and partly differed in sign from genetic correlations. The genetic correlations between AMS behavior traits with other functional traits were estimated with quite large standard errors, suggesting ongoing studies with additional data.

CONCLUSIONS

This was a first pilot study to estimate genetic parameters for alternative functional traits based on data automatically generated by the AMS, free from subjective evaluations of classifiers. Furthermore, the applicability of AMS traits as indicators for dairy cow behavior and health was verified. In such perspective, with the inclusion of milk yield as a covariate in the statistical model, genetic variances of AMS traits even increased. The genetic variance and heritability increase suggests a behavior background, instead of milk yield being the only force behind these traits, e.g., AMS visits. Behavior traits delivered from data from the AMS like AMF and DUR had a moderate heritability. Heritabilities for the other AMS behavior indicator traits (INT, KO, VIS3 and VIS4) were quite small. Regarding genetic relationships, an increase of AMF, a decrease in DUR and a decrease of INT might contribute to desired behavior without losing genetic gain for production. Different EC heritabilities for different udder quarters allow udder quarter specific breeding strategies. Hence, based on the genetic (co)variance components from the present study, results are encouraging to breed cows for AMS systems based on AMS data, but it is imperative to have a precise validation based on larger datasets.

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CHAPTER 5

Estimation of genetic parameters for behavior traits reflecting the human-animal relationship in dairy cattle

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ABSTRACT

To assess dairy cows' responsiveness towards humans, four behavior traits were recorded on 33 farms located in central and northern Germany and genetic (co)variance components were estimated. Data was available from 1,761 cows for the trait tolerance to tactile interaction (TTI), 1,766 cows for the behavior during release from restraint in the feeding gate (RB) and 1,880 cows for the avoidance distances towards an unfamiliar person at the feeding place (AD). The Qualitative Behavior Assessment (QBA) was conducted on 565 cows. Further analysis utilized the cows' individual scores from the first principal component (PC1), which explained nearly 70 % of the variation (QBA_PC1) characterized by descriptors relating to relaxation/attraction/trust on the negative and descriptors relating to fear/distress/aversiveness on the positive end. Estimated heritabilities and their corresponding standard errors for behavior traits reflecting human animal interactions were 0.27 ± 0.06 for the AD, 0.04 ± 0.05 for TTI, 0.11 ± 0.06 for RB and 0.13 ± 0.17 for QBA_PC1. Estimated breeding value (EBV) correlations (r_{EBV}) for the most influential sires among the behavior traits AD, TTI, RB (restriction: sires with at least 5 daughters), and QBA_PC1 (no restriction) were moderately positive. This indicates that while the various methodologies for behavior assessment may measure slightly different aspects of animal behavior, they are indeed related and are partly influenced by a similar genetic component. Additional calculations were made for EBV correlations from sires with at least 5 daughters between the behavior traits and test day traits based on two test-days closest to the recording date (before and after) of behavior traits. The AD and somatic cell score from the test day close to the date after the measurement of the behavior trait (SCS_a) were significantly positively correlated ($r_{EBV} = 0.31$). Hence, selection towards low AD, and therefore less fearful animals, would positively impact animals' health. Significant correlations were also calculated between the breeding index functional herd life (RZN) and AD ($r_{EBV} = -0.28$) and between RZN and RB ($r_{EBV} = -0.35$). Sires with a high EBV for longevity traits tend to have daughters with lower avoidance distances and less fearful behavior. Although behavior traits depend to a large extent on the environment and management factors, the genetic component

seems to play an important role. These results support the idea of including behavior traits that reflect the human-animal relationship in breeding strategies.

Key words: dairy cattle, human-animal relationship, behavior traits, heritabilities, genetic correlations

INTRODUCTION

The human-animal relationship (**HAR**) is a common trait of modern intensive farming systems and research in a number of livestock industries has shown that the interactions between handlers and their animals can limit the productivity and welfare of livestock (Hemsworth and Coleman, 2010). Currently there are few, if any, incentives for cattle holders to improve the temperament of their cattle solely to improve animal welfare. However animal welfare considerations are becoming increasingly important. One way to improve animal welfare on farm animals is by modifying management practices in order to reduce stress. Another option may be to improve the temperament of farm animals to reduce the quantity of stress experienced during routine handling procedures. This may be achieved genetically (e.g. through selection of breeding stock for good temperament) or non-genetically (e.g. by modification of animal behavior through training programmes) (Burrow, 1997).

The behavioral response of cattle to human handling can be chosen as an indicator for the temperament of an animal (Grandin, 1993; Burrow, 1997). It can vary from docility to aggression, with docility being preferred for farming conditions. Temperament can be quantified by scoring behavior in a standardized test situation (Tulloh, 1961; Burrow et al., 1988; Le Neindre et al., 1995; Hoppe et al., 2008). Morris et al. (1994) stated that selection pressure can be exerted in a breeding programme to alter or improve temperament. For breeding purposes the assessment of dairy cow's temperament in Germany, a trait subjectively evaluated by handlers using a five-point scale from 'very nervous' to 'very quiet' is used. This trait reflects the cow's behavior during milking. For knowledge on cattle behavior and human-animal-interactions to be used in

breeding, it is necessary to assess individual behavioral traits using objective measures (Adamczyk et al., 2013).

Behavior traits which capture aspects of the HAR for breeding purposes, have primarily been evaluated in beef cattle. Hoppe et al. (2010), suggested that the categorical assessment (on a five-point scale) of beef calves' behavior in the chute and flight speed tests after release from restraint are suitable for improving temperament traits. In dairy cows, the avoidance distance (**AD**) towards an unfamiliar person at the feeding place is a common measure for assessing the quality of the HAR. Thus, aspects of reliability and validity criterion in individual animals, as well as at the herd level have repeatedly been investigated (e.g., Rousing and Waiblinger, 2004; Windschnurer et al., 2008; Ebinghaus et al., 2016). The qualitative behavior assessment (**QBA**) as developed by Wemelsfelder et al. (2000, 2001) has been adopted in recent years to assess animals' behavioral reactions and body language in various handling situations (dairy cows: Ebinghaus et al., 2016; calves: Ellingsen et al., 2014; beef cattle: Stockman et al., 2011, 2012; Sant'Anna and da Costa, 2013). These measurements, however, have not yet been investigated from a quantitative genetic perspective.

In a preceding study, the following four behavior traits for assessing dairy cows' responsiveness towards humans were identified as repeatable in terms of inter- and intra-observer reliability (Ebinghaus et al., 2016). The AD, the tolerance to tactile interaction (**TTI**), the behavior during and after release from restraint (**RB**), and the QBA in a standardized human-animal interaction, simulating linear assessment. The TTI and RB tests were developed according to beef cattle temperament tests during and after confinement in the chute (e.g., Grandin, 1993; Hoppe et al., 2010) and adapted for routine on-farm application in dairy cows. The objectives of the present study were 1) to estimate heritabilities and breeding values (**EBV**) for the behavior traits AD, TTI, RB, and QBA 2) to calculate and compare EBV correlations among behavior traits, as well as between behavior and production traits from two different points in time (before and after the behavior measurement) and 3) to calculate correlations between behavior traits and important breeding indices included in the total merit index in Germany.

MATERIALS AND METHODS

Dataset for behavior traits. Data recording was performed by seven trained observers during the winter period 2014/2015 and 2015/2016 on 33 dairy farms (25 organic and eight conventional farms) with loose housing, located in central and northern Germany for the traits AD, TTI, RB, and QBA. Nine farms used automatic milking systems (AMS), while the remaining farms milked in herringbone or tandem milking parlors. Herd sizes ranged from 29 to 530 cows (mean = 102, sd \pm 105). Data was available from 1,761 Holstein cows for the TTI, 1,766 cows for the trait RB and 1,880 cows for trait AD. The QBA was conducted on 565 cows.

The assessments began after morning feedings when most cows were restrained in the feeding gates. For the TTI and RB tests, the cows remained restrained in the feeding gate. For the TTI, the observer viewed the cow from behind, as well as the left and right-hand sides for roughly 30 seconds before subsequently approaching the cow from one side and stroking it three times along the back and down the flank. The cows' behavioral reaction was rated on a 5-point scale (1 = cow stays calm; 2 = cow steps maximum twice; 3 = cow steps maximum five times; 4 = cow steps more than five times or kicks at least once; 5 = cow reacts violently). The RB was assessed directly afterwards, during and after the feeding gate was opened by the observer; this was also rated on a 5-point scale (1 = cow stays calm; 2 = cow leaves the feeding place walking at an intermediate speed; 3 = cow leaves the feeding place walking fast; 4 = cow leaves the feeding place running or jumping; 5 = cow reacts violently, is panicking) (Table 1). The QBA was conducted in connection with the human-animal interaction of the TTI and RB tests. The observer watched the cows' body language during the entire test situation and subsequently applied the QBA. Further analysis utilized the cows' individual scores from the first principal component (PC1) which explained nearly 70 % of the variation (**QBA_PC1**). This trait was characterized by descriptors relating to relaxation/attraction/trust on the negative and descriptors relating to fear/distress/aversiveness on the positive end (Ebinghaus et al., 2017).

Pedigree, Test-day traits and important breeding indices. The pedigree file included 36,948 animals from the last 5 generations. Official test-day records of the assessed cows from two different dates (the closest test-days before and after the recording date for behavior traits) were used in the analysis. Test-day traits include milk yield (**MY**), fat percentage (**FP**), protein percentage (**PP**), fat to protein ratio (**FPR**) and somatic cell score (**SCS**). Relative breeding values from important breeding indices (included in the total merit index (**RZG**) in Germany), from the most influential sires (sires with at least 5 daughters), were used for the calculations. The breeding indices include index milk production (protein kg, fat kg, protein percent) (**RZM**), index udder health (somatic cell count) (**RZS**), index conformation (feet and legs, udder (linear assessment, classification) (**RZE**), functional herd life (**RZN**) and index female fertility (conception traits, calving to first insemination) (**RZR**).

Statistical analysis

Estimation of genetic parameters. Variance components and heritabilities for behavior, production and health traits were estimated from univariate animal models using the AI-REML procedure, as implemented in the DMU software package (Madsen and Jensen, 2000).

For the behavior traits, the following linear animal model was applied:

$$y_{ijkl} = FG_i + LN_j + YS_k + Huthcl_l + e_{ijkl}, \quad [I]$$

where

y_{ijkl} = AD, TTI, RB, QBA_PC1

FG_i = farm effect combined with performance class in the herd (lactating, high-lactating and low-lactating cows)

LN_j = lactation number at the time of the behavior observations (1 - 3 and >4)

YS_k = year (2014, 2015, 2016) and season at the time of the behavior observation (1: January - March, 2: April - June, 3: July - September, 4: October - December)

$Huthcl_l$ = days in milk in classes according to Huth (1995) (< 14 days = 1, 14 - 77 days = 2, 78- 140 days = 3, 141 - 231 days = 4, > 231 days = 5)

e_{ijkl} = random residual effect

For official test-day traits before the behavior measurement: milk yield (**MY_b**), protein percentage (**PP_b**), fat percentage (**FP_b**), fat-to-protein ratio (**FPR_b**) and somatic cell score (**SCS_b**), and for test-day traits after the behavior measurement: milk yield (**MY_a**), protein percentage (**PP_a**), fat percentage (**FP_a**), fat-to-protein ratio (**FPR_a**) and somatic cell score (**SCS_a**), the following linear animal model was applied:

$$y_{ijkl} = FG_i + LN_j + YS_k + Huthcl_l + e_{ijkl}, \quad [II]$$

where

y_{ijkl} = MY_b, PP_b, FP_b, FPR_b, SCS_b, MY_a, PP_a, FP_a, FPR_a, SCS_a

FG_i = farm effect combined with performance class in the herd (lactating, high-lactating and low-lactating cows)

LN_j = lactation number at the time of the test-day (1 - 3 and > 4)

YS_k = year and season of the test-day (1: January - March, 2: April - June, 3: July - September, 4: October - December)

$Huthcl_l$ = days in milk in classes according to Huth (1995) (< 14 days = 1, 14 - 77 days = 2, 78 - 140 days = 3, 141 - 231 days = 4, > 231 days = 5)

e_{ijkl} = random residual effect

All original breeding values (**obv**) (results from univariate models), were standardized with a mean of 100 and a genetic standard deviation of 12 points to obtain the relative breeding values (**rbv**). For traits where a low obv is preferable (avoidance distance, tolerance to tactile interaction, release from restraint and qualitative behavior assessment), the following formula was applied:

$$rbv_trait = 100 - ((obv_trait - mean_trait)/st_trait) \times 12$$

After applying this formula for all traits, bulls with the higher rbv are those which are better for selection. This formula is applied e.g., for the test-day trait SCS.

EBV correlations (r_{EBV}) (1) among estimated breeding values for behavior traits, (2) between estimated breeding values for official test-day records and behavior traits, and (3) between estimated breeding values for behavior traits and important breeding indices were calculated based on estimates from univariate runs. For this purpose, the EBVs from the most influential sires, those with at least 5 daughters, were considered: In total, 88 sires were observed for the trait AD, 82 sires for the trait TTI and 82 sires for the trait RB. For the behavior trait QBA_PC1 (260 sires), no daughter restriction was used due to the limited sample size (available records).

RESULTS

Descriptive statistics. Descriptive statistics for the measured behavior traits are shown in Table 1 and 2. Descriptive statistics for test-day traits are depicted in Table 3 and those for relative breeding values of behavior traits and important breeding indices are shown in Table 4.

The observed avoidance distance of cows was on average 0.36 m. Nearly 70 % of the evaluated cows received scores of 1 or 2 for the traits TTI and RB, indicating that the majority of cows were more or less calm, stepped maximum twice and left the feeding place walking at an intermediate speed. However, the remaining 30 % of the cows were nervous and/or agitated. The QBA_PC1 values ranged from -1.8 to 2.39; negative values describe fear/distress/aversiveness and positive values describe relaxation/attraction/trust.

Table 1. Description and frequency of cows for the traits tolerance to tactile interaction (TTI) (N = 1,766) and release from restraint (RB) (N = 1,761) modified according to Ebinghaus et al. (2016)

Trait	Score	Description	Frequency (%)
TTI	1	cow stays calm, no stepping* or kicking**	37
	2	cow lowers the hindquarters at least at the first and second stroke or steps maximum twice, no kicking	31
	3	cow steps max. five times, no kicking	20
	4	cow steps more than five times or kicks at least once	9
	5	cow is reacting violently, is panicking	3
RB	1	cow stays calm, leaves the feeding place slowly or only after some time	29
	2	cow leaves the feeding place walking in intermediate speed	42
	3	cow leaves the feeding place walking fast	23
	4	cow leaves the feeding place running or jumping	4
	5	cow reacts violently, is panicking	1

*lifting the foot maximum 15 cm, **lifting the foot more than 15 cm

Table 2. Descriptive statistics for behavior traits

Trait	N	Mean	SD	Min.	Max.	Median
AD (m)	1,880	0.36	0.43	0	2	0.20
QBA_PC1	565	-0.01	1.01	-1.80	2.39	-0.02

¹AD: avoidance distance

QBA_PC1: qualitative behavior assessment, principal component 1

Table 3. Descriptive statistics for test day records (MY = milk yield, PP = protein percentage, FP = fat percentage, FPR = fat-to-protein ratio and SCS = somatic cell score) before (_b) and after (_a) the date of measurement of the behavior traits

Trait	N	Mean	SD	Min.	Max.
MY_b	1824	25.45	9.17	6.00	53.60
MY_a	1779	25.26	9.23	6.00	55.90
PP_b	1821	3.39	0.37	2.50	4.79
PP_a	1780	3.37	0.37	2.40	4.75
FP_b	1807	4.31	0.67	2.73	6.87
FP_a	1768	4.22	0.63	2.81	6.28
FPR_b	1807	1.27	0.18	0.80	2.28
FPR_a	1768	1.25	0.16	0.80	2.20
SCS_b	1790	3.07	1.70	0.00	8.40
SCS_a	1718	3.13	1.60	0.26	8.15

Table 4. Descriptive statistics for relative breeding values (rbv) for the behavior traits and for important breeding indices for sires with at least 5 daughters (N) (for QBA_PC1 no restriction was made)

Trait ¹	N	Mean	SD	Min.	Max.
rbv_AD	88	100.00	12.00	72	134
rbv_TTI	82	100.66	13.02	71	135
rbv_RB	82	99.54	12.16	76	130
rbv_QBA_PC1	260	99.46	12.70	52	159
RZM	298	103.34	11.58	66	133
RZS	298	102.94	10.53	68	135
RZE	290	103.22	12.54	65	140
RZN	295	106.40	10.03	69	136
RZR	295	102.79	11.07	69	134

¹rbv_AD: relative breeding value for the avoidance distance

rbv_TTI: relative breeding value for tolerance to tactile interaction

rbv_RB: relative breeding value for release from restraint

rbv_QBA_PC1: relative breeding value for the principal component 1 for qualitative behavior assessment

RZM: index milk production (protein kg, fat kg, protein percent)

RZS: index udder health (somatic cell count)

RZE: index conformation (feet and legs, udder (linear assessment, classification))

RZN: functional herd life

RZR: index female fertility (conception traits, calving to first insemination)

Genetic parameters

Variance components and heritabilities for behavior traits. Table 5 shows the variance components and heritabilities for the four behavior traits that reflect the human-animal relationship. The highest heritability was estimated for AD, with a moderate range and a low standard deviation (0.27 ± 0.06). Heritabilities for RB and QBA_PC1 were lower than the heritability estimated for the AD (0.11 ± 0.06 and 0.13 ± 0.17 , respectively). The lowest heritability was estimated for the behavior trait TTI (0.04 ± 0.05).

Table 5. Variance components and heritabilities for behavior traits

Traits ¹	σ_a^2	σ_p^2	$h^2 \pm SE$
AD	405.13	1104.94	0.27 ± 0.06
TTI	0.04	1.05	0.04 ± 0.05
RB	0.08	0.65	0.11 ± 0.06
QBA_PC1	0.11	0.73	0.13 ± 0.17

¹AD: avoidance distance

TTI: tolerance to tactile interaction

RB: release behavior from restraint

QBA_PC1: qualitative behavior assessment, principal component 1

Correlations among behavior traits. Phenotypic and EBV correlations from univariate runs among behavior traits are depicted in Table 6. Phenotypic and EBV correlations were generally in accordance with one another. The EBV correlations between the behavior traits were positive and in a moderate range, with the strongest EBV correlation being calculated between AD and RB ($r_{EBV} = 0.58$). The strongest phenotypic correlation was found between QBA_PC1 and RB ($r = 0.74$), as previously published by Ebinghaus et al. (2017).

Table 6. EBV correlations (r_{EBV}) among behavior traits above the diagonal with number of sires (sires with at least 5 daughters, except for QBA_PC1) and phenotypic correlations (r_s)¹ with number of daughter records (N) below the diagonal

Traits ²		AD	TTI	RB	QBA_PC1
AD	r_s^1/r_{EBV}		0.16*	0.58***	0.35**
	N	1880/88	81	81	67
TTI	r_s^1/r_{EBV}	0.29**		0.38***	0.44***
	N	1759	1766/82	82	65
RB	r_s^1/r_{EBV}	0.48**	0.51**		0.45***
	N	1754	1759	1761/82	65
QBA_PC1	r_s^1/r_{EBV}	0.55**	0.69**	0.75**	
	N	563	560	559	565/260

*P < 0.1; ** P < 0.01; *** P < 0.001

¹based on the study Ebinghaus et al., 2017

²see Table 4

Correlations between behavior traits and production and health traits from test-day records. Table 7 shows the EBV correlations between behavior and test-day traits close to the behavior measurement. Correlations between the behavior traits and test-day traits measured before the behavior measurement were close to zero and not significant. The AD and SCS_a were significantly positively correlated ($r_{EBV} = 0.31$). The EBV correlation between TTI and FPR_a was also significant positive ($r_{EBV} = 0.34$).

Table 7. EBV correlations (r_{EBV}) between behavior traits and test-day records from two different points in time (before and after the behavior trait measurement) from sires with at least 5 daughters (N)

Traits ¹		AD	TTI	RB	QBA_PC1
MY_b	r_{ebv}	0.03	-0.01	0.02	0.02
	N	78	74	74	62
MY_a	r_{ebv}	-0.07	-0.09	-0.04	0.04
	N	78	74	74	62
FPR_b	r_{ebv}	-0.03	0.10	0.02	-0.07
	N	78	74	74	62
FPR_a	r_{ebv}	-0.10	0.34**	-0.10	0.02
	N	78	74	74	62
FP_b	r_{ebv}	0.05	0.10	0.02	-0.01
	N	78	74	74	62
FP_a	r_{ebv}	0.03	0.21	0.05	0.03
	N	78	74	74	62
PP_b	r_{ebv}	0.10	0.003	-0.03	0.01
	N	77	73	73	61
PP_a	r_{ebv}	0.15	-0.01	0.17	0.06
	N	77	73	73	61
SCS_b	r_{ebv}	0.06	-0.09	-0.07	-0.11
	N	73	69	69	60
SCS_a	r_{ebv}	0.31**	-0.21	-0.004	-0.09
	N	73	69	69	60

*P < 0.05; ** P < 0.01; *** P < 0.001

¹AD: avoidance distance

TTI: tolerance to tactile interaction

RB: release behavior from restraint

QBA_PC1: qualitative behavior assessment, principal component 1

MY_: milk yield from official test day before (_b) and after (_a) the behavior trait measurement

FPR_: fat-to-protein ratio from official test day before (_b) and after (_a) the behavior trait measurement

FP_: fat percentage from official test day before (_b) and after (_a) the behavior trait measurement

PP_: protein percentage from official test day before (_b) and after (_a) the behavior trait measurement

SCS_: somatic cell score from official test day before (_b) and after (_a) the behavior trait measurement

Correlations between important breeding complexes and behavior traits. All of the correlations between important breeding indices and behavior traits that reflect the human-animal relationship were in a low to moderate range; these correlations are depicted in Table 8. The EBV correlation between RB and the breeding index functional herd life (RZN) was significant and negative ($r_{EBV} = -0.35$). The trait QBA_PC1 was significantly positive correlated ($r_{EBV} = 0.14$) with the breeding index conformation (RZE).

Table 8. EBV correlations (r_{EBV}) between important breeding indices and behavior traits that reflect the human animal relationship for sires (N) with at least 5 daughters; except for QBA_PC1 no daughter restriction.

Traits ¹		AD	TTI	RB	QBA_PC1
RZM	r_{ebv}	-0.09	-0.13	-0.05	-0.04
	N	59	55	55	211
RZS	r_{ebv}	-0.08	0.18	0.02	0.11
	N	59	55	55	211
RZE	r_{ebv}	0.21	0.21	0.17	0.14*
	N	57	53	53	204
RZN	r_{ebv}	-0.28*	-0.17	-0.35**	-0.05
	N	59	55	55	208
RZR	r_{ebv}	-0.07	0.05	-0.16	0.11
	N	59	55	55	208

*P < 0.05; ** P < 0.01; *** P < 0.001

¹See Table 2

DISCUSSION

Descriptive statistics. The traits TTI and RB were developed based on beef cattle temperament traits, e.g., chute score. Beef cattle that respond by struggling violently and trying to escape from confinement in a chute, weigh crush or handling race are at a higher risk of injury to itself, human handlers and other animals than an animal that responds calmly (Voisinet et al., 1997). Therefore, low values for TTI and RB are desirable. For dairy cattle, a calm response to handling during e.g., artificial insemination and veterinary treatment is important to avoid injuries to animals and humans. A high percentage of cows evaluated in this study received scores of 1 or 2 for TTI and RB. Thus, they were relatively calm, stepped maximum twice and left the feeding place walking at an intermediate speed. Variability is present, indicating that a percentage of the cows were fearful and agitated during handling.

The observed mean for the AD was 0.36 m, indicating that a high percentage of the cows maintained a distance and did not accept being touched by the unfamiliar experimenter. Waiblinger et al., 2003 found that 50 % of the evaluated farms had an avoidance distance per herd within a small range of 0.15 to 0.35 m, while Windschnurer et al. (2008) reported a mean AD of 0.24 m. Cattle husbandry involves close contact between handlers and their animals. Docility in dairy cattle during milking and handling is a trait that has been under selection for generations; thus, extreme responses are rare (Haskell, et al., 2014); however, variability in the avoidance distance was found.

The QBA_PC1 values ranged from -1.8 to 2.39. The number of cows evaluated via QBA was relatively low (N = 565) compared to the sample size for the other traits, due to the high level of concentration required from the observer for evaluation of this measurement.

Genetic parameters

Variance components and heritabilities for behavior traits. The heritability estimated for the AD was moderate, indicating the potential to improve animal behavior and the human-animal relationship simultaneously via breeding.

Heritabilities for RB and QBA_PC were low, but higher than for most functional traits. Heritability for RB ($h^2 = 0.11$) was lower than those estimated for flight speed scores in beef cattle. Hoppe et al. (2010) reported $h^2 = 0.11$ for Limousin cattle and $h^2 = 0.36$ for Hereford cattle, respectively. These differences could be due to breed-specific aspects or varying management factors. The TTI trait was adapted from the chute score trait measuring the behavioral reaction on a scale from 'quiet' to 'extremely excited', as applied to beef cattle while the animal is restrained in a head gate (Hoppe et al., 2010). The lowest heritability estimated in this study was for the TTI ($h^2 = 0.04$), which was distinctively lower than heritabilities estimated by Hoppe et al. (2010) for the chute score in Limousin ($h^2 = 0.11$) and Hereford (0.33) cattle. Here, environmental factors including handlers' behavior and attitude, as well as handling and management practises may have played a much bigger role than genetics.

Correlations among behavior traits. Phenotypic correlations are discussed in detail by Ebinghaus et al. (2017). Estimated breeding value (EBV) correlations (r_{EBV}) among the behavior traits AD, TTI, RB and QBA_PC1 were moderately positive, indicating that while the various methodologies for behavior assessment may measure slightly different aspects of animal behavior, they are related and are partly influenced by a similar genetic component. All of these measures registered the responses of restrained cows at the feeding place towards an approaching human. There are, however, some differences between the measures and what they represent. AD focuses on approach, whereas the other measures are centred on forced handling; for TTI, this occurs through physical contact (Ebinghaus et al., 2017). During the TTI test, the cow was restrained, while for RB, the cow was released and could control the situation, i.e., decide to stay or to move away. Differences between TTI, RB and QBA_PC1 may result from methodology differences. While TTI and RB used observations of defined behaviors, QBA_PC1 used observations from body language in general (Ebinghaus et al., 2017).

The highest EBV correlation was between AD and RB ($r_{EBV} = 0.58$); for both evaluations, the animal had the choice to avoid close contact with the human. Although RB involved force handling, a common genetic component is likely accountable for the

behavior response. A moderate correlation between RB and TTI ($r_{EBV} = 0.38$) was found despite both traits being measured at the same location and involving forced human-animal interaction; this correlation was probably due to the animal's level of control in the situation, an aspect that is likely important for the animal (Boivin et al., 2003; Waiblinger et al., 2004). Although the cow was restrained during the TTI recording, it was released and allowed the freedom to decide its distance from the human during the RB recording. QBA was moderately correlated with RB ($r_{EBV} = 0.45$), with AD ($r_{EBV} = 0.35$) and with TTI ($r_{EBV} = 0.44$), suggesting that QBA is a good measure for describing behavior.

Correlations between behavior traits and production and health traits from test-day records. How animals perceive the presence of humans is especially important for dairy cattle because of the regular interaction with humans. Sutherland and Dowling (2014) found, for example, a positive (phenotypic) correlation between AD and milk yield. In this study, EBV correlations between the behavior traits and test-day trait measures prior to the behavior measurement were close to zero and not significant. Purcell et al. (1988) and Uetake et al. (2002) also reported low phenotypic correlations between milk production and various behaviors towards handlers (e.g., approach distance and flight distance). Significant correlations were found between behavior traits and test-day trait measures after the measurement of the behavior. AD and SCS_a were significantly positively correlated ($r_{EBV} = 0.31$). Cows with a higher AD, e.g., cows fearful of humans, can experience physiological stress reactions (Breuer et al., 2003); stress can lead to a higher SCS. Ivemeyer et al. (2011) also stated that the human-animal relationship on the farm is associated with udder health in dairy cows. Gibbons et al. (2011) indicated that animals that are highly responsive or fearful during routine management may experience increased stress as a result of their inability to cope, which has been shown to negatively affect health (Fell et al., 1999). The EBV correlation between TTI and FPR_a was also significantly positive ($r_{EBV} = 0.34$), indicating that more fearful, nervous and aggressive cows are at greater risk of potentially having sub-clinical ketosis, i.e., metabolic stress; ketosis is a trait related to energy deficiency (Rehbein et al., 2013). In this analysis, FPR was used to depict the cow's energy balance. Curley et al. (2007) stated that higher basal serum cortisol

concentrations may suggest that easily excitable cattle are chronically stressed, possibly resulting in a compromised immune system, illness, and decreased fat and protein deposition (Bates et al., 2014).

Correlations between important breeding complexes and behavior traits. Correlations between important breeding indices and behavior traits were generally insignificant. The breeding index RZE and the behavior trait QBA_PC1 were significantly positively correlated ($r_{EBV} = 0.14$). Sires with a high EBV for conformation traits tend to have daughters with a positive QBA_PC1, meaning that daughters were characterized with descriptors relating to fear/distress/aversiveness. However, this correlation is moderate to low, implying that a set assumption cannot be made and the trait should be further investigated. Significant correlations were also calculated between RZN and AD ($r_{EBV} = -0.28$), and between RZN and RB ($r_{EBV} = -0.35$), indicating that sires with a high EBV for longevity traits tend to have daughters with good behavior, e.g., low ADs and calm demeanors. Haile-Mariam et al. (2004) and Sewalem et al. (2010) also reported positive phenotypic and genetic relationships between temperament and herd survival, in that calmer cows are less likely to be culled.

CONCLUSIONS

A moderate heritability was estimated for the AD ($h^2 = 0.27$) making this trait suitable for selection. RB and QBA_PC1 had low heritabilities, yet they are higher than for some functional traits. Estimated breeding value (EBV) correlations (r_{EBV}) among the behavioral measures AD, TTI, RB and QBA_PC1 were moderately positive, indicating that while these behavior traits tend to measure different aspects of behavior, they are linked by a similar genetic component. The AD was significantly correlated with SCS_a ($r_{EBV} = 0.31$). Cows with fear of humans are likely to experience stress reactions, and stress can lead to a higher SCS. This supports the concept of including behavior traits that reflect the human-animal relationship into breeding strategies. With respect to estimated (co)variance components from all four behavior measurements, the AD would be the most suitable for inclusion in breeding programs.

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CHAPTER 6

General discussion

Introduction

Animal welfare and sustainable breeding depend to a large extent on behavior. Behavior is an important trait-complex that has thus far received little attention in breeding programs. Moreover, behavior is the primary means of an animal's adaptation to a specific environment. If selection affects the behavioral repertoire available for such adaptation, this may have profound importance for the welfare of the animal and for its ability to cope with different environmental challenges (Jensen and Andersson, 2005). Interdisciplinary work between animal breeding and ethology may offer a means of understanding the relation between behavior, animal-technic and human-animal interactions, along with other important aspects such as production, health and fertility. Additionally, it may assist to predict possible side effects of breeding programs, which in turn may help in designing more sustainable breeding in the future.

In the previous chapters (3, 4 and 5) the obtained results were analyzed and discussed in relation to results published in other studies. This chapter aims to discuss certain behavior trait definitions and some of the used statistical models more critically. Furthermore, new calculations will also be discussed not only as individual findings, but also with regard to the identification of the genetic background for behavior traits and traits reflecting the human-animal relationship. Finally, recommendations related to a possible behavior selection index will be provided and future breeding techniques will be discussed.

Behavior indicator traits

Due to the different definitions of behavior (Mathiak 2002, Urban 2007) and the fact that the assessment of animal behavior is often characterized by subjective impressions, it is difficult to make an objective assessment of this complex and multifactorial trait (Andersson and Georges 2004). In order to take behavior traits into account in breeding programs, behavior tests, or behavior indicator traits, which are suitable for farm-based standardized recording, have to be identified.

Average milk flow

In this respect, the concept of using the trait average milk flow (AMF), an objectively recorded trait, as a behavioral indicator trait has emerged. The milk ejection reflex, essential for maximum milk collection, is inhibited by stress via the release of catecholamines such as adrenaline and noradrenaline (Silankove et al., 2000; Barowicz, 1979). These hormones prevent the milk ejection reflex through either a central inhibition of oxytocin release from the pituitary gland (Sibaja and Schmidt, 1975), and a drastic reduction in mammary blood flow (Gorewit and Aromando, 1985), or by blocking the effect of oxytocin on the myoepithelial cells of the mammary gland by binding to common receptor sites (Akers and Lefcourt, 1984). Previous studies have identified significant relationships between temperament during milking and milk ejection (Dimitrov-Ivanov and Djorbineva, 2002), yet it is not known whether temperament assessed before milking can relate with milk production and the behavior of the cow once introduced to the milking parlor.

Breeding associations routinely record the milking temperament (**MT**), which is related to the cows' behavior during milking routine, to genetically improve dairy cows' manageability. The MT is subjectively assessed by the animal owner using a multi-stage scale from 1: very nervous to 5: very quiet (Adamczyk et al., 2013). Along with MT, breeding associations in Germany and other countries also record the cows' milkability (**MA**) by means of subjective classification of milking speed (**MS**), 1: very slow to 5: very fast. Alternatively, milking speed is measured as average milk flow per minute (**AMF**) (e.g. Interbull, 2009; VIT, 2016), showing a moderate genetic background (Santos et al., 2015). In Germany, there are regional differences regarding which traits are commonly recorded. If recorded in combination, AMF and MS are summarized with a weighting of 50 : 50 (VIT, 2016).

On a phenotypic level, AMF is influenced by anatomical and physiological factors, as well as by aspects of milking management, such as pre-milking operations (e.g. Bruckmaier and Blum, 1996; Sandrucci et al., 2007). It can further be argued that AMS also has a behavioral component. In order to include this trait as behavior indicator trait in breeding programs, however, further research has to be done in combination with valid, reliable measurements such as avoidance distance (**AD**), tolerance to tactile

interaction (**TTI**), release from restraint (**RB**) and qualitative behavior assessment from the first principal component analysis (**QBA_PC1**). Furthermore, correlations with cortisol values, hormone levels and heart rate measurements will bring additional benefits.

Genetic correlations among traits

In Chapter 4 heritabilities and estimated breeding values (**EBV**) for the behavior measures AD, TTI, RB, and QBA were estimated. EBV from the most influential sires, those with at least 5 daughters, were considered for the analysis: In total, 88 animals for the trait AD, 82 animals for the trait TTI, and 82 animals for the trait RB. For the behavior trait QBA_PC1 (260 animals), no daughter restriction was used due to the limited amount of records available. Data for milking temperament and average milk flow was also available, but also for a relatively few number cows. For MT, 152 cows were available, while there were only 52 for AMF.

EBV correlations between traits routinely recorded by breeding associations (MT and AMF), along with behavior measures reflecting dairy cows' responsiveness towards humans are depicted in Table 1.

Table 1. EBV correlations (r_{ebv}) between behavior traits for the number of bulls (N) with a restriction of at least five daughters for the traits AD, TTI and RB

Traits ¹		AD	TTI	RB	QBA_PC1
MT	r_{ebv}	-0.07	0.16	-0.05	-0.08
	N	33	31	31	84
AMF	r_{ebv}	-0.75*	-0.39	-0.33	-0.37
	N	10	9	9	24

¹AD: avoidance distance

TTI: tolerance to tactile interaction

RB: behavior during release from restrain

QBA_PC1: cows individual scores from the 1st principal component analysis

MT: milking temperament

AMF: average milk flow

All correlations except the correlation between MT and TTI ($r_{EBV} = 0.16$) are negative in sign, indicating a positive breeding response between the existing behavior traits recorded by breeding associations and behavior measures that reflect dairy cows' responsiveness towards humans. The strongest negative correlation was calculated between AMF and AD ($r_{EBV} = -0.75$), reinforcing the hypothesis of using the trait AMF as a behavior indicator trait. Normally, EBV correlations are an underestimation of genetic correlations (Calo et al., 1973), implying that the genetic correlation between these two traits could be even higher. Hence, given the few number of records used for the evaluation and due to the likelihood of the low accuracy of the EBV for the traits, further research in this regard is necessary.

Traits from Automatic Milking Systems (AMS)

Utilization of traits directly generated from the AMS allows for improvements to be made to the German Holstein AMS breeding index "RZRobot". Thus far, the RZRobot is based only on the following indicator traits from official recording schemes: milking speed, somatic cell count, feet and legs, udder component score, rear teat placement and teat length (DHV, 2014). The current breeding index strongly emphasizes udder-related traits. The udder of the cow and animal's health, is indeed a strong factor influencing the use of the AMS. Hence, behavior-related traits also likely play an important role here. Through the utilization of certain behavior indicator traits generated in such systems, the RZRobot could be extended. The trait knock off of the milking device (KO), for example, could be used as an indicator not only for udder morphology, but also for temperament. Fewer knock offs could indicate good udder morphology and a good animal behavior (Chapter 4). Unfortunately, the heritability for this trait is very low, making a selection for this trait more difficult. Furthermore, the trait interval between two consecutive milking (INT), which is also related to temperament, could play a role in the analysis of new traits for the index. A short INT implies more visits to the AMS, indicating that cows can exercise freewill when deciding when to access the Robot. The additional benefit of using the trait visits (VIS) was already discussed by König, et al., 2008. Furthermore, it may be possible to improve the preexisting RZRobot index by traits actually recorded by the AMS, as well

as by including traits that have some capacity to reflect the temperament of the cows in the AMS. The heritability estimates for some behavior indicator traits are sufficiently high to allow further selection on these traits. However, before behavior indicator traits can be used as a potential parameter in the genetic selection, a better knowledge of the relationship between behavior traits and for example fertility is required for effectively designing new breeding indices.

Correlation between behavior indicator traits from AMS and fertility traits

Behavior may also play a critical role in the declining reproductive performance of genetically high-producing cows. In a study of 17 commercial herds that used an electronic oestrus-monitoring system, Dransfield et al (1998) determined that a higher proportion of cows with production above herd average exhibited only low intensity and short duration oestrus when compared to lower-producing cows (24 vs. 16 %). Lopez et al (2004) also reported an unfavorable association between milk production and oestrus behavior, with shorter oestrus periods (5.5 vs. 11.1 h) in high (> 40 kg per day) relative to low (< 30 kg per day) producing cows. Emanuelson and Oltenacu (1998) found an extended interval to first breeding and to conception in herds with poorer oestrus detection. The decline in fertility also has economic consequences and several studies reported increasing reproduction costs for dairy cattle (Lindhe & Philipsson 1999; Royal et al 2000; Lucy 2001).

With data from automatic milking systems (Chapter 4), it was possible to determine genetic and EBV correlations between the fertility trait non-return rate after 90 days (NR90) and behavior indicator traits; the results of this analysis are shown in Table 2. The trait NR90 was defined as a binary trait with cows that did not have to be inseminated again after 90 days, receiving the score “1” (pregnant), or “0” (not pregnant).

NR90 was slightly negatively correlated with AMF ($r_g = -0.13$) and DUR ($r_g = -0.16$), indicating that fast milking cows and cows that spend more time in the milking box (according to our definition, cows with a good temperament), would likely have fertility problems. Positive correlations between INT and NR90 ($r_g = 0.25$) demonstrate that longer intervals between milking were associated with good female fertility. According to Cooke et al. (2012), cattle with excitable temperaments showed an

increase in the synthesis and circulating concentrations of cortisol as a stress response. Moreover, they determined that cortisol directly impaired physiological mechanisms required for fertility in beef cows. Due to variations in sign of the genetic correlations and the EBV correlations, as well as the large standard errors of the genetic correlations, it is difficult to make an appropriate conclusion. Still, it is important to look at the genetic correlations between behavior indicator traits and fertility traits; it is therefore crucial to conduct more research on this subject.

Table 2. Genetic correlations (r_g) and breeding value correlations (r_{EBV}) between behavior indicator traits and the fertility trait non-return rate after 90 days (NR90) for bulls with at least 5 daughters

Trait ¹	NR90	
	r_g	r_{EBV}
AMF (kg/min)	-0.13 (0.17)	-0.09
DUR (min)	-0.16 (0.27)	0.16
INT (hour)	0.25 (0.24)	0.01
KO	0.10 (0.32)	-0.31
VIS3	0.12 (0.29)	-0.02
VIS4	0.20 (0.35)	-0.10

¹AMF: average milk flow

DUR: milking time during a visit in the milking robot

INT: interval between two consecutive milkings

KO: knock off of the milking device from at least one udder quarter

VIS3: at least three visits to the milking robot per day

VIS4: at least four visits to the milking robot per day

In Chapter 3, a similar antagonistic genetic relationship between AMF and udder health was discussed. In that instance, it was proposed that the trait AMF could be considered as a trait with an intermediate optimum; a similar approach could be applied for the trait INT.

Statistical Models

Recursive models

The application of recursive models in the field of dairy cattle breeding is relatively new; for the current investigation, recursive models were applied in the analysis introduced in Chapter 3. Due to the ever increasing importance of functional traits in dairy cattle programs, the implementation of recursive or simultaneous models as theoretically described by Gianola and Sorensen (2004) will be continue to be applied in the future. In the past, mixed models have been used to infer genetic and environmental correlations between production (mainly milk yield) and functional traits (fertility or somatic cell score). These models, however, ignore the existence of direct relationships between two phenotypes. High milk yield may increase liability to any specific disease and, in turn, the disease may affect milk yield adversely. The possible complexity of such structural equation models is clearly described by de los Campos et al. (2006). In the case of average milk flow and e.g. milk production (MY) in dairy cows (Chapter 3) a two-way biological causal path was postulated (Figure 1).

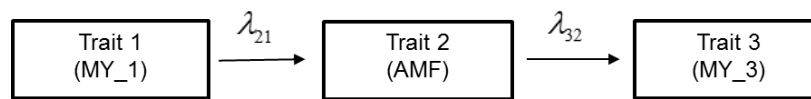


Figure 1. Biological system between AMF and two test day traits (here MY = milk production)

Where Trait 1 (e.g., MY_1) referred to the test-day record before the measurement date for AMF, AMF was Trait 2 in the biological system and Trait 3 (e.g., MY_3) was the official test-day record of the respective test-day trait after AMF recording. Here, λ_{21} is the alteration in AMF with respect to test-day trait 1. The rate of change in test-day trait 3 with respect to the change of 1 kg/min in AMF is depicted by λ_{32} .

Based on the results provided in Chapter 3, along with those from other studies (e.g., Lopez de Maturana et al., 2007; Wu et al., 2007), the use of recursive models can allow for a much more accurate representation of the real complexion among phenotypes in dairy cattle breeding. Moreover, König (2007) further supported this conclusion about recursive models.

Use of covariates

There are two reasons for choosing to include a covariate in an analytic model: Power and adjustment. Power is supplied when the covariate is highly related to the dependent variable, but unrelated to the independent variable of interest. The inclusion of the covariate in the analytic model ultimately leads to a reduction in the variance of the residuals, increasing the power of the test of the independent variables. The adjustment function emerges when the covariate is related to the independent variables. The inclusion of the covariate in the analytic model accordingly results in “adjustments” in the effect estimates associated with the independent variables (Yzerbyt et al., 2004).

A covariate and an independent variable may be correlated as a result of the three possible causal processes: The independent variable causes the covariate, the covariate causes the independent variable, and they are spuriously related because some third variable or variables causes both of them (Yzerbyt et al., 2004). A covariate is included in the model in order to examine whether the adjusted effect of the independent variable is reduced once the mediator is controlled (Judd and Kenny, 1981; Baron and Kenny, 1986).

In Chapter 4, auxiliary traits that reflect animal behavior in the milking system were defined, with the use of objectively recorded longitudinal data from automatic milking systems (AMS). The AMS traits used as behavior indicators were: AMS visits per cow and day as a binary trait, with a threshold for greater or equal to three visits (VIS3) and greater or equal to four visits (VIS4), also as a binary trait was knocking off the milking device with a threshold of at least one udder quarter (KO), milking duration of each AMS visit in min (DUR), average milk flow in kg/min (AMF) and the interval between two consecutive milkings (INT). The main question raised here is related to the role of milk yield when analyzing these traits. Could milk yield be the main driving force behind AMS visits? Does a high milk yielding cow have a longer duration in the milking box? In consideration of these questions, milk yield was included as a covariate in the statistical model. Heritabilities for behavior indicator traits resulting from models with and without milk yield as a covariate are depicted in Table 3.

Models with and without milk yield as a covariate were comparable, with slightly higher heritabilities being found for some traits in models with milk yield as a covariate (0.17 for VIS3, 0.08 for VIS4 and 0.18 for INT). These results support the conclusion that milk yield is not the only driving force behind these traits.

Selection of the most appropriate model (with or without a covariate) was based on Akaike's information criterion (Akaike, 1973). Akaike (1973) proposed a simple and useful criterion (**AIC**) for selecting the best-fit model among alternative models:

$$AIC = -2(\log \text{likelihood}) + 2(\text{number of variance parameters})$$

Here, the model with the lowest value for AIC is preferable and ideal for application. For all traits, AIC values were lower for the models with milk yield as a covariate, implying that these models are more suitable.

Table 3. Heritabilities (h^2) for behavior traits resulting from models with and without milk yield as a covariate

Traits ¹	h^2	
	with MY as covariate	without MY as covariate
AMF	0.22	0.25
INT	0.18	0.07
DUR	0.19	0.19
KO	0.03	0.03
VIS3	0.17	0.08
VIS4	0.08	0.05

¹AMF: average milk flow

INT: interval between two consecutive milkings

DUR: milking time during a visit in the milking robot

KO: knock off of the milking device from at least one udder quarter

VIS3: at least three visits to the milking robot per day

VIS4: at least four visits to the milking robot per day

Breeding program

Selection Index for behavior

Selection index theory (Hazel, 1943) provides the framework for a concrete definition of the breeding goal in terms of an aggregate genotype selected for through a correlated information index. The aggregate genotype is used to represent the genetic merit of an animal, i.e., the weighted sum of its genotypic values for several traits. To optimize relative improvement levels of aggregated genotype traits, traits are weighted by their predicted contribution to the improvement of the breeding goal.

In dairy cattle, traits influencing production efficiency are roughly characterized as production traits (milk) and functional traits. The term functional traits is used to summarize those characteristics of an animal which increase efficiency not through higher output of products, but through reduced costs of input. Major groups of breeding goal traits belonging to this category are related to health, fertility and milkability and could be complemented by behavior traits. Many authors proposed using traits related to fitness and suggested, for example, a selection index in dairy cattle based on traits such as lameness, mastitis, calving interval and lifespan as measures of health and fertility (see Lawrence et al., 2004; Oltenacu and Algers, 2005). Traits of this kind have already been recorded in practice and quantitative evaluations using real data show that, due to antagonistic relationships between production and fitness traits, a trade-off may exist between the costs of lower milk yield and the benefits of a higher health status of cows (Lawrence et al., 2004). Therefore, depending on the rate of genetic change and the weights applied to each trait, breeding for improved welfare of dairy cows may be generally profitable (Lawrence et al., 2004). Other approaches have also suggested the use of behavioral or temperament traits for breeding purposes, including, for example, fearfulness, sociality or aggression (Faure and Mills, 1998; Jones and Hocking, 1999; Kanis et al., 2004; Boissy et al., 2005; Star et al., 2008). Using temperament traits in dairy cattle breeding may be a potentially important way of implementing the results of the present thesis in practice. In contrast to more “classical” fitness traits, however, the application of temperament traits in farm animal breeding is still largely a matter of theory, which is open for discussion.

One valid reason to include underlying temperament traits in a breeding program, however, would be that these traits are capable of influencing a wide range of biological responses to a broad assortment of challenges. Conventional German dairy cattle breeding programs do not include selection for improved behavior. As shown in Chapters 3, 4 and 5, the genetic parameters of various behavior indicator traits enable the possibility for selection strategies on these traits.

The aim of this section is to compare the selection response of direct selection strategies on behavior traits (e.g., AD) with indirect approaches via indicator traits (e.g. AMF, INT) and the accuracy of the index. The first step in developing a selection index is to clearly define the goal of the genetic improvement. The general breeding goal is to improve the cow's temperament. Hence, applying selection index theory the trait in the breeding goal was AD and according to the different scenarios, the traits in the index, as well as the number of daughters used for each trait in the index, varied (Table 4).

Table 4. Investigated Scenarios

Scenario	Index-traits ¹	No. of daughters	Breeding Goal-traits ¹	r_g^2
A1	AD	5	AD	
A2	AD	50	AD	
B1	AMF	50	AD	-0.30
B2	AMF	50	AD	-0.50
B3	AMF	50	AD	-0.75
C1	AD	5	AD	-0.75
	AMF	50	AMF	
D1	AMF	50	AD	-0.75
	INT	50		0.15

¹AD: avoidance distance

AMF: average milk flow

INT: interval between two consecutive visits

² r_g : genetic correlation between index traits and traits in the breeding goal

Results from the investigated scenarios were used to recommend a suitable breeding strategy for behavior traits for cows milked in herringbone or tandem milking parlours, as well as for cows milked in AMS. The relative economic weight for the trait in the breeding

goal (AD) was always -1, to ensure that cow's with a lower avoidance distance would be selected. Scenarios A1 and A2 differed in the number of daughters, while Scenarios B1, B2 and B3 differed in the genetic correlation between AD and AMF. The problem with Scenario D1 was that the phenotypic and genetic correlation between INT and AD are nonexistent; therefore, these figures are purely speculative.

The genetic and phenotypic parameters used in this section are shown in Table 5. Most of the parameters were estimated in previous chapters (Chapters 3, 4 and 5).

Table 5. Heritabilities (diagonal), EBV correlations (above diagonal) and phenotypic correlations (below diagonal) for index traits.

Traits ²	AD	AMF	INT
AD	0.27	-0.75	0.15 ¹
AMF	0.10	0.30	0.14
INT	0.45 ¹	0.20	0.07

¹assumed correlations

²see Table 4

The negative correlation (in sign) but positive with respect to the breeding progress between AD and AMF was discussed in the previous section: behavior indicator traits. Normally, EBV correlations are an underestimation of genetic correlations (Calo et al., 1973), meaning that the genetic correlation between these two traits could be even higher. Hence, given the limited number of records used for the evaluation, and due to the likelihood of a low degree of accuracy of the EBV for these traits, especially for the trait AD, further research in this regard should be conducted. Taking this into account, three scenarios were developed with different correlations between AD and AMF.

Utilizing the parameters from the previous table, the selection response per generation (ΔG), the index weight (b) and the accuracy of the index (r_{Ti}) were calculated for different scenarios using the R Program -developed by Pimentel and König (2012); these parameters are summarized in Table 6. The selection response per generation (ΔG) is given in the traits specific unit.

Table 6. Selection response per generation (ΔG), index weights (b) and the accuracy of the index (r_{Ti}) for different Scenarios

Scenarios/ Traits ¹	r_{Ti}	ΔG		b		
		AMF (kg/min)	AD (cm)	AMF	AD	INT
Scenario A1	0.52		-8.90		-0.03	
Scenario A2	0.88		-15.29		-0.09	
Scenario B1	0.27		-4.64		2.44	
Scenario B2	0.45		-7.73		4.06	
Scenario B3	0.67		-11.60		6.09	
Scenario C1	0.91	0.16	-15.85	3.92	-0.15	
Scenario D1	0.70		-12.80	6.38		-0.90

¹see Table 4

When comparing these scenarios, Scenario C1 has the highest accuracy, even higher than scenario A2. The best breeding strategy, in order to select cows with lower avoidance distances, is to combine the traits AD and AMF in the breeding goal. For Scenarios B1, B2 and B3, where AMF was the only trait in the index, breeding success is achieved for the avoidance distance, meaning that a selection towards a faster milking speed will be accompanied with a selection response towards a shorter AD.

Scenario D1 could be seen as an example for a selection index working towards better behavior for cows in AMS. Although, due to the assumed correlations between INT and AD, intensive research is necessary to confirm this.

Interestingly, when comparing Scenario A2 and Scenario B3, the selection response in the AD per generation is similar. If the genetic correlation calculated between these two traits is accurate, it would reinforce the hypothesis that supports the use of the trait average milk flow as a behavior trait indicator.

For the B Scenarios, the accuracy of the index with 5, 50 and 100 daughters was also calculated; these results are presented in Figure 2.

Assuming a lower genetic correlation between AD and AMF, e.g., Scenario B1, an accuracy of 0.30 would be reached with a large number of daughter records (Figure 2). Thus, the best, and most realistically applicable index for selection towards cattle behavior would be an index that combines both traits, AD and AMF, as in Scenario C1.

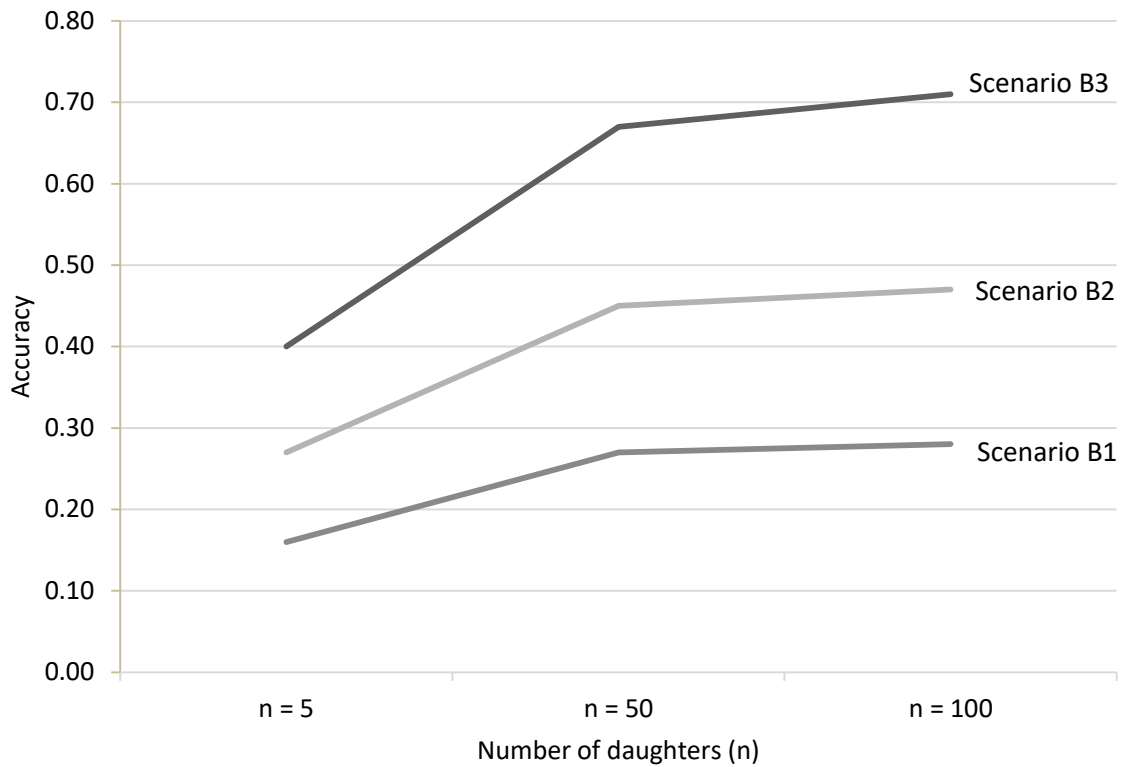


Figure 2. Accuracy of the index depending on the number of observations (n) “daughter-records” and on the Scenarios B1 (r_g between AMF and AD = -0.30), Scenario B2 (r_g between AMF and AD = -0.50) and Scenario B3 (r_g between AMF and AD = -0.75)

The results of this section highlight the additional benefit of the interdisciplinary work between animal ethology and animal breeding. The future of planning efforts for breeding programs could be revolutionized through the use of reliable and valid behavior traits and traits that reflect human-animal-interactions (as recorded by Ethologist), complemented by an emphasis on understanding the genetics from an animal breeding point of view.

Future perspectives

Marker-assisted selection

Additional gain or efficiency in dairy cattle breeding programs due to marker assisted selection was proposed mainly for traits with low heritabilities (e.g., functional and health traits), or for traits which can only be recorded late in life such as survival (Lande & Thompson, 1990). However, from the statistical point of view, the probability to find quantitative trait loci (QTL) is lower for these low heritable traits (König, 2007). Kathkar et al. (2004) gave an extensive review for QTL mapping in dairy cattle. Most of the reviewed publications (45 of 55) reported significant QTL for production traits, but relatively few studies have been reported for functional traits such as mastitis, fertility and health (e.g. Kühn et al., 2003; Ashwell et al., 2004). Also few studies reported QTL for behavior or temperament traits. Gutierrez-Gil et al. (2008), for example, searched for genomic regions (i.e., quantitative trait loci or QTLs) in a Holstein x Charolais cross cattle population, influencing temperament-related traits derived from two behavioral tests. The flight from feeder test measured the distance at which the animal moved away from an approaching human observer, while the social separation test categorized different activities which the animal engaged in when removed from its penmates. A total of 29 QTL distributed across 17 chromosomes were identified at the 5 % chromosome-wide level, with 5 of them showing effects on the flight from feeder test and the rest controlling the scoring variation of social separation variables. Notably, QTLs associated with traits assessed in different tests did not overlap, suggesting that different behavioral responses to different situations are controlled by different underlying genetic factors. These results suggest that a fearful response in different contexts has different underlying genetic causes.

Hiendleder et al., 2003 also detected a QTL for temperament that exceeded the experiment-wise significance threshold (5 % level) on chromosome 29.

Although some QTL for temperament were found, research in this area is still insufficient.

Genes underlying temperament traits

Identifying genes for complex traits would greatly enhance the understanding of these traits. Although the majority of research focused on gene identification has been centered on domestic animals, it is likely that the results of such research could also provide a practical benefit to agriculture. Traditionally, the genetics of complex traits in dairy cattle have been studied without identification of the involved genes. Selection has thus far been based on estimated breeding values calculated from phenotypic records and pedigrees, as well as on existing knowledge of the heritability of each trait. This approach has been somewhat successful, but the process is slow if the trait can only be measured in one sex (e.g., milk yield) or late in life (e.g., longevity, behavior). Therefore, to further improve on these traits, it would be advantageous to identify genes for each of them and select animals carrying the desirable alleles (Goddard and Heyes, 2009).

Following a bioinformatics approach, Gutierrez-Gil et al. (2008) searched for the possibility of coincidence related to the bovine genome between the flanking intervals of the QTL identified in their study and the location of genes that are commonly associated with stress responses and temperament traits in a variety of mammals, including cattle, or with the anxiety disorders and related personality traits in humans or mouse models.

The most namable candidate gene found in a QTL region is the DRD4 (type 4 dopamine receptor) gene. According to physical mapping studies, this gene is located at the distal end of bovine chromosome 29 (Amarante et al., 2000; Hayes et al., 2003; Everts-van der Wind et al., 2005); within the confidence interval for the genome-wide QTL, Gutierrez-Gil et al. (2008) detected the cattle trait fearfulness to human approach (FL trait). In mice, the absence of dopamine D4 receptors results in enhanced reactivity to unconditioned fear-evoking stimuli (Falzone et al., 2002), which may suggest a relationship with the trait measured by the FL test. Moreover, Glenske et al. (2011) found an association between the candidate gene DRD4 and performance in the docility test.

The proximal end of chromosome 16, where Gutierrez-Gil et al. (2008) detected a QTL for WER2, includes the gene RGS2 which regulates G-protein signaling; interestingly,

RGS2 has been associated with anxiety in mice and humans (Yalcin et al. 2004; Smoller et al. 2008). Additionally, PLXNA2, a candidate gene for schizophrenia in humans (Mah et al. 2006) maps to the flanking interval of the QTL for SA1, located at the distal end of chromosome 16. During development, PLXNA2 is essential in the formation of complex circuits required for neural function due to its functionality as a guidance molecule with the capability of directing the growth of axons along specific paths (Dickson 2002). The flanking interval of the QTL for FL1 on chromosome 20 contains the gene that is responsible for programming the prolactin precursor receptor (PRL-R). Furthermore, this gene is connected to maternal and social behavior in rodents (Leckman and Herman 2002) and has also been linked to autism in humans (Yrigollen et al. 2008). Ultimately, identifying the genes accountable for such variation will improve the general understanding of both animal and human behavior. The confirmation of strong marker-trait associations in the future could lead to these markers being used in marker-assisted selection, which has an advantage over traditional breeding schemes, especially for traits that are difficult to measure and have low heritabilities (Lande and Thompson 1990), as is the case for temperament-related traits.

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Erklärung

gemäß der Promotionsordnung des Fachbereichs 09 vom 07. Juli 2004 § 17 (2)

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.

Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus- Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

Gießen, den 19. Dezember 2017

Laura Viviana Santos