

Nutritional and physiological responses of broiler chickens to dietary supplementation with de-oiled soyabean lecithin at different metabolisable energy levels and various fat sources

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Abstract

A 42-d study was conducted to investigate the effects of an emulsifier supplementation (de-oiled soyabean lecithin (DSL)) of diets with different levels of metabolisable energy (ME) and various sources of fat on growth performance, nutrient digestibility, blood profile and jejunal morphology of broiler chickens. Diets were arranged factorially ($2 \times 2 \times 2$) and consisted of two concentrations of ME (normal and low), two fat sources (soyabean oil (SO) and poultry fat (PF)) and two levels of DSL supplementation (0 and 1 g/kg). A total of 800 1-d-old male broiler chickens were assigned to eight treatments with five replicates/treatment. The results showed the supplemental DSL caused improvements in the overall feed conversion ratio, fat digestibility and jejunal villus height: crypt depth ratio, but the magnitude of the responses was greater in the PF-containing diets, resulting in significant fat \times DSL interactions ($P < 0.05$). Abdominal fat percentage was also reduced by the PF-containing diet, but the response was greater in the normal ME diet, resulting in a significant ME \times fat interaction ($P = 0.048$). Dietary DSL supplementation also increased nitrogen-corrected apparent ME values but decreased blood TAG ($P = 0.041$) and LDL ($P = 0.049$) concentrations, regardless of the source of fat used or the ME values in the diet. In conclusion, the present study suggests that the improvements in growth performance, fat digestibility and intestinal morphology that can be achieved with DSL supplementation are highly dependent on the degree of saturation of lipid incorporated into broiler chicken diets.

Key words: Broiler chickens: De-oiled lecithin: Growth performance: Nutrient digestibility: Serum biochemistry: Intestinal morphology

Metabolisable energy (ME) in the diet is a critical factor that greatly influences the intake of all other nutrients in the poultry species⁽¹⁾. Moreover, the feed efficiency, growth performance and body composition of broiler chickens are likely to be influenced by the ME intake^(2,3). It was commonly believed that the high-ME diet for broiler chickens is produced by the addition of lipids (fats and oils) because lipids have the highest energy value compared with other nutrients⁽⁴⁾. However, some concerns regarding dietary lipid levels and digestibility have arisen in commercial poultry production, especially for young broiler chickens whose lipid utilisation is restricted due to the poor digestive ability and the absorption capacity⁽⁵⁾.

Dietary fats, including poultry fat (PF) and vegetable oils, are the main energy sources in poultry feed⁽⁶⁾. Increasing the fat digestibility may allow lowering supplemental lipid inclusion levels in the broiler chicken diet while maintaining the same

level of performance, which ultimately results in lower feed production costs⁽⁷⁾. In general, the digestibility of lipids depends on their chemical and physical characteristics, particularly the chain length of fatty acids and their saturation degree, which influence the ME content of the diet⁽⁸⁾. Ghasemi *et al.*⁽⁹⁾ indicated that the ratio of lower unsaturated fatty acids (UFA):SFA in the diet needs higher concentrations of bile salts (one kind of emulsifier), which are essential for micelle formation and, subsequently, lipid uptake. Hence, a higher degree of saturation or a lower UFA:SFA ratio led to poorer digestibility of the lipid source in broiler chickens⁽¹⁰⁾. These physiological and functional limitations of lipid digestion and absorption in different poultry species could be overcome by the supplementation of an exogenous emulsifier to the diet^(11,12).

Emulsifiers promote fatty acids' incorporation into micelles and increase the digestibility of dietary lipids in the duodenum

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; AIA, acid-insoluble ash; AME_n, apparent metabolisable energy corrected for nitrogen balance; BW, body weight; CD, crypt depth; CHOL, total cholesterol; DSL, de-oiled soyabean lecithin; FCR, feed conversion ratio; ME, metabolisable energy; PF, poultry fat; SO, soyabean oil; UFA, unsaturated fatty acids; VH, villus height; VH:CD, villus height: crypt depth ratio; VSA, villus surface area.

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of chickens^(13,14). De-oiled soyabean lecithin (DSL) is a mixture of amphiphilic phospholipids, which has good emulsification property⁽¹⁵⁾. Although there is scarce information on the use of DSL supplement in humans and animals, the application of some emulsifier agents in broiler chicken diets and their effects on growth performance and physiological response have been evaluated in several studies. Jansen *et al.*⁽¹³⁾ showed that the addition of lysolecithin in the basal diet with an SFA source (pig lard) increased nitrogen retention and apparent ME of the feed during the grower period of broiler chickens. Furthermore, supplementation of a rice-bran-derived lysolecithin increased fat digestibility and improved growth performance of broiler chickens⁽¹⁶⁾. The advantages of dietary supplementation with lysophospholipid for improving low-energy and low-nitrogenous diets were also recently observed in a study by Boontiam *et al.*⁽¹⁷⁾. Similarly, Siyal *et al.*⁽¹⁸⁾ confirmed an increased digestibility of energy due to soyabean lecithin utilisation in the broiler chicken diets, which could be an effective strategy to decrease dietary ME levels. In the study by Alzawqari *et al.*⁽¹⁹⁾, an increase in jejunal and ileal villus height (VH) for a diet supplemented with an emulsifier supplementation (desiccated ox bile) was observed in broiler chickens.

To our knowledge, no previous reports are available in the literature about the effect of dietary supplementation with DSL at different dietary energy levels or different fat sources in broiler chickens.

In the present study, we used a broiler chicken model to investigate the effects of DSL supplementation as an exogenous emulsifier on growth performance, nutrient digestibility, carcass characteristics, serum biochemical parameters and jejunal morphology at different dietary ME levels provided by two fat sources (soyabean oil (SO) or PF).

Methods

All experimental procedures involving birds adhered to the guidelines of and were approved by the Animal Management and Ethics Committee (in charge of animal welfare issues) of the Arak University (Arak, Iran).

Preparation of pure de-oiled lecithin

The pure DSL, with the commercial name of BergaPur, was obtained from Berg+Schmidt GmbH & Co. KG. and is derived from the soya plant. It is manufactured in dry powder form by extracting the oil present in the natural liquid lecithin and contained 87 % of the phospholipid complex, 10 % lysophospholipid, 2 % TAG and 1 % water. The fatty acid composition of this product includes 20 % palmitic acid, 5 % stearic acid, 9 % oleic acid, 59 % linoleic acid and 7 % linolenic acid.

Experimental design and diets

A total of 800 1-d-old male Ross 308 broilers chickens (average body weight (BW): 46.4 ± 0.39 g), were purchased from a commercial hatchery and used in a 42-d feeding trial. Broiler chickens were randomly allocated to eight treatments in five pens (replicates) of twenty birds per replicate pen, so that their initial weights were similar across all treatment groups. A three-phase

Table 1. Ingredients of the experimental diets at all stages of growth

Ingredients (g/kg)	0–10 d		10–24 d		24–42 d	
	Normal ME	Low ME	Normal ME	Low ME	Normal ME	Low ME
Maize	561.1	535.1	591.8	565.9	632.9	606.0
Soyabean meal	314.9	390.6	282.3	357.9	245.7	313.8
Maize gluten meal	56.3	9.2	53.0	5.9	41.8	0
SO or PF	20	20	30	30	40	40
Dicalcium phosphate	19.5	19.0	17.2	16.7	15.3	14.9
Common salt	2.5	2.5	2.5	2.5	2.5	2.5
CaCO ₃	11.6	11.3	10.7	10.5	10.0	12.8
Vitamin premix*	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix†	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	2.6	3.0	2.2	2.6	2.1	2.4
L-Lysine HCl	4.4	2.5	3.7	1.7	3.3	1.5
L-Threonine	2.1	1.8	1.6	1.3	1.4	1.1

ME, metabolisable energy; SO, soyabean oil; PF, poultry fat.

* The vitamin premix supplied per kg diet: vitamin A (retinol), 3600 µg; vitamin E (α-tocopheryl acetate), 63 mg; vitamin D₃ (cholecalciferol), 125 µg; vitamin K₃ (menadione), 3.5 mg; thiamine, 3 mg; riboflavin, 7.5 mg; niacin, 65 mg; pantothenate, 18 mg; pyridoxine, 4.3 mg; biotin, 0.3 mg; folic acid, 2 mg; cyanocobalamin, 0.017 mg; choline chloride, 600 mg; antioxidant, 100 mg.

† The mineral premix supplied per kg diet: Mn (MnSO₄·H₂O, 32.5 % Mn), 120 mg; Zn (ZnO, 80.4 % Zn), 33.88 g; Fe (FeSO₄·7H₂O, 20.1 % Fe), 20 g; Cu (CuSO₄·5H₂O), 16 mg; Se (NaSeO₃, 45.6 % Se), 0.3 mg, iodine (KI, 58 % iodine), 1.3 mg.

feeding schedule consisting of starter (0–10 d), grower (10–24 d) and finisher (24–42 d) diets was used in the present study. The experiment consisted of a 2 × 2 × 2 factorial arrangement of treatments including two concentrations of ME (starter: 12.55 MJ/kg for normal ME diet and 12.13 MJ/kg for low-ME diet; grower: ME = 12.97 MJ/kg for normal ME diet and 12.55 MJ/kg for low-ME diet; and finisher: ME = 13.39 MJ/kg for normal ME diet and 12.97 MJ/kg for low-ME diet), two fat sources (SO and PF) and two dietary levels of DSL supplementation (0 and 1 g/kg). All nutrients in experimental diets were formulated to meet or exceed the recommendations for broiler chickens, according to the Ross 308 nutrient specifications⁽²⁰⁾. The ingredients and nutrient composition of the experimental diets used during different growing periods are presented in Tables 1 and 2. The broiler chickens were provided with mash feed and tap water *ad libitum* during the entire experiment. All experimental groups were housed in floor pens (length 175 cm × width 170 cm) using litter top dressed with 5 cm of clean wood shavings in an environmentally controlled house. Room temperature began at 34°C for the first 3 d and was decreased gradually to 22°C until the end of the trial and the relative humidity was around 65 %. The lighting programme was standardised across all pens and consisted of 23-h light and 1-h darkness. Light source used was incandescent bulbs at light intensity of 30 lux.

Growth performance measurements

BW and feed consumption for each experimental group were recorded on days 0, 10, 24 and 42. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR; ADFI:ADG) were calculated. Mortalities and health status were also recorded daily to calibrate growth performance.

Table 2. Calculated and analysed nutrient contents of the experimental diets at all stages of growth (on as-fed basis)

	0–10 d		10–24 d		24–42 d	
	Normal ME	Low ME	Normal ME	Low ME	Normal ME	Low ME
Calculated analysis						
(g/kg unless stated otherwise)						
ME (MJ/kg)	12.55	12.13	12.97	12.55	13.39	12.97
Crude protein	230.0	230.0	215.0	215.0	195.0	195.0
Crude fat	45.2	43.6	56.0	54.5	67.0	65.5
Ca	9.6	9.6	8.7	8.7	7.9	7.9
Available P	4.8	4.8	4.35	4.35	3.95	3.95
Digestible lysine	12.8	12.8	11.5	11.5	10.3	10.3
Digestible TSAA	9.5	9.5	8.7	8.7	8.0	8.0
Digestible threonine	8.6	8.6	7.7	7.7	6.9	6.9
Analysis values*						
(g/kg unless stated otherwise)						
Crude protein	227.2	226.4	210.3	209.3	191.5	190.8
Total lysine	14.3	14.2	13.4	13.2	11.6	11.5
Total TSAA	10.6	10.7	9.7	9.8	9.1	9.3
Ca	9.3	9.3	8.5	8.4	7.8	7.8
Total P	0.74	0.72	0.66	0.65	0.61	0.60
Crude fat (SO diet)	48.1	46.4	59.1	57.4	70.3	68.6
Crude fat (PF diet)	47.1	45.4	57.6	55.9	68.3	66.6
GE (MJ/kg) (SO diet)	18.07	17.61	18.67	18.20	19.18	18.71
GE (MJ/kg) (PF diet)	17.99	17.54	18.55	18.09	19.04	18.59
Fatty acid composition						
(% of total fatty acids)						
SO diet						
16 : 0	10.93	10.79	10.76	10.60	10.63	10.46
18 : 0	3.15	3.21	3.34	3.40	3.47	3.51
18 : 1 <i>n</i> -9	22.70	22.55	22.67	22.55	22.66	22.56
18 : 2 <i>n</i> -6	56.71	56.62	56.27	56.18	55.98	55.91
18 : 3 <i>n</i> -3	4.61	4.81	5.05	5.22	5.32	5.47
<i>n</i> -6: <i>n</i> -3 PUFA ratio	12.30	11.77	11.14	10.76	10.52	10.22
Other	1.90	2.02	1.92	2.05	1.94	2.09
PF diet						
16 : 0	19.41	19.61	21.12	21.32	22.09	22.27
18 : 0	5.75	5.84	6.09	6.17	6.31	6.38
18 : 1 <i>n</i> -9	29.38	29.51	31.10	31.25	32.25	32.42
18 : 2 <i>n</i> -6	40.60	39.98	36.94	36.33	34.49	33.92
18 : 3 <i>n</i> -3	1.81	1.91	1.62	1.69	1.48	1.54
<i>n</i> -6: <i>n</i> -3 PUFA ratio	22.43	20.93	22.80	21.50	23.30	22.03
Other	3.05	3.15	3.13	3.24	3.38	3.47

ME, metabolisable energy; TSAA, total sulphur amino acids; SO, soyabean meal; PF, poultry fat; GE, gross energy.

* Mean of two samples per diet.

Sample collection and procedures

At the end of the trial (on day 42), after being fasted for 12 h, two broiler chickens from each replicate (ten birds per treatment) with BW close to the pen mean was randomly selected and weighed. Blood samples (5 ml) were collected from the wing vein in sterile test tubes without anticoagulant and incubated at 37°C for 2 h and then the serum was obtained by centrifuging at 2000 *g* at 4°C for 10 min and stored at –20°C. The broiler

chickens were killed by cervical dislocation; then the pre-slaughter, carcass, breast, leg, liver and abdominal fat weights were recorded by trained personnel. Their relative weights were expressed as the percentage of pre-slaughter live weight.

Contents of ileum (from Meckel's diverticulum to approximately 10 mm above the ileal-caecal junction) were collected in plastic zip bags. The ileal digesta of two birds in a replicate were pooled, after which a representative sample was immediately stored in a freezer at –20°C for subsequent determination of nutrient digestibility and apparent metabolisable energy (AME_n) corrected for nitrogen balance. A 2-cm section of mid-jejunum was also separated, flushed with distilled water to remove the contents and fixed in 10 % neutral-buffered formalin for morphological assessment.

Chemical analysis and calculations

Before chemical analysis, representative samples of feed and ileal digesta were ground in a laboratory mill to pass through a 1-mm screen. Samples were analysed for DM (method 930.15), crude protein (method 990.03) and crude fat (method 920.39), according to the standard procedures⁽²¹⁾. Gross energy determinations of diet and ileal digesta samples were performed in an adiabatic bomb calorimeter (Parr Instrument Company) standardised with benzoic acid. The amino acid profile of the diets was analysed by an HPLC instrument (Knauer) consisting of a K-1000 controller quaternary pump and a Shimadzu fluorescence detector (RF-551). Methionine and cysteine were analysed as methionine sulfone and cysteic acid after an overnight cold performic acid oxidation before hydrolysis⁽²²⁾.

Each of the analyses for crude fat and fatty acid profile from the diet samples was conducted, according to the method previously described by Ghasemi *et al.*⁽⁹⁾. Individual fatty acids in the diet samples were quantified by GC (Unicam 4600, equipped with a flame ionisation detector, and a BPX70 fused silica capillary column). As an endogenous indicator, acid-insoluble ash (AIA) in both diet and ileal digesta samples were analysed to calculate the nutrient digestibility and AME_n⁽²³⁾.

The digestibility of nutrients in diets was determined by the following equation:

$$\% \text{ Digestibility} = (1 - (\text{AIA}_{\text{diet}}/\text{AIA}_{\text{id}}) \times (\text{nutrient}_{\text{id}}/\text{nutrient}_{\text{diet}})) \times 100$$

where nutrient_{diet} and AIA_{diet} are the concentrations of nutrient and AIA in the diet (%) and Nutrient_{id} and AIA_{id} represent the concentrations of the same nutrient and AIA in the ileal digesta (%).

The AME_n value was calculated using the following equation⁽²⁴⁾:

$$\text{AME}_n \text{ (MJ/kg of diet)} = \text{GE}_{\text{diet}} - ((\text{GE}_{\text{id}} \times \text{IF}) + 34.44 \times (\text{N}_{\text{diet}} - \text{N}_{\text{id}} \times \text{IF}))$$

where GE_{diet} is gross energy value in diet (MJ/kg) and GE_{id} is the gross energy value in ileal digestibility (MJ/kg). Indigestibility factor (IF) = AIA_{diet}/AIA_{id}; N_{diet} is the nitrogen concentration in diet (%), N_{id} is nitrogen concentration in ileal

Table 3. Effects of different levels of metabolisable energy (ME), various fat sources and de-oiled soyabean lecithin (DSL) supplementation on body weight (BW) and average daily gain (ADG) of broiler chickens at all stages of growth up to 42 d of age (Mean values with their standard errors; *n*5)

ME	Fat source	DSL (g/kg)	BW (g)			ADG (g/bird per d)			
			10 d	24 d	42 d	0–10 d	10–24 d	24–42 d	0–42 d
Normal	SO	0	214.1	948.1	2550	17.22	52.68	90.26	59.71
Normal	SO	1	218.6	972.1	2595	17.68	54.39	93.06	60.79
Normal	PF	0	212.2	922.3	2527	17.02	50.83	88.68	59.18
Normal	PF	1	213.2	935.7	2618	17.12	51.79	93.92	61.35
Low	SO	0	207.3	914.3	2462	16.53	50.27	85.05	57.62
Low	SO	1	210.5	942.3	2456	16.84	52.26	85.08	57.49
Low	PF	0	203.3	880.3	2435	16.13	47.84	83.03	56.98
Low	PF	1	205.8	917.0	2512	16.39	50.46	87.79	58.80
SEM			3.17	14.57	39.34	0.317	1.04	2.35	0.938
Main effect means									
ME level									
Normal			214.5 ^a	944.5 ^a	2573 ^a	17.26 ^a	52.42 ^a	91.48 ^a	60.26 ^a
Low			206.7 ^b	913.5 ^b	2466 ^b	16.47 ^b	50.21 ^b	85.24 ^b	57.72 ^b
SEM			1.58	7.29	19.65	0.158	0.521	1.13	0.468
Fat source									
SO			212.6	944.2 ^a	2515	17.07	52.40 ^a	88.36	58.90
PF			208.6	913.8 ^b	2523	16.67	50.23 ^b	88.35	59.08
SEM			1.58	6.85	19.65	0.158	0.489	1.14	0.468
DSL (g/kg)									
0			209.2	916.3 ^b	2494	16.73	50.40 ^b	86.76	58.37
1			212.0	941.8 ^a	2545	17.01	52.23 ^a	89.96	59.61
SEM			1.58	6.75	19.66	0.158	0.482	1.13	0.468
Significance									
ME level			0.002	0.009	<0.001	0.001	0.009	<0.001	<0.001
Fat source			0.086	0.005	0.796	0.084	0.005	0.996	0.796
DSL			0.221	0.013	0.072	0.218	0.013	0.062	0.071
ME × fat			0.885	0.939	0.809	0.916	0.939	0.820	0.810
ME × DSL			0.972	0.476	0.556	0.981	0.477	0.603	0.557
Fat × DSL			0.651	0.963	0.260	0.647	0.961	0.252	0.259
ME × fat × DSL			0.766	0.614	0.742	0.741	0.614	0.714	0.743

SO, soyabean oil; PF, poultry fat.

^{a,b} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

digesta (%) and 34.44 is the energy equivalent (MJ/kg) of uric acid.

Blood biochemical parameters

The concentrations of glucose, TAG, total cholesterol (CHOL), HDL, LDL, total protein, albumin and uric acid in serum samples were analysed by an auto analyser apparatus (Biotechnica, BT-3000), following the instructions of the corresponding reagent kit (Pars Azmoon Co.).

Intestinal morphology

For each jejunal tissue segment, a 5- μ m cross section was made using a microtome, placed on a glass slide, stained by haematoxylin–eosin and analysed with a light microscope (Olympus, CX31, Shinjuku). Three cross-sections and ten measurements per cross section were obtained. Morphological measurements of VH (from the tip of the villus to the villus–crypt junction), villus width (VW; at the middle point of the villus) and crypt depth (CD; the depth of the invagination between adjacent villi) were made using an image-analysis software (QWinPlus v. 3.1.0; Leica Cambridge Ltd.). Data from the VH and CD were used to obtain the VH:CD ratio. The villus surface

area (VSA) was also calculated by the following formula: $2\pi \times (VW/2) \times VH$.

Statistical analysis

The data were subjected to ANOVA for a $2 \times 2 \times 2$ design using the General Linear Models procedures of SAS (v. 9.0; SAS Institute). The model included main effects of ME level, fat type and DSL level, and their interactions. The replicate pen of twenty broiler chickens for growth performance or two chickens for other responses served as the experimental unit for all statistical analyses. Data from mortality and carcass traits were transformed to arcsine for analysis. The results are presented as the least-square means with standard errors of the means. All statements of significance were considered as $P < 0.001$ or $P < 0.05$, with a trend between $P > 0.05$ and $P < 0.10$.

Results

Growth performance

The performance of broiler chickens fed the experimental diets is shown in Tables 3 and 4. The two-way interaction effects of ME × fat, ME × DSL, fat × DSL and the three-way interaction of ME × fat × DSL on BW and ADG were not significant

Table 4. Effects of different levels of metabolisable energy (ME), various fat sources and de-oiled soyabean lecithin (DSL) supplementation on average daily feed intake (ADFI) and feed conversion ratio (FCR) of broiler chickens at all stages of growth up to 42 d of age (Mean values with their standard errors; *n*5)

ME	Fat source	DSL (g/kg)	ADFI (g/bird per d)				FCR			
			0–10 d	10–24 d	24–42 d	0–42 d	0–10 d	10–24 d	24–42 d	0–42 d
Normal	SO	0	21.36	83.93	169.1	105.1	1.24	1.59	1.88	1.76
Normal	SO	1	21.46	87.13	173.8	107.7	1.22	1.60	1.86	1.77
Normal	PF	0	21.33	83.75	172.7	106.9	1.26	1.65	1.95	1.81
Normal	PF	1	21.39	86.78	168.6	106.0	1.25	1.68	1.80	1.73
Low	SO	0	21.15	85.73	173.1	108.1	1.28	1.71	2.03	1.88
Low	SO	1	21.21	87.85	171.4	107.7	1.26	1.68	2.02	1.87
Low	PF	0	21.16	85.34	170.7	107.6	1.31	1.78	2.06	1.89
Low	PF	1	21.23	86.94	169.0	106.9	1.30	1.72	1.93	1.82
SEM			0.453	2.09	3.82	1.49	0.022	0.038	0.037	0.020
Main effect means										
ME level										
Normal			21.39	85.40	171.0	106.4	1.24 ^b	1.63 ^b	1.87 ^b	1.77 ^b
Low			21.18	86.47	171.1	107.6	1.29 ^a	1.72 ^a	2.01 ^a	1.86 ^a
SEM			0.226	1.05	1.82	0.746	0.011	0.019	0.020	0.010
Fat source										
SO			21.30	86.16	171.9	107.2	1.25	1.64 ^b	1.95	1.82
PF			21.27	85.70	170.3	106.9	1.28	1.71 ^a	1.93	1.81
SEM			0.226	0.984	1.85	0.746	0.011	0.018	0.020	0.010
DSL (g/kg)										
0			21.25	84.69	171.4	106.9	1.27	1.68	1.98 ^a	1.83 ^a
1			21.32	87.18	170.7	107.1	1.25	1.67	1.90 ^b	1.80 ^b
SEM			0.226	0.971	1.83	0.746	0.011	0.018	0.020	0.010
Significance										
ME level			0.527	0.508	0.998	0.287	0.006	0.003	<0.001	<0.001
Fat source			0.944	0.749	0.562	0.793	0.062	0.023	0.649	0.470
DSL			0.832	0.083	0.796	0.899	0.258	0.629	0.005	0.019
ME × fat			0.926	0.889	0.753	0.750	0.636	0.913	0.520	0.434
ME × DSL			0.979	0.649	0.687	0.502	0.972	0.233	0.849	0.862
Fat × DSL			0.964	0.901	0.387	0.384	0.717	0.856	0.014	0.011
ME × fat × DSL			0.979	0.950	0.395	0.449	0.871	0.567	0.792	0.652

SO, soyabean oil; PF, poultry fat.

^{a,b} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

(Table 3). However, the interactions between fat source and DSL supplementation were observed for FCR during the finisher ($P = 0.014$) and the entire experimental period ($P = 0.011$), indicating that the effect of DSL on FCR was more marked in broiler chickens fed on the PF-containing diets (Table 4). Broiler chickens fed high-ME diets had higher BW and ADG ($P < 0.05$) and lower FCR ($P < 0.05$) than those fed low-ME diets during the different experimental periods. At 24 d, the BW, ADG and FCR of broiler chickens receiving the SO-containing diets were better ($P < 0.05$) compared with birds fed on the PF-containing diets, but these improvements were not reflected during the whole experimental period (0–42 d). At 10 d, there were also tendencies that SO-containing diets resulted in higher BW ($P = 0.086$), ADG ($P = 0.084$) and lower FCR ($P = 0.062$) when compared with PF-containing diets. As Table 3 indicates BW of broiler chickens receiving DSL supplementation was higher at 24 d ($P = 0.013$) and 42 d ($P = 0.072$) compared with the non-supplemented birds. Dietary DSL also increased ADG in the grower ($P = 0.013$), finisher ($P = 0.062$) and overall ($P = 0.071$) experimental periods. Furthermore, broiler chickens supplemented with DSL had lower FCR in the grower ($P = 0.005$) and the overall ($P = 0.019$) experimental period (Table 4). ME, fat source and DSL supplementation did not affect ADFI during

the whole experiment, and both two-way and three-way interactions were not significant.

Nutrient digestibility

The main effect of ME, fat source, DSL supplementation, two-way interaction effects of ME × DSL, fat × DSL and the three-way interaction of ME × fat × DSL on the ileal digestibility of DM and crude protein were not significant (Table 5). An interaction between fat sources and DSL was observed on digestibility of crude fat, which improved with DSL addition in the PF-containing diets. Supplemental DSL was also able to improve ($P \leq 0.001$) the AME_n value of diet. The PF-containing diets reduced fat digestibility compared with SO-containing diets. Broiler chickens fed on low-ME diet had lower AME_n value as compared with birds fed on the normal ME diet.

Carcass characteristics

Neither the main effects of ME, fat source and DSL supplementation, nor their interactions on the yields of carcass, breast and legs were significant (Table 6). In contrast, the low-ME diets significantly reduced ($P < 0.001$) the percentage of abdominal fat

Table 5. Effects of different levels of metabolisable energy (ME), various fat sources and de-oiled soyabean lecithin (DSL) supplementation on nutrient digestibility of broiler chickens at 38 d of age (Mean values with their standard errors; *n*5)

ME	Fat source	DSL (g/kg)	DM digestibility (%)	Crude protein digestibility (%)	Crude fat digestibility (%)	AME _n (MJ/kg)
Normal	SO	0	79.60	71.94	79.70	12.76
Normal	SO	1	80.00	73.68	80.96	13.14
Normal	PF	0	79.14	71.94	75.88	12.78
Normal	PF	1	80.14	72.62	81.08	13.30
Low	SO	0	80.22	69.50	81.50	12.60
Low	SO	1	81.16	72.34	81.94	12.82
Low	PF	0	79.70	68.56	76.92	12.20
Low	PF	1	80.40	71.38	82.12	12.52
SEM			1.220	1.703	1.389	0.203
Main effect means						
ME level						
Normal			79.72	72.54	79.40	12.99 ^a
Low			80.37	70.44	80.62	12.53 ^b
SEM			0.610	0.852	0.694	0.101
Fat source						
SO			80.24	71.86	81.03 ^a	12.83
PF			79.84	71.13	79.00 ^b	12.70
SEM			0.610	0.852	0.694	0.101
DSL (g/kg)						
0			79.67	70.49	78.50 ^b	12.59 ^b
1			80.43	72.51	81.53 ^a	12.94 ^a
SEM			0.610	0.852	0.694	0.101
Significance						
ME level			0.457	0.091	0.225	0.003
Fat source			0.646	0.543	0.047	0.372
DSL			0.385	0.103	0.004	0.017
ME × fat			0.783	0.863	0.860	0.135
ME × DSL			0.945	0.506	0.836	0.535
Fat × DSL			0.917	0.824	0.034	0.679
ME × fat × DSL			0.809	0.831	0.836	0.945

AME_n, apparent metabolisable energy; SO, soyabean oil; PF, poultry fat.

^{a,b} Mean values within a column with unlike superscript letters were significantly different (*P* < 0.05).

Table 6. Effects of different levels of metabolisable energy (ME), various fat sources and de-oiled soyabean lecithin (DSL) supplementation on carcass traits* of broiler chickens at 42 d of age (Mean values with their standard errors; *n*5)

ME	Fat source	DSL (g/kg)	Carcass	Breast	Legs	Abdominal fat	Liver
Normal	SO	0	71.60	27.88	23.86	1.250	2.392
Normal	SO	1	73.22	28.99	24.26	1.232	2.277
Normal	PF	0	72.14	27.72	25.12	1.579	2.607
Normal	PF	1	72.01	27.86	24.67	1.404	2.552
Low	SO	0	71.95	27.24	24.64	1.086	2.106
Low	SO	1	72.33	27.83	24.08	1.063	2.050
Low	PF	0	72.31	27.26	24.32	1.107	2.139
Low	PF	1	71.17	26.63	24.48	1.057	2.043
SEM			0.819	0.883	0.621	0.084	0.095
Main effect means							
ME level							
Normal			72.24	28.11	24.48	1.366 ^a	2.457 ^a
Low			71.94	27.13	24.38	1.078 ^b	2.084 ^b
SEM			0.410	0.442	0.310	0.042	0.047
Fat source							
SO			72.27	27.98	24.21	1.158 ^b	2.206
PF			71.91	27.36	24.65	1.287 ^a	2.335
SEM			0.409	0.442	0.310	0.042	0.047
DSL (g/kg)							
0			72.00	27.52	24.48	1.256	2.311
1			72.18	27.83	24.37	1.189	2.230
SEM			0.409	0.442	0.310	0.042	0.047
Significance							
ME level			0.606	0.172	0.825	<0.001	<0.001
Fat source			0.529	0.328	0.322	0.036	0.064
DSL			0.760	0.632	0.799	0.268	0.239
ME × fat			0.950	0.965	0.371	0.048	0.093
ME × DSL			0.339	0.607	0.841	0.623	0.948
Fat × DSL			0.167	0.388	0.941	0.443	0.943
ME × fat × DSL			0.919	0.920	0.382	0.587	0.711

SO, soyabean oil; PF, poultry fat.

^{a,b} Mean values within a column with unlike superscript letters were significantly different (*P* < 0.05).

* The values are expressed as a percentage of pre-slaughter live body weight.

and liver compared with normal ME diet. Moreover, the PF-containing diets resulted in higher abdominal fat deposition (*P* = 0.036) and higher liver weight (*P* = 0.064) when compared with SO-containing diets. There was an interaction of dietary ME and fat sources on abdominal fat percentage (*P* = 0.048) and a trend for an ME × fat interaction (*P* = 0.093) for relative liver weight.

Blood metabolites

Data for serum metabolites are detailed in Table 7. The main effects of dietary ME, fat source and DSL supplementation on serum glucose, TAG, total protein, albumin and globulin were not significant. Although dietary ME did not influence blood lipid profile, the broiler chickens fed on the SO-containing diets showed lower concentrations of CHOL (*P* = 0.018) and LDL (*P* = 0.003) and higher concentration of HDL (*P* = 0.035) compared with those fed on the PF-containing diets. Moreover, the DSL supplementation significantly decreased blood concentrations of TAG (*P* = 0.041), while marginally decreasing blood CHOL (*P* = 0.057) and LDL (*P* = 0.049). The two-way interaction

effects of ME × fat, ME × DSL and fat × DSL and the three-way interaction of ME × fat × DSL were not significant for all blood parameters.

Gut morphology

Morphological characteristics in the jejunum of broiler chickens fed the experimental diets are presented in Table 8. There was an interaction between fat sources and DSL supplementation on VH:CD (*P* = 0.007) in the jejunum of broiler chickens; additionally, a trend (*P* = 0.057) was observed for a fat × DSL interaction for the CD of the jejunum. However, the main effect of ME, two-way interaction effects of ME × fat and ME × DSL and the three-way interaction of ME × fat × DSL were not significant for all morphological parameters. Regarding the main effects of fat sources, the VH, VH:CD ratio and VSA were lower (*P* = 0.007, *P* < 0.001 and *P* = 0.029, respectively), whereas CD was higher (*P* = 0.060) in broiler chickens fed on the PF-containing diets compared with in birds fed on the SO-containing diets. Dietary DSL also increased the VH (*P* = 0.002) and VH:CD ratio (*P* < 0.001) and reduced CD (*P* = 0.004) in the jejunum.

Table 7. Effects of different levels of metabolisable energy (ME), various fat sources and de-oiled soyabean lecithin (DSL) supplementation on blood biochemical parameters of broiler chickens at 42 d of age (Mean values with their standard errors; *n*5)

ME	Fat source	DSL (g/kg)	Glucose	TAG	CHOL	HDL	LDL	Protein	Albumin	Uric acid
Normal	SO	0	204.8	95.22	108.3	54.90	34.40	2.46	1.32	3.58
Normal	SO	1	183.0	85.78	105.7	59.48	29.12	2.56	1.44	4.13
Normal	PF	0	199.2	101.40	125.4	49.38	55.68	2.56	1.42	4.49
Normal	PF	1	184.6	88.64	108.7	51.04	39.94	2.51	1.30	4.01
Low	SO	0	195.4	84.78	103.1	51.00	35.16	2.57	1.54	4.85
Low	SO	1	206.2	84.74	100.8	52.08	31.80	2.65	1.39	4.49
Low	PF	0	195.2	96.22	119.2	49.06	50.90	2.62	1.52	5.19
Low	PF	1	196.2	87.74	107.1	49.64	39.92	2.54	1.41	4.33
SEM			11.70	5.21	6.02	2.94	6.11	0.256	0.186	0.786
Main effect means										
ME level										
	Normal		192.9	92.62	112.0	53.70	39.70	2.52	1.37	4.05
	Low		198.3	88.37	107.6	50.45	39.45	2.60	1.47	4.71
	SEM		5.85	2.61	3.01	1.46	3.06	0.128	0.093	0.393
Fat source										
	SO		197.4	87.48	104.5 ^b	54.37 ^a	32.62 ^b	2.56	1.42	4.26
	PF		193.8	93.51	114.1 ^a	49.78 ^b	46.61 ^a	2.55	1.41	4.50
	SEM		5.85	2.61	3.01	1.46	3.06	0.128	0.093	0.393
DSL (g/kg)										
	0		198.7	94.42 ^a	114.0	51.03	44.04 ^a	2.55	1.45	4.53
	1		192.5	86.58 ^b	105.6	53.06	35.19 ^b	2.57	1.39	4.24
	SEM		5.85	2.61	3.01	1.46	3.06	0.128	0.093	0.393
Significance										
	ME level		0.522	0.257	0.302	0.127	0.939	0.692	0.469	0.242
	Fat source		0.671	0.116	0.018	0.035	0.003	0.989	0.937	0.669
	DSL		0.463	0.041	0.057	0.349	0.049	0.945	0.644	0.607
	ME × fat		0.853	0.749	0.897	0.258	0.637	0.880	0.937	0.780
	ME × DSL		0.155	0.339	0.738	0.585	0.702	0.945	0.617	0.566
	Fat × DSL		0.938	0.435	0.173	0.683	0.304	0.672	0.710	0.493
	ME × fat × DSL		0.611	0.703	0.809	0.773	0.871	0.989	0.612	0.817

CHOL, total cholesterol; SO, soyabean oil; PF, poultry fat.

^{a,b} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

Discussion

The present results indicate that DSL supplementation had a significant interaction with the fat type, and DSL significantly improved the FCR and fat digestibility of PF treatments, whereas no significant improvements were observed in the SO treatments. Therefore, it can be said the improvements that can be made with supplemental DSL are highly dependent on the lipid source incorporated in broiler chicken diets. Our results agree with those of Jansen *et al.*⁽¹³⁾ who reported that supplementing exogenous emulsifier in diets enhanced the apparent digestibility of SFA to a greater extent than that of UFA in broiler chickens. Zaefarian *et al.*⁽²⁵⁾ also reported a positive effect of lysolecithin emulsifier at the rate of 3.5 g/kg of diet on digestibility of tallow, as an SFA source. The improvement in growth performance by DSL supplementation in the PF group could be related to an increase in fat digestibility due to the emulsification property of DSL, as confirmed in the present study. Increased fat digestibility by emulsifier supplementation in broiler chickens was observed in other studies^(2,13). Poultry fat is reported to be less easy to emulsify by the native bile salts present in the digestive tract, due to its physicochemical properties^(4,26). Dietary emulsifier could enhance the emulsification process, including the stabilisation and clearance of the lipid droplet surface by bile salts, in such a way that lipase could attach at the interphase^(11,12). The supplemented emulsifier may have also a beneficial role in the

complex equilibrium of adsorption–desorption that is influenced by amphiphilic molecules, including bile salts, phospholipids and proteins, existing at the interphase⁽²⁷⁾. Therefore, it can be said that these changes induced by exogenous emulsifier could enhance the uptake of fat across the enterocyte membrane, resulting in a higher fat bioavailability of the diet.

Several studies have revealed that BW gain and feed efficiency were decreased by feeding low-energy diets, while feed intake was not affected^(2,3). This is similar to our finding that the ADG was lower and FCR was higher in the low-ME groups than the normal ME groups, which may be due to the fact that dietary energy being more easily utilised for chicken growth. Compared with the SO treatment, a lower ADG and a higher FCR observed in the PF treatment up to 24 d might be because the amounts of lipase and bile salts produced by birds are inadequate in the early growth period⁽⁵⁾. Ghasemi *et al.*⁽⁹⁾ also showed that diets with higher UFA:SFA ratio positively influenced BW gain and FCR in broiler chickens up to 28 d when dietary oils were supplemented at 50 g/kg. Hence, it can be said that the degree of fat saturation can affect the growth performance during the early and middle phases of the growth period due to better availability of energy from UFA.

In the present study, normal ME diets increased the abdominal fat deposition and liver weight at 42 d, but those values were lower when SO was used, as indicated by the interaction

Table 8. Effects of different levels of metabolisable energy (ME), various fat sources and de-oiled soyabean lecithin (DSL) supplementation on jejunum morphology of broiler chickens at 42 d of age (Mean values with their standard errors; *n* 5)

ME	Fat source	DSL (g/kg)	VH	VW	CD	VH:CD	VSA
Normal	SO	0	1535	174.4	317.5	5.41	0.842
Normal	SO	1	1564	171.6	292.4	5.45	0.844
Normal	PF	0	1407	166.7	353.5	4.01	0.729
Normal	PF	1	1577	166.1	313.2	5.22	0.830
Low	SO	0	1510	184.2	299.0	5.07	0.885
Low	SO	1	1596	173.3	306.6	5.36	0.868
Low	PF	0	1383	159.4	327.4	4.23	0.691
Low	PF	1	1492	168.9	285.3	5.33	0.826
SEM			43.58	9.31	11.94	0.254	0.058
Main effect means							
ME level							
			1521	169.7	319.1	5.02	0.811
			1495	171.4	304.5	4.99	0.817
			22.19	4.65	5.97	0.127	0.029
Fat source							
			1551 ^a	175.8	303.9	5.32 ^a	0.860 ^a
			1463 ^b	165.3	319.8	4.70 ^b	0.769 ^b
			22.19	4.65	5.97	0.127	0.029
DSL (g/kg)							
			1459 ^b	171.1	324.3 ^a	4.68 ^b	0.787
			1558 ^a	170.0	299.3 ^b	5.34 ^a	0.841
			22.19	4.65	5.97	0.127	0.029
Significance							
			0.417	0.792	0.086	0.875	0.883
			0.007	0.110	0.060	<0.001	0.029
			0.002	0.857	0.004	<0.001	0.180
			0.361	0.546	0.142	0.287	0.508
			0.982	0.944	0.362	0.848	0.922
			0.194	0.393	0.057	0.007	0.129
			0.344	0.492	0.309	0.606	0.751

VH, villus height (µm); VW, villus width (µm); CD, crypt depth (µm); VH:CD, villus height: crypt depth; VSA, villus surface area (mm²) = 2π × (VW/2) × VH; SO, soyabean oil; PF, poultry fat.

^{a,b} Mean values within a column with unlike superscript letters were significantly different (*P* < 0.05).

between ME level and fat source. Excessive fat deposition is one of the major problems for the modern poultry industry, as it not only decreases carcass yield but also causes difficulties in processing and great waste in the slaughterhouse⁽²⁸⁾. The lower abdominal fat deposition was also in line with reduced fat contents of commercial meat cuts in broiler chickens⁽²⁹⁾, thereby making the meat more acceptable for human consumption. Lower abdominal fat percentage found in the broiler chickens fed on the SO-containing diets compared with those receiving the PF-containing diets may be attributable to various metabolic uses of the absorbed dietary fats. It is hypothesised that the influence of dietary fat on tissue fat deposition may be associated with the fact that energy from the SFA is less readily used for metabolic purposes rather than PUFA and thus accumulates as body lipid⁽³⁰⁾. The higher percentage of abdominal fat in the PF-fed broilers in the present study is also consistent with a higher *n*-6:*n*-3 PUFA ratio in the PF-containing diet (Table 2), possibly suggesting the elevation of the inflammatory response. It is reported that a diet high in the *n*-6:*n*-3 PUFA ratio leads to an increase in the endocannabinoid signalling system and related mediators, which result in the enhanced energy homeostasis and production of inflammatory mediators^(31,32). There are

increasing evidence that inflammatory reactions occurring in the adipose tissue contribute to greater fat storage in humans⁽³³⁾ and broilers⁽³⁴⁾ and appear to be linked to the several metabolic syndromes⁽³³⁾. Furthermore, the decreased HDL and increased LDL levels observed in the PF-fed broilers might be attributable to the presence of metabolically induced inflammation, leading to higher abdominal adiposity. This notion is also supported by the evidence that lowering LDL and raising HDL levels have beneficial effects on inflammation, indicating anti-inflammatory effects of HDL and/or proinflammatory properties of LDL⁽³⁵⁾. Since the liver is the primary site for lipogenic process, higher dietary energy content could cause excessive TAG deposition in chicken liver⁽³⁶⁾. Hence, an enhanced liver weight in normal ME groups may be associated with increased hepatic lipogenesis activity and PF could aggravate high energy-induced negative effects on hepatic lipogenesis activity and liver weight.

According to Helkin *et al.*⁽³⁷⁾, blood TAG, CHOL, HDL and LDL are considered as key factors of lipid metabolism balance. In the present study, higher serum concentrations of CHOL and LDL and a lower concentration of HDL were found in broiler chickens fed on the PF-containing diets compared with those fed on the SO-containing diets. In general, these findings are consistent with those of recent studies^(9,38), which demonstrate modifications in blood lipid profiles of broiler chickens with the dietary fat sources differing in their degree of saturation. The decrease observed in blood TAG concentrations by replacing saturated fats in the broiler chicken diet with unsaturated fats might be related to a higher level of β-oxidation activity of UFA, resulting in a higher rate of TAG uptake from the bloodstream into body tissues⁽³⁰⁾. This can be attractive to the consumers as a high PUFA:SFA ratio has positive health benefits for humans, particularly in protection against CVD⁽³⁹⁾.

In the present study, serum concentrations of TAG and CHOL were decreased by dietary DSL, which is consistent with other studies that have shown that soyabean lecithin has TAG and CHOL-lowering capacity in rats⁽⁴⁰⁾ and humans⁽⁴¹⁾. The changes in serum lipid composition in the present study showed that DSL could improve the efficiency of the lipid utilisation in broiler chickens. Tompkins and Parkin⁽⁴²⁾ declared that the long-term ingestion of soya phospholipids by humans could decrease serum TAG concentration via increasing hepatic lipoprotein lipase activity, resulting in an improved lipid metabolism status in the liver and consequently lower TAG levels in serum. This mechanism may be due to the faster rate of clearance of chylomicrons from the blood and slower rate of its secretion into the blood. However, further studies are required to investigate the mechanism of an emulsifier affecting serum metabolite profile.

Intestinal morphology, including VH and CD as well as the VH:CD ratio are indicators of gut⁽⁴³⁾. Villi are the most likely sites of nutrient absorption and increased VH and VH:CD ratio are directly related to higher digestion and absorption⁽⁴³⁾. Morphological analysis indicates that DSL was effective in PF-containing diets, minimising the adverse effect of a higher quantity of NEFA from PF on jejunal VH:CD ratio. This suggests that the potential effects of DSL on intestinal morphology depend on the degree of saturation of lipid incorporated into broiler chicken diets. An explanation could be that DSL supplementation acts

as an emulsifier, thereby enhancing the formation of micelles and fat absorption in the intestine of broiler chickens fed on the saturated fat-containing diets⁽¹³⁾. Higher fat absorption might decrease fermentation in the small intestine, leading to lower villi surface damage and consequently better growth performance⁽⁴⁴⁾. Previous studies showed different results due to the addition of emulsifier supplementation to animal diet. For example, Boontiam *et al.*⁽¹⁷⁾ reported that the VH, CD and VH:CD ratio in the broiler jejunum were improved as the result of supplementation with lysophospholipid (0.5, 1 and 1.5 g/kg); but with respect to the duodenal morphology, only CD was decreased by lysophospholipid. Alzawqari *et al.*⁽¹⁹⁾ also showed linear increases in VH, VSA and VH:CD ratio in the jejunum and ileum, but no morphological changes in the duodenum by feeding desiccated ox bile to broilers. In another study, Mitchaothai *et al.*⁽⁴⁴⁾ showed no significant influence of dietary lecithin on morphological characteristics in the three different sites of the small intestine in young wild pigs. In the present study, the longer villi and VH:CD ratio of the jejunum observed in the DSL-fed broiler chickens indicates an increased nutrient absorption for optimal growth and production.

The morphological characteristics of intestinal mucosa can be modified by the metabolic activities of the intestinal microflora that have important consequences for human and animal health and well-being⁽⁴⁵⁾. With regard to the influence of dietary fat source, it is reported that consuming SFA source instead of UFA source led to a higher value of pH in the intestinal contents and increased levels of pathogenic bacteria, which results in intestinal tissue destruction⁽⁴⁶⁾.

In conclusion, the low-ME diets negatively affected growth performance but had no significant effects on blood metabolites and gut morphology. In contrast, the dietary fat types, SO or PF, had no effect on the overall performance of broiler chickens but influenced abdominal fat deposition, fat digestibility, serum lipid concentrations and jejunum morphology. Dietary DSL supplementation could promote feed efficiency and gut health when applied to broiler chicken diets containing saturated fat source such as PF, probably due to increasing its utilisation efficiency and consequently increasing its ME. However, the inclusion of 0.1 % DSL to the low-energy diet could not exert an extra beneficial effect in terms of growth performance and physiological traits.

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References

1. Wiseman J & Lewis CE (1998) Influence of dietary energy and nutrient concentration on the growth of body weight and carcass components of broiler chickens. *J Agric Sci* **131**, 361–371.
2. Zhao PY & Kim IH (2017) Effect of diets with different energy and lysophospholipids levels on performance, nutrient metabolism, and body composition in broilers. *Poult Sci* **96**, 1341–1347.
3. Ge XK, Wang AA, Ying ZZ, *et al.* (2019) Effects of diets with different energy and bile acids levels on growth performance and lipid metabolism in broilers. *Poult Sci* **98**, 887–895.
4. Zhang B, Haitao L, Zhao D, *et al.* (2011) Effect of fat type and lysophosphatidylcholine addition to broiler diets on performance, apparent digestibility of fatty acids, and apparent metabolizable energy content. *Anim Feed Sci Technol* **163**, 177–184.
5. Ravindran V, Tancharoenrat P, Zaefarian F, *et al.* (2016) Fats in poultry nutrition: digestive physiology and factors influencing their utilisation. *Anim Feed Sci Technol* **213**, 1–21.
6. Khonyoung D, Yamauchi K & Suzuki K (2015) Influence of dietary fat sources and lysolecithin on growth performance, visceral organ size, and histological intestinal alteration in broiler chickens. *Livest Sci* **176**, 111–120.
7. Cho JH, Zhao PY & Kim IH (2012) Effects of emulsifier and multi-enzyme in different energy density diet on growth performance, blood profiles, and relative organ weight in broiler chickens. *J Agric Sci* **4**, 161–168.
8. Smink W, Gerrits WJJ, Hovenier R, *et al.* (2010) Effect of dietary fat sources on fatty acid deposition and lipid metabolism in broiler chickens. *Poult Sci* **89**, 2432–2440.
9. Ghasemi HA, Shivazad M, Mirzapour Rezaei SS, *et al.* (2016) Effect of synbiotic supplementation and dietary fat sources on broiler performance, serum lipids, muscle fatty acid profile and meat quality. *Br Poult Sci* **57**, 71–83.
10. Rodriguez-Sanchez R, Tres A, Sala R, *et al.* (2019) Evolution of lipid classes and fatty acid digestibility along the gastrointestinal tract of broiler chickens fed different fat sources at different ages. *Poult Sci* **98**, 1341–1353.
11. Liu D & Ma F (2011) Soybean phospholipids. In *Recent Trends for Enhancing the Diversity and Quality of Soybean Products*, pp. 483–500 [D Krezhova, editor]. Rijeka, Croatia: Intech.
12. Maldonado-Valderrama J, Wilde P, Macierzanka A, *et al.* (2011) The role of bile salts in digestion. *Adv Colloid Interface Sci* **165**, 36–46.
13. Jansen M, Nuyens F, Buyse J, *et al.* (2015) Interaction between fat type and lysolecithin supplementation in broiler feeds. *Poult Sci* **94**, 2506–2515.
14. Zhang H, Dudley EG, Davidson PM, *et al.* (2017) Critical concentration of lecithin enhances the antimicrobial activity of eugenol against *Escherichia coli*. *Appl Environ Microbiol* **83**, e03467-16.
15. Van Wijk N, Balvers M, Cansev M, *et al.* (2016) Dietary crude lecithin increases systemic availability of dietary docosahexaenoic acid with combined intake in rats. *Lipids* **51**, 833–846.
16. Raju MV, Rao SV, Chakrabarti PP, *et al.* (2011) Rice bran lysolecithin as a source of energy in broiler chicken diet. *Br Poult Sci* **52**, 769–774.
17. Boontiam W, Jung B & Kim YY (2017) Effects of lysophospholipid supplementation to lower nutrient diets on growth performance, intestinal morphology, and blood metabolites in broiler chickens. *Poult Sci* **96**, 593–601.
18. Siyal FA, El-Hack MEA, Alagawany M, *et al.* (2017) Effect of soy lecithin on growth performance, nutrient digestibility and hepatic antioxidant parameters of broiler chickens. *Int J Pharmacol* **13**, 396–402.

19. Alzawqari M, Nassiri Moghaddam H, Kermanshahi H, *et al.* (2011) The effect of desiccated ox bile supplementation on performance, fat digestibility, gut morphology and blood chemistry of broiler chickens fed tallow diets. *J Appl Anim Res* **39**, 169–174.
20. Aviagen (2014) Ross 308 broiler. In *Nutrition Specification* [RB Limited, editor]. Newbridge, Midlothian, Scotland: Aviagen.
21. Association of Official Analytical Chemists (2007) *Official Methods of Analysis*, 18th ed. Washington, DC: AOAC.
22. Moore S (1963) On the determination of cystine as cysteic acid. *J Biol Chem* **238**, 235–237.
23. Vogtmann H, Pfirter HP & Prabucki AL (1975) A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. *Br Poult Sci* **16**, 531–534.
24. Tavernari FDC, Roza LF, Surek D, *et al.* (2018) Apparent metabolisable energy and amino acid digestibility of microalgae *Spirulina platensis* as an ingredient in broiler chicken diets. *Br Poult Sci* **59**, 562–567.
25. Zaeferian F, Romero LF & Ravindran V (2015) Influence of high dose of phytase and an emulsifier on performance, apparent metabolisable energy and nitrogen retention in broilers fed on diets containing soy oil or tallow. *Br Poult Sci* **56**, 590–597.
26. Guerreiro Neto AC, Pezzato AC, Sartori JR, *et al.* (2011) Emulsifier in broiler diets containing different fat sources. *Braz J Poult Sci* **13**, 119–125.
27. Singh H, Ye A & Home D (2009) Structuring food emulsions in the gastrointestinal tract to modify lipid digestion. *Prog Lipid Res* **48**, 92–100.
28. Wang HB, Li H, Wang QG, *et al.* (2007) Profiling of chicken adipose tissue gene expression by genome array. *BMC Genomics* **8**, 193.
29. Suksombat W, Boonmee T & Lounglawan P (2007) Effects of various levels of conjugated linoleic acid supplementation on fatty acid content and carcass composition of broilers. *Poult Sci* **86**, 318–324.
30. Monfaredi A, Rezaei M & Sayyahzadeh H (2011) Effect of supplemental fat in low energy diets on some blood parameters and carcass characteristics of broiler chicks. *S Afr J Anim Sci* **41**, 24–32.
31. Duan Y, Li F, Li L, *et al.* (2014) n-6:n-3 PUFA ratio is involved in regulating lipid metabolism and inflammation in pigs. *Br J Nutr* **111**, 445–451.
32. Simopoulos AP (2016) An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients* **8**, 128.
33. Scalia R (2013) The microcirculation in adipose tissue inflammation. *Rev Endocr Metab Disord* **14**, 69–76.
34. Khadem A, Soler L, Everaert N, *et al.* (2014) Growth promotion in broilers by both oxytetracycline and *Macleaya cordata* extract is based on their anti-inflammatory properties. *Br J Nutr* **112**, 1110–1118.
35. Vaziri ND, Moradi H, Pahl MV, *et al.* (2009) *In vitro* stimulation of HDL anti-inflammatory activity and inhibition of LDL pro-inflammatory activity in the plasma of patients with end-stage renal disease by an apoA-1 mimetic peptide. *Kidney Int* **76**, 437–444.
36. Sanz M, Perezde Ayala AP & Lopez-bote CJ (1999) Higher lipid accumulation in broilers fed saturated fats than in those fed unsaturated fats. *Br Poult Sci* **40**, 95–101.
37. Helkin A, Stein JJ, Lin S, *et al.* (2016) Dyslipidemia part 1 – review of lipid metabolism and vascular cell physiology. *Vasc Endovascular Surg* **50**, 107–118.
38. Zafari Naeini S, Rezvani MR, Akhlaghi A, *et al.* (2013) Effects of different dietary fat and oil supplements on performance, carcass, and blood characteristics in broiler chickens. *Eur Poult Sci* **77**, 90–95.
39. Cao Y, Lu L, Liang J, *et al.* (2014) Omega-3 fatty acids and primary and secondary prevention of cardiovascular disease. *Cell Biochem Biophys* **72**, 77–81.
40. Polichetti E, Diaconescu N, Porte PLDL, *et al.* (1996) Cholesterol-lowering effect of soyabean lecithin in normolipidaemic rats by stimulation of biliary lipid secretion. *Br J Nutr* **75**, 471–481.
41. Burgess JW, Neville TA, Rouillard P, *et al.* (2005). Phosphatidylinositol increases HDL-C levels in humans. *J Lipid Res* **46**, 350–355.
42. Tompkins RK & Parkin LG (1980) Effects of long-term ingestion of soya phospholipids on serum lipids in humans. *Am J Surg* **140**, 360–364.
43. Leonard SG, Sweeney T & Bahar B (2011) Effect of dietary seaweed extracts and fish oil supplementation in sows on performance, intestinal microflora, intestinal morphology, volatile fatty acid concentrations and immune status of weaned pigs. *Br J Nutr* **105**, 549–560.
44. Mitchaothai J, Yuangklang C, Vasupen K, *et al.* (2010) Effect of dietary calcium and lecithin on growth performance and small intestinal morphology of young wild pigs. *Livest Sci* **134**, 106–108.
45. Reid G, Sanders ME, Gaskins HR, *et al.* (2003) New scientific paradigms for probiotics and prebiotics. *J Clin Gastroenterol* **37**, 105–118.
46. Józefiak D, Świątkiewicz S, Kierończyk B, *et al.* (2016) *Clostridium perfringens* challenge and dietary fat type modifies performance, microbiota composition and histomorphology of the broiler chicken gastrointestinal tract. *Eur Poult Sci* **80**, 130.