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The Effects of Diets Supplemented with Phytase on the Productive Performance, Biochemical and Morphological Blood Indices in Broilers and Layers of Broiler Preparental Lines

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The effects of exogenous phytase in the vegetable diets on the productive performance, digestion, blood indices were studied on broilers (100 ppm, 35 birds per treatment, 1-35 days of age) and layers of three broiler preparental lines (B6, Cornish breed, B7 and B8, Plymouth Rock breed; 50 ppm, 25 birds per treatment, 21-62 weeks of age). In broilers increase in average daily weight gains by 5.7%, decrease in feed conversion ratio by 7.74%, significantly higher digestibility of crude protein by 2.23% ($P<0.05$), crude fat by 1.94% ($P<0.001$), nitrogen retention by 1.99% ($P<0.01$), phosphorus availability by 7.98% ($P<0.001$) in compare to control was found. The improvement of bone mineralization was also found. The activization of metabolism was evidenced by the significant increases ($P<0.05$) in concentrations of RBC (by 10.7% in compare to control) and hemoglobin (by 14.6%), and in hematocrit (by 10.5%). In layers slight increases in egg production and significant improvements in eggshell thickness and elastic deformation in all lines in compare to non-supplemented control treatments were found. Phytase was found to significantly ($P<0.05$) increase concentration of total protein in blood serum, decrease activity of ALP and diversely alter concentrations of glucose and total cholesterol. The changes in morphological blood profiles in certain treatments included increases in the percentages of neutrophils and basophils and decrease in the percentage of monocytes; as a result the general index of immune reactivity was higher in phytase-supplemented treatments in compare to control treatments in B6 and B7 lines while lower in B8 line.

Keywords: Broilers and preparental lines; phytase; growth efficiency; nutrient digestibility; hemoglobin; eggshell quality; immune reactivity.

INTRODUCTION

Enzyme preparations have become the intrinsic ingredients in the diets for poultry. At present over 90% of all compound feeds for poultry are supplemented with preparations of phytase, an enzyme hydrolyzing phytates from vegetable feed ingredients which cannot be digested by poultry due to the lack or shortage of endogenous phytase. Beneficial effects of exogenous (dietary) phytase include the releasing of phytate-bound phosphorus, decrease in the dietary content of expensive inorganic phosphates, decrease in the excretion of phosphorus as environmental pollutant with feces, increase in the digestibility of dietary protein and minerals (Ravindran, 1995; Singh, 2008; Trufanov, 2011).

The exclusion of animal derived ingredients from diets for poultry results in phosphorus deficiency in the diets for growing poultry. The problem is particularly acute in broilers; genetic selection of the latter for high postnatal growth

rate has led to the retardation of growth of the bones in compare to growth of the muscles often resulting in leg abnormailities. Meanwhile, growing poultry of almost all species cannot hydrolyze vegetable phytates though adult poultry (e.g. layers in the second half of their productive cycle) can cleave a larger part of the dietary phytates by endogenous phytase on the intestinal brush borders etc. Nonetheless, over 50% of dietary phytate-bound phosphorus passes the gastrointestinal tract (GIT) intact with subsequent excretion with feces (Anchikov, 2012).

The market of phytase preparations is presently diverse and saturated. The following properties of the preparations are practically important for poultry nutrition: activity (determining the dose of inclusion into diets); cost; thermal stability; efficiency in poultry (Shirley and Edwards Jr., 2003; Selle and Anchikov, 2010; Ward and Glitsoe, 2014). Preparations of phytase can be introduced into diets via premixes or directly, on the on-farm basis; in the latter case the availability of the appropriate equipment for the homogenization of supplemented feed should be considered.

Exogenous phytase can affect physical and chemical properties of chymus in the GIT of animals and poultry, primarily pH; this effect, in turn, results in the modification of the GIT microbiota and relative activity of different microbial species in it. Phytase is maximally active in acidic medium (pH 3-6), and the process of hydrolysis of phytate starts as early as in the crop and gizzard (Ward and Glitsoe, 2014). Different doses of phytase (up to 12,000 phytase units (FTU) per 1 kg of live bodyweight) in diets for broilers with lowered contents of calcium and inorganic phosphorus were reported to increase average daily weight gains (ADWG), concentrations of total phosphorus in blood serum and ash in tibia, tibial strength (Shirley and Edwards Jr., 2003). Beside these positive effects phytase can also detrimentally affect the availability of dietary amino acids: lysine, cysteine, aspartic acid, glycine, methionine, tryptophan, serine (Walk and Rama Rao, 2020). The increases in the availability of phytate-bound phosphorus and concentration of phosphorus in the jejunal digesta can be found with all species and ages of poultry and all diet types; supplementation of diets with phytase is more effective with growing poultry, in chicken especially at 14-22 days age when the active growth and mineralization of the bones occur (Babatunde et al. 2019). The efficiency of exogenous phytase was also reported to be affected by the sizes of the particles of mineral dietary ingredients (Kim et al. 2018). Combined supplementation of diets for broilers with phyase and xylanase and/or β-glucanase decreased feed consumption and colonization of the intestine by *E. coli*; no alterations were found in the GIT organs (Roofchaei et al. 2019). In certain studies the effects of phytase on the composition of the intestinal microbiota in poultry were reported (Singh, 2008); however, there's a lack of knowledge on the effects of exogenous phytase on the certain biochemical blood indices and morphological blood profiles in broilers and adult chicken, as well as on the productive performance in broiler breeders.

The aim of the study presented was the investigation of the effects of exogenous phytase on the productive performance; eggshell quality (in layers); bone mineralization, digestibility and assimilation of dietary nutrients (in broilers); biochemical and morphological blood indices in hybrid broilers and in three broiler preparental lines (Cornish and Plymouth Rock breeds).

MATERIALS AND METHODS

The study was performed on a new broiler cross recently selected by the Center for Genetics & Selection "Smena" (Moscow Province) and on layers of three preparental lines carrying the marker genes of slow and fast feathering (K-k): B6 (Cornish breed, fast-feathering, selected for growth rate, feed efficiency, meat yields, egg production); B7 (Plymouth Rock breed, fast-feathering, selected for egg production and hatchability, growth rate, feed efficiency, livability); and B8 (Plymouth Rock breed, slow-feathering, selected for egg production and hatchability, growth rate, feed efficiency, livability) (Efimov et al. 2018; Egorova, 2018; Egorova et al. 2018; Emanuylova et al., 2018; Egorova et al. 2019a,b).

The birds (35 birds for treatment for broilers, 25 birds for layers) were kept in cage batteries Big Dutchman under standard management conditions from 1 to 35 days of age (broilers) and from 21 to 62 weeks of age (layers) and fed vegetable compound feeds (corn, wheat, soybean meal) with balanced nutritive values supplemented with a phytase preparation (initial activity 10,000 FTU/g, 100 ppm for broilers and 50 ppm for layers – FTS treatments) or not supplemented (control (CON) treatments). The levels of phytase used were determined in previous experiments. The contents of metabolizable energy and crude protein in the diets for broilers were as follows: 12.98 MJ/kg and 23%, respectively, from 1 to 21 days of age, 13.19

MJ/kg and 21% from 22 to 28 days of age, and 13.40 MJ/kg and 20% from 29 to 35 days of age; in diets for layers (147-162 g/bird/day respective to age): 11.3 MJ/kg and 17% from 21 to 49 weeks of age and 11.1 MJ/kg and 16% from 50 to 62 weeks of age. The contents of crude fiber in the diets fell within the range 3.7-4.2%. The amino acid profiles of the diets were balanced on the basis of the availability from the ingredients; vitamins, minerals, and phytase (in FTS treatments) were introduced to diets via premix (1%).

The productive performance in broilers was determined (mortality, live bodyweight, ADWG, feed consumption, feed conversion ratio (FCR), dressing percentage, bone mineralization). At 30-35 days of age the balance trial was performed on 10 birds per treatment to assess the digestibility and assimilation (retention) of dietary nutrients. In layers certain parameters of productivity and eggshell quality were determined.

The blood was sampled (at 35 days of age in broilers and at 62 weeks of age in layers) from the axillary vein from the starved birds (10 birds per treatment); the solution of sodium citrate was added with subsequent centrifugation at 4,000 rpm for 3 min. The serum obtained was analyzed on the semi-automatic flow-type analyzer Sinnowa BS3000P (SINNOWA Medical Science & Technology, China) using reagent kits DIAKON-VET (Russia) to determine the concentrations of total protein, triglycerides, total cholesterol, glucose, and activity of alkaline phosphatase (ALP). The activity of trypsin in serum was determined by the kinetic method (Vertiprakhov and Grozina, 2018). Morphological blood indices were determined on automatic veterinary analyzer DF-50 with attached reagent kits (Shenzhen Dymind Biotechnology, China).

The statistical analysis of the results was performed using paired Student's t-test.

RESULTS AND DISCUSSION

Broilers

The data on the productive performance in broilers are presented in Table 1. Mortality level in all treatments was 0%.

At 21 and 35 days of age live bodyweight (LBW) in FTS treatment was insignificantly higher by 4.80 and 5.65%, respectively, in compare to control. LBW at 35 days of age in females in FTS treatment was significantly higher in compare to control by 5.13% ($P<0.05$), in males by 6.09% ($P<0.01$). ADWG in FTS treatment was higher by 5.7% in compare to control, FCR lower by 7.74%. These improvements in feed efficiency were related to better digestibility of dietary nutrients (Table 2).

Digestibility of dietary dry matter, crude protein, and crude fat in FTS treatment was significantly higher in compare to control by 1.99% ($P<0.001$), 2.23% ($P<0.05$), and 1.94% ($P<0.001$), respectively. Nitrogen assimilation (retention) was significantly higher by 1.99% ($P<0.01$) in compare to control; availability of lysine and methionine and assimilation of calcium were also significantly higher. Assimilation of dietary phosphorus in FTS treatment was higher in compare to control by 7.98% ($P<0.001$).

The improvements in the digestibility and assimilation of minerals resulted in better bone mineralization in FTS treatment: tibial ash content at 35 days of age in this treatment was 52.01% vs. 50.93% in control; calcium content 21.64 vs. 20.17%; phosphorus content 8.77 vs. 8.23%.

Biochemical and morphological blood indices in broilers are presented in Table 3. Biochemical indices were not significantly affected by the supplementation of diets with phytase. However, the increase of tryptic activity in FTS treatment indicates the enhancement of the pancreatic secretion which is known to affect metabolism in animals (Lebedev et al. 2019).

The significant decrease in the concentration of WBC in FTS treatment (by 21.1%, $P<0.05$) and increase in the percentage of monocytes (by 25.0%, $P<0.05$) were found. The concentration of RBC in FTS treatment was significantly higher by 10.7% in compare to control, concentration of hemoglobin by 14.6%, hematocrit higher by 10.5% ($P<0.05$) indicating more active growth of chicks in this treatment.

Layers

The data on the productive performance in layers of three broiler preparental lines are presented in Table 4. Mortality level in all treatments was 0%.

The supplementation of diets for preparental layers with 50 ppm of phytase resulted in slightly better egg production.

Table 1: The productive performance in broilers fed diets supplemented with phytase (FTS) (M±m, n=35)

	Treatments	
	CON	FTS
Average live bodyweight (g): at 1 day of age	42.4±0.11	42.7±0.14
7 days of age	90.2±0.33	90.4±0.40
4 days of age	180.1±1.40	189.7±1.35
14 days of age	421.7±5.70	451.3±5.91
21 days of age	850.5±13.1	891.3±14.3
35 days of age, in average	1980.4	2092.3
in males	2144.8±30.1	2275.4±35.5**
in females	1816.0±30.4	1909.2±34.4*
Average daily weight gains, g	55.37	58.56
Feed consumption, kg/bird	3.057	3.044
Feed conversion ratio, kg/kg	1.577	1.455
Dressing percentage, %	72.6	72.9

Differences with control treatment were significant at: *p<0.05; **p<0.01.

Table 2: Digestibility and assimilation of dietary nutrients (%) at 30-35 days of age by broilers fed diets supplemented with phytase (FTS) (M±m, n=10)

Digestibility	Treatments	
	CON	FTS
Dry matter	70.21±0.15	72.20±0.12***
Crude protein	91.21±0.20	93.44±0.27*
Crude fat	72.17±0.30	74.11±0.22***
Availability of lysine	82.11±0.22	83.0±0.24*
Availability of methonine	80.04±0.21	81.91±0.25*
Assimilation: nitrogen	61.23±0.13	63.22±0.12**
calcium	47.24±0.20	49.44±0.23**
phosphorus	40.14±0.15	48.12±0.20***

Differences with control treatment were significant at: *p<0.05; **p<0.01; ***p<0.001.

Table 3: Biochemical and morphological blood indices at 35 days of age in broilers fed diets supplemented with phytase (FTS) (M±m, n=10)

	Treatments	
	CON	FTS
Trypsin, U/L	33.4±0.07	38.8±3.35
ALP activity, U/L	3971±137.5	3030±435.1
Glucose, mM/L	7.9±0.45	7.0±0.25
Total protein, g/L	31.2±0.35	29.5±0.96
Triglycerides, mM/L	0.2±0.01	0.2±0.01
Total cholesterol, mM/L	1.9±0.17	1.9±0.12
WBC, 10^9/L	39.3±0.92	31±0.99*
Neutrophils, %	42.2±2.32	36.5±2.04
Lymphocytes, %	50.2±3.92	57.7±2.81
Monocytes, %	0.4±0.02	0.5±0.01*
Eosinophils, %	5.2±0.72	5.1±0.80
Basophils, %	0.2±0.04	0.2±0.02
RBC, 10^12/L	2.8±0.04	3.1±0.04*
Hemoglobin, g/L	89±1.08	102±2.86*
Hematocrit, %	21.6±0.31	24.8±0.51*
MCV, fL	123±1.50	123±0.18
MCH, pg	51.2±0.66	50.3±0.59
MCHC, g/L	416±1.70	410±4.60
RDW-CV, %	10.5±0.08	10.4±0.15
RDV-SD, %	52.4±0.27	51.9±0.78

ALP = alkaline phosphatase; WBC = white blood cells; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration;

RDW-CV = RBC distribution width - variation coefficient; RDV-SD = RBC distribution width - standard deviation. Differences with control treatment were significant at: *p<0.05.

Table 4: The productive performance in layers of three broiler preparental lines fed diets supplemented with phytase (FTS) (M±m, n=25)

	Line/Treatment					
	B6		B7		B8	
	CON	FTS	CON	FTS	CON	FTS
Average live body weight at 2 weeks, g	2420±35.1	2417±33.4	2288±36.2	2292±30.1	2277±33.8	2270±35.4
Average live bodyweight at 62 weeks, g	4480±41.9	4490±37.2	4015±43.2	4042±43.0	4030±46.2	4027±39.2
Egg production Perinitial hen, 21-62 weeks of age	117	121	155	159	153	157
Average egg weight at 30 weeks, g	61.9±0.18	62.0±0.20	60.4±0.22	60.3±0.25	60.0±0.27	60.1±0.23
Elastic deformation of eggshell, μm	22.04±0.051	20.9±0.047**	22.10±0.059	20.17±0.060***	21.95±0.047	20.14±0.054**
Average eggshell l thickness, mm	0.318±0.03	0.380±0.04***	0.320±0.02	0.392±0.03***	0.315±0.02	0.377±0.04***
Eggs suitable for incubation, %	91.1	91.5	93.5	93.8	93.4	93.6

Differences with the respective control treatments were significant at: **p<0.01; ***p<0.001.

Table 5:Biochemical and morphological blood indices at 62 weeks of age of age in layers of three broiler preparental lines fed diets supplemented with phytase (FTS) (M±m, n=10)

	Line/Treatment					
	B6		B7		B8	
	CON	FTS	CON	FTS	CON	FTS
Trypsin, U/L	243±39.5	260±13.3	247±42.3	182±18.0	181±50.0	152±4.6
ALP activity, U/L	255±24.2	172±40.9	331±50.1	159±7.3*	359±68.0	227±18.7
Glucose, mM/L	7.5±0.54	4.8±0.57*	4.1±0.61	5.9±0.29*	8.2±0.18	6.9±0.10*
Total protein, g/L	32.9±2.96	40.5±1.14*	28.7±1.97	42.3±0.95*	31.7±0.11	37.7±1.00*
Triglycerides, mM/L	4.9±1.14	5.8±0.42	3.1±0.84	3.8±0.73	1.6±0.50	4.1±0.36*
Total cholesterol, mM/L	1.6±0.02	2.6±0.26*	1.6±0.13	1.7±0.16	1.8±0.09	1.3±0.03*
WBC, 10^9/L	33.9±0.6	30±3.3	40.3±1.4	37.0±1.6	37.9±0.9	39±3.6
Neutrophils, %	40.0±3.7	44.0±9.6	47.5±6.4	41.0±3.8	38.9±4.7	51.0±0.8*
Lymphocytes, %	49.5±6.0	48.1±1.9	46.4±6.7	54±3.8	52.6±6.1	42.0±1.8
Monocytes, %	5.1±1.65	1.5±0.50	1.2±0.15	0.7±0.12*	0.7±0.14	0.8±0.26
Eosinophils, %	5.2±0.80	5.3±1.14	4.5±0.31	3.9±0.14	7.7±1.24	5.3±0.70
Basophils, %	0.2±0.13	0.5±0.16	0.3±0.13	0.2±0.09	0.1±0.02	0.7±0.19*
RBC, 10^12/L	2.1±0.02	1.9±0.14	2.2±0.04	2.0±0.08	2.2±0.03	2.2±0.11
Hemoglobin, g/L	108±0.47	100±8.10	107±0.81	108±5.20	122±1.45	113±8.30
Hematocrit, %	25.9±0.07	24.1±0.14	26.4±0.15	26.3±1.10	29.8±0.25	27.5±1.05
MCV, fL	123.0±1.8	125.6±2.8	121.0±2.6	129.8±2.8*	132.0±2.3	127.1±1.6
MCH, pg	51.4±0.75	52.2±1.20	49.4±1.21	52.9±1.67	54.1±1.20	51.9±1.36
MCHC, g/L	416±0.8	416±1.5	407±2.1	410±4.4	411±2.1	408±2.5
RDW-CV, %	9.6±0.10	12.3±0.94*	9.9±0.30	10.0±0.09	10.0±0.20	9.8±0.17
RDV-SD, %	48.7±1.02	63.2±0.40*	49.1±0.80	52.7±1.71	53.3±1.11	51.1±1.12

ALP = alkaline phosphatase; WBC = white blood cells; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW-CV = RBC distribution width - variation coefficient; RDV-SD = RBC distribution width - standard deviation. Differences with the respective control treatments were significant at *p<0.05.

Live bodyweight in the end of the trial (62 weeks of age) and average egg weight at 30 weeks of age were not affected in all three lines;

however, the parameters of eggshell quality (eggshell thickness and elastic deformation) were significantly improved. The percentages of eggs suitable for incubation were not affected by phytase and fell within the ranges 91.1-91.5% in Cornish line and 93.4-93.8% in Plymouth Rock lines.

Biochemical and morphological blood indices in broilers are presented in Table 5. Dietary phytase exerted different effects in layers of different preparental lines. E.g. concentration of glucose in blood serum in B6 and B8 lines was significantly lower in FTS treatments by 36.0 and 15.9% in compare to the respective control treatments while in B7 line higher by 43.9% ($P<0.05$). Concentration of total protein in blood serum was significantly higher in FTS treatments in compare to control treatments in all three lines: in B6 by 23.1%, in B7 by 47.4%, in B8 by 18.9%% ($P<0.05$). Concentration of total cholesterol significantly increased in FTS treatment in B6 line (by 62.5%), in B7 remained unaffected, in B8 decreased by 27.8% ($P<0.05$). The activity of ALP was significantly lower in compare to control in all FTS treatments: in B6 by 32.6%, in B7 by 52.0%, in B8 by 26.8% ($P<0.05$). The levels of triglycerides in blood serum fell within the reference values (Meluzzi et al., 1992) with the exception of B7 line; concentration of triglycerides in FTS treatments were higher compared to the control treatments: in B6 and B7 by 18.4 and 22.6%; in B8 significantly higher by 156.3% ($P<0.05$). Therefore, the increase in the availability of vegetable phosphorus from phytase-supplemented diets affected the metabolism in layers: it increased concentration of total protein in blood serum, decreased activity of ALP, and diversely altered concentrations of glucose and total cholesterol.

The changes in morphological blood profiles in certain treatments included increases in the percentages of neutrophils and basophils and decrease in the percentage of monocytes; the latter are related to the non-specific immune response. As a result the general index of immune reactivity (calculated according to Ivanov, 2014) was higher in FTS treatments in compare to control treatments in B6 and B7 lines while lower in compare to control in B8 line.

CONCLUSION

Supplementation of diets for broilers with phytase (100 ppm) resulted in the increase in ADWG by 5.7% and decrease in FCR by 7.74% in compare to control due to significantly higher digestibility of crude protein by 2.23% ($P<0.05$), digestibility of crude fat by 1.94% ($P<0.001$), nitrogen assimilation (retention) by 1.99% ($P<0.01$), phosphorus availability by 7.98% ($P<0.001$). The improvement of bone mineralization was found in phytase-supplemented treatment. The more intense metabolism in this treatment was also evidenced by the significant increases ($P<0.05$) in the concentration of RBC (by 10.7% in compare to control), concentration of hemoglobin (by 14.6%), and in hematocrit (by 10.5%).

Supplementation of diets for layers of three broiler preparental lines with phytase (50 ppm) resulted in slight increase in egg production and significant improvements in eggshell quality (eggshell thickness and elastic deformation) while egg weight and live bodyweight were unaffected. Phytase was found to affect metabolism in layers: it increased concentration of total protein in blood serum, decreased activity of ALP and diversely altered concentrations of glucose and total cholesterol. The changes in morphological blood profiles in certain treatments included increases in the percentages of neutrophils and basophils and decrease in the percentage of monocytes; as a result the general index of immune reactivity was higher in FTS treatments in compare to control treatments in B6 and B7 lines while lower in compare to control in B8 line.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

IAE designed the experiments and wrote the manuscript. TNL and VAM performed experiments on poultry. TAE performed statistical data analysis and reviewed the manuscript. VGV and AAG took the blood samples and performed the biochemical analyses. All authors read and approved the final version.

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