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Original Article

Effects of Bile Acids and Lipase Supplementation in Low-Energy Diets on Growth Performance, Fat Digestibility and Meat Quality in Broiler Chickens

■Author(s)

Arshad MA[I] https://orcid.org/0000-0001-7930-1655
Bhatti SA[I] https://orcid.org/0000-0002-5626-3809
Hassan I[II] https://orcid.org/0000-0003-1893-3266
Rahman MA[I] https://orcid.org/0000-0002-6894-1128
Rehman MS[I] https://orcid.org/0000-0001-7767-6007

[I] Institute of Animal and Dairy Sciences, Faculty of Animal Husbandry, University of Agriculture, Faisalabad-38040, Pakistan.
[II] Sharif Feed Mills (Pvt.) Ltd., Okara, Pakistan.

■Mail Address

Corresponding author e-mail address
Muhammad Aziz-ur-Rahman
Institute of Animal and Dairy Sciences, Faculty of Animal Husbandry, University of Agriculture, Faisalabad-38040, Pakistan.
Phone: +92-334-1703739
Email: drazizurrahman@uaf.edu.pk

■Keywords

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ABSTRACT

The aim of this study was to investigate the effect of bile acids and lipase supplementation in low energy (LE) diets on growth, fat digestibility, serum lipid profile and meat quality of broilers. Seven hundred one-day-old broiler chicks were divided into 5 dietary treatments with five replicates of 28 birds each. The five treatments were: i) high energy diet (HE; metabolizable energy (ME) = 3,000 and 3,170 kcal/kg for starter and finisher diet), ii) low energy diet (LE; ME = 2,900 and 3,070 kcal/kg for starter and finisher diet), iii) LE diet supplemented with 300 g/ton bile acids (LEB), iv) LE diet supplemented with 180 g/ton lipase (LEL), v) LE diet supplemented both with bile acids (300 g/ton) and lipase (180 g/ton). The experiment lasted 35 days having starter phase from days 1-21 and finisher phase from days 22-35. Dietary inclusion of both bile acids and lipase in LE diet had no effect (p>0.05) on body weight (BW) gain and feed intake. High energy diet reduced feed intake and BW gain during starter and overall period; however, during finisher phase BW gain was similar in all dietary treatments. Dietary energy level had no effect on feed conversion ratio. Fat digestibility (p>0.05) both in the starter and finisher phase was not affected by the dietary treatments. Concentration of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides were not affected by the dietary treatments (p>0.05). Meat quality of breast and thigh muscle was unaffected due to the dietary treatments (p>0.05). It is concluded that the supplementation of bile acids alone or in combination with lipase in low-energy diets did not improve broiler performance, fat digestibility, serum lipid profile and meat quality.

INTRODUCTION

Animal fats and vegetable oils are being used in broiler diets to increase energy density and improve growth performance (Leeson and Summers, 2005; Abudabos, 2014; Wu, 2018). However, fat addition negatively affects fat digestibility (Tancharoenrat *et al.*, 2013; Siyal *et al.*, 2017) especially during early broiler age (Tancharoenrat *et al.*, 2013; Ravindran *et al.*, 2016). Immature physiological function of the pancreas in broilers results in less production of bile acids and pancreatic lipase during early ages (Wiseman & Lewis, 1998; Al-Marzooqi & Leeson, 1999; Lilburn & Loeffler, 2015; Classen, 2017), which may leads to poor fat digestibility.

Recently, bile acids are getting attention as dietary emulsifier for increasing fat digestibility (Upadhaya *et al.*, 2019b) and improving broiler performance (Maisonnier *et al.*, 2003; Parsaie *et al.*, 2007). Supplementation of bile acids in broiler diet significantly improve the digestibility of fat (Nazir, 2014; Hemati Matin *et al.*, 2016; Lammasak *et al.*, 2019). Similarly, other studies (Ge *et al.*, 2018; Lai *et al.*, 2018a;

Lai *et al.*, 2018b) reported that the supplementation of bile acids improve daily weight gain, feed conversion ratio (FCR) and carcass yield in broilers.

Like bile acids, exogenous lipase also improves physiological limitation of poultry digestive system (Nagargoje *et al.*, 2016). On the other hand, Hu *et al.* (2018) reported that providing reduced energy diet had decreased ($p<0.05$) body weight (BW) gain compared to basal energy diet during a period of 14 days, however, it was compensated with the supplementation of 0.015% and 0.03% lipase. According to Wang *et al.* (2017), the supplementation of lipase in broiler diets improved FCR, growth performance and fat digestibility. On the contrary, other researchers reported that lipase supplementation had no effect on nutrient utilization and bird's performance in broiler fed wheat-based diets (Polin *et al.*, 1980; Meng *et al.*, 2004). Based on contrary results of lipase supplementation in broiler diets on performance, this study was planned to investigate the effect of supplementing bile acids and lipase in combination or alone in broiler diets on growth performance, fat digestibility, serum lipid profiles and meat quality of broiler. Our hypothesis was that feeding low energy diets supplementation with bile acids and lipase will improve broiler performance.

MATERIALS AND METHODS

All procedures carried out in this experiment were reviewed and approved by the Animal Protocol Review Committee of the University of Agriculture, Faisalabad. The experiment was conducted at the Research and Development Farm of Sharif Feed Mills (Pvt.) Limited, Okara, Pakistan.

Experimental birds, diet and housing

Seven hundred one-day-old 500 mix sex broiler chicks with an average initial BW of 45.9 ± 0.25 g were used in the trial. The chicks were randomly assigned to 5 dietary treatments with 5 replicates of 28 birds each. The five treatments were: i) high energy diet (HE; metabolizable energy (ME) = 3,000 and 3,170 kcal/kg for starter and finisher diet), ii) low energy diet (LE; ME = 2,900 and 3,070 kcal/kg for starter and finisher diet), iii) LE diet supplemented with 300 g/ton bile acids (LEB), iv) LE diet supplemented with 180 g/ton lipase (LEL), v) LE diet supplemented both with bile acids (300 g/ton) and lipase (180 g/ton). The bile acids were composed of hyocholic acid, hyodeoxycholic acid and chenodeoxycholic acid. The experiment lasted for 35 days having starter phase from days 1-21 and finisher phase from days 22-35. The composition of the experimental diets is given in Table 1. All nutrients in the experimental diets were formulated according to the nutrient requirement suggested by NRC (1994), except for ME, which was 100 kcal/kg less than recommendations for LE diets. All diets were formulated on digestible amino acids (AA) basis keeping lysine as reference AA as described in a recent study (Abdullah *et al.* 2019). Feed was offered in pellet form.

Table 1 – Ingredients and nutrient analysis of experimental diets for broiler chicks (as-fed basis).

	Starter (0-21 d)		Finisher (21-35 d)	
	HE†	LE‡	HE	LE
Ingredients (%)				
Maize	35	36.91	42.62	44.02
Wheat	18.71	19	18	19
Soybean meal,45%	37.66	37.38	29.91	29.42
Poultry fat	4.65	2.73	5.92	4
Limestone	0.98	0.99	0.76	0.77
Di-calcium phosphate	1.89	1.89	1.81	1.79
Sodium bicarbonate	0.05	0.05	0.16	0.16
Sodium chloride	0.46	0.46	0.39	0.39
Lysine sulfate, 55%	0.19	0.19	0.1	0.12
DL-Methionine, 99%	0.28	0.27	0.2	0.2
L-Threonine, 98%	0.04	0.04	0.03	0.03
Vitamin and mineral premix¶	0.10	0.10	0.10	0.10
Calculated nutrient content (%)				
ME (kcal/kg)	3000	2900	3170	3070
Crude protein	22	22	19	19
Ether extract	7.00	5.16	8.39	6.55
Crude fiber	2.88	2.9	2.61	2.64
Lys, digestible	1.18	1.18	0.95	0.95
Met + Cys, digestible	0.88	0.88	0.74	0.74
Thr, digestible	0.77	0.77	0.66	0.66
Calcium	0.98	0.98	0.85	0.85
Phosphorous, available	0.45	0.45	0.42	0.42
Nutrient composition (%) analyzed				
Dry matter	90.4	89.8	90.7	90.3
Crude protein	21.9	22	20.5	20.4
Ether extract	6.5	5.3	7.5	6.4
Crude fiber	4.6	4.6	4.3	4.1
Ash	3.6	3.5	3.8	3.8

† HE: high energy

‡ LE: low energy

¶ Provided per kg diet: 20000 KIU of vitamin A; 5400 KIU of vitamin D_3; 48000 mg of vitamin E; 4000 mg vitamin K_3; 4000 mg vitamin B_1; 9000 mg vitamin B_2; 7600 mg vitamin B_6; 20 mg vitamin B_{12}; 60000 mg niacin; 20000 mg pantothenic acid; 1600 mg folic acid; 200 mg biotin, 10000 mg of iron; 120000 mg of zinc; 140000 mg of manganese; 12000 mg of copper; 1800 mg of iodine; 400 mg of cobalt; 360 mg of selenium.

Birds in each replicate were housed in pens of 5.5'× 3.8'× 2'. Each pen had a separate tube feeder and automatic nipple drinkers. Rice husk was used for bedding material. It was ensured that the birds were not in stress and pain during the trail. Instruction of recent

studies were followed to conduct the experiment (Aziz ur Rahman *et al.,* 2017; Aziz ur Rahman *et al.,* 2019).

Data recording

To measure the feed intake, growth rate and performance parameters standard procedures were adopted as presented in the recent study (Hussain *et al.* 2018; Hussain *et al.* 2020). In brief, the birds were weighed by pen at days 1, 21 and 35 of the experiment. Weekly feed intake was calculated, body weight gain and feed intake were recorded for the overall period.

Nutrient digestibility and chemical analysis

Acid insoluble ash (AIA) was added @ 1% as internal marker in the experimental diets for determination of nutrient digestibility. Diets with AIA were offered for 4 consecutive days. Polythene sheets were placed under the floor of all pens excreta collection. Samples were placed carefully in sampling bags as described in recent studies (Iamam-ul-Haq *et al.* 2019; Shahzad *et al.* 2019). . Excreta were stored at –20 °C after the removal of the feathers and scales. Feed samples from the feeders of each pen were also collected. Before chemical analysis, excreta samples were dried at 57 °C for 72 h, after which they were ground to pass through a one-mm screen. Feed and excreta content of moisture and fat was determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2006) as decribed in literature (Muhammad *et al.* 2016; Niu *et al.* 2017; Xia *et al.* 2018). Fat contents were determined by Soxhlet apparatus. Determination of AIA was performed after ashing the samples and treating the ash with boiling hydrochloric acid (Viveros *et al.*, 2002). Digestibility procedure was followed as described in recent studies (Massuquetto *et al.*,2019).

Digestibility was calculated using the following equation:

$$\%\text{digestibility} = 1 - \left(\frac{\%marker \in feed \times \%nutrient \in feces}{\%marker \in feces \times \%nutrient \in feed}\right) \times 100$$

Organ index

On day 35, five birds (Pre-weighed) from each pen were slaughtered. After slaughtering the birds were defeathered, and the Bursa of fabricius, heart, liver (without gallbladder), gizzard (removal of content) and breast muscle were collected for calculation of the eviscerated weight as described in literature (Sharif *et al.* 2018). Organ index was expressed as a percentage of live weight.

Serum lipid profile

On day 35, ten birds were randomly selected from each treatment (2 birds per pen) for blood collection. Serum lipid profile was determined following the procedure of previous studies (*Chen et al.* 2019; He *et al.* 2018; Su *et al.* 2013). Blood samples were then centrifuged at 3,000×g for 15 min and serum was separated. The total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) in the serum samples were analyzed using a kit method on Microlab 300 (Merck, Germany).

Meat quality

Breast and thigh muscles pH was tested 24 h after slaughtering, by dipping glass-electrode in meat solution as described by (Sallam *et al.*, 2004). Color (lightness) of the breast and thigh muscles were measured by using handheld tristimulus colorimeter (Color Test Meter II, Neuhaus Neotec, 95808, Germany) by following the procedure of Sohaib *et al.* (2016).

Water holding capacity (WHC) was determined on breast and thigh muscles using the method of Zhang *et al.* (1995), with some modifications. Briefly, 15 ± 0.3 g of chopped lean muscle and 22.5 ml 0.6 N NaCl solution were inserted into a centrifuge tube. The centrifuge tube with the sample and solution was weighed (W1) after homogenizing, then centrifuged at 5000×g for 10 min at 4 °C. The centrifuge tube was then removed from the centrifuge machine, then the water was carefully removed from the tube and weighed again (W2). WHC was calculated according to the following formula:

$$\%WHC = \left(\frac{W1 - W2 - Sample\ weight}{Sample\ weight}\right) \times 100$$

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) technique under completely randomized design of Minitab Statistical Software 17 (Minitab, 2010) with the cage being considered as the experimental unit. Tukey's test was used to separate difference means among treatments. Data were assumed to be statistically significant when $p<0.05$ and variability in the data was expressed as the standard error means.

RESULTS

Growth performance

Dietary inclusion of bile acids and lipase separately or in combination in LE diet did not differ ($p>0.05$) BW

gain and feed intake in starter phase. Feed intake and BW gain was lower ($p<0.05$) in broilers fed HE diet than other dietary treatments during starter phase and in the overall period. FCR was not affected ($p>0.05$) by the dietary treatments during the starter phase of the experiment. However, during finisher and overall period HE treatment had better FCR ($p<0.05$) as compared to LE treatment (Table 2).

Table 2 – Effect of dietary bile acids and lipase enzyme supplementation on growth performance in broiler chickens.

Item	Treatments¶					SEM§	*p*-value
	HE	LE	LEB	LEL	LEBL		
1 to 21 d							
BW gain (g)	916.22[b]	1058.13[a]	1043.49[a]	1038.19[a]	1042.04[a]	12.2	0.001
Feed intake (g)	1173.89[b]	1305.52[a]	1290.74[a]	1300.31[a]	1280.42[a]	12.6	0.001
FCR	1.28	1.23	1.24	1.25	1.23	0.01	0.068
22 to 35 d							
BW gain (g)	1266.90	1328.23	1290.83	1243.65	1249.10	17.7	0.581
Feed intake (g)	1985.95[b]	2215.04[a]	2171.28[a]	2126.34[ab]	2207.37[a]	23.0	0.002
FCR	1.57[b]	1.67[ab]	1.69[ab]	1.71[ab]	1.77[a]	0.02	0.009
1 to 35 d							
BW gain (g)	2183.12[b]	2386.37[a]	2334.32[ab]	2281.84[ab]	2291.13[ab]	22.6	0.047
Feed intake (g)	3159.84[b]	3520.56[a]	3462.02[a]	3426.65[a]	3487.79[a]	32.7	0.001
FCR	1.45[b]	1.48[ab]	1.48[ab]	1.50[ab]	1.52[a]	0.01	0.034

[a-b] Means within a row with different superscripts are significantly different ($p<0.05$).

§ SEM: Standard error of mean.

¶ HE: high energy diet; LE: low energy diet; LEB: low energy diet with bile acids; LEL: low energy diet with lipase enzyme; LEBL: low energy diet with both bile acids and lipase enzyme.

Fat Digestibility

Reducing the energy in diet did not affect ($p>0.05$) fat digestibility compared to HE diet. Furthermore, the addition of bile acids and lipase alone or in combination in LE diet caused no difference ($p>0.05$) in fat digestibility both at 21 and 35 d of age (Table 3).

Table 3 – Effect of dietary bile acids and lipase enzyme supplementation on fat digestibility in broiler chickens.

Item (%)	Treatments¶					SEM§	*p*-value
	HE	LE	LEB	LEL	LEBL		
21 d							
Fat digestibility	88.36	89.26	90.36	90.99	90.29	0.84	0.89
35 d							
Fat digestibility	86.93	87.94	85.41	88.68	85.61	0.83	0.71

[a-b] Means within a row with different superscripts are significantly different ($p<0.05$).

§ SEM: Standard error of mean.

¶ HE: high energy diet; LE: low energy diet; LEB: low energy diet with bile acids; LEL: low energy diet with lipase enzyme; LEBL: low energy diet with both bile acids and lipase enzyme.

Organ index

The effect of bile acids and lipase supplementation in LE diets are presented in Table 4. Dietary treatments had no effect ($p>0.05$) on relative weights of bursa of fabricius, gizzard, heart, liver, spleen, abdominal fat and breast muscle.

Table 4 – Effect of dietary bile acids and lipase enzyme supplementation on the relative organ weights in broiler chickens.

Item (%)	Treatments¶					SEM§	*p*-value
	HE	LE	LEB	LEL	LEBL		
Bursa of fabricius	0.15	0.17	0.17	0.16	0.16	0.01	0.77
Gizzard	0.85	0.89	0.82	0.83	0.91	0.02	0.42
Heart	0.40	0.38	0.37	0.39	0.36	0.01	0.22
Liver	2.06	2.00	2.10	2.07	2.02	0.04	0.96
Spleen	0.14	0.12	0.11	0.12	0.13	0.01	0.57
Abdominal fat	1.86	2.01	1.96	2.03	1.90	0.05	0.82
Breast muscle	41.44	41.25	40.57	40.05	41.04	0.22	0.28

[a-b] Means within a row with different superscripts are significantly different ($p<0.05$).

§ SEM: Standard error of mean.

¶ HE: high energy diet; LE: low energy diet; LEB: low energy diet with bile acids; LEL: low energy diet with lipase enzyme; LEBL: low energy diet with both bile acids and lipase enzyme.

Serum lipid profile

Concentration of TC, HDL-C, LDL-C and TG were not affected (p>0.05) by all dietary treatments (Table 5).

Meat quality

Dietary treatments had no effect on WHC and pH of breast and thigh muscles. Furthermore, dietary treatments caused no difference (p>0.05) in breast and thigh muscles lightness (Table 6).

DISCUSSION

In the current study, feed intake was not affected by the supplementation of bile acids and lipase in LE diet during starter, finisher and the overall period. However, LE diet intake was more as compared to HE diet which is similar with the findings of Harrington *et al.* (2015) and Hosseini *et al.* (2018) who reported that increased dietary energy could reduce the feed intake of broilers. Similarly, Lamot *et al.* (2017) reported that increasing diet density had reduced feed intake. Broilers have the ability to control energy intake by adjusting their feed intake based on dietary energy concentration changes (Leeson *et al.*, 1996). In the current study, low intake of HE diet could be explained by the fulfillment of energy demand for broiler growth. Body weight gain was not changed by the supplementation of bile acids and lipase alone or in combination in LE diet during starter, finisher and the overall period. However, BW gain was more in LE diet compared to HE diet in starter and the overall period which is contrary to the findings of the previous researcher who reported that feeding low-energy diets reduced BW gain compared to high-energy diets during 14 d period (Zhao and Kim, 2017; Hu *et al.*, 2018) and during the period of 28 to 35 d (Ge *et al.*, 2018). In birds offered reduced energy diets, they probably compensated for lower energy intake per kilogram of feed. It can explain the increased feed intake and BW of LE diets compared to HE diets. However, bile acids and lipase in LE energy diets failed to cause any improvement in feed efficiency. Decreased BW gain in the present study evidences that feed form may influence partially to the results observed (Brickett *et al.*, 2007; Saveewonlop *et al.*, 2019).

The digestibility of fat was similar among all dietary treatments in the current study. It has been reported by Rabie *et al.* (2010) that digestibility of fat did not change by decreasing the energy level in the diet of broiler chicks. Similar digestibility coefficient of HE diets with LE diet could be explained by the decreased

Table 5 – Effect of dietary bile acids and lipase enzyme supplementation on serum lipid profile of broiler chickens at 35 d of age.

Item (mg/dl)	Treatments¶					SEM§	*p*-value
	HE	LE	LEB	LEL	LEBL		
TC	127.4	125.6	122.4	122.0	118.8	2.29	0.81
HDL-C	91.6	90.4	88.6	92.0	89.4	1.10	0.87
LDL-C	34.2	32.6	33.2	34.8	29.8	1.12	0.69
TG	90.2	80.4	86.2	83.2	82.0	1.33	0.14

[a-b] Means within a row with different superscripts are significantly different (p<0.05).

§ SEM: Standard error of mean.

¶ HE: high energy diet; LE: low energy diet; LEB: low energy diet with bile acids; LEL: low energy diet with lipase enzyme; LEBL: low energy diet with both bile acids and lipase enzyme.

Table 6 – Effect of dietary bile acids and lipase enzyme supplementation on the meat quality of breast and thigh muscle in broiler chickens.

Item	Treatments¶					SEM§	*p*-value
	HE	LE	LEB	LEL	LEBL		
Breast muscle							
pH	5.98	6.00	6.09	6.06	6.03	0.02	0.44
WHC (%)	45.46	49.47	57.92	49.76	49.79	2.05	0.44
L* (Lightness)	46.80	46.20	45.40	47.60	45.00	0.65	0.75
Thigh muscle							
pH	6.23	6.33	6.20	6.16	6.17	0.02	0.13
WHC (%)	42.77	42.42	42.57	49.43	51.72	1.38	0.06
L* (Lightness)	57.40	58.40	57.00	59.00	56.40	0.88	0.91

[a-b] Means within a row with different superscripts are significantly different (p<0.05).

§ SEM: Standard error of mean.

¶ HE: high energy diet; LE: low energy diet; LEB: low energy diet with bile acids; LEL: low energy diet with lipase enzyme; LEBL: low energy diet with both bile acids and lipase enzyme.

feed intake in HE diet. Although low intake increases digestibility, in present study this mechanism is failed due to high fat content in HE diet (Lammasak *et al.*, 2019). Our findings are similar with the findings of Dairo *et al.* (2010) and Papadopoulos *et al.* (2018) who reported that LE or HE diet did not have any effect on fat digestibility. Similarly, Hu *et al.* (2018) reported that supplementing 0.015% lipase enzyme in LE diet had similar fat digestibility compared to HE diets.

The difference in the energy level in the diet did not affect relative organ weight and breast muscle yield of broilers in the current study. Similarly, supplementation of bile acid and lipase alone or in combination in LE diet also did not affect relative organ weight and breast muscle yield of broilers. Similar organ weight and breast muscle yield of broilers in LE and HE diet are in agreement with the findings of Ge *et al.* (2018) and Upadhaya *et al.* (2019a) who reported that LE diet had no effect on liver, spleen, gizzard, abdominal fat, bursa of fabricius and breast muscle weight. Additionally, similar results were also reported by Corduk *et al.* (2007) and Rabie *et al.* (2010) who stated that carcass traits of broiler chicks remained unaffected by dietary energy level. However, many researchers claimed increased percentage of abdominal fat by increasing dietary energy levels (Zhao & Kim, 2017; Mohammadigheisar *et al.*, 2018). In the present study, the percentage of abdominal fat was similar among HE and LE diets which might be due to lower intake of HE diets. The remaining organ index weight was also unaffected due to dietary supplementation of bile acids and lipase in the present study, which is in agreement with previous researchers (Ge *et al.*, 2018; Hu *et al.*, 2018; Lai *et al.*, 2018a). Generally, gut health is considered important for better performance in livestock (Qiu *et al.* 2019a; Qiu *et al.* 2019b) and external feed additives are provided to improve the gut health and performance of livestock. However, in the current study, they didn't improve the bird's performance.

Generally key indicators of lipid metabolism balance are TC, HDL-C and LDL-C (Helkin *et al.*, 2016). In the current study, dietary treatments did not influence serum lipid profile of broilers. It has been reported that HE diets had no effect on serum TG, TC, HDL-C and LDL-C (Ge *et al.*, 2018). Similar results were also reported by Zhao and Kim (2017) and Hosseini *et al.* (2018) who observed that TG, TC, HDL-C and LDL-C concentrations were unaffected due to dietary energy in broilers. In the present study, bile acids supplementation in LE diet did not change serum TG, TC, HDL-C and LDL-C, which is in agreement with (Alzawqari *et al.*, 2011; Ge *et al.*, 2018; Lai *et al.*, 2018b). Similarly, lipase supplementation along with bile in LE diet had similar effect as bile acid supplementation alone in LE diet. Limited studies reported the influence of lipase supplementation on blood serum profile, however, Zhao & Kim (2017) noted that supplementing 0.05% emulsifier caused no effect on serum TG, TC, HDL-C and LDL-C.

In our study, HE, LE and LE supplementation with bile acid alone or in combination with lipase had similar meat quality of breast and thigh muscles. In agreement with our results, the low-energy diet had no effect on the breast and thigh muscle color (lightness), pH value and WHC in broilers fed HE diet (Upadhaya *et al.*, 2017; Hu *et al.*, 2018; Upadhaya *et al.*, 2019a). However, limited research has been done to evaluate the quality of breast and thigh muscle in broilers fed LE diet supplemented with bile and lipase, therefore further research is needed to cross check the mechanism for the effects of bile acids on the meat quality in broilers.

CONCLUSIONS

Overall, results suggest that bile acids and lipase supplementation at 300 g/ton and 150g/ton of feed in LE diets did not affect broiler growth, digestibility, serum lipid profile and meat quality.

CONFLICT OF INTEREST

No potential conflict of interest declared.

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