South African Journal of Animal Science 2021, 51 (No. 5)

Early phase dietary supplementation of lipase and lecithin affects performance, haematology and immunology of broilers

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(Submitted 6 September 2021; Accepted 24 October 2021; Published 9 November 2021)

Abstract

A total of 192 one-day-old Ross 308 chicks were weighed individually (42.0 ± 0.8 g live weight) and randomly assigned to four dietary groups, each with three replicates of 16 birds. One group was a control (CON) and fed a starter diet without supplementation. The other three groups were fed the same starter diet during the starter phase (1 - 10 days old) supplemented with lecithin (LEC) at 0.05 g/kg diet or lipase (LIP) at 0.05 g/kg, or a combination (LEC+LIP) at 0.05 + 0.05 g/kg. No significant effects of supplementation with LEC and LIP on feed intake of broilers were observed during the starter phase, whereas bodyweight gain increased after the combined addition of these supplemental ingredients. Thus, final bodyweight was greater in LEC+LIP broilers compared with the other groups. Dietary supplementation with LEC or LIP had a positive effect on final bodyweight. However, the effect was less than the simultaneous supplementation of LEC+LIP. Moreover, the feed conversion ratio in the LEC+LIP group improved (P <0.05) in the finisher phase (25 - 42 days old). Carcass traits and blood parameters were not influenced significantly by treatments, whereas supplementing LEC+LIP stimulated the immune system of broilers significantly. Thus, it can be concluded that supplementing LEC and LIP in early-stage broilers supported their performance and immune response positively up to finisher rearing phase.

Keywords: broiler production health, meat, nutrition
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Introduction

Chicken meat has many desirable nutritional characteristics, such as low intramuscular fat content and relatively high concentrations of polyunsaturated fatty acids. It therefore enjoys an exceptional position among the consumer preferences (Simopoulos, 2000; Laudadio *et al.*, 2021; Tufarelli *et al.*, 2016). Nowadays, the poultry industry faces constant challenges to maintain productivity, improve feed efficiency and reduce production costs.

Animal fats and vegetable oils are usually used as agents that increase feed energy concentration in broiler diets (Sevim *et al.*, 2020). However, the assimilation of dietary fats by young birds is limited because of their reduced capacity to produce and secrete bile salts and LIP until their gastrointestinal tract matures (10 - 14 days old) (Noy & Sklan, 1998). As a result, reduced rates of mixed micelles are formed in the intestinal lumen, leading to a decrease of fat digestion and absorption of nutrients (Leeson & Atteh, 1995) with negative effects on growth performance. This hypothesis was supported by Sato and Akiba (2002), who found that lower expression of LIP mRNA in two-week-old chickens compared with older chickens. It could be expected that LIP activity and fatty acid incorporation into the micelles would be improved by adding supplemental LIP enzymes or exogenous emulsifiers such as LEC.

In general, LIP supplementation in the early stages of bird life (0 - 21 days) had a direct negative effect on feed intake and growth rate, but these effects were not observed at later ages (Al-Marzooqi & Leeson, 2000). Nor did LEC supplementation have a significant effect on growth performance of broilers (Azman & Ciftci, 2004; Huang *et al.*, 2007). Although the effects of LEC and LIP supplementation on broiler growth parameters had been studied, there were no data about their combined effects. Therefore, the aim of the

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v51i5.14

present study was to investigate the effects of LEC and LIP dietary supplementation during the starter phase (1 - 10 days old) on productive performance, and haematological and immunological parameters of broiler chickens.

Materials and Methods

The experimental trial was approved by the Animal Care Committee of Islamic Azad University (approval # 9311-1393-04-08) and performed in accordance with recommendations of the Iranian Council for Control of Animal Experimentation. A total of 192 one-day-old chicks (Ross-308) were weighed individually (42.0 ± 0.8 g live weight) and randomly assigned to four groups, each with three replicates of 16 birds (eight males and eight females). One group served as control (CON) and was fed a commercial starter diet without supplementation, whereas the other three groups were fed the same starter diet during the starter phase, supplemented with LEC (LEC) at 0.05 g/kg diet, LIP (LIP) at 0.05 g/kg, or LEC+LIP) at 0.05 + 0.05 g/kg. Chicks in the four groups were offered a diet without LEC or LIP after the 10th day and until 42 days old. The diets were formulated to meet or exceed broiler nutrient requirements (NRC, 1994). Table 1 shows the ingredients and composition of the starter (1 - 10 d), grower (11 - 24 d), and finisher (25 - 42 d) diets fed to broilers during the feeding trial.

Table 1 Feed ingredients and nutrient calculated analysis of the starter, grower, and finisher diets

Ingredients, %	Starter	Grower	Finisher
Corn	46.81	50.22	54.78
Corn gluten meal	7.00	4.00	4.00
Poultry fat	4.00	4.00	4.00
Soybean meal (44% crude protein)	34.99	34.96	30.53
DL-Methionine	0.30	0.26	0.21
L-Lysine-HCl	0.44	0.25	0.19
L-Threonine	0.11	0.06	0.04
Choline chloride	1.00	1.00	1.00
Dicalcium phosphate	2.30	1.99	1.83
Calcium carbonate	1.23	0.99	0.97
Sodium bicarbonate	0.38	0.36	0.32
Sodium chloride	0.08	0.10	0.12
Vitamin-mineral premix[1]	0.50	0.50	0.50
Wheat bran	0.85	1.33	1.51
Chemical analysis, %			
ME (kcal/kg)	3,009	2,995	3,050
Crude protein	22.81	21.02	19.46
Crude fat	4.51	4.45	4.62
Crude fibre	3.51	3.50	3.28
Lysine	1.29	1.13	0.99
Methionine	0.62	0.55	0.49
Methionine+cysteine	0.92	083	0.75
Threonine	0.81	0.72	0.65
Calcium	1.04	0.88	0.81
Av. phosphorous	0.49	0.44	0.41
Sodium	0.16	0.16	0.16
Chloride	0.32	0.29	0.29

[1]Calcium pantothenate: 4 mg/g; niacin: 15 mg/g; vitamin B6: 13 mg/g; cu: 3 mg/g; zn: 15 mg/g; Mn: 20 mg/g; fe: 10 mg/g; K: 0.3 mg/g; vitamin A: 5000 IU/g; vitamin D3: 500 IU/g; vitamin E: 3 mg/g; vitamin K3: 1.5 mg/g; vitamin B2: 1 mg/g [in full. Tables must stand alone]

Each replicate was housed in a floor pen (1.0 × 1.7 m), where room temperature was maintained at 30 to 33 °C for the first week and was gradually reduced by 2.8 °C every week until 20 °C. (No further artificial heating was provided.) Room temperature was monitored by three thermometers, which were placed in the middle and at the two ends of the broiler house. Light regime was regulated as 24 hours light (1^{st} - 7^{th} day), 23 hours light and 1 dark (8^{th} - 21^{st} days) and 22 hours light and 2 hours dark (22^{nd} - 42^{nd} days). Birds were vaccinated against bronchitis (1 and 12 days old), Newcastle disease (8th, 15th, and 21st days old), influenza (8 days old), and Gumboro disease (18 and 21 days old) following standard protocols. Feed and water were provided ad libitum by feeders and conical drinkers, apart from the first week when feeder trays were used.

Bodyweight and feed intake were measured weekly. Feed conversion ratio was calculated as feed intake divided by bodyweight gain for each replicate. At 42 days old five chicks per replicate were randomly selected and sacrificed. Feet were separated from the carcass at the tibiotarsal joint. Breast, drumsticks, abdominal fat, stomach, gizzard, heart and kidneys were removed and weighed. The weight of thymus, liver, bursa of Fabricius and spleen (avian immune system-related organs) and the length of intestine parts (duodenum, jejunum, ileum, rectum) were recorded. Blood samples were collected from the same birds to evaluate haematological [what? components?] (uric acid, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, total protein, albumin, and glucose) and anti-viral titers against Newcastle disease and infectious bronchitis.

A blood sample of 5 ml was collected from the wing vein of five birds per replicate (on slaughtered birds) at the age of 42 days and put in a tube containing anticoagulant ethylene diamine tetra acetic acid (EDTA). Samples were centrifuged at 2000 g × 20 min, and plasma was stored at -20 °C for further analyses. Blood was collected in the early morning to minimize the circadian variations in these serum parameters. For the same reason, feed was removed for four hours before sampling (Pourhossein *et al.*, 2015).

Total plasma cholesterol and triglycerides levels were determined with enzymatic methods (TeifAzmoon Pars, Co., Tehran, Iran) according to Schmid and Von Forstner (1986). Cholesterol fractions (HDL and LDL) were established with HDL-C and LDL-C diagnostic kits (TeifAzmoon Pars Co, Tehran, Iran). The colorimetric index of cholesterol was assessed using the cholesterol oxidase procedure (Schmid & Von Forstner, 1986). Plasma glucose was measured using a glucose oxidase kit based on the oxidase-peroxidase procedure (TeifAzmoon Pars, Co., Tehran, Iran), first described by Trinder (1969) and Barham & Trinder (1972). Plasma uric acid was measured with a uric acid-uricase enzyme kit (TeifAzmoon Pars, Co., Tehran, Iran) according to the uricase-TOOS method (Kato *et al.*, 2000). Finally, albumin was determined according to the bromocresol green method (Maxwell *et al.*, 1990), and levels of total protein by the Biuret method (Maxwell *et al.*, 1990).

Humoral immune response to Newcastle vaccinations at the age of 42 days was determined based on hemagglutination-inhibition method (Seidavi *et al.*, 2014; Pourhossein *et al.*, 2015). Antibody responses to infectious bronchitis virus (IBV) were measured with an enzyme-linked immunosorbent assay (ELISA) test (Kemeny, 1991). Briefly, serum was separated with centrifugation (3000 *g* × 15 min) and antibody titers against IBV were measured by commercial ELISA kits (Bio-check BV, Gouda, Holland), according to the manufacturer's instructions. The absorbance of controls and samples was read at 405 nm with an ELISA reader (Bio-Tek Instruments Inc. ELX 800, Winooski, VT, USA).

Data were analysed according to a completely randomized experimental design involving four treatments with the general linear model procedure of Statistical Analysis System version 8 (SPSS Inc., Chicago, Illinois, USA). Significant differences were assessed via Duncan's multiple range test at 0.05 significance level.

Results and Discussion

No significant effect was observed of dietary supplementation with LEC and LIP between one and ten days old on feed intake of broilers (Table 2). On the other hand, bodyweight gain increased after LEC + LIP (P <0.05) (Table 3).

Table 2 Mean daily feed intake (g) of broilers as affected by lecithin and lipase dietary supplementation

Groups	Experimental period, days				
	1 - 10	11 - 24	25 - 42	1 - 24	1 - 42
CON	27.60	84.40	175.21	56.70	113.70
LEC	28.10	81.20	173.20	57.08	111.40
LIP	28.80	78.40	165.40	56.60	110.20
LEC+LIP	28.30	82.60	163.09	57.40	112.60
P–value	0.81	0.12	0.11	0.76	0.76
SE	0.23	0.96	2.20	0.29	1.08

CON: control, without supplementation, LEC: supplemented with lecithin at 0.05 g/kg DM of feed, LIP: supplemented with lipase at 0.05 g/kg DM of feed, LEC+LIP: supplemented with both lecithin and lipase at 0.05 + 0.05 g/kg DM of feed, between the 1st and 10th days old.

Table 3 Mean daily bodyweight gain (g) of broilers as affected by lecithin and lipase dietary supplementation

Groups	Experimental period (days)				
	1 - 10	11 - 24	25 - 42	1 - 24	1 - 42
CON	24.70^{b}	54.40^{b}	82.50^{c}	39.50^{b}	63.21^{c}
LEC	25.40^{ab}	55.06^{ab}	84.20^{bc}	40.20^{b}	64.68^{bc}
LIP	25.10^{b}	53.40^{b}	84.10^{b}	39.70^{b}	64.90^{b}
LEC+LIP	26.07^{a}	57.60^{a}	87.20^{a}	41.80^{a}	66.80^{a}
P-value	0.001	0.004	0.001	0.003	0.001
SE	0.17	0.52	0.56	0.33	0.41

CON: control, without supplementation, LEC: supplemented with lecithin at 0.05 g/kg DM of feed, LIP: supplemented with lipase at 0.05 g/kg DM of feed, LEC+LIP: supplemented with both lecithin and lipase at 0.05 + 0.05 g/kg DM of feed, between the 1st and 10th days old
^{a,,b,c} Means within each column with a common superscript did not differ significantly at P <0.05

Feed conversion ratio in LEC+LIP appeared to improve between the 25th and 42nd day (P <0.05) (Table 4). Final bodyweight was greater in LEC+LIP broilers compared with the other groups (Table 5).

Table 4 Mean feed conversion ratio of broilers as affected by lecithin and lipase dietary supplementation

Groups	Experimental period (days)				
	1 - 10	11 - 24	25 - 42	1 - 24	1 - 42
CON	1.11	1.55	2.05^{ab}	1.43	1.68
LEC	1.10	1.47	2.10^{a}	1.41	1.70
LIP	1.08	1.46	1.90^{bc}	1.42	1.72
LEC+LIP	1.06	1.43	1.87^{c}	1.37	1.68
P-value	0.443	0.352	0.011	0.156	0.329
SE	0.007	0.021	0.032	0.014	0.012

CON: control, without supplementation, LEC: supplemented with lecithin at 0.05 g/kg DM of feed, LIP: supplemented with lipase at 0.05 g/kg DM of feed, LEC+LIP: supplemented with both lecithin and lipase at 0.05 + 0.05 g/kg DM of feed, between the 1st and 10th days old
^{a,,b,c} Means within each column with a common superscript did not differ significantly at P <0.05

Table 5 Final bodyweight (g), mortality rate (%) and production index of broilers as affected by lecithin and lipase dietary supplementation

Groups	Final bodyweight, g	Mortality rate,%	European production index
CON	2655.30 [c]	3.09	312.60 [c]
LEC	2729.60 [b]	3.10	333.20 [b]
LIP	2729.01 [b]	3.05	340.50 [a]
LEC+LIP	2801.10 [a]	2.90	335.60[ab]
P-value	0.01	0.40	0.01
SEM	17.05	0.02	5.45

CON: control, without supplementation, LEC: supplemented with lecithin at 0.05 g/kg DM of feed, LIP: supplemented with lipase at 0.05 g/kg DM of feed, LEC+LIP: supplemented with both lecithin and lipase at 0.05 + 0.05 g/kg DM of feed, between the 1st and 10th days old

[a,,b,c] Means within each column with a common superscript did not differ significantly at $P<0.05$

Supplementation with LEC and LIP between one and ten days old had no positive effect on final bodyweight. Moreover, Table 6 shows that broilers fed diets supplemented with LIP and LEC+LIP had a reduction of the relative weight of the thymus compared with the control. Finally, carcass traits were not significantly different among the experimental groups (Table 7).

Supplemental LEC+LIP (P <0.01) stimulated the immune system of broilers (Table 8) in terms of higher anti-Newcastle disease (ND) and anti-infectious bronchitis virus (IBV) titers.

There is a dearth of studies that demonstrate the effect of dietary LEC on the performance of broilers. The replacement of soybean oil with LEC at levels of 25 - 50% in broiler diets did not improve fat assimilation in the gastrointestinal tract and the apparent metabolizable energy of dietary fats significantly. As a result, growth performance parameters were not affected (Azman & Ciftci, 2004). Nor did Huang *et al.* (2007) find any effect of soy LEC supplementation (0.5-1.0 g/kg) on final bodyweight.

Cantor *et al.* (1997) reached to the same conclusions after the use of soybean LEC as a replacement for blended animal-vegetable fat (2.5 - 5.0%) in broiler diets. Nor was there a significant effect on final bodyweight, cumulative feed intake and feed conversion ratio.

Table 6 Relative weight (% bodyweight) of thymus, liver, bursa of Fabricius and spleen in broilers as affected by lecithin and lipase dietary supplementation

Groups	Thymus	Liver	Bursa of Fabricius	Spleen
CON	0.29 [a]	2.20	0.17	0.13
LEC	0.25 [a]	2.41	0.19	0.15
LIP	0.18 [b]	2.57	0.15	0.14
LEC+LIP	0.17 [b]	2.28	0.16	0.12
P-value	0.004	0.422	0.145	0.623
SE	0.011	0.082	0.006	0.007

CON: control, without supplementation, LEC: supplemented with lecithin at 0.05 g/kg DM of feed, LIP: supplemented with lipase at 0.05 g/kg DM of feed, LEC+LIP: supplemented with both lecithin and lipase at 0.05 + 0.05 g/kg DM of feed, between the 1st and 10th days old

[a,,b] Means within each column with a common superscript did not differ significantly at P <0.05

Table 7 Relative weight (% hot carcass weight) of meat cuts and organs in broilers as affected by lecithin and lipase dietary supplementation

Groups	Breast	Drumsticks	Abdominal fat	Gizzard	Heart	Kidneys
CON	28.04	23.80	1.10	1.81	0.49	0.38
LEC	32.60	28.70	1.20	1.85	0.61	0.39
LIP	31.80	25.70	1.07	1.78	0.44	0.40
LEC+LIP	29.90	26.90	0.85	1.41	0.53	0.42
P-value	0.062	0.133	0.061	0.223	0.346	0.949
SE	0.30	0.72	0.05	0.08	0.01	0.01

CON: control, without supplementation, LEC: supplemented with lecithin at 0.05 g/kg DM of feed, LIP: supplemented with lipase at 0.05 g/kg DM of feed, LEC+LIP: supplemented with both lecithin and lipase at 0.05 + 0.05 g/kg DM of feed, between the 1st and 10th days old

Table 8 Anti-viral titers against Newcastle disease and infectious bronchitis in broilers as affected by lecithin and lipase dietary supplementation

Groups	Newcastle disease	Infectious bronchitis
CON	1.70 ^{c}	765.30 ^{b}
LEC	2.80 ^{b}	898.90 ^{b}
LIP	3.30^{ab}	1135.30 ^{b}
LEC+LIP	4.20 ^{a}	2331.10 ^{a}
P-value	0.001	0.003
SE	0.29	197.40

CON: control, without supplementation, LEC: supplemented with lecithin at 0.05 g/kg DM of feed, LIP: supplemented with lipase at 0.05 g/kg DM of feed, LEC+LIP: supplemented with both lecithin and lipase at 0.05 + 0.05 g/kg DM of feed, between the 1st and 10th days old

^{a,,b} Means within each column with a common superscript did not differ significantly at P <0.05

The age of the bird plays an important role during LEC supplementation. Gradual replacement of dietary fat with soybean LEC (25%, 50% and 100%) in the diets of 21-day-old chicks caused a substantial increase of bod weight, an effect that was not observed when LEC was used at the 39 days old (Cox *et al.*, 2000).

At the same time, the addition of LIP (0.2 g/kg feed) to a wheat-based diet did not have an effect on growth performance and nutrient utilization in broilers (Meng *et al.*, 2004; Tufarelli *et al.*, 2007). No significant effects were found after the inclusion of LIP in a rice-based diet, either (Mulyantini *et al.*, 2005). On the other hand, LIP supplementation at the level of 4% and 8% decreased feed intake and bodyweight gain, although diet metabolizable energy and apparent fat digestibility were improved (Al-Marzooqi & Leeson, 1999).

The inclusion of LEC and LIP in the diets of broilers between one and ten days old did not have a significant effect on the weight of the internal organs, apart from the thymus, which were lighter in LEC+LIP and LIP compared with LEC and CON groups. Al-Marzooqi and Leeson (2000) found no effect either of LIP addition (0 to 21 days) on liver size in 42-day-old chickens, although the percentage weight of the liver was significantly greater after LIP dietary supplementation at the level of 1.0% in 21-day-old chickens. The authors did not find a significant effect of dietary supplementation with LEC and LIP between one and ten days old on the length of the parts of the intestine. Similarly, Al-Marzooqi and Leeson (2000) failed to detect any difference in gut structure, and there was no apparent adverse effect on gastric motility, as was shown by the histological examination of the small intestine of birds fed diets supplemented with LIP enzyme. Broiler blood parameters were not influenced significantly by dietary treatments.

Conclusions

Supplementing LEC and LIP together the during starter phase of feeding broiler chicks may positively impact productive traits, and especially immune response; effects that persisted through the finishing phase.

Acknowledgments

This manuscript is part of the MSc thesis of the first author. The authors are grateful to Islamic Azad University, Sanandaj Branch, Sanandaj, Iran, for its support. The authors also thank Dr Panagiotis Simitzis for his assistance during the preparation of this manuscript.

Authors' Contributions

All the authors approved the final version of the manuscript. Methodology, AS and VT; validation, AS and VT; formal analysis, MNS, DD and LE; investigation, MNS and DD; data curation, AS and VT, writing original draft preparation, MNS, AS and VT; writing review and editing, AS, VT and VL

Conflict of Interest Declaration

The authors declare there are no conflicts of interest.

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