Evaluation of microwave-dried black soldier fly (*Hermetia illucens*) larvae meal as a dietary protein source in broiler chicken diets

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Received: 29 June 2021 / Accepted: 3 August 2021

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OPEN ACCESS

RESEARCH ARTICLE

Abstract

Here, the effect of microwave-dried black soldier fly (*Hermetia illucens*) larvae meal (HILM) as a dietary protein source on the growth performance and health of broilers was evaluated. A total of 126 male broilers were randomly allocated to three treatments (six replicates of seven birds per group): control diet (CON) of corn and soybean meal and two experimental diets in which soybean meal was replaced with 25% (25HILM) or 50% (50HILM) HILM. The broilers were slaughtered at 35 d to determine the apparent ileal digestibility (AID), volatile fatty acid (VFA) profiles, and length and weight of the small intestine. Blood samples were collected from 36 randomly selected birds (12 broilers per treatment) to determine serum parameters and haematological traits. Average daily gain, feed intake, and AID of protein were decreased ($P < 0.05$) in the 50HILM group compared with those in the CON group. Dietary HILM increased ($P < 0.05$) the relative length of the entire digestive tract and total VFAs in the caecal digesta. Serum low-density lipoprotein cholesterol was the lowest ($P < 0.01$), but the triglyceride levels were the highest ($P < 0.05$) in the 50HILM group. The serum phosphorus level was lower in both HILM diet-fed groups ($P < 0.01$), whereas the creatine phosphokinase level was lower in the 50HILM group than in the CON group. In contrast, the 50HILM group showed higher ($P < 0.05$) monocyte and red blood cell distribution width-standard deviation. In conclusion, dietary microwave-dried HILM positively influenced the VFA and cholesterol levels; however, growth performance was negatively affected by malnutrition caused by low protein digestibility in the 50HILM group. Microwave-dried HILM is a valuable ingredient for broiler diets, with up to 25% substitution showing no detrimental effects on growth performance and health. Further studies are needed to improve HILM digestibility.

Keywords: digestibility, protein source, soybean meal, volatile fatty acid

1. Introduction

Soybean meal (SBM) is the main protein source in animal feed, and increasing demand for protein sources (e.g. soybean) has led to competition for resources (Schiavone *et al.*, 2017). Therefore, a sustainable alternative feed source with high protein content and amino acid profiles comparable to that of SBM is required. Insects contain high-quality proteins and are considered as an alternative protein source for poultry (Bovera *et al.*, 2015). Particularly, black soldier fly (*Hermetia illucens*, HI) larvae may be useful as a feed ingredient and can be used as a countermeasure to control increasing organic waste in the future (Surendra *et al.*, 2016). In contrast to their transforming ability from

food waste biomass to valuable feed ingredients, there is a concern in terms of safety such as bioaccumulation of heavy metals in their body from contaminated rearing substrates (Diener *et al.*, 2015; Gasco *et al.*, 2020). Studies have shown that hazardous heavy metal concentrations in animal products such as chicken meats and eggs have no negative effects (Park *et al.*, 2021; Schiavone *et al.*, 2019).

HI larvae contain high levels of protein (35 to 57%) and fat (35%) and HI larvae meal (HILM) has a relatively higher crude protein (CP) content (61.24 vs 45.76%) than SBM (Park *et al.*, 2021; Veldkamp *et al.*, 2012). However, they also have a considerably high chitin content in their exoskeletons (Marono *et al.*, 2017; Nafisah *et al.*, 2019;

Park *et al.*, 2021). Chitin is indigestible and can bind to proteins and decrease their digestibility (Borrelli *et al.*, 2017; Longvah *et al.*, 2011). Although birds naturally consume insects and can hydrolyse chitin in their gut (Tabata *et al.*, 2017), HILM can also negatively affect nutrient digestibility, as poor growth performance was observed when HILM was included in bird feed (Cutrignelli *et al.*, 2018; Dabbou *et al.*, 2018; De Marco *et al.*, 2015; Suzuki *et al.*, 2002). However, chitin may also act as a potential indigestible prebiotic to increase the production of volatile fatty acids (VFAs) and modulate the gut microbiota (Borrelli *et al.*, 2017; Cutrignelli *et al.*, 2018; Kawasaki *et al.*, 2019). Furthermore, chitin has been shown to stimulate an immune response without negatively affecting growth performance (Bovera *et al.*, 2015) and has positive effects on the health of laying hens; a higher globulin value and lower albumin-to-globulin ratio (A/G), which are indicators of better disease resistance, were observed in hens fed a chitin-rich diet (Bovera *et al.*, 2018; Marono *et al.*, 2017).

HILM also contains a higher lipid content than SBM, and fatty acid (FA) profiles are characterised by high and low levels of saturated FAs and unsaturated FAs (UFAs), respectively, in HILM (Bovera *et al.*, 2018; Schiavone *et al.*, 2019). Additionally, it has been reported that the FA profiles of chicken breast meat from HILM-fed broilers showed increased UFA and polyunsaturated FA ratios (Schiavone *et al.*, 2019). HILM has been suggested as a valuable protein source for broilers; however, the FA profile of HILM should also be considered in the context of providing healthy chickens for modern consumers (Schiavone *et al.*, 2019). Therefore, further studies are required to determine the effects of HILM on the growth performance and overall health of broilers in terms of both chitin and FA profiles.

A recent study showed that the drying method affected the structures of proteins in HILM (Huang *et al.*, 2019; Schiavone *et al.*, 2017). It has also been suggested that structural changes in proteins caused by microwave drying can decrease the digestibility of HILM (Huang *et al.*, 2019). However, the microwave drying method has advantages to manage moisture content in the HI larvae and to produce products time efficiently compared to other drying methods (Khodifad *et al.*, 2020; Lenaerts *et al.*, 2018). Nevertheless, the effects of microwave-dried HILM on the growth and health of broilers are not well-understood. Thus, in this study, we examined the effects of microwave-dried HILM on the growth performance and health of broiler chickens. The effects of two different substitution levels (25 and 50%) of SBM by HILM on growth performance, apparent ileal digestibility (AID), caecal VFA production, and blood traits were evaluated.

2. Materials and methods

Insect meal

After hatching (3–4 days; 27 °C), the HI larvae were reared (10–15 days, 27±3 °C) on household food waste. Before manufacturing, the HI larvae were fasted for two days and cleaned to remove waste from their bodies. The cleaned HI larvae were dehydrated and dried using a microwave-drying oven (70–80 °C; 30 min). The dried HI larvae were defatted at 45–48 °C using a cold press oil machine (NF-80; Karaerler, Ankara, Turkey) to preserve nutritional quality from heat. The experimental diets were mixed using the pulverised HI larvae and other feed ingredients.

Birds, diets, and experimental design

This study was approved by the Institutional Animal Care and Use Committee of the Rural Development Administration (no. NIAS-2020-497) and conducted in the poultry facility of the National Institute of Animal Science of South Korea.

A total of 126 male broiler chicks (Ross 308; one-day-old) were randomly assigned to three dietary treatments (six replicates of seven birds per treatment). Three experimental diets were formulated to meet or exceed the nutrient requirements for three phases (NIAS, 2017): starter (days 1–7), grower (days 7–21), and finisher (days 21–35). The control group (CON) was fed a diet based on corn and SBM. In each phase, 25 and 50% of SBM were partially replaced with the HILM (25HILM and 50HILM groups, respectively) in the treated groups; the inclusion levels of the HILM were 7.5, 7, and 6.5% in the 25HILM group and 15, 14, and 13% in the 50HILM group. The feed ingredients and chemical composition of the experimental diets are reported in Table 1. Furthermore, the chemical composition and the FA profile of HILM and SBM were analysed as shown in our previous study (Kim *et al.*, 2021). Feed and water were provided *ad libitum* throughout the trial. An indigestible marker (chromic oxide; Cr₂O₃) was added to all diets at a level of 2.5 g/kg to estimate the AID of nutrients.

Growth performances

The initial body weight (d 1), live body weight, and feed intake were recorded at the end of each growth phase (d 7, 21, and 35). These parameters were used to calculate the average daily gain and average daily feed intake. The feed conversion ration (FCR) was also determined for each growth phase and overall experimental period. Health status and mortality were monitored and recorded daily.

Table 1. Compositions and nutrient levels of the experimental diets (as-fed basis).¹

Item	Starter phase ²			Grower phase			Finisher phase		
	CON	25 HILM	50 HILM	CON	25 HILM	50 HILM	CON	25 HILM	50 HILM
Ingredients, %									
Corn	52.69	57.21	63.15	56.03	60.89	65.72	59.32	63.10	67.86
Soybean meal, 45.8%	30.00	21.50	12.00	28.00	19.00	11.00	26.00	18.50	11.00
HILM	-	7.50	15.00	-	7.00	14.00	-	6.50	13.00
Corn gluten meal	8.00	7.00	6.00	7.00	6.00	5.00	6.00	5.00	4.00
Soybean oil	4.50	3.00	1.00	4.50	3.50	1.50	4.50	3.50	1.50
Dicalcium phosphate	1.95	1.80	1.60	1.80	1.70	1.60	1.60	1.56	1.54
Limestone	1.50	0.70	-	1.40	0.70	-	1.40	0.70	-
L-lysine	0.45	0.41	0.39	0.36	0.32	0.30	0.30	0.28	0.26
DL-methionine	0.16	0.13	0.11	0.16	0.14	0.13	0.13	0.11	0.09
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin-mineral premix ³	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated composition									
ME, kcal/kg	3,119	3,119	3,119	3,149	3,149	3,149	3,177	3,177	3,177
Lysine, %	1.47	1.47	1.47	1.33	1.33	1.33	1.21	1.21	1.21
Methionine, %	0.55	0.55	0.55	0.53	0.53	0.53	0.48	0.48	0.48
Calcium, %	0.97	0.97	0.97	0.91	0.91	0.91	0.85	0.85	0.85
Total phosphorus, %	0.79	0.79	0.79	0.75	0.75	0.75	0.70	0.70	0.70
Analysed composition, %									
Crude protein	23.23	23.67	23.60	21.96	21.76	22.08	19.53	19.97	19.80
NDF	8.67	10.03	11.08	7.90	8.76	9.99	7.35	7.47	7.90
ADF	3.36	4.03	4.38	3.73	3.67	4.02	3.10	3.59	3.74
Ash	8.34	7.50	8.20	6.90	7.46	7.18	7.46	7.43	7.57

¹ ADF = acid detergent fibre; HILM = *Hermetia illucens* larvae meal; ME = metabolisable energy; NDF = neutral detergent fibre.

² CON = control diet; 25HILM and 50HILM = HILM groups in which soybean meal was replaced with 25 and 50% of HILM, respectively.

³ The premix provided following nutrients per kg of diets: vitamin A, 1,600,000 IU; vitamin D₃, 300,000 IU; vitamin E, 800 IU; vitamin K₃, 132 mg; vitamin B₁, 97 mg; vitamin B₂, 500 mg; vitamin B₆, 200 mg; vitamin B₁₂, 1.2 mg; nicotinic acid, 2,000 mg; pantothenic acid, 800 mg; folic acid, 60 mg; choline chloride, 35,000 mg; Mn, 12,000 mg; Zn, 9,000 mg; Fe, 4,000 mg; Cu, 500 mg; I, 250 mg; Co, 100 mg; Se, 50 mg.

Slaughtering procedures

At 35 days of age, three birds were randomly chosen per pen (18 broilers per treatment) based on their final body weights and slaughtered by cutting the carotid artery. The digestive tract was excised, and each segment of the small intestine was identified. The segments were emptied, and their length and weight were recorded to calculate the relative length and weight. The ileal digesta samples from 20 mm after Meckel's diverticulum to 40 mm proximal to the ileocolic junction were gently squeezed and flushed into plastic containers for the digestibility analysis. A pair of ceca was also separated to collect caecal digesta for VFA analysis.

Digestibility trial and calculation

The collected ileal digesta samples from three birds were pooled (six pools per treatment), frozen immediately, and freeze-dried. The dried ileal digesta samples were ground through a 1-mm screen before chemical analysis.

Dry matter (DM), CP, and energy content were analysed in both the diet and ileal digesta samples based on previously described methods for the AID of the DM, CP, and energy (AOAC, 2005). The diets and ileal samples were analysed to determine the DM content. The energy content and CP were measured using bomb calorimetry (Parr 6300; Parr Instrument, Moline, IL, USA) and the Kjeldahl method (VAPO45; Gerhardt Ltd., Königswinter, Germany), respectively. The concentrations of Cr₂O₃ in the diets and ileal samples were determined by graphite furnace atomic absorption spectrometry. The AID of the DM, CP, and energy was calculated using the following equation (Stein

et al., 2007): $100 - 100 \times [(\% \text{ nutrient in the ileal digesta} / \% \text{ nutrient in the diet}) \times (\text{Cr}_2\text{O}_3 \text{ in the diet} / \text{Cr}_2\text{O}_3 \text{ in the ileal digesta})]$.

Volatile fatty acids analysis

At the time of slaughter (day 35), the caecal digesta samples were collected from one bird per pen (six broilers per treatment) and stored at -80 °C until analysis. Approximately 1 g of caecal digesta sample was diluted with 1 ml of distilled water and centrifuged at 5,000×g at 4 °C for 10 min. The supernatant was added to 200 µl of metaphosphoric acid and centrifuged (10 min at 5,000×g). The clear supernatant was used to measure VFA concentrations in the caecal digesta samples by gas chromatography (6890N Agilent Technologies) equipped with a capillary column (15 m × 0.53 mm × 0.5 µm; Supelco, Inc., Bellefonte, PA, USA).

Blood analysis

Before slaughter (day 35), blood samples were collected from the wing vein of two birds per pen (12 broilers per treatment) and placed in EDTA tubes and serum-separating tubes. The serum samples were separated by centrifugation at 1,800×g for 15 min and stored at -20 °C until analysis. Glucose, total protein, albumin, globulin, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), phosphorus, calcium, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, total bilirubin, blood urea nitrogen, creatinine, and creatine phosphokinase (CPK) were measured using an automatic biochemical analyser (Catalyst Dx, IDEXX Labs, Inc., Westbrook, ME, USA). The A/G was then calculated. EDTA-anticoagulated blood samples were analysed for blood cell count (white and red blood cells), haemoglobin, haematocrit, and red blood cell distribution width-coefficient of variation (RDW-CV) and -standard deviation (RDW-SD) using an automatic blood analyser (BC-5300Vet, Mindray, Hamburg, Germany).

Statistical analysis

All data were processed using the GLM procedure of SAS version 9.4 (SAS, Inc., Cary, NC, USA). For growth performance, the experimental unit was the pen; for AID, the experimental unit was a pooled ileal digesta sample from three birds; for VFA concentrations and blood parameters, the experimental unit was an individual bird. Statistical differences among treatments were determined using Tukey’s multiple comparison test using the SAS software (SAS, 2009). Data are reported as mean ± standard error of the mean. Significance and tendency were considered at $P<0.05$ and $0.05<P<0.10$, respectively.

3. Results

Growth performance

The growth performance of the broilers is summarised in Table 2. The initial body weight did not differ ($P>0.05$) following the different dietary treatments. The final body weight, average daily feed intake, and average daily gain decreased ($P<0.0001$) in the 50HILM group compared with those in the CON and 25HILM groups. The FCR tended to increase ($P=0.0615$) based on the inclusion level of HILM.

Ileal digestibility

As presented in Table 3, the AID of CP was lower ($P<0.05$) in the 50HILM group than in the CON group. However, the AID of DM and energy was not affected ($P>0.05$) by dietary HILM.

Relative length and weight of small intestine

The results of the relative length and weight of the small intestine are shown in Table 4. The relative length of the entire digestive tract significantly ($P<0.01$) increased in the 50HILM group compared with that in the CON and 25HILM groups. The relative length of the ileum was significantly ($P<0.05$) different in the 50HILM group compared with that in the CON and 25HILM groups. Moreover, the relative length of the duodenum ($P=0.0510$) and jejunum ($P=0.0593$) tended to increase with HILM inclusion. The relative weight of the entire digestive tract was not affected by HILM. However, the relative weight of the duodenum significantly ($P<0.05$) increased in the 25HILM group compared with that in the CON group.

Table 2. Effect of dietary *Hermetia illucens* larvae meal (HILM) inclusion level on the growth performance of broiler chickens.^{1,2}

Item	Dietary treatment ³			SEM	P-value
	CON	25HILM	50HILM		
IBW (g, d 1)	42.74	43.24	42.81	0.26	0.4011
FBW (g, d 35)	1,700.37 ^a	1,619.50 ^a	1,368.80 ^b	36.01	<0.0001
ADFI (g)	70.30 ^a	70.69 ^a	62.07 ^b	0.89	<0.0001
ADG (g)	47.36 ^a	45.04 ^a	37.89 ^b	1.03	<0.0001
FCR (g/g)	1.49	1.57	1.65	0.05	0.0615

¹ ADFI = average daily feed intake; ADG = average daily gain; FBW = final body weight; FCR = feed conversion ratio; IBW = initial body weight; SEM = standard error of the mean.

² Values with different superscripts in the same row are significantly different ($P<0.01$).

³ CON = control diet; 25HILM and 50HILM = HILM groups in which soybean meal was replaced with 25 and 50% of HILM, respectively.

Table 3. Apparent ileal digestibility of nutrients in broilers fed diets supplemented with *Hermetia illucens* larvae meal (HILM).¹

Item	Dietary treatment ²			SEM ³	P-value
	CON	25HILM	50HILM		
Dry matter (%)	61.89	55.29	49.84	5.30	0.437
Crude protein (%)	70.87 ^a	60.46 ^{ab}	51.38 ^b	3.85	0.040
Energy (%)	65.89	58.37	52.51	5.37	0.375

¹ Values with different superscripts in the same row are significantly different ($P<0.05$).

² CON = control diet; 25HILM and 50HILM = HILM groups in which soybean meal was replaced with 25 and 50% of HILM, respectively.

³ SEM, standard error of the mean.

VFA concentration in caecal digesta

The concentrations of VFAs in the caecal digesta content are presented in Table 5. The absolute concentrations of all VFAs, except for those of butyrate, increased ($P<0.05$) in the 50HILM group compared with those in the CON and 25HILM groups. Moreover, short-chain FAs (SCFAs) and branched-chain FAs (BCFAs) were higher ($P<0.05$) in the 50HILM group than in the CON and 25HILM groups. The relative concentrations of propionate, isobutyrate, valerate, and isovalerate in the 50HILM group significantly increased ($P<0.05$), but the concentration of butyrate decreased ($P<0.05$) with the inclusion of HILM.

Serum parameters and haematological traits

The results of the serum parameters are shown in Tables 6 and 7. Dietary HILM did not significantly affect serum parameters related to glucose and proteins (Table 6). The serum LDL-C level was lower, whereas the TG level was higher in the 50HILM group ($P<0.05$ and <0.01 , respectively) than in the CON group. The serum parameters of liver function, including aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, and total bilirubin, were not affected by HILM (Table 7). The serum phosphorus level was lower ($P<0.01$) in both HILM groups than in the CON group. The CPK activity was the lowest ($P<0.01$) in the 50HILM group among the other groups.

Haematological traits in broiler chickens are shown in Table 8. Monocyte levels in the blood samples were higher ($P<0.05$) in the 50HILM group than in the other groups. RDW-CV tended to be higher ($P=0.0790$) in the 50HILM group and RDW-SD was higher ($P<0.05$) in the 50HILM group than in the CON group.

4. Discussion

As shown in our previous study, the DM and ether extract (EE) of the microwave-dried HILM were 98.53 and 6.16%, respectively (Kim *et al.*, 2021). According to Schiavone *et al.* (2017), DM and EE of partially defatted HILM were 94.2 and 18%, on the contrary, highly defatted HILM contains 98.5% of DM and 4.6% of EE. Therefore, the HILM that was used in this study can be considered as highly defatted

Table 4. Effects of dietary *Hermetia illucens* larvae meal (HILM) inclusion level on the relative length and weight of the small intestine in broiler chickens at 35 d of age.¹

Item	Dietary treatment ²			SEM ³	P-value
	CON	25HILM	50HILM		
Relative length (cm/kg LW)					
Entire digestive tract	97.24 ^b	94.79 ^b	110.58 ^a	3.36	0.0093
Duodenum	14.79	15.95	18.19	0.85	0.0510
Jejunum	42.58	38.85	44.26	1.50	0.0593
Ileum	39.86 ^b	39.99 ^b	48.12 ^a	1.87	0.0105
Relative weight (g/kg LW)					
Entire digestive tract	21.68	22.31	23.93	1.13	0.4385
Duodenum	4.23 ^b	5.41 ^a	4.68 ^{ab}	0.29	0.0372
Jejunum	9.87	9.33	10.15	0.61	0.6549
Ileum	7.58	7.57	9.10	0.52	0.1023

¹ Values with different superscripts in the same row are significantly different ($P<0.05$).

² CON = control diet; 25HILM and 50HILM = HILM groups in which soybean meal was replaced with 25 and 50% of HILM, respectively.

³ SEM, standard error of the mean.

Table 5. Volatile fatty acid (VFA) levels in caecal contents of broiler chickens fed *Hermetia illucens* larvae meal (HILM).^{1,2}

Parameter	Dietary treatment ³			SEM	P-value
	CON	25HILM	50HILM		
Absolute value (mmol/g)					
Acetate	75.42 ^b	82.48 ^b	111.25 ^a	7.18	0.0194
Propionate	6.55 ^b	5.76 ^b	13.84 ^a	0.92	0.0002
Butyrate	20.10	21.16	21.57	2.28	0.9236
Isobutyrate	0.55 ^b	0.48 ^b	1.47 ^a	0.12	0.0003
Valerate	1.10 ^b	1.41 ^b	2.73 ^a	0.26	0.0041
Isovalerate	0.67 ^b	0.45 ^b	1.66 ^a	0.20	0.0032
BCFA ⁴	2.31 ^b	2.26 ^b	5.57 ^a	0.52	0.0009
Total SCFA ⁵	104.39 ^b	111.56 ^b	152.53 ^a	9.75	0.0181
Relative value (% of total VFAs)					
Acetate	72.13	73.77	73.02	0.77	0.4310
Propionate	6.21 ^{ab}	5.05 ^b	9.17 ^a	0.77	0.0108
Butyrate	19.43 ^a	19.20 ^a	14.01 ^b	1.38	0.0450
Isobutyrate	0.52 ^b	0.43 ^b	0.96 ^a	0.07	0.0013
Valerate	1.05 ^b	1.21 ^{ab}	1.76 ^a	0.14	0.0207
Isovalerate	0.65 ^{ab}	0.40 ^b	1.08 ^a	0.16	0.0306

¹ Values with different superscripts in the same row are significantly different ($P<0.05$).
² BCFA = branched-chain fatty acid; SCFA = short-chain fatty acids; SEM = standard error of the mean.
³ CON = control diet; 25HILM and 50HILM = HILM groups in which soybean meal was replaced with 25 and 50% of HILM, respectively.
⁴ BCFAs were isobutyrate, valerate, and isovalerate.
⁵ Total SCFAs were acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate.

larvae meal. It has also been reported that the partially and highly defatted HILM can be a suitable feed ingredient in broiler diets (Schiavone *et al.*, 2017). However, they suggested that the highly defatted HILM has advantages of nutritional composition and storage time due to its higher protein and lower EE content than partially defatted HILM (Schiavone *et al.*, 2017). In this study, the microwave-dried HILM contained 98.53% DM, 61.24% CP, and 6.16% EE. Hence, the microwave-dried HILM can be a suitable feed ingredient in broiler diets; therefore, in this study, we evaluated the microwave-dried HILM as a dietary protein source for broilers.

In the present study, feeding of HILM negatively affected the growth performance of broilers; however, a low inclusion level of HILM may be suitable. Dabbou *et al.* (2018) also reported that increasing the level of HILM in broiler diets resulted in a linear decrease in growth performance and that the inclusion of 15% HILM had negative effects on the

Table 6. Effect of dietary *Hermetia illucens* larvae meal (HILM) inclusion level on the serum glucose, proteins, and lipids of broiler chickens.^{1,2}

Parameter	Dietary treatment ³			SEM	P-value
	CON	25HILM	50HILM		
Glucose (mg/dl)	268.40	271.38	278.50	9.23	0.7499
Proteins					
Total proteins (g/dl)	2.75	2.60	2.64	0.12	0.7224
Albumin (g/dl)	1.35	1.30	1.31	0.08	0.9187
Globulin (g/dl)	1.40	1.30	1.33	0.06	0.5332
A/G	1.05	1.00	1.11	0.12	0.8539
Lipids					
Cholesterol (mg/dl)	144.70	134.63	139.25	7.25	0.6988
HDL-C (mg/dl)	115.10	107.00	105.50	4.15	0.2813
LDL-C (mg/dl)	29.00 ^a	12.00 ^{ab}	10.50 ^b	4.30	0.0161
TG (mg/dl)	27.80 ^b	36.13 ^{ab}	54.33 ^a	5.19	0.0058

¹ Values with different superscripts in the same row are significantly different ($P<0.05$).
² A/G = albumin-to-globulin ratio; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SEM = standard error of the mean; TG = triglycerides.
³ CON = control diet; 25HILM and 50HILM = HILM groups in which soybean meal was replaced with 25 and 50% of HILM, respectively.

FCR and intestinal morphology. They suggested that a low inclusion level of HILM could improve body weight and feed intake during the starter period (Dabbou *et al.*, 2018). We observed that the group with a low substitution level (25%) of HILM presented a higher average daily gain than the CON group during the first week of the experiment (data not shown). However, the CON group showed better growth performance during the growing and finishing periods. This low growth of HILM-fed broilers may be explained by the low digestibility of nutrients due to the presence of chitin in HILM.

Chitin, a component of the insect exoskeleton, can decrease the digestibility of DM by decreasing the digestibility of protein (Bovera *et al.*, 2018; De Marco *et al.*, 2015). The negative effect of HILM on intestinal morphology may also contribute to the lowering of protein digestibility (Dabbou *et al.*, 2018). Here, the chitin intake by broilers fed HILM during the experimental period was estimated using the following equation (Marono *et al.*, 2015): chitin (%) = ash-free ADF (%) – ADF-linked protein (%). The calculated amount of chitin in the HILM in the present study was 5.70%, and the 25HILM and 50HILM groups ingested approximately 0.28 and 0.50 g/d chitin, respectively, considering the feed intake and inclusion levels of HILM in the diets. Bovera *et al.* (2018) also reported that laying hens

Table 7. Effect of dietary *Hermetia illucens* larvae meal (HILM) inclusion level on the electrolytes, liver, renal, and muscle function of the broiler chickens.^{1,2}

Parameter	Dietary treatment ³			SEM	P-value
	CON	25HILM	50HILM		
Electrolytes					
Phosphorus (U/l)	6.98 ^a	3.06 ^b	3.57 ^b	0.79	0.0079
Calcium (mg/dl)	9.17	9.09	9.10	0.93	0.9982
Liver function					
AST (U/l)	189.40	182.63	182.75	9.34	0.8675
ALT (U/l)	1.00	0.50	0.92	0.21	0.3374
GGT (U/l)	15.60	12.50	12.00	1.69	0.3323
ALP (U/l)	1,298.50	1,323.63	1,500.17	103.22	0.3697
Total bilirubin (mg/dl)	0.03	0.04	0.03	0.01	0.5272
Renal and muscle function					
BUN (mg/dl)	0.72	0.51	0.71	0.09	0.3464
Creatinine (mg/dl)	0.27	0.18	0.25	0.05	0.5567
CPK (U/l)	4,575.70 ^a	3,005.39 ^{ab}	1,800.81 ^b	541.92	0.0072

¹ Values with different superscripts in the same row are significantly different ($P < 0.01$).

² ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CPK = creatine phosphokinase; GGT = gamma-glutamyl transferase; SEM = standard error of the mean.

³ CON = control diet; 25HILM and 50HILM = HILM groups in which soybean meal was replaced with 25 and 50% of HILM, respectively.

Table 8. Effects of dietary *Hermetia illucens* larvae meal (HILM) inclusion level on the haematological traits of broiler chickens.^{1,2}

Parameter	Dietary treatment ³			SEM	P-value
	CON	25HILM	50HILM		
WBC ($\times 10^9/l$)	21.53	17.87	18.34	1.56	0.2556
Heterophils (%)	32.88	25.65	29.98	2.63	0.2625
Lymphocytes (%)	49.07	58.02	48.48	3.75	0.2643
Monocytes (%)	12.66 ^b	11.67 ^b	16.41 ^a	1.03	0.0148
Eosinophils (%)	5.27	4.48	4.97	0.64	0.7553
Basophils (%)	0.12	0.18	0.16	0.06	0.8113
H/L	0.79	0.46	0.66	0.11	0.1858
RBC ($\times 10^{12}/l$)	2.43	2.19	2.11	0.11	0.1048
Haemoglobin (g/l)	12.17	11.12	10.71	0.56	0.1902
Haematocrit (%)	32.50	29.73	29.36	1.53	0.3210
RDW-CV	8.69	8.63	9.09	0.14	0.0790
RDW-SD	48.58 ^b	49.23 ^{ab}	52.50 ^a	1.03	0.0317

¹ Values with different superscripts in the same row are significantly different ($P < 0.05$).

² H/L = heterophils to lymphocytes ratio; RBC = red blood cells; RDW-SD = red blood cell distribution width-coefficient of variation; RDW-SD = red blood cell distribution width-standard deviation; SEM = standard error of the mean; WBC = white blood cells.

³ CON = control diet; 25HILM and 50HILM = HILM groups in which soybean meal was replaced with 25 and 50% of HILM, respectively.

(16 weeks old) that ingested approximately 0.47 g/d chitin showed lower protein digestibility than those fed SBM-based diets. Furthermore, we focused on structural changes in the manufacturing process of HILM. Although previous studies did not report the method of HILM manufacture, the HILM used in this study was microwave-dried. The microwave drying method makes HILM more compact via polymerisation of the protein particles but decreases protein digestibility compared with HILM manufactured using conventional drying methods (Huang *et al.*, 2019). Thus, the AID of the protein was substantially decreased by the substitution of HILM. Furthermore, the reduced AID of DM and energy (-19.47 and -20.31%, respectively) in the 50HILM group compared with that in the CON group was can be ascribed to strongly reduced protein digestibility (-27.50%).

Decreased nutrient digestibility is also related to the length and weight of the digestive intestinal tract. As the ileum length increased, the relative length of the entire small intestinal tract increased in the 50HILM group. An increase in the ileum length of broilers and laying hens fed insect meals has been reported previously (Bovera *et al.*, 2016, 2018). These results are typically ascribed to the effects of undigestible substances (Borin *et al.*, 2006). However, in this study, the compensative effect for increasing nutrient absorption via elongation of the ileum in HILM-fed broilers appeared to be greater than the peristaltic activity caused by chitin (Borin *et al.*, 2006; Bovera *et al.*, 2018). Moreover,

the elongation effect of the intestinal tract by HILM seems to be greater in the early growth stage than in other stages (Bovera *et al.*, 2018).

HILM-fed broilers showed higher production of VFAs in the caecal content caused by chitin. Chitin has been suggested as a potential prebiotic substance because it is undegradable and unabsorbable (Bovera *et al.*, 2015). In the present study, 50HILM treatment increased the absolute value of total VFAs, except that of butyrate. However, 25HILM treatment did not increase the total SCFA production, which agrees with the findings of Moniello *et al.* (2019). Thus, chitin intake was not sufficient to have a prebiotic effect. Unlike previous study results, the propionate level was also increased in the 50HILM group (Cuttrignelli *et al.*, 2018; Moniello *et al.*, 2019). Kim *et al.* (2020) reported that black soldier fly larvae oil mostly affected propionate level. This result was ascribed to the inhibitory activities of medium-chain FAs (MCFAs), which are present in high quantities in black soldier fly larvae oil. Here, the analysis of the FA profile of HILM revealed a high percentage of MCFAs. Therefore, the difference in the FA profiles of the ingredients (HILM vs SBM) may have affected the propionate level.

Increased VFA levels are also related to feed intake via the control of satiety and hunger, as it has been reported that elevated propionate levels reduce eating behaviour by affecting satiety through brain striatal pathways (Bedford *et al.*, 2020; Byrne *et al.*, 2016). Therefore, in the present study, the decreased feed intake may have been affected by the elevated caecal propionate level; infusion of propionate altered the feeding behaviour and decreased energy intake, supporting this prediction (Oba and Allen, 2003).

Although an increase in the total SCFA levels in HILM-fed broilers indicates an improvement in the gut health, a concern is that the relative value of BCFAs (isobutyrate, valerate, and isovalerate) also increased. This increase can be promoted by protein fermentation in the gut, generating protein fermentation products (e.g. ammonia, amines, and phenol) (Diether and Willing, 2019; Gilbert *et al.*, 2018; Rios-Covian *et al.*, 2020). Undigested protein and amino acids caused by poor ileal digestibility may also promote fermentation in the cecum, which could decrease the relative value of butyrate (Russell *et al.*, 2011). Increased propionate and decreased butyrate levels could also be ascribed to the flux of butyrate to propionate by decreased feed intake (Bedford *et al.*, 2020).

The effects of chitin and different FA profiles of HILM and SBM on blood traits were investigated by examining the serum protein and lipid levels. It has been shown that the prebiotic effect of chitin lowers A/G, which indicates a better immune response and disease resistance (Bovera *et al.*, 2015; Marono *et al.*, 2017). However, in the present

study, the A/G was not altered, because of the lower chitin intake by broilers, in contrast to the results of a previous study (Marono *et al.*, 2017). Commercial chitin has also been reported to decrease lipid absorption by binding to lipids; therefore, it lowers plasma cholesterol (El-Gobary *et al.*, 2016). Moreover, the effects of HILM in terms of lowering serum cholesterol and TG levels in laying hens have been attributed to chitin (Bovera *et al.*, 2018; Marono *et al.*, 2017). However, here, we observed that the TG level was increased by HILM. Although the reason for this is not clear, the high level of MCFAs in HILM may have affected serum lipids such as LDL-C and TG, because of lower chitin ingestion by broilers in our study than by laying hens in their studies. MCFAs have a smaller molecular size and hence have a lower weight than long-chain FAs, facilitating rapid absorption into the portal vein (Baltić *et al.*, 2017; Hanczakowska, 2017). This characteristic may lead to a decrease in the level of LDL-C, and several previous studies have shown that MCFA decreased LDL-C and increased TG (Baltić *et al.*, 2019; Hill *et al.*, 1990; Sung *et al.*, 2018). Here, the relative weight of the duodenum in the 25HILM group was increased, possibly because of its higher relative length compared with that in the CON group. However, the cause of this increased relative weight may be the proliferation of the intestinal mucosa because of the fast-absorbing property of MCFAs in HILM (Zeitz *et al.*, 2015).

Clinical signs in chickens were not observed during the experiment; they were verified by the serum parameters that indicated liver, renal, and muscle functions. However, phosphorus was decreased in HILM-fed broilers, in contrast to previous study result, that is, phosphorous showed high bioavailability and was obtained from insects (Dabbou *et al.*, 2018). It has been reported that chitosan, which is a deacetylated form of chitin, reduced serum phosphorus in rats (Baxter *et al.*, 2000). According to Pap *et al.* (2020), chitosan can bind to phosphorus via its amine group ($-NH_2$), and the similar structure of chitin with chitosan may explain the observed decrease in serum phosphorus. The CPK level decreased in the 50HILM group, which may be ascribed to the low muscle mass caused by the lower final body weight of HILM-fed broilers (Garcia and Creatine, 1974; Yen *et al.*, 2017).

Information regarding the effect of HILM on RDW in broilers is limited. We observed that RDW-CV and RDW-SD were slightly and significantly increased, respectively, with the inclusion level of HILM. The increased RDW level has been reported to be related to the size of red blood cells, which may be linked to problems such as dysfunction, malnutrition, and chronic liver disease (Reddy *et al.*, 2018). The reason for the significantly higher levels of monocytes in the blood of HILM-fed broilers is not completely clear. Additionally, there was no difference in monocyte levels in HILM-fed laying hens compared with those in SBM-fed laying hens in a previous study (Marono

et al., 2017). However, it has been reported that protein energy malnutrition increases blood monocyte levels in mice (Corware *et al.*, 2014). Therefore, malnutrition caused by low nutrient digestibility in the 50HILM group may have increased the monocyte and RDW-SD levels.

5. Conclusions

We determined the effects of microwave-dried HILM, in terms of chitin content and FA profiles, on the growth performance and health of broilers. HILM substitution in broiler diets showed positive effects on the SCFA concentrations. Additionally, HILM affected serum lipid parameters; a higher MCFA content was observed in broilers fed HILM than those fed SBM. However, poor results in terms of growth performance and digestibility in HILM-fed broilers suggest that the protein source in HILM was polymerised during drying and that undigested protein affected the BCFA concentrations in the caecal digesta. Furthermore, the analysis of the haematological traits suggested that HILM-fed broilers suffered from malnutrition due to the low digestibility of nutrients. Therefore, further studies to overcome the limitations of microwave-dried HILM and improve protein digestibility by improving the manufacturing process (e.g. control of energy output and drying time) are needed.

Acknowledgements

This research was supported by the 'Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01456002)' and the 2021 RDA Fellowship Program of the National Institute of Animal Science, Rural Development Administration, Republic of Korea. We gratefully acknowledge all team members who helped either directly or indirectly when conducting this study.

Conflict of interest

The authors declare no conflict of interest.

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