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Impact of high-oil diets with bile acids or bovine bile extracts on growth performance and histomorphology of liver and intestine in grower broilers

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ABSTRACT

This study investigated the effects of supplementing high-fat starter diets with bovine bile extract (BBE) and commercial bile acid (CBA) on growth performance, organ morphology, histomorphology of the small intestine and liver, fat digestibility, and economic outcomes in broilers during the starter phase. A total of 336 Cobb strain day-old chicks were randomly assigned to eight dietary regimens: BF (basal fat), HF (high fat), HF+CBA (200, 400, and 600 mg/kg), and HF+BBE (200, 400, and 600 mg/kg), with six replicates per regimen. The feeding trial was conducted from days 7 to 21. Data were analyzed using orthogonal contrasts and polynomial analysis. Results showed that CBA at low levels significantly increased body weight gain ($P = 0.02$). Additionally, BBE supplementation significantly increased body weight gain ($P = 0.05$), performance index ($P = 0.03$), and reduced feed conversion ratio (FCR) ($P = 0.04$). HF+CBA (200) and HF+BBE (600) treatments significantly reduced FCR ($P = 0.01$) and improved the performance index ($P = 0.02$) by day 21. CBA also significantly increased pancreatic weight ($P = 0.02$) and villus height ($P = 0.01$). BBE significantly reduced bile weight ($P = 0.03$), increased ileum weight ($P = 0.04$), and extended the length of the large intestine ($P < 0.01$). Economically, the treatments BF vs HF HF+CBA (200) and HF+BBE (600) increased revenue by 5.25% and IOFC by 12.29%, while HF+CBA (200) vs HF+BBE (600) raised IOFC by 9.71%. In conclusion, supplementing high-fat diets with CBA and BBE improved growth performance, digestive organ efficiency, and economic outcomes, and BBE at 600 mg/kg was identified as the optimal dose for supplementation.

Introduction

Including oil or fat in poultry feed is a well-established practice aimed at increasing energy density, enhancing feed palatability, and improving feed intake, which ultimately improves production performance in broilers (Saminathan *et al*., 2022). Feeds containing oil or fat offer better energy efficiency, with higher energy gains and reduced energy losses, than those without these components (Moura, 2003). Crude palm oil (CPO), derived from the mesocarp of the oil palm fruit, is one of the most used oils in broiler diets (Baiocchi *et al*., 2019). However, the digestibility of CPO in broilers is influenced by several factors, including the level of saturation, unesterified fatty acid content, and the age of the birds (Palmquist, 2004). These factors pose challenges for chicks, as their limited production of endogenous emulsifiers restricts their ability to efficiently utilize dietary fat (Ravindran *et al*., 2016). Diets containing 4% CPO lead to efficient weight gain, efficient feed intake, and high feed conversion ratio (FCR) (Nooraida and Abidah, 2020).

Excessive energy intake from high-fat diets can cause fat accumulation, particularly in the abdominal region in which it disrupts gut microbiota and increases intestinal permeability. This can lead to metabolic disorders, such as fatty liver disease, which burden the liver and intestines (Sanz *et al*., 1999; Crespo and Esteve-Garcia, 2001; Murphy *et al*., 2015). Efficient fat digestion and absorption in broilers depend on the presence of bile acids produced by the liver and lipase produced by the pancreas, both of which are present at low levels in young chicks (Noy and Sklan, 1999). Therefore, feed additives that can enhance fat digestion without compromising performance are crucial. Bile extract supplementation is a promising option to improve fat utilization in poultry diets.

Bovine bile, a by-product of slaughterhouses, has emulsifying properties similar to those of endogenous bile acids (Lammasak *et al*., 2018). Bile acids play a critical role in the emulsification, digestion, and absorption of fats, particularly during the early stages of broiler growth (Arshad *et al*., 2021). Bile acid supplementation in poultry feed substantially improves feed intake and weight gain in broilers (Parsaie *et al*., 2007). Consequently, this study was performed to compare the efficacy of commercial bile acids (CBA) and bovine bile extract (BBE) in broiler diets, especially in high-fat feeding regimes, in which fat digestibility, organ morphology, and economic efficiency are the major issues.

Materials and methods

All animal procedures were approved by the research ethics committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (approval number: 19/EC-FKH/int./2024).

Bovine bile extraction

Bovine bile was collected from the gallbladders of freshly slaughtered cattle at a local slaughterhouse (Giwangan, Yogyakarta, Indonesia). The collected bile was frozen until extraction. Before extraction, the bile was dried in an oven at 60°C until it reached a constant weight. The dried bile (1 g) was dissolved in 10 ml of methanol and extracted twice using ultrasonication at 50°C for 15 min with occasional stirring. The resulting extract was filtered and concentrated under low pressure to obtain dried bile extract (Yaman and Chahyadi, 2020).

Experimental protocol, birds, and diets

A total of 336 day-old Cobb strain broiler chicks (DOC) were randomly placed in metabolite cages, divided into eight groups, and given the following diets: basal fat diet, basal fat diet plus bile acid (BF + BA), high-fat diet (HFD), high-fat diet plus commercial bile acid at levels of 200, 400, and 600 mg/kg (HFD + CBA), and high-fat diet plus bovine bile extract at levels of 200, 400, and 600 mg/kg (HFD + BBE). Each group consisted of six replicates with seven birds per replicate. Commercial feed (PT. Cargill Indonesia) was used in the starter phase (1–7 days). The diet provided during the starter phase (7–21 days) was formulated on the recommendation of the National Research Council (NRC) (1994) (Table 1). Water was provided ad libitum. The broilers were housed in battery cages measuring 75 cm in length, 45 cm in width, and 50 cm in height. Lighting was provided 24 h a day using LED lamps.

Growth performance

Body weight (g/bird) was measured after 7, 14, and 21 days. Weight gain was observed from day 7 to day 21 and was calculated by subtracting the initial weight from the previous weight (g/bird). Feed intake (g/bird) was calculated by subtracting the remaining feed from the total feed provided. The FCR was calculated by dividing the total feed intake by the weight gain from day 7 to day 21. Mortality was recorded daily from days 7 to 21 by noting the number of dead chickens. Mortality was calculated as a percentage of the initial populations or total number of birds raised. The performance index (PI) was calculated by multiplying the percentage of live chickens by the average weight (kg), dividing by the average age at harvest (days), multiplying by the feed conversion ratio, and then multiplying by 100%.

Internal organ morphology

One broiler from each replication group was slaughtered on day 22 for internal organ morphology measurements. The weights of the proventriculus, gizzard, pancreas, small intestine, cecum, bile, and liver were measured after the organs were cleaned of their contents using a digital scale with a 0.01-g accuracy. The small intestine was separated into the jejunum, ileum, and duodenum, before each part was weighed. Organ weight data were expressed as a percentage by dividing the broiler's body weight and multiplying by 100. The lengths of the digestive organs, including the duodenum, jejunum, ileum, cecum, and large intestine segments, were measured.

Fat digestibility

Excreta were collected from each treatment using trays attached to the metabolism cages from day19 to day 21. The excreta were dried by

Table 1. Ingredients and chemical composition of experimental broiler starter phase (7-21 days old).

Ingredients	BF	HF	HF+CBA level (g/kg)			HF+BBE level (g/kg)		
			200	400	600	200	400	600
Corn	43.94	31.91	31.83	31.75	31.67	31.83	31.75	31.67
Soybean Meal	31.75	38.68	38.62	38.55	38.49	38.62	38.55	38.49
Rice Bran	12.61	15	15	15	15	15	15	15
Crude Palm Oil	6.69	8	8	8	8	8	8	8
Palm Kernel Meal	3	2.21	2.8	3.39	3.98	2.8	3.39	3.98
Limestone	0	1.91	1.52	1.13	0.74	1.52	1.13	0.74
Premix	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
DL Methionine	0.38	0.39	0.37	0.35	0.33	0.37	0.35	0.33
L Lysine	0.29	0.27	0.24	0.22	0.2	0.24	0.22	0.2
Salt	0.14	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Sodium Bicarbonate	0.17	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dicalcium Phosphate	0	0.19	0.18	0.17	0.15	0.18	0.17	0.15
L Threonine	0.18	0.18	0.16	0.14	0.12	0.16	0.14	0.12
Choline Powder 60	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Commercial Bile Acid	0	0	0.02	0.04	0.06	0	0	0
Bovine Bile Extract	0	0	0	0	0	0.02	0.04	0.06
Total	100	100	300	500	700	300	500	700
Chemical composition								
Moisture	9.92	9.92	9.93	9.95	9.97	9.93	9.95	9.97
Ash	6.77	6.77	6.41	6.05	5.69	6.41	6.05	5.69
Fiber	4.08	4.08	4.19	4.30	4.42	4.19	4.30	4.42
Fat	11.21	11.21	11.27	11.32	11.38	11.27	11.32	11.38
Linoleic Acid	2.01	2.01	2.01	2.01	2.01	2.01	2.01	2.01
Protein	22	22	22	22	22	22	22	22
Metabolizable Energy	2.94	2.94	3.00	3.06	3.12	3.00	3.06	3.12
Calcium	1	1	1	1	1	1	1	1
Phosphorus	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Available Phosphor	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Sodium	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Chlorine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DIG LYS	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26
DIG MET	0.66	0.66	0.65	0.63	0.62	0.65	0.63	0.62
DIG M+C	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
DIG TRY	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
DIG THR	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
DIG ARG	1.38	1.38	1.3	1.39	1.4	1.38	1.39	1.40

incubation in an oven at 55°C for 48 h. The dried excreta were weighed and replicated for determination of fat digestibility using the method of the Association of Official Analytical Chemists (AOAC 2006). Fat digestibility was calculated according to Aya *et al*. (2013).

Histomorphology of the small intestine and liver

One broiler from each replication group was sacrificed on day 22 to collect samples from the jejunum segment of the small intestines and liver samples. The samples were stored in a cream pot containing 10% formalin awaiting histomorphological analysis of the villi (using a 2-cm section of the jejunum) and of liver tissue (using a 2-g tissue sample). The samples were placed in a sterile bottle and fixed in 10% neutral buffered formalin for four days. The tissue samples were dehydrated by passing them through a graded series of alcohol concentrations starting from 70%, 80%, 90%, and 100%. The samples were clarified with a xylene solution, before they were embedded in paraffin. After embedding, the tissues were sectioned with a microtome 5-μm sections, which were placed on glass slides. The preparations on the slides were examined and measured using a light microscope with computer assistance at 40x magnification. Measurements included villus height, villus width, crypt depth, and the villus height-to-crypt depth ratio in the jejunum segment. Additionally, histological changes and lipid droplets in the liver were investigated (Dono, 2012; Hu *et al*., 2024).

Feed cost, revenue, and Income Over Feed Cost (IOFC)

Revenue, feed costs, and Income Over Feed Cost (IOFC) are included in the economic aspects (Sutanto *et al*., 2020). IOFC is calculated by subtracting feed expenses from total revenue. Total revenue was calculated by multiplying farm production yield by the prevailing market price per kilogram of live weight, and feed expenditure was calculated based on the cost incurred to produce one kilogram of live poultry. Feed intake, feed ingredient prices, and the prevailing market price of broilers influenced the IOFC value.

Statistical analysis

Data were analyzed using polynomial contrast analysis to test the linear, quadratic, and cubic properties, and orthogonal contrast analysis to compare the three treatment groups, namely, control group, commercial bile acid group, and bovine bile extract group. Data analysis was conducted using the statistical programme IBM SPSS Statistics 26 (SPSS Inc., USA).

Results

Growth performance

Results on effect of CBA and BBE supplementation are presented in (Fig. 1) From day 7 to day 14, CBA and BBE supplementation at low to high levels did not significantly affect body weight (BW), weight gain (WG), feed intake (FI), FCR, or performance index (PI) (P > 0.05). However, from days 7 to 21, supplementation with low-dose CBA showed a linear increase in WG (P = 0.02), whereas BBE supplementation linearly increased WG (P = 0.05), PI (P = 0.03), and reduced FCR (P = 0.04).

Orthogonal contrast analysis showed significant differences in growth performance between the BF and HF, HF + CBA (200), and HF+BBE (600) groups (P < 0.05; Table 2). Specifically, broilers in the HF + BBE (600) group exhibited higher BW, WG, and PI and lower FCR than those in the HF + CBA (200) group. Additionally, there were significant improvements in BW and WG, with the 600 mg/kg BBE group showing better performance than the 200 mg/kg CBA group (P < 0.05).

Internal organ morphology

The effect of CBA and BBE supplementation on the internal organ morphology is presented in Table 3. CBA supplementation at low levels significantly affected pancreatic weight (P = 0.02), whereas no significant effects were observed in other organs. However, BBE supplementation had significant cubic and linear effects on gallbladder weight (P = 0.03) and ileum length (P = 0.04).

Orthogonal contrast analysis revealed significant differences (P < 0.05; Table 4) in the relative weights and lengths of several organs between the BF group and the other groups HF, HF + CBA (200), and HF + BBE (600). No significant differences in organ morphology (P > 0.05) were recorded between HF + CBA (200) and HF + BBE (600).

Fat digestibility

As shown in (Fig. 2), fat digestibility significantly increased as the level of BBE in the diet increased (P = 0.005). However, CBA supplementation

Table 2. Orthogonal polynomial and orthogonal contrast p-value of broiler growth performance.

Variables	Contras Orthogonal			CBA-Polynomial Orthogonal			BBE-Polynomial Orthogonal		
	C1	C2	C3	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
Day 14									
BW	0.42	0.75	0.85	0.21	0.83	0.89	0.87	0.2	0.6
WG	0.63	0.62	0.76	0.07	0.82	0.78	0.69	0.16	0.6
FI	0.47	0.41	0.04	0.31	0.83	0.44	0.04	0.15	0.31
FCR	0.52	0.43	0.18	0.06	0.77	0.52	0.27	0.1	0.38
Mortality	0.57	0.42	0.17	0.45	0.57	0.8	0	0	0
PI	0.38	0.43	0.27	0.06	0.76	0.65	0.37	0.13	0.55
Day 21									
BW	0.02	0.1	0.07	0.12	0.6	0.91	0.06	0.09	0.67
WG	0.03	0.07	0.06	0.02	0.59	0.78	0.05	0.11	0.65
FI	0.44	0.81	0.91	0.44	0.57	0.8	0.74	0.15	0.69
FCR	0.01	0.01	0.01	0.64	0.52	0.84	0.04	0.64	0.32
Mortality	0.57	0.42	0.17	0.45	0.57	0.8	0	0	0
PI	0.01	0.02	0.01	0.33	0.49	0.72	0.03	0.23	0.4

FI: feed intake, BW: body weight, WG: body weight gain, FCR: feed conversion, PI: performance index, C1: BF vs HF; HF+CBA; HF+BBE, C2: HF vs HF+CBA; HF+BBE, C3: HF+CBA vs HF+BBE, HF+CBA: high-fat content diet with added commercial bile acid at 0, 200, 400, 600 mg/kg, HF+BBE: high-fat content diet with added bovine bile extract at 0, 200, 400, 600 mg/kg.

did not significantly affect fat digestibility (P > 0.05). Orthogonal contrast analysis showed that the HF + BBE (600) group had significantly higher fat digestibility than the HF + CBA (200) group (P < 0.05; Table 4).

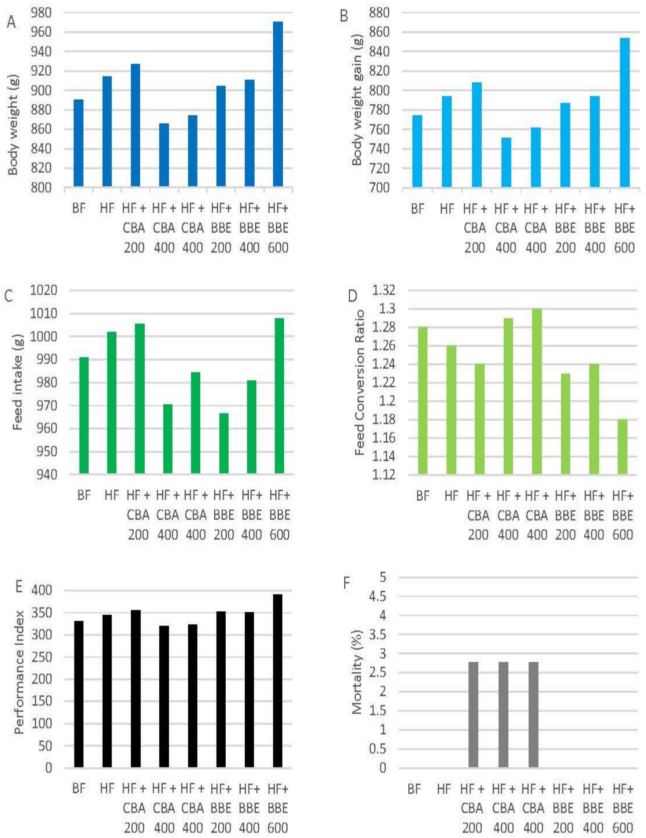

Fig. 1. Growth performance of broiler at d 21; BF: basal-fat content diet, HF: high-fat content diet, HF+CBA: high-fat content diet with added commercial bile acid, HFD+BBE: high-fat content diet with added bovine bile extract, FI: feed Intake; BW: body weight; BWG: body weight gain; FCR: feed conversion; PI: performance index.

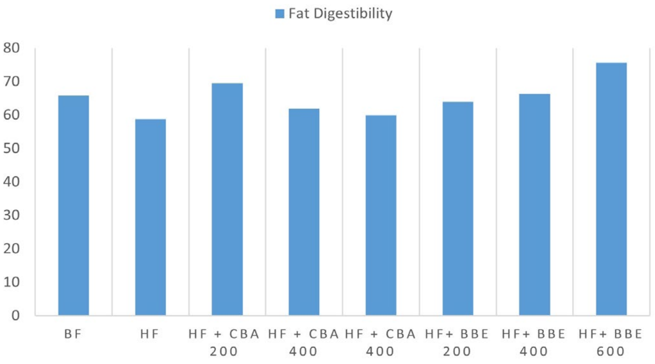

Fig. 2. Fat digestibility of broiler at 21 days. BF: basal-fat content diet, HF: high-fat content diet, HF+CBA: high-fat content diet with added commercial bile acid, HFD+BBE: high-fat content diet with added bovine bile extract.

Small intestinal and liver histomorphology

The histomorphological effects of CBA and BBE supplementation on the small intestine are shown in (Fig. 3) and (Fig. 4). Whereas CBA supplementation showed a significant linear effect on villus height (P = 0.01), BBE supplementation did not significantly affect crypt depth, villus width, villus height, or the villus-to-crypt ratio (P > 0.05).

Contrast analysis indicated that there were no significant effects of CBA and BBE supplementation on villus width or crypt depth across the treatment groups (P > 0.05). However, the 600 mg/kg BBE group had a significantly higher villus width than the 200 mg/kg CBA group (P = 0.01). Histological analysis (Fig. 4) revealed no significant changes in the liver tissue structure. These findings suggest that high-fat diets supplemented with CBA or BBE did not significantly affect the integrity of the liver tissue.

Feed costs, revenue, and IOFC of broilers

The economic impact of CBA and BBE supplementation on feed costs, revenue, and income over feed cost (IOFC) is presented in (Fig. 6).

Table 3. Effect of supplementation of commercial bile acids and bovine bile extract in high-oil starter broiler diets on the relative weights of broiler internal organs.

Variables	BF	HF	HF+CBA level (g/kg)			HF+BBE level (g/kg)			CV
			200	400	600	200	400	600	
Live BW (g.)	883.83	935.33	896.2	846.67	887.5	946.17	893.17	1009.67	
Proventriculus (g)	4.92	5.53	5.19	5.63	5.01	4.63	5.39	5.86	
Proventriculus (%)	0.56	0.59	0.58	0.67	0.56	0.49	0.6	0.58	8.39
Gizzard (g)	18.89	18.81	16.93	16.62	18.72	17.66	18.08	19.74	
Gizzard (%)	2.14	2.01	1.92	1.96	2.1	1.87	2.03	1.95	4.49
Pancreas (g)	3.22	3.13	3.23	3.27	2.85	3.13	3.45	3.58	
Pancreas (%)	0.36	0.33	0.36	0.39	0.32	0.33	0.39	0.35	6.91
Bile (g)	0.61	0.6	0.69	0.61	0.76	0.89	0.73	0.79	
Bile (%)	0.04	0.02	0.03	0.04	0.04	0.03	0.05	0.02	19.18
Duodenum (g)	6.64	7.23	7.57	6.21	8.14	7.53	7.28	7.53	
Duodenum (%)	0.76	0.78	0.86	0.74	0.91	0.8	0.82	0.74	7.65
Jejunum (g)	10.46	12.4	11.74	10.43	12.27	12.39	12	12.56	
Jejunum (%)	1.18	1.33	1.33	1.23	1.38	1.32	1.35	1.24	5.32
Ileum (g)	9	10.47	9.73	8.88	10.2	10.03	11.07	11.5	
Ileum (%)	1.02	1.12	1.11	1.05	1.15	1.07	1.24	1.14	6.23
Large Intestine (g)	1.42	1.51	1.63	1.45	1.72	1.9	1.74	1.81	
Large Intestine (%)	0.16	0.16	0.18	0.17	0.19	0.2	0.19	0.18	8.4
Cecum (g)	3.96	4.6	4.52	3.83	4.41	4.47	4.51	4.43	
Cecum (%)	0.45	0.5	0.51	0.45	0.5	0.47	0.5	0.44	5.84
Liver (g)	20.31	22.87	21.77	20.71	21.72	22.29	22.56	23.12	
Liver (%)	2.29	2.45	2.46	2.44	2.44	2.36	2.52	23.29	3.36

BF: basal-fat content diet, HF: high-fat content diet, HF+CBA: high-fat content diet with added commercial bile acid, HFD+BBE: high-fat content diet with added bovine bile extract, % live BW: calculation by dividing by live weight multiplied by 100, g: grams.

Supplementation with 600 mg/kg BBE resulted in the highest IOFC of Rp 10,766.91, and supplementing with 200 mg/kg CBA produced an IOFC of Rp 9,814.42. Orthogonal contrast analysis showed that BF vs the HF, HF + CBA (200), and HF + BBE (600) groups significantly increased revenue by 5.25% and IOFC by 12.29% ($P < 0.05$; Table 5). Additionally, HF+BBE (600) caused a more pronounced significant increase in IOFC than HF + CBA (200) ($P < 0.05$).

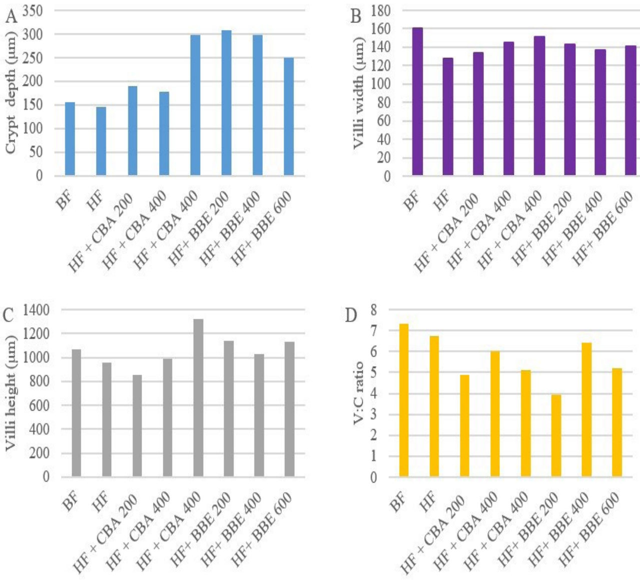

Fig. 3. Crypt depth, villus width, villus height and villus height/crypt depth ratio of broiler jejunum villi at 21 days. BF: basal-fat content diet, HF: high-fat content diet, HF+CBA: high-fat content diet with added commercial bile acid, HFD+BBE: high-fat content diet with added bovine bile extract, V: villi, C: crypts.

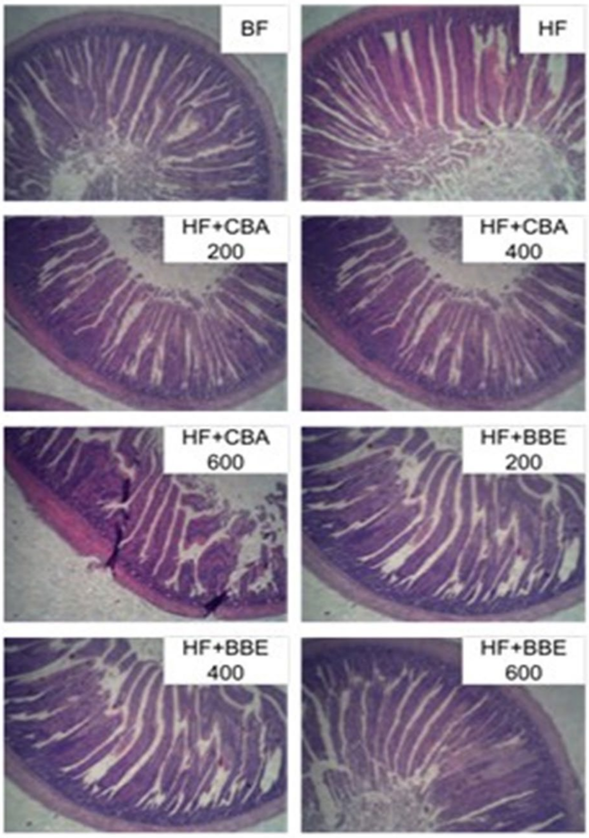

Fig. 4. Histology of the small intestine, jejunum segment of broiler at 21 days. Magnification X40. BF: basal fat diet; HF: high-fat diet; HF+CBA: high-fat diet supplemented with commercial bile acid at levels of 200, 400, 600 mg/kg; HF+BBE: high-fat diet supplemented with bovine bile extract at levels of 200, 400, 600 mg/kg.

Table 4. Orthogonal polynomial and orthogonal contrast p-value of internal organ weight, internal organ length, fat digestibility, and jejunum histomorphology of broilers.

Variables	Contras Orthogonal			CBA-Polynomial Orthogonal			BBE-Polynomial Orthogonal		
	C1	C2	C3	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
Organ weight									
Proventriculus	0.49	0.82	0.92	0.98	0.13	0.06	0.67	0.37	0.06
Gizzard	0.12	0.57	0.82	0.43	0.23	0.97	1	0.78	0.21
Pancreas	0.53	0.3	0.76	0.84	0.02	0.31	0.24	0.51	0.14
Bile	0.48	0.77	0.76	0.82	0.71	0.66	0.72	0.59	0.03
Duodenum	0.6	0.8	0.19	0.45	0.55	0.14	0.75	0.54	0.83
Jejunum	0.4	0.77	0.56	0.93	0.52	0.46	0.63	0.67	0.71
Ileum	0.31	0.98	0.78	0.96	0.55	0.68	0.55	0.81	0.18
Large Intestine	0.53	0.43	0.8	0.28	1	0.33	0.59	0.1	0.69
Cecum	0.47	0.67	0.21	0.82	0.7	0.35	0.46	0.6	0.4
Liver	0.13	0.45	0.1	0.92	0.91	0.88	0.47	0.4	0.08
Intestine length									
Duodenum	0.13	0.24	0.92	0.51	0.74	0.4	0.45	0.45	0.4
Jejunum	0.09	0.34	0.9	0.95	0.46	0.34	0.64	0.83	0.29
Ileum	0.08	0.04	0.49	0.97	0.31	0.27	0.04	0.92	0.57
Large Intestine	0.81	0.1	0.74	0.3	0.86	0.21	0.3	0.43	0
Cecum	0.44	0.69	0.49	0.77	0.58	0.99	0.88	0.51	0.66
Fat Digestibility									
Fat Digestibility	0.24	0	0.01	0.81	0.13	0.20	0.01	0.59	0.56
Histomorphology of jejunum									
Kripta Depth (µm)	0.55	0.86	0.9	0.1	0.2	0.28	0.59	0.17	0.05
Villi width (µm)	0.29	0.95	0.01	0.01	0.06	0.16	0.17	0.39	0.69
Villi height (µm)	0.77	0.18	0.63	0.24	0.34	0.21	0.23	0.1	0.67
V:C ratio	0.15	0.17	0.83	0.38	0.61	0.25	0.66	0.47	0.08

C1: BF vs HF; HF+CBA; HF+BBE, C2: HF vs HF+CBA; HF+BBE, C3: HF+CBA vs HF+BBE.

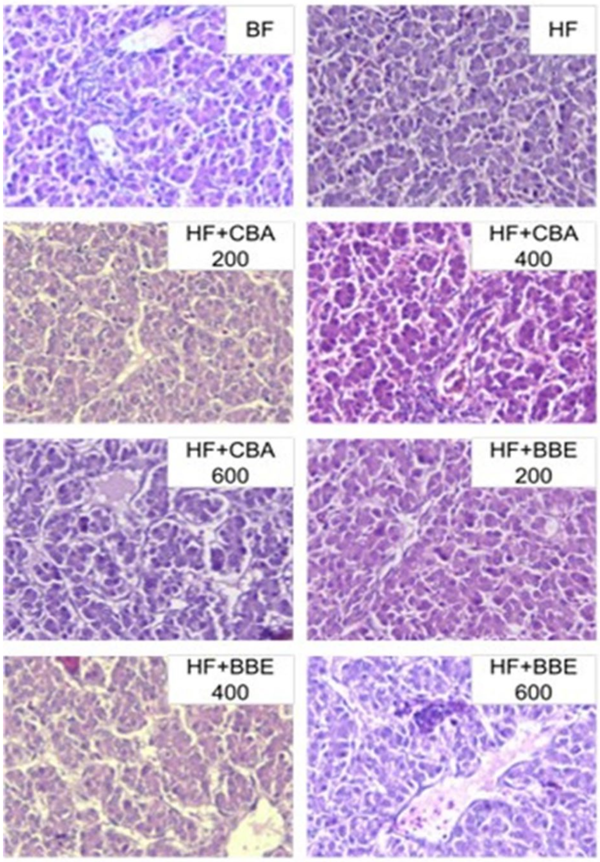

Fig. 5. Histology of broiler liver at 21 days. Magnification X40. BF: basal fat diet; HF: high-fat diet; HF+CBA: high-fat diet supplemented with commercial bile acid at levels of 200, 400, 600 mg/kg; HF+BBE: high-fat diet supplemented with bovine bile extract at levels of 200, 400, 600 mg/kg.

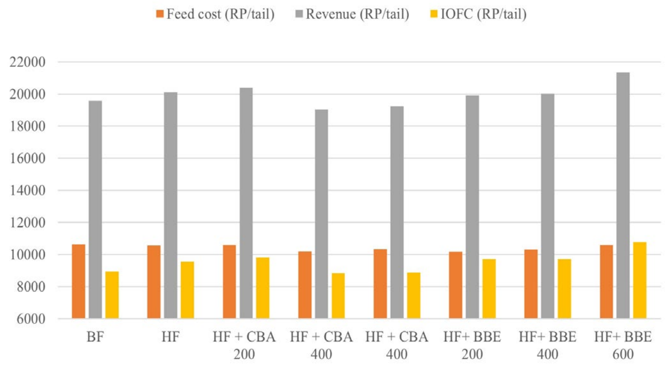

Fig. 6. Feed cost, revenue, IOFC and feed price of broiler at 21 days.BF: basal-fat content diet, HF: high-fat content diet, HF+CBA: high-fat content diet with added commercial bile acid, HFD+BBE: high-fat content diet with added bovine bile extract.

Discussion

This study confirms that supplementing high-oil-content diets with CBA at low levels enhances WG in broilers by 21 days of age. Additionally, increasing BBE levels also improved the WG and PI, but reduced the FCR. These findings are consistent with those of a previous study by Maisonnier *et al.* (2003), which demonstrated that 0.3% bile salt supplement significantly enhanced WG in broilers during the starter phase. Similarly, Parsaie *et al.* (2007) observed that supplementing feed with bile acids at 500 mg/kg significantly improved WG and FI in broilers between days 1 and 18.

The high-oil-content in the feed plays a critical role in boosting growth performance during the broiler starter phase. In this study, the groups receiving HF, HF + CBA (200), and HF + BBE (600) exhibited significant improvements in BW, WG, and PI, but had a lower FCR than the BF group during the 7–21 day feeding period. Khatun *et al.* (2018) confirmed that oil is an essential component of high-energy feed formulations in poultry, contributing significantly to improved growth performance. Oil is commonly used as a metabolizable energy (ME) source in feed to increase energy density, which can be easily stored as adenosine triphosphate (ATP) in non-ruminant animals (Jalali *et al.*, 2015).

Supplementing high-oil diets for starter broilers with BBE at 200 mg/kg CBA or 600 mg/kg yielded significantly higher feed efficiency and growth performance than that of the HF group without bile acid supplementation at 21 days. These findings suggest that lack of bile acid supplementation in high-oil diets may result in suboptimal energy absorption, as high concentrations of crude palm oil (CPO) can be difficult to digest efficiently. The improved performance of broilers fed diet supplemented with bile acid can be attributed to enhanced absorption of metabolizable energy (ME), especially when nutrients contain palmitic and oleic acids, which are better emulsified with cholic acid (Azman *et al.*, 2005). Furthermore, lower fat digestibility in young broilers can be explained by insufficient endogenous bile salts and an underdeveloped bile salt recycling system (Leeson & Summers, 2005).

Supplementation of diet with BBE at 600 mg/kg produced more favorable results than supplementation with CBA at 200 mg/kg. This superior performance of diet supplemented with BBE can be linked to the higher concentration of cholic acid (317.38 mg/g) in the bovine bile extract (Yaman and Cahyadi, 2022). Cholic acid plays a crucial role in emulsifying dietary fats, which enhances fat digestion and absorption. Polin *et al.* (1980) observed that supplementation with cholic acid at 0.04% and chenodeoxycholic acid improved WG, FCR, and reduced FI in broilers during the early growth phase (days 1-7). In contrast, Lammasak *et al.* (2018) reported that supplementation with BBE in a diet containing 6% CPO did not significantly affect BW and WG, but it showed a tendency toward lower FCR over 21 days. This discrepancy in the results may be attributed to variations in dietary composition and the specific fatty acid profiles of various fat sources.

Digestive organs, particularly the pancreas, play a vital role in the secretion of digestive enzymes, such as amylase, lipase, and trypsin, all of which are essential for nutrient digestion and absorption (Denbow, 2015). The observed increase in pancreatic weight in broilers fed a high-oil diet supplemented with CBA may indicate high enzymatic activity, especially of lipase, which is responsible for hydrolyzing dietary fats into absorbable fatty acids. Krogdahl and Sell (1984) noted that whereas lipase activity increases more slowly than that of other enzymes, its role becomes increasingly important in fat-rich diets. The significant increase in pancreatic weight associated with CBA supplementation underscores the crucial role of the pancreas in fat metabolism, particularly as it responds to the high fat content of the diet and the need for high lipase production.

Increasing levels of BBE supplementation significantly influenced the

Table 5. Orthogonal polynomial and orthogonal contrast p-value of feed cost, revenue, and Income Over Feed Cost (IOFC) in response to supplementation of commercial bile acids and bovine bile extract in high oil content starter broiler rations.

Variables	Contras Orthogonal			CBA-Polynomial Orthogonal			BBE-Polynomial Orthogonal		
	C1	C2	C3	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
Feed Cost	0.72	0.92	0.99	0.42	0.84	0.48	0.78	0.11	0.54
Revenue	0.02	0.10	0.07	0.06	0.92	0.13	0.06	0.09	0.67
IOFC	0.00	0.02	0.01	0.05	0.75	0.13	0.03	0.24	0.34

C1: BF vs HF; HF+CBA; HF+BBE, C2: HF vs HF+CBA; HF+BBE, C3: HF+CBA vs HF+BBE.

relative weight of the gallbladder and led to the elongation of both the ileum and colon. These adaptations most likely reflect the ileum's critical function as the primary site for bile acid reabsorption, which is key to the enterohepatic circulation of bile acids. Approximately 95% of bile acids are reabsorbed in the terminal ileum (Fang *et al*., 2019), thus, the length of the ileum is important in optimizing this process. Whereas the extension of the colon may represent an adaptive response to enhance both nutrient absorption and water retention, which is especially important in fat-rich diets. About 5% of bile salts enter the colon, where they are further modified by bacterial enzymes (Devlin, 2006), further highlighting the importance of colonic length in facilitating efficient digestion and absorption.

The increase in villi in the small intestine of broilers is closely related to improved digestive and absorptive functions by expanding the surface area for nutrient absorption throughout the body (Awad *et al*., 2014). Supplementing starter broiler diets with CBA at low levels increases jejunum villi height, the effect is, on average, less pronounced than that of BBE. This indicates that adding bile acids to the feed can enhance nutrient absorption capacity, which is closely related to villi height (Mantis *et al*., 2011). The F-contrast tests revealed that supplementing HFD with CBA at 200 mg/kg or BBE at 600 mg/kg significantly increased jejunal villi width ($p = 0.01$). Greater villi width increases the absorptive surface area, which is crucial in optimizing nutrient absorption in the small intestine. Higher nutrient absorption through active and passive mechanisms supports the growth and production efficiency of broilers (Denbow, 2015). These findings are consistent with those of previous studies showing that bile acid supplementation can affect intestinal morphology, particularly in enhancing villi dimensions, thereby expanding the absorptive area (Ruttanavut *et al*., 2009).

Metabolic processes in the liver involve various pathways, such as glycolysis and beta-oxidation, which are essential for energy production. In young broilers, glucose for glycolysis generally comes from endogenous sources, whereas lipids may come from the diet or endogenous processes, such as adipogenesis and lipogenesis (Salway 2004; Dridi *et al*., 2022). Use of bile salt can reduce liver fat deposition caused by high-energy diets (Buyse *et al*., 2004). However, in this study, liver histopathology analysis showed that high-oil-content, and CBA and BBE supplementation did not cause significant changes in the structure of liver tissue (Fig. 5).

Although HF content in the diet may increase the risk of hepatic steatosis, supplementation with emulsifiers, such as BBE at 600 mg/kg, can help reduce liver fat accumulation by directing lipids to more efficient body depots (Dersjant-Li and Peisker 2005; Roy *et al*., 2010). Use of bile salt does not affect the histopathology of organs, such as the liver, kidneys, and intestines, but increases the rate of lipid removal from the liver, thereby reducing the risk of excessive fat accumulation (Roy *et al*., 2010; Lai *et al*., 2018;).

The significant increase in fat digestibility associated with supplementing HFD with high levels of BBE suggests that BBE is more effective than CBA in enhancing fat digestion and absorption. These findings are supported by previous studies, such as Alzawqari *et al*. (2011), who demonstrated that bovine bile supplementation significantly improved fat digestibility in broilers. These improvements indicate the ability of BBE to promote fat metabolism, which is important in high-oil diets where the emulsification and absorption of fats are critical. Additionally, supplementing HF diet with both CBA and BBE significantly enhanced fat digestibility, indicating a pivotal role of bile acids in fat metabolism. In line with these findings, Ge *et al*. (2019) reported that bile acid supplementation in HFDs improves fat digestibility and modulates gut microbiota composition, further contributing to enhanced lipid metabolism and overall feed efficiency. Polin *et al*. (1980) emphasized that bile acids and bile salts are necessary in enhancing the absorption of saturated fatty acids, particularly in high-fat dietary conditions. BBE is more effective than CBA in improving fat digestibility, suggesting that the quality and quantity of supplementation significantly influence the effectiveness of bile acids in fat digestion.

The economic analysis of the treatments showed that both CBA and BBE supplementation increased revenue and IOFC. Supplementing diet with BBE at 600 mg/kg produced the highest IOFC, even surpassing that of CBA at 200 mg/kg. This suggests that although both additives are effective, BBE at higher concentrations provides superior economic returns due to its greater impact on growth performance and feed efficiency. These results highlight the potential of BBE as a cost-effective additive in broiler diets, particularly in high oil feeding regimes. Nwoche *et al*. (2003) reported similar results highlighting that supplementation of optimal levels of oil improved feed efficiency and economic performance, supporting the economic benefits of incorporating fat-digesting additives, such as BBE in broiler production systems.

Conclusion

This study provides practical insights into the use of BBE and CBA as diet supplements to improve growth performance, fat digestibility, and economic outcomes in broilers fed high-fat diets. The findings suggest that supplementing broiler starter diets with BBE at 600 mg/kg can lead to higher feed efficiency and economic returns, making it a promising additive for large-scale broiler production. Future research should explore the effects of these additives in the various production phases, including their potential to improve meat quality and carcass composition in finisher diets.

Conflict of interest

The authors have no conflict of interest to declare.

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