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Enhancement of growth performance and some blood constituents of broilers chickens by using of probiotic and enzymes

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Abstract

This study was conducted on broiler chicks (Ross-308) to evaluate the influences of using probiotics and enzymes in diets on growth performance and some blood parameters. A total number of 135 one-day-old broiler chicks were randomly obtained and divided into three equal groups; each group consisted of three replicates of 15 chicks. Experiment was lasted to 35 days of age. First group (C) designated as control and was given only basal diet (Table 1), while the 2nd (P) and the 3rd (E) groups were received probiotic **(**Guardizen-M**)** and enzymes (Fra®Multizyme) at the levels of 1g and 0.5g/kg in diets, respectively. Data indicated that body weight BW and feed conversion ratio FCR were significant (P<0.05) better for birds in P group, followed by those in E group, while the lowest (P<0.05) values were noticed in control one. Feed consumption FC was significantly (P<0.05) decreased in P group, followed in E group, while the highest (P<0.05) value were noticed in control one. Moreover, P and E groups had the highest (P<0.05) body weight gain BWG as compared to control group. All studied blood parameters significantly (P<0.05) improved in group of P, followed by E group compared with control one. Finally, it could be concluded that adding of 1g probiotic **(**Guardizen-M**)** or 0.5g enzymes (Fra®Multizyme)/kg in diets of broiler chicks (Ross-308) could be recommend for high growth performance and improve blood parameters.

Keywords: performance, broilers, blood parameters, probiotic, enzymes.

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1. Introduction

Probiotic microorganisms are responsible for the creation of vitamin B complex and digestive enzymes, which help to stimulate gut immunity and increase protection against pathogenic organism toxins (Rahman *et al.*, 2013). In broiler chickens, probiotics demonstrated have been shown to improve daily weight gain, feed intake, feed conversion ratio and mortality rate when compared to non-supplemented diets and are as effective as antibiotic growth promoters (Mountzouris *et al.,* 2010). *Lactobacillus, Bifidobacterium, Bacillus, Streptococcus, Pediococcus, Enterococcus,* and *yeast* such as Saccharomyces cerevisiae and *Saccharomyces boulardii* are among the probiotics utilized in poultry, such as broiler chickens (Kabir *et al.*, 2004). Non-starch polysaccharides in cereal grains like barley, wheat, and corn can have a suppressive and anti-nutritional effect on broiler performance, which can be alleviated by adding enzymes (Annison and Choct, 1991). The addition of enzymes to broiler diets is done to improve the efficiency of meat production. Because chickens cannot produce some enzymes, such as galactosidases, corn-soy bean-based diets without additional enzymes like xylanases and pectinases may cause gas accumulation in the intestine and diarrhea (Wu *et al.*, 2005). Galactosidase, amlyase, β-glucanase, cellulase, protease, pectinase, amylase, mannanase, phytase and xylanase are enzymes that cleave non-starch polysaccharides in cereals and vegetable meals and are widely employed in the feed industry. The objective of the present study was to evaluate how adding probiotics (Guardizen-M) and enzymes mixture (Fra®Multizyme) to diets for broiler chickens affected their growth performance, blood parameters.

2. Materials and methods

2.1 Place and objectives of study

During the period from February 2020 to March 2020, this study was carried out at Poultry Farm, Animal Production Department, Faculty of Agriculture, Al-Azhar University (Assuit branch), Egypt.

2.2 Experimental design and Birds husbandry

A total number of 135 healthy, one day old broiler chicks (Ross-308), unsexed were selected and randomly distributed into three experimental groups of 45 chicks. Each group contains three replicates of 15 chicks each. The first group (C) was fed only basal diet and saved as control, while the second (P) group was received probiotic (Guardizen-M) at level of 1g/kg diet and the third (E) group was received enzymes (Fra®Multizyme) at the level 0.5g/kg in diets. Probiotic (Guardizen-M) Country of Origin: South Korea. Manufacture Company: DONG BANG CO., LTD. Head office: Kumsung Bldg. 3-5F, Dogok 2-dong, Gangnam-gu, Seoul. Imported Company: BIO POULTRY, Dakahlia, Egypt. Each kg of (Guardizen-M) contains mixed probiotics concentrates 5.6 g (a minimum 1×10^{10}

CFU) *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus Enterococcus faecium*, *Aspergillus oryzae*, *Candida pintolopesii* and Lactose or Dextrose 994.4 g. Enzymes mixture (Fra®Multizyme) manufacture Company: FRAMELCO BV, Country of Origin: Holanda, Imported Company: Abu El Naga Trading Company, Tanta, Gharbia, Egypt. Each 1g contains: Xylanase 16,000 BXU/g, 1,3(4) ß-glucanase 2,400 BU/g, Pectinase 210 U/g, Alpha-amylase 2,100 IU/g, Mannanase 3,000 MNU/g, Protease 7 mg and Phytase 1,000 FTU/g. All birds were placed in floor pens at the same space (Dimensions of nest per replicate are Length = 2 m, Width= 1.5 m, Height= 2.5 m), at stocking density of 5 birds/m^2 from 1-35 days of age, using the sawdust as litter at 7 cm deepness, in opened house under similar hygienic and normal environmental conditions with natural ventilation. Feed and water were offered to the bird's *ad-libitum* during the whole experimental period. Feed was accessible throughout the experiment, and the same feeder space was used. Fresh tap water is also available at all times through the standard waters. The composition and analysis of the basal diet are shown in Table (1).

Table (1): The composition and proximate chemical analysis of the basal diet.

Ingredients	Starter (%) (1-14 days)	Grower (%) (15-35 days)
Yellow corn (8.8%CP)	58.00	58.80
Corn Gluten (60%CP)	8.58	6.28
Soybean meal (46% CP)	28.10	26.00
Limestone	1.00	0.90
Wheat bran	0	2.00
Soya Oil	1.20	3.20
Di-phosphate calcium	2.30	2.00
DL – Methionin	0.07	0.07
L-Lysine	0.15	0.15
NaCl	0.30	0.30
Vitamins minerals mixture*	0.30	0.30
Total	100	100
Calculated analysis**		
Metabolizable energy (kcal/kg died)	3003.88	3100.29
Crude protein, (%)	23.01	21.04
Crude fiber, (%)	3.44	3.49
Ether extract, (%)	3.84	5.86
Calcium, (%)	1.05	0.93
Available phosphorus, (%)	0.51	0.45
Methionine, (%)	0.53	0.48
Lysine, (%)	1.20	1.13

*Vitamins and minerals premix provided per kilogram of the diet: Vit A, 1000 IU; D3 2000 ICU; Vit E, 10 mg; Vit K, 1mg; B1, 10 mg; B2, 5 mg; B6, 1500 mg; B12, 10mg Pantothenic acid, 10 mg; Nicotinic acid, 30 mg; Folic acid, 1mg; Biotin, 50 mcg; Chloride, 500 mg; copper, 10 mg; iron, 50 mg; Manganese, 60 mg; Zinc, 50mg, and selenium, 0.1 mg. **Calculated according to NRC (1994).

The birds during the first three days of age were exposed to the continuous lighting program for 24 hour light per day, from the 4th day of age, birds were exposed to the lighting program of 23 hour light and one hour dark per day to the end of experiment; which depend on natural light and supposed by artificial lighting by using incandescent lambs, 60 watt hanged at a level of 2 m from the floor. Temperature was set initially at 35 ^{o}C and gradually reduced at a rate of 2-5 ^{o}C per 3 days till reach 22^{o}C at the fourth week, afterword, at the 5th week, the temperature was kept at 22^{o}C. All birds were vaccinated by vaccination program as recommended for broilers from 1 to 35 days of age.

2.3 Studied Parameters

2.3.1 Growth performance

Each group's live body weight (LBW), Body weight gain (BWG), feed consumption (FC), and feed conversion ratio (FCR) were measured individually. Chicks were weighed at one day-old and weekly during the experimental period (1 to 35 days of age) and recorded to the nearest gram. The live body weight gain was calculated every week by subtracting the initial weight from the initial body weight from its present body weight every week. Then, it divided the result on the number of days to calculate daily body weight gain. Each group of chicks received a certain amount of feed each week, with the remaining feed weighed at the end of each week. It was estimated the difference between the feed given and the rest feed. The average amount of feed consumed was computed by dividing the weekly feed consumption by the number of birds in each group during that week. Feed conversion ratio (FCR) was calculated weekly by dividing the average of feed consumption per bird to the average body weight gain per bird.

2.3.2 Blood Parameters

At 35 days of age, 5 birds were randomly obtained from each replicate, and then slaughtered for blood parameters. During slaughtering, blood samples were collected from each bird in heparinized and un-heparinized tubes. The blood samples were cooled to approximately 4^{o}C using icepacks and were transferred to the laboratory within two hours after blood collection. The whole blood in heparinized tube was used to determine the blood plasma physical characteristics. While, the blood in the un-heparinized tube was centrifuged at 3000 round per minute for 10 minutes to obtain blood serum and was separated in Eppendorf's tubes using micropipette. Then the serum was stored at -80°C till biochemical analysis.

2.3.2. Hematocrit (PCV %).

The microchematocrit procedure was used to make two determinates from each sample to determine the Hematocrit value (packed cell volume): Two heparinized capillary tubes were filled with blood, the ends of each tube were sealed, and the tubes were centrifuged for ten minutes at 1200 round per minute in a micro capillary centrifuge. A circular reader was used to determine the PCV measurements (Daice and Lewis, 1991).

2.3.2 Biochemical Determinations

Using assay kits supplied by Bio Med Chemical Company, Egypt, serum total protein was determined according to Doumas *et al.* (1981), and serum albumin was determined according to Doumas *et al.* (1972). Albumin values were subtracted from total protein values to get serum globulin values. According to Trinder (1969), serum glucose was determined by using assay kits supplied by Diamond Chemical Company, Germany. Serum cholesterol was determined according to Watson (1960) using assay kits supplied by Bio Med Chemical Company, Egypt. Serum aspartate aminotransferase (AST) and alanine transaminase (ALT) were determined according to Reitman and Frankel (1957) using assay kits supplied by Bio Med Chemical Company, Egypt.

2.4. Statistical analysis

Data obtained were subjected to statistical analysis using one way analysis of variance (ANOVA) with treatment group effect by using the General Liner Model (GLM) procedure of SAS (1998) according to the following model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where; Y_{ij} = an observation, μ = overall mean, T_i = effect of treatment, i= 1,2,…., E_{ij} = random error. Differences among means of the experimental groups were testified for significance by Duncan's multiple range test (Duncan, 1955). A level of probability (*P*. value) of <0.05 was considered significant.

3. Results and discussion

3.1. Growth performance

There are a significant (P<0.05) differences in body weight, body weight gain, feed consumption, and feed conversion ratio among all groups as shown in Tables (2 and 3)**.**

3.1.1 Body weight (BW) and body weight gain (BWG)

Data showed that birds in P group had significant (P<0.05) higher BW as compared to other groups during the whole experimental period, followed by those in E group as compared to those in control one. The obtained results indicated that the differences were not significant (P>0.05) between E and C group at the 1st, 2nd and 3rd weeks of age. BWG was significantly (P<0.05) increased in P group compared with other groups during the periods of (0-1), (1-2) and (2-3) weeks of age. At final periods of (3-4) and (4-5) weeks of age, P and E groups had the highest (P < 0.05) BWG compared with control group. There were no significant (P > 0.05) differences between C and E groups during the periods of (0-1), (1-2) and (2-3) weeks, also between P and E groups at (3-4) and (4-5) weeks of age. The obtained results are in agreement with observations of Algedawy *et al.* (2011), who found that dietary supplementation of probiotic significantly ($P<0.05$) increased BW and BWG for broilers reared from one-day to 6 weeks of age as compared to adding of multi enzymes mixture. Additionally, Bai *et al.* (2017) found that final BW and BWG were significantly ($P<0.05$) increased for broilers fed diets supplemented with probiotic (*Bacillus subtilis*) compared with control during the period of 1-42 days of age. Concerning, enzymes effects Chimote *et al.* (2009) found that supplementation of enzymes in feed significantly ($P<0.01$) improved BW and BWG as compared to control for Japanese quails during the growth period.

Table (2): The effect of probiotics and enzymes on live body weight and body weight gain (g).

Age (week)	Treatment			SEM[1]	*P. value*	*Sig.*[2]
	C	P	E			
Body weight (g)						
0	43.8	43.9	43.9	0.5	0.9912	NS
1	160.2^{b}	176.2^{a}	158.1^{b}	2.5	0.0001	***
2	346.4^{b}	420.4^{a}	351.6^{b}	7.0	0.0001	***
3	766.7^{b}	890.8^{a}	754.5^{b}	15.3	0.0001	***
4	1238.6^{c}	1448.0^{a}	1306.8^{b}	25.2	0.0001	***
5	1736.3^{c}	1998.1^{a}	1863.8^{b}	33.2	0.0001	***
Body weight gain (g)						
0-1	16.6^{b}	18.9^{a}	16.3^{b}	0.3	0.0001	***
1-2	26.6^{b}	34.9^{a}	27.6^{b}	0.8	0.0001	***
2-3	60.0^{b}	67.2^{a}	57.6^{b}	1.4	0.0001	***
3-4	67.5^{b}	79.3^{a}	80.3^{a}	2.1	0.0001	***
4-5	71.1^{b}	78.6^{a}	79.6^{a}	2.9	0.0001	***

[a,b and c] Means with different superscripts in the same row are significantly different ($P< 0.05$). C= Control, P= Probiotic, E= Enzymes, [1]SEM= Stander error of means, Sig.[2]= Significance, NS= not significant, ***=Very highly significant.

On the other hand, Flores *et al*. (2016) demonstrated that BW was not significantly ($P>0.05$) affected by adding of probiotic in feed for broilers as compared to control diets during the period of 1-42 day of age. The action of Probiotics on intestinal microflora and increasing the digestibility, absorbability, and utilize ability of different nutrients in the gastrointestinal tract by probiotics product, enzymes of cellulose, amylase, and protease, as well as the action of exogenous enzymes on improving nutrient digestibility and nitrogen and phosphorus reduction, could be attributed to the improvement in BW and BWG for broilers (Bedford, 2000). Previous research has also shown that adding of enzymes in diets result in enhanced nutrition utilization, which leads to better body weight growth (Costa *et al.*, 2008).

3.1.2 Feed Consumption (FC) and Feed conversion ratio (FCR)

Total feed consumption (TFC) was significant ($P<0.05$) higher in control group as compared with P and E treatments (Table 3). During (0-1) and (2-3) periods, differences in FC was not significant (P>0.05) among all groups. The best (P<0.05) values of FCR were observed in P group, followed E group as compared to control one (Table 4). However, the differences were not significant (P>0.05) between E and C groups at (1-2) and (2-3) weeks of age, also between P and E treatments at (3-4) weeks of age. Similar observations were also reported by Chimote *et al.* (2009), who showed that FC was significantly (P<0.01) decreased by supplementation of probiotics in diets for Japanese quails during the growth period, who added that there were no significant (P>0.01) differences between using of enzymes and control diet in FC. In contrast, Ahmed *et al.* (2014) found that the addition of probiotics in diet of broilers significantly (P<0.05) increased feed intake as compared to control during the period from 1 to 35 day of age. With regard to FCR, Algedawy *et al.* (2011) reported that dietary supplementation of probiotics significantly (P<0.05) improved FCR for broilers reared from one-day to 6 weeks of age as compared to adding of multi enzymes mixture group.

Table (3): The effect of probiotics, enzymes on daily feed consumption (g/bird/day) and feed conversion ratio (g feed/g gain).

Period (week)	Treatment			SEM[1]	*P. value*	*Sig.*[2]
	C	P	E			
Feed consumption (g)						
0-1	18.2	17.0	17.3	0.5	0.2322	NS
1-2	49.2[a]	46.9[b]	47.3[b]	0.2	0.0005	**
2-3	82.6	79.2	80.9	1.6	0.2121	NS
3-4	115.3[a]	106.9[c]	110.1[b]	0.9	0.0001	***
4-5	132.2[a]	113.6[c]	124.5[b]	2.0	0.0004	***
TFC	2782.4[a]	2541.4[c]	2661.1[b]	24.2	0.0002	***
Feed conversion ratio						
0-1	1.096[a]	0.899[c]	1.061[b]	0.02	0.0006	**
1-2	1.849[a]	1.344[b]	1.713[a]	0.15	0.0021	**
2-3	1.377[a]	1.178[b]	1.404[a]	0.08	0.0097	*
3-4	1.721[a]	1.347[b]	1.371[b]	0.19	0.0036	**
4-5	1.859[a]	1.445[c]	1.564[b]	0.27	0.0003	***

[a,b and c] Means with different superscripts in the same row are significantly different (P< 0.05). TFC=total feed Consumption, C=Control, P=Probiotic, E=Enzymes, [1]SEM= Stander error of means, Sig.[2]= Significance, NS= not significant, *= significant, **= highly significant, ***= Very highly significant.

Also, Pourakbari *et al.* (2016) stated that supplementation of probiotics in diet for broilers significantly (P<0.01) improved FCR during the period of 1-6 weeks of age as compared to control diet. As for enzymes effects on FCR, Seifi (2014) noticed that adding of enzyme complex in diets significantly (P<0.05) improved FCR compared with control group in broilers during the period from 1 to 47 days of age. On the other hand, Khan *et al.* (2019) indicated that FCR was not significantly (P>0.05) affected by adding of enzyme in diets for broilers during the period of 1- 35 day of age. Chimote *et al.* (2009) showed that there were no significant (P>0.01) differences between supplementation of probiotics or enzymes in FCR for Japanese quails during the growth period. Probiotic increase the population of useful microflora in the intestines and promote a better flora balance (Kabir *et al.*, 2004). The activity of microflora on the alimentary tract, which has a significant effect on broiler health and performance, could be linked to the improvement in FCR for birds given probiotics in their diets (Alkhalf *et al.*, 2010). This may lead to better capacity for absorption of available nutrients. Exogenous enzymes that hydrolyze the NSP of vegetable constituents in monogastric diets improve energy availability and nutrient utilization, hence improving FCR (Shirmohammad and Mehr, 2011).

3.2 Blood parameters

As shown in Table (4), results revealed that the highest values of serum total protein, albumin, globulin and PCV% were obtained in P group as compared to C and E groups. The lowest (P<0.05) levels of and cholesterol were noticed in P and E groups compared with control. Data indicated that adding of probiotics or enzymes had not any significant (P>0.05) effects on glucose, ALT and AST concentrations in blood of broiler chicks. The obtained results in harmony with results observed by Pourakbari *et al.* (2016) who found that supplementation of probiotics in diet for broilers significantly (P<0.05) increased albumin concentration in blood during the period of 1-6 weeks of age as compared to control diet. Also, Bai *et al.* (2017) found that serum globulin levels were significantly (P<0.05) increased for male broilers fed diets supplemented with probiotics compared with control during the period of 1-42 days of age. Furthermore, Alkhalf *et al.* (2010) indicated that the serum cholesterol concentrations were significantly (P<0.05) decreased for broilers fed probiotics in their diet as compared to control group at 1-42 days of age. In addition, Djouvinov *et al.* (2005) observed that the probiotics supplementation in diet for duckling shad not significant (P<0.05) effects on AST and ALT concentrations in blood during the period of 54-93 days of age as compared to control diet. As well as, Algedawy *et al.* (2011) found that there were no significant (P>0.05) differences among birds received multi enzymes mixture and those received probiotics in items of plasma AST and ALT compared with control group in broiler chickens reared from one-day to 6 weeks of age. Our findings disagreement with Khan *et al.* (2011) who reported that the concentration of serum cholesterol was significantly (P<0.05) increased in layers fed enzyme supplementing in diets at 40 weeks of age. Additionally, Ndazigaruye *et al.* (2019) indicated that using exogenous enzymes had not significant (P>0.05) effect on serum cholesterol, total protein, albumin and globulin levels for broilers at 1-35 days of age. Adding probiotics to broiler diets increases blood total protein, albumin, and globulin levels, indicating that their use has no negative impact on the birds' hematological and health state (Ismail *et al.*, 2002). According to Azoz and Al-Kholy (2006), blood globulin levels are good indications of immune response, while albumin levels reflect liver function. The greater PCV in the birds fed a diet supplemented with probiotics could be attributed to an acidic gastrointestinal tract generated by probiotics, resulting in improved iron salt absorption from the small intestine Lactobacillus is also thought to lower blood cholesterol levels by deconjugating bile salts in the intestine, preventing them from acting as precursors in cholesterol synthesis (Abdulrahim *et al.*, 1996).

Table (4): The effect of probiotics and enzymes on blood parameters.

Parameters	Treatment			SEM[1]	*P. value*	*Sig.*[2]
	C	P	E			
Total Protein (g/dl)	5.04[c]	6.78[a]	5.56[b]	0.10	0.0001	***
Albumin (g/dl)	2.08[b]	2.96[a]	2.39[b]	0.13	0.0001	***
Globulin (g/dl)	2.95[b]	3.82[a]	3.16[b]	0.15	0.0001	*
Glucose (mg/dl)	192.96	189.16	191.49	2.14	0.5257	*NS*
Cholesterol (mg/dl)	205.71[a]	174.69[b]	180.61[b]	5.07	0.0001	***
PCV (%)	32.06[c]	41.49[a]	38.41[b]	1.03	0.0001	***
ALT (U/L)	8.81	8.28	8.58	0.36	0.7838	*NS*
AST (U/L)	127.20	126.73	124.46	5.83	0.9885	*NS*

[a,b] and [c] Means with different superscripts in the same column are significantly different (P<0.05). C= Control P= Probiotic, E= Enzymes, [1]SEM= Stander error of means, Sig.[2]= Significance, NS= Not significant, *= Significant, ***= Very Highly significant PCV= Packed cell volume, ALP= Alkaline phosphatase, ALT= Alanine aminotransferase, AST= Aspartate transaminase.

4. Conclusion

From the results obtained in this study, it can be concluded that the using of 1g probiotic (Guardizen-M)/kg diet is recommended to obtain high growth performance and improve blood parameters, followed by adding of 0.5g enzymes (Fra®Multizyme)/kg diet as compared to control diet for broiler chickens during the period of 1-35 days of age.

References

Abdulrahim, S. M., Haddadin, M. S. Y., Hashlamoun, E. A. R. and Robinson, R. K. (1996), "The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk", *British Poultry Science*, Vol. 37 No. 2, pp. 341–346.

Ahmed, S. T., Islam, M. M., Mun H. S., Sim, H. J., Kim, Y. J. and Yang, C. J. (2014), "Effects of *Bacillus amyloliquefaciens* as a probiotic strain on growth performance, cecalmicroflora, and fecal noxious gas emissions of broiler chickens", *Poultry Science*, Vol. 93 No. 8, pp. 1963–1971.

Algedawy, S. A., Soliman, M. K., Elashmawy, I. M. and El-Sayed, Y. S. (2011), *Effect of commercial probiotic and exogenous enzymes on the growth performance, immune response, and hemato-biochemical parameters of broiler chickens*, In Proceedings of the 4th Scientific Conference of Animal Wealth Research in the Middle East and North Africa, Foreign Agricultural Relations (FAR), Egypt, pp. 430–445.

Alkhalf, A., Alhaj, M. and Al-Homidan, I. (2010), "Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens", *Saudi Journal of Biological Sciences*, Vol. 17 No. 3, pp. 219–225.

Annison, G., and Choct, M. (1991), "Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects", *World's Poultry Science Journal*, Vol. 47 No. 3, pp. 232–242.

Azoz, A. A. and Al-Kholy, K. H. (2006), "Reproductive performance and blood constituents of V-line and Bauscat female rabbits under middle Egypt conditions", *Egyptian Journal of Rabbit Science*, Vol. 16 No. 1, pp. 139–160.

Bai, K., Huang, Q., Zhang, J., He, J., Zhang, L. and Wang, T. (2017), "Supplemental effects of probiotic *Bacillus Subtilis* fmbJ on growth performance, antioxidant capacity, and meat quality of broiler chickens", *Poultry Science*, Vol. 96 No. 1, pp. 74–82.

Bedford, M. (2000), "Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimize subsequent problems", *World's Poultry Science Journal*, Vol. 56 No. 4, pp. 347–365.

Chimote, M. J., Barmase, B. S., Raut, A. S., Dhok, A. P. and Kuralkar, S. V. (2009), "Effect of supplementation of probiotic and enzymes on performance of Japanese quails", *Veterinary World*, Vol. 2 No. 6, pp. 219–220.

Costa, F. G. P., Goulart, C. C., Figueiredo, D. F., Oliveira, C. F. S. and Silva, J. H. V. (2008), "Economic and environmental impact of using exogenous enzymes on poultry feeding", *International Journal of Poultry Science*, Vol. 7 No. 4, pp. 311–314.

Daice, S. J. and Lewis, S. M. (1991), *Practical hematology*, 7th ed., Churchill Livingstone, London, England, pp. 143–174.

Djouvinov, D., Boicheva, S., Simeonova, T. and Vlaikova, T. (2005), "Effect of feeding lactina probiotic on performance, some blood parameters and caecal microflora of mule ducklings", *Trakia Journal of Sciences*, Vol. 3 No. 2, pp. 22–28.

Doumas, B. T., Biggs, H. G., Arends, R. L. and Pinto, P. V. (1972), "Determination of serum albumin", In *Standard Methods of Clinical Chemistry*, Vol. 7, Academic Press, USA, pp. 175–188.

Doumas, B. T., Bayse, D. D., Carter, R. J., Peters, T. and Schaffer, R. (1981), "A candidate reference method for determination of total protein in serum, I. Development and validation", *Clinical Chemistry*, Vol. 27 No. 10, pp. 1642–1650.

Duncan, D. B. (1955), "Multiple range and multiple tests", *Biometrics*, Vol. 11, pp. 1–42.

Flores, C., Williams, M., Pieniazek, J., Dersjant-Li, Y., Awati, A. and Lee, J. T. (2016), "Direct-fed microbial and its combination with xylanase, amylase, and protease enzymes in comparison with AGPs on broiler growth performance and foot-pad lesion development", *Journal of Applied Poultry Research*, Vol. 25 No. 3, pp. 328–337.

Ismail, A. M., Abou-El-Ellaa, M. A. and Sedki, A. A. (2002), "Blood metabolites, growth and reproductive performance and immune responsiveness in growing and doe rabbits as affected by Acitrol treatments", *Egyptian Journal of Agriculture Research*, Vol. 80, pp. 1789–1808.

Kabir, S. M. L., Rahman, M. M., Rahman, M. B., Rahman, M. M. and Ahmed, S. U. (2004), "The dynamics of probiotics on growth performance and immune response in broilers", *International Journal of Poultry Science*, Vol. 3 No. 5, pp. 361–364.

Khan, S. H., Atif, M., Mukhtar, N., Rehman, A. and Fareed, G. (2011), "Effects of supplementation of multi-enzyme and multi-species probiotic on production performance, egg quality, cholesterol level and immune system in laying hens", *Journal of Applied Animal Research*, Vol. 39 No. 4, pp. 386–398.

Khan, K., Zaneb, H., Rehman, Z. U., Maris, H. and Rehman, H. (2019), "Effect of phytase supplementation on growth performance in broiler chickens", *Pakistan Journal of Zoology*, Vol. 51 No. 2, pp. 731–735.

Mountzouris, K. C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G. and Fegeros, K. (2010), "Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition", *Poultry science*, Vol. 89, No. 1, pp. 58–67.

NRC, (1994), *Nutrient Requirements of Poultry*, 9th Review Edition, National Academy Press, Washington D.C., USA.

Ndazigaruye, G., Kim, D. H., Kang, C. W., Kang, K. R., Joo, Y. J., Lee, S. R. and Lee, K. W. (2019), "Effects of low-protein diets and exogenous protease on growth performance, carcass traits, intestinal morphology, cecal volatile fatty acids and serum parameters in broilers", *Animals*, Vol. 9 No. 5, Article ID: 226.

Pourakbari, M., Seidavi, A., Asadpour, L. and Martínez, A. (2016), "Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers", *Anais da Academia Brasileira de Ciências*, Vol. 88, pp. 1011–1021.

Rahman, M. S., Mustari, A., Salauddin, M. and Rahman, M. M. (2013), "Effects of probiotics and enzymes on growth performance and haematobiochemical parameters in broilers", *Journal of the Bangladesh Agricultural University*, Vol. 11, pp. 111–118.

Reitman, S. and Frankel, S. (1957), "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases", *American Journal of Clinical Pathology*, Vol. 28 No. 1, pp. 56–63.

SAS, (1998), *SAS Users Guide: Statistics*, SAS Institute Inc., Cary, NC, USA.

Seifi, S. (2014), "An investigation of the effects of using an enzyme-probiotic combination on broilers performance", *Iranian Journal of Veterinary Medicine*, Vol. 7 No. 4, pp. 299–304.

Shirmohammad, F. and Mehri, M. (2011), "Effects of dietary supplementation of multi-enzyme complex on the energy utilization in rooster and performance of broiler chicks", *African Journal of Biotechnology*, Vol. 10 No. 38, pp. 7541–7547.

Trinder, P. (1969), "Enzymatic method of glucose estimation", *Annals of Clinical Biochemistry*, Vol. 6 No. 24, Article ID: e33.

Watson, D. (1960), "A simple method for the determination of serum cholesterol", *Clinicachimicaacta*, Vol. 5 No. 5, pp. 637–643.

Wu, G., Bryant, M. M., Voitle, R. A. and Roland Sr, D. A. (2005), "Effects of β-mannanase in corn-soy diets on commercial leghorns in second-cycle hens", *Poultry Science*, Vol. 84 No. 6, pp. 894–897.