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RESEARCH ARTICLE

Growth, Serum Biochemical Indices, Antioxidant Status and Meat Quality of Broiler Chickens Fed Diet Supplemented with Sodium Stearoyl-2 Lactylate

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ABSTRACT

This experiment was performed to examine the impact of sodium stearoyl-2-lactylate (SSL) on growth, antioxidants enzymes and meat quality of broilers fed low energy diets. A total of 240 Arbor Acre broilers were randomly distributed into four groups in complete randomized experimental design. The experimental groups were as: 1) PC: control was fed with the diet without adding any emulsifier, 2) P1: Low energy diet (LED) + 0.025% SSL, 3) P2: LED + 0.05% SSL and 4) P3: LED + 0.1% SSL, respectively. Findings in our study demonstrated that chicks fed with P2 diet had greater ($P<0.05$) average daily feed intake (ADFI), daily weight gain (ADG) and feed conversion ratio (FCR) compared to PC during 0-21 days. Moreover, during 21-42 days and overall experiment, the ADG and ADFI were statistically higher ($P<0.05$) in P3 as compared to other groups. The better FCR during whole experimental period was observed in P3 comparing to control and other treatments. Weights of pancreas and thymus were significantly ($P<0.05$) improved in P2 and P3, while spleen weight was higher ($P\leq0.05$) in P3 followed by P2 then P1 compared to PC. The birds treated with P3 showed the lowest ($P\leq0.05$) value of serum total cholesterol at 42 days of age. Values of breast muscle color were statistically ($P\leq0.05$) differed due to treatments. The group of P3 showed significantly decreased lightness (L*) and increased redness of breast muscle compared to other groups. In addition, serum concentration of malondialdehyde (MDA) depressed ($P\leq0.05$) and GSH-Px activity increased ($P\leq0.05$) in P3 comparing with other groups. It can be concluded that dietary supplementation of SSL (0.025-0.1%) in low energy diets exhibited similar or more effective effects on growth performance than the high metabolizable energy diet. However, the use of 0.1% SSL can be effective to improve the growth performance, meat quality and antioxidant status of broiler.

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INTRODUCTION

Dietary fats and its inclusion level affect lipids lipoproteins digestibility and triacyglycerols concentration in chickens' blood (Viveros *et al.,* 2009). However, digestion of fat and its absorption varies with bird age, since immature chickens have physiological restrictions to absorb the nutrient (Lima *et al.,* 2003). Therefore, such problems can be avoided by using exogenous emulsifiers to enhance dietary fat utilization. Emulsifier encourage the fatty acids assimilation into micelles, therefore emulsifiers addition might results in improved digestion of fat and growth performance of broilers (Polin, 1980). Thus, using synthetic emulsifiers into the diets of poultry is one of the modern practices compared to other dietary additives.

Using bile salt as poultry diet Supplement can improve fat digestibility and formation of emulsion (Kussaibati *et al.,* 1982). Generally, emulsifiers have positive effect on performance and feed efficiency of birds; however, some diversified results have also been

reported in previous studies (Van Heugten and Odle, 2000; Daněk *et al.*, 2005) and this could be due to different methodologies used in experiments.

Sodium stearoyl-2 lactylate (SSL) is a salt of sodium with long-chained carboxylic acid and have two linkages of ester and widely used in modern food industry. In previous studies, SSL has effectively been used as emulsifier (Gómez *et al.*, 2004) and conditioning agent (Armero and Collar, 1998). However, the related studies which examined the impact of SSL on the activities of anti-oxidation enzymes in broiler are limited.

Furthermore, metabolizable energy (ME) level in the feed is one of major factors to obtain proper growth of broiler. In addition, study regarding the supplementation of SSL in varying ME diets has not been reported, hence this study aimed to examine the impacts of sodium stearoyl-2 lactylate on growth performance, serum biochemical indices, antioxidant enzyme activities and carcass quality assessment of broiler chickens.

MATERIALS AND METHODS

Design, birds, diets and management: The experiment was performed as per suggestions of Animal Care and Use Committee of Nanjing Agricultural University, China. Total of 240 Arbor Acre chicks one-day old (average body weight=45.97±3g) were used in a 42-day experiment using complete randomized design. Broilers were assigned into four groups with five replicates per group and 12 birds per replicate. The experimental groups were as follows: 1) PC: the control which fed the basal diet without any added emulsifier, 2) P1: low energy diet (LED) + 0.025% sodium stearoyl-2 lactylate (SSL), 3) P2: LED + 0.05% SSL and 4) P3: LED + 0.1% SSL, respectively. Level of energy decreased by minimizing level of palm oil as illustrated in Table 1. The starter diets were fed to birds up to 21 d followed by finisher diets during 21 to 42 d of age. Birds were allowed to consume feed and water *ad libitum* in an environmentally controlled room with temperature ranging from 34-36°C through the period of 1-14 day of age and later on progressively decreased to 26°C up to finishing the experiment.

Sampling and measurements: Birds were weighed at the days of 21 and 42 by cages. Average daily Feed intake (ADFI) was recorded to calculate average daily gain (ADG) as well as feed conversion ratio (FCR). At the end of feeding trial, the chickens were prohibited from feed for 12 h with free access to water. Afterward, one bird for each replicate randomly selected to weight and sacrificed by severing of jugular vein. Samples of blood were collected then centrifuged at 3,000 × g for 15 min at 4°C to separate the serum. Samples were frozen at −25°C until analysis. Serum content of total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were calculated by via diagnostic kits provided by Nanjing Jiancheng Bioengineering Institute, China. While, serum total protein, urea, creatinine and glucose were determined by means of automatic biochemical analyzer. The visceral organs (liver, kidney, thymus, lung, pancreas, spleen and bursa) were weighed after collection. Relative weights of above menthe aforementioned organs were determined as:g of the organ/kg of body weight.

Breast meat quality evaluation: The pH and color values of breast meat samples were evaluated at the time of slaughter. Digital pH meter (Hanna, Italy) was used to measure the pH values, while color values were evaluated for redness (a*), yellowness and (b*) lightness (L*) by Konica Minolta colorimeter CR-400 (Japan) by the method of (Pi *et al*., 2005)**.** Breast muscle drip loss was calculated at the slaughtering time, 24 and 48 hrs post slaughter by using the suspension method (Honikel, 1998). The breast muscles were cut to a 40 g strip (40 × 10 × 80 mm). After weighing, strip was hanged in a plastic zip lock bag and kept in a cabinet having controlled temperature at 2°C for 24 h. extra moisture was then lightly removed from the surface of muscle and samples were reweighed. In this way drip loss was calculated and loss in weight was mentioned as a proportion of initial weight.

Table 1: Formulation and calculated composition of diet (as-fed basis)

Ingredient	Starter (0-21 days of age)				Finisher (22-42 days of age)			
	PC	P1	P2	P3	PC	P1	P2	P3
Corn	54.10	55.08	55.05	55.00	64.15	65.12	65.10	65.05
Soybean meal 49.8%	35.64	35.64	35.64	35.64	27.75	27.75	27.75	27.75
Palm oil	5.51	4.51	4.51	4.51	4.16	3.16	3.16	3.16
Dicalcium phosphate	1.87	1.87	1.87	1.87	1.45	1.45	1.45	1.45
Limestone 39%	1.24	1.24	1.24	1.24	1.01	1.01	1.01	1.01
Salt	0.38	0.38	0.38	0.38	0.28	0.28	0.28	0.28
Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride 60%	0.08	0.08	0.08	0.08	0.05	0.05	0.05	0.05
BS premix	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine HCl	0.16	0.16	0.16	0.16	0.18	0.18	0.18	0.18
DL-Methionine	0.26	0.26	0.26	0.26	0.20	0.20	0.20	0.20
L-Threonine	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02
Pellet binder	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Toxin binder	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Cygro	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
SSL	0.00	0.025	0.05	0.10	0.00	0.025	0.05	0.10
Total	100	100	100	100	100	100	100	100
Analyzed composition (%)								
CP	22.30	22.30	22.30	22.30	20.10	20.10	20.10	20.10
Ether Extract	5.48	5.48	5.48	5.48	6.13	6.13	6.13	6.13
Dry matter	89.74	89.74	89.74	89.74	88.12	88.12	88.12	88.12

Provided per kg of diet: Iron, 60 mg; copper, 7.5 mg; zinc, 65 mg; manganese, 110 mg; iodine, 1.1 mg; selenium. 0.4 mg; Bacitracin Zinc, 30 mg; vitamin A, 4500 IU; vitamin D3, 1,000 IU; vitamin E, 20 mg; vitamin K, 1.3 mg; vitamin B1, 2.2 mg; vitamin B2, 10 mg; vitamin B3, 10 mg; choline choride, 400 mg; vitamin B5, 50 mg; vitamin B6,4 mg; Biotin, 0.04 mg; vitamin B11, 1mg; vitamin B12, 1.013 mg.

Measurement of antioxidant enzymes: The enzyme activity of antioxidants such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and glutathione peroxidase (GSH-PX) content in the serum were evaluated spectrophotometrically with help of the commercial kits which purchased from Nanjing Jiancheng Bioengineering Institute, China. Activities of CAT, SOD and GSH-PX were expressed as units (U) per milliliter whereas MDA content was measured into nanomoles per milliliter.

The Chemical composition of feed samples: In hot air oven, samples of feed were dried at 65°C for 24 hours and then grounded through small lab mill fitted with one mm screen. After drying, samples were evaluated for dry matter (DM), crude protein (CP) and ether extract (EE) according to (AOAC, 1990). Samples were kept air dried oven at 65°C for 48 h for determination of dry matter (DM). The ether extract (EE) of samples was extracted with methanol by using soxhlet apparatus (Method 945.16). Crude protein (CP) was determined by Kjeldahl (Method 984.13) with Kjeltec 2300 apparatus of Foss USA.

The statistical analysis: Using one-way ANOVA, data were analyzed. Also, the test of Duncan was used to define significant difference (P<0.05) between the mean values by using SPSS software (Ver.20).

RESULTS

Growth performance: As shown in Table 2, from days 0-21, chicks fed with P2 showed higher (P<0.05) ADG, ADFI and FCR comparing with PC. During the finisher phase (21-42 days) ADG and ADFI were significantly improved (P<0.05) in P3, while FCR was non-significant among the various treatments. Moreover, from days 0-42, chicken fed on theP3 diet has the best (P<0.05) ADG, ADFI and FCR compared to other treatments.

Relative organ weights: Relative weight of pancreas and thymus were statistically better (P<0.05) in P2 and P3 comparing with PC and P1 treatments, while spleen weight was found to be higher (P<0.05) in P3, P2 and P1 other than PC. Moreover, bursa index was in significantly improved in SSL treated groups in a dose dependent manner. Similarly, relative weights of liver, lung and kidney were unaffected due to various treatments (Table 3).

Serum biochemical indices: Influence of dietary sodium stearoyl 2 lactylate on blood biochemical indices of broiler is summarized in Table 4. No significant effects (P>0.05) on serum triglycerides as well as high density lipoprotein cholesterol were reported. However, feeding SSL resulted in lower (P<0.05) total cholesterol in P3 compared to PC and P1 groups. While low density lipoprotein cholesterol remained unaffected between various treatments. Comparing to PC, the circulating level of total protein (TP) was increased (P<0.05) in P3 and P2 whilst serum glucose was improved (P<0.05) in all SSL supplementary groups.

Table 2: Effect of sodium stearoyl-2 lactylate on growth performance of broiler chickens (birds/group=60)

Items	PC	P1	P2	P3	SEM
Starter phase (days 0-21)					
ADG (g/d)	31.23^{b}	33.00^{ab}	33.43^{a}	33.07^{ab}	0.605
ADFI (g/d)	45.61^{b}	48.31^{ab}	48.47^{a}	48.03^{ab}	0.876
FCR (g feed/ g gain)	1.46^{a}	1.46^{a}	1.44^{b}	1.45^{ab}	0.020
Finisher phase (days 22-42)					
ADG (g/d)	56.97^{b}	61.39^{b}	60.27^{b}	68.03^{a}	1.914
ADFI (g/d)	120.4^{c}	129.1^{ab}	125.0^{bc}	133.7^{a}	1.618
FCR (g feed/ g gain)	2.13	2.10	2.08	1.96	0.059
Overall experiment (days 0-42)					
ADG (g/d)	44.10^{c}	47.23^{b}	46.85^{b}	50.51^{a}	0.372
ADFI (g/d)	83.05^{c}	88.60^{ab}	86.75^{b}	91.02^{a}	0.856
FCR	1.88^{a}	1.87^{a}	1.85^{a}	1.80^{b}	0.012

PC (basal diet), P1 (LED + 0.025% SSL); P2 (LED + 0.05% SSL); P3 (LED + 0.1% SSL), LED (low energy diet); ^{abc}Means in the same row with different superscripts differ (P<0.05).

Table 3: Effects of sodium stearoyl-2 lactylate on relative organ weight (g/kg of body weight) of broiler chicken at 42 days of age (birds/group =60)

Items	PC	P1	P2	P3	SEM
Liver	50.93	51.01	51.77	51.49	1.367
Pancreas	4.25^{bc}	4.18^{c}	5.08^{a}	4.84^{ab}	0.208
Thymus	3.61^{b}	3.99^{b}	5.10^{a}	5.74^{a}	0.227
Bursa of fabricius	1.67	1.80	1.91	2.10	0.138
Spleen	3.16^{b}	4.00^{a}	3.94^{a}	4.22^{a}	0.202
Kidney	4.52	5.07	5.16	5.16	0.207
Lung	4.91	4.73	5.11	5.18	0.243

PC (basal diet), P1 (LED + 0.025% SSL); P2 (LED + 0.05% SSL); P3 (LED +0.1% SSL); LED (low energy diet); ^{abc}Means in the same row with different superscripts differ (P<0.05).

Table 4: Effects of sodium stearoyl-2 lactylate on blood biochemical indices of broiler at 42 days of age (birds/group=60).

Items	PC	P1	P2	P3	SEM
Triglyceride (mmo/L)	0.64	0.68	0.75	0.72	0.045
Total cholesterol (mmo/L)	2.60^{a}	2.57^{a}	2.43^{ab}	2.38^{b}	0.061
HDL-C (mmo/L)	2.00	2.06	2.06	2.05	0.031
LDL-C (mmo/L)	1.19	1.08	0.77	0.74	0.135
Total protein (g/L)	36.96^{c}	37.81^{bc}	40.16^{b}	43.66^{a}	0.842
Glucose (mmo/L)	12.97^{b}	15.38^{a}	14.54^{a}	15.25^{a}	0.366
Urea (mmo/L)	0.51	0.54	0.54	0.51	0.012
Creatinin (umol/L)	35.88	37.62	37.66	35.67	0.893

PC (basal diet), P1 (LED + 0.025% SSL); P2 (LED + 0.05% SSL); P3 (LED + 0.1% SSL); LED (low energy diet); LDL-C (low density lipoprotein cholesterol); HDL-C (high density lipoprotein cholesterol); ^{abc}Means in the same row with different superscripts differ (P<0.05).

Table 5: Effect of sodium stearoyl 2-lactylate on serum antioxidant enzymes activity of broiler at days of age (birds/group=60)

Items	PC	P1	P2	P3	SEM
GSH-Px(U/ml)	247^{b}	332^{ab}	361^{ab}	428^{a}	3.77
CAT (U/ml)	2.29	2.27	2.31	2.34	0.211
SOD (U/ml)	422	406	413	426	2.67
MDA (nmol/ml)	4.17^{a}	3.38^{a}	2.41^{b}	2.31^{b}	0.255

PC (basal diet), P1 (LED + 0.025% SSL); P2 (LED + 0.05% SSL); P3 (LED + 0.1% SSL); LED (low energy diet); SOD (superoxide dismutase); GSH-Px (glutathione peroxidase; CAT (catalase), and MDA (malondialdehyde); ^{abc}Means in the same row with different superscripts differ (P<0.05).

Table 6: Effects of sodium stearoyl-2 lactylate on breast meat color, pH and Drip loss of broiler at 42 days of age (birds/group=60)

Items	PC	P1	P2	P3	SEM
pH	6.51	6.56	6.49	6.50	0.025
Lightness (L)	50.55^{a}	49.70^{a}	48.30^{ab}	46.72^{b}	0.781
Redness (a)	3.18^{b}	3.16^{b}	3.80^{ab}	4.13^{a}	0.256
Yellowness (b)	12.31	12.85	12.45	12.47	0.531
DL% at slaughtering time	3.70	3.67	3.65	3.72	0.066
DL% 24 hrs post slaughter	3.86	3.78	3.81	3.90	0.069
DL% 48 hrs post slaughter	3.85	3.88	3.84	3.74	0.063

PC (basal diet), P1 (LED + 0.025% SSL); P2 (LED + 0.05% SSL); P3 (LED + 0.1% SSL); LED (low energy diet); DL (drip loss). ^{abc}Means in the same row with different superscripts differ (P<0.05).

Serum antioxidant enzymes activities: As shown in Table 5, use of P3 diet significantly increased (P<0.05) serum GSH-Px compared with PC, however, SOD and CAT were not statistically affected. Furthermore, serum MDA content found to be low in P3 and P2 (P<0.05) other than PC and P1 groups.

Quality of breast meat: Dietary supplementation of SSL had no (P>0.05) effect on breast meat pH and drip loss during slaughtering time, 24 and 48 hrs post slaughter (Table 6). On the other hand, breast muscle lightness (L) decreased (P<0.05) and redness (a) were significantly improved in P3 treatment compared to PC and P1 groups, however, b values remained unaffected between birds fed diets supplemented with sodium staeroyl-2 lactylate or the control diet (PC).

DISCUSSION

Growth performance and relative organ weight: Birds consumed diet enriched with P2 (SSL0.05%) showed better ADG, ADFI and FCR for 0-21 days. Furthermore, during grower phase and overall experiment birds in the group of P3 (SSL0.1%) showed highest ADG and ADFI while improved FCR during 0-42 days of age. This is consistent with previous results of (Udomprasert and Rukkwamsuk, 2006) who found beneficial effects of exogenous emulsifiers on BW, ADFI and FCR of broiler chickens. Furthermore, enhanced utilization of dietary fat, better live weight gain and feed efficiency were observed with supplementing emulsifier into broiler diet (Dierick and Decuypere, 2004) which is in accordance with our results. The better effects by adding emulsifier to the diets on growth could be attributed to better feed palatability which resulted in more consumption of feed and energy (Øverland and Sundstøl, 1995). It is clear that emulsifier addition into diet of broilers has improved growth and digestion in the beginning stage of life (0 to 21 days). Insufficient production and lipase activity in young broilers which reaches at high point after 40 and 56 days of age could be the reason for this result (Krogdahl and Sell, 1989). In contrast, Azman and Ciftci (2004) found non-significant effect on body weight of groups fed with lecithin at 21 and 35 days and those of the control group. According to Wieland *et al.* (1993), emulsifiers efficiency to increase medium-chain triglycerides absorption varies. Therefore, we assumed that variation in growth performance results might be because of fat source and emulsifier type used therefore chickens can extract more nutrients and could sustain their growth rate under low energy density diet.

Relative weight of pancreas and thymus was significantly improved in P2 and P3 while relative weight of spleen was found to be higher in P3, P2 and P1 other than PC. Moreover, bursa index was improved in SSL treated groups in a dose dependent manner but effect was non- significant. Similar findings were confirmed by Cho *et al.*(2012) who found that the weight of bursa of fabricus did not affect by supplementation of SSL into broiler diet.

Blood biochemical indices: Sodium stearoyl 2 lactylate have no impact on serum HDL-C, LDL-C and TG. However, feeding SSL resulted in lower (P<0.05) the concentration of TC in P3 comparing with PC and P1 groups. Our results are in agreement with reports of Spilburg *et al.* (2003) who found that soy lecithin powder decreased cholesterol absorption and increased fecal sterol excretion when polyunsaturated phosphatidylcholine (PC) was added to diet (Greten *et al.*, 1980). On the contrary, (Guerreiro Neto *et al.*, 2011) observed insignificant effect on total cholesterol level with addition of emulsifier. It's unclear by which mechanism SSL induced serum cholesterol minimizing property. This may be possibly that due to the decreased cholesterol absorption in small intestine according to Iwata *et al.*(1992).

Our results showed that serum concentration of TP increased (P<0.05) in P3 and P2 and glucose was elevated (P<0.05) in all SSL supplementary groups compared to PC. Such improvement in serum concentration of glucose might be attributed to the utilization of the majority of available glucose for growth and production in SSL supplemented groups. Whereas, elevated total serum protein in the birds of P3 and P2 group could be related to improved protein digestibility and increased availability of amino acid precursors for protein synthesis.

Meat quality assessment: Table 5; shows effects of SSL on meat quality assessment of broiler after 42 days of age. Decreased *L* values and increased *a* values indicate less pale and more red meat that reflect desirable traits by the consumers. In our study, broilers received the P3 diet showed significantly decreased *L* and increased *a* values in breast meat compared with other groups. In addition, dietary SSL had no effect on breast meat pH and drip loss during slaughtering time, 24 and 48hrs post slaughter. Similar to our findings, Akit *et al.* (2014) demonstrated that dietary emulsifier improved *a* values and decreased *L* values but has no effect on pork meat pH and drip loss. In contrast, Kim *et al.* (2008) found no differences between meat color from the pigs fed lecithin and the control diets. The increase in redness (*a*) might be expected due to possible increase in myoglobin content. A simultaneous increase of redness (*a*) and decrease of lightness (*L*) were demonstrated in meat that contained increased myoglobin content (Gil *et al.*, 2001).

Serum antioxidant enzymes activities: Broilers are prone to oxidative stress under certain unsuitable physiological and environmental conditions, especially in cases of immunological stress. Such damages can be prevented by using antioxidants. In our study, supplementation of SSL into broiler diet increased activity of serum GSH-Px and decreased MDA content in P3 group. Our findings are also in harmony with previous studies (Al-Daraji *et al.*, 2010; Ahmed *et al*., 2016; Butt *et al*., 2016) who found that lecithin can increase activities of GSH, GPx, SOD and GST whereas decreased TBARS in the blood and seminal plasma of rabbit bucks. Furthermore, antioxidant effect of emulsifier depends on the oil type or its tocopherols and fatty acid composition of oil (Judde *et al*., 2003; Ilyas *et al*., 2016; Mashkoor *et al*., 2016). Therefore, it could be imagine that the synergistic effect of SSL occurred with γ- and δ- forms of tocopherols or tocotrienols that are naturally found in palm oil. Taken together, both decreased level of MDA and the elevated GSH-Px activity in the serum showed that SSL can protect the bird against oxidative stress and to improve immunity.

Conclusions: It can be concluded that dietary supplementation of SSL (0.025-0.1%) in low energy diets exhibited similar or more effective effects on growth performance than the high metabolizable energy diet. However, the use of 0.1% SSL can be effective to improve the growth performance, meat quality and antioxidant status of broiler.

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