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The effects of using a Rovabio® Plus multi-enzyme on production and incubation performance, blood parameters and duodenum morphology of broiler breeders at age of 45 - 60 weeks

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Abstract

The aim of this study was to investigate the effects of using a Rovabio® Plus multi-enzyme on production and reproductive performance, duodenum morphology and biochemical parameters in 45 - 60 weeks old broiler breeders. For this purpose, 260 broiler breeders of Ross 308 strain were used in a completely randomized design with four treatments and five replications (13 hens and one rooster in each replication). Experimental treatments included: 1) Positive control diet (diet with standard energy and phosphorus and without multi-enzyme), 2) Negative control diet (diet in terms of energy 5.00% and phosphorus 50.00% less than the positive control diet, 3) Negative control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme and 4) Positive control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme. The results showed that negative control diet caused a significant decrease in the egg production percentage, egg weight, egg mass, settable eggs, fertile eggs, hatchability and one grade chickens, increased the feed conversion ratio and embryonic losses. Addition of 1.00 g kg^{-1} of multi-enzyme to negative control diet made the above parameters similar to the positive control treatment, however, the positive control treatment supplemented with multi-enzyme had a better performance compared to other treatments. This research showed that dietary supplementation of Rovabio® Plus multi-enzyme in broiler breeders at the age of 45 - 60 weeks led to the improvement of productive performance and incubation in negative control diet.

Introduction

The broiler breeder breeding guide[1] states that the flocks economic age range is from 21 to 64 weeks. In commercial flocks the egg production gradually declines after the peak point of production period, which occurs after about 40 weeks of their age as the age of the hens increases.[2] The physiological performance and activity of digestive enzymes decline with hen age and this is invariably linked to the alteration of the gut microbiota following the laying peak, which results in a large loss of economic value.[3] Numerous studies including those on nutritional programs have been carried out to enhance performance and guard against production decrease in older hens.[4] Multi-enzyme supplementation appears to be beneficial for enhancing older broiler breeder performance.[5] Exogenous enzyme addition is thought to accelerate the breakdown of dangerous macromolecules and aid in the breakdown of protein and starch.[3] It goes without saying that enzymes are frequently used in chicken feed. However, a number of parameters such as the source, kind, features, dose, composition of the enzymes as well as the diet's structure, composition and physiological condition affect the enzymes function in the feed digestion, performance and health of chickens.[5]

The main source of energy in a chickens diets is grain. However, according to Slominski *et al.,*[6] these materials especially have a high concentration of anti-nutritional components such as non-starch polysaccharides (NSPs) which vary from 8.30 to 9.80%. The primary NSPs present in wheat and corn are arabinoxylan chains which make up 7.30 and 4.70% of the dry matter, respectively.[7] For a long time, wheat-based diets for poultry have included carbohydrases, particularly xylanases which degrade NSPs.[8] Proteases can lessen the negative effects of trypsin inhibitors or heat-stabilized lectins and boost the digestion

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of protein and amino acids. As such, they enhance the quality of the diet.[9] and the presence of proteases in the diet of poultry leads to a notable improvement in the digestibility of protein and amino acids.[10] Exogenous enzymes can, in general, interact with the host through non-digestible food components and supply nutrients to control immunological, digestive and antioxidant functions to promote host output and financial gain.[11] Furthermore, phytase addition in low available phosphorus diets has increased eggshell ash, eggshell thickness and eggshell weight significantly in laying hens.[12] Recent studies have also demonstrated the synergistic effects of phytase and xylanase on growth performance following simultaneous inclusion in diets based on wheat.[13]

In general, when a multiple enzyme complex is added to respective poultry diets, it usually results in several beneficial effects such as increased utilization of nutrients (e.g. fat and protein), improved apparent metabolizable energy, increased growth rate, improved feed conversion ratio (FCR), reduction of intestinal viscosity, reduction of sticky material excretion, improvement of bedding conditions, and reduction of environmental pollution are due to reduction of fertilizer production and gases such as ammonia.[14] Rovabio® Max is a stabilized multi-enzyme composition that is naturally produced by the non-genetically modified fungus *Penicillium funicullosum* and contains xylanases, β-glucanases, proteases, cellulases and phytase. It is claimed that this multi-enzyme breaks down NSPs, reduces the viscosity of intestinal contents and improves nutrient utilization.[15]

The use of multi-enzymes in the diet of broiler breeders always brings the challenge of increasing the cost of feed, so the use of enzymes in diluted diets can be economically justified in addition to increasing efficiency. Based on this, diet dilution with the use of commercial enzyme in the diet of broiler breeders was investigated. Therefore, according to the assumption that Rovabio® Plus multi-enzyme can possibly improve the performance of old broiler breeders by affecting feed digestibility and the state of the digestive system, the present study aimed to investigate the effects of using the Rovabio® Plus multi-enzyme on production performance, reproductive traits, intestinal morphology and blood parameters in broiler breeders in 45 - 60 weeks of age.

Materials and Methods

Birds, diets, and management. This experiment was conducted using 260 broiler breeders of Ross 308 strain with the same weight (3825 ± 13.00 g) in a completely randomized design with four treatments and five replications (13 hens and one rooster *per* replication) for 90 days. Broiler breeding instructions, including control of exposure, ventilation, drinking water and vaccination, were implemented for all treatments in the same way and based on the standard conditions of the strain.[1] Experimental treatments included: 1) Positive control (diet with standard energy and phosphorus and without multi-enzyme), 2) Negative control (diet in terms of energy 5.00% and phosphorus 50.00% less than the standard and without multi-enzyme), 3) Negative control with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme and 4) Positive control with 1.00 g kg^{-1} multi-enzyme. The multi-enzyme used in this experiment was Rovabio® Plus (Adisseo, Paris, France), with the formulation of 12,500 unit g^{-1} xylanase, 8,600 unit g^{-1} glucanase and 10,000 unit g^{-1} phytase, which was used at the rate of 100 g *per* ton in the test diets. The rations were the same in terms of the composition of nutrients except for energy and phosphorus. The feed consumption in the experimental groups was calculated according to the standards of the company[1] and provided to the birds (162 g *per* bird from weeks 45 to 60) (Table 1). The study adhered to the guidelines set by the Iranian Council of Animal Care for the Care and Use of Experimental Animals, vol. 1, as implemented at Isfahan University of Technology, Isfahan, Iran.[16]

Performance parameters. During the experimental period, the number of egg production, egg weight and mortality were checked on a daily basis. Egg mass (g *per* hen *per* day) and FCR were calculated weekly. The percentage of egg production was evaluated by dividing the total number of eggs produced in each repetition by the hen day. The egg mass was estimated multiplying the average egg weight of each replicate by the percentage of egg production. In addition, weekly feed intake was divided by egg mass to estimate FCR.

Incubation performance. Every 21 days, the number of settable eggs, percentage of hatchability, percentage of fertile eggs, embryonic losses (percentage of fertilized eggs) and percentage of grade one chicks were checked. Fertility percentage was checked using nose light (candling) on the 12^{th} day after laying in the incubator. The hatched chicks were divided into grade one chicks and second chicks according to freshness, umbilical cord condition, movement problems and some other appearance characteristics.

Duodenum morphology. At the end of the week 60, two hens form each replicate were selected for duodenum morphology evaluation. Briefly, after slaughtering 1.00 cm sections from the middle portion of the duodenum tissue was fixed in 10.00% formaldehyde phosphate buffer after being washed with 0.10 M phosphate-buffered saline and the fixated sections were processed, dehydrated, embedded in paraffin wax, sectioned at 5.00 μm and stained with the Hematoxylin-Eosin. Using a digital camera microscope (Olympus, Tokyo, Japan) in histological sections, villus height (VH), villus width and crypt depth (CD) on 10 well-oriented villi and 10 muscular thicknesses were examined from each segment. The ratio of VH/CD was calculated subsequently.[17]

Table 1. Composition of the basal diet (as-fed basis).[1]

Ingredient (g kg^{-1})	Positive control	Negative control
Corn grain	625	522
Soybean meal	165	135
Wheat grain	40.00	40.00
Barley grain	0.00	30.00
Wheat bran	40.00	155
Soybean oil	12.00	4.00
Di-calcium phosphate	16.40	5.40
Bentonite	7.10	7.75
Calcium carbonate	77.20	82.00
Vitamin premix[a]	2.50	2.50
Mineral premix[b]	2.50	2.50
Salt	2.20	2.20
Sodium bicarbonate	2.20	2.20
Potassium bicarbonate	1.50	1.50
DL-methionine	1.80	2.00
L-lysine HCL	0.60	1.10
L-threonine	0.80	1.65
Choline chloride	1.00	1.00
Antioxidants	0.20	0.20
Toxin binder	1.00	1.00
Antiammonium extra	1.00	1.00
Complex enzymes	1.00	0.00
Total	1,000	1,000
Calculated composition		
Metabolizable energy (kcal kg^{-1})	2,800	2,660
Crude protein (%)	13.52	13.53
Crude fiber (%)	2.99	3.96
Calcium (%)	3.20	3.20
Available phosphorus (%)	0.34	0.17
Total phosphorus (%)	0.60	0.49
Lysine (SID)	0.60	0.60
Methionine (SID)	0.38	0.38
Methionine + Cysteine (SID)	0.61	0.60
Threonine (SID)	0.55	0.55
DCAB (mEq kg^{-1})	203	210

Positive control, diet with standard energy and phosphorus; Negative control, diet in terms of energy 5.00% and phosphorus 50.00% less than the positive control diet.

The multi-enzyme used in this experiment was Rovabio® Plus with the formulation of 12,500 VU g^{-1} xylanase, 8,600 VU g^{-1} gluconase and 10,000 FTU g^{-1} phytase which was used at the rate of 100 g *per* ton in the test diets.

[a] Vitamin premix provided *per* kilogram of diet: Vitamin A, 15,000 IU; Vitamin D_3, 5,000 IU; Vitamin E, 130 IU; Vitamin K_3, 9.00 mg; Vitamin B_1, 6.00 mg; Vitamin B_2, 20.00 mg; Vitamin B_6, 8.00 mg; Vitamin B_9, 5.00 mg; Vitamin B_{12}, 0.07 mg; Biotin, 0.60 mg; Niacin, 70.00 mg; Pantothenic acid, 25.00 mg.

[b] Mineral premix provided *per* kilogram of diet: Fe, 50.00 mg; Cu, 16.00 mg; Mn, 120 mg; Zn, 120 mg; I, 3.00 mg; Se, 0.30 mg.

SID: Standardized ileal digestibility; and DCAB: Dietary cation-anion balance.

Biochemical parameters. At the end of the week 60, 5.00 mL of blood was collected from the left wing vein of two birds of *per* replication using gauge needles in labeled screw-top tubes to obtain plasma containing 200 µL of ethylenediaminetetraacetic acid as an anticoagulant to study various blood parameters as recommended by Zhou *et al.*[18] Blood samples, were centrifuged at 3,500 *g* for 10 min at 4.00 ˚C in the laboratory to obtain plasma. The obtained heparinized plasma samples were stored in a freezer at – 20.00 ˚C until further analyses of glucose, calcium, phosphorus, cholesterol, triglycerides, alkaline phosphatase by commercial enzymatic kits (Pars Azmoon Inc., Tehran, Iran) with a plasma autoanalyzer (Abbott Laboratories, Chicago, USA).

Statistical analysis. Data normality was tested with UNIVARIATE method and Shapiro-Wilk using SAS Software (version 9.4; SAS Institute, Cary, USA). To analyze repeated measure ANOVA for productive performance, reproductive and egg quality and generalized linear model method for biochemical parameters were used. Means were compared with each other by Tukey's method at the significance level of 5.00%.

Results

Productive performance. The results related to the effect of multi-enzyme addition to the diets on the productive performance of broiler breeders are shown in Table 2. According to the results, it was found that egg laying percentage, egg weight, egg mass and FCR were affected by treatment, time and interaction effect of treatment and time. Use of negative control diet, caused a significant decrease in egg laying percentage, egg weight, egg mass and increased FCR compared to the positive control diet ($p < 0.05$). The addition of 1.00 g kg^{-1} of multi-enzyme to negative control diet made the above parameters similar to the control treatment. However, the positive control treatment supplemented with multi-enzyme had a higher performance compared to other treatments ($p < 0.05$).

Incubation performance. Table 3 shows the effect of multi-enzyme in the diet on the reproductive performance of broiler breeders. The effects of treatment, time and the interaction effect of treatment and time on reproductive traits were significant ($p < 0.05$). The percentage of settable eggs, fertility, hatchability and grade one chickens were decreased in the negative control diet ($p < 0.05$). However, supplementation of multi-enzyme to negative and positive control diets improved the mentioned parameters and this increase was higher in positive control treatment ($p < 0.05$).

Duodenum morphology. The results related to the effect of Rovabio® Plus multi-enzyme in the diet on duodenum morphology of broiler breeders are given in Table 4. The results showed that the VH of the duodenum was affected by experimental treatments, and the chickens fed on the positive control diet containing multi-enzymes had higher VH than the chickens without multi-enzymes diets ($p < 0.05$). Dietary supplementation of 1.00 g kg^{-1} of multi-enzyme to positive control diet increased the VH/CD ratio compared to negative control treatment ($p < 0.05$).

Table 2. Effect of Rovabio® Plus multi-enzyme in experimental diets on the production performance of broiler breeders at the age of 45 - 60 weeks (Ross 308).

Items		Percent of egg production	Egg weight (g)	Egg mass (g)	Feed conversion ratio
Treatments	T1	69.26[b]	65.61[c]	45.37[b]	3.52[b]
	T2	64.64[c]	64.70[d]	41.77[c]	3.84[a]
	T3	69.05[b]	66.17[b]	45.63[b]	3.50[b]
	T4	71.57[a]	68.25[a]	48.77[a]	3.26[c]
Standard error of means		0.17	0.06	0.11	0.01
***p*-value**	Treat	<0.001	<0.001	<0.001	<0.001
	Time	<0.001	<0.001	<0.001	<0.001
	Treat × Time	<0.001	<0.001	<0.001	<0.001

T1: Positive control diet (diet with standard energy and phosphorus and without multi-enzyme), T2: Negative control diet (diet in terms of energy 5.00% and phosphorus 50.00% less than the positive control diet, T3: Negative control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme and T4: Positive control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme.
[ab] Means within same column with different letters differ significantly ($p < 0.05$).

Table 3. Effect of Rovabio® Plus multi-enzyme in experimental diets on the incubation performance of broiler breeders at the age of 45 - 60 weeks (%), (Ross 308).

Items		Settable eggs	Fertile egg	Hatchability of settable egg	Embryonic losses of fertile egg	Grade one chicks
Treatments	T1	92.94[b]	84.39[c]	77.11[c]	8.36[b]	98.12[a]
	T2	87.23[c]	82.43[d]	74.21[d]	10.03[a]	97.32[b]
	T3	92.45[b]	86.78[b]	79.13[b]	8.84[b]	97.94[ab]
	T4	94.99[a]	88.20[a]	80.34[a]	8.91[b]	98.19[a]
Standard error of means		0.46	0.19	0.19	0.18	0.08
***p*-value**	Treat	<0.001	<0.001	<0.001	<0.001	<0.001
	Time	<0.001	<0.001	<0.001	<0.001	<0.001
	Treat × Time	<0.001	<0.001	<0.001	0.05	<0.001

T1: Positive control diet (diet with standard energy and phosphorus and without multi-enzyme), T2: Negative control diet (diet in terms of energy 5.00% and phosphorus 50.00% less than the positive control diet, T3: Negative control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme and T4: positive control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme.
[ab] Means within same column with different letters differ significantly ($p < 0.05$).

Table 4. Effect of Rovabio® Plus multi-enzyme in experimental diets on duodenum morphology of broiler breeders at the age of 60 weeks (Ross 308).

Items		Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height/Crypt depth
Treatments	T1	787.21[b]	155.53	196.27	4.01[ab]
	T2	762.24[b]	163.25	210.06	3.61[b]
	T3	821.06[ab]	159.19	206.64	3.98[ab]
	T4	837.29[a]	148.31	192.68	4.35[a]
Standard error of means		28.77	8.96	5.15	0.23
***p*-value**		0.05	0.38	0.19	0.04

T1: Positive control diet (diet with standard energy and phosphorus and without multi-enzyme), T2: Negative control diet (diet in terms of energy 5.00% and phosphorus 50.00% less than the positive control diet, T3: Negative control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme and T4: positive control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme.
[ab] Means within same column with different letters differ significantly ($p < 0.05$).

Table 5. Effect of Rovabio® Plus multi-enzyme in experimental diets on biochemical parameters of broiler breeders at the age of 60 weeks (Ross 308).

Treatments	Triglyceride (mg dL^{-1})	Cholesterol (mg dL^{-1})	Calcium (mg dL^{-1})	Phosphorus (mg dL^{-1})	Glucose (mg dL^{-1})	Alkaline phosphatase (U L^{-1})
T1	3,145.80	238.20	14.29	5.29[b]	144.03[b]	1,819.30[a]
T2	2,951.30	211.59	15.05	5.51[b]	140.10[b]	1,805.10[a]
T3	2,777.20	206.90	14.47	6.58[ab]	156.80[ab]	1,797.20[a]
T4	2,698.60	215.80	15.10	6.89[a]	178.30[a]	1,736.30[b]
SEM	97.28	18.51	1.45	1.34	8.55	37.97
***p*-value**	0.25	0.86	0.50	0.02	0.02	0.001

T1: Positive control diet (diet with standard energy and phosphorus and without multi-enzyme), T2: Negative control diet (diet in terms of energy 5.00% and phosphorus 50.00% less than the positive control diet, T3: Negative control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme and T4: positive control diet with 1.00 g kg^{-1} Rovabio® Plus multi-enzyme.
[ab] Means within same column with different letters differ significantly ($p < 0.05$).

Biochemical parameters. The results related to the effect of Rovabio® Plus multi-enzyme on biochemical parameters of broiler breeders are given in Table 5. Phosphorus and glucose concentrations were affected by the experimental treatments, and the chickens fed on the positive control diet containing multi-enzymes had higher concentrations compared to those of the chickens without multi-enzymes diets ($p < 0.05$). Dietary addition of 1.00 g kg^{-1} of multi-enzyme to positive control diet decreased the concentration of alkaline phosphatase compared to those of other treatments ($p < 0.05$).

Discussion

The positive effects of the enzyme in the diets were predictable because the amount of NSPs in these diets was high due to the use of wheat. The findings of this experiment and several other studies[19] showed the positive effects of NSPs degrading enzymes on egg laying performance. Reports show that the water-soluble part of NSPs in diets may disrupt the process of digestion and absorption in the gastrointestinal tract by increasing the viscosity of the intestinal contents and reduce the digestibility of nutrients.[20] The inclusion of NSP-degrading enzymes (especially xylanase) in the diet affects the fibrous structures of the diet and reduces the viscosity of the intestinal contents. These interactions will eventually improve the digestibility of nutrients.[21] In addition, the use of NSP-degrading enzymes can help reduce the anti-nutritional effects of NSPs in the diet by releasing the cellular contents of cereal grains and increasing the level of contact with internal digestive enzymes.[22] In study of Zheng *et al.*,[23] egg production and egg mass in laying hens fed on low-energy diets supplemented with enzymes were similar to those fed with standard diets without enzymes. Evaluating the effects of enzymes has shown that they can increase the energy value of diets when used in the diet of laying hens.[24] It can be speculated that the improvement of energy utilization by multi-enzyme supplementation may partially improve production performance. However, it cannot fully explain the beneficial effects shown in the present study. It has been shown that the addition of enzymes improves the digestibility of nutrients such as crude fiber, amino acids digestibility and phosphorus digestibility.[25] Also, the improvement of egg laying performance with multi-enzyme can be attributed to the reduction of pathogens, Gram-positive cocci and enterococci in the intestinal microbiota, the improvement of intestinal absorption capacity and the reduction of digestive viscosity attributed.[26]

Regarding the average egg weight, it was found that negative control diet led to a decrease in egg weight, however, by supplementing the negative control diet with 1.00 g kg^{-1} of enzyme, the egg weight was significantly higher than the positive and negative control treatments. Phytase supplementation (300 FTU microbial phytase) to a low phosphorus diets has positive effects on egg production, egg mass and egg weight by improving phosphorus use and this may be due to improvement in availability of some other nutrients such as energy and amino acids.[27] Falakodin *et al.*,[28] showed that the use of multi-enzyme up to 1.00 g kg^{-1} in laying hens led to a significant improvement in egg weight which was consistent with the findings of this research. However, Choct stated that the use of 1.00 g kg^{-1} of multi-enzyme in standard diets did not have effect on the weight of eggs produced.[26] In the review of various studies, it has been shown that the weight of eggs in many studies had a high dependence on the age of the bird, therefore, the differences in the results of the mentioned research might be due to the difference in the age of the bird and the basic diet.[5]

The main application of commercial multi-enzymes in poultry feed is to complement the effects of internal enzymes in reducing the effects of food inhibitors and increasing the digestion and absorption of nutrients in the feed which in turn improves the quality properties of eggs.[28] Negative control diet led to a decrease in the egg albumen height and egg shell weight. Also, the addition of multi-enzyme to the negative control diet led to the improvement of these parameters. In agreement with our results, Liu *et al.*[5] reported that the addition of multi-enzyme could significantly increase the albumin height and Haugh unit indicating that the addition of multi-enzyme improves egg freshness. The increase in shell weight in the standard treatment supplemented with enzyme can be caused by the higher egg weight in this treatment. The results of Liolopoulou *et al.*[29] showed the improvement of egg shell properties due to the increase in the bioavailability of calcium and phosphorus due to the use of xylanase in the diet. One of the important features of NSP is their partial solubility in water which leads to the formation of viscous gel solutions. These results are attributed to increased viscosity of the intestinal digesta which may impair the function of digestive enzymes, decrease transit rate and interfere with nutrient absorption.[30] As a result, the digestibility of nutrients and the utilization of minerals, especially calcium, may be significantly reduced.[31] On the other hand, Ebrahimnezhad *et al.*[27] reported that phytase supplementation increased egg shell thickness and dry shell weight in laying hens. It may due to increase in availability of phosphorus also, phosphorus is apart from the phytic acid complex, and at the same time, was released from molecule by microbial phytase. It seems, when phosphorus is limiting to maintain physiological takes more phosphorus remains in the body, as a result, less phosphorus is excreted through the feces.[32]

Intestinal morphology (villi length, CD and their ratio) can be used as an index for nutrient absorption. Longer villi and reduced CD are indicators for better nutrient absorption and lower mucosal tissue turnover rate.[33] The ratio of VH to CD is considered as one of the most relevant indicators for intestinal health.[34] However, the underlying mechanisms of the effect of corn and soybean meal-based diet supplementation with enzymes on intestinal morphology are not fully understood. In the present study, the reduction of dietary energy and phosphorus reduced the ratio of VH to CD and multi-enzyme supplementation compensated this reduction. Similar findings were reported by Alqhtani *et al.*[35] with multi-enzyme supplementation on the improvement of villi height. However, in contrast to our study, energy level did not affect intestinal morphology. This was in agreement with the study of Karimi and Zhandi[36] who found that the height of the duodenal villus was increased through dietary supplements of beta-glucanase or beta-mannanase, but was not affected by the energy level of the diet. However, Zou *et al.*[37] did not find any effect of energy level or multi-enzyme on VH to CD ratio in duodenum. For the absorption of nutrients, the small intestine is the main part of the digestive system. Intestinal cells can differentiate into mucin producing, digestive and absorptive roles.[38] The poor performance in birds fed on diluted rations is probably due to the poor absorption of nutrients due to shorter villi and deeper crypts, which leads to increased secretion of water and electrolytes in the digestive tract and thus to the risk performance falls.[39]

The obtained results showed that the use of multi-enzyme in the diets increased the serum phosphorus concentration, but no difference was observed in the positive control and negative control diets (50.00% reduction of phosphorus). In fact, diets with low phosphorus increase the release of parathyroid hormone and intensify the ionization of calcium in the plasma.[40] With the inhibitory effect of parathyroid hormone on renal tubules, phosphate reabsorption is reduced and subsequently, calcium absorption from digestive tubules increases. Plasma calcium and phosphorus concentrations are regulated through their absorption from the gastrointestinal tract, storage or retrieval from bones, excretion through feces and urine or reabsorption from the kidneys. Balancing calcium and phosphorus is done with the involvement of vitamin D_3 and the effect of hormones such as parathormone and calcitonin on the small intestine, kidneys and bones.[40] Some researchers[41] observed a significant increase in blood phosphorus levels in Japanese quail by adding phytase to the diet. By forming insoluble salts, phytate makes calcium, phosphorus and protein unavailable. In acidic conditions, the phosphate groups of phytic acid establish an electrostatic bond with the amino groups at the end of proteins, thus, forming a ternary combination of protein-cation-phytate which has a negative effect on the digestibility of protein and minerals.[42] Phytase enzyme increases the digestibility of protein and minerals by reducing the formation of double and triple protein complexes with phytate and minerals as well as reducing the inhibitory effect of phytate on digestive enzymes, as a result, the concentration of phosphorus in the blood serum increases.[42] Daramola,[43] reported that increasing the availability of phosphorus during the use of phytase may reduce the birds need to use endogenous phosphatases, therefore, the amount of alkaline phosphatase in the blood decreases with the use of phytase. In the report of Nourmohammadi *et al.,*[44] adding phytase enzyme to poultry diet decreased the concentration of liver alkaline phosphatase enzyme. Contradictory results have been reported about the effect of enzyme on blood cholesterol and triglyceride concentration. Zarghi and Golian,[45] showed that chickens fed on commercial enzyme cocktail had higher cholesterol concentration compared to those treated without enzyme. In contrast, Al-Kassie *et al.*[46] did not observe any significant difference in the concentration of cholesterol and total lipid with the addition of enzyme which was consistent with the results of the present study.

In conclusion, this research showed that the use of 1.00 g kg^{-1} of Rovabio® Plus multi-enzyme in broiler breeders at the age of 45 - 60 weeks led to the improvement of productive performance, reproduction and quality characteristics of eggs in negative control diet. So that performance of the birds in positive control fed on multi-enzyme were higher than that of the positive control treatment in many production parameters, incubation and biochemical indices.

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Conflicts of interest

There is not conflict of interest with any person or institute/organization regarding this manuscript.

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