Aus dem Institut für Tierernährung und Ernährungsphysiologie

der Justus-Liebig-Universität Gießen

Beeinflussung ernährungsphysiologischer Prozesse durch alternative Futterquellen in der Mastgeflügelfütterung

INAUGURAL-DISSERTATION

zur Erlangung des Doktorgrades (Dr. agr.)

im Fachbereich Agrarwissenschaften, Ökotrophologie und

Umweltmanagement der Justus-Liebig-Universität Gießen

vorgelegt von

M. Sc. Lea Schäfer

aus Lindenfels

Gießen, 2025

Mit Genehmigung des Fachbereichs Agrarwissenschaften,

Ökotrophologie und Umweltmanagement der

Justus-Liebig-Universität Gießen

Prüfungskommission:

1. Gutachter: Prof. Dr. Klaus Eder

2. Gutachter: Prof. Dr. Martin Rühl

Prüfer: Prof. Dr. Robert Ringseis

Prüfer: Prof. Dr. Uwe Wenzel

Vorsitzende: Prof. Dr. Gesine Lühken

Tag der Disputation: 10.06.2025

„Dein Wissen und Können kann dir niemand nehmen!“

Inhalt

1. Einführung

Nach den letzten Schätzungen der Organisation für wirtschaftliche Zusammenarbeit und Entwicklung (OECD) wird die Weltbevölkerung bis zum Jahr 2032 auf 8,6 Milliarden Menschen anwachsen. Dieser Anstieg der Weltbevölkerung und der daraus resultierende Bedarf an Nahrungsmitteln ist die zentrale Triebkraft der steigenden Nachfrage nach Agrarrohstoffen (OECD/FAO, 2023). Analog zu einer wachsenden Bevölkerung steigt auch die Nachfrage an proteinreichen Nahrungsmitteln, um den Proteinbedarf der Bevölkerung decken zu können. Eine erhöhte Produktion von pflanzlichen und tierischen Proteinquellen ist daher nötig.

Aus einer erhöhten Produktion von tierischen Proteinquellen resultiert auch ein steigender Bedarf an Futtermitteln für landwirtschaftliche Nutztiere, was unter anderem aufgrund der Nahrungskonkurrenz zum Menschen, der begrenzten Verfügbarkeit von Ressourcen wie Ackerfläche und Wasser sowie der Berücksichtigung von Klimaschutzzielen, zunehmend schwieriger wird (United Nations, 2017; Wu et al., 2014). Laut OECD wird die globale Produktion an tierischen Produkten im nächsten Jahrzehnt um ca. 10 % ansteigen. Für diesen Anstieg ist vorrangig Geflügelfleisch, insbesondere Hähnchenfleisch, der Hauptwachstumstreiber, weil Produktionskosten, Futtereffizienz und Verbraucherpreise deutlich günstiger sind als bei Rind, Schwein und Schaf (OECD/FAO, 2023). Außerdem besitzt Geflügelfleisch, im Vergleich zu Rind- oder Schweinefleisch, eine für die menschliche Ernährung günstige Zusammensetzung. Es weist einen hohen Proteingehalt und gleichzeitig einen niedrigen Fettgehalt auf (Wu et al., 2014). Aufgrund des hohen Gehaltes an einfach ungesättigten Fettsäuren ist das Fettsäuremuster im Geflügelfleisch für die menschliche Ernährung vorteilhaft. Vor diesem Hintergrund steht die Tierernährung somit vor der Herausforderung alternative Futtermittel zu finden, die mit einer größeren Nachhaltigkeit produziert werden können und gleichzeitig die endlichen Ressourcen schützen.

In dieser Dissertation sollen zwei alternative Futtermittel als Rationskomponenten und deren Auswirkungen auf die Wachstumsleistung und auf tiefergehende ernährungsphysiologische Prozesse von Mastbroilern betrachtet werden. Untersucht wurden zum einen das Insektenfett der schwarzen Soldatenfliege (*Hermetia illucens*), welches als Nebenprodukt der Insektenmehlherstellung anfällt, und zum anderen das Mycel des Austernpilzes *Pleurotus sapidus*, welches biotechnologisch hergestellt wurde. Das übergeordnete Ziel dieser Dissertation ist es, alternative Futtermittel zu finden, welche die Wachstumsleistung und auch die Gesundheit der Broiler positiv bzw. zumindest nicht negativ beeinflussen, um somit die Broilermast nachhaltiger zu gestalten.

In den folgenden Unterkapiteln sollen nun die Hintergründe der jeweiligen Futtermittel näher beschrieben werden.

1.1 Insektenfett in der Tierernährung

Zurzeit fungieren Insekten für ca. zwei Milliarden Menschen in Asien, Lateinamerika und Afrika als Nahrungsquelle. In den westlichen Ländern hingegen spielt die Zucht von essbaren Insekten zur Herstellung eines alternativen Proteinmehls für die Tierfütterung und nicht als Nahrungsquelle für die Bevölkerung eine immer wichtigere Rolle (Grau et al., 2017; Mwangi et al., 2018). Aus ökologischer und wirtschaftlicher Sicht bringt die Zucht der Insekten viele Vorteile mit sich: im Gegensatz zu landwirtschaftlichen Nutztieren wachsen Insekten schneller, weisen eine bessere Futterverwertung auf und produzieren niedrigere Treibhausgasemissionen. Außerdem ist eine großtechnische industrielle Massenaufzucht ohne zusätzlichen Ackerflächenbedarf möglich (Belluco et al., 2013; Gessner et al., 2019; Oonincx und Boer, 2012; Oonincx et al., 2015; van Huis, 2016; Pinotti et al., 2019). Ein weiterer Vorteil besteht darin, dass die Insekten nicht in Nahrungskonkurrenz zum Menschen oder zu landwirtschaftlichen Nutztieren stehen, weil sie auf sekundären Abfällen wie geringwertigen pflanzlichen Nebenprodukten gehalten werden können (Churchward-Venne et al., 2017; Grau et al., 2017). In der EU sind mittlerweile sieben Insektenarten als Nutzinsekten zur Verwendung als Futtermittel zugelassen, diese sind: die Soldatenfliege (*Hermetia illucens*), die Stubenfliege (*Musca domestica*), der Mehlkäfer (*Tenebrio molitor*), der Getreideschimmelkäfer (*Alphitobius diaperinus*), das Heimchen (*Acheta domesticus*), die Kurzflügelgrille (*Gryllodes sigillatus*) und die Steppengrille (*Gryllus assimilis*) (Verordnung EU 2017/893). Abhängig vom Substrat auf dem die Insekten gehalten werden und ihrem Entwicklungsstadium zum Zeitpunkt der Ernte weisen sie zwischen 42,0 % und 54,1 % Rohprotein und 11,8 - 34,8 % Rohfett auf Trockenmassebasis auf (Astuti et al., 2018; Kroeckel et al., 2012).

In der Tierernährung hat sich mittlerweile die schwarze Soldatenfliege (*H. illucens*) aufgrund ihrer geringen Haltungsanforderungen und ihres schnellen Wachstums etabliert. Der Rohproteingehalt im *H. illucens* Larvenmehl schwankt allerdings sehr stark zwischen 21,6 % und 65,5 % in der Trockenmasse (Lu et al., 2022). Die Schwankungen lassen sich darauf zurückführen, auf welchem Substrat die Larven gehalten wurden, in welchem Entwicklungsstadium sie sich zum Zeitpunkt der Ernte befanden und ob bzw. auch mit welcher Methode das Larvenmehl entfettet wurde. Beim Rohfett schwanken die Gehalte bei nicht entfetteten Mehlen zwischen 29,4 % und 51,3 % (Lu et al., 2022). Nach einer Entfettung liegt der Rohfettgehalt im Mittel bei ungefähr 6,90 % (Lu et al., 2022). Während der Einsatz von *H. illucens* Larvenmehlen in der Tierernährung schon gut erforscht ist, liegen zurzeit nur wenige Daten über den Einsatz des Insektenfettes, welches als Nebenprodukt der Insektenmehlherstellung anfällt, vor. Das Fett stellt nach dem Protein die zweitgrößte Komponente in Insekten dar (Cerletti et al., 2021; Benzertiha et al., 2020). Wie beim Protein ist der Fettanteil und die Qualität davon abhängig, in welchem Entwicklungsstadium sich die Insekten beim Erntezeitpunkt befanden und auf welchem Substrat sie gehalten wurden (Barragan-Fonseca et al., 2018; Fontaneto et al., 2011; Oonincx et al., 2015). Die im Fett vorherrschenden Lipidspezies sind zu 99 % Triglyceride und die dominierende Fettsäure im *H. illucens* Larvenfett ist die Laurinsäure, eine gesättigte, mittelkettige Fettsäure, deren Anteil bei ca. 45 % der Gesamtfettsäuren liegt (Marschall et al., 2024; Tzompa-Sosa et al., 2021). Der hohe Anteil der gesättigten Fettsäuren führt zu einer festen Konsistenz des Fettes bei Raumtemperatur.

Studien zeigen, dass mittelkettige Fettsäuren antimikrobielle und antivirale Wirkungen haben (Isaacs et al., 1995; Matsue et al., 2019), wodurch die Vermutung nahe liegt, dass der Einsatz von *H. illucens* Larvenfett eine antimikrobielle und antivirale Wirkung im Darm von Monogastern aufweisen könnte. Als eine Quelle von gesättigten Fettsäuren, welche aufgrund der fehlenden Doppelbindungen besser gegen Lipidperoxidation geschützt sind, und der durch vorangegangenen Studien belegten Einlagerung bzw. Widerspiegelung des Fettsäureprofils des *H. illucens* Larvenfettes in verschiedene Gewebe, wie Leber, Plasma und Brustmuskelgewebe, besteht die Hypothese, dass auch diese Gewebe aufgrund eines erhöhten Anteils an gesättigten Fettsäuren eine höhere Oxidationsstabilität aufweisen könnten (Kierończyk et al., 2018; Kierończyk et al., 2023; Kim et al., 2020b). Bei der Erhitzung des Brustmuskels kommt es durch die Lipidperoxidation der ungesättigten Fettsäuren zu der Bildung von Cholesterol- und Phytosteroloxidationsprodukten. Diese Oxidationsprodukte begünstigen die Entstehung von Arteriosklerose, Krebserkrankungen und haben negative Auswirkungen auf den Stoffwechsel, wie etwa auf den Lipid- und Glucose-Metabolismus (Alemany et al., 2014; Kulig et al., 2016; Lin et al., 2016). Daher kann eine erhöhte Oxidationsstabilität des Muskelgewebes auch für den Verbraucher positive Auswirkungen mit sich bringen.

Das *H. illucens* Larvenfett wurde schon in der Fütterung von Broilern erfolgreich als Austausch von Fettquellen, wie Sojaöl, eingesetzt, wobei der Fokus dieser Studien vorrangig auf den Wachstumsleistungen der Broiler lag (Benzertiha et al., 2019; Kierończyk et al., 2018; Kim et al., 2020b; Schiavone et al., 2017; Schiavone et al., 2018). Die Auswirkungen des Insektenfettes auf tiefergehende Stoffwechselparameter wie das Transkriptom, das Lipidom oder das Darmmikrobiom ist noch weitestgehend unbekannt. Die durchgeführte Studie soll einen Einblick dahingehend geben.

1.2 Pilzmycele als Präbiotikum

In Hinblick auf Nachhaltigkeit sind Nebenerzeugnisse aus der Verarbeitung pflanzlicher Rohstoffe für die Tierfütterung besonders interessant. Diese Nebenprodukte haben oft einen begrenzten Nährwert für die menschliche Ernährung und können kaum anderweitig ressourcenschonend genutzt werden. Beispiele hierfür sind verschiedene Trester, wie Apfel-, Wein-, Zitrus- und Karottentrester, sowie Biertreber. Aufgrund ihres hohen Gehalts an verdaulichkeitshemmender Rohfaser werden sie in der Fütterung monogastrischer Tiere wie Geflügel eher selten und meist nur in konzentrierter Form als Quelle sekundärer Pflanzenstoffe wie Polyphenole eingesetzt (Huang and Lee., 2018). Durch eine indirekte Nutzung als Substrat zur Kultivierung von essbaren Pilzen, wie etwa dem Austernpilz *Pleurotus sapidus*, können diese Nebenströme dennoch in der Geflügelfütterung eingesetzt werden. In der Humanernährung sind essbare Pilze schon seit Jahrhunderten aufgrund ihres niedrigen Energie- und Kohlenhydratgehalts und ihres hohen Gehalts an Faser, Mikronährstoffen und biologisch aktiven Proteinen, sowie ihrer anti-inflammatorischen, antioxidativen, präbiotischen und antimikrobiellen Eigenschaften relevant (Chuang et al., 2020; Ma et al., 2018; Rizzo et al., 2021). In der Tierernährung ist ihr Einsatz bislang noch sehr gering (Das et al., 2010). Ihre gesundheitlichen Aspekte sind aber auch für die Gesundheit und Leistung von Mastbroilern vorteilhaft, da auch beim Geflügel lokale (im Darm) und systemische Entzündungsprozesse eine Rolle spielen und einen negativen Einfluss auf die Leistungen haben (Gessner et al., 2017).

Während die Produktion von Pilzfruchtkörpern ein langer und zeitaufwendiger Prozess ist, ist die Produktion von Pilzmycelen im industriellen Maßstab mit Hilfe einer biotechnologischen Umwandlung eines Nebenstroms ein effizienter und kostengünstiger Prozess, da viel Masse in einer relativ geringen Zeit hergestellt werden kann (Lee et al., 2008; Rathore et al., 2019; Wan-Mohtar et al., 2016).

Pilzmycele weisen einen hohen Gehalt an präbiotischen Polysacchariden wie den β-Glucanen auf, welche die mikrobielle Darmgemeinschaft, die Darmgesundheit und Darmintegrität fördern (Maheshwari et al., 2020). Die β-Glucane in Pilzen sind aus D-Glucose Monomeren aufgebaut, welche über den β-Typ der glycosidischen Bindungen (1,3 und 1,6) miteinander verknüpft sind (Cerletti et al., 2021). Eine Studie mit Laborratten, welche eine Diät mit einem Mycel aus der Familie der Austernpilze (*Pleurotus* spp.) erhielten, zeigte eine günstige Beeinflussung der mikrobiellen Darmgemeinschaft, wie auch der Darmintegrität und -gesundheit, zudem wurde eine Hemmung des systemischen Entzündungsgeschehens beobachtet (Maheshwari et al., 2021). Bislang fehlen allerdings Studien zur Wirkung eines nachhaltig erzeugten Futtermittels auf Basis von Pilzmycelen in der Broilerfütterung, was die Wichtigkeit der im Rahmen dieser Dissertation durchgeführten Studie nochmals unterstreicht. Denn auch in der Broilerfütterung ist die Darmintegrität und -gesundheit von herausragender Bedeutung für die systemische Gesundheit und Wachstumsleistung der Tiere, gerade in Bezug auf die industrielle Bodenhaltung, bei der Erkrankungen eine größere Rolle spielen. Ebenso ist die biotechnologische Umwandlung eines kostengünstigen Nebenstroms aus der Verarbeitung pflanzlicher Rohstoffe, welche nicht oder nur begrenzt in der Tierfütterung eingesetzt werden können, ein vielversprechender Ansatz zur nachhaltigen Gestaltung der Broilermast.

2. Ziele der Studien

Im folgenden Kapitel sollen die Ziele und Hypothesen der beiden Fütterungsstudien aufgezeigt werden.

2.1 Austausch von Sojaöl durch das Fett von *Hermetia illucens* Larven

Die erste Fütterungsstudie, die in den **Publikationen 1** und **2** veröffentlicht wurde, beschäftigte sich mit einem teilweisen und vollständigen Austausch des Sojaöls als Fettquelle durch das *H. illucens* Larvenfett in Broilerdiäten.

2.1.1 Wirkung des Austauschs von Sojaöl durch *Hermetia illucens* Larvenfett auf die Leistung, die Verdaulichkeit, das Darmmikrobiom, das Lebertranskriptom und das Leber- und Plasmalipidom von Mastbroilern

Das Ziel des ersten Teils dieser Studie, die in **Publikation 1** veröffentlicht wurde, war es, den teilweisen (50 %) sowie vollständigen (100 %) Austausch von Sojaöl durch *H. illucens* Larvenfett auf den Stoffwechsel bei Mastbroilern der Rasse Cobb 500 zu untersuchen. Sojaöl wurde in der Studie durch ein Nebenprodukt ersetzt. Neben der Erfassung der Leistungsdaten und der Fettverdaulichkeit wurden auch verschiedene Screening-Technologien verwendet, um tiefergehende Erkenntnisse zum Stoffwechselgeschehen auf Ebene des Darmmikrobioms im Caecum, des Transkriptoms der Leber und des Lipidoms in Plasma und Leber zu gewinnen. Die Hypothese, welche aufgestellt wurde, bestand darin, dass der Austausch von Sojaöl durch das *H. illucens* Larvenfett keine nachteiligen Stoffwechselwirkungen ausübt und somit das Wachstum sowie die Tiergesundheit nicht beeinträchtigt.

<u>Publikation 1</u>

Schäfer, L.; Grundmann, S. M.; Maheshwari, G.; Höring, M.; Liebisch, G.; Most, E.; Eder, K.; Ringseis, R. (2023): Effect of replacement of soybean oil by *Hermetia illucens* fat on performance, digestibility, cecal microbiome, liver transcriptome and liver and plasma lipidomes of broilers. Journal of Animal Science and Biotechnology 14 (1): 20

2.1.2 Ein Austausch von Sojaöl durch das Fett von *Hermetia illucens* Larven in Broilerdiäten verändert das Brustmuskellipidom und verringert die Lipidoxidation des Brustmuskels während der Hitzebehandlung

Der zweite Teil dieser Studie, welcher in **Publikation 2** veröffentlicht wurde, hatte das Ziel zu untersuchen, inwieweit der Austausch des Sojaöls durch das *H. illucens* Larvenfett die Oxidationsstabilität des Brustmuskels der Broiler beeinflusst. Es wurde die Hypothese aufgestellt, dass das *H. illucens* Larvenfett, welches einen hohen Anteil an gesättigten Fettsäuren enthält, die Entstehung von Lipidperoxidationsprodukten, wie Cholesterol- und Phytosteroloxidationsprodukten, im erhitzten Brustmuskel reduziert. Eine langfristige Aufnahme von Cholesterol- und Phytosteroloxidationsprodukten kann ein gesundheitliches Risiko darstellen. Um den Gehalt an Cholesterol- und Phytosteroloxidationsprodukten im gegarten Brustmuskel zu messen, wurde ein Teil des Brustmuskels der Broiler für 50 min bei 170 °C erhitzt.

<u>Publikation 2</u>

Schäfer, L.; Grundmann, S. M.; Friedrichs, S.; Lütjohann, D.; Höring, M.; Liebisch, G.; Most, E.; Ringseis, R.; Eder, K. (2023): Replacement of soybean oil by *Hermetia illucens* larvae fat in broiler diets alters the breast muscle lipidome and reduces lipid oxidation of the breast muscle during heat-processing. Archives of Animal Nutrition 77 (2): 121-140

2.2 Einsatz eines biotechnologisch produzierten *Pleurotus sapidus* Mycels

In der zweiten Fütterungsstudie, welche in den **Publikationen 3** und **4** veröffentlicht wurde, erhielten Broiler eine 2,5 und 5 prozentige Zulage von *P. sapidus* Mycel in den Diäten. Die Herstellung des Mycels erfolgte biotechnologisch mit Melasse als Nährmedium.

2.2.1 Auswirkungen eines biotechnologisch hergestellten *Pleurotus sapidus* Mycels auf das Darmmikrobiom, das Lebertranskriptom und das Plasmametabolom von Mastbroilern

Das Ziel dieser Studie, welche in **Publikation 3** veröffentlicht wurde, bestand darin, ob ein biotechnologisch hergestelltes *P. sapidus* Mycel auf Basis eines pflanzlichen Nebenstroms in Broilerdiäten zur nachhaltigen Gestaltung der Broilermast eingesetzt werden kann. Untersucht wurden die Wachstumsleistung der Broiler, die Zusammensetzung der Darmmikrobiota sowie verschiedene physiologische Merkmale wie die Darmintegrität, die Nährstoffverdaulichkeit, die Leberlipide, das Lebertranskriptom und das Plasmametabolom. Zu diesem Zweck wurden auch in dieser Studie verschiedene Screening-Technologien wie die Transkriptomik und Metabolomik sowie die metagenomische Analyse der Darmmikrobiota eingesetzt. Die Hypothese der Studie lautete, dass die β-Glucane, welche in hohem Maße im *P. sapidus* Mycel vorhanden sind und die nachweislich eine präbiotische Wirkung besitzen, positive Auswirkungen auf die mikrobielle Gemeinschaft im Darm und die Integrität des Darms haben, sowie die Stoffwechselgesundheit und die Leistung positiv beeinflussen.

<u>Publikation 3</u>

Schäfer, L.; Grundmann, S. M.; Rühl, M.; Zorn, H.; Seel, W.; Simon, M.-C.; Schuchardt, S.; Most, E.; Ringseis, R.; Eder, K. (2024): Effects of a biotechnologically produced *Pleurotus sapidus* mycelium on gut microbiome, liver transcriptome and plasma metabolome of broilers. Poultry Science 103 (9): 103975

2.2.2 Wirkung eines biotechnologisch hergestellten *Pleurotus sapidus* Mycels auf die Expression von Genen, die an der Proteinsynthese und dem Proteinabbau im Brustmuskel von Mastbroilern beteiligt sind

Der zweite Teil der Studie, welcher in **Publikation 4** veröffentlicht wurde, beschäftigte sich damit, eine Erklärung für den reduzierten relativen Brustmuskelanteil, welcher bei den Broilern auftrat, die das *P. sapidus* Mycel in ihren Diäten erhielten, zu finden. Es wurde die Hypothese aufgestellt, dass das *P. sapidus* Mycel und der hohe Gehalt an β-Glucanen den relativen Brustmuskelanteil reduzieren, indem die Expression von Genen verringert wird, die an der Proteinsynthese beteiligt sind, und die Expression von Genen erhöht wird, die am Proteinabbau beteiligt sind.

Publikation 4

Schäfer, L.; Herrero-Encinas, J.; Rühl, M.; Zorn, H.; Most, E.; Eder, K.; Ringseis, R. (2024): Research note: Effect of a biotechnologically produced *Pleurotus sapidus* mycelium on expression of genes involved in protein synthesis and degradation in breast muscle of broilers. Poultry Science 103 (12): 104450

3. Originalarbeiten

3.1 Wirkung eines Austauschs von Sojaöl durch *Hermetia illucens* Larvenfett auf die Leistung, die Verdaulichkeit, das Darmmikrobiom, das Leber-transkriptom und das Leber- und Plasmalipidom von Mastbroilern

Effect of replacement of soybean oil by *Hermetia illucens* fat on performance, digestibility, cecal microbiome, liver transcriptome and liver and plasma lipidomes of broilers

Schäfer *et al.*
Journal of Animal Science and Biotechnology (2023) 14:20
https://doi.org/10.1186/s40104-023-00831-6

Journal of Animal Science and Biotechnology

RESEARCH Open Access

Effect of replacement of soybean oil by *Hermetia illucens* fat on performance, digestibility, cecal microbiome, liver transcriptome and liver and plasma lipidomes of broilers

Lea Schäfer[1], Sarah M. Grundmann[1], Garima Maheshwari[2], Marcus Höring[3], Gerhard Liebisch[3], Erika Most[1], Klaus Eder[1] and Robert Ringseis[1*]

Abstract

Background In contrast to protein-rich insect meal, the feed potential of insect fat is generally less explored and knowledge about the suitability of insect fat as a fat source specifically in broiler diets is still limited. In view of this, the present study aimed to comprehensively investigate the effect of partial (50%) and complete replacement of soybean oil with insect fat from Hermetia illucens (HI) larvae in broiler diets on performance, fat digestibility, cecal microbiome, liver transcriptome and liver and plasma lipidomes. Thus, 100 male, 1-day-old Cobb 500 broilers were randomly assigned to three groups and fed three different diets with either 0 (group HI-0, $n = 30$), 2.5% (group HI-2.5, $n = 35$) or 5.0% (HI-5.0, $n = 35$) *Hermetia illucens* (HI) larvae fat for 35 d.

Results Body weight gain, final body weight, feed intake, and feed:gain ratio during the whole period and apparent ileal digestibility coefficient for ether extract were not different between groups. Cecal microbial diversity did not differ between groups and taxonomic analysis revealed differences in the abundance of only four low-abundance bacterial taxa among groups; the abundances of phylum Actinobacteriota, class Coriobacteriia, order Coriobacteriales and family Eggerthellaceae were lower in group HI-5.0 compared to group HI-2.5 ($P < 0.05$). Concentrations of total and individual short-chain fatty acids in the cecal digesta were not different between the three groups. Liver transcriptomics revealed a total of 55 and 25 transcripts to be differentially expressed between groups HI-5.0 vs. HI-0 and groups HI-2.5 vs. HI-0, respectively ($P < 0.05$). The concentrations of most lipid classes, with the exception of phosphatidylethanolamine, phosphatidylglycerol and lysophosphatidylcholine in the liver and cholesterylester and ceramide in plasma ($P < 0.05$), and of the sum of all lipid classes were not different between groups.

Conclusions Partial and complete replacement of soybean oil with HI larvae fat in broiler diets had no effect on growth performance and only modest, but no adverse effects on the cecal microbiome and the metabolic health of broilers. This suggests that HI larvae fat can be used as an alternative fat source in broiler diets, thereby, making broiler production more sustainable.

*Correspondence:
Robert Ringseis
Robert.ringseis@ernaehrung.uni-giessen.de
Full list of author information is available at the end of the article

Keywords Broilers, Cecal microbiota, *Hermetia illucens*, Insect fat, Liver lipidome, Medium-chain fatty acids

Introduction

In recent years, insect biomass has been increasingly recognized as an alternative and sustainable source of feed for monogastric livestock [1–3]. Consequently, the use of processed insect biomass as a feed for poultry and pigs has been authorized by the European commission in 2021 [4], with the aim of improving sustainability of food systems and securing food supply. Owing to its high protein content (40%–50% of dry matter (DM) depending on the insect species [5] and provided that the rearing substrate is suitable [6, 7]), research dealing with the feed potential of insect biomass has primarily focused on its role as a source of protein. In this context, a large number of studies in different monogastric livestock species demonstrated that insect larvae meal obtained from different edible insect species suitable for large-scale production, such as *Tenebrio molitor* (TM) and *Hermetia illucens* (HI), can replace conventional protein sources, like soybean meal, without impairing performance, metabolic health or product quality [8–11]. Apart from protein, insect larvae contain a significant amount of fat (up to 47% and 43% of DM in HI and TM, respectively, depending on the rearing substrate [12]), which can be obtained by a defatting process during insect meal production in order to increase the protein content and improve the storage stability of the insect meal. In contrast to insect meal, the feed potential of insect fat is far less explored. Despite that several studies have demonstrated that TM or HI fat has no negative impact on performance, gut morphology, selected blood parameters and product quality in broilers, laying hens and turkeys [13–21], knowledge about the suitability of insect fat as a fat source in broiler diets is still limited. In particular, in-depth analysis of the effects of insect fat on the gut microbiome and intermediary metabolism is lacking.

Despite that fatty acid composition of insect larvae is influenced by the fatty acids in the rearing substrate [5], the fatty acid composition of TM and HI fat are completely different. While TM fat consists mainly of unsaturated fatty acids (up to 80%), most of which are long-chain fatty acids (LCFA; C18:1 and C18:2; [11]), the majority of total fatty acids in HI fat are saturated fatty acids (SFA) [5, 16]. This explains that HI fat, but not TM fat has a hard consistency at room temperature. A further characteristic of HI fat is its high proportion of the medium chain-fatty acid (MCFA) lauric acid (C12:0) (≈ 40%; [16]). Interestingly, MCFA including lauric acid have been reported to exert antimicrobial effects (e.g., against Gram-positive cocci and *Escherichia coli*) in the gut of broilers [22, 23], and, thus, dietary inclusion of HI fat might alter the gut microbiota composition. Considering recent evidence that feed efficiency in broilers is affected by the gut microbiota composition through different mechanisms, such as a more complete digestion of substrates, an increased production of short-chain fatty acids (SCFA) or a decreased stimulation of the intestinal immune system [24, 25], an alteration of the gut microbiota might be of great relevance to broiler´s performance. In addition, convincing evidence exists that the gut microbiota has also a profound influence on energy metabolism and feeding behavior of the host due to the ability of the gut microbiota to communicate with the host along the gut-liver axis via different gut-derived compounds [26]. Moreover, MCFA-rich dietary fats might also exert a direct effect on energy metabolism, because triglyceride (TG)-bound MCFA are considered to be more efficiently absorbed than TG-bound LCFA due to easier emulsification and less dependence on pancreatic lipase [27, 28], thereby increasing digestible energy intake to support a higher growth performance. Against this background, the present study aimed to comprehensively investigate the effect of partial (50%) and complete replacement of soybean oil, the most commonly used fat source in commercial broiler diets, with HI larvae fat in broiler diets on performance, fat digestibility, cecal microbiome, liver transcriptome and liver and plasma lipidomes.

Methods

Animals and diets

The 35-d feeding trial was approved by the Animal Welfare Officer of the Justus Liebig University Giessen (approval no.: JLU 786_M). All experimental procedures described followed established guidelines for the care and handling of laboratory animals. The experiment included 100 male, 1-day-old broiler chickens (Cobb 500, Cobb, Wiedemar, Germany), which were randomly assigned to three groups (5 broilers/cage, group 1: 6 cages, group 2 and group 3: 7 cages). The mean initial body weight (BW) (44.9 ± 2.4 g; mean ± SD) was similar across the groups. The broilers were kept in 2.1 m^2 cages equipped with nipple drinkers and feed automates and had free access to feed and water. At the floor of the cages there were cardboards, which were covered with litter to allow scratching, pecking and dustbathing. Cardboards and litter were exchanged 2 times per week during the first two weeks and every 2 d during the last three weeks of the trial. In addition, broilers were provided with perches in elevated position for resting and sleeping. Light intensity was constantly at 40 Lux and the light regime was 24 h:0 h, 23 h:1 h, 22 h:2 h, 21 h:3 h, 20 h:4 h, 19 h:5 h (light:dark) at d 1, 2, 3, 4, 5, 6, and 18 h:6 h from d

7 onward, as recommended by the breeder [29]. The room temperature decreased from 28–29 °C on d 1, measured at pen height, to 23–24 °C on d 35. During the first 6 d, infrared lamps (Albert Kerbl GmbH, Buchbach, Germany) were used as additional heat sources in order to adjust the temperature at the cage floor to 34 °C. Mean relative humidity was 60.0% ± 1.9%. The groups (group 1: HI-0, group 2: HI-2.5, group 3: HI-5.0) were fed three different diets, which varied only in the fat source (HI-0: 0 HI fat and 5% soybean oil, HI-2.5: 2.5% HI fat and 2.5% soybean oil, HI-5.0: 5.0% HI fat and 0 soybean oil), in a three-phase feeding system (starter diet form d 1 to 10, grower diet from d 11 to 21, finisher diet from d 22 to 35). The HI fat was purchased from Madebymade (Pegau, Germany) and stored at −20 °C until diet preparation. Prior to diet preparation, the stability of HI fat was assessed by determining the acid value and the percentage of polar compounds [30]. Low values for both parameters indicated no significant oxidation of the HI fat. Company's details on the rearing conditions (substrate, duration) and further processing of the HI larvae are not available for reasons of confidentiality. The composition of the three diets are shown in Table 1. The finisher diets contained 0.5% titanium dioxide as indicator in order to calculate the apparent ileal digestibility (AID) coefficient for ether extract by the indicator method [31]. The diets met the broiler's requirements of nutrients and energy according to the breeder's recommendations [32]. For diet preparation, three different basal feed mixtures for the starter, grower and finisher period, respectively, were produced from all feed components except the fat source using mixing machines (V100 and V250, Diosna, Germany). The experimental diets were obtained by mixing the different basal feed mixtures with the intended amounts of fat sources (soybean oil, HI fat) using the mixing machine. Prior to adding the HI fat, the HI fat was melted in an electric oven at 50 °C. Subsequently, the experimental diets were pelleted using a pelleting device (V3/30 C, Simon-Heesen, Boxtel, Netherlands) and aliquots of all diets were stored at −20 °C for analysis of diet composition. Diets were fed in crumbled form during the first 3 d, and in pellet form (2 mm diameter) from d 3 until the end of the trial. Body weight (individually) and feed intake (per cage) were determined on d 1, 10, 21 and 35, and the feed:gain ratio was calculated from feed intake and body weight gains on cage basis.

Analysis of diet composition

Concentrations of DM, crude protein, crude ash, ether extract, crude fiber and amino acids in the main diet

Table 1 Composition of the broiler diets, g/kg

Item	Starter diets			Grower diets			Finisher diets		
	HI-0	HI-2.5	HI-5.0	HI-0	HI-2.5	HI-5.0	HI-0	HI-2.5	HI-5.0
Maize	300	300	300	280	280	280	280	280	280
Soybean meal (44% CP)	380	380	380	320	320	320	300	300	300
Wheat	205	205	205	283.5	283.5	283.5	308.65	308.65	308.65
Soybean oil	50	25	-	50	25	-	50	25	-
HI fat	-	25	50	-	25	50	-	25	50
Mineral & vitamin mix – Starter*	20	20	20	-	-	-	-	-	-
Mineral & vitamin mix – Grower*	-	-	-	20	20	20	-	-	-
Mineral & vitamin mix – Finisher*	-	-	-	-	-	-	20	20	20
Monocalciumphosphate	15	15	15	15	15	15	9	9	9
Calciumcarbonate	15.5	15.5	15.5	15.5	15.5	15.5	15	15	15
Sodiumchloride	4	4	4	4	4	4	4	4	4
DL-Methionine	3.2	3.2	3.2	3	3	3	2.6	2.6	2.6
L-Lysine	2.1	2.1	2.1	2.5	2.5	2.5	1.8	1.8	1.8
L-Threonine	1.1	1.1	1.1	0.9	0.9	0.9	0.4	0.4	0.4
L-Arginine	1.4	1.4	1.4	1.3	1.3	1.3	0.5	0.5	0.5
L-Valine	1.5	1.5	1.5	2.5	2.5	2.5	1.6	1.6	1.6
L-Isoleucine	1.2	1.2	1.2	1.8	1.8	1.8	1.45	1.45	1.45
TiO_2	-	-	-	-	-	-	5	5	5

* The mineral & vitamin mix supplied the following minerals and vitamins per kg diet (starter/grower/finisher): Fe, 40/40/40 mg; Cu, 15/15/15 mg; Mn, 100/100/100 mg; Zn, 100/100/100 mg; I, 1/1/1 mg; Se, 0.35/0.35/0.35 mg; vitamin A, 10,000/10,000/10,000 IU; vitamin D_3, 5000/5000/5000 IU; vitamin K_3, 3/3/3 mg; vitamin E, 80/50/50 IU; vitamin B_1, 3/2/2 mg; vitamin B_2, 9/8/6 mg; vitamin B_6, 4/3/3 mg; vitamin B_{12}, 0.02/0.015/0.015 mg; biotin, 0.2/0.18/0.18 mg; folic acid, 2/2/1.5 mg; nicotinic acid, 60/50/50 mg; choline chloride, 500/400/350 mg; pantothenic acid, 15/12/10 mg

The experimental diets did not contain any technological (e.g., emulsifier) or zootechnical feed additives (e.g., feed enzymes) components (wheat, maize, soybean extraction meal) and the experimental diets were determined by official methods [33]. Concentrations of sugar and starch were analyzed in the experimental diets by official methods (sugar according to method 7.2.1 and starch according to method 7.1.3 [33]). Total lipid fatty acid composition of HI fat, soybean oil and diets were analyzed as described below. Apart from total lipid fatty acid composition, the detailed composition (all major lipid classes and individual lipid species) of the HI fat was comprehensively analyzed using lipidomics, as described for liver and plasma (see below). The apparent N-corrected metabolizable energy (AME_N) content of the diets was calculated according to the formula of the World's Poultry Science Association (WPSA) for poultry compound feed [34]:

$$AME_N\ (\text{MJ/kg}) = \left[(0.01551 \times \text{crude protein}) + (0.03431 \times \text{ether extract}) + (0.01669 \times \text{starch}) + (0.01301 \times \text{sugar})\right]$$

Considering that the AME and AME_N content of HI larvae fat for broiler chickens was shown to be similar to that of soybean oil [35], the formula is appropriate to calculate the ME contents of the experimental diets.

Sample collection

A total of 12 (group HI-0) and 14 (groups HI-2.5 and HI-5.0) broilers per group (two broilers from each cage), whose body weights represented the mean body weight of the whole group, were selected for sample collection in order to avoid that effects were biased by random selection of broilers with very low or very high body weights. All analyses described below were carried out in these animals. The animals were killed by bleeding (opening of *Vena jugularis* and *Arteria carotis*) under electrical anesthesia using a BTG-40A stunning device (Westerhoff Geflügeltechnik, Hoogstede, Germany) in accordance with the European legislation for euthanasia of animals [36]. Whole blood was collected into ethylenediaminetetraacetic acid-coated polyethylene tubes (9 mL S-Monovette, Sarstedt, Nümbrecht, Germany). Plasma was prepared by centrifugation (1100 × *g*, 10 min) at 4 °C and stored at −20 °C. The liver was excised, washed in ice-cold NaCl solution (0.9%), weighted and small aliquots were snap-frozen in liquid nitrogen and stored at −80 °C. The gastrointestinal tract was removed and digesta from the ileum (segment between Meckel´s diverticulum and the ileo-cecal junction) and the cecum was collected. Tissue and digesta samples were snap-frozen in liquid nitrogen and stored at −80 °C pending analysis.

Determination of AID coefficient for ether extract

The AID coefficient for ether extract was determined at the end of the experiment by the indicator method using titanium dioxide as an inert marker [31]. Prior to analysis, ileal digesta samples were freeze-dried and ground using a centrifugal mill (Retsch, Haan, Germany). Ileal digesta concentration of the indigestible indicator TiO_2 was determined by the method of Brandt and Allam with slight modifications [37]. Concentration of ether extract in the ileal digesta was determined by official methods as described above. Based on the ileal concentrations of indicator, the AID coefficient for ether extract was calculated according to the following formula:

$$\text{AID coefficient } (\%) = 100 - [(TiO_{2\_Diet}/TiO_{2\_Digesta}) \times (EE_{\_Digesta}/EE_{\_Diet}) \times 100],$$

in which TiO_{2_Diet} is the TiO_2 concentration in the diet (% DM), $TiO_{2\_Digesta}$ is the TiO_2 concentration in the ileal digesta (% DM), $EE_{\_Digesta}$ is the ether extract concentration in ileal digesta (% DM), and $EE_{\_Diet}$ is the ether extract concentration in the diet (% DM).

Determination of microbiota composition and diversity in the cecal digesta

Metagenomic DNA was isolated from approximately 180–200 mg of cecal digesta using genomic DNA columns (Macherey-Nagel, Düren, Germany) according to Lagkouvardos et al. [38]. V3-V4 regions of the 16S rRNA genes were amplified using bacteria-specific primers following a two-step procedure according to the Illumina sequencing protocol as described [38]. Amplicons were sequenced using a MiSeq system (Illumina, Inc., San Diego, CA, USA). Further processing of raw sequences was carried out as described recently [39]. Finally, sequences with a relative abundance > 0.1% in at least one sample were sorted, merged and operational taxonomic units (OTU) were picked at a threshold of 97% similarity. Taxonomic classification to the OTU was assigned using the SILVA database [40]. Further downstream analyses were done using Rhea (https://lagkouvardos.github.io/Rhea/). The differential abundance analysis of taxa was performed on the aggregated data at the different taxonomic levels as described [38]. For estimation of diversity within samples (α-diversity), the Shannon and Simpson indices, the most common indices to compare diversity, were calculated and transformed to the corresponding effective number of species according to Jost [41], because they are better suited at indicating the true diversity between samples and are minimally affected by the number of rare species. To measure the similarity between different microbial profiles, the β-diversity was determined by calculating generalized UniFrac distances with PERMANOVA statistical test as described previously [38]. Visualization of bacterial profiles among different groups was done by computation of non-metric multidimension distance scaling (NMDS) [42].

Determination of SCFA concentrations in the cecal digesta

Cecal digesta SCFA concentrations were determined as described previously [43]. In brief, 50 mg aliquots of cecal digesta were mixed with 0.5 mL 5% o-phosphoric acid containing internal standard (0.15 mg/mL crotonic acid). Extraction was carried out by vortexing for 3 min and subsequent centrifugation at 21,100 × g at 4 °C for 10 min. Prior to injection, the supernatant was centrifuged again at 21,100 × g at 4 °C for 5 min. 1 µL of the extract was injected into a gas chromatograph (Clarus 580 GC system, Perkin Elmer, Waltham, USA) equipped with a polar capillary column (10 m free fatty acid phase, 0.32 mm internal diameter, 0.25 µm film thickness; Macherey and Nagel, Düren, Germany) and a flame ionisation detector.

RNA extraction and hepatic transcript profiling

Total RNA from liver aliquots (20 mg) were isolated using TRIzol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. RNA quantity and quality were assessed spectrophotometrically using an Infinite 200 M microplate reader equipped with a NanoQuant plate (both from Tecan, Mainz, Germany). The average RNA concentration and the A_{260}/A_{280} ratio of all total RNA samples ($n = 40$, means ± SD) were 421 ± 48 ng/µL and 1.90 ± 0.02. For hepatic transcript profiling, total RNA samples from six randomly selected broilers/group were sent on dry-ice to the Genomics Core Facility "KFB—Center of Excellence for Fluorescent Bioanalytics" (Regensburg, Germany). Following a further RNA quality check using an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany), which revealed an average RNA integrity number (RIN) value of 8.23 ± 0.19 for all samples ($n = 18$, means ± SD), total RNA samples were processed using an Affymetrix GeneChip Array (Chicken Gene 1.0 ST), which covers 18,214 genes represented by 439,582 probes, according to the Applied Biosystems™ GeneChip™ Whole Transcript (WT) PLUS Reagent Kit User Guide (Thermo Fisher Scientific, Waltham, MA, USA). Following scanning of the processed GeneChips, cell intensity files, which provided a single intensity value for each probe cell, were generated from the image data using the Command Console software (Affymetrix). The compressed array image files (CEL files) were imported into the Applied Biosystems™ Transcriptome Analysis Console (TAC) (v. 4.0.2) software (Thermo Fisher Scientific) for calculation of summarized probe set signals (in log2 scale) using the Robust Multichip Analysis algorithm, comparison fold changes (FC) and significance P-values (ANOVA). Annotation of the microarrays was performed with the "ChiGene-1_0-st-v1.na36.galgal3.transcript.csv" annotation file. The microarray data of this study have been deposited in MIAME compliant format in the NCBI´s Gene Expression Omnibus public repository [44]. Owing to the rather moderate differences in the hepatic transcriptomes between groups HI-5.0 vs. HI-0 and groups HI-2.5 vs. HI-0, the differentially expressed transcripts were filtered based on a fold change > 1.5 or < −1.5 and a P-value < 0.05. Identical or similar filter criteria were also applied in several recent studies [45, 46]. Filtering of differentially expressed transcripts using the Benjamini & Hochberg false discovery rate adjustment method could not be applied, because the adjusted P-values for all transcripts were > 0.05.

Gene set enrichment analysis (GSEA) was performed with the identified differentially expressed transcripts in order to identify enriched Gene Ontology (GO) terms within GO category biological process using the Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.8 bioinformatic resource [47, 48]. Biological process and molecular function terms were considered as enriched if $P < 0.05$.

Validation of microarray data using qPCR analysis

Microarray data of 16 differentially expressed transcripts were validated by qPCR. For qPCR analysis, total RNA from all broilers ($n = 12$–14/group) was used. Synthesis of cDNA and qPCR analysis was performed with a Rotor-Gene Q system (Qiagen, Hilden, Germany) as described recently in detail [49]. Gene-specific primers were synthesized by Eurofins MWG Operon (Ebersberg, Germany). Characteristics of primers are listed in Additional file 1: Table S1. Normalization was carried out using multiple reference genes as described recently [50].

Tissue homogenization and lipid extraction

Frozen liver tissue was homogenized in methanol/water (50/50, v/v) with addition of 1% sodium laurylsulfate using bead-based homogenization at a concentration of 0.05 mg/µL [51]. Lipid class specific, non-endogenous internal standards were added prior to lipid extraction. An amount of 2 mg liver (wet weight) or a volume of 10 µL plasma was subjected to lipid extraction according to the protocol by Bligh and Dyer [52]. A volume of 0.5 mL of the chloroform phase was recovered by a pipetting robot and vacuum dried. The residue was dissolved in 1.2 mL chloroform/methanol/2-propanol (1:2:4, v/v/v) with 7.5 mmol/L ammonium formate.

Lipidomic analysis of major lipid classes by mass spectrometry

The analysis of lipids was performed by direct flow injection analysis (FIA) using a high-resolution Fourier Transform (FT) hybrid quadrupole-Orbitrap mass spectrometer (FIA-FTMS) [53]. TG, diglycerides (DG) and cholesteryl esters (CE) were recorded in positive ion mode as $[M+NH_4]^+$ in *m/z* range 500–1000 and a target resolution of 140,000 (at *m/z* 200). CE species were corrected for their species-specific response [54]. Ceramides (Cer), phosphatidylcholines (PC), ether PC (PC O), phosphatidylethanolamines (PE), ether PE (PE O), phosphatidylglycerols (PG), phosphatidylinositols (PI), and sphingomyelins (SM) were analyzed in negative ion mode in *m/z* range 520–960; lysophosphatidylcholines (LPC) and lysophosphatidylethanolamine (LPE) in *m/z* range 400–650. Multiplexed acquisition (MSX) was applied for free cholesterol (FC) and the internal standard FC[D7] [54]. Lipid annotation is based on the latest update of the shorthand notation [55].

The datasets from liver and plasma lipidomes were subjected to principal component analysis (PCA) using the MetaboAnalystR 3.2 package for R version 4.2.1. For the PCA, the relative metabolite composition of individual lipid species within the different lipid classes were used. Prior to the PCA, variables with missing values were either excluded from the analyzes if more than 50% of the samples were missing or the missing values were replaced by the limit of detection (1/5 of the minimum positive value of each variable). After normalization by log transformation and autoscaling the remaining values were used for the PCA.

Determination of fatty acid composition of total lipids of the diets and the liver

Fatty acid composition of total lipids of the liver and the diets was determined by gas chromatography-flame ionization detection (GC-FID). Briefly, total lipids were extracted from 75 mg liver aliquots with a 3:2 (v/v)-mixture of n-hexane and isopropanol containing C19:0 (50 mg/mL) as internal standard. After extraction, samples were centrifuged ($1200 \times g$, 10 min) and an aliquot of the supernatant was evaporated under a stream of N_2 at 37 °C. Lipids were subsequently transmethylated using trimethylsulfonium hydroxide solution (Sigma-Aldrich) and the resulting fatty acid methyl esters (FAME) were separated by a GC-FID system described in detail recently [56].

Statistical analysis

Statistical analysis was conducted with SPSS 27 software (IBM, Armonk, NY, USA). The cage served as the experimental unit for feed intake and feed:gain ratio and the individual animal for all other data. All parameters were tested with the Shapiro–Wilk test for normal distribution and with the Levene's test for homoscedasticity. When the normal distribution was followed only after a log transformation, the log transformed data were used for statistical analysis. Differences between the three groups were analyzed by one-way analysis of variance (one-way ANOVA) followed by a Tukey's post-hoc test when the data were normally distributed and the variances were homogeneous. If the data showed heterogeneity of variance, the means of the three groups were analyzed using Welch's ANOVA in conjunction with the post-hoc Games-Howell test. If the normal distribution was not followed, a Kruskal–Wallis one-way ANOVA was performed using the Mann–Whitney U test with Bonferroni correction as post-hoc test. For all tests, a *P*-value < 0.05 was considered statistically significant.

Results

Lipid composition of the HI fat

Analysis of the composition of major lipid classes of the HI fat revealed that HI total lipids consisted almost completely of TG (99.2% of total fat). All other lipid classes detected (PG, PC, DG, PE and SM) made up < 0.3% of total lipids (Table 2). Analysis of individual TG species demonstrated that TG 36:0, TG 38:0, TG 40:0 and TG 42:2 were the most abundant TG species, whereas all other TG species contributed < 5% of all TG species (Table 2). The majority of TG species of the HI fat was saturated (56.7%), while TG species with one, two and three or more double bonds made up 14.0%, 17.3% and 12.0%, respectively. Due to the low proportions of PG, PC, DG, PE, PC-O, SM and LPC in the HI fat, the individual lipid composition of these non-TG lipid classes is not reported. Analysis of the fatty acid composition of HI fat by GC-FID revealed that SFA were the dominating fatty acids, with C12:0 (57.2%), C14:0 (8.7%) and C16:0 (10.9%) contributing to 76.8% of total fatty acids (data not shown). The essential fatty acids C18:2 n-6 and C18:3 n-3 made up 10.6% and 0.8%, of total fatty acids, respectively.

Composition of the experimental diets

The three experimental diets within each feeding phase had similar concentrations of crude nutrients, sugar, starch and energy (Table 3) and amino acids (Additional file 1: Table S2), but substantially differed in the fatty acid composition of dietary total lipids (Table 3). With increasing replacement of soybean oil by HI fat, the proportions of C10:0, C12:0 and C14:0 markedly increased, while those of C18:0, C18:1, C18:2 n-6 and C18:3 n-3 decreased. As a consequence, the dominating fatty acids in the HI-5.0 diet were in decreasing

Table 2 Lipid composition of the *Hermetia illucens* larvae fat

Item	Content
Lipid class, % of total lipids	
TG	99.20 ± 0.02
PG	0.28 ± 0.01
PC	0.18 ± 0.01
DG	0.17 ± 0.01
PE	0.12 ± 0.02
SM	0.02 ± 0.01
TG species, % of total TG species[a]	
34:0	2.06 ± 0.04
36:0	30.06 ± 0.49
37:0	0.60 ± 0.31
38:0	12.25 ± 0.51
38:1	0.59 ± 0.01
40:0	7.76 ± 0.39
40:1	2.40 ± 0.10
42:0	2.11 ± 0.22
42:1	3.91 ± 0.08
42:2	6.04 ± 1.04
42:3	0.73 ± 0.07
44:0	0.92 ± 0.10
44:1	2.10 ± 0.21
44:2	1.65 ± 0.09
46:1	2.54 ± 0.43
46:2	4.80 ± 0.21
46:3	0.95 ± 0.03
48:1	0.92 ± 0.21
48:2	1.79 ± 0.15
48:3	1.78 ± 0.07
48:4	1.89 ± 0.18
50:2	1.21 ± 0.06
50:3	0.86 ± 0.03
52:2	0.56 ± 0.09
52:3	1.13 ± 0.02
52:4	1.30 ± 0.17
Sum DB0	56.73 ± 1.16
Sum DB1	13.99 ± 0.98
Sum DB2	17.29 ± 1.26
Sum DB3	6.33 ± 0.16
Sum DB4	4.18 ± 0.39
Sum DB5	0.94 ± 0.11
Sum DB6	0.45 ± 0.08
Sum DB7	0.09 ± 0.01

Abbreviations: *DB* Double bond, *DG* Diglycerides, *LPC* Lysophosphatidylcholine, *PC* Phosphatidylcholine, *PC O* PC-ether, *PE* Phosphatidylethanolamine, *PG* Phosphatidylglycerol, *SM* Sphingomyelin, *TG* Triglycerides

[a] Only TG species > 0.5% are shown. Data are means ± SD, $n = 4$ samples

order: C12:0, C18:2 n-6, C18:1, C16:0 and C14:0; in the HI-2.5 diet the dominating fatty acids were in decreasing order: C18:2 n-6, C18:1, C12:0, C16:0 and C18:3 n-3; in the HI-0 diet the dominating fatty acids were in decreasing order: C18:2 n-6, C18:1, C16:0, C18:3 n-3 and C18:0. Monounsaturated fatty acids and polyunsaturated fatty acids in the HI fat total lipids made up 9.4% and 11.4%, respectively.

Performance and ether extract digestibility

Performance data (body weight gain, final body weight, feed intake, and feed:gain ratio) during the whole period were not different between groups. However, body weight gain and feed intake during the starter period were higher in groups HI-2.5 and HI-5.0 than in group HI-0 ($P < 0.05$, Table 4). No differences of performance data between groups were found during the grower and the finisher period. AID coefficient for ether extract also did not differ between groups.

Cecal microbiota diversity and composition

In order to identify alterations of the cecal microbiota structure of the broilers, 16S rRNA-based high-throughput sequencing was applied. Following quality check, chimera check and filtering, the high-quality sequences obtained from the cecum digesta samples of the 40 broilers were delineated into 90 OTUs at 97% sequence identity (Additional file 1: Table S3). Treatment effect on microbial diversity was evaluated by the use of different diversity metrics. None of the metrics used to describe α-diversity (effective species richness, shannon effective, simpson effective, evenness) differed between groups (Fig. 1a). In addition, β-diversity of cecal bacterial community calculated based on generalized UniFrac distances did not differ among groups. The MetaNMDS plot generated to visualize the difference in β-diversity of cecal bacterial community among groups shows that no clustering was most visible among groups (Fig. 1b).

To analyze the effect on the microbiota composition, the microbial community was analyzed at different taxonomic levels (phylum, class, order, family, genus). In total, only the relative abundances of four different bacterial taxa differed among groups. At the phylum level, only the relative abundance of the least abundant bacterial phylum, Actinobacteriota, contributing less than 0.15% to total bacterial phyla was different among groups. While the relative abundance of this phylum was lower in group HI-5.0 than in group HI-2.5 ($P < 0.05$), it did not differ between group HI-5.0 and HI-0 and between group HI-2.5 and HI-0 (Fig. 1c). Amongst five different bacterial classes identified in the cecal digesta of the broilers, only the relative abundance of Coriobacteriia, which belong to the phylum Actinobacteriota, was affected by treatment; while the relative abundance of this phylum was lower in group HI-5.0 than in group HI-2.5 ($P < 0.05$), it did not differ between group HI-5.0 and HI-0 and between group HI-2.5 and HI-0. At

Table 3 Concentrations of nutrients and energy in the broiler diets

Item	Starter diets			Grower diets			Finisher diets		
	HI-0	HI-2.5	HI-5.0	HI-0	HI-2.5	HI-5.0	HI-0	HI-2.5	HI-5.0
Analyzed crude nutrient content									
Dry matter, % FM	87.5	87.5	87.4	87.6	87.3	87.7	87.5	87.4	88.1
Crude protein, % DM	21.6	21.6	21.6	19.8	20.0	20.0	19.1	19.0	19.3
Ether extract, % DM	7.3	7.1	7.2	7.6	7.4	7.4	7.6	7.4	7.2
Crude ash, % DM	5.6	5.9	5.9	5.5	5.5	5.6	5.5	5.4	5.5
Crude fiber, % DM	3.5	3.6	3.5	3.9	3.9	3.9	4.2	4.3	4.0
Sugar, % DM	3.3	3.0	2.7	3.8	3.0	2.7	2.9	3.0	3.4
Starch, % DM	35.7	35.9	35.5	37.2	36.4	37.7	36.4	38.6	38.3
AME_N[#], MJ/kg DM	12.2	12.2	12.1	12.4	12.1	12.3	12.0	12.3	12.3
Fatty acids[a], % of total fatty acids									
C10:0	-	0.41	0.82	-	0.46	0.81	-	0.44	0.80
C12:0	0.42	18.23	37.65	0.22	19.06	38.09	0.50	19.06	38.03
C14:0	0.18	2.93	5.89	0.15	3.09	5.97	0.20	3.08	6.05
C16:0	11.82	11.88	12.17	11.44	12.06	12.19	11.59	12.12	12.26
C16:1	0.22	1.00	1.90	0.16	1.02	1.92	0.16	1.07	1.92
C18:0	3.99	3.10	1.85	3.98	3.09	1.86	3.89	3.09	1.87
C18:1	24.00	19.44	12.42	25.57	18.65	12.60	25.00	19.00	12.56
C18:2 n-6	51.32	37.73	22.94	50.88	36.83	23.13	50.93	37.26	23.02
C18:3 n-3	5.66	3.74	1.65	5.48	3.55	1.64	5.55	3.56	1.62
C20:0	0.51	0.38	0.28	0.46	0.37	0.28	0.45	0.34	0.28

Abbreviations: AME_N Apparent N-corrected metabolizable energy, *DM* Dry matter, *FM* Fresh matter

[a] Only fatty acids > 0.5% of total fatty acids are shown. [#]The AME_N content of the feed was calculated according to the formula of the World´s Poultry Science Association for poultry compound feed

the order level, the relative abundance of Coriobacteriales (class Coriobacteriia) was identified as the only bacterial order differing between groups; its relative abundance was lower in group HI-5.0 than in group HI-2.5 ($P < 0.05$), but was not different between groups HI-5.0 and HI-2.5 (Fig. 1c). At the family level, the only taxon identified to be altered was the Eggerthellaceae (order Coriobacteriales), whose abundance was lower in group HI-5.0 than in group HI-2.5 ($P < 0.05$), but was not different between group HI-5.0 and HI-0 and between group HI-2.5 and HI-0 (Fig. 1c). No differences between groups were found regarding the relative abundances of bacterial genera.

Cecal concentrations of short-chain fatty acids

The concentrations of total and individual SCFA [acetic acid (C2:0), propionic acid (C3:0), isobutyric acid (iC4:0), butyric acid (C4:0), isovaleric acid (iC5:0) and valeric acid (C5:0)] in the cecal digesta were not different between the three groups (Fig. 2).

Liver transcriptome

According to the filter criteria applied ($P < 0.05$; fold change > 1.5 and < −1.5), a total of 55 transcripts were identified as differentially expressed (upregulated: 31, downregulated: 24) in the liver between groups HI-5.0 and HI-0. In Fig. 3a, the differentially expressed transcripts between groups HI-5.0 and HI-0 are illustrated as red dots in the Volcano plot. Amongst the upregulated genes, only three genes (*BDKRB1*, *XDH*, *IGJ*) exhibited a regulation > 2.0-fold. The top 10 upregulated transcripts were in decreasing order of their fold change (in brackets): *BDKRB1* (2.57), *XDH* (2.36), *IGJ* (2.10), *DPP4* (1.90), *ENDOUL* (1.83), *UPP2* (1.82), *EMB* (1.81), *BASP1* (1.76), *SIK1* (1.71) and *B3GALT2*. Amongst the downregulated genes, five genes (*IL22RA2*, *ANKRD22*, *LOC101749538*, *ATP2B2*, *SLC6A13*) were regulated < −2.0-fold. The top 10 downregulated transcripts were in increasing order of their fold change: *IL22RA2* (−5.09), *ANKRD22* (−3.76), *LOC101749538* (−2.55), *ATP2B2* (−2.15), *SLC6A13* (−2.12), *DDO* (−1.89), *SCAP* (−1.88), *SPATA4* (−1.84), *CA4* (−1.82) and *ULK1* (−1.81). The fold change and *P*-value of all differentially expressed transcripts between group HI-5.0 vs. HI-0 are shown in Additional file 1: Table S4.

Considering the same filter criteria as for the comparison of groups HI-5.0 vs. HI-0, a total of 25 transcripts were identified as differentially expressed (upregulated: 19, downregulated: 6) in the liver between groups

Table 4 Performance data and apparent ileal digestibility (AID) coefficient for ether extract of broilers fed diets with either 0 (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) *Hermetia illucens* (HI) larvae fat for 35 d

Item	HI-0	HI-2.5	HI-5.0	*P*-value
Whole period (d 1 to 35)				
Initial BW, g	44.8 ± 0.4	44.7 ± 0.9	45.1 ± 0.9	0.719
Final BW, g	2740 ± 139	2696 ± 237	2738 ± 92	0.860
BW gain, g	2696 ± 138	2651 ± 237	2693 ± 92	0.861
Feed intake, g	3745 ± 183	3696 ± 236	3719 ± 127	0.898
Feed:Gain ratio, g/g	1.39 ± 0.02	1.40 ± 0.11	1.38 ± 0.03	0.537
Starter period (d 1 to 10)				
BW gain, g	272 ± 17^{b}	290 ± 10^{a}	287 ± 12^{a}	0.049
Feed intake, g	290 ± 19^{b}	307 ± 8^{a}	308 ± 11^{a}	0.042
Feed: Gain ratio, g/g	1.09 ± 0.02	1.09 ± 0.01	1.08 ± 0.01	0.437
Grower period (d 11 to 21)				
BW gain, g	823 ± 37	830 ± 61	819 ± 34	0.901
Feed intake, g	1068 ± 53	1081 ± 61	1065 ± 43	0.842
Feed: Gain ratio, g/g	1.30 ± 0.01	1.30 ± 0.04	1.30 ± 0.03	0.926
Finisher period (d 22 to 35)				
BW gain, g	1601 ± 91	1522 ± 210	1587 ± 79	0.560
Feed intake, g	2387 ± 124	2309 ± 181	2346 ± 98	0.612
Feed: Gain ratio, g/g	1.49 ± 0.03	1.54 ± 0.23	1.48 ± 0.06	0.822
AID coefficient for EE, %	89.0 ± 1.6	88.6 ± 1.5	88.8 ± 2.8	0.950

Data are means ± SD, $n = 6–7$ cages/groups. Abbreviation: *BW* Body weight, *EE* Ether extract

HI-2.5 and HI-0 (Fig. 3b). Only three genes (*NSMF*, *CHIA*, *BDKRB1*) were regulated > 2.0-fold and no genes were regulated < −2.0-fold. The 10 most strongly upregulated transcripts were in decreasing order of their fold change (in brackets): *NSMF* (2.29), *CHIA* (2.16), *BDKRB1* (2.04), *ENPEP* (1.80), *PFKFB3* (1.80), *MIR1790* (1.72), *SIK1* (1.71), *DPP4* (1.71), *LOC395159* (1.69) and *CP* (1.66). The 6 downregulated transcripts were in increasing order of their fold change: *GUCY2C* (−1.53), *ATP2B2* (−1.53), *INDOL1* (−1.51), *MIR17* (−1.51), *DDO* (−1.51) and *PROM1* (−1.50). The fold change and *P*-value of all differentially expressed transcripts between groups HI-2.5 vs. HI-0 are shown in Additional file 1: Table S5.

Microarray data of 16 differentially expressed transcripts between groups HI-5.0 and HI-0 were validated by qPCR. As shown in Additional file 1: Table S6, the effect direction (positive or negative fold change) was the same between microarray and qPCR for all validated transcripts, whereas the effect size (value of fold change) differed to some extent for the validated transcripts between microarray and qPCR. Statistical analysis of qPCR data revealed that twelve transcripts were regulated either significantly (*CA4*, *DDO*, *IL22RA2*, *HMGCS1*, *SCAP*, *SH2D4A*, *ULK1*, *XDH*) or at a *P*-value < 0.1 (*AKR1D1*, *ANKRD22*, *MTHFS*, *OGN*), whereas four transcripts (*DPP4*, *FANCL*, *SIK1*, *SPATA4*) were not regulated.

In order to extract biological meaning from the transcripts differentially expressed between groups HI-5.0 and HI-0, GSEA was performed using GO category biological process. Due to the low number of differentially expressed transcripts, GSEA was not conducted for the comparison of groups HI-2.5 and HI-0. Within GO category biological process, the most enriched biological process terms assigned to the transcripts regulated between groups HI-5.0 and HI-0 were (in increasing order of their *P*-values): small molecule biosynthetic process, cholesterol metabolic process, secondary alcohol metabolic process, sterol metabolic process, lipid biosynthetic process, steroid biosynthetic process, cholesterol biosynthetic process and secondary alcohol biosynthetic process (Fig. 3c).

Liver and plasma lipidomes

The major lipid classes in the liver were in decreasing order: TG, PC, PE, FC, PI, DG, SM, CE, PE O, Cer, LPC, LPE, PC O and PG. Despite that the sum of total lipid classes in the liver were not different between groups (HI-0: 47.6 ± 8.9 μmol/g, HI-2.5: 42.4 ± 8.9 μmol/g, HI-5.0: 47.7 ± 6.5 μmol/g, mean + SD, $n = 12–14$/group, $P = 0.170$), hepatic concentrations of PE, LPC, LPE and PG differed between groups ($P < 0.05$), even though only slightly (Fig. 4a). Hepatic concentrations of LPE and PG

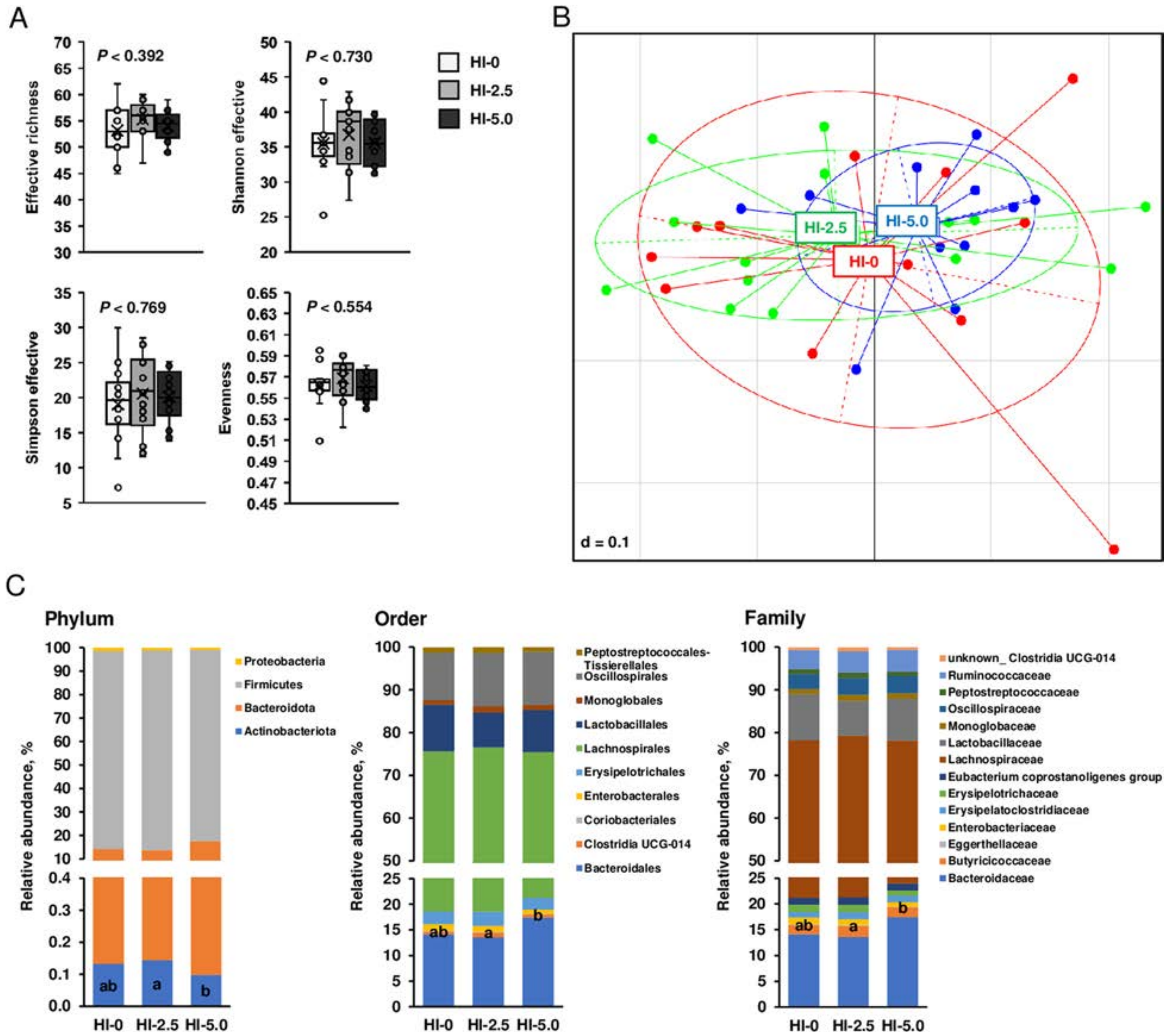

Fig. 1 Analysis of the cecal microbiome. Indicators of α-diversity (effective richness, shannon effective, simpson effective, evenness) of the cecal bacterial community (**A**), visualization of the difference in β-diversity of cecal bacterial community between groups by non-metric multidimension distance scaling plot (**B**), and distribution of cecal bacteria at different taxonomic levels (phylum, order, family) (**C**) of broilers fed diets with either 0 (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) *Hermetia illucens* (HI) larvae fat for 35 d. **A**: Box and whisker plots for $n = 12–14$ broilers/group; **C**: Data are means for $n = 12–14$ broilers/group. [a,b]Means without a common letter differ across the groups, $P < 0.05$

were higher in group HI-5.0 than in groups HI-0 and HI-2.5 ($P < 0.05$). Concentrations of PE and LPC in the liver were higher in group HI-5.0 than in group HI-2.5 ($P < 0.05$), but not compared to group HI-0.

In contrast to the slight or absent differences in the concentrations of the lipid classes in the liver between groups, pronounced differences between groups were seen with regard to the composition of individual lipid species within the different lipid classes. Regarding TG, the dominating lipid class in the liver, relative proportions of species with zero and one double bonds were higher in group HI-5.0 than in group HI-0, while those with five and six double bonds were lower in group HI-5.0 than in group HI-0 ($P < 0.05$, Fig. 4b). With regard to PC, the second most abundant hepatic lipid class, relative proportions of species with one and three double bonds were higher and those with four, five and six double bonds were lower in group HI-5.0 than in group HI-0 ($P < 0.05$, Fig. 4c). Within PE, the third most abundant lipid class in the liver, relative proportions of species with two and three double bonds were higher and those with four, six and eight double bonds were lower in group

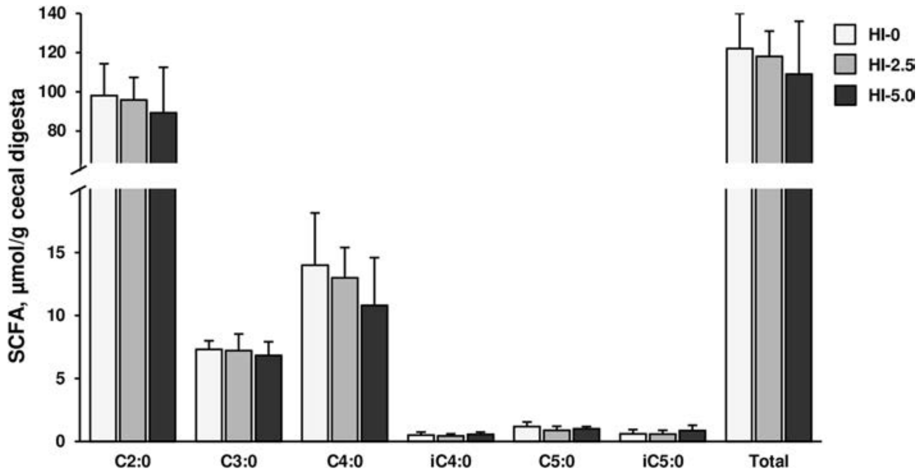

Fig. 2 Analysis of microbial fermentation profile in the gut. Concentrations of individual [acetic acid (C2:0), propionic acid (C3:0), butyric acid (C4:0), isobutyric acid (iC4:0), valeric acid (C5:0), isovaleric acid (iC5:0)] and total (= sum of individual) short-chain fatty acids (SCFA) in the cecal digesta of broilers fed diets with either 0 (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) *Hermetia illucens* (HI) larvae fat for 35 d. Data are means ± SD, $n = 12–14$ broilers/group

HI-5.0 than in group HI-0 ($P < 0.05$, Fig. 4d). The proportions of all individual TG, PC and PE species are shown in Additional file 1: Table S7.

In plasma, the major lipid classes were in decreasing order: PC, CE, FC, TG, PE, SM, LPC, PC O, PE O, DG, LPE and Cer (Fig. 5a). Like the concentrations of most lipid classes (PC, FC, TG, PE, SM, LPC, PC O, PE O, DG, LPE), the sum of total lipid classes in plasma did not differ among groups (HI-0: 7992 ± 1423 µmol/L, HI-2.5: 7683 ± 1742 µmol/L, HI-5.0: 8550 ± 752 µmol/L, mean + SD, $n = 12–14$/group, $P = 0.358$). In contrast, the plasma concentrations of CE and Cer differed among groups ($P < 0.05$), but only slightly; plasma concentrations of CE and Cer were higher in group HI-5.0 than in group HI-0. Plasma concentration of Cer in group HI-5.0 was also higher than in group HI-2.5 ($P < 0.05$), whereas plasma concentration of CE did not differ between group HI-5.0 and group HI-2.5. Similar as in the liver, pronounced differences among groups were observed regarding the composition of individual lipid species within the different lipid classes in plasma. With regard to CE, the dominating lipid class in the plasma, relative proportions of species with zero and one double bonds were higher in group HI-5.0 than in group HI-0, while those of species with two, three, four, five and six double bonds were lower in group HI-5.0 than in group HI-0 ($P < 0.05$, Fig. 5b). Regarding PC, the second most abundant plasma lipid class, the relative proportions of PC species with zero, one and three double bonds were higher, whereas those with four, five, six and seven double bonds were lower in group HI-5.0 than in group HI-0 ($P < 0.05$, Fig. 5c). With regard to TG, relative proportions of species with zero, one, and two double bonds were higher and those with four, five, six, seven and eight double bonds were lower in group HI-5.0 than in group HI-0 ($P < 0.05$, Fig. 5d). The proportions of all individual CE, PC and TG species in plasma are shown in Additional file 1: Table S8.

The pronounced differences among groups with regard to the composition of individual lipid species within the different lipid classes in liver and plasma were reflected by the results from PCA analysis which was carried out with the relative proportions of individual lipid species of all lipid classes. The dimensional reduction of the lipidome datasets from the liver (Fig. 6a) and the plasma (Fig. 7a) revealed a clear separation (rightward shift) between the three feeding groups with increasing dietary levels of HI fat. For the liver lipidome the cumulative proportion is 54.6% with principal component 1 accounting for more than 40% of the variance of the dataset. In the plasma, the cumulative proportion adds up to 51.8% with principal component 1 accounting for 38.8%. The loading plot for the liver lipidome dataset shows that the left shift of the HI-0 group was mainly caused by individual TG and PC lipid species with three or more double bonds and the right shift of the HI-2.5 and HI-5.0 groups was mainly caused by individual TG, PC and PE lipid species with less than 3 double bounds (Fig. 6b). In the plasma, the right shift of the HI-2.5 and HI-5.0 groups was largely driven by individual TG, PC and PE species with less than three double bounds, while the left shift of the HI-0 group is driven by individual TG, PC and CE species with more than 3 double bounds (Fig. 7b).

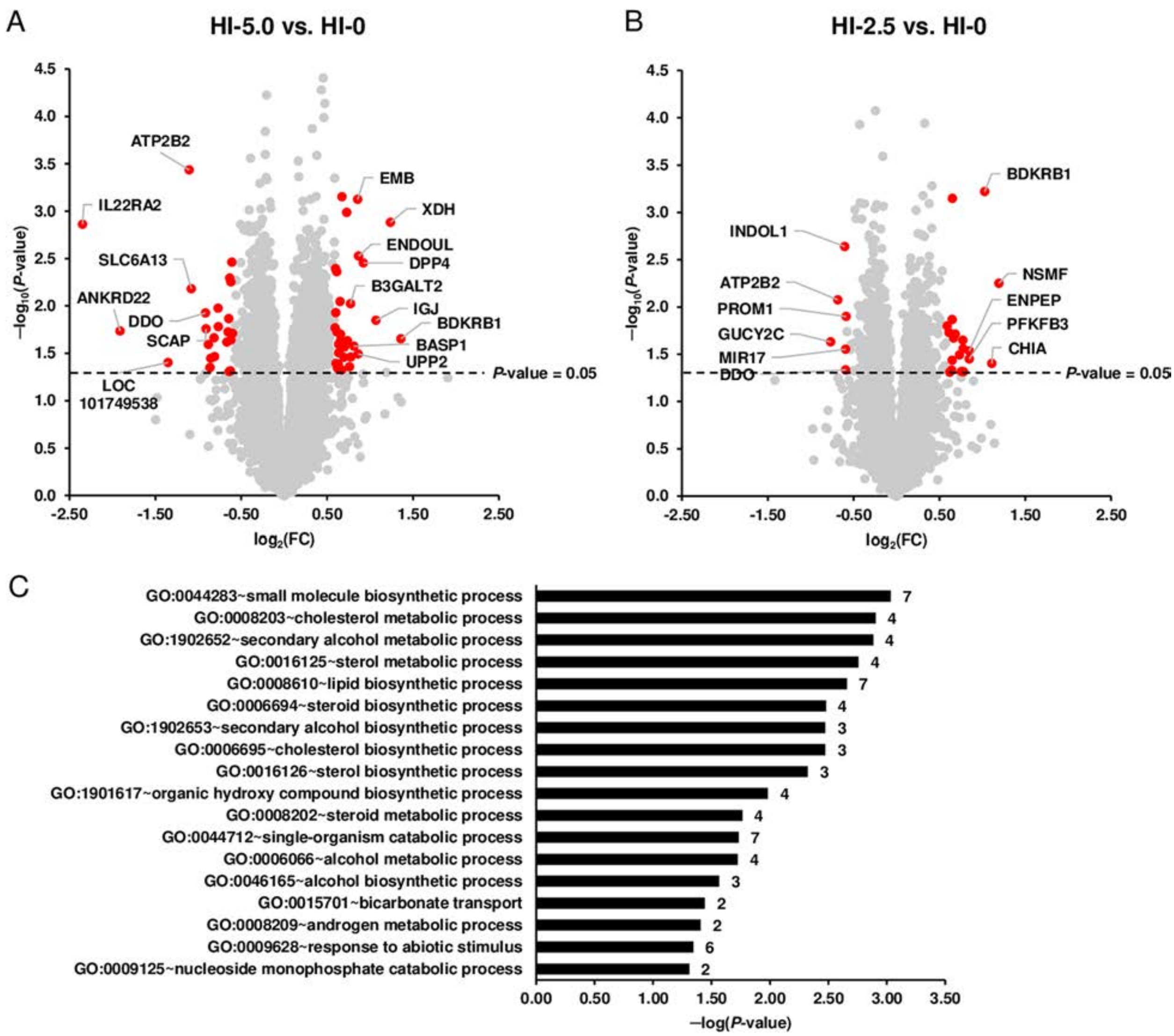

Fig. 3 Differential transcriptome analysis of the liver. Volcano plot illustrating the differentially expressed transcripts in the liver of broilers between group HI-5.0 vs. HI-0 (**A**) and group HI-2.5 vs. HI-0 (**B**). The double filtering criteria are indicated by horizontal (*P*-value < 0.05) and vertical (fold change > 1.5 or < −1.5) dashed lines. Red dots in the upper left and the upper right corner represent the downregulated and the upregulated transcripts, respectively. **C** Most enriched gene ontology (GO) biological process terms associated with the transcripts differentially expressed between group HI-5.0 vs. HI-0. GO terms are sorted by their enrichment *P*-values (EASE score) (top: lowest *P*-value, bottom: highest *P*-value)

Fatty acid composition of liver total lipids

As expected, fatty acid composition of hepatic total lipids was affected by dietary inclusion of HI fat. Proportions of C12:0, C14:0 and C16:1 were higher in group HI-5.0 than in groups HI-2.5 and HI-0, whereas proportion of C18:3 n-3 was lower in groups HI-5.0 and HI-2.5 than in group HI-0 ($P < 0.05$, Table 5).

Discussion

In the present study, the effect of replacement of soybean oil with HI larvae fat in broiler diets on the cecal microbiome and metabolic health was comprehensively investigated using omics technologies. Since no study existed in the literature reporting the lipid composition of HI fat in detail, with the exception of total lipid fatty acid composition, lipidomics was applied for the first time to fully characterize the HI fat. Our lipidomic analysis showed that the HI larvae fat used consisted almost completely (> 99%) of TG, whereas PG, PC, DG, PE and SM were only minor lipid classes. Amongst the TG species, saturated ones (34:0, 36:0, 38:0, 40:0, 42:0) made up approximately 60% of all TG species. TG species with one or two double bonds contributed to nearly 30% of all TG species, while those with three or more

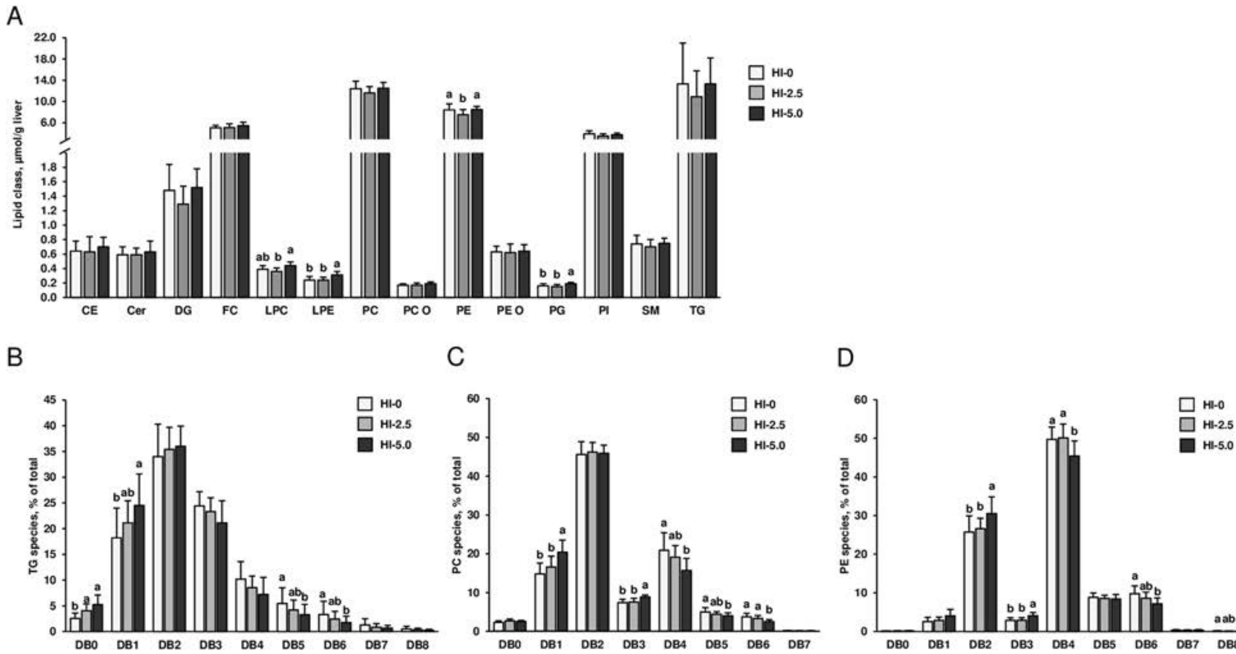

Fig. 4 Analysis of the liver lipidome. Concentrations of the major lipid classes in the liver (**A**) and lipid species composition within the three most abundant lipid classes in the liver, TG (**B**), PC (**C**) and PE (**D**), of broilers fed diets with either 0 (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) *Hermetia illucens* (HI) larvae fat for 35 d. Lipid species were grouped according to the number of double bonds (DB). Data are means ± SD, $n = 12–14$ broilers/group. Abbreviations: CE, cholesteryl ester; Cer, ceramide; DG, diglycerides; FC, free cholesterol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PC O, PC-ether; PE, phosphatidylethanolamine; PE O, PE-ether; PG, phosphatidylglycerol; PI, phosphatidylinositol; SM, sphingomyelin; TG, triglycerides

double bonds contributed less than 10% of all TG species. In line with the high proportion of saturated TG species and TG species with a low number of double bonds, additional fatty acid analysis of HI larvae fat total lipids revealed that C12:0, C16:0, C14:0 and C10:0 in decreasing order of their proportions made up nearly 80% of total fatty acids. Our observation that saturated TG species made up 60% of all TG species, while saturated fatty acids made up 80% of all fatty acids in HI fat total lipids, however, is not a discrepancy. This is likely attributed to the fact that saturated fatty acids are not exclusively found in TG species with zero double bonds, but are also present in TG species with 1 or more double bonds. Regardless of this, our data from lipidomics and fatty acid analysis clearly show that the HI fat used in the present study contained large amounts of saturated fatty acids, which largely explains the hard consistency of the HI fat at room temperature. Despite the marked difference in the fatty acid profile between soybean oil, the commonly used fat source in broiler diets, and HI larvae fat, partial or complete replacement of soybean oil with HI larvae fat had no effect on performance parameters (feed intake, body weight gain, feed:gain ratio) of the broilers over the whole 35 d-period. In line with the unaltered growth performance, ileal digestibility of ether extract determined during the finishing period did not differ among groups. This indicates that soybean oil replacement with HI larvae fat has neither beneficial nor adverse effects on fat digestibility, feed utilization and growth performance of broilers. However, our study revealed that feed intake and body weight gain, but not feed efficiency were increased by dietary inclusion of HI larvae fat during the starter period, whereas no effect was found during the grower and the finisher period. This suggests that feed acceptance was obviously improved in broilers during the early d of life by the use of HI larvae fat. The reason underlying this observation is unclear, but we exclude the possibility that the taste of the soybean oil used in the HI-0 diet was impaired due to fat deterioration during storage or diet preparation, because the same batch of soybean oil was also used for the diet of group HI-2.5, despite feed intake of this group was similar as in group HI-5.0. Unfortunately, comparable data from other studies are not available to explain our observation. While in two other studies dealing with the use of HI larvae fat as an alternative fat source in broiler diets performance data were reported either for the whole period [16] or only the finishing period [13], a further study did not provide any performance data [17]. However, the latter study is not suitable due to inadequate control of nutrient composition of the

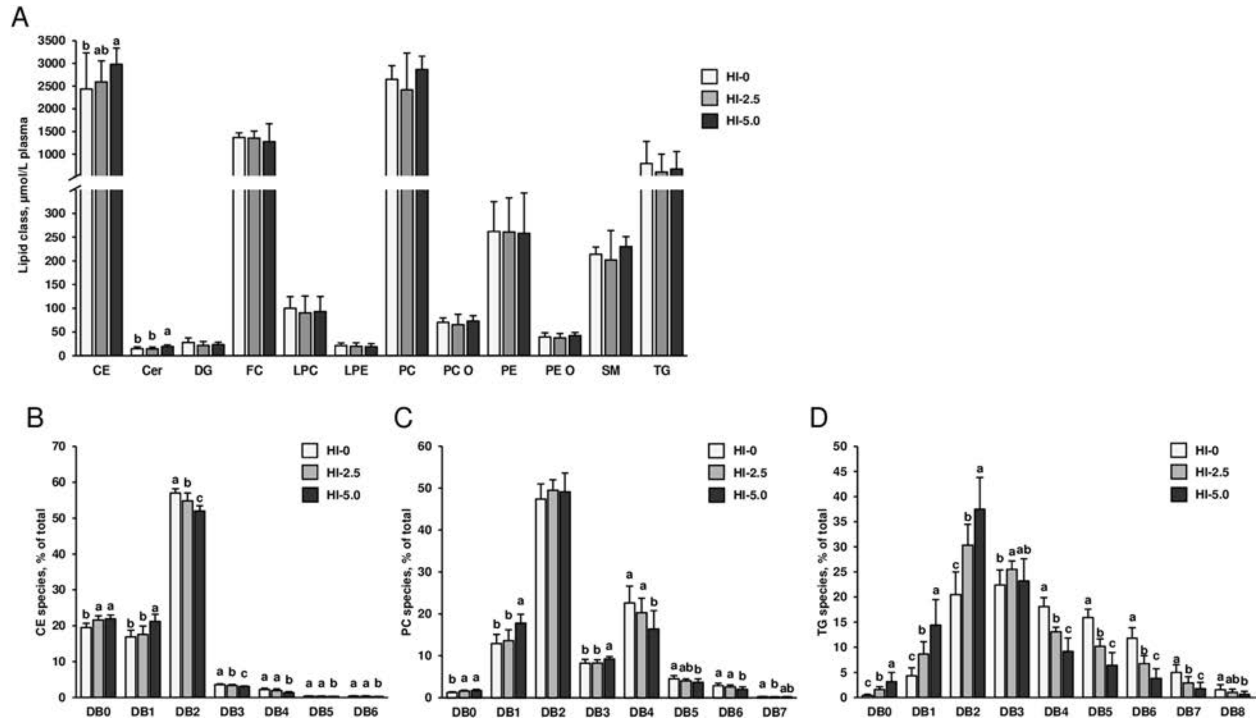

Fig. 5 Analysis of the plasma lipidome. Concentrations of the major lipid classes in the plasma (**A**) and lipid species composition within the three most abundant lipid classes in plasma, CE (**B**), PC (**C**) and TG (**D**), of broilers fed diets with either 0 (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) *Hermetia illucens* (HI) larvae fat for 35 d. Lipid species were grouped according to the number of double bonds (DB). Data are means ± SD, $n = 12–14$ broilers/group. Abbreviations: CE, cholesteryl ester; Cer, ceramide; DG, diglycerides; FC, free cholesterol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PC O, PC-ether; PE, phosphatidylethanolamine; PE O, PE-ether; SM, sphingomyelin; TG, triglycerides

diets as evident from the marked difference of crude fat content between the starter diets (3.6% and 5.9% in the control diet and HI larva fat diet, respectively).

Despite studies demonstrating antimicrobial effects of MCFA in the intestine of broilers at dosages between 0.35% and 1.4% in the feed [22, 23], the present study, in which the dietary concentration of MCFA (sum of C10:0 and C12:0) was about 1.25% and 2.5% in groups HI-2.5 and HI-5.0, respectively, showed only a very little impact of dietary inclusion of HI larvae fat on the cecal microbiome. This was evident from the observation that cecal microbial diversity (α and β) did not differ between groups and taxonomic analysis revealed differences in the abundance of only four low-abundance bacterial taxa among groups; namely, the relative abundances of the phylum Actinobacteriota, the class Coriobacteriia, the order Coriobacteriales and the family Eggerthellaceae (all belonging to Actinobacteriota) were lower in group HI-5.0 compared to group HI-2.5, but the abundances of these bacterial taxa did not differ between groups HI-5.0 and HI-0 and between groups HI-2.5 and HI-0. No differences at all were found between groups in the abundances of bacterial families belonging to the two main phyla, Bacteroidota and Firmicutes, together accounting for approximately 99% of all bacteria in the three groups. Considering this and the rather small reduction in the abundances of the abovementioned bacterial taxa suggests that the impact of HI larvae fat on the cecal microbiota structure was very low. One important reason might be that the amount of MCFA reaching the cecum was not sufficient to exert pronounced antimicrobial effects. This assumption is supported by our observation that the ileal digestibility of crude fat was very high (approximately 90% in all groups) and the fact that TG-bound MCFA are hydrolysed and absorbed more rapidly than TG-bound long-chain fatty acids due to easier emulsification and less dependence on pancreatic lipase activity [27, 28]. In agreement with this, an increased ileal fat digestibility has been observed in a recent study with broilers by dietary substitution of soybean oil by HI fat [20]. Nevertheless, reports exist demonstrating that short-term administration of specific MCFA, such as caprylic acid (C8:0) and 1-monoglyceride of capric acid (C10:0), via the feed or the drinking water reduces cecal colonization of pathogenic bacteria, such as *Campylobacter jejuni*, after artificial infection [22, 23, 57].

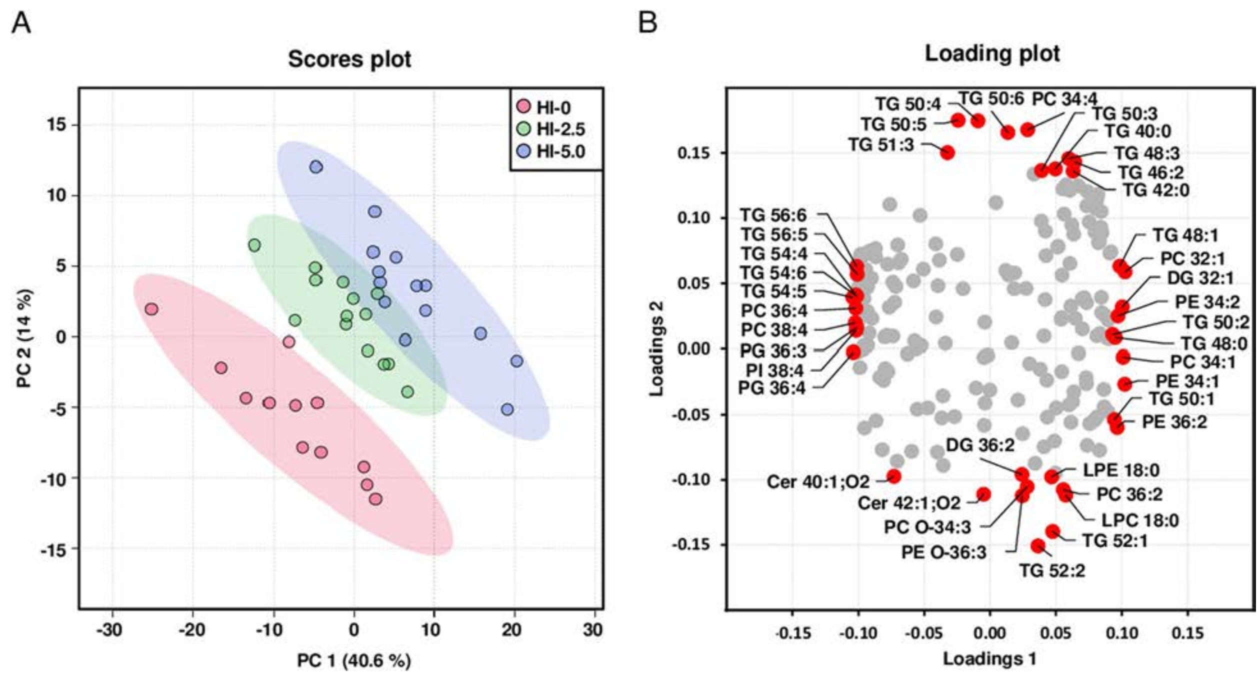

Fig. 6 Principal component analysis of liver lipidome. Scores plot with plotted 5% confidence interval (**A**) and associated loading plot (**B**) of principal component analysis (PCA) of the liver lipidome of the broilers. Broilers were fed diets with either 0 (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) *Hermetia illucens* (HI) larvae fat for 35 d. Data are principal components (PC 1 or PC 2) and their loadings calculated based on the relative abundances of the lipid species within their lipid classes, $n = 12–14$ broilers/group. Abbreviations: Cer, ceramide; DG, diglycerides; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PC O, PC-ether; PE, phosphatidylethanolamine; PE O, PE-ether; PG, phosphatidylglycerol; PI, phosphatidylinositol; TG, triglycerides

However, contradictory results have been reported from another study, in which addition of either non-coated or coated specific MCFA (C6:0, C8:0, C10:0) to the feed at comparable concentrations as in the studies from Solis de los Santos et al. [22, 23] did not reduce cecal *Campylobacter* colonization in broilers [58]. The reason for these contradictory results is not clear, but differences between studies in the formulation of the MCFA in the feed may explain that the MCFA were less absorbed in the small intestine, thereby, reaching the ceca at higher concentrations in the one study than in the other. Thus, it is possible that the formulation of the diets containing HI larvae fat in the present study allowed an efficient absorption of MCFA in the small intestine, but a low passage of MCFA into the ceca of the broilers.

Owing to differences between bacterial families of different taxa with regard to the use of fermentation substrates and the metabolic pathways engaged in substrate utilization, a shift in the gut microbial community is typically accompanied by an altered profile of microbial fermentation products, such as SCFA, in the gut. Since dietary inclusion of HI larvae fat into the broiler diets did not cause a substantial shift in the bacterial community of the cecum, the unaltered concentrations of total and individual SCFA in the cecal digesta among the three groups are not surprising and are a further indication of the limited impact of HI larvae fat on the cecum microbiota of the broilers. In agreement with our results, a recent study demonstrated that complete replacement of 5% corn oil in the diet with HI larvae fat did not affect the concentrations of total and individual SCFA and viable counts of *Clostridium perfringens* in the ileum and cecum of broilers during a 30-d-feeding period [16]. In addition, feeding a diet containing about 1% MCFA as lauric acid had no influence on the DNA copy numbers of some beneficial bacteria (*Lactobacillus* and *Bifidobacterium* spp.), opportunistic pathogens (Enterobacteriaceae, *Escherichia coli*) and the pathogen *Campylobacter jejuni* in the jejunal digesta of broilers [59].

Consistent with our finding that the dietary fats including the HI larvae fat were readily absorbed in the small intestine, the present study revealed some effects of dietary inclusion of HI larvae fat on the hepatic and plasma lipidomes of the broilers. However, the effects of dietary inclusion of HI larvae fat were mainly related to the individual lipid species composition of the lipid classes, whereas the concentrations of the different lipid classes in liver and plasma were either not or only marginally (liver: PE, PG, LPC and LPE; plasma: CE and Cer) affected. In line with this, the sum of total lipids in

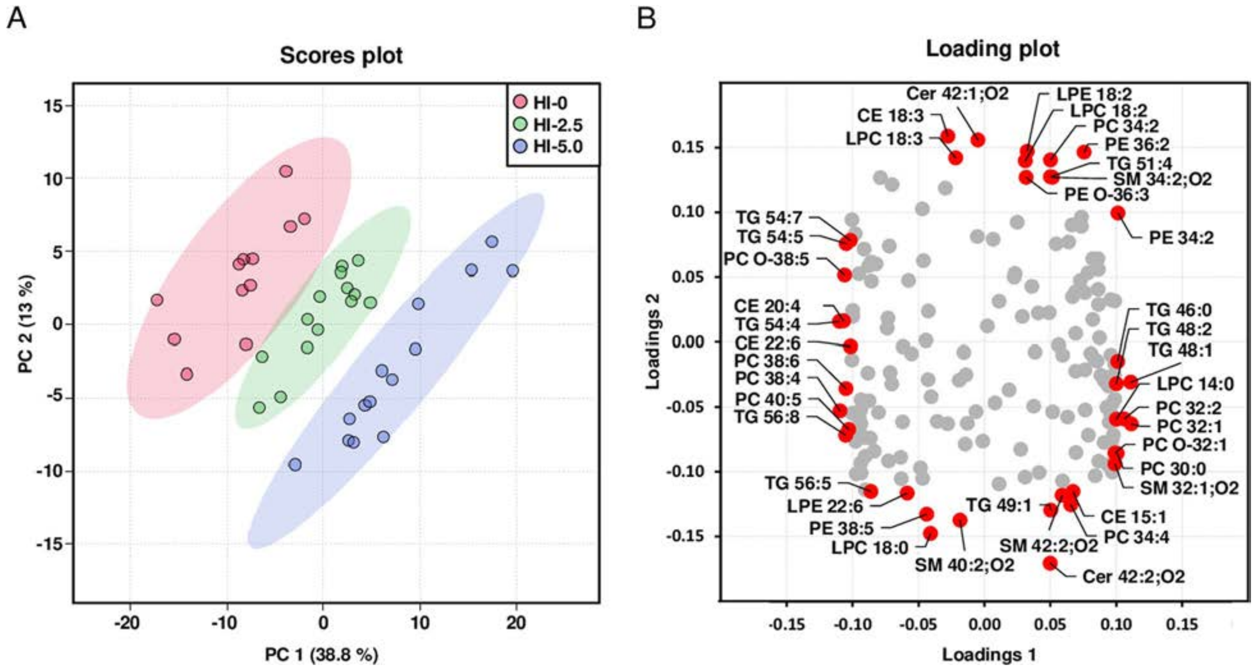

Fig. 7 Principal component analysis of plasma lipidome. Scores plot with plotted 5% confidence interval (**A**) and associated loading plot (**B**) of principal component analysis (PCA) of the plasma lipidome of the broilers. Broilers were fed diets with either 0 (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) *Hermetia illucens* (HI) larvae fat for 35 d. Data are principal components (PC 1 or PC 2) and their loadings calculated based on the relative abundances of the lipid species within their lipid classes, $n = 12$–14 broilers/group. Abbreviations: CE, cholesteryl ester; Cer, ceramide; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PC O, PC-ether; PE, phosphatidylethanolamine; PE O, PE-ether; SM, sphingomyelin; TG, triglycerides

Table 5 Fatty acid composition of hepatic total lipids of broilers fed diets with either 0 (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) *Hermetia illucens* (HI) larvae fat for 35 d

Fatty acid*, g/100 g total fatty acids	HI-0	HI-2.5	HI-5.0	*P*-value
C12:0	0.56 ± 0.17^{c}	1.03 ± 0.2^{b}	1.53 ± 0.34^{a}	0.000
C14:0	0.48 ± 0.18^{c}	1.18 ± 0.41^{b}	1.89 ± 0.53^{a}	0.000
C16:0	22.0 ± 2.7	22.4 ± 2.1	22.3 ± 2.4	0.945
C16:1	1.93 ± 0.68^{b}	1.81 ± 0.85^{b}	2.62 ± 0.55^{a}	0.012
C18:0	21.5 ± 2.9	21.5 ± 2.4	19.7 ± 2.2	0.108
C18:1 n-9	21.6 ± 4.4	20.4 ± 4	23.1 ± 3.3	0.204
C18:2 n-6	20.5 ± 2.6	20.4 ± 2.2	19.3 ± 2.9	0.436
C18:3 n-3	0.61 ± 0.09^{a}	0.50 ± 0.11^{b}	0.43 ± 0.10^{b}	0.000
C20:3 n-6	0.87 ± 0.31	0.97 ± 0.33	0.95 ± 0.15	0.352
C20:4 n-6	8.52 ± 2.09	8.46 ± 1.74	7.10 ± 1.34	0.065
C22:6 n-3	0.92 ± 0.37	0.83 ± 0.34	0.67 ± 0.18	0.035

Data are means ± SD, $n = 12$–14 broilers/group. *Only fatty acids > 0.5 g/100 g total fatty acids are shown

liver and plasma were not influenced by dietary treatment. With regard to the individual lipid species composition of the different lipid classes, the most striking observation was that the proportions of lipid species with zero or few double bonds (e.g., in the liver: TG species with 0 and 1 double bonds, PC species with 1 and 3 double bonds, PE species with 2 and 3; in plasma: TG species with 0, 1 and 2 double bonds) were increased but those with four or more double bonds (e.g., in the liver: TG species with 5 and 6 double bonds, PC species with 4, 5 and 6 double bonds, PC species with 4, 6 and 8 double bonds; in plasma: TG species with 4, 5, 6, 7 and 8 double bonds) were decreased in group HI-5.0 compared to group HI-0. These alterations in the individual lipid species composition of lipid classes likely reflected the higher percentage of saturated fatty acids in the HI-5.0 broiler diets (59%–60%) than in the HI-2.5 (37%–38%) and the HI-0 (16%–17%) broiler diets, thereby, resulting in a higher intake of saturated fatty acids from HI larvae fat. Although most of the alterations in the plasma and liver lipidomes among the treatment groups were probably attributed to the marked difference in fatty acid composition between soybean oil and HI larvae fat, certain changes in the liver or plasma lipidomes might also reflect specific metabolic effects of the characteristic fatty acids, such as MCFA, contained in the HI larvae fat. For instance, the reason for the slight increase of CE by HI larvae fat might be based on the fact that MCFA, such as capric acid and lauric acid, and the long-chain saturated fatty acid myristic acid and palmitic acid exert hypercholesterolemic effects and increase blood levels of low-density lipoprotein (LDL) [60–62], in which CE makes up approximately 50%, at least in humans. Considering this, it appears possible that the raise in plasma CE levels in group HI-5.0 resulted from elevated LDL levels. The latter might be explained by an effect of HI larvae fat on different aspects of lipoprotein metabolism, such as the catabolism of very-LDL (VLDL) particles by lipoprotein lipase in extrahepatic tissues (white adipose tissue, skeletal muscle), hepatic clearance of LDL particles from plasma or the exchange of CE between lipoproteins which is governed by CE transfer protein.

In order to provide a deeper insight into the metabolic effects of HI larvae fat in broilers, differential transcriptome analysis of the liver was carried out. The finding that only 55 annotated genes out of more than 19,000 genes screened were found to be differentially expressed between group HI-5.0 and group HI-0 and the number of genes regulated greater twofold was very low, suggests that the impact of HI larvae fat inclusion on the hepatic transcriptome was modest. As expected, the impact of HI larvae fat inclusion on the hepatic transcriptome was less at the lower dietary inclusion level of HI larvae fat; i.e., only 25 annotated genes were identified as differentially expressed between groups HI-2.5 and HI-0, from which 7 genes were also differentially expressed between groups HI-5.0 and HI-0. Bioinformatic GSEA of these 55 differentially expressed hepatic genes revealed a particular involvement of the encoded proteins in biological processes dealing with sterol metabolism, such as cholesterol metabolic process, cholesterol biosynthetic process, sterol metabolic process, steroid biosynthetic process and lipid biosynthetic process. This observation was likely explained by the finding that several genes involved in sterol synthesis, such as *HMGCS1* (encoding 3-hydroxy-3-methylglutaryl-CoA synthase 1), *SCAP* (encoding SREBF chaperone), *AKR1D1* (encoding aldo–keto reductase family 1 member D1), *FDFT1* (encoding farnesyl-diphosphate farnesyltransferase 1) and *HSD17B7* (encoding hydroxysteroid 17-beta dehydrogenase 7), were amongst the genes differentially expressed between groups HI-5.0 or HI-2.5 and HI-0. Despite that the proteins encoded by *HMGCS1*, *FDFT1* and *SCAP* are involved in cholesterol synthesis [63], the unaltered levels of free cholesterol and CE in the liver between groups suggests that the regulation of these genes by HI larvae fat inclusion had no impact on hepatic cholesterol levels. Plausible reasons might be that the regulation of these genes occurred either only at the transcriptional level or at a too weak level to substantially alter the abundance of the encoded proteins. In any case, our findings from hepatic transcriptome analysis indicate that the effect of soybean oil replacement with HI larvae fat on intermediary metabolism of broilers is relatively weak.

Conclusion

Comprehensive analysis of the effect of partial and complete replacement of soybean oil with HI larvae fat in broiler diets on growth performance, cecal microbiome, liver transcriptome and liver and plasma lipidomes revealed only very modest, but not any adverse effects of dietary HI larvae fat inclusion. Interestingly, growth performance of the broilers during the starter period was even improved by dietary inclusion of HI larvae fat. The findings of this study suggest that HI larvae fat can be used as an alternative fat source in broiler diets, which makes broiler production more sustainable through the exclusion of soybean oil and the utilization of HI larvae fat from regional production.

Abbreviations

AID	Apparent ileal digestibility
AKR1D1	Aldo–keto reductase family 1 member D1
CE	Cholesteryl esters
Cer	Ceramides
DG	Diglycerides
DM	Dry matter
FAME	Fatty acid methyl esters
FC	Free cholesterol
FDFT1	Farnesyl-diphosphate farnesyltransferase 1
GO	Gene ontology
GSEA	Gene set enrichment analysis
HI	*Hermetia illucens*
HMGCS1	3-Hydroxy-3-methylglutaryl-CoA synthase 1
HSD17B7	Hydroxysteroid 17-beta dehydrogenase 7
LCFA	Long-chain fatty acids
LDL	Low-density lipoprotein
LPC	Lysophosphatidylcholines
LPE	Lysophosphatidylethanolamines
MCFA	Medium-chain fatty acids
NMDS	Non-metric multidimension distance scaling
OUT	Operational taxonomic units
PC	Phosphatidylcholines
PC O	Ether PC
PCA	Principal component analysis
PE	Phosphatidylethanolamines
PE O	Ether PE
PG	Phosphatidylglycerols
PI	Phosphatidylinositols
SCAP	SREBF chaperone
SCFA	Short-chain fatty acids
SFA	Saturated fatty acids
SM	Sphingomyelins
TG	Triglycerides
TM	Tenebrio molitor
VLDL	Very low-density lipoprotein
WPSA	World´s Poultry Science Association

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40104-023-00831-6.

Additional file 1. Table S1 Characteristics of Gallus gallus gene-specific primers used for qPCR analysis. **Table S2** Concentrations of amino acids in the broiler diets. **Table S3** Operational taxonomic units (OTU) identified in cecum digesta of the broilers. **Table S4** List of differentially expressed transcripts in the liver of broilers between group HI-5.0 vs. HI-0. **Table S5** List of differentially expressed transcripts in the liver of broilers between group HI-2.5 vs. HI-0. **Table S6** qPCR validation of microarray data. **Table S7** Individual lipid species composition of triacylglycerols (TG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the liver of broilers fed diets with either 0% (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) Hermetia illucens (HI) larvae fat for 35 d. **Table S8** Individual lipid species composition of cholesteryl esters (CE), phosphatidylcholine (PC) and triacylglycerols (TG) in plasma of broilers fed diets with either 0% (HI-0), 2.5% (HI-2.5) or 5.0% (HI-5.0) Hermetia illucens (HI) larvae fat for 35 d.

Acknowledgements
Not applicable.

Author contributions
RR and KE designed research and coordinated the study. LS performed the experiment, the laboratory analysis and statistical analysis of all data. SMG performed principal component analysis. GM performed analysis of the microbiome data. MH and GL performed lipidomics analysis. RR performed bioinformatic analysis of transcriptomic data. LS, KE and RR interpreted the data. LS wrote the paper. KE and RR reviewed and edited the paper. All authors read and approved the final manuscript.

Funding
Open Access funding enabled and organized by Projekt DEAL. Lea Schäfer was financially supported by H. Wilhelm Schaumann foundation.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. The microarray data of this study have been deposited in MIAME compliant format in the NCBI´s Gene Expression Omnibus public repository.

Declarations

Ethics approval and consent to participate
The animal study trial was approved by the Animal Welfare Officer of the Justus Liebig University Giessen (approval no.: JLU 786_M).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany. [2]Institute of Nutritional and Food Sciences, Molecular Food Technology, University of Bonn, Friedrich-Hirzebruch-Allee 7, 53115 Bonn, Germany. [3]Institute of Clinical Chemistry and Laboratory Medicine, University Hospital of Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany.

Received: 2 September 2022 Accepted: 3 January 2023
Published online: 01 March 2023

References

1. van Huis A. Potential of insects as food and feed in assuring food security. Annu Rev Entomol. 2013;58:563–83.
2. Gasco L, Biasato I, Dabbou S, Schiavone A, Gai F. Animals fed insect-based diets: state-of-the-art on digestibility, performance and product quality. Animals (Basel). 2019;9:170.
3. Sogari G, Amato M, Biasato I, Chiesa S, Gasco L. The potential role of insects as feed: a multi-perspective review. Animals (Basel). 2019;9:119.
4. EUR-Lex - 32021R1372. Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals. URL: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32021R1372. Site accessed at August 10 2022.
5. Makkar HPS, Tran G, Heuzé V, Ankers P. State-of-the-art on use of insects as animal feed. Anim Feed Sci Technol. 2014;197:1–33.
6. Pinotti L, Giromini C, Ottoboni M, Tretola M, Marchis D. Review: Insects and former foodstuffs for upgrading food waste biomasses/streams to feed ingredients for farm animals. Animal. 2019;13:1365–75.
7. Pinotti L, Ottoboni M. Substrate as insect feed for bio-mass production. J Insects Food Feed. 2021;7:585–96.
8. Bovera F, Piccolo G, Gasco L, Marono S, Loponte R, Vassalotti G, et al. Yellow mealworm larvae (*Tenebrio molitor*, L.) as a possible alternative to soybean meal in broiler diets. Br Poult Sci. 2015;56:569–75.
9. Bovera F, Loponte R, Marono S, Piccolo G, Parisi G, Iaconisi V, et al. Use of larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. J Anim Sci. 2016;94:639–47.
10. Meyer S, Gessner DK, Braune MS, Friedhoff T, Most E, Höring M, et al. Comprehensive evaluation of the metabolic effects of insect meal from *Tenebrio molitor* L. in growing pigs by transcriptomics, metabolomics and lipidomics. J Anim Sci Biotechnol. 2020;11:20.
11. Hartinger K, Greinix J, Thaler N, Ebbing MA, Yacoubi N, Schedle K, et al. Effect of graded substitution of soybean meal by Hermetia illucens larvae meal on animal performance, apparent ileal digestibility, gut histology and microbial metabolites of broilers. Animals (Basel). 2021;11:1628.
12. Benzertiha A, Kierończyk B, Rawski M, Mikołajczak Z, Urbański A, Nogowski L, et al. Insect fat in animal nutrition – a review. Ann Anim Sci. 2020;20:1217–40.
13. Schiavone A, Dabbou S, De Marco M, Cullere M, Biasato I, Biasibetti E, et al. Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source. Animal. 2018;12:2032–9.
14. Benzertiha A, Kierończyk B, Rawski M, Kołodziejski P, Bryszak M, Józefiak D. Insect oil as an alternative to palm oil and poultry fat in broiler chicken nutrition. Animals (Basel). 2019;9:116.
15. Cullere M, Schiavone A, Dabbou S, Gasco L, Dalle Zotte A. Meat quality and sensory traits of finisher broiler chickens fed with black soldier fly (Hermetia illucens l.) larvae fat as alternative fat source. Animals (Basel). 2019;9:140.
16. Kim YB, Kim DH, Jeong SB, Lee JW, Kim TH, Lee HG, et al. Black soldier fly larvae oil as an alternative fat source in broiler nutrition. Poult Sci. 2020;99:3133–43.
17. Kim B, Bang HAT, Jeong JY, Kim M, Kim KH, Chun JL, et al. Effects of dietary supplementation of black soldier fly (Hermetia illucens) larvae oil on broiler health. J Poult Sci. 2021;58:222–9.
18. Sypniewski J, Kierończyk B, Benzertiha A, Mikołajczak Z, Pruszyńska-Oszmałek E, Kołodziejski P, et al. Replacement of soybean oil by Hermetia illucens fat in turkey nutrition: effect on performance, digestibility, microbial community, immune and physiological status and final product quality. Br Poult Sci. 2020;61:294–302.
19. Heuel M, Sandrock C, Leiber F, Mathys A, Gold M, Zurbrügg C, et al. Black soldier fly larvae meal and fat can completely replace soybean cake and oil in diets for laying hens. Poult Sci. 2021;100:101034.
20. Hartinger K, Fröschl K, Ebbing MA, Bruschek-Pfleger B, Schedle K, Schwarz C, et al. Suitability of Hermetia illucens larvae meal and fat in broiler diets: effects on animal performance, apparent ileal digestibility, gut histology, and microbial metabolites. J Anim Sci Biotechnol. 2022;13:50.

21. Kim B, Kim M, Jeong JY, Kim HR, Ji SY, Jung H, et al. Black soldier fly (*Hermetia illucens*) larvae oil as an alternative fat ingredient to soybean oil in laying hen diets. Anim Biosci. 2022;35:1408–17.
22. Solis de los Santos F, Donoghue AM, Venkitanarayanan K, Metcalf JH, Reyes-Herrera I, Dirain ML, et al. The natural feed additive caprylic acid decreases Campylobacter jejuni colonization in market-aged broiler chickens. Poult Sci. 2009;88:61–4.
23. Solis de los Santos F, Donoghue AM, Venkitanarayanan K, Dirain ML, Reyes-Herrera I, Blore PJ, et al. Caprylic acid supplemented in feed reduces enteric Campylobacter jejuni colonization in ten-day-old broiler chickens. Poult Sci. 2008;87:800–4.
24. Huang Y, Lv H, Song Y, Sun C, Zhang Z, Chen S. Community composition of cecal microbiota in commercial yellow broilers with high and low feed efficiencies. Poult Sci. 2021;100:100996.
25. Lv H, Huang Y, Wang T, Zhai S, Hou Z, Chen S. Microbial composition in the duodenum and ileum of yellow broilers with high and low feed efficiency. Front Microbiol. 2021;12: 689653.
26. Ringseis R, Gessner DK, Eder K. The gut-liver axis in the control of energy metabolism and food intake in animals. Annu Rev Anim Biosci. 2020;8:295–319.
27. Hanczakowska E. The use of medium-chain fatty acids in piglet feeding–a review. Ann Anim Sci. 2017;17:967–77.
28. Shah ND, Limketkai BN. The use of medium-chain triglycerides in gastrointestinal disorders. Practical Gastroenterol. 2017;160:20–5.
29. Cobb-Vantress.com. Broiler management guide. URL: https://www.cobb-vantress.com/assets/5c7576a214/Broiler-guide-R1.pdf. Site accessed at August 15 2021.
30. Keller U, Brandsch C, Eder K. Supplementation of vitamins C and E increases the vitamin E status but does not prevent the formation of oxysterols in the liver of guinea pigs fed an oxidised fat. Eur J Nutr. 2004;43:353–9.
31. Short FJ, Gorton P, Wiseman J, Boorman KN. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim Feed Sci Technol. 1996;59:215–21.
32. Cobb-Vantress.com. Cobb500 broiler performance & nutrition supplement. URL: https://www.cobb-vantress.com/assets/5a88f2e793/Broiler-Performance-Nutrition-Supplement.pdf. Site accessed at August 15 2021.
33. VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten), Die chemische Untersuchung von Futtermitteln. VDLUFA-Methodenbuch. Band III, Ergänzungslieferungen von 1983, 1988, 1992, 1997, 2004, 2006, 2007, VDLUFA-Verlag, Darmstadt, Germany; 2007.
34. WPSA (World's Poultry Science Association). The prediction of apparent metabolizable energy values for poultry in compound feeds. World's Poult Sci J. 1984;40:181–2.
35. Kierończyk B, Rawski M, Stuper-Szablewska K, Józefiak D. First report of the apparent metabolisable energy value of black soldier fly larvae fat used in broiler chicken diets. Animal. 2022;16: 100656.
36. EUR-Lex - 32009R1099. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. URL: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32009R1099. Site accessed at October 11 2022.
37. Brandt M, Allam SM. Analytik von TiO2 im Darminhalt und Kot nach Kjeldahlaufschluss. Arch Anim Nutr. 1987;37:453–4.
38. Lagkouvardos I, Kläring K, Heinzmann SS, Platz S, Scholz B, Engel KH, et al. Gut metabolites and bacterial community networks during a pilot intervention study with flaxseeds in healthy adult men. Mol Nutr Food Res. 2015;59:1614–28.
39. Meyer S, Gessner DK, Maheshwari G, Röhrig J, Friedhoff T, Most E, et al. Tenebrio molitor larvae meal affects the cecal microbiota of growing pigs. Animals (Basel). 2020;10:1151.
40. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73:5261–7.
41. Jost L. Partitioning diversity into independent alpha and beta components. Ecology. 2007;88:2427–39.
42. Minchin PR. Simulation of multidimensional community patterns: towards a comprehensive model. Vegetatio. 1987;71:145–56.
43. Fiesel A, Gessner DK, Most E, Eder K. Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. BMC Vet Res. 2014;10:196.
44. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 2002;30:207–10.
45. Gessner DK, Schwarz A, Meyer S, Wen G, Most E, Zorn H, et al. Insect meal as alternative protein source exerts pronounced lipid-lowering effects in hyperlipidemic obese zucker rats. J Nutr. 2019;149:566–77.
46. Ringseis R, Zeitz JO, Weber A, Koch C, Eder K. Hepatic transcript profiling in early-lactation dairy cows fed rumen-protected niacin during the transition from late pregnancy to lactation. J Dairy Sci. 2019;102:365–76.
47. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009;37:1–13.
48. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4:44–57.
49. Keller J, Ringseis R, Koc A, Lukas I, Kluge H, Eder K. Supplementation with l-carnitine downregulates genes of the ubiquitin proteasome system in the skeletal muscle and liver of piglets. Animal. 2012;6:70–8.
50. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3:research0034.1.
51. Höring M, Krautbauer S, Hiltl L, Babl V, Sigruener A, Burkhardt R, et al. Accurate lipid quantification of tissue homogenates requires suitable sample concentration, solvent composition, and homogenization procedure-a case study in murine liver. Metabolites. 2021;11:365.
52. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37:911.
53. Höring M, Ejsing CS, Krautbauer S, Ertl VM, Burkhardt R, Liebisch G. Accurate quantification of lipid species affected by isobaric overlap in Fourier-transform mass spectrometry. J Lipid Res. 2021;62:100050.
54. Höring M, Ejsing CS, Hermansson M, Liebisch G. Quantification of cholesterol and cholesteryl ester by direct flow injection high-resolution fourier transform mass spectrometry utilizing species-specific response factors. Anal Chem. 2019;91:3459–66.
55. Liebisch G, Fahy E, Aoki J, Dennis EA, Durand T, Ejsing CS, et al. Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures. J Lipid Res. 2020;61:1539–55.
56. Schlegel G, Ringseis R, Shibani M, Most E, Schuster M, Schwarz FJ, et al. Influence of a rumen-protected conjugated linoleic acid mixture on carcass traits and meat quality in young Simmental heifers. J Anim Sci. 2012;90:1532–40.
57. Hilmarsson H, Thormar H, Thráinsson JH, Gunnarsson E, Dadadóttir S. Effect of glycerol monocaprate (monocaprin) on broiler chickens: an attempt at reducing intestinal Campylobacter infection. Poult Sci. 2006;85:588–92.
58. Hermans D, Martel A, Van Deun K, Verlinden M, Van Immerseel F, Garmyn A, et al. Intestinal mucus protects Campylobacter jejuni in the ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids. Poult Sci. 2010;89:1144–55.
59. Zeitz JO, Fennhoff J, Kluge H, Stangl GI, Eder K. Effects of dietary fats rich in lauric and myristic acid on performance, intestinal morphology, gut microbes, and meat quality in broilers. Poult Sci. 2015;94:2404–13.
60. Denke MA, Grundy SM. Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. Am J Clin Nutr. 1992;56:895–8.
61. Dreon DM, Fernstrom HA, Campos H, Blanche P, Williams PT, Krauss RM. Change in dietary saturated fat intake is correlated with change in mass of large low-density-lipoprotein particles in men. Am J Clin Nutr. 1998;67:828–36.
62. Tholstrup T, Ehnholm C, Jauhiainen M, Petersen M, Høy CE, Lund P, et al. Effects of medium-chain fatty acids and oleic acid on blood lipids, lipoproteins, glucose, insulin, and lipid transfer protein activities. Am J Clin Nutr. 2004;79:564–9.
63. Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog Lipid Res. 2001;40:439–52.

3.2 Ein Austausch von Sojaöl durch das Fett von *Hermetia illucens* Larven in Broilerdiäten verändert das Brustmuskellipidom und verringert die Lipidoxidation des Brustmuskels während der Hitzebehandlung

Replacement of soybean oil by *Hermetia illucens* larvae fat in broiler diets alters the breast muscle lipidome and reduces lipid oxidation of the breast muscle during heat-process

Diese Originalarbeit wurde aus der elektronischen Version der Dissertation entfernt.

Veröffentlicht wurde der Artikel online am 11.05.2023 im *Archives of Animal Nutrition* (Volume 77, Issue 2, Pages: 121-140) und ist unter folgender DOI verfügbar: 10.1080/1745039X.2023.2190297

This publication has been removed from the electronic version of the dissertation.

The article was published online on 11.05.2023 in the *Archives of Animal Nutrition* (Volume 77, Issue 2, Pages: 121-140) and is available under the following DOI: 10.1080/1745039X.2023.2190297

3.3 Auswirkungen eines biotechnologisch hergestellten *Pleurotus sapidus* Mycels auf das Darmmikrobiom, das Lebertranskriptom und das Plasmametabolom von Mastbroilern

Effects of a biotechnologically produced *Pleurotus sapidus* mycelium on gut microbiome, liver transcriptome and plasma metabolome of broilers

Poultry Science

Effects of a biotechnologically produced *Pleurotus sapidus* mycelium on gut microbiome, liver transcriptome and plasma metabolome of broilers

Lea Schäfer,[*] Sarah M. Grundmann,[*] Martin Rühl,[†,‡] Holger Zorn,[†,‡] Waldemar Seel,[§] Marie-Christine Simon,[§] Sven Schuchardt,[#] Erika Most,[*] Robert Ringseis,[*,‖] and Klaus Eder[*,‖,1]

[*]*Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, Giessen, Germany;* [†]*Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Giessen, Germany;* [‡]*Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Giessen, Germany;* [§]*University of Bonn, Nutrition and Microbiota, Institute of Nutrition and Food Sciences, Bonn, Germany;* [#]*Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany; and* [‖]*Center for Sustainable Food Systems, Justus Liebig University Giessen, Giessen, Germany*

ABSTRACT Submerged cultivation using low-value agro-industrial side streams allows large-scale and efficient production of fungal mycelia, which has a high nutritional value. As the dietary properties of fungal mycelia in poultry are largely unknown, the present study aimed to investigate the effect of feeding a *Pleurotus sapidus* (**PSA**) mycelium as a feed supplement on growth performance, composition of the cecal microbiota and several physiological traits including gut integrity, nutrient digestibility, liver lipids, liver transcriptome and plasma metabolome in broilers. 72 males, 1-day-old Cobb 500 broilers were randomly assigned to 3 different groups and fed 3 different adequate diets containing either 0% (PSA-0), 2.5% (PSA-2.5) and 5% (PSA-5.0) *P. sapidus* mycelium in a 3-phase feeding system for 35 d. Each group consisted of 6 cages (replicates) with 4 broilers/cage. Body weight gain, feed intake and feed:gain ratio and apparent ileal digestibility of crude protein, ether extract and amino acids were not different between groups. Metagenomic analysis of the cecal microbiota revealed no differences between groups, except that one α-diversity metric (Shannon index) and the abundance of 2 low-abundance bacterial taxa (Clostridia UCG 014, Eubacteriales) differed between groups ($P < 0.05$). Concentrations of total and individual short-chain fatty acids in the cecal digesta and concentrations of plasma lipopolysaccharide and mRNA levels of proinflammatory genes, tight-junction proteins, and mucins in the cecum mucosa did not differ between groups. None of the plasma metabolites analyzed using targeted-metabolomics differed across the groups. Hepatic transcript profiling revealed a total of 144 transcripts to be differentially expressed between group PSA-5.0 and group PSA-0 but none of these genes was regulated greater 2-fold. Considering either the lack of effects or the very weak effects of feeding the *P. sapidus* mycelium in the broilers it can be concluded that inclusion of a sustainably produced fungal mycelium in broiler diets at the expense of other feed components has no negative consequences on broilers´ performance and metabolism.

Key words: broiler, liver transcriptome, plasma metabolome, gut microbiome, mycelium

2024 Poultry Science 103:103975
https://doi.org/10.1016/j.psj.2024.103975

INTRODUCTION

According to the latest estimates, the world population will increase from 7.9 billion people in 2022 to 8.6 billion people in 2032. The rise in world population will result in a global increase in the consumption of animal products which is associated with an increased requirement for animal feed (OECD/FAO, 2023). The strongest increase in animal production will be in poultry meat that is affordable compared to other livestock meat and has a favorable combination of a high protein and a low lipid concentration. However, feed production is not only complicated by the fact that natural resources such as farmland and water are becoming increasingly limited but also that the production of feed for poultry as a monogastric species competes with human nutrition (Wu et al., 2014). It is therefore of great importance to produce animal feed with increased sustainability compared to conventionally produced feed using feedstuffs which do not compete with human nutrition.

Agricultural side streams with a high fiber content fulfill these demands, because they are used in human

Received April 17, 2024.
Accepted June 8, 2024.

[1]Corresponding author: klaus.eder@ernaehrung.uni-giessen.de

nutrition only to a limited extent. In monogastric animals, such side streams with a high fiber content, like brewer's grain, sugar beet pulp and pomace, have also a very limited nutritional value due to their low digestibility (Huang and Lee, 2018). However, a potential strategy to increase the nutritional value of side streams with a low digestibility is to use them as a substrate for the production of edible mushrooms. Mushrooms possess a unique enzyme system enabling them to degrade a wide array of non-starch polysaccharide- and lignin-containing substrates contained in the abovementioned side streams and, thereby, to upcycle low-value side streams into high-value fungal biomass. The most commonly cultivated mushrooms include *Agaricus bisporus, Lentinula* and *Pleurotus spp.* (Chang and Buswell, 1996). Mushrooms have a high nutritional value due to their high concentrations of protein, fiber, vitamins and minerals, in combination with a low concentration of lipids (Mattila et al., 2001; Valverde et al., 2015). While commercial mushroom products are obtained from fruiting bodies, which are produced in a time-exhausting process, submerged cultivation has the potential to produce mycelial biomass at large-scale and in an efficient manner (Papaspyridi et al., 2012). Apart from providing protein, fiber, vitamins and minerals, the mycelium of *Pleurotus spp.* could be interesting for poultry nutrition due to its high concentration of β-glucans. The β-glucans are well-known to act as potent prebiotics in monogastric animals (Lam and Chi-Keung Cheung, 2013). Prebiotics beneficially modulate the structure of the gut microbial community through promoting the growth of beneficial commensal bacteria, such as *Lactobacillus* spp. and *Bifidobacterium* spp.. The commensals protect the outer mucin layer from colonization by opportunistic pathogens, thereby, strengthening the intestinal barrier function, which strongly relies on tight-junction proteins, for example, claudins (**CLDN**) and occludin (**OCLN**), sealing the space between adjacent intestinal epithelial cells and different mucin (**MUC**) glycoproteins, for example, MUC2 and MUC5AC, preventing the direct bacterial contact with the intestinal epithelial cells. Apart from modulating the gut microbiota structure, prebiotics have been shown to exert several beneficial metabolic effects including blood and liver lipid-lowering and anti-inflammatory effects (Cheung, 1998; Manzi, 2000; Maheshwari et al., 2020). The latter effects are supposed to be mediated by several gut-derived compounds, such as short-chain fatty acids (**SCFA**), and the improved gut barrier function, which prevents the translocation of pro-inflammatory bacterial components, for example, lipopolysaccharide (**LPS**), into the systemic circulation.

As the dietary properties of fungal mycelia in poultry feeding are largely unknown, the present study aimed to investigate the effect of feeding *P. sapidus* mycelium as a dietary supplement, included into the diet at the expense of wheat, on growth performance, composition of the cecal microbiota and several physiological traits including gut integrity, nutrient digestibility, liver lipids, liver transcriptome and plasma metabolome in broilers. For this end, several screening technologies, such as transcriptomics, metabolomics, and metagenomic analysis of the gut microbiota were applied. The hypothesis of the study was that the β-glucans, which have been documented to exert a prebiotic effect, in the *P. sapidus* mycelium have beneficial effects on the gut microbial community and influence gut integrity, metabolic health and performance in a favorable manner.

MATERIAL AND METHODS

Biotechnological Production of P. sapidus *Mycelium*

P. sapidus (German Collection of Microorganisms and Cell Cultures DSMZ, strain number 8266) was used for the production of vegetative mycelium. All cultivation procedures were performed in a sterile environment. The chemicals utilized were purchased from Carl Roth (Karlsruhe, Germany) and Sigma-Aldrich (Merck, Darmstadt, Germany). The stock culture was cultivated according to the method of Ahlborn et al. (2019). A mixture of 30 g/L malt extract and 3 g peptone was used for the precultures. After a 6-d cultivation period at 24°C in the dark on a rotary shaker at 110 rpm (KS 4000 i control, IKA, Staufen, Germany), the preculture was transferred to a main culture. The medium of the main culture contained yeast extract, magnesium sulfate hydrate, potassium dihydrogen phosphate, and different trace elements as described by Trapp et al. (2018). In addition, the main culture media contained isomaltulose molasses (**ISM**) from Südzucker AG (Offstein, Germany). After another 6-d cultivation period at 24°C in the darkness on a rotary shaker, the main culture was homogenized using Art-Miccra D-8 (ART Labortechnik, Müllheim, Germany) and transferred to a 150 L bioreactor (Biostat D 100, B. Braun International, Melsungen, Germany) for fermentation (Maheshwari et al., 2020). The bioreactor was equipped with a Rushton-type impeller, a pH electrode, and a temperature sensor. The main culture was grown at 24°C and 150 rpm with an air-flow rate of 3.0 NL per min. After 6 d, *P. sapidus* mycelium was harvested using a 250-micron sieve, thoroughly washed with water, freeze-dried, and stored at -20°C (Maheshwari et al., 2020).

Chemical Analysis of P. sapidus *Mycelium*

Crude protein (**CP**), crude ash (**CA**), ether extract (**EE**), crude fiber (**CF**), sugar, starch, fatty acids and amino acids in the *P. sapidus* mycelium were analyzed according to the official methods (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, VDLUFA, 2012). For the measurement of glucans, the enzyme assay kit for mushroom and yeast β-glucan from Megazyme (Ireland) was used in accordance with the manufacturer's protocol. The chitin content was determined by a colorimetric assay with modifications described recently (Ahlborn et al., 2019; Maheshwari et al., 2020).

Animals, Experimental Design, and Diets

The 5-wk feeding trial with 72 males, 1-day-old broiler chickens (Cobb 500, Cobb-Vantress, Weidemar, Germany) was approved by the Animal Welfare Officer of the Justus Liebig University Giessen (approval no.: JLU 843_M). All experimental procedures described followed established guidelines for laboratory animals care and handling. The broilers were randomly assigned to 3 different groups (4 broilers/cage, 6 cages/group), with a similar mean initial body weight (38.1 ± 2.9 g; mean ± SD; $N = 72$) across the groups. The broilers were housed in 2.1 m^2 cages with nipple drinkers and feed automates and had free access to feed and water. The floor was covered with hemp-based litter (Hemparada, HempFlax Group B.V., Oude Pekela, Netherlands) which allowed scratching, pecking and dustbathing and was exchanged 2 times per week during the first 2 wk and every 2 d during the last 3 wk of the trial. In addition, broilers were provided with perches in elevated position for resting and sleeping. Light regime followed a schedule of 24 h:0 h, 23 h:1 h, 22 h:2 h, 21 h:3 h, 20 h:4 h, 19 h:5 h (light: dark) at d 1, 2, 3, 4, 5, 6, and 18 h:6 h from d 7 onward and the light intensity was constantly 40 Lux, as recommended by the breeder. The room temperature decreased from 28 to 29°C on d 1, measured at pen height, to 23 to 24°C on d 35. Infrared lamps (Albert Kerbl GmbH, Buchbach, Germany) were used as additional heat sources during the first 6 d to adjust the temperature at the cage floor to 34°C. Mean relative humidity was 60.0 ± 1.9%. The groups were fed 3 different nutrient adequate diets containing either 0% (group PSA-0), 2.5% (group PSA-2.5) or 5% (group PSA-5.0) *P. sapidus* mycelium in a 3-phase feeding system (starter diet form d 1 to 10, grower diet from d 11 to 21, finisher diet from d 22 to 35). The composition of the 3 diets is shown in Table 1. The composition of the diets met the broiler's requirements of nutrients and energy according to the breeder's recommendations (Cobb-Vantress, 2022). Diets were provided in crumbled form during the first 3 d, and in pellet form (2 mm diameter) from d 3 until the end of the trial. On d 1, 10, 21, and 35 body weight (individually) and feed intake (per cage) were determined. The feed:gain ratio was calculated from feed intake and body weight gains on cage basis.

Sample Collection

On d 35, all animals were killed by bleeding (opening of *Vena jugularis* and *Arteria carotis*) under electrical anesthesia using a BTG-40A stunning device (Westerhoff Geflügeltechnik, Hoogstede, Germany) in accordance with the European legislation for euthanasia of animals (EUR_-Lex, 09.01.2024). For determination of the metabolic parameters twelve broilers per group (2 broilers from each cage), whose body weight represented the mean values of the whole group, were selected. Whole blood was individually collected into tubes containing ethylenediaminetetraacetic acid (9 mL S-Monovette, Sarsted, Numbrecht, Germany) as an anti-coagulant. For plasma preparation, whole blood was centrifuged (1,100 × *g*, 10 min) at 4°C and stored at -80°C for further analysis. The liver was excised, weighted and aliquots were snap-frozen in liquid nitrogen and stored at -80°C until analysis. The gastrointestinal tract was removed and digesta and mucosa from the ileum and the cecum were collected. Mucosa and digesta samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis. Feed samples were collected after feed manufacturing and stored at -20°C.

Table 1. Composition of the broiler diets supplemented with 0% (PSA-0), 2.5% (PSA-2.5) or 5% (PSA-5.0) of *Pleurotus sapidus* mycelium.

	Starter diets			Grower diets			Finisher diets		
	PSA-0	PSA-2.5	PSA-5.0	PSA-0	PSA-2.5	PSA-5.0	PSA-0	PSA-2.5	PSA-5.0
Components (g/kg)									
Maize	283.7	281.9	284	280	280	280	320	320	320
Soybean meal (42% CP)	373	370	370	310	310	310	270	270	270
Wheat	203.7	193.7	176.7	268.7	254.6	240	265.6	251	236.6
P. sapidus mycelium	0	25	50	0	25	50	0	25	50
Soybean oil	50	50	50	50	50	50	50	50	50
Cellulose	20	10	0	20	10	0	20	10	0
Mineral & vitamin mix[1]	20	20	20	20	20	20	20	20	20
Monocalcium phosphate	15	15	15	15	15	15	15	15	15
Calcium carbonate	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5
Sodium chloride	4	4	4	4	4	4	4	4	4
DL-Methionine	3.8	3.8	3.8	3.6	3.6	3.6	3.4	3.4	3.3
L-Lysine	4.4	4.3	4.2	4.6	4.5	4.3	4.4	4.2	4.1
L-Threonine	2	2	2	1.8	1.7	1.6	1.5	1.5	1.4
L-Arginine	1.9	1.8	1.8	2	1.9	1.8	1.8	1.7	1.6
L-Valine	2	2	2	3	2.7	2.7	2.2	2.2	2.1
L-Isoleucine	1	1	1	1.8	1.5	1.5	1.6	1.5	1.5
Titanium dioxide	0	0	0	0	0	0	5	5	5

[1]The mineral & vitamin mix supplied the following minerals and vitamins per kg diet (starter/grower/finisher): Fe, 40/40/40 mg; Cu, 15/15/15 mg; Mn, 100/100/100 mg; Zn, 100/100/100 mg; I, 1/1/1 mg; Se, 0.35/0.35/0.35 mg; vitamin A, 10,000/10,000/10,000 IU; vitamin D3, 5,000/5,000/5,000 IU; vitamin K3, 3/3/3 mg; vitamin E, 80/50/50 IU; vitamin B1, 3/2/2 mg; vitamin B2, 9/8/6 mg; vitamin B6, 4/3/3 mg; vitamin B12, 0.02/0.015/0.015 mg; biotin, 0.2/0.18/0.18 mg; folic acid, 2/2/1.5 mg; nicotinic acid, 60/50/50 mg; choline chloride, 500/400/350 mg; pantothenic acid, 15/12/10 mg.

Determination of the Chemical Composition of the Diets

Concentrations of dry matter (**DM**), CP, CA, EE, CF, sugar, starch, amino acids and fatty acids in the main diet components (wheat, maize, soybean extraction meal) and the experimental diets were determined using the official methods (Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten, VDLUFA, 2012). To calculate the CP content of the *P. sapidus* mycelium, a specific, chitin corrected *N*-to-protein conversion factor of 4.17 (Souci et al., 2016) was used, for all other components and for the experimental diets a *N*-to-protein conversion factor of 6.25 were used. To calculate the apparent *N*-corrected metabolizable energy (**AME$_N$**) content of the diets, the formula of the World's Poultry Science Association for poultry compound feed was used (WPSA, 1984):

$$\mathrm{AME_N(MJ/kg)} = [(0.01551 * \text{crude protein}) + (0.03431 * \text{crude lipids}) + (0.01669 * \text{starch}) + (0.01301 * \text{sugar})]$$

Determination of Apparent Ileal Digestibility

The determination of the apparent ileal digestibility (**AID**) of EE, CP and amino acids at the end of the experiment was performed with the indicator method using titanium dioxide (**TiO_2**) as an inert marker in the finisher diets (d 22−35) (Short et al., 1996). Prior to analysis, ileal digesta samples were freeze-dried and manually grounded under nitrogen with a mortar. The concentration of the indigestible indicator TiO_2 in the ileal digesta was determined according to the method of Brandt and Allam (1987) with slight modifications. Concentrations of EE, CP and amino acids in the ileal digesta were determined by official methods as described above. Based on the ileal concentrations of indicator, the AID of EE, CP and amino acids were calculated according to the following formula:

$$\mathrm{AID}(\%) = 100 - [(\mathrm{TiO_{2\_Diet}}/\mathrm{TiO_{2\_Digesta}}) \times (\mathrm{Nutrient_{\_Digesta}}/\mathrm{Nutrient_{\_Diet}}) \times 100],$$

in which TiO_{2_Diet} is the TiO_2 concentration in the diet (% DM), $TiO_{2\_Digesta}$ is the TiO_2 concentration in the ileal digesta (% DM), $Nutrient_{\_Digesta}$ is the nutrient (EE, CP, amino acids) concentration in ileal digesta (% DM), and $Nutrient_{\_Diet}$ is the nutrient (EE, CP, amino acids) concentration in the diet (% DM).

Determination of Microbiota Composition and Diversity in the Cecal Digesta

16S rRNA gene amplicon sequencing was performed at Life & Brain as described recently in detail (Beller et al., 2024). QIIME 2 version 2022.8 was used to process the 16S sequencing data (Bolyen et al., 2019). Raw sequencing data were deposited as FASTQ files at the NCBI's Sequence Read Archive (**SRA**) repository under BioProject accession number PRJNA1120330. DADA2 was deployed for sequencing quality control, including filtering for PhiX reads and chimeric sequences, and denoising (Callahan et al., 2016). To identify amplicon sequencing variants (**ASV**) with >99% similarity, a pretrained SILVA classifier (silva-138-nr99-16S-V3-V4-classifier) was used. The α-diversity, β-diversity, and relative abundance were analyzed with the MicrobiomeAnalyst platform using the default settings (Dhariwal et al., 2017; Chong et al., 2020).

Determination of SCFA Concentrations in the Cecal Digesta

SCFA concentrations in cecal digesta were determined as previously described (Fiesel et al., 2014). In brief, 50 mg aliquots of cecal digesta were mixed with 0,5 mL 5% o-phosphoric acid containing internal standard (0.15 mg/mL crotonic acid). Extraction was performed by vortexing for 3 min followed by centrifugation at 21,000 × g for 10 min at 4°C. One μL of the extract was injected into a gas chromatograph (Clarus 580 GC system, Perkin Elmer, Waltham, MA) equipped with a polar capillary column (30 m free fatty acid phase, 0.32 mm internal diameter, 0.25 μm film thickness; Macherey and Nagel, Düren, Germany) and a flame ionisation detector.

Concentration of LPS in Plasma

The concentration of LPS in plasma was analyzed using an ELISA Kit (Chicken Lipopolysaccharide ELISA Kit, LOT: 2K235N; Assay Genie, Dublin, Ireland) according to the manufacturer's protocol.

Total RNA Extraction From Liver and Cecum Mucosa and qPCR Analysis

Aliquots from the liver (20−30 mg) and aliquots from cecum mucosa (30−50 mg) were used for total RNA extraction using TRIzol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. Total RNA was analyzed for quantity and quality using an Infinite 200M microplate reader with a NanoQuant plate (both from Tecan, Mainz, Germany). The mean RNA concentration and A260/A280 ratio of all total RNA samples were 385 ± 64 μg/mL and 1.91 ± 0.02 ($N = 36$ or $n = 12$/group) and 466 ± 62 μg/mL and 1.93 ± 0.02 ($N = 36$ or $n = 12$/group) for liver and cecum mucosa, respectively. The synthesis of cDNA was performed as described previously (Chiappisi et al., 2017). qPCR analysis of target and reference genes was performed with a Rotor-Gene Q system (Qiagen) using the KAPA SYBR FAST qPCR Mastermix (Merck, Darmstadt, Germany) and gene-specific primer pairs from Eurofins MWG Operon (Ebersberg, Germany), as recently described (Meyer et al., 2019). The properties of primers are listed in Supplemental Table S1 for cecum mucosa. The qPCR data were normalized using 4 reference genes (*ACTB, GAPDH, SDHA* and *YWHAZ*) (Vandesompele et al., 2002). Also, qPCR analysis was carried out for validation of microarray data (17 genes) of the liver. The characteristics of these primers are listed in Supplemental Table S2.

Determination of Triglyceride and Cholesterol Concentration in Plasma and Liver

The plasma and liver concentrations of triglycerides (**TG**) and cholesterol (**Chol**) were determined by using enzymatic reagent kits from DiaSys (Triglycerides FS LOT: 60161285; Cholesterol FS LOT: 50302128/34859, both from DiaSys Diagnostic Systems GmbH, Holzheim, Germany). The concentration of TG and Chol in the liver were measured by preparing liver lipid extracts using a mixture of n-hexane and isopropanol (3:2, v/v) according to Hara and Radin (1978). Afterwards, extracted lipids were dried and dissolved with chloroform and Triton X-100 (1:1, v/v). Chloroform was evaporated by a stream of nitrogen. The test reagents were added to the lipids dissolved in Triton X-100 (Eder and Kirchgessner, 1994).

Hepatic Transcript Profiling

Following a further RNA quality check by an Agilent 2100 Bioanalyzer, which revealed an average RNA integrity number value of 7.49 ± 0.48 for all samples ($N = 18$, means ± SD), total RNA samples were processed using an Affymetrix GeneChip Array (Chicken Gene 1.0 ST), according to the Applied Biosystems GeneChip Whole Transcript (**WT**) PLUS Reagent Kit User Guide (Thermo Fisher Scientific, Waltham, MA). After scanning of the processed GeneChips, cell intensity files, where a single intensity value for each cell is provided, were generated from the image data using the Command Console software (Affymetrix). The compressed array image files (CEL files) were imported into the Applied Biosystems Transcriptome Analysis Console (v. 4.0.2) software (Thermo Fisher Scientific) for calculation of summarized probe set signals (in log2 scale) using the Robust Multichip Analysis algorithm, comparison fold changes (**FC**) and significance *P*-values (**ANOVA**). Gene names were annotated with the “ChiGene-1_0-st-v1.na36.galgal3.transcript.csv” annotation file. The microarray data of this study have been deposited in MIAME compliant format in the NCBI's Gene Expression Omnibus (**GEO**) public repository under GEO accession number GSE269154 (Edgar et al., 2002). The differentially expressed transcripts were filtered based on a FC > 1.3 or < -1.3 and a *P*-value < 0.05 between groups PSA-5.0 vs. PSA-0 and groups PSA-2.5 vs. PSA-0. Identical or similar filter criteria were also applied in several recent studies (Ringseis et al., 2019; Grundmann et al., 2023; Schäfer et al., 2023). Gene set enrichment analysis (**GSEA**) was performed with the identified differentially expressed transcripts in order to identify enriched Gene Ontology (**GO**) terms within GO category biological process (**BP**), molecular function (**MF**) and cellular component (**CC**) and Kyoto Encyclopedia of Genes and Genomes (**KEGG**) pathways using the Database for Annotation, Visualization and Integrated Discovery bioinformatic resource 6.8 (Huang et al., 2009; Sherman et al., 2022). GO terms were considered as enriched if $P < 0.05$.

Targeted Plasma Metabolomics

Targeted quantification of 1019 plasma metabolites was performed using a combination of liquid chromatography (Agilent 1290 infinity II LC, Santa Clara, CA) and mass spectrometry (SCIEX 5500 QTrap MS, Darmstadt, Germany) with the MxP Quant 500XL kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) as described recently in detail (Marschall et al., 2023). Prior to the analysis, variables with missing values were either excluded from the analyses if > 50% of the samples were missing, or the missing values were replaced by the limit of detection (1/5 of the minimum positive value of each variable). Metabolites were filtered based on their interquartile range. After normalization by logarithmic transformation and autoscaling, the remaining values (507) were used for statistical analysis and principal component analysis (**PCA**).

Statistical Analysis

Statistical analysis was performed using SPSS 28 software (IBM, Armonk, NY). The cage served as the experimental unit for feed intake and feed:gain ratio and the individual animal for all other data. All parameters were tested for normal distribution by Kolmogorov-Smirnov test for initial body weight, final body weight and body weight gain (all animals alive at the end of the trail, $N = 65$ broilers or $n = 21$-22 broilers/group) and the Shapiro-Wilk test for feed intake, feed:gain ratio (cage basis, $N = 18$ cages or $n = 6$ cages/group) and biochemical and qPCR data (2 animals per cage, $N = 36$ or $n = 12$ broilers/group). For test of homoscedasticity the Levene’s test was used. If normal distribution was only observed after a log-transformation, the log-transformed data were used for statistical analysis. Differences between the 3 groups were analyzed using one-way analysis of variance (one-way **ANOVA**) followed by a Tukey’s post-hoc test for normally distributed data with homogeneous variances. For data with heterogeneity of variance, means of the 3 groups were analyzed using Welch’s ANOVA in conjunction with the Games-Howell post-hoc test. In the case that data were not-normally distributed, a Kruskal−Wallis one-way ANOVA was performed using the Mann-Whitney U test with Bonferroni correction as post-hoc test. For all the abovementioned tests, a *P*-value < 0.05 was considered statistically significant. Statistical analysis of the metabolomics dataset was performed using MetaboAnalyst version 6.0 (Xia et al., 2009). Metabolites with a false discovery rate (**FDR**) < 0.05 were considered significantly different.

RESULTS

Chemical Composition of P. sapidus *Mycelium*

After 6 d of cultivation in a 150 L stirred bioreactor the *P. sapidus* mycelium contained (per kg DM) 105 g CP, 18 g EE, 36 g CA, and 780 g carbohydrates. The concentration of total glucans, β- and α-glucans (per kg DM) were 366 g, 312 g and 54 g, respectively. The chitin concentration of the mycelium was 53 g per kg DM. The concentrations of amino acids are shown in Supplemental Table S3. The highest concentrations within amino acids were detected for glutamine + glutamic acid and alanine. Concentrations of most of the other amino acids (arginine, asparagine + aspartic acid, cysteine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine) were below 10 g/kg mycelium (on a DM base). The concentration of tryptophan was below a concentration of 1 g/kg mycelium (on a DM base). Fatty acid analysis showed that eight fatty acids were present with a concentration greater than 0.1% of total fatty acids in the mycelium. The highest concentration was found for C18:2, followed by C18:1, C16:0, C18:0 and C18:3. The concentrations of C12:0, C14:0 and C17:0 were below 1% of total fatty acids.

Chemical Composition of the Experimental Diets

The concentration of nutrients of the 3 experimental diets fed during the 3 phases are shown in Table 2. Within one phase, the concentrations of crude nutrients, AME_N, fatty acids and amino acids were similar within the 3 diets (PSA-0, PSA-2.5, PSA-5.0).

Performance and AID of Nutrients

Final body weight ($P = 0.881$), body weight gain ($P = 0.866$), feed intake ($P = 0.515$), and feed:gain ratio

Table 2. Concentrations of nutrients and energy in the broiler diets supplemented with 0% (PSA-0), 2.5% (PSA-2.5) or 5% (PSA-5.0) of *Pleurotus sapidus* mycelium.

	Starter diets			Grower diets			Finisher diets		
	PSA-0	PSA-2.5	PSA-5.0	PSA-0	PSA-2.5	PSA-5.0	PSA-0	PSA-2.5	PSA-5.0
Analyzed crude nutrient and energy content									
DM (% FM)	86.1	86.4	86.6	86.6	86.5	86.9	86.8	86.5	86.9
CP (% DM)	24.4	24.3	24.4	22.9	22.9	22.9	21.1	21.2	21.2
EE (% DM)	9.1	8.9	8.9	8.5	8.5	8.3	8.8	9.1	8.7
CA (% DM)	6.5	6.6	6.6	6.1	6.2	6.3	6.3	6.4	6.5
CF (% DM)	5.8	5.5	5.1	5.2	4.7	4.8	5.1	4.1	3.9
Sugar (% DM)	3.8	2.8	5.0	3.0	4.8	5.4	3.2	3.7	5.3
Starch (% DM)	38.3	38.4	35.5	41.2	39.1	40.1	44.9	44.0	44.5
Calculated energy content									
AME_N (MJ/kg DM)	13.8	13.6	13.4	13.7	13.6	13.8	14.2	14.2	14.4
Fatty acids (% of total fatty acids)[1]									
C16:0	11.54	11.56	11.60	11.48	11.37	11.64	11.57	11.47	11.56
C18:0	3.63	3.86	4.25	3.55	3.94	4.31	4.04	3.96	4.11
C18:1	26.39	25.19	23.79	26.53	24.15	23.75	23.71	23.80	23.79
C18:2	52.34	53.21	53.67	52.56	54.05	53.88	54.28	54.33	54.11
C18:3	4.62	5.02	5.36	4.58	5.33	5.35	5.27	5.31	5.32
C20:0	0.40	0.37	0.42	0.38	0.35	0.37	0.35	0.35	0.36
C20:1	0.31	0.25	0.31	0.33	0.29	0.21	0.31	0.30	0.28
C22:0	0.43	0.39	0.34	0.43	0.34	0.33	0.31	0.31	0.32
Amino acids (g/kg diet)									
Alanine	8.96	9.05	9.19	8.31	8.33	8.81	7.80	8.07	8.27
Arginine	15.46	14.21	14.68	13.68	13.33	13.51	12.15	12.50	12.45
Asparagine/Aspartic acid	18.93	17.58	17.82	16.09	15.91	16.52	14.66	15.08	14.94
Cysteine	3.19	3.30	3.04	3.02	2.85	2.97	2.76	3.12	3.14
Glutamine/Glutamic acid	36.93	35.97	36.11	35.57	34.59	35.40	33.14	32.98	32.69
Glycine	7.95	8.01	8.09	7.56	7.40	7.60	6.89	6.97	7.00
Histidine	5.14	4.78	4.87	4.51	4.52	4.45	4.20	4.26	4.25
Isoleucine	8.77	8.63	8.90	8.83	8.67	8.92	8.06	8.14	7.97
Leucine	15.77	15.20	15.29	14.50	14.47	14.56	13.76	13.72	13.62
Lysine	14.84	13.63	13.78	13.09	12.96	12.96	12.01	12.20	11.89
Methionine	6.39	6.37	6.29	6.21	6.39	6.21	5.61	6.03	5.70
Phenylalanine	10.34	9.41	9.59	9.08	9.00	9.04	8.40	8.31	8.43
Proline	13.62	13.61	13.48	14.16	13.54	13.21	13.07	12.78	12.73
Serine	10.25	9.96	10.11	9.63	9.31	9.62	8.75	8.87	8.86
Threonine	9.11	9.29	9.43	8.88	8.50	8.81	7.85	8.13	7.98
Tryptophan	3.47	3.42	3.12	2.82	2.86	2.76	2.82	2.91	2.96
Tyrosine	6.59	6.52	6.37	5.72	5.77	5.21	5.39	5.47	4.85
Valine	10.14	9.61	10.13	10.28	10.01	10.08	9.01	9.03	8.92

[1]Only fatty acids > 0.1% of total fatty acids are shown.
Abbreviations: CA, crude ash; CF, crude fiber; CP, crude protein; DM, dry matter; EE, ether extract; FM, fresh matter.

Table 3. Performance data of broilers fed diets with either 0% (PSA-0), 2.5% (PSA-2.5) or 5.0% (PSA-5.0) *Pleurotus sapidus* mycelium for 35 d.

	PSA-0	PSA-2.5	PSA-5.0	*P*-value
Whole period (d 1−35)				
Initial BW (g)	38.1 ± 3.0	38.0 ± 2.9	38.1 ± 2.9	0.995
Final BW (g)	2870 ± 328	2940 ± 243	2911 ± 300	0.881
BW gain (g)	2832 ± 328	2902 ± 242	2873 ± 300	0.866
Feed intake (g)	3827 ± 238	3960 ± 154	3885 ± 245	0.515
Feed:gain ratio (g/g)	1.35 ± 0.08	1.37 ± 0.06	1.36 ± 0.03	0.846
Mortality (%)	8.3	4.2	4.2	-
Starter period (d 1−10)				
BW gain (g)	245 ± 35	261 ± 29	260 ± 27	0.171
Feed intake (g)	254 ± 27	282 ± 15	273 ± 23	0.165
Feed:gain ratio (g/g)	1.03 ± 0.08	1.08 ± 0.05	1.05 ± 0.04	0.203
Grower period (d 11−21)				
BW gain (g)	821 ± 77	855 ± 78	841 ± 81	0.361
Feed intake (g)	1001 ± 117	1146 ± 105	1072 ± 40	0.062
Feed:gain ratio (g/g)	1.22 ± 0.13	1.35 ± 0.15	1.27 ± 0.02	0.402
Finisher period (d 22−35)				
BW gain (g)	1761 ± 285	1784 ± 204	1760 ± 260	0.820
Feed intake (g)	2572 ± 193	2531 ± 131	2540 ± 230	0.979
Feed:gain ratio (g/g)	1.47 ± 0.10	1.43 ± 0.04	1.46 ± 0.05	0.556

Data are means ± SD, $n = 21$-22 broilers/group (initial BW, final BW, BW gain) and $n = 6$ cages/group (feed intake, feed:gain ratio).
Abbreviation: BW, body weight.

($P = 0.846$) of the broilers during the whole period did not differ between the 3 groups of broilers (Table 3). The mortality of the broilers was 8.3% in group PSA-0 and 4.2% in groups PSA-2.5 and PSA-5.0. The AID of CP ($P = 0.769$), EE ($P = 0.316$) and amino acids (lowest *P*-value: 0.347 for cysteine, highest *P*-value: 0.997 for tryptophan) did also not differ between the 3 groups (Table 4).

Diversity and Composition of the Cecum Microbiota

After normalizing the data and a subsequent filtering step, a total of 80 ASV was used to analyze the diversity and composition of the microbiota. The 4 indicators Richness, Chao1, Shannon-Index and Simpson-Index were used to analyze the α-diversity (Figure 1A). Richness and Chao1, which are both indicators of the microbial community richness, showed no significant differences between the groups. The Shannon-Index, which considers both the richness and the evenness, differed significantly ($P < 0.05$) between group PSA-0 and group PSA-2.5, but not between group PSA-0 and PSA-5.0, while no effect was found for the Simpson-Index. To assess β-diversity, 3 metrics (Bray-Curtis Index, Jensen-Shannon divergence, Jaccard Index) were calculated and the data were visualized using nonmetric multidimensional scaling (**NMDS**) plots (Figure 1B). The β-diversity metrics indicated no significant difference between the groups. The effects on the composition of

Table 4. Apparent ileal digestibility (AID) of ether extract (EE), crude protein (CP) and amino acids of broilers fed diets with either 0% (PSA-0), 2.5% (PSA-2.5) or 5.0% (PSA-5.0) *Pleurotus sapidus* mycelium for 35 d.

AID	PSA-0	PSA-2.5	PSA-5.0	*P*-value
EE (%)	89.7 ± 1.5	89.7 ± 1.9	88.5 ± 2.2	0.316
CP (%)	76.1 ± 2.6	75.2 ± 4.5	74.7 ± 5.1	0.769
Amino acids (%)				
Alanine	74.5 ± 4.5	73.2 ± 7.3	74.3 ± 5.6	0.845
Arginine	88.7 ± 1.9	87.9 ± 33	86.6 ± 4.7	0.394
Asparagine/Aspartic acid	80.7 ± 2.3	80.6 ± 3.4	80.5 ± 4.0	0.983
Cysteine	70.5 ± 3.9	73.7 ± 4.1	72.5 ± 6.5	0.347
Glutamine/Glutamic acid	87.4 ± 2.3	86.6 ± 3.7	86.2 ± 4.7	0.746
Glycine	76.6 ± 3.2	75.8 ± 5.3	76.5 ± 5.6	0.923
Histidine	83.5 ± 2.8	82.0 ± 5.1	82.5 ± 5.4	0.742
Isoleucine	84.1 ± 2.3	83.3 ± 4.5	83.2 ± 5.0	0.860
Leucine	83.2 ±.2.9	82.0 ± 4.6	81.9 ± 5.2	0.783
Lysine	87.9 ± 2.1	87.1 ± 3.9	86.8 ± 4.2	0.731
Methionine	89.3 ± 2.0	89.5 ± 3.2	88.8 ± 4.2	0.863
Phenylalanine	84.0 ± 2.9	82.7 ± 4.8	82.6 ± 5.6	0.732
Proline	87.0 ± 3.1	86.3 ± 4.0	86.6 ± 4.2	0.830
Serine	79.9 ± 3.2	79.4 ± 5.2	79.6 ± 5.8	0.972
Threonine	79.2 ± 3.4	78.8 ± 6.0	79.4 ± 5.8	0.862
Tryptophan	82.9 ± 2.5	83.1 ± 3.2	81.9 ± 7.0	0.997
Tyrosine	82.3 ± 2.8	80.9 ± 5.8	81.3 ± 5.9	0.828
Valine	84.3 ± 2.6	83.2 ± 5.0	83.7 ± 4.8	0.850

Data are means ± SD, $n = 12$ broilers/group.

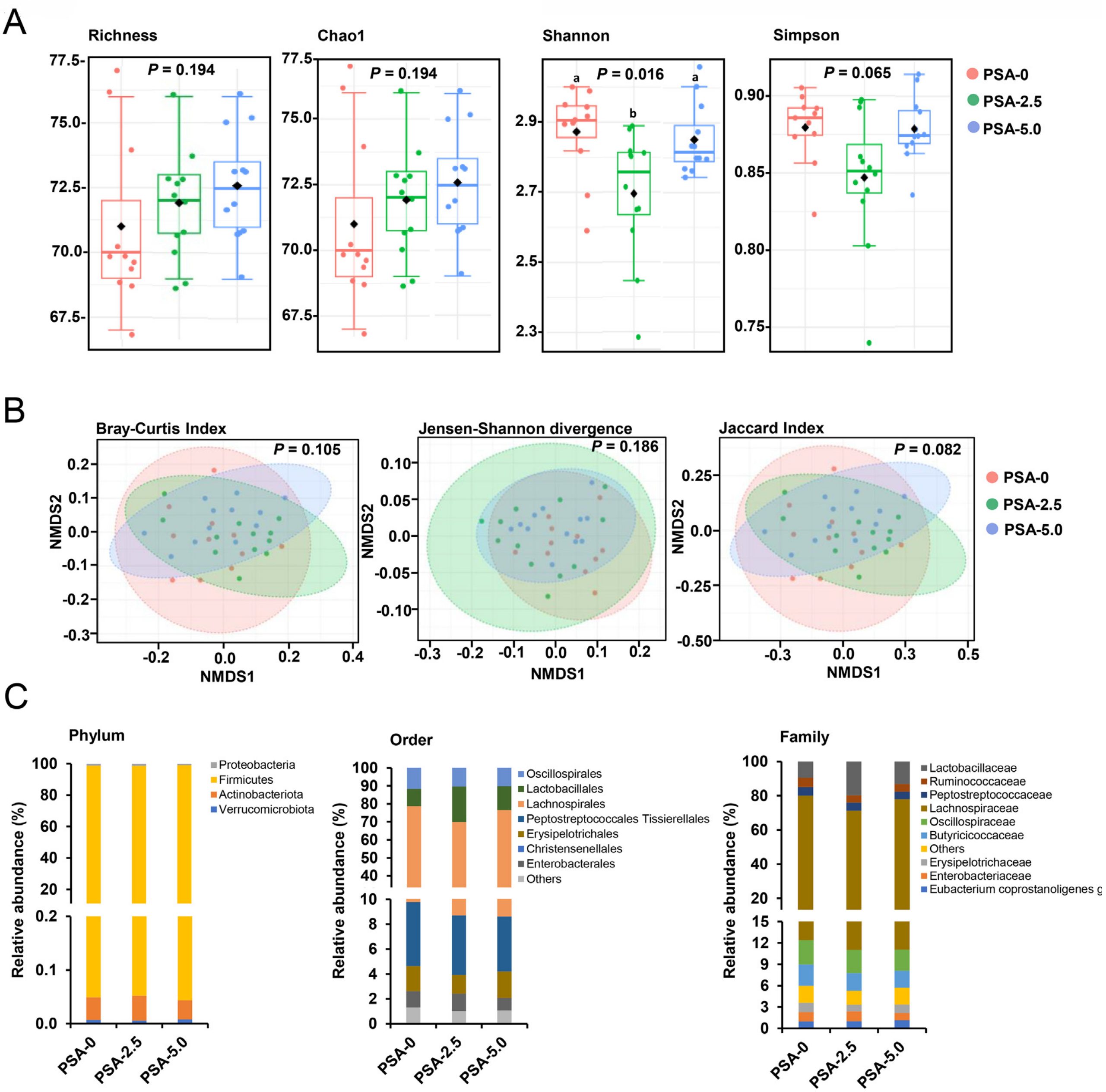

Figure 1. Analysis of the cecal microbiota. Indicators of α-diversity (Richness, Chao1, Shannon Index and Simpson Index) of the cecal bacterial community **(A),** visualization of the difference in the β-diversity (Bray-Curtis Index, Jenson-Shannon divergence and Jaccard Index) of cecal community between the groups by NMDS plots **(B)** and taxonomic composition (phylum, order, family) **(C)** of the cecal bacteria of broilers fed diets with either 0% (PSA-0), 2.5% (PSA-2.5) or 5% (PSA-5.0) *P. sapidus* mycelium for 35 d. (**A**) box plot for $n = 12$ broilers/group; (**B**) NMDS plots for $n = 12$ broilers/group; (**C**) Data are means for $n = 12$ broilers/group.

the microbiota were analyzed at different taxonomic levels (Figure 1C). The analysis at the phylum level showed that the cecal digesta samples contained bacteria from only 4 different phyla, whose abundance did not differ between the 3 groups. The predominant phylum was Firmicutes with > 98% in all groups, followed by Proteobacteria (1.01−1.40% in all groups), Actinobacteriota (0.03−0.04% in all groups) and Verrumicrobiota (< 0.01% in all groups). At the order level, 14 orders were identified with Lachnospirales (60−67% in all groups), Oscillospirales (11−13%) and Lactobacillaes (9-19%) as dominant taxa. However, only 2 low-abundance bacterial taxa (Clostridia UCG 014 and Eubacteriales, both belonging to the phylum Firmicutes) differed across the groups ($P < 0.05$). Clostridia UCG 014 showed higher abundance in the group PSA-0 and group PSA-5.0 than in group PSA-2.5, while the abundance of Eubacteriales was higher in group PSA-5.0 than in the other 2 groups and higher in group PSA-2.5 than in group PSA-0. At the family level, 23 different families were identified and only 1 low-abundance bacterial family (Anaerofustaceae), belonging to the class Clostridia and phylum Firmicutes, was affected by treatment ($P < 0.05$). The abundance of Anaerofustaceae was higher in group PSA-5.0 compared to the other 2 groups and higher in group PSA-2.5 compared to group PSA-0. The abundance of all bacterial taxa in the cecal digesta of the broilers is shown in Supplemental Table S4.

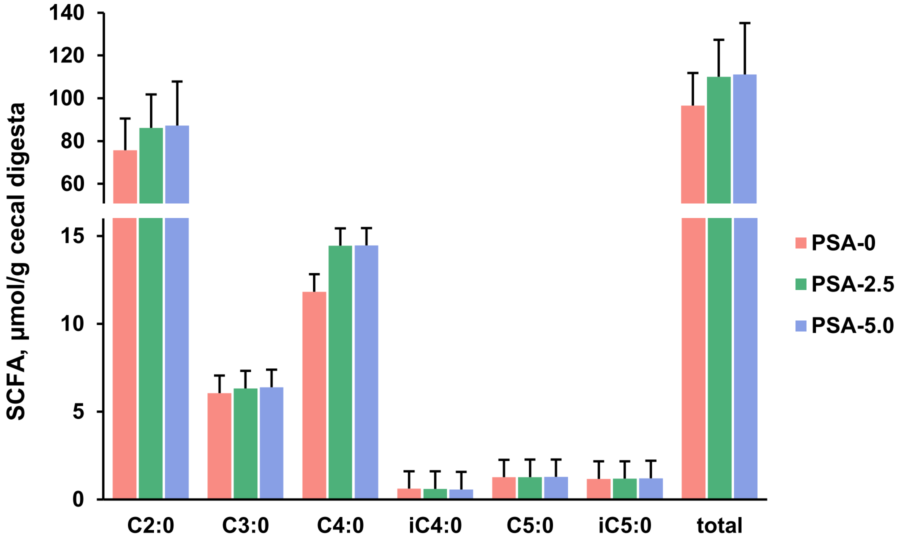

Figure 2. Concentrations of individual (acetic acid (C2:0), $P = 0.227$; propionic acid (C3:0), $P = 0.739$; butyric acid (C4:0), $P = 0.253$; isobutyric acid (iC4:0), $P = 0.896$; valeric acid (C5:0), $P = 0.983$; isovaleric acid (iC5:0), $P = 0.958$) and total (= sum of all individual, $P = 0.157$) short-chain fatty acids (**SCFA**) in cecal digesta of broilers fed diets with either 0% (PSA-0), 2.5% (PSA-2.5) or 5% (PSA-5.0) *P. sapidus* mycelium for 35 d. Data are means ± SD, $n = 12$ broilers/group.

Cecal Digesta Concentrations of SCFA

The concentrations of total and individual SCFA (C2:0, acetic acid; C3:0, propionic acid; C4:0, butyric acid; iC4:0, isobutyric acid; C5:0, valeric acid; iC5:0, isovaleric acid) in the cecal digesta were not different between the 3 groups of broilers (Figure 2).

Concentration of LPS in Plasma and mRNA Levels of Genes Involved in Gut Integrity in Cecum Mucosa

The concentration of LPS in plasma did not differ between the 3 groups of broilers. The mRNA levels of genes encoding tight-junction proteins (*CLDN3, CLDN5, JAM2* and *OCLN*), mucins (*MUC2, MUC5AC* and *MUC13*) and pro-inflammatory genes (*IL1b, IL8L1, IL8L2, TLR4* and *VCAM1*) in cecum mucosa were not different between the 3 groups (Table 5).

Liver Weight and Concentrations of TG and Chol in Liver and Plasma

Liver weights and concentrations of TG and Chol in liver and plasma are shown in Table 6. The liver weight was significantly higher in broilers of group PSA-5.0 than in broilers of group PSA-0 ($P < 0.05$), but did not differ between group PSA-5.0 and group PSA-2.5 and between group PSA-2.5 and group PSA-0. The relative liver weight did not differ between the 3 groups. While the concentrations of TG and Chol in plasma and concentration of Chol in liver did not differ between the groups, the concentration of TG in liver was higher in broilers of group PSA-5.0 than in broilers of group PSA-2.5 ($P < 0.05$). The concentration of TG in the liver did

Table 5. Concentrations of lipopolysaccharides in plasma and mRNA levels of genes in cecum mucosa of broilers fed diets with either 0% (PSA-0), 2.5% (PSA-2.5) or 5.0% (PSA-5.0) *Pleurotus sapidus* mycelium for 35 d.

	PSA-0	PSA-2.5	PSA-5.0	*P*-value
Lipopolysaccharides (pg/ml)	22.9 ± 20.4	17.0 ± 12.0	8.21 ± 6.01	0.155
Normalized mRNA level (fold of PSA-0)				
Tight-junction proteins				
CLDN3	1.00 ± 0.69	0.92 ± 0.70	0.76 ± 0.31	0.747
CLDN5	1.00 ± 0.55	1.36 ± 1.09	1.17 ± 0.69	0.848
JAM2	1.00 ± 0.86	0.93 ± 0.66	1.1 ± 0.94	0.973
OCLN	1.00 ± 0.42	0.96 ± 0.56	1.17 ± 0.44	0.558
Mucins				
MUC2	1.00 ± 0.72	1.52 ± 0.97	1.22 ± 0.73	0.384
MUC5AC	1.00 ± 0.57	0.88 ± 0.25	0.71 ± 0.44	0.531
MUC13	1.00 ± 0.50	1.25 ± 0.62	1.13 ± 0.55	0.590
Proinflammatory genes				
IL1B	1.00 ± 0.69	1.24 ± 0.40	1.07 ± 0.52	0.268
IL8L1	1.00 ± 0.58	0.93 ± 0.56	0.96 ± 0.54	0.831
IL8L2	1.00 ± 0.57	1.02 ± 0.57	0.99 ± 0.42	0.970
TLR4	1.00 ± 0.77	1.12 ± 0.59	0.83 ± 0.43	0.568
VCAM1	1.00 ± 0.95	1.22 ± 0.73	1.16 ± 0.55	0.344

Data are means ± SD, $n = 12$ broilers/group.
[a,b]Means without a common letter differ across the groups, $P < 0.05$.

Table 6. Liver weight and triglycerides and cholesterol in liver and plasma of broilers fed diets with either 0% (PSA-0), 2.5% (PSA-2.5) or 5.0% (PSA-5.0) *Pleurotus sapidus* mycelium for 35 d.

	PSA-0	PSA-2.5	PSA-5.0	*P*-value
Liver weight (g)	49.9 ± 4.8^{b}	54.0 ± 6.8^{ab}	57.3 ± 4.6^{a}	0.044
Liver weight (% of BW)	1.73 ± 0.16	1.83 ± 0.23	1.92 ± 0.14	0.059
Liver				
Triglycerides (μmol/g)	13.6 ± 2.8^{ab}	13.0 ± 2.3^{b}	16.7 ± 4.0^{a}	0.021
Cholesterol (μmol/g)	12.4 ± 0.8	11.7 ± 1.9	13.3 ± 2.0	0.158
Plasma				
Triglycerides (mmol/L)	0.57 ± 0.26	0.53 ± 0.22	0.74 ± 0.27	0.121
Cholesterol (mmol/L)	2.90 ± 0.71	2.88 ± 0.47	3.10 ± 0.45	0.276

Data are means ± SD, $n = 12$ broilers/group.
[a,b]Means without a common letter differ across the groups, $P < 0.05$. Abbreviation: BW, body weight.

not differ between broilers of group PSA-0 and broilers of group PSA-5.0.

Liver Transcriptome

According to the filter criteria applied ($P < 0.05$; FC >1.3 and < - 1.3), a total of 144 transcripts were identified as differentially expressed (upregulated: 94, downregulated: 50) in the liver between group PSA-5.0 and group PSA-0. The volcano plot (Figure 3A) illustrates the differentially expressed transcripts between group PSA-5.0 and group PSA-0 as red dots. The top 10 upregulated transcripts in decreasing order of their FC (in brackets) were: *TOPAZ1* (1.97), *MYH1A* (1.63), *LOC429206* (1.59), *XCL1* (1.58), *LOC101750249* (1.56), *C3AR1* (1.55), *LOC422924* (1.55), *GJD2* (1.55), *ASL1* (1.53) and *PLACL2* (1.53). The top 10 downregulated transcripts in increasing order of their FC (in brackets) were: *KIF20B* (-1.73), *B3GALT5* (-1.68), *MXRA8* (-1.64), *ANGPTL2* (-1.58), *NSMF* (-1.58), *CAMK1D* (-1.54), *RNF138* (-1.51), *FGL1* (-1.51), *SEMA5A* (-1.49) and *PXDN* (-1.46). The FC and *P*-value of all differentially expressed transcripts between group PSA-5.0 vs. PSA-0 are shown in Supplemental Table S5.

Considering the same filter criteria as for the comparison of group PSA-2.5 and group PSA-0, a total of 58 transcripts were identified as differentially expressed (upregulated: 34, downregulated: 24) in the liver (Figure 3B). Only one gene (*GUCY2C*) was regulated > 2.0-fold and one gene (*LDHB*) < -2.0-fold. The 10 most strongly upregulated transcripts in decreasing order of their FC (in brackets) were: *GUCY2C* (2.27), *LOC422513* (1.97), *TMEM27* (1.78), *ACE2* (1.69), *C11ORF34* (1.66), *LOC100859846* (1.65), *DNAJC6* (1.64), *CYP2K1L* (1.61), *OVSTL* (1.57) and *LOC428289* (1.49). The 10 most strongly downregulated transcripts in increasing order of their FC (in brackets) were: *LDHB* (-2.26), *CACNA1D* (-1.87), *NR0B1* (-1.70), *ADGRD1* (-1.60), *LOC768786* (-1.59), *MIR6578* (-1.54), *ACAA1* (-1.44), *SLC16A1* (-1.44), *GAL2* (-1.44) and *TMEM186* (-1.43). The FC and *P*-value of all differentially expressed transcripts between group PSA-2.5 and group PSA-0 are shown in Supplemental Table S6.

Microarray data of 17 differentially expressed transcripts between groups PSA-5.0 and PSA-0 were validated by qPCR. As shown in Supplemental Table S7, the effect direction (positive or negative FC) was the same between microarray and qPCR for all validated transcripts, whereas the effect size (value of FC) differed to some extent for the validated transcripts between microarray and qPCR. Statistical analysis of qPCR data revealed that only 2 transcripts were regulated significantly (*P*-value < 0.05) (*FYB1, PSTPIP2*), whereas 3 transcripts showed a *P*-value < 0.1 (*MYH1A, TOPAZ1, XCL1*). The other twelve transcripts were not regulated (*ANGPTL2, C3AR1L, CCNB1, HTR7, KITLG, PHC1, PLEK, SMC2, TBXAS1, TOP2A, TSHZ2, WWTR1*).

In order to extract biological meaning from the transcripts differentially expressed between groups PSA-5.0 and PSA-0, GSEA was performed using GO terms and KEGG-pathways. Due to the low number of differentially expressed transcripts, GSEA was not conducted for the comparison of groups PSA-2.5 and PSA-0. The GSEA identified 13 enriched terms within the upregulated genes. “Neuroactive ligand-receptor interaction” was the most strongly enriched GO term belonging to GO category cellular component (Figure 3C). The most strongly enriched KEGG-pathway was “integral component of plasma membrane pathway” ($P = 0.041$). Fourteen terms were identified to be enriched within the downregulated transcripts. “Cell division” was the most strongly enriched GO term (Figure 3D). Within the downregulated transcripts, “cell cycle”, “progesterone-mediated oocyte maturation” and “melanogenesis” were identified as enriched KEGG-pathways.

Plasma Metabolome

The detected metabolites belonged to the group of TG (188), followed by phosphatidylethanolamines (59), phosphatidylcholines (49), phosphatidylinositols (38), amino acids (38), phosphatidylglycerols (26), Chol esters (18), phosphatic acids (17), diacylglycerols (11), fatty acids (11), ceramides (8), sphingomyelins (6), biogenic amines (5), carboxylic acids (4), monoacylglycerols (4), bile acids (3), glucosylceramides (3), sphingobases (3), indoles (2), nucleobases related (2), cresols (1), phosphatidylserines (1), sugars (1), and vitamins and cofactors (1). The concentration of all the analyzed metabolites did not differ between the 3 groups of broilers according

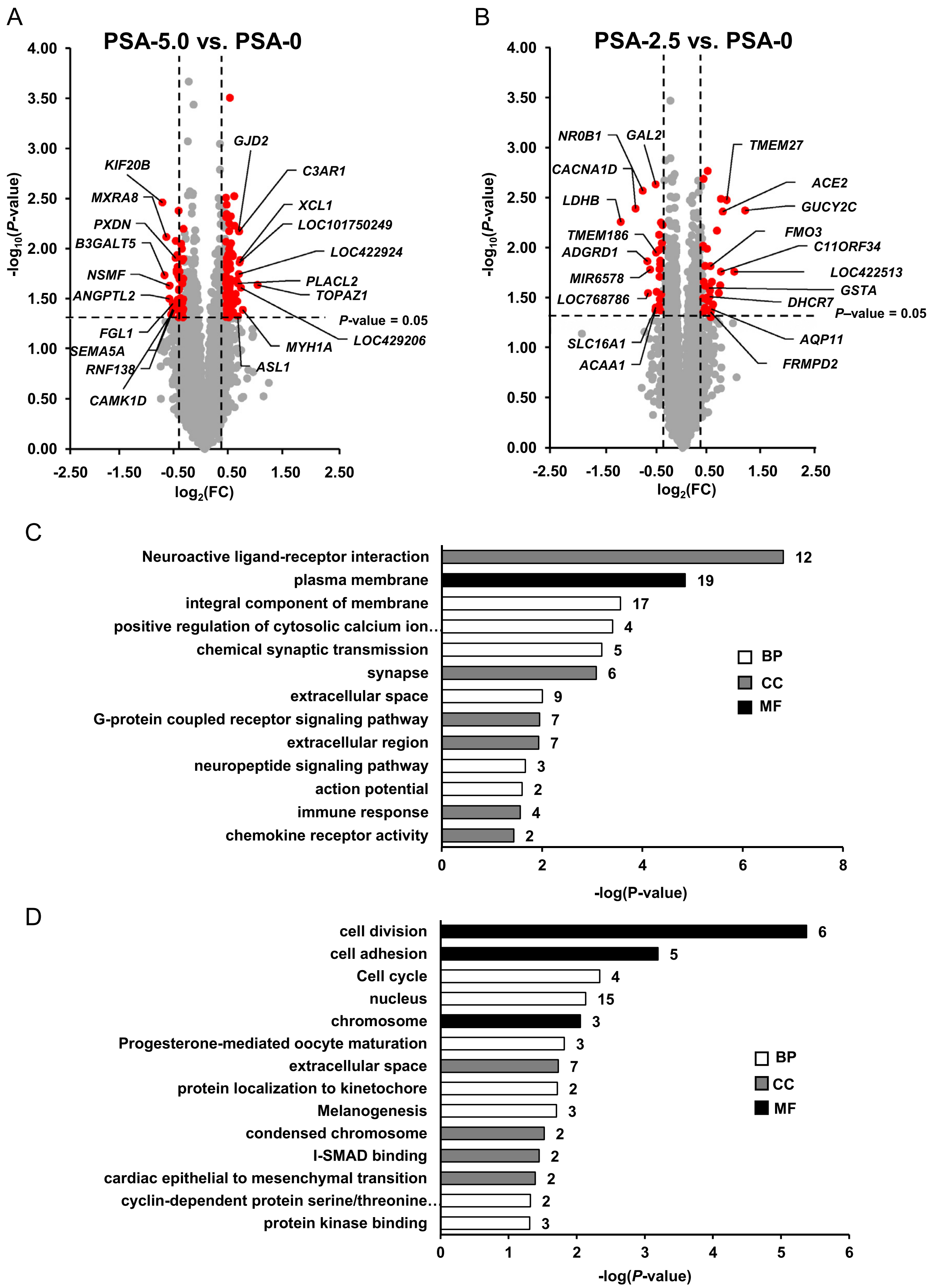

Figure 3. Differential transcriptome analysis in the liver ($n = 6$ microarrays/group). Volcano plot illustrating the differentially expressed transcripts in the liver of broilers between group PSA-5.0 vs. PSA-0 **(A)** and group PSA-2.5 vs. PSA-0 **(B)**. The filtering criteria are indicated by horizontal (P-value = 0.05) and vertical (fold change >1.3 or < -1.3) dashed lines. Red dots in the upper left and upper right corner represent the downregulated and upregulated transcripts. Most enriched gene ontology (GO) terms (biological process (BP), cellular component (CC) and molecular function (MF)) associated with the differentially expressed upregulated **(C)** and downregulated **(D)** transcripts between group PSA-5.0 vs. PSA-0. GO terms are sorted by their enrichment P-values (EASE score) (top: lowest P-value, bottom: highest P-value).

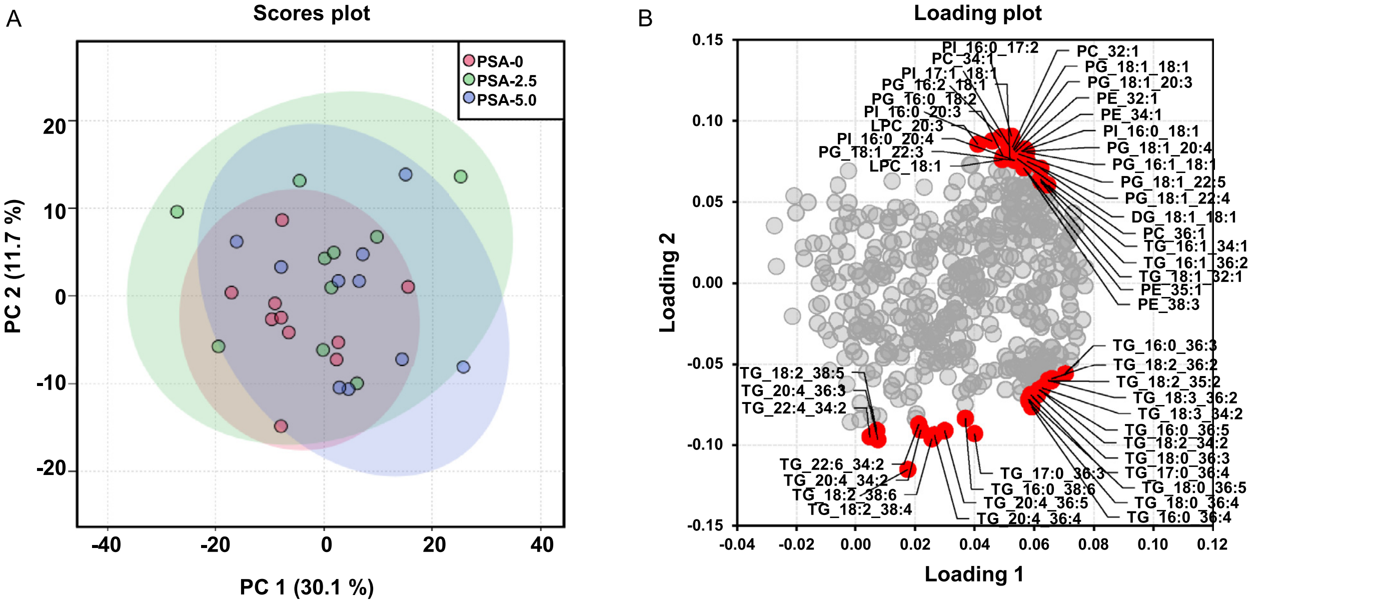

Figure 4. Principal component analysis (**PCA**) of plasma metabolome. Scores plot with plotted 5% confidence interval **(A)** and associated loading plot **(B)** of PCA of broilers plasma metabolome. Broilers were fed diets with either 0% (PSA-0), 2.5% (PSA-2.5) or 5.0% (PSA-5.0) *P. sapidus* mycelium for 35 d. Data are principal components (PC 1 or PC 2) and their loadings. The upper 10 % of the parameters with the greatest distance to zero are marked in red, $n = 10$ broilers/group. Abbreviations: DG, Diacylglycerols; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, Phosphatidylinositols; TG, triglycerides.

to the FDR < 0.05. The score plot of the PCA also showed no separate clustering of the 3 groups (Figure 4). All analyzed metabolites are shown in Supplemental Table S8.

DISCUSSION

Several studies have already reported beneficial effects (e.g., hypolipidemic, antioxidant, antibacterial, immune system-enhancing) of fungal fruiting bodies for humans and animals (Mattila et al., 2001; Hu et al., 2006; Valverde et al., 2015). However, knowledge about the effect of fungal mycelia produced by submerged cultivation as a part of feed for poultry is limited. Therefore, the present study was conducted to investigate the effects of a biotechnologically produced *P. sapidus* mycelium on performance, digestibility of nutrients, gut microbiota structure and intermediary metabolism in broilers.

Chemical analysis of the produced *P. sapidus* mycelium showed that carbohydrates were the dominating nutrient fraction making up nearly 80% of the mycelial DM. Similar contents of carbohydrates have been reported for other *P.* mycelia, for example, *P. eous* (former *P. sajor-caju*), produced by submerged cultivation (Maheshwari et al., 2020). Within the carbohydrate fraction, the β-glucans were the dominant compound reaching similar levels as reported for *P. eous* (Manzi, 2000; Maheshwari et al., 2020). Regarding that certain β-glucans are well-known to act as potent prebiotics in monogastric animals (Lam and Chi-Keung Cheung, 2013), the *P. sapidus* mycelium used in the present study could be considered as a rich source of prebiotics. The dietary inclusion level of 5% of the *P. sapidus* mycelium was chosen based on findings of a recent study in which the same dose of *P. eous* mycelium in the diet exhibited beneficial effects on hepatic lipid metabolism and gut function in an obese rat model (Maheshwari et al., 2020). The *P. sapidus* mycelium was also included in a lower dose (2.5%) in order to evaluate if the effects observed are dose-dependent. The fungal mycelium was included into the diets at the expense of wheat in order to keep the energy content of the 3 diets comparable. Isoenergetic inclusion of the fungal mycelium would have been also possible by replacing maize, but we are not aware of any reason that study results would have been substantially different when the mycelium was replaced at the expense of maize.

In the present study, dietary inclusion of 2.5% and 5% *P. sapidus* mycelium did not exert any effects on growth performance, feed intake, feed conversion and AID of EE, CP and amino acids of the broilers. Despite improvements of performance and nutrient digestibility have been documented in several studies with broilers fed β-glucans or other prebiotic oligosaccharides (Chae et al., 2006; Richards et al. 2020; Al-Baadani et al., 2022), there are also studies demonstrating no effect of β-glucans on broiler performance (Cox et al., 2010; Moon et al., 2016). These contradictory results have been attributed to the fact that the structures of different β-glucans can vary significantly which strongly affects physicochemical properties including solubility, viscosity, gelation, binding ability, bulking ability, and fermentability (Singh and Kim, 2021). Considering that improvements of broiler performance in response to feeding of prebiotic-enriched diets in the abovementioned studies were associated with positive effects on gut integrity and/or gut microbiota composition (Richards et al. 2020; Al-Baadani et al., 2022), we also studied the effect of dietary inclusion of the *P. sapidus* mycelium and, thus, a high content of β-glucans on the bacterial community structure and diversity in the gut of the broilers.

Evaluation of the effect of dietary inclusion of *P. sapidus* mycelium on the gut microbiota composition and diversity of the broilers based on metagenomic analysis of the cecum digesta revealed only marginal effects. Amongst the α-diversity metrics investigated, only 1 (Shannon-Index) out of 4 was found to be slightly affected, while all β-diversity indicators did not differ among groups. Likewise, analysis of the microbiota composition of the broilers at different taxonomic levels showed almost no effect of dietary treatment. The only exception was that the abundance of 2 low-abundance bacterial taxa belonging to the phylum Firmicutes (Clostridia UCG 014, Eubacteriales) was altered in the groups fed the *P. sapidus* mycelium. Thus, the lack of effect of the *P. sapidus* mycelium on broiler performance in the present study could be explained by its very weak effect on the gut microbiota structure, which might be caused by a limited fermentability of the β-glucans or other carbohydrates of the fungal mycelium. The latter assumption is supported by the observation that the concentrations of total and all individual SCFA in the cecal digesta of the broilers did not differ among groups. It can therefore be concluded that, in contrast to our assumption, the high β-glucan content of the *P. sapidus* mycelium had only marginal effects on the structure of the cecum microbiota community in healthy broilers. Despite these marginal effects, the lack of any untoward changes of the gut microbiota structure, like increases in the abundance of obligate pathogens, suggests that the *P. sapidus* mycelium can be fed to broilers without impairment of the gut microbial community.

In contrast to these weak effects of the *P. sapidus* mycelium on the gut microbiota in broilers, feeding a *P. eous* mycelium-enriched diet to obese rats caused a strong effect on the gut microbiota structure with increases in the abundance of beneficial bacterial taxa, such as *Lactobacillaceae, Bifidobacterium, Roseburia*, and *Blautia*, and a decrease in the abundance of the pathogenic genus *Escherichia–Shigella* (Maheshwari et al., 2021). In addition, the favorable effect of dietary *P. eous* mycelium on the gut microbiota in the obese rat model was associated with an improved gut barrier function as evidenced from decreased levels of the bacterial endotoxin LPS (Maheshwari et al., 2021). It is well-known that obese animals develop gut dysbiosis – a term used to describe a perturbation of the commensal gut bacteria. Gut dysbiosis causes an impairment of the gut barrier function due to gut mucosa inflammation, decreased production of mucins leading to thinning of the protective mucin layer and reduced production of tight-junction proteins, which tightly connect adjacent intestinal epithelial cells in order to prevent paracellular passage of bacteria from the gut lumen into the portal vein (Ringseis et al., 2020). Consequently, gut dysbiosis is associated with hyperpermeability of the gut and induction of endotoxemia and metabolic derangements (Ringseis et al., 2020).

In the present broiler study, no evidence was gained that gut integrity was affected by dietary inclusion of the *P. sapidus* mycelium. This assumption is based on the observation that plasma levels of LPS and the mRNA levels of proinflammatory genes (*IL1B, IL8L1, IL8L2, TLR4, VCAM1*), tight-junction proteins (*CLDN3, CLDN5, JAM2, OCLN*), and mucins (*MUC2, MUC5AC, MUC13*) in the cecum mucosa did not differ among groups. These findings indicated that the key protective elements of the gut barrier were not impaired and no substantial translocation of bacterial compounds occurred in response to dietary inclusion of the *P. sapidus* mycelium in the broilers. This effect of feeding the *P. sapidus* mycelium can be considered as favorable considering that no impairment of gut integrity is beneficial and an improvement of gut integrity in healthy broilers with a normal gut microbiota structure is unlikely. In contrast to healthy broilers, feeding a *P. eous* mycelium to obese rats suffering from gut dysbiosis is able to restructure the perturbed gut microbiota, thereby, improving gut integrity.

Apart from marginal or absent effects of feeding the β-glucan-rich *P. sapidus* mycelium on the gut microbiota and gut integrity, our study revealed only very few effects on the intermediary metabolism of the broilers. The gut microbiota is well-known to strongly affect animal's intermediary metabolism and feeding behavior *via* the gut-liver-brain axis, and gut-derived compounds, such as SCFA or bile acids, serve as the communication signals between gut microbes and all key metabolic tissues (Ringseis et al., 2020). In order to evaluate if the intermediary metabolism of the broilers was affected by feeding the *P. sapidus* mycelium, we carried out genome-wide hepatic transcript profiling. According to this analysis, the *P. sapidus* mycelium had only a very moderate effect on the hepatic transcriptome, which was particularly evident from the very weak regulation of the genes identified to be differentially expressed between group PSA-5.0 and PSA-0. None of these genes was regulated greater 2-fold and *TOPAZ1* (1.97-fold) and *KIF20B* (-1.73-fold) were the top up- and down-regulated genes, respectively. Considering the above-mentioned connection between gut microbiota and host metabolism, the weak effect of the *P. sapidus* mycelium on the hepatic transcriptome is in agreement with the very weak effect on the cecal microbiota and the lack of effect on cecal SCFA concentrations. Despite the very weak effect on the hepatic transcriptome, liver weights and liver TG concentration but not liver Chol concentration were slightly higher in the broilers of group PSA-5.0 compared to group PSA-0. Since hepatic transcript profiling did not reveal an up-regulation of hepatic genes involved in lipid synthesis, such as *ACACA, FASN, SCD, HMGCR*, and genes involved in inflammation and stress-response, such as *VCAM1, TLR4, IL8L1*, in group PSA-5.0 compared to group PSA-0, the mechanism underlying the increase of hepatic TG concentration is unknown. However, considering that the hepatic TG concentration in broilers of group PSA-5.0 was still within the physiological range, this slight increase should not be overstated. Based on this, the effect of the *P. sapidus* mycelium on hepatic metabolism can be regarded as very moderate. Our assessment of a weak effect of the *P. sapidus* mycelium on the intermediary metabolism of broilers is supported by the results from targeted metabolomics of blood plasma and from the measurement of plasma TG and Chol concentrations. According to plasma metabolomics, the concentrations of none of the large set of metabolites analyzed differed across the groups. This was also reflected by PCA showing no separation of the metabolomes of the different groups. Despite our study revealed no obvious improvements of intermediary metabolism of the broilers due to dietary inclusion of the *P. sapidus* mycelium, the lack of any adverse metabolic effects, which is a prerequisite for the use of novel feed components in animal nutrition, can be considered as beneficial.

CONCLUSIONS

The present study shows that dietary inclusion of 2.5% and 5% of a biotechnologically produced *P. sapidus* mycelium in broiler diets does not affect growth performance and nutrient digestibility. In addition, feeding of the *P. sapidus* mycelium exhibited only a negligible effect on the cecal microbiota community but did not affect the concentrations of microbial fermentation products and did not impair gut barrier function. In line with this, liver transcriptomic and plasma metabolomics revealed only weak or not any effects, respectively, of feeding the *P. sapidus* mycelium in the broilers. Based on these findings it can be concluded that inclusion of a sustainably produced fungal mycelium in broiler diets at the expense of other feed components, such as wheat, has no negative consequences on broilers´ performance and metabolism. However, it cannot be excluded that higher doses of *P. sapidus* mycelium achieve different results.

ACKNOWLEDGMENTS

Lea Schäfer was financially supported by H. Wilhelm Schaumann foundation (Hamburg, Germany).

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.103975.

REFERENCES

Ahlborn, J., A. Stephan, T. Meckel, G. Maheshwari, M. Rühl, and H. Zorn. 2019. Upcycling of food industry side streams by basidiomycetes for production of a vegan protein source. Int. J. Recycl. Org. Waste Agricult. 8:447–455.

Al-Baadani, H. H., R. A. Alhotan, A. A. Al-Abdullatif, I. A. Alhidary, A. S. Alharthi, S. I. Al-Mufarrej, M. A. Al-Garadi, M. M. Qaid, A. A. Al-Sagan, K. E. Ibrahim, and M. M. Azzam. 2022. The effect of gum arabic supplementation on growth performance, blood indicators, immune response, cecal microbiota, and the duodenal morphology of broiler chickens. Animals (Basel) 12:2809.

Beller, S., S. M. Grundmann, K. Pies, E. Most, S. Schuchardt, W. Seel, M.-C. Simon, K. Eder, and R. Ringseis. 2024. Effect of replacing soybean meal with *Hermetia illucens* meal on cecal microbiota, liver transcriptome and plasma metabolome of broilers. Poult. Sci. 103:103635.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:852–857.

Brandt, M., and S. M. Allam. 1987. Analytik von TiO2 im Darminhalt und Kot nach Kjeldahlaufschluss. Arch. Anim. Nutr. 37:453–454.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. methods. 13:581–583.

Chae, B. J., J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, and T.-W. Hahn. 2006. Effects of supplementation of β-glucan on the growth performance and immunity in broilers. Res. Vet. Sci. 80:291–298.

Chang, S. T., and J. A. Buswell. 1996. Mushroom nutriceuticals. World J. Microbiol. Biotechnol. 12:473–476.

Cheung, P. C. K. 1998. Functional properties of edible mushrooms. J. Nutr. 128:1512–1516.

Chiappisi, E., R. Ringseis, K. Eder, and D. K. Gessner. 2017. Effect of endoplasmic reticulum stress on metabolic and stress signaling and kidney-specific functions in Madin-Darby bovine kidney cells. J. Dairy Sci. 100:6689–6706.

Chong, J., P. Liu, G. Zhou, and J. Xia. 2020. Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nat. Protoc. 15:799–821.

Cobb-Vantress. Cobb 500 broiler performance & nutrition supplement 2022.

Cox, C. M., L. H. Stuard, S. Kim, A. P. McElroy, M. R. Bedford, and R. A. Dalloul. 2010. Performance and immune responses to dietary β-glucan in broiler chicks. Poult. Sci. 89:1924–1933.

Dhariwal, A., J. Chong, S. Habib, I. L. King, L. B. Agellon, and J. Xia. 2017. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res 45:W180–W188.

Eder, K., and M. Kirchgessner. 1994. Dietary fat influences the effect of zinc deficiency on liver lipids and fatty acids in rats force-fed equal quantities of diet. J. Nutr. 124:1917–1926.

Edgar, R., M. Domrachev, and A. E. Lash. 2002. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 30:207–210.

EUR_Lex. 09.01.2024. EUR-Lex - 32009R1099. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing, (https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32009R1099). Accessed Jan. 2024.

Fiesel, A., D. K. Gessner, E. Most, and K. Eder. 2014. Effects of dietary polyphenol-rich plant products from grape or hop on proinflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. BMC Vet. Res. 10:196.

Grundmann, S. M., K. Ress, L. Zimmermann, M. Höring, G. Liebisch, E. Most, R. Ringseis, and K. Eder. 2023. A high-phosphorus diet moderately alters the lipidome and transcriptome in the skeletal muscle of adult mice. Nutrients 15:3734.

Hara, A., and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90:420–426.

Hu, S. H., Z. C. Liang, Y. C. Chia, J. L. Lien, K. S. Chen, M. Y. Lee, and J. C. Wang. 2006. Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. J. Agric. Food Chem. 54:2103–2110.

Huang, C. M., and T. T. Lee. 2018. Immunomodulatory effects of phytogenics in chickens and pigs - a review. Asian-Australas. J. Anim. Sci. 31:617–627.

Huang, D. W., B. T. Sherman, and R. A. Lempicki. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4:44–57.

Lam, K.-L., and P. Chi-Keung Cheung. 2013. Non-digestible long chain beta-glucans as novel prebiotics. Bioact. Carbohydr. Diet Fibre 2:45–64.

Maheshwari, G., D. K. Gessner, S. Meyer, J. Ahlborn, G. Wen, R. Ringseis, H. Zorn, and K. Eder. 2020. Characterization of the nutritional composition of a biotechnologically produced oyster mushroom and its physiological effects in obese zucker rats. Mol. Nutr. Food Res. 64:e2000591.

Maheshwari, G., D. K. Gessner, K. Neuhaus, E. Most, H. Zorn, K. Eder, and R. Ringseis. 2021. Influence of a biotechnologically produced oyster mushroom (*Pleurotus sajor-caju*) on the gut microbiota and microbial metabolites in obese Zucker rats. J. Agric. Food Chem. 69:1524–1535.

Manzi, P. 2000. Beta-glucans in edible mushrooms. Food Chem 68:315–318.

Marschall, M. J. M., S. M. Grundmann, W. Seel, M.-C. Simon, S. Schuchardt, E. Most, D. K. Gessner, G. Wen, R. Ringseis, and K. Eder. 2023. Fat from *Hermetia illucens* alters the cecal gut microbiome and lowers hepatic triglyceride concentration in comparison to palm oil in obese zucker rats. J. Nutr. 154:455–468.

Mattila, P., K. Könkö, M. Eurola, J. M. Pihlava, J. Astola, L. Vahteristo, V. Hietaniemi, J. Kumpulainen, M. Valtonen, and V. Piironen. 2001. Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. J. Agric. Food Chem. 49:2343–2348.

Meyer, S., D. K. Gessner, G. Wen, E. Most, G. Liebisch, H. Zorn, R. Ringseis, and K. Eder. 2019. The antisteatotic and hypolipidemic effect of insect meal in obese Zucker rats is accompanied by profound changes in hepatic phospholipid and 1-carbon metabolism. Mol. Nutr. Food Res. 63:e1801305.

Moon, S. H., I. Lee, X. Feng, H. Y. Lee, J. Kim, and D. U. Ahn. 2016. Effect of dietary beta-glucan on the performance of broilers and the quality of broiler breast meat. Asian-Australas. J. Anim. Sci. 3:384–389.

OECD/FAO. 2023. OECD-FAO Agricultural Outlook 2023-2032. OECD Publishing, Paris.

Papaspyridi, L.-M., N. Aligiannis, E. Topakas, P. Christakopoulos, A.-L. Skaltsounis, and N. Fokialakis. 2012. Submerged fermentation of the edible mushroom *Pleurotus ostreatus* in a batch stirred tank bioreactor as a promising alternative for the effective production of bioactive metabolites. Molecules (Basel, Switzerland) 17:2714–2724.

Richards, P. J., G. M. Flaujac Lafontaine, P. L. Connerton, L. Liang, K. Asiani, N. M. Fish, and I. F. Connerton. 2020. Galacto-oligosaccharides modulate the juvenile gut microbiome and innate immunity to improve broiler chicken performance. mSystems 5 e00827-19.

Ringseis, R., D. K. Gessner, and K. Eder. 2020. The gut-liver axis in the control of energy metabolism and food intake in animals. Annu. Rev. Anim. Biosci. 8:295–319.

Ringseis, R., J. O. Zeitz, A. Weber, C. Koch, and K. Eder. 2019. Hepatic transcript profiling in early-lactation dairy cows fed rumen-protected niacin during the transition from late pregnancy to lactation. J. Dairy Sci. 102:365–376.

Schäfer, L., S. M. Grundmann, G. Maheshwari, M. Höring, G. Liebisch, E. Most, K. Eder, and R. Ringseis. 2023. Effect of replacement of soybean oil by Hermetia illucens fat on performance, digestibility, cecal microbiome, liver transcriptome and liver and plasma lipidomes of broilers. J. Anim. Sci. Biotechnol. 14:20.

Sherman, B. T., M. Hao, J. Qiu, X. Jiao, M. W. Baseler, H. C. Lane, T. Imamichi, and W. Chang. 2022. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res 50:W216–W221.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Singh, A. K., and W. K Kim. 2021. Effects of dietary fiber on nutrients utilization and gut health of poultry: a review of challenges and opportunities. Animals (Basel) 11:181.

Souci, S. W., W. Fachmann, H. Kraut, and G. Andersen. 2016. Food Composition and Nutrition Tables. 8th Revised and Completed Edition - 8., revidierte und ergänzte Auflage - 8e édition, revue et complétée. MedPharm Scientific Publishers, Stuttgart Wissenschaftliche Verlagsgesellschaft.

Trapp, T., M. Zajul, J. Ahlborn, A. Stephan, H. Zorn, and M. A. Fraatz. 2018. Submerged cultivation of pleurotus sapidus with molasses: aroma dilution analyses by means of solid phase microextraction and stir bar sorptive extraction. J. Agric. Food Chem. 66:2393–2402.

Valverde, M. E., T. Hernández-Pérez, and O. Paredes-López. 2015. Edible mushrooms: improving human health and promoting quality life. Int. J. Microbiol. 2015:376387.

Vandesompele, J., K. de Preter, F. Pattyn, B. Poppe, N. van Roy, A. de Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3 RESEARCH0034.

VDLUFA. 2012. Die chemische Untersuchung von Futtermitteln. Dritte Auflage. VDLUFA-Verlag, Darmstadt.

WPSA. World's Poultry Science Association. 1984. The prediction of apparent metabolizable energy values for poultry in compound feeds. World's Poult. Sci. J. 40:181–182.

Wu, G., J. Fanzo, D. D. Miller, P. Pingali, M. Post, J. L. Steiner, and A. E. Thalacker-Mercer. 2014. Production and supply of high-quality food protein for human consumption: sustainability, challenges, and innovations. Ann. New York Acad. Sci. 1321:1–19.

Xia, J., N. Psychogios, N. Young, and D. S. Wishart. 2009. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic Acids Res 37:W652–W660.

3.4 Research note: Wirkung eines biotechnologisch hergestellten *Pleurotus sapidus* Mycels auf die Expression von Genen, die an der Proteinsynthese und dem Proteinabbau im Brustmuskel von Mastbroilern beteiligt sind

Research note: Effect of a biotechnologically produced *Pleurotus sapidus* mycelium on expression of genes involved in protein synthesis and degradation in breast muscle of broilers

103 (2024) 104450

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Research Note

Research note: Effect of a biotechnologically produced *Pleurotus sapidus* mycelium on expression of genes involved in protein synthesis and degradation in breast muscle of broilers

Lea Schäfer [a], Javier Herrero-Encinas [a,b], Martin Rühl [c,d], Holger Zorn [c,d], Erika Most [a], Klaus Eder [a,e], Robert Ringseis [a,e,*]

[a] *Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, Giessen, Germany*
[b] *ETS Ingenieria Agronómica, Alimentaria y de Biosistemas, Departamento de Pruducción Agraria, Universidad Politécnica de Madrid, Madrid, Spain*
[c] *Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Giessen, Germany*
[d] *Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Giessen, Germany*
[e] *Center for Sustainable Food Systems, Justus Liebig University Giessen, Giessen, Germany*

ARTICLE INFO

Key words:
Broilers
Breast muscle
Fungal mycelium
Protein synthesis
Protein degradation

ABSTRACT

Recently, feeding a fungal mycelium from *Pleurotus sapidus* was found to reduce relative breast muscle weight of broilers. The present study tested the hypothesis that dietary inclusion of *P. sapidus* mycelium modulates the expression of genes involved in protein anabolic and protein catabolic pathways in breast muscle of broilers. The study included 72 male, 1-day-old Cobb 500 broilers which were randomly assigned to three groups fed three different diets containing either 0 (PSA-0), 25 (PSA-25) and 50 (PSA-50) g/kg diet *P. sapidus* mycelium in a three-phase feeding system for 35 days. Within the somatropic axis, the mRNA level of *GHR* was higher and that of *IGF1R* was lower in group PSA-25 than in group PSA-0 ($P < 0.05$). Within the mTOR signaling pathway, the mRNA level of *S6K1* was higher in group PSA-25 than in group PSA-0 ($P < 0.05$). Within muscle growth-related genes, the mRNA level of *MYOG* was lower in groups PSA-25 and PSA-50 than in group PSA-0 ($P < 0.05$). The relative phosphorylation of proteins involved in protein anabolic pathways (S6K1, RPS6, eIF2a, AKT) did not differ across the three groups. The mRNA of most genes involved in molecular pathways of protein degradation and inhibition of protein synthesis, such as the GCN/eIF2a pathway, the ubiquitin-proteasome pathway, and the autophagy-lysosomal pathway, showed no differences across the three groups. Only the mRNA level of *ATG9A* was higher in group PSA-25 compared to group PSA-0 ($P < 0.05$). These observations suggest that a modulation of these signaling pathways is unlikely to explain the reduced relative breast muscle weight in broilers. Nevertheless, future studies are necessary to exclude an effect of feeding *P. sapidus* mycelium on other less prominent pathways affecting skeletal muscle mass.

Introduction

Low-value agro-industrial by-products as feed for monogastric animals are reasonable to improve resource efficiency and sustainability of livestock farming. However, the use of such by-products is often limited by their poor nutrient digestibility, which is largely explained by a high crude fiber content (Huang and Lee, 2018). One strategy to improve the utilization of these by-products in poultry feed is their valorization by edible fungi, such as *Pleurotus sapidus*, which are capable of degrading a wide range of non-starch polysaccharides and lignin-containing substrates (Valverde et al., 2015). While commercial mushroom products are usually obtained in a time-exhausting process from the fruiting bodies, the submerged cultivation of mushrooms allows a fast and efficient production of mycelial biomass (Papaspyridi et al., 2012). *P. sapidus* mycelia could be of interest for poultry as a source of prebiotic polysaccharides, such as β-glucans, which support intestinal integrity and overall gut and systemic health (Maheshwari et al, 2020).

Considering that knowledge about the use of fungal mycelia in poultry feeding is scarce, we have recently explored the effect of feeding diets with 25 and 50 g/kg diet *P. sapidus* mycelium in broilers (Schäfer et al., 2024). This study demonstrated only marginal effects on the gut microbiome, the liver transcriptome and the plasma metabolome

* Corresponding author at: Justus Liebig University Giessen, Germany.
E-mail address: Robert.ringseis@ernaehrung.uni-giessen.de (R. Ringseis).

https://doi.org/10.1016/j.psj.2024.104450
Received 17 July 2024; Accepted 28 October 2024
Available online 29 October 2024

(Schäfer et al., 2024). Despite feed intake and feed:gain ratio did not differ between broilers fed the diets without and with *P. sapidus* mycelium, unpublished data of this study showed a reduction of the relative breast muscle weight in broilers fed the diet with 50 g/kg diet *P. sapidus* mycelium indicating that protein turnover in breast muscle might has been affected.

Protein turnover in skeletal muscle is the result of the activity of protein synthetic and protein catabolic pathways (Ferreira and Duarte, 2023). A key signaling pathway stimulating protein synthesis is the mammalian target of rapamycin (**mTOR**) pathway. In addition, the ribosomal protein S6 kinase (**S6K1**), a down-stream target of mTOR, activates protein synthesis. Furthermore, protein anabolism in skeletal muscle is stimulated by insulin-like growth factor 1 (**IGF1**) and hepatic growth hormone receptor (**GHR**) *via* the somatotropic axis. While the alpha serine/threonine-protein kinase (**AKT**) acts as an upstream activator of the protein anabolic mTOR pathway, AKT negatively regulates the forkhead box protein O1 (**FOXO1**) transcription factor, which stimulates protein degradation. Protein degradation by FOXO1 involves induction of F-box only protein 32 (**FBXO32**) encoding the E3 ligase atrogin-1 mediating protein degradation *via* the ubiquitin-proteasome pathway. The E3 ligase atrogin-1 is also induced by pro-inflammatory stimuli which explains that protein degradation *via* the ubiquitin-proteasome pathway is stimulated during inflammatory conditions. A further protein catabolic pathway is the general control nonderepressible 2 (**GCN2**)/eukaryotic translation initiation factor 2A (**eIF2a**) pathway, which inhibits the mTOR pathway (Métayer et al., 2008), and stimulates protein degradation *via* the autophagy-lysosomal pathway (Finkbeiner, 2020).

Against the abovementioned indications that *P. sapidus* mycelium might has affected protein turnover in breast muscle of broilers, the present study tested the hypothesis that dietary inclusion of *P. sapidus* mycelium modulates the expression of genes involved in protein synthesis and muscle growth as well as protein degradation in breast muscle of broilers.

Materials and methods

Animals and diets

For this investigation, breast muscle samples from a recently published animal study involving 72 one-day-old broiler chickens (Cobb 500; Cobb-Vantress, Wiedemar, Germany) were utilized (Schäfer et al., 2024). The five-week feeding trial was authorized by the Animal Welfare Officer of the Justus Liebig University Giessen (Approval no.: JLU 843_M). All experimental procedures followed established guidelines for the care and handling of laboratory animals. The broilers (total $N = 72$) were randomly assigned to three different groups (6 cages/group with 4 broilers each) with similar body weights (**BW**), and fed three different nutrient adequate diets containing either 0 (PSA-0), 25 (PSA-25) and 50 (PSA-50) g/kg diet of a biotechnologically produced *P. sapidus* mycelium in a three-phase feeding system (starter diet: day 1-10, grower diet: day 11-21, finisher diet: day 22-35). The composition of the different experimental diets, which had similar concentrations of crude nutrients, sugar, starch, amino acids, fatty acids and metabolizable energy within each feeding phase, and details about the production of *P. sapidus* mycelium *via* submerged fermentation, animal housing conditions, light regime, feeding regime and animal killing procedure can be found in our recent publication (Schäfer et al., 2024).

Sample collection

After slaughtering the animals as recently described (Schäfer et al., 2024), the left and right thigh and the left and right breast muscle were dissected. Thigh and breast muscle weights were obtained from weighing the left and the right part together. For the measurement of expression of genes involved in protein synthesis and protein degradation pathways, small portions of the right breast muscle from 12 broilers per group (2 broilers/cage), whose BW corresponded to the mean of the whole group, were collected, snap-frozen in liquid nitrogen, and stored at –80°C for later analysis.

Total RNA extraction and qPCR in breast muscle

Total RNA extraction from frozen breast muscle portions (40-50 mg) was performed using TRIzol reagent (Invitrogen, Karlsruhe, Germany). The total RNA concentration and the A260/A280 ratio of all samples was 515 ± 46 ng/μL and 1.92 ± 0.02 (total $N = 36$ or $n = 12$/group), respectively. Analysis of RNA quantity and quality, synthesis of cDNA, qPCR analysis using a Rotor-Gene Q system (Qiagen, Hilden, Germany), and calculation of relative mRNA levels with the comparative Ct method using the sample with the lowest Ct value as reference Ct value were carried out as described in a recent publication (Chiappisi et al., 2017). Characteristics of gene-specific primers (Eurofins MWG Operon, Ebersberg, Germany) are available on request from the corresponding author.

Western blot analysis in breast muscle

Approximately 30 mg of frozen breast muscle tissue was homogenized in radioimmunoprecipitation assay buffer containing phosphatase and protease inhibitors. The homogenates were centrifuged, and the supernatants were stored at -20ºC. Following determination of protein concentration of the supernatants, protein separation using SDS-PAGE (10% separating gel, 6% stacking gel) was carried out with different amounts of protein depending on the target antibody [100 μg for phosphorylated and total S6K1 (p-S6K1, S6K1), phosphorylated and total eIF2a (p-eIF2a, eIF2a), phosphorylated and total alpha serine/threonine-protein kinase (p-AKT, AKT); 30 μg for phosphorylated and total ribosomal protein S6 (**p-RPS6**, RPS6)]. In each gel, a molecular mass marker was included (10-170 kDa, PageRuler prestained protein ladder; Thermo Scientific, Dreieich, Germany). Subsequently, the proteins were electro-transferred for 1.5 h at 100 V and 300 mA to a nitrocellulose membrane (Pall, Pensacola, FL, USA) using a blotting buffer with 20% methanol. Ponceau S (Carl Roth, Karlsruhe, Germany) staining was performed to verify equal loading. Afterwards, the membranes were blocked using 5% skimmed milk powder (for p-S6K1, S6K1, p-RPS6 and RPS6) or 5% bovine serum albumin (for p-eIF2a, eIF2a, p-AKT, and AKT) in TBST buffer. The following primary antibodies were used for the detection of p-S6K1, S6K1, p-RPS6, RPS6, p-eIF2a, eIF2a, p-AKT, and AKT: Phospho-p70 S6 Kinase (Thr389) (108D2) rabbit monoclonal antibody (cat. no. 9234), p70 S6 kinase antibody (cat. no. 9202), Phospho-S6 ribosomal protein (Ser235/236) antibody (cat. no. 2211), S6 ribosomal protein (5G10) rabbit monoclonal antibody (cat. no. 2217), Phospho-eIF2α (Ser51) antibody (cat. no. 9721), eIF2α antibody (cat. no. 9722), Phospho-Akt (Ser473) (D9E) XP rabbit monoclonal antibody (cat. no. 4060), Akt (pan) (11E7) rabbit monoclonal antibody (cat. no. 4685; all from Cell Signaling Technology Europe B.V. (CST), Frankfurt, Germany). Vinculin recombinant rabbit monoclonal antibody (42H89L44; Invitrogen) was used as reference antibody. Primary antibodies were diluted 1:1,000 in the blocking buffer and the membranes were incubated with the primary antibody solution overnight at 4°C. After washing, a horseradish peroxidase-conjugated secondary polyclonal goat anti-rabbit IgG antibody (A0545, Sigma-Aldrich, Taufkirchen, Germany) for all primary antibodies was added in 1:10,000 dilution in TBST buffer to the membranes and incubated for 1.5 h at room temperature. After washing, the blots were developed using the ECL Select western blotting detection reagent (GE Healthcare, Munich, Germany) for 5 min at room temperature. The intensities of the specific bands were detected using a Bio-Imaging system (G:Box, Syngene, Cambridge, UK) and quantified using Syngene Gene Tools 4.3.1 software.

Statistical analysis

Statistical analysis was performed using SPSS 28 software (IBM, Armonk, NY, USA). The cage served as the experimental unit for feed intake and feed:gain ratio ($n = 6$ cages/group; published in Schäfer et al. (2024)) and the individual animal for all other data. Because carcass characteristics and breast muscle and thigh weights were obtained for all animals alive at the end of the trail (total animals alive = 65), the means from these data represented $n = 21$-22 broilers/group. Data from qPCR and Western blotting were calculated from a total of 36 animals ($n = 12$ broilers/group), because only 2 broilers/cage (6 cages/group) were used for these analyses. All data were tested for normal distribution using the Kolmogorov-Smirnov test for carcass characteristics and breast muscle and thigh weights and Shapiro-Wilk test for qPCR and Western blotting data. For evaluating homoscedasticity, the Levene's test was used. If the normal distribution was only achieved after log-transformation, the log-transformed data were used for statistical analysis. Differences between the three groups were analyzed using one-way analysis of variance (one-way **ANOVA**) followed by a Tukey's post-hoc test when the data were normally distributed, and the variances were homogeneous. If the variance was heterogenous, the means of the three groups were analyzed using Welch's ANOVA and the Games-Howell post-hoc test. If data were not-normally distributed, a one-way ANOVA according to Kruskal-Wallis was performed using the Mann-Whitney U test with Bonferroni correction as post-hoc test. A P-value < 0.05 was considered statistically significant for all analyses.

Results and discussion

Despite that growth performance (reported in Schäfer et al. (2024)), carcass characteristics, and absolute breast and thigh weights of the broilers was not affected by dietary inclusion of *P. sapidus* mycelium (Table 1), the relative breast muscle weight of broilers was lower in group PSA-50 than in group PSA-0 ($P < 0.05$, Table 1). In order to clarify this effect, we analyzed the expression of several key genes involved in critical pathways regulating protein turnover in breast muscle of these broilers. In addition, we analyzed the phosphorylation of selected proteins, namely S6K1, RPS6, eIF2a and AKT, playing a key role in muscle protein synthesis (Ferreira and Duarte, 2023).

Within genes of the mTOR pathway, the mRNA level of *GHR* was higher in group PSA-25 than in group PSA-0, whereas the mRNA level of *IGF1R* was lower in group PSA-25 than in group PSA-0 ($P < 0.05$, Table 2). The mRNA levels of *GHR* and *IGF1R* did not differ between group PSA-50 and the other two groups. Amongst the genes belonging to the mTOR pathway, the mRNA level of *S6K1* was higher in group PSA-25 than in group PSA-0 ($P < 0.05$), whereas the *S6K1* mRNA level was not different between group PSA-50 and groups PSA-25 and PSA-0 (Table 2). The mRNA levels of two other genes of the mTOR pathway, *MTOR* and *4EBP1*, were not different among groups (Table 2). Within the muscle growth-related genes investigated, the mRNA level of *MYOG* was lower in groups PSA-25 and PSA-50 than in group PSA-0 ($P < 0.05$), whereas the mRNA levels of *MYOD1* and *MYF5* did not differ among groups (Table 2). The effect on *MYOG* is noteworthy because MYOG is involved in myotube differentiation (Sabourin and Rudnicki, 2000), which indicates a general inhibitory effect of feeding *P. sapidus* mycelium on muscle cell differentiation of broilers. However, given that the mRNA levels of other muscle growth-related genes in breast muscle were not different between groups, an effect on myotube differentiation is questionable and more research is necessary to clarify this issue.

The relative phosphorylation of S6K1, RPS6, eIF2a and AKT did not differ across the three groups (data not shown). The mRNA levels of most genes involved in pathways of protein degradation and inhibition of protein synthesis, such as the GCN/eIF2a, the ubiquitin-proteasome and the autophagy-lysosomal pathways, showed no differences across the three groups (Table 2). Only the mRNA level of *ATG9A* was higher in group PSA-25 compared to group PSA-0 ($P < 0.05$), but did not differ between group PSA-50 and the other two groups (Table 2). While no effect was seen regarding the mRNA levels of pro-inflammatory genes between groups PSA-50 and PSA-0, the mRNA levels three (*IL1B, TLR4, TNFA*) out of four pro-inflammatory genes were lower in group PSA-25 than in group PSA-0 ($P < 0.05$, Table 2). This anti-inflammatory effect of *P.* spp. mycelia, which has been also documented for rats (Maheshwari et al., 2020), can be considered as beneficial because pro-inflammatory stimuli like IL1β and TNFα promote protein degradation *via* targeting the ubiquitin-proteasome pathway, thereby, impairing protein accretion. Nevertheless, the anti-inflammatory effect seen at the transcriptional level in group PSA-25 obviously had no relevance for the breast muscle gain. Albeit being speculative, one reason might be that the effect was not translated to the protein level keeping the concentrations of pro-inflammatory mediators unaffected.

Table 1
Carcass characteristics and breast muscle and thigh weights of broilers fed diets with either 0 (PSA-0), 25 (PSA-25) or 50 (PSA-50) g/kg diet *P. sapidus* mycelium for 35 days.

	PSA-0	PSA-25	PSA-50	Pooled SEM	P-value
Eviscerated carcass weight, g	2154	2158	2154	93	0.984
Dressing percentage, %	75.1	73.3	73.9	1.5	0.703
Breast muscle, g	674	659	646	34	0.612
Breast muscle, % of BW	23.5^{a}	22.4^{a,b}	22.1^{b}	0.7	0.044
Thigh, g	574	579	577	26	0.967
Thigh, % of BW	20.0	19.7	19.8	0.5	0.736

Data are means, $n = 21$-22 broilers/group. ^{a,b} Means without a common letter differ across the groups, $P < 0.05$. BW, body weight

Table 2
Relative mRNA levels of selected genes involved in protein anabolic and protein catabolic pathways in breast muscle of broilers fed diets with either 0 (PSA-0), 25 (PSA-25) or 50 (PSA-50) g/kg diet *P. sapidus* mycelium for 35 days.

	PSA-0	PSA-25	PSA-50	Pooled SEM	P-value
	Relative mRNA level, fold of PSA-0				
Somatotropic axis					
GHR	1.00^{b}	1.62^{a}	1.41^{ab}	0.28	0.034
IGF1	1.00	1.13	0.91	0.25	0.550
IGF1R	1.00^{a}	0.67^{b}	0.81^{ab}	0.15	0.024
IGFBP2	1.00	0.73	0.80	0.24	0.457
mTOR pathway					
MTOR	1.00	1.17	1.21	0.13	0.124
S6K1	1.00^{b}	1.88^{a}	1.67^{ab}	0.37	0.019
4EBP1	1.00	1.00	1.02	0.19	0.970
Muscle growth					
MYOD1	1.00	0.76	0.79	0.18	0.256
MYF5	1.00	0.92	1.24	0.21	0.174
MYOG	1.00^{a}	0.56^{b}	0.63^{b}	0.15	0.006
GCN/eIF2a pathway					
SQSTM1	1.00	1.05	1.00	0.18	0.765
Ubiquitin-proteasome pathway					
FBXO32	1.00	1.06	0.78	0.24	0.271
FOXO1	1.00	1.13	1.21	0.16	0.241
MURF1	1.00	1.21	1.12	0.25	0.587
Autophagy-lysosomal pathway					
ATG5	1.00	0.88	0.94	0.16	0.644
ATG9A	1.00^{b}	1.39^{a}	1.26^{ab}	0.17	0.033
BECN1	1.00	1.26	1.24	0.21	0.246
Inflammation					
IL1B	1.00^{a}	0.61^{b}	1.00^{ab}	0.19	0.008
TLR4	1.00^{a}	0.58^{b}	0.77^{ab}	0.15	0.007
TNFA	1.00^{a}	0.58^{b}	0.97^{ab}	0.26	0.031
VCAM1	1.00	0.97	1.17	0.15	0.220

Data are means, $n = 12$ broilers/group. ^{a,b} Means without a common letter differ across the groups, $P < 0.05$.

In overall, considering that only the mRNA level of one muscle growth-related gene (*MYOG*) in breast muscle differed between group PSA-50 and PSA-0, whereas the expression of genes from all other pathways and phosphorylation levels of S6K1, RPS6, eIF2a and AKT did not differ between these two groups, a modulation of these pathways is not very likely to explain the reduced relative breast muscle weight in broilers of group PSA-50. However, we cannot exclude the possibility that feeding 50 g/kg diet *P. sapidus* mycelium affected other genes of these pathways, which were not considered in this study. In addition, it might be possible that feeding *P. sapidus* mycelium affected other less prominent pathways, such as Wnt/β-catenin, Notch and Hippo signaling, which are also involved in the regulation of protein turnover but whose contribution to skeletal muscle mass regulation is less clear (Ferreira and Duarte, 2023). Future studies using genome-wide transcript profiling of breast muscle could help to identify with greater certainty the signaling pathways involved in the regulation of skeletal muscle mass which were affected by feeding *P. sapidus* mycelium.

A rather unexpected finding was that a significant number of genes from the above-mentioned signaling pathways including *IL1B, TLR4, TNFA, GHR, IGF1R, S6K1, MYOG* and *ATG9A* was found to be affected by feeding the low dose of *P. sapidus* mycelium, although relative breast muscle weights did not differ between groups PSA-25 and PSA-0. However, with the exception of pro-inflammatory genes, the regulation of these genes between group PSA-25 and group PSA-0 was very inconsistent. This was evident from the observation that one gene involved in the protein anabolic mTOR pathway (*S6K1*) and one gene involved in the protein catabolic autophagy-lysosomal pathway (*ATG9A*) were simultaneously up-regulated in group PSA-25 compared to group PSA-0. This inconsistency in the effect of feeding 25 g/kg diet *P. sapidus* mycelium was also seen in the somatotropic axis, in which one gene each was up- (*GHR*) and down-regulated (*IGF1R*) in breast muscle of group PSA-25 compared to group PSA-0, whereas relative phosphorylation of proteins involved in protein anabolic pathways in breast muscle did not differ between these two groups (data not shown). Given this inconsistent regulation of genes between group PSA-25 and group PSA-0 and the lack of difference in the relative breast muscle weights between groups PSA-25 and PSA-0, the observed effects on gene expression are obviously not relevant to breast muscle gain. It is not unlikely that the latter is the consequence of the overall weak effects on gene expression as reflected by the less than 2-fold regulation of these genes between groups.

In conclusion, the present findings suggest that a modulation of the protein anabolic or protein catabolic pathways considered in this study is unlikely to explain the reduced relative breast muscle weight in broilers fed 50 g/kg diet *P. sapidus* mycelium. However, it cannot be excluded that feeding *P. sapidus* mycelium affected other less prominent pathways which have not been considered in this study but which are also involved in the regulation of protein turnover.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgments

Lea Schäfer was financially supported by H. Wilhelm Schaumann foundation (Hamburg, Germany). Javier Herrero-Encinas received a Margarita Salas grant from the Ministerio de Universidades (RD 289/2021) of the Spanish Government financed by the European Union-Next Generation EU (UP2021-035).

References

Chiappisi, E., Ringseis, R., Eder, K., Gessner, D.K., 2017. Effect of endoplasmic reticulum stress on metabolic and stress signaling and kidney-specific functions in Madin-Darby bovine kidney cells. J. Dairy Sci. 100, 6689–6706.

Ferreira, R.P., Duarte, J.A., 2023. Protein turnover in skeletal muscle: looking at molecular regulation towards an active lifestyle. Int. J. Sports Med. 44, 763–777.

Finkbeiner, S., 2020. The autophagy lysosomal pathway and neurodegeneration. Cold Spring Harb. Perspect. Biol. 12, a033993.

Huang, C.M., Lee, T.T., 2018. Immunomodulatory effects of phytogenics in chickens and pigs - a review. Asian-Australas. J. Anim Sci. 31, 617–627.

Maheshwari, G., Gessner, D.K., Meyer, S., Ahlborn, J., Wen, G., Ringseis, R., Zorn, H., Eder, K., 2020. Characterization of the nutritional composition of a biotechnologically produced oyster mushroom and its physiological effects in obese Zucker rats. Mol. Nutr. Food Res. 64, e2000591.

Métayer, S., Seiliez, I., Collin, A., Duchêne, S., Mercier, Y., Geraert, P.A., Tesseraud, S., 2008. Mechanisms through which sulfur amino acids control protein metabolism and oxidative status. J. Nutr. Biochem. 19, 207–215.

Papaspyridi, L.M., Aligiannis, N., Topakas, E., Christakopoulos, P., Skaltsounis, A.L., Fokialakis, N., 2012. Submerged fermentation of the edible mushroom *Pleurotus ostreatus* in a batch stirred tank bioreactor as a promising alternative for the effective production of bioactive metabolites. Molecules 17, 2714–2724.

Sabourin, L.A., Rudnicki, M.A., 2000. The molecular regulation of myogenesis. Clin. Genet. 57, 16–25.

Schäfer, L., Grundmann, S.M., Rühl, M., Zorn, H., Seel, W., Simon, M.C., Schuchardt, S., Most, E., Ringseis, R., Eder, K., 2024. Effects of a biotechnologically produced *Pleurotus sapidus* mycelium on gut microbiome, liver transcriptome and plasma metabolome of broilers. Poult. Sci. 103, 103975.

Valverde, M.E., Hernández-Pérez, T., Paredes-López, O., 2015. Edible mushrooms: improving human health and promoting quality life. Int. J. Microbiol. 2015, 376387.

4. Diskussion

Diese Dissertation erforscht die Auswirkungen von zwei alternativen Futterquellen auf die ernährungsphysiologischen Prozesse in der Mastgeflügelfütterung. Hauptziel dieser Arbeit war es, alternative Futtermittel zu finden, die sich positiv bzw. neutral auf die Wachstumsleistung und den Stoffwechsel der Broiler auswirken, um somit zur nachhaltigen Gestaltung der Broilermast beizutragen. Untersucht wurden die Auswirkungen eines teilweisen sowie vollständigen Austauschs von Sojaöl durch das *H. illucens* Larvenfett (**Publikation 1** und **Publikation 2**), wie auch die Auswirkungen einer Zulage eines biotechnologisch hergestellten *P. sapidus* Mycels **(Publikation 3** und **Publikation 4)**, auf die Wachstumsleistung, sowie auf Metaboliten und Lipide des Plasmas, Leberlipide, das Transkriptom der Leber, Parameter der Darmintegrität und das Mikrobiom von Mastbroilern. Die beiden Futterquellen wurden ausgewählt, da sowohl die *H. illucens* Zucht als auch die biotechnologische Herstellung des *P. sapidus* Mycels mit Reststoffen vollzogen werden kann, die sowohl in der menschlichen Ernährung als auch in der Geflügelfütterung nur begrenzt eingesetzt werden können und somit in keiner Nahrungskonkurrenz zu Mensch oder Tier stehen. Ausnahme war in dieser Studie die biotechnologische Umwandlung eines Nebenstroms zur Mycelherstellung. Als Nährmedium für das *P. sapidus* Mycel wurde in dieser Arbeit Melasse verwendet, um zunächst einen Eindruck zu erlangen, welche Auswirkungen die Fütterung des Mycels auf die Leistung und Gesundheit der Broiler hat. Melasse als Nährmedium wurde auch deswegen gewählt, da diese Methode schon erfolgreich zur Mycelherstellung genutzt wurde (Maheshwari et al., 2020). Die Verwendung von Melasse zur Mycelherstellung ist unter dem Aspekt der Nachhaltigkeit kritisch zu betrachten, da diese ebenfalls als Futtermittel und Lebensmittel genutzt wird. Der Fokus wurde aber in dieser Dissertation zunächst auf eine erfolgreiche Mycelherstellung gelegt und der Beantwortung der Frage, inwieweit ein Futtermittel auf Basis von Pilzmycel überhaupt in der Broilerfütterung eingesetzt werden kann. Unter dem Aspekt der Nachhaltigkeit wären rohfaserreiche Nebenströme wie Biertreber, Apfeltrester etc., welche nur geringfügig als Futtermittel genutzt werden, als Nährmedium von Pilzmycel besser geeignet. Deren Einsatz für eine erfolgreiche Mycelherstellung muss allerdings noch in weiteren Schritten untersucht werden. Aus der Praxis ist bereits bekannt, dass sich das Mycel auch auf Holzspänen und Stroh kultivieren lässt.

Die Verfütterung beider Futterquellen führte zu keiner Beeinträchtigung der Leistung und der Gesundheit der Tiere. In beiden Studien wurde eine Zulage bis zu 5 % vorgenommen:

Bei der Fütterungsstudie mit der Zulage des *P. sapidus* Mycels wurde sich an vorangegangenen Studien von Maheshwari et al. (2020, 2021) orientiert (**Publikation 3** und **4**). Im Falle des Austauschs der Fettfraktion im Futter (**Publikation 1** und **2**) wurde 5 % als Höchstdosis gewählt, zur Gewährleistung des von Cobb 500 empfohlenen Energiegehalts in den Diäten (Cobb-Vantress, 2022). Außerdem wurde eine niedrigere Dosis von 2,5 % bei beiden Studien gewählt, um eine Dosis-Wirkungs-Beziehung abbilden zu können.

Zunächst wird die Studie betrachtet, welche sich mit einem teilweisen und vollständigen Austausch von Sojaöl durch *H. illucens* Larvenfett in der Broilerration beschäftigte. Es wurde neben den Analysen in den Geweben (Plasma, Leber, Muskel, Darm) der Broiler, auch die Zusammensetzung des eingesetzten *H. illucens* Larvenfetts analysiert, um Auswirkungen auf die Gewebe erklären zu können.

Die Charakterisierung des *H. illucens* Larvenfetts mittels einer Lipidomanalyse zeigte, dass 99 % der Lipidspezies Triglycerid-Spezies waren und davon über 50 % gesättigte Spezies (**Publikation 1**). Die genaue Betrachtung des Fettsäureprofils ergab, dass Laurinsäure, eine mittelkettige Fettsäure, den höchsten Anteil mit ca. 57 % der Gesamtfettsäuren ausmachte (**Publikation 1**). Andere Autoren zeigten eine vergleichbare Charakterisierung des Fettsäureprofils des *H. illucens* Larvenfetts und zeigten, dass es über mehrere verschiedene Larvenstadien hinweg stabil bleibt (Kim et al., 2020a; Georgescu et al., 2022). Beim Vergleich des Fetts der *H. illucens* Larven mit dem Öl von *Tenebrio molitor* Larven liegt der größte Unterschied im Aggregatzustand bei Raumtemperatur. So ist das *H. illucens* Larvenfett, aufgrund seines hohen Anteils an gesättigten Fettsäuren (bis zu 70 % der Gesamtfettsäuren), bei Raumtemperatur fest. Der Anteil von einfach ungesättigten Fettsäuren liegt im Mittel bei 15,2 % und der von mehrfach ungesättigten Fettsäuren bei 12,7 % der Gesamtfettsäuren (Benzertiha et al., 2020). Mit einem Anteil von nur ca. 29,3 % der Gesamtfettsäuren an gesättigten Fettsäuren liegt das *T. molitor* Öl bei Raumtemperatur in flüssiger Form vor. Den höchsten Gehalt machen dort die einfach ungesättigten Fettsäuren mit ca. 48,2 % der Gesamtfettsäuren aus. Mehrfachungesättigte Fettsäuren liegen zu ca. 21,7 % der Gesamtfettsäuren vor (Benzertiha et al., 2020, Ugur et al., 2021). Sowohl das *H. illucens* Larvenfett als auch das *T. molitor* Larvenöl wurden schon erfolgreich in der Geflügelfütterung, wie z.B. bei Broilern, Legehennen und Puten, als Fettquelle eingesetzt, ohne negative Auswirkungen auf die Leistungen der Tiere zu haben (Benzertiha et al., 2019; Heuel et al., 2019; Kierończyk et al., 2018; Schiavone et al., 2018; Schiavone et al., 2017; Sypniewski et al., 2020). Auch in dieser Dissertation zeigte sich beim teilweisen und vollständigen Austausch von Sojaöl als Fettquelle durch das *H. illucens* Larvenfett keine

Beeinflussung der Wachstumsleistungen der Broiler. Lediglich in der Starterphase konnte ein signifikanter Anstieg des Körpergewichts und der Futteraufnahme in den beiden Versuchsgruppen beobachtet werden (**Publikation 1**). Die Leistungsdaten aus **Publikation 1** über die gesamte Versuchsperiode sind vergleichbar mit den Daten von anderen Autoren. Dort wurden ebenfalls bei einem teilweisen oder vollständigen Austausch der Fettquelle (Sojaöl, Maisöl, Kokosnussöl oder Palmöl) in Broilerdiäten keine Effekte auf das Körpergewicht beschrieben (Benzertiha et al., 2019; Kierończyk et al., 2018; Kierończyk et al., 2020; Kim et al., 2020b; Schiavone et al., 2017; Schiavone et al., 2018). Eine reduzierte Futterverwertung, die von Kim et al. (2020b) und Kierończyk et al. (2020) beobachtet wurde, konnte in **Publikation 1** nicht nachgewiesen werden. Auffällig waren ein höheres Brustmuskelgewicht und ein signifikant höherer Brustmuskelanteil (bezogen auf das Körpergewicht) in den beiden Versuchsgruppen im Vergleich zur Kontrollgruppe (**Publikation 2**), wohingegen alle anderen Leistungs- und Schlachtungsparameter unbeeinflusst waren. Das gesteigerte Brustmuskelgewicht könnte in Verbindung mit den günstigen Wirkungen der Laurinsäure stehen. Bei der Studie von Zeitz et al. (2015), welche Laurinsäure in Broilerdiäten eingesetzt haben, wurde ebenfalls ein gesteigertes Brustmuskelgewicht aufgezeigt. Auch beim Einsatz von *H. illucens* Insektenmehl als Proteinquelle konnte ein signifikant höheres Brustmuskelgewicht beobachtet werden (Beller et al., 2024). In dieser Studie zeigte sich im Fettsäureprofil der Diäten auch ein höherer Anteil an Laurinsäure in den beiden Versuchsgruppen im Vergleich zur Kontrollgruppe (Beller et al., 2024), weshalb davon ausgegangen werden kann, dass die Laurinsäure das Brustmuskelwachstum in einem positiven Maße beeinflusst. Es ist aber nicht auszuschließen, dass das erhöhte Brustmuskelgewicht auf einen anderen Aspekt bzw. Inhaltsstoff des *H. illucens* Larvenfetts zurückzuführen ist, der allerdings nicht untersucht wurde.

Dass sich mittelkettige Fettsäuren auch positiv auf die mikrobielle Darmgemeinschaft, die Darmintegrität und Darmgesundheit von Broilern auswirken, konnten schon einige Untersuchungen bestätigen (Solis de los Santos et al., 2008; Solis de los Santos et al., 2009). Dies lässt den Schluss zu, dass auch in dieser Studie der hohe Gehalt an Laurinsäure im *H. illucens* Larvenfett (**Publikation 1**) die mikrobielle Darmgemeinschaft positiv beeinflusst haben könnte, indem pathogene Bakterien, wie *Campylobacter jejuni, Gram positiv cocci* oder *Escherichia coli,* reduziert wurden. Allerdings konnte in **Publikation 1** keine Beeinflussung der α- und β-Diversität und der flüchtigen Fettsäuren, welche die Fermentationsprodukte der Mikroben darstellen, festgestellt werden. Lediglich ein Taxon mit einer geringen Häufigkeit auf *Phylum* Ebene (*Actinobacteriota*) und jeweils eins auf *Order* (*Coriobacteriales*) und *Family* Ebene (*Eggerthellaceae*) wurde beeinflusst. Der hohe Gehalt an Laurinsäure hatte dementsprechend keine wesentlichen Auswirkungen auf die Darmgemeinschaft. Es konnten aber auch keine negativen Effekte beobachtet werden.

Aufgrund der Aufnahme der spezifischen Fettsäuren des *H. illucens* Larvenfetts in die Leber, das Plasma und das Brustmuskelgewebe, welche auch schon in anderen Studien beobachtet wurde (Cullere et al., 2019; Heuel et al., 2021; Daszkiewicz et al., 2022), und dessen hohen Anteil an gesättigten Fettsäuren (**Publikation 1** und **2**), zeichnete sich im gegarten Brustmuskel eine höhere Lipidperoxidationsstabilität ab, was zu einer Reduzierung der Entstehung gesundheitsschädlicher Cholesterol- und Phytosteroloxidationsprodukten führte (**Publikation 2**). Bei der Fütterung von Sojaöl oder dem Öl von *T. molitor* Larven wird zusätzlich ein Antioxidans (z.B. Vitamin E) benötigt, um die Gewebe vor Oxidation zu schützen. Die Reduzierung der Cholesterol- und Phytosteroloxidationsprodukte, welche koronare Herzerkrankungen und Krebserkrankungen bei einer zu hohen Aufnahme begünstigen können (Alemany et al., 2014; Kulig et al., 2016), ist aus ernährungsphysiologischer Sicht günstig. Allerdings ist ein hoher Gehalt an gesättigten Fettsäuren eher ungünstig, da ein hoher Gehalt von gesättigten Fettsäuren mit kardiovaskulären Krankheiten und verschiedenen Krebsarten beim Menschen in Verbindung gebracht wird (Ringel et al., 2020; Sacks et al., 2017; Wood et al., 2008). Aufgrund des geringen Fettgehalts von Broilerfleisch (ca. 3 %) (Oliveira et al., 2016) im Vergleich zu Rind- (10 %) und Schweinefleisch (10-22 %) (Vicente und Pereira, 2024), spielt dieser Aspekt jedoch eine untergeordnete Rolle.

Nicht nur die Untersuchung des Fettsäureprofils oder die Lipidomanalyse in der Leber (**Publikation 1**) bestätigte die Aufnahme der im *H. illucens* Larvenfett vorhandenen Fettsäuren in das Gewebe, sondern auch die Transkriptomanalyse. Es wurde eine Auswirkung festgestellt, die hauptsächlich Gene beeinflusste, welche mit dem Lipidstoffwechsel assoziiert werden können. Insbesondere zeigte sich eine veränderte Expression von Genen, die in der Regulation der Lipidsynthese, sowie bei Cholesterol- und Sterolsyntheseprozessen beteiligt sind. Diese Veränderungen deuten darauf hin, dass das *H. illucens* Larvenfett den Stoffwechselweg der Lipide in der Leber auf zellulärer Ebene moduliert.

Im Gegensatz zu einem 5 prozentigen Austausch von Sojaöl durch das *H. illucens* Larvenfett als Fettquelle in Broilerdiäten, zeigte der Einsatz von 5 % *P. sapidus* Mycel in den Diäten keine Auswirkungen, weder negative noch positive, auf die Wachstumsleistung und die

Gesundheit der Broiler. Die chemische Charakterisierung des *P. sapidus* Mycels ergab, dass die Kohlenhydratfraktion mit etwa 78 % die Hauptkomponente in der Myceltrockenmasse darstellt (**Publikation 3**). Innerhalb dieser Fraktion waren etwa 31 % β-Glucane enthalten. Diese Erkenntnisse decken sich mit der Charakterisierung eines andern Mycels aus der *Pleurotus* Familie. Das Mycel des *P. eous* (früher *P. sajor-caju*), welches ebenfalls biotechnologisch mit Melasse hergestellt wurde, wies ebenfalls etwa 78 % Kohlenhydrate und 25 % β-Glucane in der Trockenmasse auf (Maheshwari et al., 2020). Aufgrund des hohen Gehalts an β-Glucanen, kann das *P. sapidus* Mycel als eine gute Präbiotikum-Quelle angesehen werden (Lam und Chi-Keung Cheung, 2013). Die Datenlage zu einer Auswirkung von β-Glucanen auf die Wachstumsleistung von Broilern ist jedoch widersprüchlich. Einige Autoren zeigten positive Effekte auf die Wachstumsleistungen (Chae et al., 2006; Richards et al., 2020), während andere keine Effekte auf die Wachstumsleistungen beobachteten (Cox et al., 2010; Hahn et al., 2006; Moon et al., 2016; Morales-López et al., 2009). In der vorliegenden Studie hatte das *P. sapidus* Mycel keine Effekte auf die Wachstumsleistung der Broiler (**Publikation 3**). Auch konnte keine Beeinflussung der ilealen Nährstoffverdaulichkeit beobachtet werden, was die Untersuchungen von Chae et al. (2006) bestätigt. Bei Schweinen konnten Hahn et al. (2006) allerdings eine verbesserte Verdaulichkeit der Nährstoffe durch eine β-Glucan angereicherte Diät feststellen.

In **Publikation 4** war auffällig, dass trotz unveränderter Körpergewichte in der Gruppe mit 5 % *P. sapidus* Mycel Zulage eine Reduzierung des relativen Brustmuskelanteils (bezogen auf das Körpergewicht) im Vergleich zur Kontrollgruppe vorlag. In anderen Studien wurden bei β-Glucanen in Broilerdiäten keine Auswirkungen auf das Brustmuskelwachstum festgestellt (Kovitvadhi et al., 2019; Zhang et al., 2020). Um die Ursache dieser Reduzierung nachvollziehen zu können, wurden verschiedene Gene aus dem anabolen und katabolen Proteinstoffwechsel gemessen, da der Proteinturnover im Muskel ein Resultat der Aktivierung verschiedener anabolen und katabolen Proteinstoffwechselwege ist (Ferreira und Duarte, 2023). Dafür wurde die Expression einzelner Gene gemessen, welche im „mammalian target of rapamycin“ (mTOR) Stoffwechselweg, dem Hauptsignalweg der Proteinsynthese, beteiligt sind. Da einige Gene aus dem „mammalian target of rapamycin“ Stoffwechselweg Gene aus dem Ubiquitin-Proteasom Metabolismus und dem GCN/elf2a Stoffwechselweg beeinflussen, wurde auch die Expression von Genen aus den beiden letzteren Stoffwechselwegen betrachtet. Auch Gene der somatotrophen Achse, welche eine wichtige Rolle im Stoffwechsel und bei physiologischen Prozessen spielt, wurden untersucht (Renaville et al., 2002), wie auch die Expression ausgewählter Gene, die in Verbindung mit dem Muskelwachstum stehen, und pro-inflammatorische Gene wurden gemessen. Lediglich ein Gen (*MYOG*), welches mit dem Muskelwachstum in Verbindung steht, wurde beeinflusst. Das relative Brustmuskelgewicht der Broiler aus der Gruppe mit 2,5 % *P. sapidus* Mycel Zulage war im Vergleich zur Kontrollgruppe nicht niedriger, dennoch waren Gene aus den verschiedenen Stoffwechselwegen beeinflusst worden (**Publikation 4**). Es lag somit eine Inkonsistenz vor, die in der Arbeit nicht erklärt werden konnte. In der Gruppe mit 2,5 % *P. sapidus* Mycel Zulage konnte ein anti-inflammatorischer Effekt im Brustmuskel nachgewiesen werden, welcher schon in einer vorangegangenen Studie mit fettleibigen Laborratten beobachtet wurde (Maheshwari et al., 2020). Dieser Effekt ist für schnell wachsende Broiler positiv, da pro-inflammatorische Stimuli den Proteinabbau über den Ubiquitin-Proteasom-Stoffwechselweg fördern und dadurch die Proteinakkumulation beeinträchtigen können (Lecker et al., 2006). Allerdings steht dieser Effekt im Widerspruch zum reduzierten Brustmuskelanteil in der Gruppe mit 5 % *P. sapidus* Mycel Zulage, da ein anti-inflammatorischer Effekt den Proteinabbau verlangsamen und somit das Brustmuskelgewicht fördern müsste. Ein möglicher Grund für die unterschiedlichen Beobachtungen zwischen der in dieser Dissertation dargelegten Studie und der Studie mit Laborratten hinsichtlich des anti-inflammatorischen Effekts könnte darin liegen, dass es sich um einen Vergleich zwischen gesunden Broilern und Laborratten mit einem pathologischen Stoffwechsel handelte.

Präbiotika-angereicherte Diäten führen in einigen Untersuchungen auch zu einer Veränderung des Darmmikrobioms und der Darmintegrität (Guo et al., 2023; Wang et al., 2022). Wang et al. (2022) konnten eine positive Beeinflussung der mikrobiellen Darmgemeinschaft bei Broilern und eine Zunahme nützlicher Bakterien wie *Alistipes, Bacteroides* und *Faecalibacterium* feststellen. Zusätzlich wiesen Guo et al. (2023) einen positiven Effekt von β-Glucanen auf die Leistung und die Darmgesundheit bei mit *Clostridium perfringens* infizierten Broilern nach. Mit β-Glucanen angereicherte Broilerdiäten weisen bei infizierten Broilern, was in der industriellen Haltung häufiger vorkommt, höhere Effekte auf als in der in dieser Dissertation dargelegten Studie, die unter Versuchsbedingungen stattgefunden hat, bei denen Erkrankungen seltener auftreten. Hervorzuheben ist, dass es nur wenige Studien gibt, die sich mit dem Einsatz von β-Glucanen bei gesunden Broilern, wie dies in **Publikation 3** der Fall war, beschäftigen. In **Publikation 3** konnten keine nennenswerten Veränderungen in der mikrobiellen Darmgemeinschaft oder der Taxonomie festgestellt werden. Auch die Konzentrationen der flüchtigen Fettsäuren, welche die Fermentationsprodukte der Mikroben darstellen, blieben unbeeinflusst (**Publikation 3**). Deshalb traten auch keine nennenswerten Auswirkungen durch die Zulage des *P. sapidus* Mycels auf die mikrobielle Darmgemeinschaft auf.

Untersuchungen der Darmintegrität, wie die Genexpression ausgewählter Mucine und *tight-junction* Proteine, zeigten keine Regulation durch die Zulage des *P. sapidus* Mycels. Die Konzentration der Lipopolysaccharide, die als Bestandteile der äußeren Zellwand von Gram-negativen Bakterien pro-inflammatorisch wirken, waren ebenfalls unbeeinflusst (**Publikation 3**). Diese Erkenntnisse zusammengefasst zeigen, dass die Darmbarriere nicht geschädigt wurde. Im Gegensatz dazu stehen die Ergebnisse aus einer Studie mit fettleibigen Laborraten, welche eine Zulage von 5 % *P. eous* Mycel in den Diäten erhielten (Maheshwari et al., 2021). In dieser Studie wurden sowohl die mikrobielle Darmgemeinschaft als auch die Darmintegrität beeinflusst (Maheshwari et al., 2021). Dies deutet darauf hin, dass eine Zulage eines β-Glucan-reichen Pilzmycels auf Laborratten mit einem pathologischen Stoffwechsel einen stärkeren Einfluss hat als auf gesunde Broiler.

Das *P. sapidus* Mycel hatte keine Auswirkungen auf die mikrobielle Darmgemeinschaft und Darmintegrität und somit kam es auch zu keiner Beeinflussung des Stoffwechsels. Auch hatte das *P. sapidus* Mycel anderweitig keine Auswirkungen auf den Stoffwechsel (**Publikation 3**). Eine Metabolomanalyse des Plasmas zeigte keine Auswirkungen auf die Konzentration von ca. 500 gemessenen Metaboliten durch das *P. sapidus* Mycel. Die Transkriptomanalyse in der Leber zeigte, dass lediglich 144, davon 94 hoch- und 50 herunterreguliert, von ca. 20.000 Genen beeinflusst waren. Insgesamt waren die Gene in ihrer Expression nur geringfügig verändert (**Publikation 3**). Auffällig war allerdings, dass trotz der geringen Beeinflussung auf Genebene ein erhöhtes Lebergewicht beobachtet wurde (**Publikation 3**). Das Gewicht der Gruppe mit 5 % *P. sapidus* Mycel Zulage lag um ca. 14 % höher als das der Kontrollgruppe. Auch der Triglycerid-Gehalt in der Leber war in der Gruppe mit 5 % *P. sapidus* Mycel Zulage um ca. 23 % erhöht. Die Ursache für diesen Anstieg konnte in der vorliegenden Arbeit jedoch nicht ermittelt werden.

5. Schlussfolgerung

Abschließend lässt sich zusammenfassen, dass die **Publikationen 1** und **2** positiv aufgezeigt haben, dass das *H. illucens* Insektenfett als eine alternative Fettquelle in der Broilerfütterung eingesetzt werden kann. Es wurden keine negativen Auswirkungen auf das Wachstum der Broiler in den Studien beobachtet. Es konnte ein gesteigertes Brustmuskelwachstum nachgewiesen werden. Wodurch dieser Anstieg des Brustmuskelwachstums hervorgerufen wurde, bedarf noch weiterer Forschung. Die in **Publikation 2** aufgestellte Hypothese konnte bestätigt werden. Die spezifischen Fettsäuren des *H. illucens* Larvenfettes führten zu einer reduzierten Lipidperoxidation und somit zu einer reduzierten Entstehung von Cholesterol- und Phytosteroloxidationsprodukten. Eine zu hohe Aufnahme dieser Produkte kann langfristig ein gesundheitliches Risiko darstellen. Der ernährungsphysiologische Aspekt, dass der Brustmuskel von Broilern, welche *H. illucens* Larvenfett in ihrer Diät erhielten, einen höheren Anteil an gesättigten Fettsäuren aufwies, kann aus dem Grunde etwas außer Acht gelassen werden, da der Gesamtfettgehalt von Broilerfleisch generell ziemlich gering ist und unter dem Fettgehalt von Rind- und Schweinefleisch liegt.

Die **Publikationen 3** und **4** zeigten, dass der Einsatz eines biotechnologisch hergestellten *P. sapidus* Mycels keinerlei Auswirkungen auf die Leistung und die Stoffwechselparameter der Broiler hatte, obwohl ein hoher Gehalt an β-Glucanen nachweislich die Zusammensetzung der mikrobiellen Darmgemeinschaft und die Darmgesundheit und Darmintegrität fördern soll. Das *P. sapidus* Mycel führte zu keinen Effekten auf Stoffwechselebene. Beobachtet wurde ein reduzierter Brustmuskelanteil, welcher mit den durchgeführten Analysen nicht erklärt werden konnte. Die Studie, als eine der wenigen, welche sich mit dem Einsatz von Pilzmycelen in der Nutztierfütterung beschäftigte, zeigte das Potenzial von mit Hilfe von Melasse als Nährmedium gezogenen *P. sapidus* Mycel. Dieses kann in einer Dosierung von 2,5 % ohne Bedenken in den Diäten von Broilern zugelegt werden kann. Eine Zulage über 5 % des *P. sapidus* Mycels bedarf noch weiterer Forschung. Das Ziel weiterer Forschung sollte es sein, Pilzmycele auf rohfaserreichen Nebenströmen zu züchten, um diese der Geflügelernährung zugänglich zu machen und somit die Broilermast nachhaltig zu gestalten.

Diese Dissertation hat aufgezeigt, dass die Fütterung des *H. illucens* Larvenfettes und des *P. sapidus* Mycels dazu führen können, die Broilermast nachhaltiger zu gestalten und Ressourcen effizienter zu nutzen, ohne dabei Einbußen bei der Gesundheit und Leistung der Tiere in Kauf nehmen zu müssen.

6. Zusammenfassung

Die vorliegende Dissertation befasste sich mit der Frage, wie nachhaltige Futtermittel zur Verbesserung der Ressourceneffizienz in der Broilermast beitragen können. In Anbetracht des Bevölkerungswachstums und des steigenden Bedarfs an tierischen Produkten stellt die Entwicklung nachhaltiger Futtermittel eine zentrale Herausforderung der Tierernährung dar. Diese Dissertation untersuchte zwei alternative Futtermittel: das Fett der Schwarzen Soldatenfliege (*Hermetia illucens*) und das biotechnologisch hergestellte Mycel des Austernpilzes *Pleurotus sapidus*. Ziel war es, deren Auswirkungen auf das Wachstum und verschiedene Stoffwechselprozesse im Darm, der Leber, dem Brustmuskel und dem Plasma von Mastbroilern zu analysieren und herauszufinden, ob sie als Ersatz für Sojaöl als Lipidquelle bzw. als eine Zulage geeignet sind.

Die erste Studie beschäftigte sich mit den Auswirkungen eines teilweisen und vollständigen Austauschs des Sojaöls durch das *H. illucens* Larvenfett. Das Insektenfett, ein Nebenprodukt der Insektenmehlproduktion, ist reich an gesättigten Fettsäuren, insbesondere der Laurinsäure. In der Studie wurden verschiedene Parameter untersucht, darunter die Wachstumsleistung, die Fettverdaulichkeit, das Darmmikrobiom, das Lebertranskriptom sowie das Lipidom von Leber und Plasma der Broiler.

Die Ergebnisse zeigten, dass der teilweise (50 %) und vollständige (100 %) Austausch von Sojaöl durch das *H. illucens* Larvenfett keine negativen Auswirkungen auf das Wachstum der Tiere hatte und es zu einer Widerspiegelung der spezifischen Fettsäuren des *H. illucens* Larvenfetts in der Leber, dem Plasma und dem Brustmuskel kam. Zusätzlich führte die Fütterung des *H. illucens* Larvenfetts zu einem signifikanten Anstieg des Brustmuskelgewichts. Dieser Anstieg könnte mit dem hohen Anteil an Laurinsäure im *H. illucens* Fett zusammenhängen. Eine weitere positive Wirkung des *H. illucens* Larvenfetts war die erhöhte Oxidationsstabilität des Brustmuskels bei einer Hitzehandlung. Das führte zu einer reduzierten Entstehung von gesundheitsschädlichen Cholesterol- und Phytosteroloxidationsprodukten, die beim Erhitzen von Fleisch entstehen können. Diese Ergebnisse deuten darauf hin, dass der Einsatz von *H. illucens* Fett in der Broilerfütterung nicht nur die Nachhaltigkeit verbessert, sondern auch potenzielle gesundheitliche Vorteile für den Verbraucher bietet.

Die zweite Studie befasste sich mit der Zulage eines biotechnologisch hergestellten Mycels des Austernpilzes *P. sapidus*, welches auf Melasse als Nährmedium gezüchtet wurde. Pilzmycele enthalten einen hohen Anteil an β-Glucanen, die als Präbiotikum bekannt sind und positive Effekte auf die Darmgesundheit und das Immunsystem haben können. Ziel der Studie war es zu untersuchen, ob eine Zulage von 2,5 % oder 5 % des *P. sapidus* Mycels in die Diät der Broiler zu einer Verbesserung der Wachstumsleistung, der Zusammensetzung der mikrobiellen Darmgemeinschaft oder der Stoffwechselprozesse führt.

Die Ergebnisse zeigten jedoch, dass die Zulage des Mycels keine signifikanten Auswirkungen auf die Wachstumsleistung hatte. Auch das Darmmikrobiom, das Transkriptom in der Leber und die Konzentration der Metaboliten im Plasma waren unverändert. Die Fütterung des *P. sapidus* Mycels führte in der Gruppe mit 5 % *P. sapidus* Mycel Zulage im Vergleich zur Kontrollgruppe zu einer Reduzierung des Brustmuskelanteils. Dies war aber nicht auf eine Veränderung von anabolen und katabolen Stoffwechselwegen im Brustmuskel zurückzuführen.

Zusammenfassend zeigt die Dissertation, dass das *H. illucens* Larvenfett eine vielversprechende alternative Fettquelle in der Broilerfütterung darstellt. Es unterstützt das Wachstum der Tiere und verbessert die Oxidationsstabilität des Fleisches. Das *P. sapidus* Mycel zeigte hingegen in dieser Studie keine signifikanten Effekte auf die Broilerleistung oder deren Stoffwechselparameter, obwohl es aufgrund seines hohen β-Glucan-Gehalts als potenzielles Präbiotikum angesehen wird. Der Einsatz des Mycels könnte jedoch in weiteren Studien untersucht werden, insbesondere wenn es auf anderen, nachhaltigen Nährmedien wie faserreichen Nebenströmen anstelle von Melasse kultiviert und somit ressourcenschonender produziert wird.

Die Dissertation trägt maßgeblich zur Erforschung nachhaltiger Futtermittel in der Tierernährung bei und zeigt auf, dass alternative Futtermittel wie Insektenfett und Pilzmycel dazu beitragen können, die Broilermast ressourceneffizienter und nachhaltiger zu gestalten.

7. Summary

This dissertation focused on how sustainable feed can contribute to improving resource efficiency in broiler fattening. In the context of population growth and the increasing demand for animal products, the development of sustainable feed sources is a critical challenge in animal nutrition. This dissertation investigated two alternative feed ingredients: the fat of the black soldier fly (*Hermetia illucens*) and the biotechnologically produced mycelium of the oyster mushroom *Pleurotus sapidus*. The aim was to analyze their effects on growth and various metabolic processes in the intestine, liver, breast muscle, and plasma of broilers and to determine whether they could serve as substitutes for soybean oil as a lipid source (*H. illucens* larvae fat) or as dietary supplements (*P. sapidus* mycelium).

The first study investigated the effects of partially and completely replacing soybean oil with *H. illucens* larvae fat. This insect fat, a by-product of insect meal production, is rich in saturated fatty acids, particularly lauric acid. Various parameters were determined, including growth performance, fat digestibility, gut microbiome, liver transcriptome, as well as the lipidome of the liver and plasma of the broilers.

The results showed that partial (50 %) or complete (100 %) replacement of soybean oil with *H. illucens* larvae fat had no negative effects on the growth of the animals. The specific fatty acid profile of the *H. illucens* larvae fat was reflected in the liver, plasma and breast muscle of the broilers. Additionally, feeding *H. illucens* larvae fat resulted in a significant increase in breast muscle weight, likely linked to the high lauric acid content of the fat. Another positive effect of *H. illucens* fat was the increased oxidative stability of the breast muscle after cooking. This led to a reduction in harmful cholesterol and phytosterol oxidation products, which can form during meat cooking. These findings suggest that using *H. illucens* fat in broiler diets not only enhances sustainability but also offers potential health benefits for consumers.

The second study focused on the addition of biotechnologically produced mycelium of *P. sapidus*, which was cultivated on molasses as a nutrient medium. Mushroom mycelia contain a high proportion of β-glucans, which are known prebiotics with potential benefits for gut health and the immune system. The study aimed to investigate whether adding 2.5 % or 5 % *P. sapidus* mycelium to the broiler diet would improve growth performance, the composition of the gut microbial community, or metabolic processes.

However, the results showed that the addition of the mycelium had no significant effects on growth performance. The gut microbiome, liver transcriptome, and plasma metabolite

concentrations also remained unchanged. Feeding 5 % *P. sapidus* mycelium led to a reduction in relative breast muscle weight compared to the control group, but this reduction was not associated with alterations in anabolic or catabolic metabolic pathways in the breast muscle.

In conclusion, the dissertation demonstrates that *H. illucens* larvae fat is a promising alternative fat source in broiler diets. It supports animal growth and increases the oxidative stability of meat. In contrast, feeding *P. sapidus* mycelium showed no significant effects on broiler performance or metabolic parameters in this study, although its high β-glucan content makes it a potential prebiotic. Future studies could explore its application, particularly if it is cultivated on other sustainable nutrient media, such as fiber-rich by-products, instead of molasses.

This dissertation makes an important contribution to research into sustainable feedstuffs in animal nutrition and shows that alternative feedstuffs, such as insect fat and fungal mycelium, can help to make broiler fattening more resource-efficient and sustainable.

8. Literaturverzeichnis

Alemany, L.; Barbera, R.; Alegría, A.; Laparra, J. M. (2014): Plant sterols from foods in inflammation and risk of cardiovascular disease: a real threat? Food and Chemical Toxicology 69: 140–149.

Astuti, D. A.; Damanik, R. H.; Anggraeny, A.; Aidismen, Y. D. P. (2018): Utilization of insect as a protein alternative for goat rations. Proceeding of the 4th International Asian-Australasian Dairy Goat Conference. Vietnam 17–19 October 2018.

Barragan-Fonseca, K. B.; Dicke, M.; van Loon, J. J. A. (2018): Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (Hermetia illucens). Entomologia Experimentalis et Applicata 166: 761–770.

Beller, S.; Grundmann, S. M.; Pies, K.; Most, E.; Schuchardt, S.; Seel, W.; Simon, M.-C.; Eder, K.; Ringseis, R. (2024): Effect of replacing soybean meal with Hermetia illucens meal on cecal microbiota, liver transcriptome, and plasma metabolome of broilers. Poultry Science 103 (5): 103635.

Belluco, S.; Losasso, C.; Maggioletti, M.; Alonzi, C. C.; Paoletti, M. G.; Ricci, A. (2013): Edible Insects in a Food Safety and Nutritional Perspective: A Critical Review. Comprehensive Reviews in Food Science and Food Safety 12: 296–313.

Benzertiha, A.; Kierończyk, B.; Rawski, M.; Kołodziejski, P.; Bryszak, M.; Józefiak, D. (2019): Insect Oil as An Alternative to Palm Oil and Poultry Fat in Broiler Chicken Nutrition. Animals 9 (3): 116.

Benzertiha, A.; Kierończyk, B.; Rawski, M.; Mikołajczak, Z.; Urbański, A.; Nogowski, L.; Józefiak, D. (2020): Insect Fat in Animal Nutrition – A Review. Annals of Animal Science 20 (4): 1217–1240.

Cerletti, C.; Esposito, S.; Iacoviello, L. (2021): Edible Mushrooms and Beta-Glucans: Impact on Human Health. Nutrients 13 (7): 2195.

Chae, B. J.; Lohakare, J. D.; Moon, W. K.; Lee, S. L.; Park, Y. H.; Hahn, T.-W. (2006): Effects of supplementation of beta-glucan on the growth performance and immunity in broilers. Research in Veterinary Science 20 (3): 291–298.

Chuang, W. Yang; Hsieh, Y. Chen; Lee, T.-T. (2020): The Effects of Fungal Feed Additives in Animals: A Review. Animals 10 (5): 805.

Churchward-Venne, T. A.; Pinckaers, P. J. M.; van Loon, J. J. A.; van Loon, L. J. C. (2017): Consideration of insects as a source of dietary protein for human consumption. Nutrition Reviews 75 (12): 1035–1045.

Cobb-Vantress.com. Broiler management guide.URL: https://www.cobbgenetics.com/assets/Cobb-Files/2022-Cobb500-Broiler-Performance-Nutrition-Supplement.pdf. Seite aufgerufen 06.12.2024

Cox, C. M.; Stuard, L. H.; Kim, S.; McElroy, A. P.; Bedford, M. R.; Dalloul, R. A. (2010): Performance and immune responses to dietary beta-glucan in broiler chicks. Poultry Science 89 (9): 1924–1933.

Cullere, M.; Schiavone, A.; Dabbou, S.; Gasco, L.; Dalle Zotte, A. (2019): Meat Quality and Sensory Traits of Finisher Broiler Chickens Fed with Black Soldier Fly (Hermetia Illucens L.) Larvae Fat as Alternative Fat Source. Animals 9 (4): 140.

Das, S. Kumar; Masuda, M.; Sakurai, A.; Sakakibara, M. (2010): Medicinal uses of the mushroom Cordyceps militaris: current state and prospects. Fitoterapia 81 (8): 961–968.

Daszkiewicz, T.; Murawska, D.; Kubiak, D.; Han, J. (2022): Chemical Composition and Fatty Acid Profile of the Pectoralis major Muscle in Broiler Chickens Fed Diets with Full-Fat Black Soldier Fly (Hermetia illucens) Larvae Meal. Animals 12 (4): 464.

Ferreira, R. P., Duarte J. A. (2023): Protein Turnover in Skeletal Muscle: Looking at Molecular Regulation towards an Active Lifestyle. International Journal of Sports Medicine 44 (11): 763–777.

Fontaneto, D.; Tommaseo-Ponzetta, M.; Galli, C.; Risé, P.; Glew, R. H.; Paoletti, M. G. (2011): Differences in fatty acid composition between aquatic and terrestrial insects used as food in human nutrition. Ecology of Food and Nutrition 50 (4): 351–367.

Georgescu, B.; Boaru, A. Mihaela; Muntean, L.; Sima, N.; Struți, D. Ioan; Păpuc, T. Andrei; Georgescu, C. (2022): Modulating the Fatty Acid Profiles of Hermetia illucens Larvae Fats by Dietary Enrichment with Different Oilseeds: A Sustainable Way for Future Use in Feed and Food. Insects 13 (9): 801.

Gessner, D. K.; Ringseis, R.; Eder, K. (2017): Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. Journal of Animal Physiology and Animal Nutrition 101 (4): 605–628.

Gessner, D. K.; Schwarz, A.; Meyer, S.; Wen, G.; Most, E.; Zorn, H.; Ringseis, R.; Eder, K. (2019): Insect Meal as Alternative Protein Source Exerts Pronounced Lipid-Lowering Effects in Hyperlipidemic Obese Zucker Rats. Journal of Nutrition 149 (4): 566–577.

Grau, T.; Vilcinskas, A.; Joop, G. (2017): Sustainable farming of the mealworm Tenebrio molitor for the production of food and feed. Zeitschrift für Naturforschung C, Journal of Biosciences 72 (9-10): 337–349.

Guo, W.; Di Han; Zhang, F.; Zhan, Q.; Liu, Y.; Peng, Q.; Huang, S.; Xue, Z.; Yang, X. (2023): Effects of dietary β-1,3-glucan addition on the growth performance, mRNA expression in jejunal barrier, and cecal microflora of broilers challenged with Clostridium perfringens. Poultry Science 102 (2): 102349.

Hahn, T.-W.; Lohakare, J. D.; Lee, S. L.; Moon, W. K.; Chae, B. J. (2006): Effects of supplementation of beta-glucans on growth performance, nutrient digestibility, and immunity in weanling pigs. Journal of Animal Science 84 (6): 1422–1428.

Heuel, M.; Sandrock, C.; Leiber, F.; Mathys, A.; Gold, M.; Zurbrügg, C.; Gangnat, I. D. M.; Kreuzer, M.; Terranova, M. (2021): Black soldier fly larvae meal and fat can completely replace soybean cake and oil in diets for laying hens. Poultry Science 100 (4): 101034.

Heuel, M.; Sandrock, C.; Mathys, A.; Gold, M.; Zurbrügg, C.; Kreuzer, M.; Terranova, M. (2019): Performance of laying hens when replacing soybean cake and oil by insect larval protein meal and fat. Energy and protein metabolism and nutrition EAAP Scientific Series 138: 179–180.

Huang, C. M.; Lee, T. T. (2018): Immunomodulatory effects of phytogenics in chickens and pigs - A review. Asian-Australasian Journal of Animal Sciences 31 (5): 617–627.

Isaacs, C.E.; Litov, R.E.; Thormar, H. (1995): Antimicrobial activity of lipids added to human milk, infant formula, and bovine milk. Journal of Nutritional Biochemistry 6 (7): 362–366.

Kierończyk, B.; Rawski, M.; Józefiak, A.; Mazurkiewicz, J.; Świątkiewicz, S.; Siwek, M.; Bednarczyk, M.; Szumacher-Strabel, M.; Cieślak, A.; Benzertiha, A.; Józefiak, D. (2018): Effects of replacing soybean oil with selected insect fats on broilers. Animal Feed Science and Technology 240: 170–183.

Kierończyk, B.; Rawski, M.; Mikołajczak, Z.; Szymkowiak, P.; Stuper-Szablewska, K.; Józefiak, D. (2023): Black Soldier Fly Larva Fat in Broiler Chicken Diets Affects Breast Meat Quality. Animals 13 (7): 1137.

Kierończyk, B.; Sypniewski, J.; Rawski, M.; Czekała, W.; Świątkiewicz, S.; Józefiak, D. (2020): From Waste to Sustainable Feed Material: The Effect of Hermetia Illucens Oil on the Growth Performance, Nutrient Digestibility, and Gastrointestinal Tract Morphometry of Broiler Chickens. Annals of Animal Science 20 (1): 157–177.

Kim, B.; Bang, H. Tae; Kim, K. Hyun; Kim, M. Ji; Jeong, J. Young; Chun, J. Lan; Ji, S. Yun (2020a): Evaluation of black soldier fly larvae oil as a dietary fat source in broiler chicken diets. Journal of Animal Science and Technology 62 (2): 187–197.

Kim, Y. Bhin; Kim, D.-H.; Jeong, S.-B.; Lee, J.-W.; Kim, T.-H.; Lee, H.-G.; Lee, K.-W. (2020b): Black soldier fly larvae oil as an alternative fat source in broiler nutrition. Poultry Science 99 (6): 3133–3143.

Kovitvadhi, A.; Chundang, P.; Tirawattanawanich, C.; Prathumpai, W.; Methacanon, P.; Chokpipatpol, K. (2019): Effects of dietary supplementation with different levels and molecular weights of fungal β-glucan on performances, health and meat quality in broilers. Asian-Australasian Journal of Animal Sciences 32 (10): 1548–1557.

Kroeckel, S.; Harjes, A.-G.; Roth, I.; Katz, H.; Wuertz, S.; Susenbeth, A.; Schulz, C. (2012): When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (Hermetia illucens) as fish meal substitute — Growth performance and chitin degradation in juvenile turbot (Psetta maxima). Aquaculture 364–365: 345–352.

Kulig, W.; Cwiklik, L.; Jurkiewicz, P.; Rog, T.; Vattulainen, I. (2016): Cholesterol oxidation products and their biological importance. Chemistry and Physics of Lipids 199: 144–160.

Lam, K.-L.; Chi-Keung Cheung, P. (2013): Non-digestible long chain beta-glucans as novel prebiotics. Bioactive Carbohydrates and Dietary Fibre 2 (1): 45–64.

Lecker, S. H.; Goldberg, A. L.; Mitch, W. E. (2006): Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. Journal of the American Society of Nephrology 17 (7): 1807–1819.

Lee, J. Yeon; Kim, J. Young; Lee, Y. Gyu; Rhee, M. Hee; Hong, E. Ki; Cho, J. Youl (2008): Molecular mechanism of macrophage activation by Exopolysaccharides from liquid culture of Lentinus edodes. Journal of Microbiology and Biotechnology 18 (2): 355–364.

Lin, Y.; Knol, D.; Trautwein, E. A. (2016): Phytosterol oxidation products (POP) in foods with added phytosterols and estimation of their daily intake: A literature review. European Journal of Lipid Science and Technology 118 (10): 1423–1438.

Lu, S.; Taethaisong, N.; Meethip, W.; Surakhunthod, J.; Sinpru, B.; Sroichak, T.; Archa, P.; Thongpea, S.; Paengkoum, S.; Purba, R. Aprilia Patindra; Paengkoum, P. (2022): Nutritional Composition of Black Soldier Fly Larvae (Hermetia illucens L.) and Its Potential Uses as Alternative Protein Sources in Animal Diets: A Review. Insects 13 (9): 831.

Ma, G.; Yang, W.; Zhao, L.; Pei, F.; Fang, D.; Hu, Q. (2018): A critical review on the health promoting effects of mushrooms nutraceuticals. Food Science and Human Wellness 7 (2): 125–133.

Maheshwari, G.; Gessner, D. K.; Meyer, S.; Ahlborn, J.; Wen, G.; Ringseis, R.; Zorn, H.; Eder, K. (2020): Characterization of the Nutritional Composition of a Biotechnologically Produced Oyster Mushroom and its Physiological Effects in Obese Zucker Rats. Molecular Nutrition & Food Research 64: e2000591.

Maheshwari, G.; Gessner, D. K.; Neuhaus, K.; Most, E.; Zorn, H.; Eder, K.; Ringseis, R. (2021): Influence of a Biotechnologically Produced Oyster Mushroom (Pleurotus sajor-caju) on the Gut Microbiota and Microbial Metabolites in Obese Zucker Rats. Journal of Agricultural and Food Chemistry 69: 1524–1535.

Marschall, M. J. M.; Grundmann, S. M.; Seel, W.; Simon, M.-C.; Schuchardt, S.; Most, E.; Gessner, D. K.; Wen, G.; Ringseis, R.; Eder, K. (2024): Fat from Hermetia illucens Alters the Cecal Gut Microbiome and Lowers Hepatic Triglyceride Concentration in Comparison to Palm Oil in Obese Zucker Rats. Journal of Nutrition 154 (2): 455–468

Matsue, M.; Mori, Y.; Nagase, S.; Sugiyama, Y.; Hirano, R.; Ogai, K.; Ogura, K.; Kurihara, S.; Okamoto, S. (2019): Measuring the Antimicrobial Activity of Lauric Acid against Various Bacteria in Human Gut. Cell Transplantation 28 (12): 1528–1541.

Moon, S. Hee; Lee, I.; Feng, X.; Lee, H. Yong; Kim, J.; Ahn, D. Uk (2016): Effect of Dietary Beta-Glucan on the Performance of Broilers and the Quality of Broiler Breast Meat. Asian-Australasian Journal of Animal Sciences 29 (3): 384–389.

Morales-López, R.; Auclair, E.; García, F.; Esteve-Garcia, E.; Brufau, J. (2009): Use of yeast cell walls; beta-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. Poultry Science 88 (3): 601–607.

Mwangi, M. N.; Oonincx, D. G. A. B.; Stouten, T.; Veenenbos, M.; Melse-Boonstra, A.; Dicke, M.; van Loon, J. J. A. (2018): Insects as sources of iron and zinc in human nutrition. Nutrition Research Reviews 31 (2): 248–255.

OECD/FAO (2023): OECD-FAO Agricultural Outlook 2023-2032. OECD Publishing, Paris.

Oliveira, J. de; Avanço, S. V.; Garcia-Neto, M.; Ponsano, E. (2016): Composition of broilers meat. Journal of Applied Poultry Research 25 (2): 173–181.

Oonincx, D. G. A. B.; Boer, I. J. M. de (2012): Environmental impact of the production of mealworms as a protein source for humans - a life cycle assessment. PlOS One 7 (12): e51145.

Oonincx, D. G. A. B.; van Broekhoven, S.; van Huis, A.; van Loon, J. J. A. (2015): Feed Conversion, Survival and Development, and Composition of Four Insect Species on Diets Composed of Food By-Products. PlOS One 10 (12): e0144601.

Pinotti, L.; Giromini, C.; Ottoboni, M.; Tretola, M.; Marchis, D. (2019): Review: Insects and former foodstuffs for upgrading food waste biomasses/streams to feed ingredients for farm animals. Animal 13 (7): 1365–1375.

Rathore, H.; Prasad, S.; Kapri, M.; Tiwari, A.; Sharma, S. (2019): Medicinal importance of mushroom mycelium: Mechanisms and applications. Journal of Functional Foods 54: 182–193.

Renaville, R.; Hammadi, M.; Portetelle, D. (2002): Role of the somatotropic axis in the mammalian metabolism. Domestic Animal Endocrinology 23 (1-2): 351–360.

Richards, P. J.; Flaujac Lafontaine, G. M.; Connerton, P. L.; Liang, L.; Asiani, K.; Fish, N. M.; Connerton, I. F. (2020): Galacto-Oligosaccharides Modulate the Juvenile Gut Microbiome and Innate Immunity To Improve Broiler Chicken Performance. mSystems 5 (1): e00827–19.

Ringel, A. E.; Drijvers, J. M.; Baker, G. J.; Catozzi, A.; García-Cañaveras, J. C.; Gassaway, B. M.; Miller, B. C.; Juneja, V. R.; Nguyen, T. H.; Joshi, S.; Yao, C.-H.; Yoon, H.; Sage, P. T.; LaFleur, M. W.; Trombley, J. D.; Jacobson, C. A.; Maliga, Z.; Gygi, S. P.; Sorger, P. K.; Rabinowitz, J. D.; Sharpe, A. H.; Haigis, M. C. (2020): Obesity Shapes Metabolism in the Tumor Microenvironment to Suppress Anti-Tumor Immunity. Cell 183 (7): 1848–1866.

Rizzo, G.; Goggi, S.; Giampieri, F.; Baroni, L. (2021): A review of mushrooms in human nutrition and health. Trends in Food Science & Technology 177: 60–73.

Sacks, F. M.; Lichtenstein, A. H.; Wu, J. H. Y.; Appel, L. J.; Creager, M. A.; Kris-Etherton, P. M.; Miller, M.; Rimm, E. B.; Rudel, L. L.; Robinson, J. G.; Stone, N. J.; van Horn, L. V. (2017): Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. Circulation 136 (3): e1–e23.

Schiavone, A.; Cullere, M.; Marco, M. de; Meneguz, M.; Biasato, I.; Bergagna, S.; Dezzutto, D.; Gai, F.; Dabbou, S.; Gasco, L.; Dalle Zotte, A. (2017): Partial or total replacement of soybean oil by black soldier fly larvae (Hermetia illucens L.) fat in broiler diets: effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. Italian Journal of Animal Science 16 (1): 93–100.

Schiavone, A.; Dabbou, S.; Marco, M. de; Cullere, M.; Biasato, I.; Biasibetti, E.; Capucchio, M. T.; Bergagna, S.; Dezzutto, D.; Meneguz, M.; Gai, F.; Dalle Zotte, A.; Gasco, L. (2018): Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source. Animal 12 (10): 2032–2039.

Solis de los Santos, F.; Donoghue, A. M.; Venkitanarayanan, K.; Dirain, M. L.; Reyes-Herrera, I.; Blore, P. J.; Donoghue, D. J. (2008): Caprylic acid supplemented in feed reduces enteric Campylobacter jejuni colonization in ten-day-old broiler chickens. Poultry science 87 (4): 800–804.

Solis de los Santos, F.; Donoghue, A. M.; Venkitanarayanan, K.; Metcalf, J. H.; Reyes-Herrera, I.; Dirain, M. L.; Aguiar, V. F.; Blore, P. J.; Donoghue, D. J. (2009): The natural feed additive caprylic acid decreases Campylobacter jejuni colonization in market-aged broiler chickens. Poultry Science 88 (1): 61–64.

Sypniewski, J.; Kierończyk, B.; Benzertiha, A.; Mikołajczak, Z.; Pruszyńska-Oszmałek, E.; Kołodziejski, P.; Sassek, M.; Rawski, M.; Czekała, W.; Józefiak, D. (2020): Replacement of soybean oil by Hermetia illucens fat in turkey nutrition: effect on performance, digestibility, microbial community, immune and physiological status and final product quality. British Poultry Science 61 (3): 294–302.

Tzompa-Sosa, D. A.; Dewettinck, K.; Gellynck, X.; Schouteten, J. J. (2021): Replacing vegetable oil by insect oil in food products: Effect of deodorization on the sensory evaluation. Food Research International 141: 110140.

Ugur, A. Erdem; Bolat, B.; Oztop, M. Halil; Alpas, H. (2021): Effects of High Hydrostatic Pressure (HHP) Processing and Temperature on Physicochemical Characterization of Insect Oils Extracted from Acheta domesticus (House Cricket) and Tenebrio molitor (Yellow Mealworm). Waste and Biomass Valorization 12 (4): 1–10.

United Nations, Department of Economic and Social Affairs, Population Division (2017): World Population Prospects: The 2017 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP/248.

van Huis, A. (2016): Edible insects are the future? Proceedings of the Nutrition Society 75 (3): 294–305.

VERORDNUNG (EU) 2017/ 893 DER KOMMISSION - vom 24. Mai 2017 - zur Änderung der Anhänge I und IV der Verordnung (EG) Nr. 999/ 2001 des Europäischen Parlaments und des Rates sowie der Anhänge X, XIV und XV der Verordnung (EU) Nr. 142/ 2011 der Kommission in Bezug auf die Bestimmungen über verarbeitetes tierisches Protein (europa.eu) 26.07.2024.

Vicente, F.; Pereira, P. C. (2024): Pork Meat Composition and Health: A Review of the Evidence. Foods 13 (12): 1905.

Wang, J.; Zheng, Z.; Yang, H.; Chen, J.; Xiao, Y.; Ji, X.; Zhang, Z.; He, H.; Ding, B.; Tang, B. (2022): Effect of β-1,3/1,6-glucan on gut microbiota of yellow-feathered broilers. AMB Express 12: 115.

Wan-Mohtar, W. Abd Al Qadr Imad; Ab Kadir, S.; Saari, N. (2016): The morphology of Ganoderma lucidum mycelium in a repeated-batch fermentation for exopolysaccharide production. Biotechnology Reports 11: 2–11.

Wood, J. D.; Enser, M.; Fisher, A. V.; Nute, G. R.; Sheard, P. R.; Richardson, R. I.; Hughes, S. I.; Whittington, F. M. (2008): Fat deposition, fatty acid composition and meat quality: A review. Meat Science 78 (4): 343–358.

Wu, G.; Fanzo, J.; Miller, D. D.; Pingali, P.; Post, M.; Steiner, J. L.; Thalacker-Mercer, A. E. (2014): Production and supply of high-quality food protein for human consumption: sustainability, challenges, and innovations. Annals of the New York Academy of Sciences 1321 (1): 1–19.

Zeitz, J. O.; Fennhoff, J.; Kluge, H.; Stangl, G. I.; Eder, K. (2015): Effects of dietary fats rich in lauric and myristic acid on performance, intestinal morphology, gut microbes, and meat quality in broilers. Poultry Science 94 (10): 2404–2413.

Zhang, S.; Ou, J.; Luo, Z.; Kim, I. Ho (2020): Effect of dietary β-1,3-glucan supplementation and heat stress on growth performance, nutrient digestibility, meat quality, organ weight, ileum microbiota, and immunity in broilers. Poultry Science 99 (10): 4969–4977.

Eidesstattliche Erklärung

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

Lea Schäfer

Der Lebenslauf wurde aus der elektronischen Version der Dissertation entfernt.

The curriculum vitae was removed from the electronic version of the dissertation.

Danksagung

An dieser Stelle möchte ich mich bei all denjenigen bedanken, die mir in den vergangenen Jahren zur Seite gestanden haben und mich auf meinem Weg der Promotion begleitet haben.
Mein besonderer Dank gilt Herrn Prof. Dr. Klaus Eder für die Überlassung dieses Themas und die Möglichkeit an seiner Professur promovieren zu dürfen, wie auch für seine Betreuung und sein Vertrauen in meine Arbeit.
Ein großer Dank geht auch an Herrn Prof. Dr. Martin Rühl für seine geduldige und präzise Einweisung in die Arbeit am Fermenter und für die Übernahme des Zweitgutachtens dieser Dissertation.
Ebenso möchte ich Herrn Prof. Dr. Robert Ringseis meinen Dank aussprechen für seine Ratschläge und Unterstützung, die eine wertvolle Hilfe für mich waren.
Besonders hervorheben möchte ich an dieser Stelle auch die großzügige finanzielle Unterstützung durch die H. Wilhelm Schaumann Stiftung. Dank dieser Förderung konnte ich mich voll und ganz auf meine Forschung konzentrieren.
Ein großes Dankeschön geht an die gesamte Arbeitsgruppe Tierernährung für die Unterstützung in jeder Phase meiner Arbeit – sei es bei der Durchführung der Versuche, den Probenentnahmen oder den Analysen. Ich konnte mich stets auf eure Hilfe verlassen und wusste, dass ich in jedem Moment auf euch zählen kann. Besonders schätze ich euer offenes Ohr für all meine Fragen sowie die vielen aufmunternden und unterhaltsamen Pausen, die mir oft die nötige Energie für die nächsten Schritte gegeben haben.
Liebe Anja, dir möchte ich Danken für deine Geduld und Unterstützung, insbesondere bei der Mycelherstellung und der Arbeit am Fermenter. Du warst eine wichtige Stütze in dieser intensiven Zeit und ich bin dir für all deine Hilfe sehr dankbar.
Abschließend möchte ich meiner Familie und meinen Freunden von ganzem Herzen danken! Besonders meinen Eltern und Patrick: Ihr habt mir zu jeder Zeit den Rücken gestärkt, mich motiviert und mir den Glauben an mich selbst vermittelt, auch wenn es manchmal schwierig war. Eure Zuversicht und euer Vertrauen in mich haben mir Kraft gegeben, weiterzumachen. Ohne euch wäre ich diesen Weg nicht so erfolgreich gegangen.