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ABSTRACT

The objective of this experiment was to investigate the effect of supplementation of an exogenous emulsifier (lyso-phospholipid) in the diet of growing broilers on growth performance and digestibility. A total of 1224 Ross-308 ten day old broiler chicks were distributed into two experimental treatments in such a way that each treatment had twelve replicates with fifty-one birds per replicate. Two experimental diets were formulated with and without emulsifier supplementation according to the nutrition standards of Ross 308. Feed intake and body weight gain of the broilers were measured on a daily basis and feed conversion ratio was also calculated. Nutrient digestibility was determined on the 25th day of age. Analysis of variance under completely randomized design technique was used to analyze the data. Feed intake was increased ($p < 0.05$) by supplementation of emulsifier in the broiler diet on the 12th, 13th, 21th, 22th, 23th, 24th, and 25th days. Bodyweight gain was not affected ($p > 0.05$) with or without emulsifier supplementation in the broiler diet during 11-25 days of life. However, feed conversion ratio was affected ($p < 0.05$) by emulsifier supplementation and increased from days 21-25th. Nutrient digestibility (dry matter, fat, and crude protein) in the grower phase was decreased ($p < 0.05$) by supplementation of emulsifier in the diet. It can be concluded that supplementation of an exogenous emulsifier in the diet did not show positive effect on the growth performance during the grower phase of broilers, while nutrient digestibility showed adverse effect. Emulsifier supplementation should be tested after 25 days of the life of broilers.

INTRODUCTION

Poultry production has gained special attention in the growth of economy of the world especially in developing countries. However, many challenges (health and production) still need to be addressed to achieve maximum output (Abbas *et al.*, 2019; Mehmood *et al.*, 2019; Rahman *et al.*, 2019; Shehata *et al.*, 2019; Khater *et al.*, 2020). Good feed with proper nutrient optimization is the primary concern in broiler diet for proper growth, development and good economics (Ashraf *et al.*, 2019; Asad *et al.*, 2019; Sarwar *et al.*, 2019; Mujahid *et al.*, 2019). Among other nutrients, energy supply shows considerable effect on the cost of feed formulation. To fulfill the demand of energy, different fats and oils are being added in the feed to reduce the dustiness, improve the palatability, efficiency of feed and growth performance of broilers (Nayebpor *et al.*, 2007; Febel *et al.*, 2008; Azman & Ciftci, 2014; Mehmood *et al.*, 2020). However, the digestibility of fat reduces due to the addition of fat in the diet during early age (Siyal *et al.*, 2017; Ward *et al.*, 2019). For the absorption of fatty acids from the small intestine, fatty acids agglomerate and form lipid micelles. This



mechanism is resolved by the natural emulsifier in the digestive tract of broilers like bile salts and pancreatic lipase (Noy & Sklan, 1998). During the early age of broilers, absorption of fats is less due to the immaturity of digestive organs and less secretions of bile salts and lipases (Noy & Sklan, 1995). This incapacity of the organs leads to less digestion and absorption of fats due to the insufficiency of the development of mixed micelles in the small intestine of the broilers (Leeson & Atteh, 1995; Raheel *et al.*, 2019).

Different emulsifiers are added in the feed of broilers to improve the emulsification process by enhancing the activity of lipases and consolidate the fatty acids into lipid micelles, which directly improve the digestion and absorption of fats (Zhang *et al.*, 2011). Zaho & Kim, (2017) reported that supplementation of exogenous emulsifiers in the diet improved the digestibility of fats and the performance of broilers. Similarly, it was reported that Lysophospholipid based emulsifier in the diet enhances the efficiency of feed, digestibility and productive performance of the chicken (Zampiga *et al.*, 2016; Zainb *et al.*, 2019).

Similarly, Zaefarian *et al.* (2015) publicized that lysolecithin emulsifier supplemented with different fat sources enhanced body weight gain (BWG) and improved feed conversion ratio (FCR) during 1-21 days of broilers. In contrast, Khonyoung *et al.* (2015) reported that there was an increase in the BWG and a decrease in the FCR of broilers by the addition of exogenous emulsifiers in the diet during the 7th -21st days only. Boontiam *et al.* (2017) concluded that the addition of a natural emulsifier (lysophospholipids) increased BWG and improved feed intake (FI) and FCR of broilers during starter (0-7 days), grower (8-21 days) and finisher (22-35 days) phases.

Based on the evidences from previous researchers, emulsifiers positively effect on the feed intake and improved the growth performance of broilers (Mohammadigheisar *et al.*, 2018; Papadopoulos *et al.*, 2018; Li *et al.*, 2020). But no study had yet been reported in the literature to check the effect of emulsifier in the broiler diet during the 11th -25th days (grower phase). Therefore, a study was planned to elaborate the impact of a natural exogenous emulsifier in the diet of broilers during the 11th -25th days life only.

MATERIALS AND METHODS

The experiment was conducted at the Research and Development Farm of Shamim Feed Mills (Pvt. Ltd.) Samma Satta Link Road, Bahawalpur, Pakistan.

Experimental design

A total of 1224 Ross 308 broiler chicks were raised. Total birds were divided into two experimental groups. Each group was assigned with twelve replicates with fifty-one birds per replicate. All the chicks were raised on concrete floor with rice husks litter material and floor space of 0.6 square feet. The birds were placed in the metal pens with dimensions of 9.2 × 4.2 × 2.5 cubic feet. All pens were equipped with automatic nipple drinkers and feeders. All the facilities were provided for the normal behavior as described by Rahman *et al.*, (2019). A natural emulsifier Lysoforte EXTEND (lyso-phospholipids) was used in the diet. During the starter phase (1-10 days), feed was formulated without an emulsifier. However, during the grower phase (11-25 days), two experimental diets were formulated with and without emulsifier (lyso-phospholipids). Both diets were formulated according to the feeding standards of Ross 308 guidelines. All diets were formulated on digestible amino acids basis keeping lysine as reference

Table 1 – Ingredients and nutrient composition (g/kg) of broiler diets.

Ingredients	Starter	Grower	Finisher
Corn (¹ CP 7.5%)	51.96	55.64	56.38
Soybean meal (CP 45%)	35.96	26.04	18.41
Canola meal (CP 38%)	5	10	15
Tallow	2.5	3.8	5.4
Mono-dicalcium phosphate	-	1.2	1.1
Dicalcium phosphate	1.5	-	-
Limestone	1.15	1.3	1.2
L-lysine	0.43	0.5	0.74
DL-Methionine	0.41	0.41	0.51
L-Threonine	0.19	0.2	0.32
L-Tryptophan	-	0.01	0.04
NaHCO ₃	0.1	0.1	0.1
Salt	0.3	0.3	0.3
² Vitamin premix	0.2	0.2	0.2
³ Mineral premix	0.2	0.2	0.2
Choline	0.1	0.1	0.1
Total	100	100	100
Chemical Composition			
Metabolizable Energy (kcal/kg)	3000	3100	3200
CP (%)	23.76	21.28	19.98
Crude Fat (%)	4.82	6.31	8.02
Lysine (%)	1.5	1.28	1.25
Methionine (%)	0.72	0.66	0.71
Phosphorous (%)	0.45	0.42	0.4
Calcium (%)	0.92	0.84	0.8
Emulsifier Lysoforte Extend (Lyso-phospholipids) @500 g/ton			

¹Crude protein, ²Vitamin mixture MNVIT-96 supplies (per kilogram of mixture): 5,600,000 IU vitamin A (acetate), 1,760,000 IU vitamin D3 \ (cholecalciferol), 16,800 IU vitamin E (acetate), 0.7 g menadione dimethyl pyrimidinol bisulfite, 3.2 g riboflavin, 6.4 g d-calcium pantothenate, 36 g niacin, 7.2 mg vitamin B12, 0.7 g folic acid, 2.0 g pyridoxine, and 80 mg biotin, ³Mineral premix: Mn, 88 mg; Cu, 66 mg; Fe, 8.5 mg; Zn, 88 mg; Se, 0.30 mg/kg.



amino acid as described in recent studies (Hussain *et al.*, 2018; Hussain *et al.*, 2020). Feed and water were provided ad libitum to the birds and light was provided to the birds for 24 hours as described in the recent study by (Rahman *et al.*, 2015). During the 1st week temperature of 33-35 °C was maintained using brooder then the temperature was reduced by 3 °C per week.

Performance Parameters

Feed intake and BWG of birds of each pen were calculated from days 11-25 on a daily basis for the calculation of FCR. Mortality was noted on a daily basis.

Nutrient Digestibility and Chemical Analysis

Dry matter (DM), crude protein (CP), and ether extract (EE) of feces were calculated for digestibility. For digestibility assay, 1% celite was used as an internal marker and was mixed in the feed for the last three days, from days 22-25. A polythene sheet was used on the litter material for fecal collection. After three days of collection, a composite sample was obtained, litter material was removed and stored in the polythene sampling bags. Then the samples were dried in the oven and used for nutrient digestibility (DM, CP, and EE), ash and AIA as methods explained by the AOAC (2000) with some modifications as describe by researchers (Muhammad *et al.*, 2016; Niu *et al.*, 2017; Xia *et al.*, 2018). Then digestibility was calculated by the following formula:

$$\text{Digestibility \%} = 100 - \frac{(100 \times \% \text{ marker in feed} \times \% \text{ nutrient in feces})}{\% \text{ marker in feces} \times \% \text{ nutrient in feed}} \times 100$$

Statistical Design

Data were evaluated by using Analysis of Variance (ANOVA) under Completely Randomized Design (CRD) of statistical software of Minitab 17 (Minitab, 2010). Standard Error of Mean (SEM) was calculated and results were declared according to the *p* values.

RESULTS

The effect of natural exogenous emulsifiers in the diet of broilers on FI during 11-25 days is shown in table 2. Data showed that FI was affected (*p*<0.05) by supplementation of emulsifier in the diet on the 12th and 13th days. Similar trend was found on the 21st, 22th, 23th, 24th, and 25th days (*p*<0.05) of the trial. Feed intake was increased (*p*<0.05) in those broilers supplemented with emulsifier in the diet as compared to the broilers without supplementation in the diet. However, emulsifier supplementation (ES) showed no significant result (*p*>0.05) on the 11th, 14th, 15th, 16th, 17th, 18th, 19th, 20th days. Effect of the addition of emulsifier on BWG in the diet of broiler the broiler diet is shown in table 3. Results revealed that ES in the grower phase has no effect (*p*>0.05) on BWG. Although there was no statistical effect of ES on BWG but numerically ES showed better BWG than a diet that was not supplemented with an emulsifier. The effect of the inclusion of the exogenous emulsifier in the diet of the broilers on FCR during the 11th -25th days is shown in the table 4. The results showed that supplementation of natural emulsifiers in the diet did not show any effect (*p*>0.05) on FCR of the broilers during the 11th -20th days of the trial. However, from

Table 2 – Effect of Emulsifier on Feed Intake (g/bird) of broilers during 11-25 days.

Days	Without Emulsifier	With Emulsifier	¹ SEM	² <i>p</i> Value
Day 11	50.617	49.896	0.266	0.068
Day 12	55.400 ^b	56.567 ^a	0.313	0.015
Day 13	61.013 ^b	62.571 ^a	0.358	0.006
Day 14	67.883	67.318	0.416	0.347
Day 15	73.354	73.146	0.392	0.710
Day 16	78.771	78.238	0.626	0.553
Day 17	84.146	84.275	0.410	0.826
Day 18	89.408	90.196	0.495	0.273
Day 19	94.717	94.767	0.540	0.183
Day 20	100.992	102.142	0.628	0.209
Day 21	106.271 ^b	109.067 ^a	0.826	0.026
Day 22	111.496 ^b	114.385 ^a	0.717	0.009
Day 23	117.492 ^b	120.937 ^a	0.785	0.005
Day 24	122.971 ^b	126.564 ^a	0.789	0.008
Day 25	127.492 ^b	130.796 ^a	0.793	0.007

¹Standard error of the mean of 12 replicates,

²a, b Means with different superscripts within a row differ significantly (*p*<0.05).



Table 3 – Effect of Emulsifier on Body weight gain (g/bird) of broilers during 11-25 days.

Days	Without Emulsifier	With Emulsifier	¹ SEM	² p Value
Day 11	42.308	42.388	0.567	0.922
Day 12	45.504	45.575	0.565	0.930
Day 13	48.721	48.921	0.644	0.828
Day 14	52.062	52.367	0.637	0.738
Day 15	55.508	55.783	0.590	0.745
Day 16	58.958	59.212	0.479	0.711
Day 17	62.592	63.075	0.393	0.394
Day 18	66.479	66.900	0.326	0.372
Day 19	69.825	70.117	0.211	0.338
Day 20	73.413	73.642	0.239	0.504
Day 21	77.163	76.808	0.288	0.394
Day 22	80.329	79.938	0.420	0.516
Day 23	83.525	82.983	0.522	0.471
Day 24	86.792	86.771	0.480	0.976
Day 25	90.271	90.275	0.720	0.997

¹Standard error of the mean of 12 replicates, ²a, b Means with different superscripts within a row differ significantly ($p < 0.05$).

Table 4 – Effect of Emulsifier on Feed conversion ratio of broilers during 11-25 days.

Days	Without Emulsifier	With Emulsifier	¹ SEM	² p Value
Day 11	1.20	1.18	0.0152	0.385
Day 12	1.22	1.24	0.0137	0.231
Day 13	1.25	1.28	0.0140	0.184
Day 14	1.31	1.29	0.0133	0.345
Day 15	1.32	1.31	0.0107	0.512
Day 16	1.34	1.32	0.0068	0.133
Day 17	1.34	1.34	0.0062	0.334
Day 18	1.35	1.35	0.0060	0.716
Day 19	1.36	1.37	0.0071	0.373
Day 20	1.38	1.39	0.0076	0.307
Day 21	1.38 ^b	1.42 ^a	0.0080	0.001
Day 22	1.39 ^b	1.43 ^a	0.0071	0.000
Day 23	1.41 ^b	1.46 ^a	0.0078	0.000
Day 24	1.42 ^b	1.46 ^a	0.0078	0.001
Day 25	1.41 ^b	1.45 ^a	0.0112	0.031

¹Standard error of the mean of 12 replicates,

²a,b Means with different superscripts within a row differ significantly ($p < 0.05$).

the 21th to 25th day, better FCR was achieved in broiler birds with the diet, which was not supplemented with emulsifier as compared with the diet supplemented with an emulsifier. The effect of the emulsifier in the broiler diet broilers on the digestibility of DM, CP, and EE is shown in table 5. Results showed that there was considerable effect ($p < 0.05$) of the addition of exogenous emulsifier in the broiler diet on DM %, CP

% and EE % digestibility. The % digestibility of DM, CP, and EE was increased in the broiler's diet, which was not supplemented with an emulsifier.

DISCUSSION

Previous researchers described that the physiological potential for the digestion and absorption of fats

Table 5 – Effect of emulsifier on nutrient digestibility of broiler o day 25th.

Item	Without Emulsifier	With Emulsifier	¹ SEM	² p Value
Grower Phase (At Day 25 th)				
DM Digestibility (%)	95.51 ^a	92.93 ^b	0.516	0.005
CP Digestibility (%)	67.12 ^a	52.38 ^b	3.710	0.018
Fat Digestibility (%)	90.34 ^a	85.16 ^b	1.060	0.006

¹Standard error of the mean of 12 replicates.

²a,b Means with different superscripts within a row differ significantly ($p < 0.05$).



is reduced during the early age of broilers and improved during 1.5 to 3.5 weeks of life (Freeman, 1984; Krogdahl, 1985). Many researchers focused on providing exogenous emulsifiers in the diet for better digestion and absorption of fats (Siyal *et al.*, 2017). However in the current study, ES decreased the digestibility of fat. In the present study, BWG was not affected by the supplementation of the emulsifier in the diet during the 11th -25th days. Similar results were reported by Upadhaya *et al.* (2017) that exogenous ES in the diet did not show any effect on BWG during the grower phase of the broilers. In the current study with age and development of secretory organs in broilers, digestion of fat was improved with the diet without supplementation of the emulsifier. The emulsifier intended to perform the function of fat digestion similar to that of the secretory organs, showed no significant effect in the grower phase. In contrast to the findings of the current study, Cho *et al.* (2012) and Wang *et al.* (2016) publicized that ES in the broiler diet improved the growth performance.

Feed intake was not affected during the early days of the trial but FI was increased from day 21 to 25 of age. Similar results were documented by Khonyoung *et al.* (2015) that FI was improved by supplementation of the emulsifier in the diet. This was due to better palatability of feed, which positively affected FI (Febel *et al.*, 2008; Noy & Sklan, 1998). In contrast to the findings of current studies, Zhang *et al.* (2011) reported that FI was not affected by the addition of supplementation of exogenous emulsifier in the broiler dietbroilers. Likewise, Upadhaya *et al.* (2017) reported that FI was not affected by the supplementation of emulsifier in diet during grower phase of the broilers.

Ho Cho *et al.* (2012) described that FCR was not influenced by the supplementation of the emulsifier in the broiler diet. Similar results were obtained during 11-20 days in the current study. However, during the 21th to 25th days of age, FCR was increased by supplementation of an emulsifier as compared to the diet without supplementation of emulsifier. In the current study, higher FCR in broiler supplemented with emulsifier could be justified by BWG and FI. In the present study, BWG was not improved with ES in broilers, but FI was increased, which resulted in higher FCR.

Digestibility of DM, CP, and EE was decreased by supplementation of the emulsifier during the 11th -25th days of the trial. This may be due a reduced functioning of the digestive organs. While the results of Park *et al.* (2018) and Neto *et al.* (2011) of ES on digestibility were

in contrast to the current study that the digestibility of EE was increased, but DM and CP digestibility were not affected by ES in the diet. Similar results were reported by Ho Cho *et al.* (2012) and Dražbo *et al.* (2019) that nutrient digestibility was not affected by supplementation of the emulsifier in the broiler diet. Lesser nutrient digestibility in the birds fed diet with ES represents that ES could have an adverse effect on digestive enzymes or digestive organs that result in lower digestibility of nutrients.

CONCLUSION

In conclusion, supplementation of natural exogenous emulsifiers in the diets did not show positive effect on the growth performance during the grower phase of broilers, while nutrient digestibility showed adverse impact. Emulsifier supplementation should be tested after 25 days of broiler's life of broilers.

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