INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–1457/2021/25–6–1197–1202
DOI: 10.17957/IJAB/15.1780
http://www.fspublishers.org

Full Length Article

Dietary Replacement with Food Waste and Black Soldier Fly Larvae Supplementation Improved Growth Performance, Nutrient Digestibility and Intestinal Microbial Population in Broilers

Mohammed Al-Qazzaz[1†], Anjas Asmara Samsudin[2], Lokman Hakim Idris[3], Dahlan Ismail[2] and Henny Akit[2,4*]
[1]*Animal Resources Department, Faculty of Agriculture, University of Baghdad, Baghdad, Iraq*
[2]*Animal Science Department, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Department of Veterinary Pre-Clinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[4]*Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[*]For correspondence: henny@upm.edu.my; mohfa77@hotmail.com
[†]Contributed equally to this work and are co-first authors
Received 13 October 2020; Accepted 05 January 2021; Published 10 May 2021

Abstract

This study was aimed to evaluate the effect of conventional ingredients replacement with alternative ingredients on growth performance, carcass quality, nutrient digestibility and intestinal microbial of broilers. One hundred twenty Cobb500 broiler chicks were randomly assigned to four diets. Corn, soybean meal and fish meal were replaced with rice waste, meat and bone waste and black soldier fly larvae (BSFL) at 0, 10, 30 and 50% to form four treatments. Body weight gain, feed conversion ratio and digestibility of crude protein and fat were improved in broilers fed the replacement diets. Feed intake was not affected by the treatments suggesting that the replacement diets were well accepted by the chicken. *Escherichia coli* was decreased in the cecum and *Lactobacillus* were increased in the intestines of broilers fed the replacement diets. The fiber and chitin contents in the replacement diets may alter intestinal bacterial fermentation leading to improved nutrient digestibility. However, abdominal fat percentage increased in broilers fed the replacement diets. In conclusion, conventional ingredients can be replaced with up to 50% rice waste, meat and bone waste and BSFL in the diets with promising effect on growth performance, nutrient digestibility and intestinal microbial populations. © 2021 Friends Science Publishers

Keywords: Black soldier fly larvae; Broiler; Food waste; Microbial population; Nutrient digestibility

Introduction

Food waste is disposed in landfills and decomposition of food waste in landfills contributes to the greenhouse gas emission. Utilization of food waste as part of the chicken diet can divert food waste from landfills and offer viable solution to mitigate its negative impact on the environment. In the current study, food waste is defined as leftover food not consumed by consumers sourced from restaurants (Parfitt *et al.* 2010). Depending on the demographic and consumer's eating behavior, the leftover typically consists of meat and bone, rice, vegetables, fruits, meat trimmings and grease. Food wastes have adequate but highly variable nutritional values depending on the sourced leftovers; dry matter (85.3 to 91.6%), crude protein (14.4 to 28.3%) and ether extract (9.1 to 31.5%). The fiber content can vary from 3.7 to 14.5% (Chen *et al.* 2007, 2015; Nadia *et al.* 2016) depending on the content of fruit and vegetables in the leftovers. The growth performance of chickens improved with inclusion of black soldier fly larvae (BSFL) in the diets (Oluokun 2000; Marono *et al.* 2017). The exoskeleton of BSFL contains chitin that could promote diverse gut bacterial communities, improving the gut health of hens (Borrelli *et al.* 2017). Owing to the properties of BSFL, we hypothesized that the dietary combinations of food wastes and BSFL could improve the growth performance of broilers. To test the hypothesis, corn, soybean meal and fish meal were replaced with rice waste, meat and bone waste as well as BSFL, respectively. The aim of the study was to evaluate the effect of replacing conventional feed ingredients with alternative ingredients on growth performance, nutrient digestibility, carcass quality and intestinal microbial population of broilers.

Materials and Methods

Animals and diets

The approved protocol of the Institutional Animal Care and Use Committee of the Universiti Putra Malaysia

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To cite this paper: Al-Qazzaz M, AA Samsudin, LH Idris, D Ismail, H Akit (2021). Dietary replacement with food waste and black soldier fly larvae supplementation improved growth performance, nutrient digestibility and intestinal microbial population in broilers. *Intl J Agric Biol* 25:1197–1202

(UPM/IACUC/AUP-R053/2017) was applied in all animal handling procedures. Food wastes were collected and pooled daily from the restaurants around Serdang, Malaysia. The food wastes were then separated into two parts. One part contained rice waste as an energy source, while the other part contained meat and bone waste as a protein source. Then, the food wastes were processed daily by washing using tap water, followed by immersing in hot water at 90˚C for 10 min and then oven-drying at 60˚C for two days (Hossein and Dahlan 2015). Live BSFL younger than seven days old were oven-dried at 60˚C for five days. A total of 120 Cobb 500 female broiler chicks (one day old) were randomly allocated to four treatment groups with six replicates per treatment (five birds per replicate). The control treatment (T1) consisted of corn, soybean meal and fish meal with 0% replacement with alternative ingredients. The second treatment (T2) consisted of corn replaced with 10% rice waste, soybean meal replaced with 10% meat and bone waste and fish meal replaced with 10% BSFL. The third treatment (T3) contained conventional ingredients replaced with 30% alternative ingredients and the fourth treatment (T4) contained conventional ingredients replaced with 50% alternative ingredients. The experimental diets were formulated to be isonitrogenous and isocaloric across treatments (Table 1). Feed and water were provided at *ad libitum*. Vaccinations against Newcastle and Gumboro diseases were administered to the birds at 7 and 21 days old.

Growth performance and carcass quality

Body weight gain and feed intake of the birds were measured weekly and subsequently, feed conversion ratio (FCR) was calculated. Two birds from each replicate were randomly selected and slaughtered on 21 and 42 days of age. Dressing percentage was determined by dividing the carcass weight over the live weight. *Pectoralis major* muscle (breast) percentage was calculated by dividing a percentage of the breast weight over the live weight. Abdominal fat percentage was calculated by dividing a percentage of the abdominal fat weight over the live weight.

Nutrient digestibility

Titanium dioxide (TiO_2) was added to all diets as an indigestible marker at 5 g/kg, four days prior to slaughtering. Digesta samples were pooled from the ileum (Merckel's diverticulum to the ileal-cecal junction), then oven-dried at 60˚C for two days prior to storing at -20˚C. Proximate analysis of dry matter, crude protein, ash, ether extract, crude fiber, gross energy and TiO_2 were conducted on the homogenized samples of the diets and ileal digesta (AOAC 2005). Apparent nutrient digestibility was determined using the following equation;

$$\text{Apparent nutrient digestibility (\%)} = 100 - [100 \times (\%\ TiO_2 \text{ in feed}/\%\ TiO_2 \text{ in digesta}) \times (\%\text{ nutrient in digesta}/\%\text{nutrient in feed})]\times 100.$$

Quantification of the bacterial populations in the ileum and caecum

On day 42, eight birds from each treatment were slaughtered for collection of digesta samples from the cecum and ileum prior to storing at -20˚C. The samples were then subjected to DNA extraction using the QIAamp® Fast DNA Stool Mini kit (Qiagen, Valencia, CA, U.S.A.). A total of 200 mg sample was collected in a microcentrifuge tube that was placed on ice. Then, 1 mL InhibitEX Buffer was added to the sample and suspended by vortex for 1 min. The samples were subjected to follow the manufacturer's instructions.

Quantitative real time-polymerase chain reaction (RT-PCR)

Specific primers (10 ng/mL concentration) of different bacterial populations were used for bacterial quantification (Table 2). The amplification reactions were conducted using BioRad CFX96 Touch® (BioRad, Hercules, C.A., U.S.A.). The extracted DNA of the samples was used as a template in the PCR assay. The reaction volume was 25 μL, consisting of 1 μL of DNA, 12.5 SYBR Green, 1 μL forward primer, 1 μL reverse primer, and 9.5 μL RNaes-free water. The amplification conditions were set at 94°C for 5 min, followed by 40 cycles of 94°C × 20 s, primer annealing at 58, 50, 60 and 50°C × 30 s for *Lactobacillus*, *Enterococcus*, *Bifidobacterium* and *Escherichia coli*, respectively, which was then extended to 72°C × 20 s.

Statistical analysis

One-way ANOVA analysis was applied using the GLM procedure of SAS software (SAS Institute Inc., Cary, NC, USA) for all data. The differences among treatments were determined using the Duncan's new multiple range test, and the significant differences among treatment means were determined at $P < 0.05$.

Results

Growth performance and carcass quality

The effect of replacement diets on the growth performance of broilers is shown in Table 3. In the starter period, dietary treatments had no effect on the feed intake ($P > 0.05$). Body weight gain was higher in birds fed the replacement diets than the control diet ($P < 0.01$). Body weight was the highest in the birds fed 30 and 50% replacement diets ($P < 0.05$). Feed conversion ratio was the lowest in the birds fed 10 and 30% replacement diets ($P < 0.05$). In the finisher period, treatments had no effect on the feed intake and live weight ($P > 0.05$, respectively). Body weight gain was the highest in the birds fed 30 and 50% replacement diets ($P < 0.05$) and FCR was the lowest in the 10 and 30% replacement diet groups ($P < 0.05$). In the

Table 1: Composition of experimental diets

Diet composition (%)	Starter (1–21 days)				Grower (22–42 days)			
	T1	T2	T3	T4	T1	T2	T3	T4
Corn	55.60	51.01	43.50	37.90	57.40	52.88	45.46	39.63
Rice waste[a]	0	5.10	13.05	18.95	0	5.29	13.64	19.82
Soybean meal	28.90	25.45	21.58	19.10	25.60	21.30	16.69	14.70
Meat & bone waste[b]	0	2.55	6.47	9.55	0	2.13	5.01	7.35
Fish meal	4	4	3	2	3	4	4	3
Black soldier fly larvae[c]	0	0.4	0.9	1.0	0	0.4	1.2	1.5
Rice bran	1	1	1	1	1	1	1	1
Palm kernel cake	1	1	1	1	3	3	3	3
Methionine	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Lysine	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Palm oil	5	5	5	5	5	5	5	5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Limestone	2.8	2.8	2.8	2.80	3.5	3.5	3.5	3.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin-mineral premix[d]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calculated chemical composition								
Crude protein (g/kg)	19.90	20.04	20.22	20.32	18.36	18.47	18.57	18.63
Metabolizable energy (kcal/kg)	3066.0	3086.0	3109.5	3130.9	3058.6	3081.3	3095.6	3103.7
Calcium (g/kg)	1.53	1.56	1.55	1.53	1.74	1.81	1.84	1.82
Phosphors (g/kg)	0.71	0.66	0.58	0.53	0.71	0.66	0.57	0.52
Methionine (g/kg)	0.56	0.57	0.58	0.57	0.47	0.48	0.50	0.50
Lysine (g/kg)	1.29	1.29	1.28	1.26	1.11	1.12	1.12	1.11
Ash (g/kg)	90.97	90.71	90.20	90.98	91.32	90.65	91.60	92.81
Fat (g/kg)	7.54	7.55	8.20	8.95	7.59	7.42	8.62	9.05
Crude fiber (g/kg)	2.76	2.49	2.52	1.66	2.21	2.14	1.88	1.94

T1: control diet. T2: replacement of conventional ingredients with 10% alternative ingredients. T3: replacement of conventional ingredients with 30% alternative ingredients. T4: replacement of conventional ingredients with 50% alternative ingredients.

[a] Rice waste replaced corn at 0% (T1), 10% (T2), 30% (T3) and 50% (T4).

[b] Meat & bone waste replaced soybean meal at 0% (T1), 10% (T2), 30% (T3) and 50% (T4).

[c] Black soldier fly larvae replaced fish meal at 0% (T1), 10% (T2), 30% (T3) and 50% (T4).

[d] Vitamin-mineral premix supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8000 IU;
vitamin B1, 2 mg; vitamin B2, 5 mg; vitamin B6, 2 mg; vitamin B12, 0.01 mg; niacin, 30 mg; vitamin C, 50 mg; d-pantothenate, 8 mg, folic acid, 0.5 mg; vitamin D3 (cholecalciferol), 1000 IU; D-biotin, 0.045 mg; vitamin E (DL-a-tocopherol), 30 IU; vitamin K3 (menadione dimethylpyrimidinol), 2.5 mg; ;

Table 2: Primer sequences used for quantitative real-time polymerase chain reactions

Reference	Organism	Amplicon (base pairs)	Primer sequence (5’-3’)[a]
Wang *et al*. (1996)	*Lactobacillus*	341	F: CATCCAGTGCAAACCTAAGAG R: GATCCGCTTGCCTTCGCA
Frahm and Obst (2003)	*Escherichia coli*	82	F: GTGTGATATCTACCCGCTTCGC R: GAACGCTTTGTGGTTAATCAGGA
Rinttilä *et al*. (2004)	*Enterococcus*	144	F: CCCTTATTGTTAGTTGCCATCATT R: ACTCGTTGTACTTCCCATTGT
Bartosch *et al*. (2004)	*Bifidobacterium*	440	F: GGGTGGTAATGCCGGATG R: TAAGCCATGGACTTTCACACC

[a] F: forward; R: reverse

overall period, treatments had no effect on the feed intake and live weight ($P > 0.05$, respectively). Body weight gain was the highest in the birds fed 30 and 50% replacement diets ($P < 0.01$) and FCR was the lowest in the replacement diet groups ($P < 0.05$). The effect of replacement diets on the carcass quality is shown in Table 4. Dietary treatments had no effect on the carcass weight, dressing percentage and breast muscle percentage ($P > 0.05$, respectively). Abdominal fat weight was higher in the birds fed 30 and 50% replacement diets than the control diet ($P < 0.05$).

Nutrient digestibility

The effect of replacement diets on nutrient digestibility is shown in Table 5. Birds fed the replacement diets showed higher digestibility of dry matter, crude protein, ether extract and ash compared to the control diet in both the starter and finisher periods ($P < 0.05$, respectively). However, treatments had no effect on crude fiber digestibility in both periods ($P > 0.05$, respectively). Crude protein digestibility was the highest in the birds fed 50% replacement diet, followed by 10% replacement diet in both periods ($P < 0.05$, respectively). Ether extract digestibility was the highest in the birds fed 30 and 50% replacement diets in both periods ($P < 0.05$, respectively).

Intestinal microbial population

The effect of replacement diets on the ileal and cecal microbial populations is shown in Table 6. *Bifidobacterium*

Table 3: Effect of conventional ingredients replacement with 10, 30 and 50% of alternative ingredients on the growth performance of broiler chickens

Variables	T1	T2	T3	T4	SEM	*p* Value
0-21 days						
Feed intake (g)	995.2	952.9	1035.5	1052.7	0.13	ns
Body weight gain (g)	608.8^{b}	669.4^{a}	710.3^{a}	685.6^{a}	0.39	0.01
Feed conversion ratio (g/g)	1.6^{a}	1.4^{b}	1.5^{b}	1.5^{ab}	0.11	0.01
Body weight (g)	631.2^{b}	703.5^{a}	697.4^{ab}	723.4^{a}	0.30	0.05
21-42 days						
Feed intake (g)	2244.9^{a}	2236.5^{ab}	1940.3^{b}	2008.3^{ab}	1.94	0.05
Body weight gain (g)	1199.7^{b}	1226.9^{ab}	1262.6^{a}	1262.4^{a}	1.43	0.05
Feed conversion ratio (g/g)	1.9^{a}	1.8^{ab}	1.5^{b}	1.6^{bc}	0.18	0.05
Body weight (g)	2076.3	2087.5	2118.0	2099.0	1.57	ns
0-42 days						
Feed intake (g)	3240.1	3129.7	3120.1	3249.6	1.05	ns
Body weight gain (g)	1808.5^{b}	1896.3^{ab}	1929.2^{a}	1979.2^{a}	4.91	0.01
Feed conversion ratio (g/g)	1.8^{a}	1.7^{b}	1.6^{b}	1.6^{b}	0.07	0.05

Superscripts (a-c) show significant differences among treatments in each row ($P < 0.05$). NS: non-significant. SEM: standard error of means.

T1: control diet. T2: replacement of conventional ingredients with 10% alternative ingredients. T3: replacement of conventional ingredients with 30% alternative ingredients. T4: replacement of conventional ingredients with 50% alternative ingredients.

Table 4: Effect of conventional feed ingredients replacement with 10, 30 and 50% of alternative ingredients on carcass quality

Variables	T1	T2	T3	T4	SEM	*p* Value
Carcass weight (g)	1771.1	1725.0	1857.1	1789.9	1.32	ns
Dressing (%)	84.2	86.7	85.8	85.4	1.30	ns
Breast muscle (%)	25.9	24.5	26.3	24.8	2.93	ns
Abdominal fat (%)	1.1^{b}	1.6^{ab}	2.2^{a}	2.0^{a}	0.65	0.05

Superscripts (a-b) show significant differences among treatments in each row ($p<0.05$). NS: non-significant. SEM: standard error of means. T1: control diet. T2: replacement of conventional ingredients with 10% alternative ingredients. T3: replacement of conventional ingredients with 30% alternative ingredients. T4: replacement of conventional ingredients with 50% alternative ingredients

Table 5: Effect of conventional feed ingredients replacement with 10, 30 and 50% of alternative ingredients on nutrient digestibility

Nutrient digestibility (%)	T1	T2	T3	T4	SEM	*p* Value
0-21 days						
Dry matter	89.0^{d}	90.9^{c}	92.8^{a}	91.8^{b}	0.04	0.001
Crude protein	83.5^{c}	85.7^{ab}	84.4^{b}	86.8^{a}	0.84	0.001
Ether extract	81.3^{c}	85.9^{b}	90.3^{a}	89.5^{a}	1.04	0.001
Ash	88.6^{d}	90.5^{c}	91.9^{a}	91.2^{b}	0.13	0.001
Crude fibre	77.2	82.6	87.5	84.6	0.85	ns
21-42 days						
Dry matter	89.0^{d}	91.0^{c}	92.8^{a}	91.6^{b}	0.09	0.001
Crude protein	88.8^{d}	92.2^{b}	91.3^{c}	94.6^{a}	0.30	0.001
Ether extract	92.5^{b}	91.5^{b}	96.6^{a}	96.2^{a}	0.70	0.001
Ash	89.0^{c}	90.6^{b}	92.2^{a}	90.9^{b}	0.10	0.001
Crude fibre	74.8	78.0	79.8	74.3	0.54	ns

Superscripts (a-c) show significant differences among treatments in each row ($p<0.05$). NS: non-significant. SEM: standard error of means.T1: control diet. T2: replacement of conventional ingredients with 10% alternative ingredients. T3: replacement of conventional ingredients with 30% alternative ingredients. T4: replacement of conventional ingredients with 50% alternative ingredients

Table 6: Effect of conventional feed ingredients replacement with 10, 30 and 50% of alternative ingredients on ileal and cecal bacteria in broilers at 42 day of age

Microbial population ($\log^{10}$ copy n/mL DNA extract)	Organ	T1	T2	T3	T4	SEM	*P* Value
Bifidobacterium	Ileum	7.9	10.9	6.6	7.4	3.49	ns
	Cecum	9.7	7.9	9.1	7.8	4.72	ns
Enterococcus	Ileum	6.9^{c}	6.8^{c}	7.4^{b}	7.9^{a}	0.13	0.001
	Cecum	6.6^{b}	6.2^{b}	6.7^{b}	7.6^{a}	0.48	0.01
Escherichia coli	Ileum	3.7^{b}	4.6^{a}	4.7^{a}	4.6^{a}	0.53	0.05
	Cecum	6.2^{a}	5.0^{c}	6.2^{b}	5.3^{b}	0.21	0.001
Lactobacillus	Ileum	5.7^{c}	6.5^{a}	6.0^{b}	6.1^{b}	0.15	0.001
	Cecum	5.8^{c}	5.9^{b}	6.0^{b}	6.3^{a}	0.20	0.01

Superscripts (a-c) show significant differences among treatments in each row ($P < 0.05$). NS: non-significant. SEM: standard error of means

T1: control diet. T2: replacement of conventional ingredients with 10% alternative ingredients. T3: replacement of conventional ingredients with 30% alternative ingredients. T4: replacement of conventional ingredients with 50% alternative ingredients

populations in the ileum and cecum of broilers ($P > 0.05$, respectively) were not affected by treatments. Birds fed the 30 and 50% replacement diets had higher *Enterococcus* population in the ileum compared to the control diet ($P < 0.05$). The population of *Enterococcus* in the cecum recorded the highest number in the birds fed 50% replacement diet ($P < 0.05$). *E. coli* population increased in the ileum ($P < 0.05$) but decreased in the cecum of birds fed the replacement diets ($P < 0.001$). Birds fed the replacement diets had increased *Lactobacillus* populations in both the ileum ($P < 0.001$) and cecum ($P < 0.01$) compared to the control diets.

Discussion

Body weight gain and FCR improved in broilers fed mixtures of food wastes and BSFL at 30 and 50% replacement diets. In contrast, previous studies showed that dietary inclusion of 20 to 30% of food waste had no effect on body weight gain and FCR of broilers (Saki *et al.* 2006; Viana *et al.* 2006). Chen *et al.* (2007) reported no difference in the weight gain of Taiwan Native chicken fed 20% food waste and the conventional diet. In the current study, broilers fed diet containing 10% BSFL replacement had similar growth performance to the control group. On the other hand, broilers fed higher levels of BSFL at 30 and 50% replacements resulted in improved growth performance. The improved growth performance was a result of improved crude protein and fat digestibility in the broilers that received the replacement diets. Improvement of FCR with no changes in the feed intake suggests that the nutrients were utilized for body weight gain. No differences in the feed intake between treatments suggest that the replacement diets were as palatable as the control diet.

Dietary fiber plays an important role in the microbial fermentation in the cecum of chicken (Dunkley *et al.* 2007). The type and quantity of dietary fiber can alter the microbial populations in the guts of broilers (Mateos *et al.* 2012). Chen *et al.* (2007) reported that the crude protein digestibility was increased but energy digestibility was decreased in chickens fed 20% food waste. In the above-mentioned study, the reduced energy digestibility was suggested to be due to the high fiber content of food waste

(10.8% crude fiber) which contained an average of 51.2% fruit and vegetables. In addition, Sadeghi *et al.* (2015) indicated that dietary fiber above 3% could have a negative effect on growth performance of broilers. In the current study, fruit and vegetable residuals were not included in the food waste and the dietary crude fiber was below 3%. In fact, the performance of broilers improved with lower amount of fiber in the basal diet (Jiménez-Moreno *et al.* 2009, 2013).

E. coli population was decreased in the cecum and *Lactobacillus* populations were increased in the intestines of broilers fed the replacement diets. BSFL contained chitin at 50 to 96 g/kg dry matter of BSFL (Kroeckel *et al.* 2012; Schiavone *et al.* 2017). Chitin may be responsible for the alteration of bacteria populations that could have favorable effects on nutrients digestibility in the current study. Chitin content in BSFL could be the key factor in modifying microbial fermentation (Borrelli *et al.* 2017). Increased butyrate and acetate levels in the caeca of hens proved that chitin was used a substrate for intestinal bacterial fermentation (Cutrignelli *et al.* 2018). It has been speculated that the high butyric acid level may partly be responsible for inhibiting *E. coli*, with no inhibition of beneficial bacteria such as *Lactobacillus*. Another possible explanation for the alteration of microbial populations could be due to the antimicrobial properties of BSFL (Spranghers *et al.* 2017). Lauric acid is the major component of BSFL that ranges from 21 to 68% of the total lipid depending on its rearing substrates (DiGiacomo *et al.* 2019). Lauric acid is a natural antimicrobial agent that suppressed the growth of *E. coli* (Dierick *et al.* 2002) with less impact on *Lactobacilli* (Spranghers *et al.* 2017). However, it should be noted that the impacts of chitin and lauric acid contents could be minimum considering the low level of dietary BSFL inclusion in the current study. There is limited report on the effect of food wastes and BSFL on the broiler's intestinal microbiome and this warrants further investigation.

The broilers that received the replacement diets had higher abdominal fat weight, with no changes in carcass weight and breast muscle percentage, suggesting that the increased body weight gain could be a result of increased fat deposition. It is well documented that dietary fatty acid profile could influence abdominal fat deposition. The broilers fed dietary fats rich in saturated fatty acid (SFA) had higher abdominal fat deposition compared to fats rich in polyunsaturated fatty acid (PUFA) (Crespo and Esteve-Garcia 2001; Khatun *et al.* 2017). Meat and bone waste contained 20.39% crude fat (Alqazzaz *et al.* 2019). Hossein (2015) reported relatively high SFA in restaurant wastes composed of meat and chicken bones. Although not measured, the replacement diets in the current study may be rich in SFA, which is less readily available for energy production compared to PUFA. Hence, SFA was stored as adipose tissue that resulted in increased abdominal fat deposition (Velasco *et al.* 2010).

Conclusion

Combinations of food waste and BSFL improved the body weight gain and feed efficiency of broilers. Alteration of intestinal microbial population and improvement of crude protein and fat digestibility in the broilers that received the replacement diets could be because of a reasonable amount of fiber in the food wastes as well as chitin content and antimicrobial properties of BSFL. Rice waste and meat and bone waste could become partial substitutes for conventional ingredients that could offer viable solution to mitigate food waste's negative impact on the environment.

Acknowledgements

This research was sponsored by Putra Grant (IPS) from the Universiti Putra Malaysia. This research was funded by GP-IPS from Universiti Putra Malaysia

Author Contributions

All authors designed the experiment. MA and HA performed the experiments. MA, HA and AAS analyzed the data. All authors reviewed and offered critical comments on the manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

All datasets presented in this study are included in the article

Ehics Approval

The approved protocol of the Institutional Animal Care and Use Committee of the Universiti Putra Malaysia (UPM/IACUC/AUP-R053/2017) was applied in all animal handling procedures.

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