Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2021) 45: 148-157
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doi:10.3906/vet-2008-52

Effect of lipase and bile acids on growth performance, nutrient digestibility, and meat quality in broilers on energy-diluted diets

Muhammad SHOAIB, Shaukat Ali BHATTI*, Haq NAWAZ, Muhammad SAIF-UR-REHMAN
Institute of Animal and Dairy Sciences, Faculty of Animal Husbandry, University of Agriculture, Faisalabad, Punjab, Pakistan

Received: 17.08.2020 • **Accepted/Published Online:** 20.12.2020 • **Final Version:** 23.02.2021

Abstract: The objective of this study was to evaluate the effect of bile acid and lipase alone and in combination form on growth performance and meat quality parameter of broilers reared on low-energy diet. Five hundred and twenty day-old broiler birds were divided into eight treatments with 5 replicates of 13 birds each to evaluate the effects of lipase alone and its combination with bile acids as emulsifier in broilers reared on 75 and 150 kcal/kg reduced-energy diets. Eight diets—PC (positive control), NC1 (negative control 1), NC1L (NC1 + lipase at 0.015%), NC1LB (NC1 + lipase at 0.015% + bile acids at 0.05%), NC2 (negative control 2), NC2L (NC2 + lipase at 0.015%), NC2LB (NC2 + lipase at 0.015% + bile acids at 0.05%), and NC2 (2LB) (NC2 + 2x (lipase at 0.03% + bile acids at 0.1%))—were formulated. Weight gain was higher ($P < 0.05$) in birds of the NC1L and NC1BL groups and it was lower ($P < 0.05$) in birds of the NC2 and NC2L groups. Protein efficiency ratio, European production efficiency factors, and feed conversion ratio were better ($P < 0.05$) in birds of the NC1L, NC1LB, and NC2 (2BL) groups and poor in birds of the NC2 group, whereas feed intake was not affected ($P > 0.05$) by dietary treatments. Dressing percentages and water holding capacity were higher ($P < 0.05$) and cooking loss was lower ($P < 0.05$) in birds of the NC1BL group, whereas lower ($P < 0.05$) dressing percentage and water holding capacity and higher ($P < 0.05$) cooking loss were recorded in birds of the NC2 group. Birds of the PC and NC1BL groups had higher ($P < 0.05$) crude protein and ether extract digestibility, while lower ($P < 0.05$) digestibilities of ether extract and crude protein were recorded in the NC2 groups. In conclusion, addition of lipase alone or combined with bile acids as emulsifier improved growth performance, nutrient digestibility, and meat quality in broilers reared on 75 kcal reduced energy diet.

Key words: Lipase, bile acids, energy-diluted diet, growth performance, meat quality, serum biochemistry

1. Introduction

Fats and vegetable oils are used in broiler diets to increase their energy density [1,2]. Energy is considered a major dietary component, which can affect the utilization of nutrients [3]. Adding fat has some negative impacts due to the limited digestive ability of fat by broilers, which compromises its function of promoting growth [4,5]. Poor digestion and absorption of lipids have been observed in broiler chickens during early age [6]. A negative impact on other nutrients' intake and body composition of broilers has been confirmed due to high dietary fat. Immature physiological function at an early age of broilers can lead to low production of bile acids and pancreatic lipase [7]. Fat utilization is not efficient in broilers due to low lipase activity until it reaches the optimum level between 40 and 56 days of age. That is why it is important to improve fat utilization of broilers.

Fat utilization in broiler chickens has been improved by using lecithin and lysolecithin as emulsifier for decades. Recently, bile acids have been used as a dietary emulsifier in poultry production. The utilization of bile acids during the early stage in chicks has more potential to improve fat digestion and absorption than older ones [8–10]. Similar to bile acids, exogenous lipase may also improve the physiological capacity of the poultry digestive system. Lipase production is widespread among yeasts having different properties. Using yarrowia lipolytic lipase in broiler diets improved feed conversion ratio (FCR) and had no adverse effect on feed intake for a 42-day period [11]. Supplementation of dietary lipase in broilers fed low-fat diet resulted in a better response on growth performance and fat digestibility [12]. Meat quality and health status of broilers were also improved with supplementing emulsifier or multienzyme [13]. Feeding low-energy diets to broiler chickens resulted in reduced performance but supplementing emulsifier (bile acids) or lipase in low-energy diets alleviated the negative effects. Therefore, this study was planned to evaluate the effects of lipase and bile

* Correspondence: shaukat.ali@uaf.edu.pk

acids on production performance in a reduced-energy diet.

2. Materials and methods

The present study was conducted at Research House, Animal Nutrition Center, University of Agriculture, Faisalabad with prior approval from the animal care and use committee of the University of Agriculture, Faisalabad via letter no. 15497-500.

2.1. House cleaning and preparation

Before chick arrival, the house was cleaned, washed, fumigated, and closed to minimize the microbial load. The experimental trial was conducted under all hygienic and standard conditions. Birds were vaccinated with ND+IB (day 1), IBD (day 8), IBD (day 18), and ND (day 25) vaccine.

2.2. Experimental birds and diet

The objective of this experiment is to evaluate the efficacy of lipase and bile acids in broilers reared on energy-diluted diet. Five hundred and twenty day-old broiler birds were divided in to eight treatments with five replicates of 13 birds each. Eight diets—PC (positive control), NC1 (negative control 1), NC1L (NC1 + lipase at 0.015%), NC1LB (NC1 + lipase at 0.015% + bile acids at 0.05%), NC2 (negative control 2), NC2L (NC2 + lipase at 0.015%), NC2LB (NC2 + lipase at 0.015% + bile acids at 0.05%), and NC2 (2LB) (NC2 + 2x (lipase at 0.03% + bile acids at 0.1%))—were formulated. The bile acids were composed of hyocholic acid, hyodeoxycholic acid, and chenodeoxycholic acid (Table 1).

2.3. Data collection

2.3.1. Growth performance

Data on the following parameters were recorded every 7 days. Body weights of birds were measured at the end of each subsequent week. Feed intake was calculated by subtracting feed refusal from feed offered. Feed conversion ratio (FCR) was calculated by dividing feed intake (g) with weight gain (g). Protein efficiency ratio was calculated by dividing weight gain (g) with protein intake [14]. Energy efficiency ratio was calculated by dividing weight gain by energy consumed (kcal) and multiplying the result with 100 [14]. European production efficiency factor was calculated by multiplying livability and live weight (kg) with 100 and dividing the result by FCR and age (days) [15].

2.3.2. Nutrient digestibility

The indirect marker method was used for determining nutrient digestibility. For this purpose, acid insoluble ash (Celite) was included in experimental diets at 1%. Feces were collected on days 21 and 35 of the experiment. Flex sheets were placed under each pen and droppings were collected three times a day. Proximate analysis of feed and feces were determined [16].

2.3.3. Slaughter parameters

On day 35, two birds (preweighed) from each pen were slaughtered. After slaughtering, the birds were unfeathered and heart, liver (without gallbladder), gizzard (removal of content), and breast muscle were collected for calculation of the eviscerated weight as described in the literature [17].

2.3.4. Meat quality parameters

Meat samples from breast meat were collected at the end of trial and frozen for further analysis. To measure pH of breast muscles, approximately 1.5 g of ground breast meat was homogenized in 10-mL water and pH was measured using a pH meter (Milwaukee MW102) [18] at 3 h postslaughtering. A weighed meat sample (15 g) was centrifuged at 5000 rpm for 15 min at 4 °C in a stainless steel tube. Water was decanted off immediately to stop its reabsorption. Meat sample was reweighed to determine liquid loss [19]. Approximately 40 g or 2 × 5 cm meat cut was taken and cooked to an internal temperature of 75 ± 1 °C in water bath (80 ± 0.5 °C) for 30 to 35 min. After cooling the meat, cooking loss was calculated as weight loss from meat [20]. Meat samples from breast fillet were dried to measure dry matter, crude protein, crude fat, and crude ash according to the procedures described by AOAC [21].

2.3.5. Blood sampling

Blood samples were collected from wing vein at the end of the experiment. Each blood sample was divided into two equal parts. One part was transferred to EDTA-containing vacutainer and the other one was used for plasma collection by centrifuging the sample at 6000 rpm for 10 min. The supernatant plasma was collected, placed in plastic Eppendorf tubes, and stored at –20 °C until further analysis (urea, uric acid, triglycerides, cholesterol, HDL, LDL, and atherogenic index (LDH/HDL)) [22].

2.3.6. Economics

Expenditures incurred on chicks, feed, litter, and medication were used for calculation of cost of production [15].

2.4. Statistical analysis

The obtained data were subjected to statistical analysis using analysis of variance technique by completely randomized design using Minitab 17 and treatment means were compared using Tukey's test [23].

3. Results

Weight gain was higher ($P < 0.05$) in birds of NC1L and NC1LB and lower ($P < 0.05$) in birds of the NC2 and LC2L groups. Protein efficiency ratio, European production efficiency factor, and FCR were improved ($P < 0.05$) in birds of NC1L, NC1LB, and NC2 (2LB) whereas they were poor ($P < 0.05$) in birds of the NC2 group while feed intake was not affected ($P > 0.05$) by addition of lipase and bile

Table 1. Ingredients composition of experimental diets.

Ingredients	Starter phase			Finisher phase		
	PC (Recommended energy)[1]	NC1 (75 kcal RE)[2]	NC2 (150 kcal RE)[3]	PC (Recommended energy)[1]	NC1 (75 kcal RE)[2]	NC2 (150 kcal RE)[3]
Corn	52.38	54.04	54.87	55.79	57.61	59.44
Soybean meal 45%	38.94	38.63	38.4	34.6	34.27	33.93
Molasses	0	0	0.89	0	0	0
Oil	3.78	2.33	1	6.12	4.62	3.12
Calcium carbonate	0.9	0.91	0.89	0.73	0.73	0.73
Dicalcium phosphate	2.16	2.16	2.16	1.75	1.75	1.75
Sodium chloride	0.39	0.47	0.37	0.32	0.32	0.32
Sodium bicarbonate	0.31	0.31	0.26	0.04	0.04	0.04
L-lysine sulphate	0.35	0.36	0.37	0.08	0.09	0.1
DL-methionine	0.37	0.37	0.37	0.26	0.26	0.26
L-threonine	0.11	0.11	0.11	0	0	0
Vitamin premix*	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix**	0.15	0.15	0.15	0.15	0.15	0.15
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100	100
Nutrient (calculated)						
Dry matter fed	88.63	88.48	88.19	88.11	88.95	88.79
Metab. energy	3000	2925	2850	3200	3125	3050
Crude protein	22.00	22.00	22.00	20.00	20.00	20.00
Ether extract	5.98	4.60	3.30	8.39	6.96	5.52
Crude fiber	2.94	2.96	2.99	2.80	2.82	2.84
Ash	4.91	4.99	4.96	4.34	4.33	4.32
Calcium	0.96	0.96	0.96	0.79	0.79	0.79
Available phosphorus	0.48	0.48	0.48	0.40	0.40	0.40
Sodium	0.25	0.28	0.24	0.15	0.15	0.15
Potassium	0.88	0.88	0.91	0.81	0.81	0.81
Chlorine	0.30	0.35	0.30	0.26	0.26	0.26
DEB	250	250	250	200	200	200
Dig. lysine	1.28	1.28	1.28	1.03	1.03	1.03
Dig. methionine	0.67	0.67	0.67	0.54	0.54	0.54
Dig. met + cys	0.95	0.95	0.95	0.80	0.80	0.80
Dig. threonine	0.86	0.86	0.86	0.69	0.69	0.69
Dig. tryptophan	0.25	0.25	0.25	0.23	0.23	0.23
Dig. arginine	1.40	1.40	1.40	1.28	1.28	1.28
Dig. leucine	1.70	1.70	1.70	1.59	1.60	1.60
Dig. isoleucine	0.86	0.86	0.86	0.79	0.79	0.78
Dig. valine	0.92	0.92	0.92	0.85	0.85	0.85
Dig. histidine	0.53	0.53	0.53	0.49	0.49	0.49
Dry matter fed	88.63	88.48	88.19	88.11	88.95	88.79

Table 1. (Continued).

Nutrients (analyzed)						
DM	89.58	89.12	88.86	90.30	90.5	90.69
CP	21.28	22.10	22.09	20.3	19.79	19.95
EE	5.87	5.29	5.13	7.81	7.03	6.46
AIA	1.34	1.28	1.30	1.28	1.24	1.31

*Vitamins premix provides 10,000 IU Vitamin A, 5 mg riboflavin, 12 mg Ca pantothenate, 2.2 mg thiamin, 1.55 mg folic acid, 44 mg nicotinic acid, 2.2 mg vitamin B_6, 12.1 µg vitamin B_{12}, 250 mg choline chloride, 0.11 mg d-biotin, 1100 IU vitamin D_3, 11.0 IU vitamin E, 1.1 mg vitamin K per kg of diet.
**Mineral premix provides 30 mg Fe, 50 mg Zn, 5 mg Cu, 60 mg Mn, 0.1 mg Co, 0.3 mg I and 1 mg Se per kg of diet.
Dig.: digestible
[1]PC (positive control),
[2]NC1 (negative control 1), NC1L (NC1 + lipase at 0.015%), NC1LB (NC1 + lipase at 0.015% + bile acids at 0.05%),
[3]NC2 (negative control 2), NC2L (NC2 + lipase at 0.015%), NC2LB (NC2 + lipase at 0.015% + bile acids at 0.05%) and NC2 (2LB) (NC2 + 2x (lipase at 0.03% + bile acids at 0.1%))

acids in energy-diluted diets. Mortality percentage was higher (P < 0.05) in birds of the NC2 group (Table 2).

Dressing percentages were higher (P < 0.05) in birds of the NC1LB group and they were lower (P < 0.05) in birds of the NC2 group, while breast and thigh meat yield were not affected (P > 0.05) by dietary treatments. Weight of relative organs includes heart, liver, gizzard, and abdominal fat pad. The results showed that dietary treatments had no effect (P > 0.05) on weights of relative organs (Table 3).

Water holding capacity of broiler breast meat was higher (P < 0.05) in birds of the NC1LB group while it was lower (P < 0.05) in the NC2LB group. The pH was not affected (P > 0.05) among dietary treatments while cooking loss was higher (P < 0.05) in birds of the NC2 group and lower (P < 0.05) in birds of the NC1LB group. Ether extract content of broiler breast meat was higher (P < 0.05) in birds of the PC group and it was lower (P < 0.05) in birds of the NC1, NC1LB, NC2LB, and NC2 (2LB) groups. However, moisture, ash, and CP percentage were not affected (P > 0.05) by different dietary treatments (Table 4).

Digestibilities of Dry matter (DM), ether extract (EE), and crude protein (CP) were calculated through an indirect marker method using Celite. EE digestibility was higher (P < 0.05) in birds of the NC1LB group and lower in birds of the NC1, NC2, and NC1L groups on day 21; however, digestibilities of DM and CP were not affected (P > 0.05) by treatments. CP digestibility was higher (P < 0.05) in birds of the NC1LB group and EE digestibility was greater (P < 0.05) in birds of the PC and NC1LB groups and birds of the NC2 group had lower (P < 0.05) digestibilities of CP and EE on day 35 (Table 5).

Serum biochemistry analysis contains total proteins, albumins, globulins, albumin/globulins, triglycerides, cholesterol, HDL, LDL, and atherogenic index test. Atherogenic index was lower (P < 0.05) in birds of NC1LB and higher in the NC2 group, while other serum biochemistry parameters were not affected among different treatments (Table 6). Blood hematology parameters (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT) were not affected (P > 0.05) by dietary treatment (Table 7). Production cost per kg live weight was lower (P < 0.05) in birds of NC1L, NC1LB, and NC2 (2LB) whereas it was higher in birds of the NC2 group (Table 8).

4. Discussion

Weight gain was higher (P < 0.05) in birds of NC1L and NC1LB and lower (P < 0.05) in birds of the NC2 and LC2L groups. This might be because bile acid and lipase increase the energy value of oil used in this experiment. Our results are consistent with those of Hu et al. [12] who concluded that the use of 0.03% lipase in broilers fed lower-energy diet had improved FCR, but body weight gain was not affected. Soya lecithin (50% of oil in basal diet) and lipase (100,000 IU/ton) in broiler diet had higher weight gain, feed consumption, and better FCR [24]. Maisonnier et al. [8] showed that addition of 0.3% bile salts yielded better (P < 0.05) body weight gain (440 vs 399 g) during 7–21 days in broiler chickens. However, Al-Marzooqi and Leeson [25] evaluated the different levels of supplementary lipase enzyme (0, 0.37%, 0.75%, 1.12%) and reported that with increasing level of lipase enzyme, FCR was improved (P < 0.05). In contrast, Wang et al. [11] tested the effect of dietary lipase supplementation of three levels of lipase enzyme (0, 4U/g, and 6U/g) on broilers and concluded that lipase did not have any effect on growth rate and final body weight in broilers during 42 days. Nazir [26] tested the effect of dietary supplementation of three levels of bile acids (0, 0.03% and 0.06%) on broilers and concluded that bile acids did not affect growth rate in broilers during 35 days. Lipase addition at 0.02% did not affect the

Table 2. Effects of lipase alone and in combination with emulsifier on growth performance in broilers reared on energy-diluted diet.

Treatments	Feed intake (g)	Weight gain (g)	FCR	PER	EER	EPEF
PC	3289.07	2061.94[ab]	1.60[b]	3.02[a]	2.02[ab]	371.96[a]
NC1	3283.41	2018.10[ab]	1.63[ab]	2.96[a]	1.98[ab]	358.08[ab]
NC1 + lipase	3363.65	2120.99[a]	1.59[b]	2.99[a]	2.03[ab]	379.94[a]
NC1 + bile acids + lipase	3383.62	2146.62[a]	1.58[b]	3.08[a]	2.05[a]	386.01[a]
NC2	3473.14	1961.68[b]	1.77[a]	2.63[b]	1.82[b]	304.35[b]
NC2 + lipase	3273.92	1963.02[b]	1.67[ab]	2.93[ab]	1.93[ab]	343.72[ab]
NC2 + bile acids + lipase	3413.99	1977.22[b]	1.72[ab]	2.79[ab]	1.87[ab]	334.75[ab]
NC2 + 2x (bile acids + lipase)	3254.27	2055.92[ab]	1.58[b]	3.01[a]	2.04[a]	368.84[a]
SEM	81.2	28.5	0.04	0.07	0.05	13.3
P-value	0.493	0.0001	0.005	0.001	0.007	0.002

SEM: Standard error of the mean
P > 0.05 (Nonsignificant), P < 0.05 (Significant)
Values with different superscript letters in the same column differ significantly
PER: Protein efficiency ratio, EER: Energy efficiency ratio, EPEF: European production efficiency factor
PC: Recommended energy, NC1: 75 kcal.kg lower than the recommended energy, NC2: 150 kcal.kg lower than the recommended energy

Table 3. Effects of lipase alone and in combination with emulsifier on carcass characteristics in broilers reared on energy-diluted diet.

Treatments	Dressing percentage	Breast yield*	Thigh yield*	Heart weight**	Gizzard weight**	Liver weight**	Abdominal fat weight**
PC	62.39[ab]	63.70	36.30	0.50	1.85	1.89	2.01
NC1	61.05[ab]	61.39	38.61	0.54	1.68	2.27	1.84
NC1 + lipase	60.70[ab]	61.73	38.27	0.54	1.65	2.02	1.51
NC1 + bile acids + lipase	64.17[a]	61.39	38.61	0.46	1.59	1.92	1.47
NC2	61.12[ab]	61.76	38.24	0.53	1.61	2.20	1.83
NC2 + lipase	61.88[ab]	61.14	38.86	0.40	1.65	1.95	1.50
NC2 + bile acids + lipase	59.94[ab]	60.71	39.29	0.44	1.46	2.07	1.80
NC2 + 2x (bile acids + lipase)	59.07[b]	62.23	37.77	0.44	1.45	2.24	2.07
SEM	1.01	0.86	0.86	0.04	0.12	0.22	0.18
P-value	0.045	0.375	0.375	0.071	0.433	0.854	0.121

P > 0.05: Nonsignificant, P < 0.05: Significant
*Breast and thigh yield (% to carcass weight)
**Relative organ (liver, gizzard, and heart) weight and abdominal fat (% to live weight)
SEM: Standard error of the mean
P > 0.05 (Nonsignificant), P < 0.05 (Significant)
Values with different superscript letters in the same column differ significantly
PC: Recommended energy, NC1: 75 kcal.kg lower than the recommended energy, NC2: 150 kcal.kg lower than the recommended energy

production performance of broilers fed different sources of oil (beef tallow and canola oil) [27]. Piekarski et al. [28] reported that supplementing 0.01% and 0.5% bile acids (chenodeoxycholic acid) lowered body weight 3–6% and 7–11%, respectively, compared to the control group.

Dressing percentages was higher (P < 0.05) in birds of the NC1LB group and it was lower (P < 0.05) in birds of the NC2 group, while breast and thigh meat yield were not affected (P > 0.05) by dietary treatments. Weight of relative organs includes heart, liver, gizzard, and abdominal fat pad. Increased dressing percentage in broilers fed diet containing bile acids + lipase in NC1 diet may be due to the addition of fat which increases the edible portion of meat. Our results are consistent with those of the

Table 4. Effects of lipase alone and in combination with emulsifier on meat quality and meat proximate in broilers reared on energy-diluted diet**.**

Treatments	Meat quality parameters			Proximate composition (%)			
TRT	WHC %	pH	Cooking loss	Moisture	Ash	CP	EE
PC	63.72^{ab}	6.00	26.09^{ab}	74.59	4.66	20.87	1.63^{a}
NC1	60.10^{ab}	6.01	29.44^{ab}	74.29	4.19	19.28	1.28^{b}
NC1 + lipase	61.90^{ab}	5.96	29.77^{ab}	74.13	4.67	19.85	1.32^{ab}
NC1 + bile acids + lipase	64.28^{a}	5.93	25.89^{b}	74.25	4.22	20.34	1.28^{b}
NC2	58.01^{ab}	5.98	32.08^{a}	73.38	4.44	19.39	1.36^{ab}
NC2 + lipase	62.06^{ab}	6.00	29.69^{ab}	73.21	4.41	20.85	1.44^{ab}
NC2 + bile acids + lipase	56.59^{b}	5.96	31.17^{ab}	73.82	3.90	20.09	1.25^{b}
NC2 + 2x (bile acids + lipase)	59.52^{ab}	5.96	28.71^{ab}	72.71	4.60	20.52	1.24^{b}
SEM	1.56	0.05	1.26	0.49	0.26	0.08	0.47
P-value	0.033	0.950	0.032	0.159	0.376	0.016	0.156

SEM: Standard error of the mean
P > 0.05 (Nonsignificant), P < 0.05 (Significant)
Values with different superscript letters in the same column differ significantly
PC: Recommended energy, NC1: 75 kcal.kg lower than the recommended energy, NC2: 150 kcal.kg lower than the recommended energy

Table 5. Effects of lipase alone and in combination with emulsifier on nutrient digestibility in broilers reared on energy-diluted diet.

Treatments	Day 21			Day 35		
TRT	DM (%)	EE (%)	CP (%)	DM (%)	EE (%)	CP (%)
PC	89.23	63.71^{ab}	66.67	83.27	69.25^{a}	65.98^{ab}
NC1	88.15	61.54^{b}	63.64	84.56	64.47^{ab}	63.55^{b}
NC1 + lipase	89.10	61.19^{b}	66.60	85.15	62.69^{ab}	68.32^{ab}
NC1 + bile acids + lipase	88.75	68.44^{a}	67.47	84.03	67.65^{a}	69.32^{a}
NC2	89.86	60.63^{b}	64.46	85.28	59.70^{b}	63.55^{b}
NC2 + lipase	87.91	64.61^{ab}	63.47	85.32	59.83^{b}	66.62^{ab}
NC2 + bile acids + lipase	88.21	64.30^{ab}	64.89	85.57	61.03^{b}	66.14^{ab}
NC2 + 2x (bile acids + lipase)	89.39	65.97^{ab}	65.63	84.55	59.65^{b}	67.00^{ab}
SEM	0.511	1.33	1.51	0.87	1.44	1.15
P-value	0.119	0.003	0.473	0.588	0.001	0.013

SEM: Standard error of the mean
P > 0.05 (Nonsignificant), P < 0.05 (Significant)
Values with different superscript letters in the same column differ significantly
PC: Recommended energy, NC1: 75 kcal.kg lower than the recommended energy, NC2: 150 kcal.kg lower than the recommended energy

following studies. Supplementing bile acids (0.008%) increased carcass weight/body weight (93.02% vs 90.25%) as compared to the control [29]. Soya lecithin (50% of oil in basal diet) and lipase (100,000 IU/ton) treatments had higher carcass yield in broilers than the control group, while muscle pH was not affected by treatments [24].

Water holding capacity was higher (P < 0.05) in birds of the NC1LB group and lower in birds of the NC2LB group.

Table 6. Effects of lipase alone and in combination with emulsifier on serum biochemistry in broilers reared on energy-diluted diet.

Treatments	Total protein	Albumin	Globin	Albumin: globin	Triglycerides	Cholesterol	HDL	LDL	Atherogenic index
	(g/L)	(g/L)	(g/L)		(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)	
PC	5.41	1.70	3.57	0.49	151.33	118.67	52.33	36.07	0.69^{ab}
NC1	5.37	1.77	3.67	0.49	135.33	132.33	63.00	42.27	0.67^{ab}
NC1 + lipase	5.03	1.53	3.77	0.41	127.67	133.33	68.33	39.47	0.60^{ab}
NC1 + bile acids + lipase	5.07	1.83	3.07	0.61	131.33	117.33	65.00	26.07	0.45^{b}
NC2	5.06	1.47	3.27	0.45	137.00	139.67	59.33	52.93	0.88^{a}
NC2 + lipase	5.13	1.87	3.27	0.57	130.00	128.00	62.00	40.00	0.65^{ab}
NC2 + bile acids + lipase	4.90	1.57	3.23	0.49	125.33	121.33	58.67	37.60	0.64^{ab}
NC2 + 2x (bile acids + lipase)	4.90	1.57	3.07	0.51	119.00	127.33	63.00	40.53	0.65^{ab}
SEM	0.12	0.13	0.19	0.06	18.8	12.5	8.74	5.67	0.08
P-value	0.069	0.312	0.118	0.377	0.961	0.895	0.944	0.177	0.011

SEM: Standard error of the mean
P > 0.05 (Nonsignificant), P < 0.05 (Significant)
Values with different superscript letters in the same column differ significantly
PC: Recommended energy, NC1: 75 kcal.kg lower than the recommended energy, NC2: 150 kcal.kg lower than the recommended energy

Table 7. Effects of lipase alone and in combination with emulsifier on blood hematology in broilers reared on energy-diluted diet.

Treatments	WBC 10^3/µL	RBC 10^6/µL	HGB g/dL	HCT (%)	MCV (+FL)	MCH (+pg)	MCHC (g/dL)	PLT 10^3/µL
PC	16.87	1.47	10.58	31.50	149.63	50.27	31.57	13.53
NC1	15.26	1.55	12.63	43.57	154.77	46.70	35.03	15.46
NC1 + lipase	16.47	1.57	12.33	31.47	149.83	51.07	33.97	16.93
NC1 + bile acids + lipase	18.15	1.69	11.33	32.40	143.23	49.03	32.13	18.53
NC2	15.18	1.51	11.97	28.13	141.37	47.32	30.80	17.12
NC2 + lipase	17.23	1.59	11.92	25.67	151.10	47.47	33.63	20.91
NC2 + bile acids + lipase	15.55	1.33	12.40	31.77	146.30	48.93	33.23	15.50
NC2 + 2x (bile acids + lipase)	15.97	1.81	12.40	32.00	150.30	45.03	31.47	16.30
SEM	1.36	0.198	1.27	3.68	4.19	2.28	1.22	3.03
P-value	0.756	0.810	0.949	0.119	0.407	0.633	0.256	0.795

SEM: Standard error of the mean
P > 0.05 (Nonsignificant), P < 0.05 (Significant)
PC: Recommended energy, NC1: 75 kcal.kg lower than the recommended energy, NC2: 150 kcal.kg lower than the recommended energy
White blood count (WBC); Red blood count (RBC); Hemoglobin (HGB); Hematocrit count (HCT); Mean corpuscular volume (MCV); Mean corpuscular hemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC); Platelet count (PLT)

This might be due to the increased metabolic activity of lipid utilization. The pH was not affected (P > 0.05) by dietary treatments. Cooking loss was higher (P < 0.05) in birds of NC2 and lower (P < 0.05) in birds of the NC1LB group. In contrast, Arshad et al. [30] reported that lipase at 0.018% and bile acid at 0.03% addition in low-energy diet had no effect on WHC and pH of breast meat. Adding 0.015% and 0.03% lipase enzyme to reduced-energy diet did not affect muscle pH, drip loss, and water holding capacity [12].

CP digestibility was higher (P < 0.05) in birds of the NC1LB group and EE digestibility was greater (P < 0.05) in birds of the PC and NC1LB groups and birds of the NC2 group had lower (P < 0.05) digestibilities of CP and EE. This might be due to the addition of emulsifier-accelerated micelle formation leading to an increase in fat absorption.

Table 8. Effects of lipase alone and in combination with emulsifier on economics efficiency in broilers reared on energy-diluted diets.

Production cost (Rs.)	PC	NC1	NC1L	NC1LB	NC2	NC2L	NC2LB	NC2 (2LB)	SEM	P-value
Starter phase (1–21 days)										
Bird cost	18	18	18	18	18	18	18	18	-	-
Average feed intake (g)	1595.1	1526.3	1579.3	1600.3	1658.1	1523.7	1612.4	1558.4	36.2	0.184
Feed cost per kg	58.8	57.2	57.4	57.8	55.6	55.7	56.1	56.7	-	-
Feed cost per bird	93.8	87.3	90.6	92.5	92.1	84.9	90.5	88.4	2.07	0.076
Finisher phase (22–35 days)										
Average feed intake (g)	1694.0	1757.1	1784.3	1783.3	1815.1	1750.2	1801.6	1695.9	58.1	0.742
Feed cost per kg	58.2	56.6	56.7	57.2	55.0	55.1	55.6	56.1	-	-
Feed cost per bird	98.6	99.4	101.2	102.0	99.8	96.4	100.1	95.2	3.27	0.833
Overall Period (1–35 days)										
Feed cost per bird	192.4	186.8	191.8	194.5	191.9	181.3	190.6	183.6	4.58	0.420
Miscellaneous[1]	30	30	30	30	30	30	30	30	-	-
Production cost per bird[2]	240.4	234.8	239.8	242.5	241.9	229.3	238.6	231.8	4.51	0.348
Average body weight (g)	2104.0[ab]	2059.9[ab]	2163.7[a]	2188.4[a]	2003.9[b]	2004.9[b]	2019.5[b]	2098.6[ab]	28.6	0.0001
Production cost per kg	114.3[ab]	114.2[ab]	110.9[b]	110.8[b]	120.7[a]	114.4[ab]	118.1[ab]	110.5[b]	2.10	0.015

[1] Miscellaneous cost include vaccination cost, farm preparation, and brooding expenditures
[2] Production cost per bird = Bird cost + Feed cost per bird + Miscellaneous
Values with different superscript letters in the same column differ significantly
PC: Recommended energy, NC1: 75 kcal.kg lower than the recommended energy, NC2: 150 kcal.kg lower than the recommended energy

Similar findings were noted by Hu et al. [12] who showed that the addition of 0.03% lipase enzyme had higher dry matter and ether extract digestibility than the control and reduced-energy diet [12]. Alzawqari et al. [9] reported that fat digestibility was improved ($P < 0.05$) from 51.9% to 68.9% and 78.8% with supplementation of 0.25% and 0.50% desiccated ox bile, respectively, during starter phase. The addition of lipase improved apparent ileal digestibility of organic matter, DM, CP, ash, and minor fatty acids [31]. Al-Marzooqi and Leeson [32] reported that supplementation of 0.0714% lipase enzyme had a positive ($P < 0.05$) effect on fat digestibility (83.5% vs 75.6%). In contrast, lipase addition at 0.02% in broiler diet did not affect fat, starch, and nitrogen digestibility of broiler fed various sources of oil (beef tallow and canola oil) [27]. Dierick and Decuypere [31] reported that addition of lipase and lysoforte (emulsifier) did not produce any significant improvement in fat digestibility while there was an increase in digestibility of some minor fatty acids.

Atherogenic index was lower ($P < 0.05$) in birds of NC1LB and higher ($P < 0.05$) in birds of the NC2 group. Bile acids, in addition to increased cholesterol, lead to a decrease in the solubilization of cholesterol. Birds fed diet containing 0.03% lipase enzyme in a reduced-energy diet had lower triglycerides and low-density lipoprotein than the control diet [12]. According to Ge et al. [33] supplementation of bile acids caused a decrease in TG (0.40 mmol/L vs 0.50 mmol/L and LDL cholesterol (1.01 vs 1.29 mmol/L). Hemati Matin et al. [34] reported that supplementation of bile acids did not have any effect on total serum cholesterol, triglycerides, and HDL. However, the amount of LDL seemed to be reduced ($P < 0.05$). Lipase alone or with a combination of enzymes in reduced-energy diets did not affect ($P > 0.05$) blood hematology parameters. According to Ge et al. [33], high-energy diet had no effect on serum TG, TC, HDL, and LDL when compared to the control.

In conclusion, birds reared on 75 and 150 kcal/kg reduced-energy diet had poor growth performance; however, addition of lipase alone or in combination with bile acids as emulsifier improved growth performance, nutrient digestibility, meat quality, and economic efficiency in broilers reared on 75 kcal reduced-energy diet.

Acknowledgment
The authors acknowledge the fellowship provided by the Higher Education Commission Pakistan (HEC) to Muhammad Shoaib under the framework of HEC Indigenous PhD Fellowship Program

Conflict of interest
We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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