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The effect of insect or microalga alternative protein feeds on broiler meat quality

Brianne A Altmann,* Ruth Wigger, Marco Ciulu and Daniel Mörlein

Abstract

BACKGROUND: In order to combat environmental and food security concerns associated with the increasing demand for soymeal related to increasing meat consumption, this study determines the chicken meat quality derived when soymeal is substituted for either partially de-fatted *Hermetia illucens* larval meal or spirulina (*Arthrospira platensis*) in broiler diets. Physicochemical parameters, sensory traits, and fatty acid composition of the meat are investigated, as well as an experiment to evaluate the impact of highly oxygenated atmosphere *versus* vacuum-bag packaging on shelf life was conducted.

RESULTS: *Hermetia illucens* did not compromise quality; meat was slightly more yellow (higher *b), had a slightly decreased pH, and was less adhesive during chewing compared to the soy-fed control. Furthermore, *Hermetia illucens* resulted in higher saturated fatty acids proportions in thigh meat. Spirulina resulted in redder (higher *a**) and more yellow (higher *b**) meat with a slightly increased umami and chicken flavour. Spirulina-fed chicken meat had higher lipid oxidation levels compared to the control after being packaged in a highly oxygenated atmosphere; although, differences between the spirulina-fed and control fatty acid composition in thigh meat were minor.**

CONCLUSION: Both alternative protein feeds show potential to replace soymeal in broiler diets; however, they do result in moderately altered products.
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Supporting information may be found in the online version of this article.

Keywords: *Hermetia illucens*; black soldier fly; spirulina; *Arthrospira platensis*; sensory profiling; fatty acid composition

INTRODUCTION

Increasing population and incomes have driven up demand for animal-based proteins, leading to an increased need for animal feed.[1] For poultry, incorporating a high quality protein source in feed is important to ensure efficient production.[2] Conventionally, soybean meal is used as the main protein source in poultry production; however, environmental[3–6] and food security[7] concerns associated with the production of soybeans in South America have driven European research and policy to focus on alternative protein sources.[8] Two promising protein sources that could be produced in Europe include *Hermetia illucens* (HI) larvae, also known as black soldier fly, and the microalga spirulina (SP) (*Arthrospira platensis*).

HI larvae are relatively simple to reproduce and rear.[9,10] HI can be raised on various substrates, such as municipal organic[11] and vegetable[12] waste, animal waste,[13,14] and even faecal waste[15] enabling a more sustainable[16] and advantageous production compared to other insects, such as crickets which have a lower feed efficiency rate.[17] In addition, they are considered a non-pest species and therefore the environmental risk of production is minimal.[18] Finally, when considering the viability of feeding insect meal to chickens, it should be kept in mind that chicken naturally eat insect larvae[9,19] and in some regions larvae have been traditionally cultivated as a poultry protein source.[20] HI has an amino acid composition suitable for animal feed along with high levels (between 20 and 30 g kg^{-1}) of essential amino acids lysine, valine, and arginine; the larvae contain approximately 40% crude protein in dry matter.[12] However, currently in the European Union (EU) insect feed must be produced on regulated feedstuffs as a substrate, coming into direct competition with poultry production, and insect feed is only allowed in aquaculture production;[21] although permission for use in poultry production is expected in the near future. The current legal limitations mean that HI will likely remain more expensive than the current standard protein feed used in broiler production, soybean meal. Yet improvements in animal performance parameters could compensate for the additional cost of feed inputs.[22]

SP can be produced independent of arable land in bioreactors or open pond systems.[23] Currently, SP is produced to replace expensive fishmeal and fish oil supplies needed in aquaculture

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markets; however, improvements are still required to make it cost-effective as a feed ingredient at a large scale in aquaculture, and therefore in poultry production as well.[24] Bioreactors remain difficult to up-scale in production[24] and unfortunately to date SP production continues to have a larger environmental impact compared to arable crops.[25] Accordingly, much research is being invested into improving production efficiency. Examples are the integration of biogas effluent[26] or swine waste water[27] as nutrient sources and coupling production facilities to biogas reactors in order to utilize otherwise lost thermal energy.[23] The search to improve SP production is driven by its high protein content, over 60% protein in dry matter (DM),[28–30] as well as it being a source of essential fatty acids, such as linoleic acid, γ-linolenic acid, and arachidonic acid.[28,31]

The two protein sources were evaluated from a meat production perspective, monitoring parameters relevant from an industry and consumer perspective. This study investigates the effect of the inclusion of either a partially defatted HI larval meal or SP in the diets for broiler chickens on: (i) carcass traits, meat physicochemical and sensory quality; (ii) the shelf-life of meat under different packaging conditions.

MATERIALS AND METHODS

Birds and diets

Ross-308 broiler chicks were divided amongst three experimental groups; the broilers were raised on amino acid supplemented diets where 75% (starter diets) and 50% (grower diets) of the soymeal was substituted by either partially defatted HI larval meal or SP, or where no soymeal was substituted (C). One-day old broiler chicks were housed until slaughter (35 days of age) at the Division Animal Nutrition Physiology, University of Göttingen, Germany, on wood shavings in seven floor pens (seven chicks per pen at study-start; stocking density 5.8 birds m^{-2}) per treatment group. Birds were fed *ad libitum* with continuous access to water.[32] Animals were held in accordance with article 4 of Germany's Animal Welfare Regulation[33] and the study was approved (#33.9-42502-04-15/2027) by the Ethics Committee of the Lower Saxony Federal Office for Consumer Protection and Food Safety (LAVES), Germany.

The defatted HI meal was produced in Germany; whereas the SP was sourced from Myanmar. The HI meal contained 55 g kg^{-1} moisture, 141 g kg^{-1} lipids, and 605 g kg^{-1} crude protein in DM.[32] A conversion factor of 6.25 was applied to calculate crude protein content from nitrogen content. Since the time of this experiment, more accurate conversion factors have been validated for HI. A more appropriate conversion factor would be 4.76 and should be applied in future studies.[34] The SP composition included 34 g kg^{-1} moisture, 43 g kg^{-1} lipids, and 588 g kg^{-1} crude protein in DM.[32] The diets were aimed to be balanced according to the ideal amino acid ratio[35] and overall metabolizable energy.[36] This study is an extension of already published research and the diet composition in Table 1 was previously published as experiment 2 in Neumann *et al.*[32]

Dietary phosphorus (P) and calcium (Ca) contents were analysed according to standardized procedures for the chemical analysis of feedstuffs.[37] Fatty acid composition of diets was determined with freeze-dried milled (1 mm) feed samples using the procedure by Palmquist and Jenkins[38] with some modifications. In brief, 3 mL of 3 mol L^{-1} methanolic hydrochloric acid (HCl) was added to 0.5 g of feed and the mixture was incubated for 120 min at 60 °C in a water bath prior to being centrifuged at 4000 × *g* and 10 °C for 5 min. Next, 1 mL of the supernatant was removed to a test tube and 1 mL of hexane was added. The sample was gently mixed and 0.2 mL of the upper phase was used for the gas chromatography flame ionization detector (GC-FID) analysis of fatty acid methyl esters (FAME). The gas chromatograph (TRACE™ 1310) was equipped with an autosampler (AS1310) and FID; equipment was sourced from Thermo Fischer Scientific (Waltham, MA, USA). A Supelcowax™-10 (30 m × 0.32 mm × 0.25 µm; Sigma-Aldrich Chemie GmbH, Munich, Germany) capillary column was employed for the separation. Oven temperature was held at 160 °C for 1 min, increased until 220 °C (heating rate: 10 °C min^{-1}), maintained at 220 °C for 3 min, increased again until 250 °C (heating rate: 10 °C min^{-1}), and as a last step was held at 250 °C for 3 min. Each sample was injected adopting a 1:50 split ratio, at 250 °C, using hydrogen as the carrier gas with a flow rate of 1.2 mL min^{-1}. The FID operated at 260 °C with an air flow of 350 mL min^{-1}, hydrogen flow of 35 mL min^{-1}, and makeup gas flow of 40 mL min^{-1}. Fatty acids were identified using the Supelco® 37 Component FAME Mix (Sigma-Aldrich, Munich, Germany) and relative areas were analysed using the Chromeleon Chromatography Data System (Version 7.2 SR9; Thermo Fischer Scientific). All the analyses were performed in duplicate.

Sample collection

All surviving broilers were pooled according to treatment group (HI $n = 45$; SP $n = 43$; C $n = 44$) and slaughtered at 35 days of age at the University of Göttingen poultry slaughterhouse, which is regulated by article 4 of the EU's (EG) NR. 853/2004.[39] The birds were weighed, electrically stunned, slaughtered by decapitation, scalded (60 °C), defeathered, and gutted. Immediately following slaughter, the carcasses were weighed and dissected, where the breasts (*Pectoralis major*) and legs (thigh including drumstick) were skinned and cooled to 4 °C (5 h) until further analysis. The material was divided per treatment group as follows: the first 28 broilers' breasts were assigned for physicochemical analysis and the remaining eight broilers' breasts were assigned for sensory evaluation. Additional carcasses (HI $n = 9$; SP $n = 7$; C $n = 8$) were allocated for sensory panel training. Samples were stored at −18 °C for 2 months prior to sensory analysis.

The effect of highly oxygenated modified atmosphere packaging (HiOxMAP) compared to vacuum-sealed polyamide/polyethylene bags (VAC) was tested by equalling subdividing 36 birds' legs per treatment group amongst three packaging times (3, 7, and 14 days) where the right leg was VAC and the left leg was HiOxMAP packaged. The HiOxMAP packaging consisted of polypropylene (PP) plastic trays lined with a moisture absorbent pad and heat-sealed using oriented polyethylene terephthalate (OPET)/PP film (< 3 cm^3 m^{-2}/24 h/bar oxygen (O_2) transmission rate; < 12 cm^3 m^{-2}/24 h/bar carbon dioxide (CO_2) transmission rate), trapping a 80% O_2/20% CO_2 modified atmosphere. All leg samples were stored without illumination at 4 °C. The experimental unit used in analysis of meat quality was the individual bird.

Physicochemical analysis

Meat pH was recorded at 20 min and at 24 h *post mortem* using a portable pH meter (Knick Portamess 911, Berlin, Germany) equipped with a glass electrode and metal thermometer probe inserted 1 cm into the superior portion of the left breast muscle. Meat composition, including protein, moisture, and fat content were estimated using the left chicken breast trimmed of extra fat using a FOSS FoodScan™ analyser according to Anderson.[40]

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Table 1. Starter and grower diet composition and analysed nutrient content as previously reported by Neumann *et al.*, experiment 2[32]

	Starter diets 75% replacement			Grower diets 50% replacement		
Diets	C	HI	SP	C	HI	SP
Ingredients (g kg^{-1} as-fed)						
Wheat	326.7	390.3	392.5	360.2	396.5	398.8
Corn	163.4	195.1	196.2	180.1	198.3	199.4
Soymeal	390	97.5	97.5	330.0	165.0	165.0
Hermetia meal	—	217.1	—	—	122.5	—
Spirulina meal	—	—	221.0	—	—	124.7
Soybean oil	78.5	58	52	91.0	80.0	76.0
Premix[a]	10.0	10.0	10.0	10.0	10.0	10.0
DCP 40	11.0	8.0	11.0	10.0	8.0	9.0
$CaCO_3$	11.0	11.0	9.0	8.0	8.0	7.0
NaCl	3.0	1.0	0.8	2.5	1.5	1.0
Wheat starch	—	—	—	3.0	3.0	3.0
L-Lysine HCl	2.5	4.2	5.8	1.8	2.8	3.6
DL-Methionine	3.6	4.2	3.5	2.6	2.9	2.5
L-Threonine	0.3	0.1	—	0.1	0.03	—
L-Arginine	—	3.5	0.2	—	1.5	—
L-Histidine	—	—	0.6	—	—	—
L-Valine	—	—	—	0.7	—	—
Analysed crude nutrients (g kg^{-1} DM)						
Phosphorus (P)	6.24	6.61	6.35	5.80	6.11	6.27
Calcium (Ca)	4.90	5.39	5.07	4.57	4.71	4.85
Crude protein	247.8	268.6[c]	262.2	236.9	224.4[c]	254.9
Ether extract	102.2	111.0	85.2	117.1	120.6	114.5
AME_N (MJ kg^{-1} DM)[b]	14.4	15.3	15.3	15.0	15.5	15.5

[a] Added per kilogram of final diet: 2.1 g calcium, 0.8 g sodium, 5000 IU vitamin A, 1000 IU vitamin D3, 30 mg vitamin E, 2.6 mg vitamin B1, 4.8 mg vitamin B2, 3.2 mg vitamin B6, 20 µg vitamin B12, 3 mg vitamin K3, 50 mg nicotinic acid, 10 mg calcium pantothenate, 0.9 mg folic acid, 100 µg biotin, 1000 mg choline chloride, 50 mg Fe as iron(II) sulphate, monohydrate, 15 mg Cu as copper(II) sulphate, pentahydrate, 120 mg Mn as manganese(II) oxide, 70 mg Zn as zinc oxide, 1.4 mg I as calcium iodate, hexahydrate, 0.28 mg Se as sodium selenite, 0.55 mg Co as alkaline cobalt(II) carbonate, monohydrate and 100 mg butylhydroxytoluol.
[b] N corrected apparent metabolizable energy, calculated according to WPSA.[36]
[c] Conversion factor of 6.25 applied; crude protein is likely reduced based on a validated reduced conversion factor of 4.76 for *Hermetia illucens* larvae.[34]

Conventional drip loss was monitored at 2 °C according to Honikel[41] using the right breast muscle hung in a polyvinyl chloride (PVC) container with a lid. The muscle was removed once at 24 h to measure lean colour development with a portable spectrophotometer (model: CM 600d, Konica Minolta, Tokyo, Japan). The average across three colour measurements was used in further statistical analysis. After 72 h *post mortem* the muscles were weighed and drip loss was expressed as a percentage of the initial sample weight.

Further, the breast was used to determine cooking loss and shear force. Trimmed of exterior fat, the breast was cooked *sous vide* at 77 °C for 60 min in a hot water bath (incubation/deactivation bath, Gesellschaft für Labortechnik mbH (GFL), Burgwedel, Germany) to reach a core temperature of approximately 75 °C. Samples were cooled to room temperature outside the vacuum bag and cooking loss was expressed as the percentage of the initial weight. Afterwards, samples were stored overnight at 4 °C until shear force was measured using the razor blade method according to Xiong *et al.*[42] The method entailed the following modifications: a TA.XTplus Texture Analyser (Stable Micro Systems, Godalming, UK) was set to penetrate 15 mm and samples were cut three times. The average over the three measurements per sample was used in further statistical analysis. Shear force was recorded as the highest peak in Newtons (N).

Thigh lean colour was measured on the lateral side of the thigh prior to and immediately after opening a stored package with a portable spectrophotometer (model: CM 600d, Konica Minolta). As with the breast muscle, the average across three colour measurements was used in further statistical analysis. Legs were weighed prior to packaging and after storage; storage loss was expressed as a percentage of the initial weight.

The 2-thiobarbituric acid reactive substances (TBARS) method from Bruna *et al.*[43] was adjusted and applied to assess lipid oxidative stability of packaged thigh meat. First, 5 mL of 20% trichloroacetic acid (TCA) were pipetted to a 0.5 g sample; 250 µL of 0.19 mol L^{-1} butylated hydroxytoluene (BHT) was added. The mixture was homogenized with an Ultra-Turrax (T18 basic, IKA®-Werke GmbH & Co. KG, Staufen, Germany) and centrifuged at 3000 × *g* and 6 °C for 6 min. Then the contents were filtered and 2000 µL of filtrate was pipetted together with 2000 µL 0.02 mol L^{-1} 2-thiobarbituric acid (TBA), shaken with a vortex mixer and incubated for 30 min at 100 °C in a water bath. After cooling for 5 min on ice, the samples were remixed, rested for 5 min at room temperature and divided into three cuvettes. The absorbance was measured using a spectrophotometer (Libra

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S22, Biochrom GmbH, Berlin, Germany) at 532 nm and malonaldehyde (MDA) concentration was expressed as μg g^{-1} of meat.

Descriptive sensory profiling

Sensory evaluation was conducted in the University of Göttingen Sensory Laboratory, which fulfils requirements set out by ISO 8589[44] and was approved by the Veterinary and Consumer Protection Agency for the Municipality and City of Göttingen. A trained panel of 12 assessors, selected according to ISO 8586-1,[45] carried out descriptive sensory profiling of the three chicken breast products: SP-fed, HI-fed, soy (C)-fed. Assessors where first exposed to the products during a training period of four 2 h sessions, where a list of attributes was collectively defined by the assessors. In total, 22 attributes were defined to be evaluated according to Siekmann *et al.*;[46] the trained panel defined three additional attributes: umami taste, fattiness; malleability (Supporting Information Table S2). Sensory evaluation was conducted using a 10 cm unmarked line-scale and data were electronically recorded by EyeQuestion survey software (Logic8 BV, Elst, The Netherlands). Products were evaluated eight times across four sessions in a complete block design. The assessors evaluated samples in a sequential monadic manner using two randomly allocated set orders per day, i.e. six assessors received the same sample order. All assessors evaluated only one sample per bird. The samples were cooked *sous vide* according to the cooking loss procedure and were cut into approximately 1 cm^2 cubes. Each sample consisted of two warm cubes on a warmed plate that was assigned a randomly allotted three-digit code.

Fatty acid composition in thigh meat

Ten of the leg samples per dietary treatment group were homogenized without adipose fat for the determination of fatty acid composition in thigh meat. The samples were prepared according to Du *et al.*[47] and the fatty acid profile was determined using GC (TRACE™ 1310) equipped with an autosampler (TriPlus RSH™) and FID sourced from Thermo Fischer Scientific. The FID was heated to 260 °C with an air flow of 400 mL min^{-1}, hydrogen (H_2) flow of 40 mL min^{-1} and a makeup gas flow of 30 mL min^{-1}. A TG-WaxMS capillary column (30 m × 0.32 mm × 0.25 μm; Thermo Fischer Scientific), was injected with 1 μL of sample with an injector temperature of 260 °C, a 1:50 split, and using helium as the carrier gas at a rate of 1.2 mL min^{-1}. The oven temperature was held at 160 °C for 1 min, increased by 20 °C min^{-1} to 200 °C, maintained for 5 min, and then increased by 10 °C min^{-1} to 230 °C (maintained for 5.5 min). The column was baked out at 30 °C min^{-1} until 250 °C for 3 min after every run. Fatty acids were identified and analysed as stated in the Birds and diets section.

Statistical analyses

One-way analysis of variance (ANOVA) was conducted to compare breast parameters and thigh fatty acid relative area means. General linear regression models were used to analyse all other leg parameter means between dietary treatments and packaging types over time; here, package system was a within-subject variable, and dietary treatment and storage time, with their interaction effect, were between-subject variables. A mixed model was applied to sensory data, where dietary treatment was the fixed effect and bird and assessor were input as random variables. Variance analyses were conducted using SPSS software (Version 24.0, IBM Corporation, Armonk, NY, USA) and statistical significance was determined with $P < 0.05$. Furthermore, principal component analysis (PCA) was used to analyse fatty acid data, as often these data are likely to be inter-correlated.[48] The data were standardized and the PCA was computed using R (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) with the FactoMineR package.[49] Animal is the experimental unit of analysis used in this study.

RESULTS

Physicochemical parameters

Overall, the dietary treatment HI affected live and carcass weight, with HI-fed broilers being the heaviest and resulting in heavier carcasses (Table 2). SP resulted in redder (higher a^*) meat than the other two dietary treatment groups. Both alternative feeds also resulted in marginally higher b^* values (more yellow),

Table 2. Chicken breast meat physicochemical parameter means ± standard deviations across dietary protein treatments: soy (control group; C)-fed, *Hermetia illucens* (HI) larval meal-fed and spirulina (SP)-fed

Parameter	C ($n = 28$)	HI ($n = 28$)	SP ($n = 28$)
Live weight (kg)[a]	2.28 ± 0.41[b]	2.48 ± 0.29[a]	2.26 ± 0.36[b]
Carcass weight (kg)[a]	1.73 ± 0.34[b]	1.89 ± 0.24[a]	1.70 ± 0.30[b]
$pH_{20\ min}$	6.79 ± 0.12[a]	6.65 ± 0.17[b]	6.71 ± 0.13[ab]
$pH_{24\ h}$	5.96 ± 0.15[a]	5.84 ± 0.12[b]	5.99 ± 0.10[a]
L^*	57.0 ± 2.6	57.9 ± 1.8	57.4 ± 2.7
a^*	1.79 ± 1.35[b]	1.95 ± 1.15[b]	3.81 ± 1.18[a]
b^*	13.1 ± 1.4[b]	14.5 ± 1.0[a]	15.1 ± 1.5[a]
Moisture[b] (g kg^{-1})	754.0 ± 12.9	758.0 ± 10.6	749.5 ± 10.7
Protein[b] (g kg^{-1})	215.4 ± 11.2	209.6 ± 9.4	216.3 ± 10.0
Intramuscular fat[b] (g kg^{-1})	27.0 ± 5.0	29.5 ± 4.1	27.0 ± 4.8
Drip loss (%)	1.88 ± 0.76	1.86 ± 0.54	1.86 ± 1.01
Cooking loss (%)	24.56 ± 2.76	27.27 ± 6.05	25.89 ± 2.83
Shear force (N)	10.60 ± 2.04	9.88 ± 1.62	10.22 ± 2.27

[a] C ($n = 44$); HI ($n = 45$); SP ($n = 43$).
[b] $n = 14$ per treatment group.
[c] $n = 27$.
Different lowercase superscript letters indicate statistical differences between feed groups ($P < 0.05$).

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compared to the control. Water-holding capacity parameters, i.e. drip loss and cooking loss, as well as shear force remained unaffected by dietary treatment.

Packaging trial with chicken thigh and attached drumstick

Lightness (*L**) values were minimally different between the different packaging systems according to dietary treatment (Fig. 1(a)). However, lipid oxidation (TBARS) levels were drastically different between the dietary treatments in HiOxMAP compared to relatively similar values in VAC (Fig. 1(b)). SP-fed samples had elevated TBARS values in HiOxMAP. Redness (*a**) colour development over time was also affected by dietary treatment (Fig. 2). Yellowness (*b**) was influenced by a combination of factors (interaction effect): packaging, storage time, and dietary treatment (Table 3). Overall SP-fed samples were the reddest throughout the entire storage period, and the control and HI-fed samples behaved similarly until the control sample redness values decreased more rapidly than those of the HI-fed samples between day 7 and day 14 (Fig. 2).

In both packaging systems, *L** values increased over time; however, values increase more intensively in VAC between 3 and 7 days, and then decreased by day 14; whereas HiOxMAP samples maintained stable *L** values between 7 and 14 days of storage (Fig. 3 (a)). Redness values were considerably higher in HiOxMAP packages across the entire storage time. Although both packaging systems values climaxed at 7 days, the differences in values between storage times are more intense, i.e. steeper slopes, in HiOxMAP (Fig. 3(b)). In addition, TBARS values peaked at 7 days for HiOxMAP packages and then decreased by 14 days; whereas VAC packaging lead to a rather steady and incremental increase in TBARS over the entire period (Fig. 3(c)). Finally, storage losses increased over time for both packaging systems, where HiOxMAP packaged samples had cumulatively higher losses until day 14 (Fig. 3(d)).

Sensory profiling

Dietary treatment affected the chicken breast sensory profile (Table 4). Chicken breast produced with SP scored higher in terms of umami and chicken flavour. It also had a reduced off-odour (barn odour). In addition, chicken breast produced with HI was less adhesive during chewing.

Fatty acid in thigh meat

HI protein feed has a significant impact on the proportion of saturated fatty acids (SFAs) found in the intramuscular fat of the chicken thighs. This increased proportion is mostly due to the increased amounts of lauric acid (C12:0) and myristic acid (C14:0), which are 100 and 10 times larger, respectively, than the thigh meat of SP-fed and control broilers (Table 5). Opposite the HI samples, SP and control thigh meat is characterized by a high proportion of polyunsaturated fatty acids (PUFAs); the control group has the highest levels of PUFAs. The superior proportions of PUFAs in the SP-fed and control samples, compared to the HI group, are largely due to an increased share of linoleic acid (C18:2 n-6).

Figure 4 highlights the characterization of each treatment group based on their respective fatty acid composition. The score plot illustrates that the HI samples are unequivocally unique based on fatty acid composition; whereas there is overlap between the SP and control samples. The correlation loading plot illustrates which fatty acids correspond to which treatment group. Here it is clear that SFA content, specifically lauric acid (C12:0) and myristic acid (C14:0) are highly correlated with the HI treatment, and a high PUFA content is negatively correlated to HI feeding. Finally, the correlation loading plot depicts the unimportance played by numerous fatty acids in thigh meat intramuscular fat characterization; C20:0, C22:0, C20:3 n-6; C18:3 n-6, C22:1 n-9 are all dark in colour illustrating that their weak correlation with principal components 1 and 2.

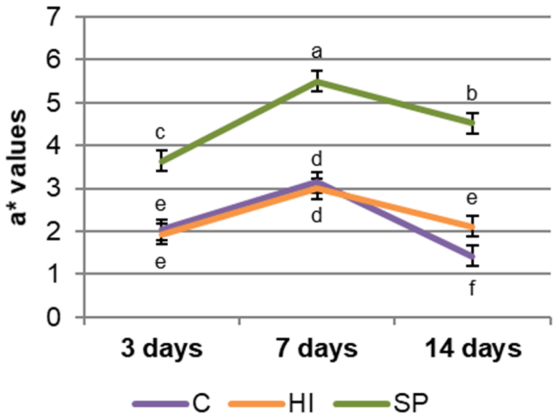

Figure 2. Estimated marginal means with standard error bars for the redness (*a**) development of leg meat samples from chickens fed *Hermetia illucens* (HI), spirulina (SP) or a soy-based control (C) feed stored up to 14 days. Different letters discern significant differences between means.

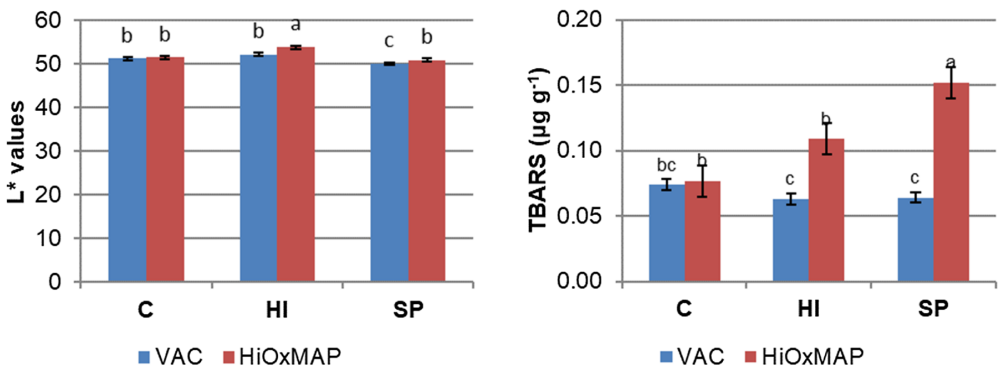

Figure 1. Estimated marginal means with standard error bars for *L** values (a) and TBARS (b) of leg meat samples from chickens fed *Hermetia illucens* (HI), spirulina (SP) or a soy-based control (C) protein source feed packaged in highly oxygenated modified atmosphere packaging (HiOxMAP) compared to vacuum-sealed bags (VAC). Different letters discern significant differences between means.

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Table 3. Estimated marginal means of *b** (yellowness) values for leg pieces, originating from soy (control group; C)-fed, *Hermetia illucens* (HI) larval meal-fed, or spirulina (SP)-fed broilers, stored in vacuum-sealed bags (VAC) or highly oxygenated modified atmosphere packaging (HiOxMAP) for 3, 7, or 14 days

Package	VAC (SE = 0.38)			HiOxMAP (SE = 0.37)		
Days	C	HI	SP	C	HI	SP
3	6.87^{Bb}	7.12^{Cb}	8.33^{Ba}	10.07^{Bb}	10.46^{Cb}	11.77^{Ba}
7	8.87^{Ab}	10.56^{Aa}	10.42^{Aa}	12.32^{Ab}	11.77^{Bb}	14.64^{Aa}
14	7.87^{Ac}	9.67^{Bb}	11.15^{Aa}	10.29^{Bb}	13.14^{Aa}	13.81^{Aa}

Standard error (SE).
The VAC and HiOxMAP samples are all significantly different within each dietary treatment.
Different uppercase superscript letters indicate differences within a column (storage time).
Different lowercase superscript letters indicate significant differences within a dietary treatment for a specific packaging system (row).

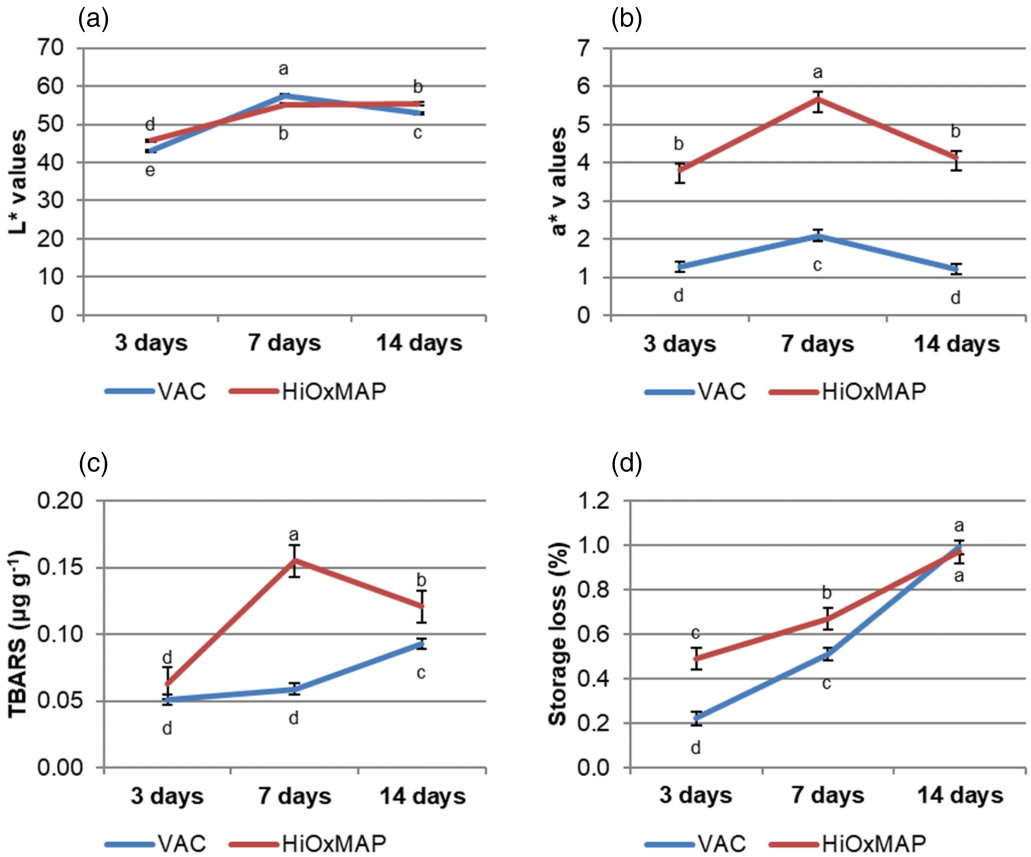

Figure 3. Estimated marginal means with standard error bars of *L** values (a), *a** values (b), TBARS (c) and storage loss (d) of leg meat in highly oxygenated modified atmosphere (HiOxMAP) or vacuum-sealed bag (VAC) packaging over 3, 7, and 14 days of storage. Different letters discern significant differences between means.

Table 4. Estimated means and standard error (SE) for sensory attributes that are significantly different between dietary protein treatments: soy (control group; C)-fed, *Hermetia illucens* (HI) larval meal-fed and spirulina (SP)-fed

Attribute	C ($n = 8$)	HI ($n = 8$)	SP ($n = 8$)	SE
Barn odour	14.9^{a}	15.8^{a}	11.1^{b}	2.5
Umami	18.6^{b}	18.7^{b}	21.8^{a}	3.9
Chicken flavour	56.0^{b}	55.7^{b}	59.1^{a}	5.9
Adhesiveness	47.8^{a}	43.5^{b}	48.3^{a}	6.7

Different lowercase superscript letters indicate statistical differences between feed groups ($P < 0.05$).

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Table 5. Relative area (%) means ± standard deviation for identified fatty acids in thigh intramuscular fat according to dietary protein treatments: soy (control group; C)-fed, *Hermetia illucens* (HI) larval meal-fed and spirulina (SP)-fed

	C ($n = 9$)[a]	HI ($n = 10$)	SP ($n = 9$)
Saturated fatty acids (SFAs)	24.9 ± 0.5[b]	29.42 ± 0.7[a]	25.17 ± 1.1[b]
C10:0	ND	0.049 ± 0.007[a]	ND
C12:0	0.019 ± 0.015[b]	3.143 ± 0.420[a]	0.033 ± 0.015[b]
C14:0	0.169 ± 0.061[b]	1.223 ± 0.136[a]	0.168 ± 0.057[b]
C15:0	0.059 ± 0.006	0.059 ± 0.007	0.063 ± 0.005
C16:0	13.3 ± 0.43[b]	15.0 ± 0.67[a]	14.9 ± 0.80[a]
C17:0	0.226 ± 0.025[b]	0.154 ± 0.018[c]	0.357 ± 0.032[a]
C18:0	9.81 ± 0.86[a]	8.49 ± 0.67[b]	8.60 ± 0.72[b]
C20:0	0.037 ± 0.044	0.031 ± 0.032	0.034 ± 0.029
C22:0	0.070 ± 0.063	0.040 ± 0.062	0.062 ± 0.060
C23:0	0.331 ± 0.635	0.672 ± 0.749	0.204 ± 0.610
C24:0	1.036 ± 0.164	0.883 ± 0.162	0.862 ± 0.132
Monounsaturated fatty acids (MUFAs)	21.6 ± 0.8[b]	24.4 ± 1.0[a]	23.6 ± 1.3[a]
C14:1	0.021 ± 0.016[c]	0.190 ± 0.036[a]	0.052 ± 0.011[b]
C15:1	0.014 ± 0.022[b]	0.042 ± 0.022[a]	0.017 ± 0.026[b]
C16:1	0.447 ± 0.204	0.275 ± 0.088	0.517 ± 0.488
C17:1	0.036 ± 0.022[b]	0.035 ± 0.020[b]	0.080 ± 0.011[a]
C18:1 n-9	20.7 ± 0.9[b]	23.5 ± 1.0[a]	22.6 ± 1.2[a]
C20:1 n-9	0.113 ± 0.032	0.106 ± 0.046	0.097 ± 0.024
C22:1 n-9	0.238 ± 0.124	0.260 ± 0.148	0.291 ± 0.160
Polyunsaturated fatty acids (PUFAs)	53.5 ± 1.1[a]	46.2 ± 1.3[c]	51.2 ± 1.8[b]
C18:2 n-6	39.0 ± 0.8[a]	33.7 ± 0.8[c]	37.4 ± 1.1[b]
C18:3 n-6	2.96 ± 0.32	3.15 ± 0.72	3.24 ± 1.04
C18:3 n-3	3.43 ± 0.31[a]	2.95 ± 0.12[b]	3.06 ± 0.39[b]
C20:2 n-6	0.542 ± 0.086	0.376 ± 0.331	0.466 ± 0.049
C20:3 n-6	0.097 ± 0.093	0.117 ± 0.052	0.134 ± 0.101
C20:4 n-6	6.55 ± 0.87[a]	5.24 ± 0.83[b]	6.14 ± 0.94[ab]
C20:3 n-3	0.057 ± 0.015	0.061 ± 0.026	0.039 ± 0.023
C20:5 n-3	0.169 ± 0.033[a]	0.145 ± 0.023[ab]	0.124 ± 0.028[b]
C22:2 n-6	0.046 ± 0.137	0.056 ± 0.167	0.008 ± 0.016
C22:6 n-3	0.716 ± 0.261[a]	0.379 ± 0.113[b]	0.571 ± 0.261[ab]
Sum n-6	49.2 ± 0.8[a]	42.7 ± 1.2[c]	47.4 ± 1.7[b]
Sum n-3	4.37 ± 0.37[a]	3.53 ± 0.07[b]	3.80 ± 0.47[b]
n-6/n-3 ratio	11.3 ± 1.0	12.1 ± 0.4	12.7 ± 1.9

[a] One sample removed from analysis due to extreme deviations in fatty acid profile and composition despite running multiple aliquots.
ND, not detected.
Different lowercase superscript letters indicate statistical differences between feed groups ($P < 0.05$).

DISCUSSION

In terms of live and carcass weights, HI-fed birds were superior compared to the other two treatment groups. This finding is not supported by Pieterse *et al.*,[50] nor Onsongo *et al.*,[51] who both observed no differences in animal weights. One explanation could be imperfectly balanced energy contents between diets in this study. In a previous study, Altmann *et al.*[52] also reported increased weights for HI-fed broilers; as well as Oluokun[53] has also observed increased growth rates when feeding HI diets in compared to full-fat soybean diets. The contradictory findings could be due to unobserved differences in HI-meal composition, such as differing mineral contents or estimated crude protein contents. HI larvae contain chitin which biases standard nitrogen-to-protein conversion rates (usually 6.25 for organic matter). Janssen *et al.* have established a conversion rate of 4.76 to be more suitable for estimating crude protein content from measured nitrogen levels.[34] In the case of this study, the crude protein content of the HI grower diet is already, even with a conversion rate of 6.25, below that of the other experimental diets. Therefore, we can assume the crude protein content in diet HI to be considerably lower; nonetheless, no negative effects on neither weights nor other meat quality aspects were observed. Future studies should focus on using a conversion factor of 4.76 to verify the effects of HI on animal growth. HI larvae can also contain high amounts of P[17] and Ca,[12,54] two important minerals that need to be carefully balanced for efficient poultry development. Our HI starter diets contained slightly higher levels (increase of approximately 0.5 g kg^{-1}) of P and Ca, yet this too did not contribute to a loss in meat quality. Further research should turn its focus towards HI meal composition, expanding on knowledge regarding amino acid composition[12] and the nitrogen-to-protein conversion factor,[34] in order to best incorporate HI into poultry diets for efficient meat production.

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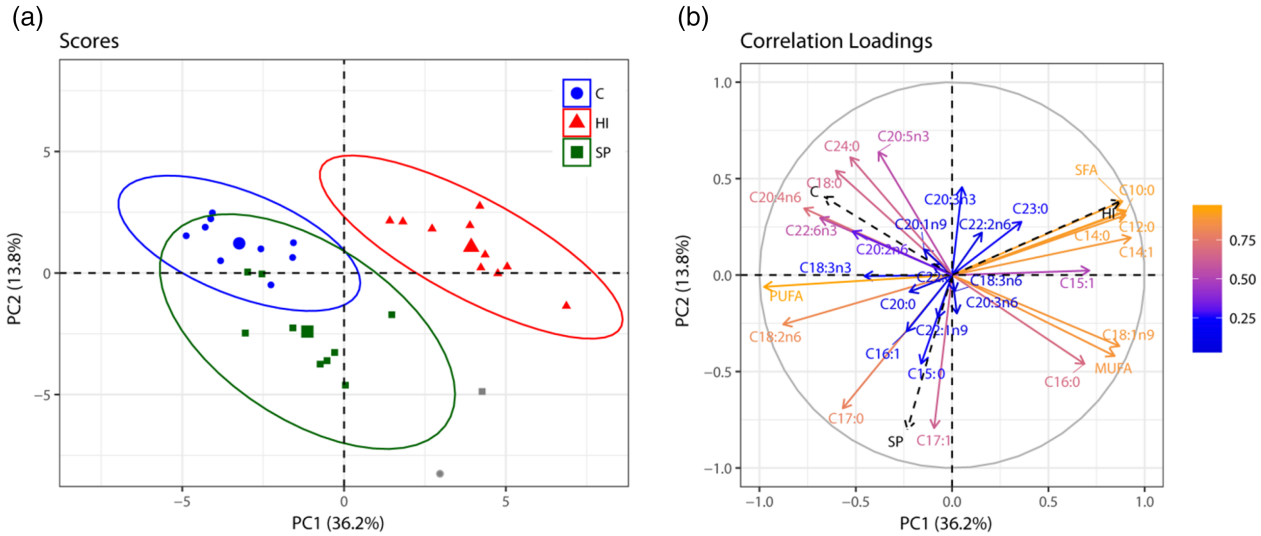

Figure 4. Score plots (a) and loading plots (b) derived from principal component analysis of fatty acid composition data from thigh meat for *Hermetia illucens* (HI), spirulina (SP) and control (C) dietary treatment groups. Variable contribution to the principal components is illustrated by arrow colour. Outliers excluded from analysis are identified in the score plot in grey.

Dietary treatment played a relatively minor role in influencing physicochemical parameters. The pH values, although significantly different between the treatment groups, remained within acceptable levels for fast-growing chicken meat.[55] In this study, chicken breast produced with HI had significantly lower pH values after 24 h *post mortem*. This is in agreement with Cullere *et al.*,[56] who reported lower ultimate pH levels for quails fed black soldier flies. Despite lower pH values, no differences between treatment groups were observed in water-holding capacity, as recorded per drip loss and cooking loss.

Lean colour is one of the noticeable physicochemical differences between the treatment groups. Venkataraman *et al.*[57] and Toyomizu *et al.*[58] have long since established that a high inclusion of SP in poultry diets significantly influences the red and yellow hues in poultry meat colour. This study confirms their results. Therefore, further research should turn its focus on the implications concerning consumer acceptance of raw chicken breast colour. In addition, chicken breasts from HI-fed broilers exhibit increased yellow (b^*) hues; this has already been observed by Altmann *et al.*[52] in raw chicken breasts. In the cooked chicken breast these differences were not discernable by a trained panel;[52] whereas Secci *et al.*[59] have determined increased b^* values for cooked Barbary partridge meat produced using HI feed.

Additionally, protein source affected lean colour values in HiOxMAP. To the best of our knowledge, Altmann *et al.*[52] remains the single study to investigate the effect of HI or SP dietary treatments throughout an industrial packaging scenario; however, that study used chicken breast instead of chicken thigh. Altmann *et al.*[52] only observed an interaction of dietary treatment with storage time for ultimate pH (not recorded in the current study), and did not observe an interaction between storage time and dietary treatment for colour parameters. The statistical differences observed in the current study remain minimal (L^* values) to moderate (TBARS) and are likely unobserved in Altmann *et al.*[52] due to the relatively small sample size. Additionally, the increased lipid oxidation levels of SP samples in HiOxMAP may be of concern, as samples could develop an oxidized flavour[60] and values observed in this study could be interpreted as above acceptable values pertaining to 'good meat quality.'[61] Unexpectedly, this increased level of lipid oxidation does not correspond with the fatty acid composition results. The fatty acid composition in intramuscular fat (IMF) of SP-fed broiler's thigh meat has a lower PUFA content than the control group. This goes to show that further research is required in determining the biochemical pathways effecting lipid oxidation in HiOxMAP packaging of poultry meat produced with SP. Protein oxidation is another important aspect that should be investigated in future studies; investigating protein quality and oxidation can also assist in this regard.[62] Moreover, it should be noted that TBARS values are method-sensitive and cannot directly be compared.[63] The other reported effects associated with HiOxMAP have already been well documented and established throughout the literature.[62,64–67]

In accordance with Pieterse *et al.*,[50] we determined no drastic differences in eating quality between the chicken breast produced with HI-fed and the control sample in eating quality when stored frozen prior to sensory evaluation. The only distinguishable difference found by our trained panel was a slightly less adhesive texture during chewing. Cullere *et al.*[68] also reported no sensory differences for broiler quail fed HI. However, some research does suggest that insect-fed meat products may have a more intensive aroma and increased juiciness attributes.[52,69]

More interestingly, higher umami and chicken flavour values were reported for SP-fed samples. Likely, these two attributes are associated. Umami is usually linked to a meaty flavour[70] and SP's profile includes a strong umami taste.[71] Increased chicken flavour and umami taste is likely favourable in chicken meat products. Limited research has investigated the consumer acceptance of the eating quality resulting from SP as a feed in poultry diets; yet, in red sea bream Mustafa *et al.*[72] indicate that taste is improved with just a 2% contribution of SP in the fish diet. However, Nandeesha *et al.*[73] report no discernable difference in flavour. More profound studies need to incorporate sensory profiling coupled with consumer sensory tests in order to better understand the effects of SP protein feed on eating quality.

Finally, HI significantly affected the fatty acid composition in chicken thigh meat. The high levels of C12:0 and C14:0 found in

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the feed (Table S1) are reflected in the fatty acid composition of the thigh meat. Just as in Cullere *et al.*[68] with quail broilers, Secci *et al.*[59] with Barbary partridge, and Schiavone *et al.*[74] with broiler chickens fed HI fat (not meal), C12:0 and C14:0 levels increased in our study compared to a soy-based control group. In addition, 14:1 also characterized HI-fed samples; whereby Secci *et al.*[59] found no differences in this fatty acid in the raw or cooked samples of Barbary partridge, yet Cullere *et al.*[68] and Schiavone *et al.*[74] also reported increased C14:1 levels. Surprisingly, Pieterse *et al.*[50] did not report any significant differences in the fatty acid composition of cooked chicken broiler meat produced with diets containing HI.

Finally, although SP is often marketed as a good source of PUFAs, the SP-fed chicken in our study did not produce thigh meat with the highest PUFA content. In fact, the control samples contained the highest proportions of PUFAs. Furthermore, the control treatment had the largest proportion of omega-3 fatty acids. SP is cited as high in γ-linolenic acid and other essential omega fatty acids;[28,30] however levels vary widely.[31] On the other hand, soy is known to have high levels of polyunsaturated fats, such as linoleic and linolenic acids.[75,76] In this regard soy is already a reasonable feed component to ensure a high content of good PUFAs. Therefore, the expectations of increasing PUFA content in chicken meat by feeding SP should not be over-estimated. Especially considering that the SP feed used in this study was high in omega-6 fatty acids, not omega-3 fatty acids. Other feed ingredients have been established to increase the omega-3 fatty acid content in poultry.[77] Particularly, Gatrell *et al.*[78] were able to successfully increase the PUFA content in chicken thigh, especially the omega-3 fatty acids content, using microalgal biomass derived from *Nannochloropsis oceanica*. SP has been shown to influence the PUFA[79] and overall fat content[80] in animal products; however, the next step should focus on increasing the omega-3 fatty acid proportion of the microalga so that SP could play a more relevant role in improving fatty acid composition of poultry products.

CONCLUSIONS

Our experiment clearly portrays the resulting poultry meat quality between a partially-defatted HI meal and SP-based diets, compared to a soy-fed control. Results show that the alternative protein sources can be viably included in broiler chicken production, as investigated from a multi-faceted meat quality perspective. However, both alternative protein sources come with their own challenges. HI-fed meat contains an increased proportion of SFAs, which may increase quality parameters, such as shelf-life, but are not positively perceived from a health point of view. In the case of SP, special attention should be paid to the intensive meat colour and increased lipid oxidation of products in HiOxMAP when planning to bring these products to market. All-in-all, no eating quality concerns were identified. HI-fed chicken mirrored the control group, and SP feed makes it taste all-the-more like chicken.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 OECD/Food and Agriculture Organization of the United Nations, *OECD-FAO Agricultural Outlook 2015*. OECD Publishing, Paris. http://dx.doi.org/10.1787/agr_outlook-2015-en (2015).
2 Waldroup PW, Influence of environmental temperature on protein and amino acid needs of poultry. *Fed Proc* **41**:2821–2823 (1982).
3 Reckmann K, Blank R, Traulsen I and Krieter J, Comparative life cycle assessment (LCA) of pork using different protein sources in pig feed. *Arch Anim Breed* **59**:27–36 (2016).
4 Mungkung R, Aubin J, Prihadi TH, Slembrouck J, van der Werf HMG and Legendre M, Life cycle assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. *J Clean Prod* **57**:249–256 (2013).
5 Prudêncio da Silva V, van der Werf HMG, Spies A and Soares SR, Variability in environmental impacts of Brazilian soybean according to crop production and transport scenarios. *J Environ Manage* **91**:1831–1839 (2010).
6 Boerema A, Peeters A, Swolfs S, Vandevenne F, Jacobs S, Staes J *et al.*, Soybean trade: balancing environmental and socio-economic impacts of an intercontinental market. *PLoS One* **11**:1–13 (2016).
7 Song B, Marchant MA, Reed MR and Xu S, Competitive analysis and market power of China's soybean import market. *Int Food Agribus Manage Rev* **12**:21–42 (2009).
8 European Commission. Communication from the commission to the European Parliament, the council, the European economic and social committee and the committee of the regions. *Innovating for Sustainable Growth: A Bioeconomy for Europe*. European Commission, Brussels (2012).
9 Jozefiak D, Jozefiak A, Kieronczyk B, Rawski M, Swiatkiewicz S, Dlugosz J *et al.*, Insects—a natural nutrient source for poultry—a review. *Ann Anim Sci* **16**:297–313 (2016).
10 Makkar HPS, Tran G, Heuzé V and Ankers P, State-of-the-art on use of insects as animal feed. *Anim Feed Sci Technol* **197**:1–33 (2014).
11 Diener S, Studt Solano NM, Roa Gutiérrez F, Zurbrügg C and Tockner K, Biological treatment of municipal organic waste using black soldier fly larvae. *Waste Biomass Valoriz* **2**:357–363 (2011).
12 Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De Meulenaer B *et al.*, Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *J Sci Food Agric* **97**:2594–2600 (2017).
13 St-Hilaire S, Cranfill K, McGuire MA, Mosley E, Tomberlin JK, Newton L *et al.*, Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *J World Aquacult Soc* **38**:309–313 (2007).
14 Sheppard DC, Larry G, Thompson SA and Savage S, A value added manure management system using the black soldier fly. *Bioresour Technol* **50**:275–279 (1994).

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15 Lalander C, Diener S, Magri ME, Zurbrügg C, Lindström A, Vinnerås B *et al*., Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*)—from a hygiene aspect. *Sci Total Environ* **458–460**:312–318 (2013).
16 Smetana S, Palanisamy M, Mathys A and Heinz V, Sustainability of insect use for feed and food: life cycle assessment perspective. *J Clean Prod* **137**:741–751 (2016).
17 Oonincx DGAB, van Broekhoven S, van Huis A and van Loon JJA, Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS One* **10**:1–20 (2015).
18 van Huis A and DGAB O, The environmental sustainability of insects as food and feed. A review. *Agron Sustainable Dev* **37**:43 (2017).
19 Sossidou ENN, Dal Bosco A, Castellini C and Grashorn MAA, Effects of pasture management on poultry welfare and meat quality in organic poultry production systems. *Worlds Poult Sci J* **71**:375–384 (2015).
20 Pomalégni SCB, Gbemavo DSJC, Kpadé CP, Kenis M and Mensah GA, Traditional use of fly larvae by small poultry farmers in Benin. *J Insects Food Feed* **3**:1–6 (2017).
21 European Commission, Commission Regulation (EU) 2017/893. Comm. Regul. 2017/893 24 May 2017 Amend. Annex. I IV to Regul. No 999/2001 Eur. Parliam. Counc. Annex. X, XIV XV to Comm. Regul. No 142/2011 as regards provisions proc. European Commission: Brussels (2017).
22 Sánchez-Muros MJ, Barroso FG and Manzano-Agugliaro F, Insect meal as renewable source of food for animal feeding: a review. *J Clean Prod* **65**:16–27 (2014).
23 Taelman SE, De Meester S, Van Dijk W, da Silva V and Dewulf J, Environmental sustainability analysis of a protein-rich livestock feed ingredient in The Netherlands: microalgae production versus soybean import. *Resour Conserv Recycl* **101**:61–72 (2015).
24 Chen J, Wang Y, Benemann JR, Zhang X, Hu H and Qin S, Microalgal industry in China: challenges and prospects. *J Appl Phycol* **28**: 715–725 (2016).
25 Ye C, Mu D, Horowitz N, Xue Z, Chen J, Xue M *et al*., Life cycle assessment of industrial scale production of spirulina tablets. *Algal Res* **34**:154–163 (2018).
26 Hultberg M, Lind O, Birgersson GGG and Asp HH, Use of the effluent from biogas production for cultivation of spirulina. *Bioprocess Biosyst Eng* **40**:625–631 (2017).
27 Olguín EJ, Galicia S, Mercado G and Pérez T, Annual productivity of spirulina (*Arthrospira*) and nutrient removal in a pig wastewater recycling process under tropical conditions. *J Appl Phycol* **15**:249–257 (2003).
28 Tokusoglu Ö and Unal MK, Biomass nutrient profiles of three microalgae: *Spirulina platensis, Chlorella vulgaris and Isochrysis galbana*. *J Food Sci* **68**:1144–1148 (2003).
29 Narasimha DLR, Venkataraman GS, Duggal SK and Eggum BO, Nutritional quality of the blue-green alga *Spirulina platensis* geitler. *J Sci Food Agric* **33**:456–460 (1982).
30 Gutiérrez-Salmeán G, Fabila-Castillo L and Chamorro-Cevallos G, Nutritional and toxicological aspects of spirulina (*Arthrospira*). *Nutr Hosp* **32**:34–40 (2015).
31 Diraman H, Koru E and Dibeklioglu H, Fatty acid profile of *Spirulina platensis* used as a food supplement. *Isr J Aquacult* **61**:134–142 (2009).
32 Neumann C, Velten S and Liebert F, The graded inclusion of algae (*Spirulina platensis*) or insect (*Hermetia illucens*) meal as a soybean meal substitute in meat type chicken diets impacts on growth , nutrient deposition and dietary protein quality depending on the extent of amino acid Supple. *Open J Anim Sci* **8**:163–183 (2018).
33 BMJV, Tierschutz-Nutztierhaltungsverordnung in der Fassung der Bekanntmachung vom 22 August 2006 (BGBl. I S. 2043), die zuletzt durch Artikel 3 Absatz 2 des Gesetzes vom 30. Juni 2017 (BGBl. I S. 2147) geändert worden ist. BMJV: Berlin (2017).
34 Janssen RH, Vincken JP, Van Den Broek LAM, Fogliano V and Lakemond CMM, Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *J Agric Food Chem* **65**:2275–2278 (2017).
35 Wecke C and Liebert F, Improving the reliability of optimal in-feed amino acid ratios assed on individual amino acid efficiency data from N balance studies in growing chicken. *Animals* **3**:558–573 (2013).
36 WPSA, The prediction of apparent metabolizable energy values for poultry in compound feeds. *Worlds Poult Sci J* **40**:181–182 (1984).
37 Naumann C and Bassler R, Die chemische Untersuchung von Futtermitteln: Methodenbuch Band III, Loseblattausgabe mit Ergänzungen 1983, 1988, 1993, 1997 und 2004, in *Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik*, VDLUFA-Verlag, Darmstadt (2004).
38 Palmquist DL and Jenkins TC, Challenges with fats and fatty acid methods. *J Anim Sci* **81**:3250–3254 (2003).
39 Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. European Commission: Brussels (2004).
40 Anderson S, Determination of fat, moisture, and protein in meat and meat products by using the FOSS FoodScan™ near-infrared spectrophotometer with FOSS artificial neural network calibration model and associated database: collaborative study. *J AOAC Int* **90**: 1073–1083 (2007).
41 Honikel KO, Reference methods for the assessment of physical characteristics of meat. *Meat Sci* **49**:447–457 (1998).
42 Xiong R, Cavitt LC, Meullenet JF and Owens CM, Comparison of Allo-Kramer, Warner-Bratzler and razor blade shears for predicting sensory tenderness of broiler breast meat. *J Texture Stud* **37**:179–199 (2006).
43 Bruna JM, Ordóñez JA, Fernández M, Herranz B and La Hoz LD, Microbial and physico-chemical changes during the ripening of dry fermented sausages superficially inoculated with or having added an intracellular cell-free extract of *Penicillium aurantiogriseum*. *Meat Sci* **59**:87–96 (2001).
44 ISO, ISO 8589:2007: Sensory analysis—General guidance for the design of test rooms. International Organization for Standardization: Geneva (2007).
45 ISO, ISO 8586-1:1993: Sensory analysis—General guidance for the selection, training and monitoring of assessors—Part 1: Selected assessors. International Organization for Standardization: Geneva (1993).
46 Siekmann L, Meier-Dinkel L, Janisch S, Altmann B, Kaltwasser C, Sürie C *et al*., Carcass quality, meat quality and sensory properties of the dual-purpose chicken Lohmann dual. *Foods* **7**:1–16 (2018).
47 Du M, Ahn D, Nam K and Sell J, Influence of dietary conjugated linoleic acid on volatile profiles, color and lipid oxidation of irradiated raw chicken meat. *Meat Sci* **56**:387–395 (2000).
48 Pethybridge HR, Parrish CC, Bruce BD, Young JW and Nichols PD, Lipid, fatty acid and energy density profiles of white sharks: insights into the feeding ecology and ecophysiology of a complex top predator. *PLoS One* **9**:e97877 (2014).
49 Lê S, Josse J and Husson F, FactoMineR: an R package for multivariate analysis. *J Stat Softw* **25**:1–18 (2008).
50 Pieterse E, Erasmus SW, Uushona T and Hoffman LC, Black soldier fly (*Hermetia illucens*) pre-pupae meal as a dietary protein source for broiler production ensures a tasty chicken with standard meat quality for every pot. *J Sci Food Agric* **99**:893–903 (2019).
51 Onsongo VO, Osuga IM, Gachuiri CK, Wachira AM, Miano DM, Tanga CM *et al*., Insects for income generation through animal feed: effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. *J Econ Entomol* **111**:1966–1973 (2018).
52 Altmann BA, Neumann C, Velten S, Liebert F and Mörlein D, Meat quality derived from high inclusion of a micro-alga or insect meal as an alternative protein source in poultry diets: a pilot study. *Foods* **7**: 1–15 (2018).
53 Oluokun JA, Upgrading the nutritive value of full-fat soyabeans meal for broiler production with either fishmeal or black soldier fly larvae meal (*Hermetia illucens*). *Niger J Anim Sci* **3** (2000).
54 Liland NS, Biancarosa I, Araujo P, Biemans D, Bruckner CG, Waagbø R *et al*., Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLoS One* **12**: 1–23 (2017).
55 Petracci M, Mudalal S, Soglia F and Cavani C, Meat quality in fast-growing broiler chickens. *Worlds Poult Sci J* **71**:363–374 (2015).
56 Cullere M, Tasoniero G, Giaccone V, Miotti-Scapin R, Claeys E, De Smet S *et al*., Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *Animal* **10**:1923–1930 (2016).
57 Venkataraman LV, Somasekaran T and Becker EW, Replacement value of blue-green alga (*Spirulina platensis*) for fishmeal and a vitamin-mineral premix for broiler chicks. *Br Poultry Sci* **35**:373–381 (1994).

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58 Toyomizu M, Sato K, Taroda H, Kato T and Akiba Y, Effects of dietary spirulina on meat colour in muscle of broiler chickens. *Br Poultry Sci* **42**: 197–202 (2001).
59 Secci G, Moniello G, Gasco L, Bovera F and Parisi G, Barbary partridge meat quality as affected by *Hermetia illucens* and *Tenebrio molitor* larva meals in feeds. *Food Res Int* **112**:291–298 (2018).
60 Fernández J, Pérez-Álvarez J and Fernández-López J, Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chem* **59**: 345–353 (1997).
61 Jensen C, Engberg R, Jakobsen K, Skibsted LH and Bertelsen G, Influence of the oxidative quality of dietary oil on broiler meat storage stability. *Meat Sci* **47**:211–222 (1997).
62 Spanos D, Tørngren MA, Christensen M and Baron CP, Effect of oxygen level on the oxidative stability of two different retail pork products stored using modified atmosphere packaging (MAP). *Meat Sci* **113**: 162–169 (2016).
63 Zhang Y, Holman BWB, Ponnampalam EN, Kerr MG, Bailes KL, Kilgannon AK *et al*., Understanding beef flavour and overall liking traits using two different methods for determination of thiobarbituric acid reactive substance (TBARS). *Meat Sci* **149**:114–119 (2019).
64 Delles RM and Xiong YL, The effect of protein oxidation on hydration and water-binding in pork packaged in an oxygen-enriched atmosphere. *Meat Sci* **97**:181–188 (2014).
65 Bao Y, Puolanne E and Ertbjerg P, Effect of oxygen concentration in modified atmosphere packaging on color and texture of beef patties cooked to different temperatures. *Meat Sci* **121**:189–195 (2016).
66 Xiong YL, Park D and Ooizumi T, Variation in the cross-linking pattern of porcine myofibrillar protein exposed to three oxidative environments. *J Agric Food Chem* **57**:153–159 (2008).
67 Muhlisin P, Kim DS, Song YR, Lee SJ, Lee JK and Lee SK, Effects of gas composition in the modified atmosphere packaging on the shelf-life of *longissimus dorsi* of korean native black pigs-duroc crossbred during refrigerated storage. *Asian-Australasian J Anim Sci* **27**:1157–1163 (2014).
68 Cullere M, Tasoniero G, Giaccone V, Acuti G, Marangon A, Dalle Zotte A *et al*., Black soldier fly as dietary protein source for broiler quails: meat proximate composition, fatty acid and amino acid profile, oxidative status and sensory traits. *Animal* **12**:640–647 (2018).
69 Khan S, Khan RUU, Alam W and Sultan A, Evaluating the nutritive profile of three insect meals and their effects to replace soya bean in broiler diet. *J Anim Physiol Anim Nutr* **102**:662–668 (2018).
70 Löliger J, Function and importance of glutamate for savory foods. *J Nutr* **130**:915S–920S (2000).
71 Grahl S, Palanisamy M, Strack M, Meier-Dinkel L, Toepfl S and Mörlein D, Towards more sustainable meat alternatives: how technical parameters affect the sensory properties of extrusion products derived from soy and algae. *J Clean Prod* **198**:962–971 (2018).
72 Mustafa MG, Umino T and Nakagawa H, The effect of spirulina feeding on muscle protein deposition in red sea bream Pagrus major. *J Appl Ichthyol* **10**:141–145 (1994).
73 Nandeesha MC, Effectson feeding *Spirulina platensis* on the growth, proximote composition and organoleptic quality of common carp *Cyprinus carpio L*. *Aquac Res* **29**:305–312 (1998).
74 Schiavone A, Cullere M, De Marco M, Meneguz M, Biasato I, Bergagna S *et al*., 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. *Ital J Anim Sci* **16**:93–100 (2017).
75 Shin E, Hwang C, Lee B, Kim H, Ko J, Baek I *et al*., Chemometric approach to fatty acid profiles in soybean cultivars by principal component analysis (PCA). *Prev Nutr Food Sci* **17**:184–191 (2012).
76 Patil AG, Oak MD, Taware SP, Tamhankar SA and Rao VS, Nondestructive estimation of fatty acid composition in soybean [*Glycine max* (L.) Merrill] seeds using near-infrared transmittance spectroscopy. *Food Chem* **120**:1210–1217 (2010).
77 Pérez-palacios T, Ruiz-carrascal J, Solomando JC and Antequera T, Strategies for enrichment in ω-3 fatty acids aiming for healthier meat products. *Food Rev Int* **35**:485–503 (2019).
78 Gatrell SK, Kim JG, Derksen TJ, O'Neil EV and Lei XG, Creating ω-3 fatty-acid-enriched chicken using defatted green microalgal biomass. *J Agric Food Chem* **63**:9315–9322 (2015).
79 Holman BWB and Malau-Aduli AEO, Spirulina as a livestock supplement and animal feed. *J Anim Physiol Anim Nutr* **97**:615–623 (2013).
80 Kashani A, Holman BW, Nichols PD and Malau-Aduli AE, Effect of dietary supplementation with spirulina on the expressions of AANAT, ADRB3, BTG2 and FASN genes in the subcutaneous adipose and *Longissimus dorsi* muscle tissues of purebred and crossbred Australian sheep. *J Anim Sci Technol* **57**:8 (2015).

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