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Impact of Emulsifier Supplementation with Varying Levels of Metabolizable Energy on Production Performance, Egg Quality and Blood Parameters of Commercial Layers

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Key words
Dietary lipid, Energy level, Laying hen, Emulsifier, Egg production

ABSTRACT

The current study investigated the effect of emulsifier supplementation, while reducing the dietary metabolizable energy (ME) to determine its impact on production performance, egg quality parameters and blood parameters of commercial laying hens. Total 7125 commercial laying hens (at the production stage 54 weeks of age) were divided randomly into five treatment groups with three replicates/treatment. Five diets were formulated according to the nutrients requirement of laying hens (Nick Chick). A basal diet T_1 with ME 2800 Kcal/kg served as a negative control, T_2 supplemented with 500 g/ton emulsifier and 2800 Kcal/kg ME, T_3 supplemented with 500 g/ton emulsifier while decreased ME 40 Kcal/kg, T_4 supplemented with 500 g/ton emulsifier and having ME 80 Kcal/kg reduced and T_5 supplemented with 500 g/ton emulsifier and decreased ME 120 Kcal/kg. Feed intake, egg production, egg weight and feed conversion ratio were recorded daily. Blood samples were collected for lipid profile estimation. The highest feed intake and egg production were observed in T_5 and T_2 groups, respectively. The egg quality parameters (egg shell strength, egg shell thickness, yolk index, and haugh unit) were not affected by the dietary energy level and emulsifier supplementation. Blood lipid profile was also affected by the supplementation of emulsifier as the highest total cholesterol, low density lipoprotein, high density lipoprotein were observed in the hens fed T_2 diet compared with the others. Overall, the emulsifier can be used in layer diet (500 g/ton) while reducing the energy content (40-80 Kcal/kg) without any adverse effect on the production performance and egg quality of laying hens.

INTRODUCTION

The addition of fats and oils has received considerable attention recently to increase the energy density of the feeds (Ahmadi-Sefat *et al*., 2022; Oketch *et al*., 2022). Such an approach might be relatively more convenient to meet the demands of modern poultry and reduce the feed cost without compromising the production performance. Additionally, nutritionists remain in continuous search for the approaches to improve the energy utilization efficiency of lipids instead of further increase in energy densities. Several strategies are used for this purpose including the feed processing through steaming, combination of saturated and unsaturated fats to maximize the natural emulsifying effects of unsaturated fats, decreased Ca concentration to prevent lipophytin synthesis, enzyme supplementation, and exogenous emulsifier (Meng *et al*., 2004; Ravindran *et al*., 2016; Oketch *et al*., 2023).

The process of fat and oil digestion is more complex than other macro-nutrients as it largely depends on the supply of bile salts, pancreatic lipase, and co-lipase. Several processes are involved in lipid digestion including the breakdown of large droplets, emulsification, lipolysis, and micelle and chylomicron formation, which are secreted through the portal system to be transported in the bird (Ravindran *et al*., 2016). Nutritional, physiological,

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and biochemical benefits of dietary lipids in broilers have been investigated previously (Ravindran *et al*., 2016; Oketch *et al*., 2022, 2023). Therefore, the interest in using the emulsifiers has been increased recently to improve the growth performance and fat utilization in broilers. Exogenous emulsifiers are known to improve the active surface area for lipase to break large fat droplets into smaller ones, facilitating the lipids absorption process (Ko *et al*., 2023). Various emulsifiers are tested in broiler feeds including sodium stearoyl 2-lactylate, lysolecithin, lysophosphatidyl choline, glycerol polyethylene glycol ricinoleate, soy-lecithin, and bile salts (Roy *et al*., 2010; Zhang *et al*., 2011; Siyal *et al*., 2017). In general, emulsifiers may improve the fat digestion and energy efficiency of feed even in the low energy diets (Saleh *et al*., 2020; Oketch *et al*., 2022). As a result, improved weight gain, feed efficiency, and lipid metabolism has been observed with exogenous emulsifier supplementation in broiler diet (Bontempo *et al*., 2018). A meta-analysis also determined that the addition of 125 and 250 g/ton emulsifier in the diets containing approximately 4.42% lipids could replace 57.9 and 73.1 Kcal/kg of feed, respectively, without compromising the growth performance of the birds (Wealleans *et al*., 2020). Several other studies supported these findings that dietary emulsifier improved the growth performance and feed utilization of chicken by increasing the fatty acid digestibility (Zhao and Kim, 2017; Siyal *et al*., 2017; San Tan *et al*., 2016).

However, most of the studies investigated the effects of dietary emulsifiers with different energy supplies in broilers (Cho *et al*., 2012; Aguilar *et al*., 2013; Oketch *et al*., 2022, 2023) and limited literature is available on the effects of dietary emulsifiers, especially with varying dietary energy supplies in layers. Therefore, the current study was planned to investigate the effects of exogenous lysophospholipids emulsifier in combination with the reducing dietary energy levels on egg production, egg quality, and serum lipid profile of layers.

MATERIALS AND METHODS

Experimental design

The experiment was carried out in a commercial cage layer farm (Roomi Poultry Pvt. Ltd. Kabirwala, Pakistan). Laying hens (n= 7125) were randomly divided into 5 treatment groups in a completely randomized design. There were three replicates of each treatment, with 475 birds in each replicate. Nick Chick strain laying hen at the of age 54 weeks and approximately 1610 g weight was used in this experiment. Birds were kept in cages (60.96×60.96×45.72 cm). All the birds had free access to fresh drinking water throughout the experiment. The birds were vaccinated against Newcastle disease (day 1, 12, 35, and 40 of age) and infectious bronchitis (day 1 and 40 of age). The experiment was conducted under the protocols approved by the ethical committee for animal welfare at the Cholistan University of Veterinary Sciences, Bahawalpur (No. ORIC/272). Total duration of the experiment was 45 days. The environmental conditions were regularly monitored and adjusted according to the birds behavior and age.

Experimental diets

The experimental diets were formulated according to the nutritional standards of nick chick white egg layers. The ingredients and chemical compositions of the treatments diets is presented in Table I. Before the start of the trial, 5 treatment groups were designed; each group comprised three replicates. The first treatment (T_1) served as a basal diet and negative control containing the optimized ME level (2800 kcal/kg). The second treatment (T_2) served as a positive control containing basal diet and addition of 500 g/ton of emulsifier, the third treatment group (T_3) was basal diet and addition of 500 g/ton of emulsifier while reduction of 40 Kcal/kg ME (2760 Kcal/kg), fourth treatment group (T_4) was basal diet plus 500 g/ton of emulsifier while decrease 80 Kcal/kg ME (2720 Kcal/kg) and fifth treatment (T_5) was 120 kcal/kg ME (2680 Kcal/kg) less than basal diet and supplemented with 500 g/ton of emulsifier (Smart LPL, Devenish, Ireland). Feed formulation and feed production were done separately for each treatment group, stocked in individual and clearly labeled bags to provide for each treatment and its replicates.

Data collection

Production performance was measured in terms of feed consumption, egg production, egg weight, and feed conversion ratio (FCR). The FCR was calculated by FCR = feed consumed (g)/ egg mass (g) following Clark *et al*. (2019). Egg quality performance of laying hens was assessed in terms of egg shell strength, egg shell thickness, Haugh unit, and yolk index at the age of 60 week. Egg shell strength was measured using egg force reader following Kang *et al*. (2018). Digital Vernier calipers were used to measure the egg shell thickness without inner and outer shell membranes in the equatorial region of each treatment group (Kang *et al*., 2018). Yolk index was determined by dividing the yolk height by the yolk diameter (Sauter *et al*., 1951). Haugh unit was calculated by haugh unit = 100 × log (albumen height – 1.7 × egg weight + 7.6) following Eisen *et al*. (1962). Blood parameters included serum total cholesterol, serum triglycerides, high-density lipoproteins (HDL), and low-density lipoproteins (LDL).

Table I. Ingredients and chemical composition of experimental diets.

Item	Diets				
	T_1	T_2	T_3	T_4	T_5
Ingredients (% of DM)					
Maize grains	51.7	51.7	52.8	60.0	64.3
Rice broken	10.0	10.0	10.0	3.67	00.0
Soya bean meal	5.00	5.00	5.00	5.00	5.00
Canola meal	6.81	6.81	5.80	6.35	6.30
Guar meal	3.08	3.08	3.70	3.00	3.22
Rape seed meal	2.00	2.00	2.00	2.00	2.00
Fish meal	6.00	6.00	6.00	6.00	6.00
Poultry meal	2.00	2.00	2.00	1.94	1.69
Lime stone	10.2	10.2	10.2	10.2	10.2
Sunflower oil	2.30	2.30	1.50	0.90	0.30
Sodium chloride	0.22	0.22	0.22	0.216	0.22
Sodium bicarbonate	0.025	0.025	0.025	0.025	0.025
Mineral premix	0.05	0.05	0.05	0.05	0.05
Vitamin premix	0.05	0.05	0.05	0.05	0.05
DL-methionine	0.18	0.18	0.19	0.18	0.19
L-threonine	0.026	0.026	0.028	0.025	0.022
L-tryptophan	0.009	0.009	0.009	0.015	0.017
L-lysine sulphate	0.23	0.23	0.24	0.24	0.24
Choline	0.08	0.08	0.08	0.08	0.08
Micro ingredients	0.10	0.10	0.10	0.10	0.10
Smart LPL (Emulsifier) %	0.00	0.05	0.05	0.05	0.05
Analyzed chemical composition (%)					
Dry matter	87.2	87.2	88.1	88.4	88.7
Crude protein	16.1	16.1	16.0	16.1	16.1
Ash	13.4	13.4	13.4	13.5	13.6
Crude fiber	2.99	2.99	3.00	3.00	3.04
Crude fat	6.06	6.06	5.31	4.90	4.43
Calculated content (%)					
Metabolizable energy (Kcal/Kg)	2800	2800	2760	2720	2680
Calcium	4.16	4.16	4.18	4.18	4.17
Total phosphorus	0.53	0.53	0.53	0.54	0.55
Lysine	0.81	0.81	0.81	0.81	0.81
Methionine	0.40	0.40	0.40	0.39	0.40
Threonine	0.50	0.50	0.50	0.49	0.50
Tryptophan	0.16	0.16	0.16	0.16	0.17
Dietary electrolyte balance (mEq/kg)	152	152	150	153	155

Blood samples (03 ml from each bird) were collected from jugular vein in gel and clot activator tube at the age of 60 week. This blood containing tubes were put in centrifugal machine at 4000 rpm for 10 min to attain serum and store in cups. For serum analysis, commercially available enzymatic kits were used for cholesterol (Artiss and Zak, 1997; Fluitest CHOL, Analyticon Biotechnologies, Lichtenfels, Germany), triglyceride (Cole *et al*., 1997; Fluitest TG, Analyticon Biotechnologies, Lichtenfels, Germany), and HDL contents (Schettler and Nussel, 1975; Fluitest HDL, Analyticon Biotechnologies, Lichtenfels, Germany). The concentration of LDL was calculated using the formula LDL = total cholesterol – (triglyceride⁄5) – HDL, as described by Friedewald *et al*. (1972).

Statistical analysis

The collected of data were analyzed using the analysis of variance (ANOVA) technique under a completely randomized design by using SPSS-20.0 for the dietary treatment as the source of variation. Statistical significance was declared at $P < 0.05$. Tukey's multiple comparison test was used to compare the means where significant treatment effects were observed (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Production performance

Laying hen's production performance was measured in terms of feed consumption, egg production, egg weight and FCR. The mean values of daily feed intake, egg production, egg weight and feed conversion ratio of the commercial layer as affected by the emulsifier (Smart LPL) are presented in Table II. Maximum feed intake (102.9 g/d) was noted in the laying hens fed diet T_5 than hens fed others diets ($P < 0.05$). Conversely, hens fed diets T_1 (without emulsifier and 2800 ME), T_2, T_3, and T_4, showed the similar result ($P > 0.05$). Highest egg production and total number of eggs were observed in hens fed diet T_2 than hens fed other diets ($P < 0.05$). Minimum egg production was noticed in hens fed diet T_5 than hens fed other diets ($P < 0.05$). Conversely, hens fed diets T_1, T_3, and T_4 showed a non-significant ($P > 0.05$) difference in egg production. Highest egg weight was observed with T2 diet while lowest egg weight was observed with T1 and T5 ($P < 0.05$). Similar initial body weight was observed in all treatment groups ($P > 0.05$). Better FCR was noted in hens fed diet T_2 than hens fed other diets ($P < 0.05$). Poor FCR was recorded for hens fed diet T_5 than hens fed other diets ($P < 0.05$). Conversely, hens fed diets T_1, T_3, and T_4 showed similar FCR ($P > 0.05$).

Table II. Impact of emulsifier with varying levels of metabolizable energy on production performance of commercial layer.

Item	Treatment groups					SEM*	P-value
	T_1	T_2	T_3	T_4	T_5		
Feed intake (g/d)	101.7[b]	101.6[b]	101.7[b]	101.8[b]	102.9[a]	0.04	0.007
Egg production (%)	72.8[b]	74.6[a]	72.8[b]	72.6[b]	69.9[c]	0.09	0.004
No. of eggs/d per replicate	344[b]	352[a]	343[b]	344[b]	332[c]	0.464	0.003
Egg weight (g)	61.5[c]	61.9[a]	61.8[ab]	61.6[bc]	61.4[c]	0.027	0.001
Feed conversion ratio	2.28[b]	2.21[c]	2.27[b]	2.28[b]	2.39[a]	0.003	0.007
Initial body weight (g)	1601	1605	1610	1613	1618	20.7	0.500

Means with different superscripts in a row are statistically non-significant (P<0.05). *SEM, Standard error mean.

Table III. Impact of emulsifier with varying levels of metabolizable energy on egg quality of commercial layer.

Item	Treatment groups					SEM*	P value
	T_1	T_2	T_3	T_4	T_5		
Egg shell strength (KgF)	3.54	3.63	3.63	3.60	3.58	0.018	0.377
Egg shell thickness (mm)	0.39	0.40	0.40	0.40	0.41	0.002	0.561
Haugh unit	83.9	84.4	83.4	83.6	83.7	0.19	0.459
Yolk index	0.32	0.31	0.33	0.31	0.33	0.005	0.338

Means with different superscripts in a row are statistically non-significant (P<0.05). *SEM, Standard error mean.

Harms *et al.* (2000), reported that feed intake was increased by lowering the dietary energy, which indicates that laying hens are sensitive to lowering the energy supplies. According to Mohsen and Mousa (2022), adding emulsifiers to the feed of laying hens improved the nutritional absorption and digestion. Celebi and Utlu (2004) observed that feed consumption dropped likely due to the increased energy density in the diet than in the control diet (2740 vs 2600 kcal/kg ME, respectively). The literature is still lacking in details about the addition of emulsifiers to the diets of laying hens. In a study by Roy *et al.* (2010), exogenous emulsifier supplementation has shown to be beneficial in low-energy diets, with broilers performing better than those delivered low-energy diets without emulsifier. According to the findings of Rovers and Excentials (2014), incorporating emulsifiers into feeds can serve as a method to enhance lipid digestibility and subsequently improve energy efficiency. Juntanapum *et al.* (2019) concluded that emulsifier supplementation improved FCR and decreased feed intake when they conducted an experiment to evaluate the effects of emulsifier supplementation in diets on productive performance of laying hens. In our study, energy content might not be sufficient for the action of emulsifier with the low energy diets to achieve the increase in egg production. Contrarily, higher egg production with emulsifier supplementation without reducing dietary energy could be a result of improved energy utilization because of its emulsifying properties, increased micelle formation, and increased nutrient absorption (Van Nieuwenhuyzen and Tomas, 2008; Zhao *et al.*, 2015; Boontiam *et al.*, 2017).

Egg quality

Egg quality performance of laying hens in terms of egg shell strength, egg shell thickness, haugh unit, and yolk index as influenced by the emulsifier (Smart LPL) are presented in Table III. Egg shell strength, haugh unit, and egg yolk index were not affected by the dietary treatments as similar egg shell strength, haugh unit, and egg yolk index were noticed in all treatment groups (P>0.05).

In the current study, no effect of emulsifier supplementation observed on egg quality were in agreement with Ferreira *et al.* (2022). According to Juntanapum *et al.* (2019), no evidence of a substantial impact of emulsifier supplementation on egg shell quality and Haugh units were observed. Even with the energy level being reduced, there were no discernible negative impacts on egg quality and it was conceivable to conclude that the effect of emulsifier on dietary lipids was advantageous in providing laying hens more energy by improving the absorption of fatty acids from low energy diets through intestinal walls (Torrico *et al.*, 2014; Hu *et al.*, 2019).

Table IV. Impact of emulsifier with varying level of metabolizable energy on serum lipid profile of commercial layer.

Item	Treatment groups					SEM*	P value
	T_1	T_2	T_3	T_4	T_5		
Total cholesterol (mg/dL)	232^{d}	315^{a}	291^{b}	251^{c}	253^{c}	8.1	0.004
LDL (mg/dL)	121^{d}	136^{a}	131^{b}	126^{c}	119^{d}	1.7	0.004
HDL (mg/dL)	39.3^{c}	63.3^{a}	49.7^{b}	41.7^{c}	40.3^{c}	2.42	0.001
Triglycerides (mg/dL)	1265^{e}	2038^{b}	1341^{d}	2194^{a}	1635^{c}	98.6	0.002

Means with different superscripts in a row are statistically non-significant (P<0.05). *SEM, Standard error mean.

Blood lipid parameters

Blood lipid parameters are presented in Table IV. Highest cholesterol level (315 mg/dL) was observed in hens fed diet T_2 than hens fed other diets (P<0.05). While, the lowest cholesterol level (232 mg/dL) was recorded in hens fed diet T_1 than hens fed other diets (P<0.05). Similar cholesterol level (P>0.05) was noted in hens fed diets T_4 and T_5. Maximum LDL level (136 mg/dL) was noticed in hens fed diets T_2 than hens fed other diets (P<0.05). Similar LDL level (P>0.05) was observed in hens fed diets T_1 and T_5. Highest HDL level (63.3 mg/dL) was observed in hens fed diet T_2 than hens fed other diets (P<0.05). Conversely, similar HDL level (P>0.05) was recorded in hens fed diets T_1, T_4 and T_5. Maximum triglycerides level (2194 mg/dL) was noticed in hens fed diet T_4 compared with those fed other diets (P<0.05). Lowest value for triglycerides (1265 mg/dL) was observed in hens fed diet T_1 than hens fed other diets (P<0.05).

The results of hens fed diets T_3, T_4 and T_5 were in agreement with those reported previously by Huang *et al*. (2007), who observed a reduction in total serum cholesterol with soy-lecithin supplementation in broiler. Present study results deviate from those reported by Melegy *et al*. (2010) and Osek *et al*. (2008), where dietary oil and emulsifier supplementation had no impact on the serum cholesterol fractions in broilers. Jankowski *et al*. (2012) reported that serum cholesterol fractions remained unaffected in turkey hens when consumed diets fortified with soybean, rapeseed, or linseed oil. According to Park *et al*. (2018), emulsifier incorporation had no impact on blood total cholesterol. However, it has also been reported that birds fed a diet enriched with emulsifier experienced lower levels of LDL in the serum (Jones *et al*., 1992). Another study demonstrated that emulsifier products based on soybean substantially decreased the levels of LDL and cholesterol (Medic *et al*., 2003). However, increased cholesterol and LDL with emulsifier supplementation without decreasing dietary energy is in agreement with previous reports, where these responses were attributed to the type of dietary fat source (vegetable vs. animal) and to the inclusion level of both dietary fat and emulsifier (Wang *et al*., 2016; Bontempo *et al*., 2018). In our study, HDL level was increased in hens fed diet T_2. Multiple potential mechanisms are involved in the regulation of lipid metabolism (Bontempo *et al*., 2018), high HDL level with diet T_2 might be due to increased digestibility of dietary fat by addition of emulsifier as reported previously (Dierick and Decuypere, 2004; Upadhaya *et al*. 2018; Ahmadi-Sefat *et al*., 2022), resulting in increased HDL absorption. The present study findings align with Huang *et al*. (2007), as they observed an improvement in serum HDL through the supplementation of soy-lecithin. Similarly, according to Celebi and Utlu (2004), layers fed linseed oil-enriched diets had considerably larger HDL fractions in their serum. Al-Daraji *et al*. (2010) found that Japanese quail fed experimental diets enriched with flaxseed oil and fish oil had noticeably increased levels of HDL fractions. Huang *et al*. (2007) reported that soy-lecithin supplementation reduced blood triglyceride levels, which is consistent with the findings of the current investigation. Contrarily, no effect of dietary emulsifier supplementation with a range of dietary fat sources (linseed oil, sunflower oil, soybean oil, and lard) has been reported in broilers (Melegy *et al*., 2010; Neto *et al*., 2011; Febel *et al*., 2008). Contradictory findings of previous studies on the changes in blood lipid parameters of broilers with dietary emulsifier addition are reported to be age related (Hoque *et al*., 2022). Various studies reported either no change (Upadhaya *et al*., 2018; Saleh *et al*., 2020; Liu *et al*., 2020) or a decrease (Cho *et al*., 2012; Zhao and Kim, 2017) in blood lipid parameters with dietary emulsifier supplementation. Nevertheless, the positive production response of laying hen to emulsifier supplementation in our study and limited knowledge in aged laying hens are indicative of further investigation with respect to different life stages in terms of blood lipid changes with dietary energy variations, supplemental emulsifier type and inclusion levels.

CONCLUSIONS

The emulsifier can be used in layer diets (500 g/ton) while reducing the dietary energy contents (40-

80 Kcal/kg) without any adverse effect on production performance and egg quality. Emulsifier supplementation helps to increase the absorption of fatty substances and other nutrients from diet. The highest egg production with best FCR was observed by emulsifier supplementation without reducing the dietary energy content. Nevertheless, the positive production response of laying hen to emulsifier supplementation in this study warrants further investigation for better understanding on fat digestion process and economic efficacy in response to emulsifier supplementation.

DECLARATIONS

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IRB approval

The study was approved by the Office of Research, Innovation, and Commercialization (ORIC) at the Cholistan University of Veterinary Sciences, Bahawalpur, Pakistan (No. ORIC/272).

Ethical statement

The experiment was conducted under the protocols approved by the ethical committee for animal welfare at the Cholistan University of Veterinary Sciences, Bahawalpur, Pakistan.

Statement of conflict of interest

The authors have declared no conflict of interest.

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