# Effect of AgpP-P phytase supplementation on productivity, nutrient availability, veterinary-sanitary assessment of meat and serum biochemical parameters of broiler chickens

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**Research**

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# Abstract

Background The major storage form of phosphorus in plant-derived feed is presents by insoluble phytates and not digested by animals. Microbial enzymes phytases are able to hydrolyze phytates and are widely used as feed additives in animal nutrition to improving the utilization of phytate-phosphorus in diets and reducing manure phosphorus excretion to the environment. However, no single phytase can perform effectively under various conditions of digestive systems and dietary compositions. That's why there is a constant search and development of new effective feed phytases preparations. The objective of this study was to study the effect of AgpP-P phytase supplementation on productivity, nutrient availability, veterinary-sanitary assessment of meat and serum biochemical parameters of Hubboard broilers.

Results Results showed that phytase supplementation increased body weight and absolute weigh gain in broilers, while reducing the total amount of feed consumed by birds ($P < 0.05$). Phytase improved the availability and absorption of organic nutrients, calcium (Ca) and phosphorus (P). However, no differences were observed for nitrogen (N) ($P < 0.05$). Phytase supplementation increased Ca and P contents, total protein (TP) level and alanine aminotransferase (ALT) activity, and reduced urea level, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities in serum, respectively ($P < 0.05$). The meat of phytase-fed broilers meets Russian State Standards (GOST) for fresh good-quality meat based on organoleptic, physicochemical and bacterioscopic characteristics.

Conclusions The results of present study demonstrated that addition of AgpP-P phytase to the diet of broiler chickens has a positive effect on the nutrient availability and productivity of birds, while reducing the total amount of feed consumed by chickens. Biochemical parameters in serum remained within the physiological norm. The meat of phytase-fed broilers met the Russian State Standards (GOST) for fresh good-quality meat. Thus, it is shown that AgpP-P phytase has the potential for use as a feed additive in poultry farming.


# Background

Phosphorus is one of the most important mineral elements in growth and development of poultry. This chemical element is necessary for normal functioning of many biological processes and it is present in phospholipids, nucleic acids, ATP molecules, coenzymes and hormones. Phosphorus deficiency can impede growth in birds and, with an additional lack of calcium and vitamin D3, cause the onset of rickets. A severe deficiency could lead to a rapid loss in appetite, fatigue and even death in fowls [1, 2].

In the modern feeding industry, the lack of digestible phosphorus is a significant problem due to the fact that in cereal grain-based diets 50–85% of phosphorus is present in the form of insoluble and indigestible phytate, which leads to significant losses of this important element [3]. In addition, phytates can form complexes with amino acids and metal ions, which further reduces their bioavailability [4]. Phytases are able to hydrolyze phytate, releasing phosphorus, and thus making it available for metabolic use [5–7].

However, poultry and other monogastric animals are not able to produce enough phytases and thus cannot effectively digest phytate [8]. Therefore, supplementation of diet with inorganic phosphorus is needed to satisfy the phosphorus requirement, which in turns leads to phosphorus excess in the manure. Consequently, the cost of feed and the environmental adverse impact are increased. In line with this, it is very important to find a way to reduce the phosphorus content in the poultry manure without reducing the amount of available phosphorus.

Currently, one of the most common methods to increase the availability of phosphorus and reduce its excretion in the environment is the use of microbial phytases as feed additives. Phytase supplementation of the broiler chicks diet has been shown to improve performance and phosphorus utilization by birds [9–11]. Phytase supplementation increased the availability of phytate-derived phosphorus and many metal ions, including $Ca^{2+}$, $Mn^{2+}$ and $Zn^{2+}$ [12, 13]. Moreover, addition of microbial phytases also improves the bioavailability of amino acids, proteins [10] and energy [14] in poultry. In addition, the use microbial phytase as feed additives improves utilization of phytate phosphorus, decreases its excretion and further phosphate pollution [15, 16].

To create feed additives, fungi (Aspergillus sp. and Penicillum sp.) and bacteria (E.coli and Citrobacter braakii), which produce 3-phytases and 6-phytases, respectively, are often used as sources of phytases [17]. These phytases belong to the histidine acid phytase family and exhibit an optimum pH between 2.5 to 5.5, which is ideally suited for maximal activity in the digestive tract of poultry. Although commercial production of phytases is currently focused on fungal phytases from Aspergillus sp., studies have shown that bacterial phytases are more promising because of their thermal stability, broader substrate specificity, greater resistance to proteolysis, and better catalytic efficiency [18].

Given the rise in prices of feed components, the use of phytase in the production of animal feed, the search for new efficient enzymes of this class and the expansion of the arsenal of commercial preparations of phytase are becoming urgent problems in fundamental biology and poultry farming. The ability of phytase to improve nutrient availability in poultry depends on the composition of the diet, the source of the phytases, the species and age of the poultry, as well as many endogenous conditions, such as the pH range of the poultry's digestive tract [19]. To date, all commercial phytase preparations available on the market are based on histidine acid phytases [20]. The AgpP phytase from Pantoea sp. 3.5.1 used in our study also belongs to this family. The enzyme has a broad substrate specificity, the pH optimum of the enzyme activity is 4.5, and the pH stability is in the range 3.0–6.0. Phytase stability is between 10 and 45 ° C with an optimum of 37 ° C [21]. Low pH values, at which the enzyme's activity and stability are maintained, allow it to work in the acidic environment of the animal’s stomach and make it possible to use AgpP phytase of Pantoea sp. 3.5.1 as a feed additive.

In our previous studies, a AgpP phytase from Pantoea sp. 3.5.1 has been expressed in Pichia pastoris (termed AgpP-P) for the production of high amounts of this enzyme (Suleimanova, In Press). In the present study, our aim was to evaluate the effects of AgpP-P phytase supplementation on productivity,

nutrient availability, veterinary-sanitary assessment of meat and serum biochemical parameters of broiler chickens.

# Methods

Enzyme preparation, birds, diets and experimental design

AgpP phytase preparation was done using recombinant yeast strain Pichia pastoris pPINK-agpP as described previously (Suleimanova, In Press).

The experiments were conducted with Hubboard broiler chickens in a vivarium of Z.I. Alimchueva's farm (Srednyaya Azyakovo village, Medvedevsky district, Republic of Mari El, Russia). Experimental procedures were carried out in accordance with the Institutional Animal Care and Use Protocol. A total of 180 1-day-old Hubboard broiler chickens were randomly divided into 2 groups (control and experimental) with 3 replicates and 30 chickens per replicate. Birds were housed in 3-tier cages made of galvanized mesh (0.5 m x 0.5 m x 0.35 m) with controlled temperature conditions. The initial temperature in the room was maintained at 32 °C, with a subsequent daily reduction by 0.2 °C. Final temperature in the room by the end of the experiment was 18 °C. Birds were subjected to a 23 h : 1 h (light : dark) photoperiod for the first 7 days, followed by 18 h : 6 h (light : dark) from 8th – 21st day and 23 h : 1 h (light : dark) for days 22–35 of the experiment. Broilers were given an access to food and water ad libitum during the duration of experiment. The ingredients and the nutrient contents of the chicken feed during different age periods are shown in Table 1. For experimental group, diet was supplemented with phytase preparation at 1000 phytase units (FTU)/kg.

Table 1. Ingredient and nutrient contents of broiler chickens diets

| Ingredients | Age | | |
|---|---|---|---|
| | 0 to 10 days | 11 to 21 days | 22 to 35 days |
| ME, kcal/100 g | 305 | 307 | 311 |
| Crude protein, % | 23.00 | 21.70 | 18.00 |
| Crude fat, % | 5.15 | 5.99 | 6.48 |
| Crude fiber, % | 3.55 | 3.73 | 4.77 |
| Lysine, % | 1.43 | 1.32 | 1.08 |
| Methionine and cysteine, % | 1.08 | 1.01 | 0.86 |
| Threonine, % | 0.98 | 0.90 | 0.77 |
| Calcium, % | 1.00 | 0.91 | 0.91 |
| Total phosphorus, % | 0.83 | 0.82 | 0.74 |
| Nonphytate phosphorus, % | 0.48 | 0.48 | 0.40 |
| Potassium, % | 0.76 | 0.71 | 0.60 |
| Sodium, % | 0.17 | 0.20 | 0.15 |
| Chlorine, % | - | - | 0.20 |
| Vitamin A, IU*$10^3$/kg | 14.40 | 12.00 | 12.00 |
| Vitamin D3, IU*$10^3$/kg | 4.80 | 4.00 | 4.00 |
| Vitamin E, mg/kg | 72.00 | 60.00 | 60.00 |
| Vitamin K3, mg/kg | 2.40 | 2.00 | 2.00 |
| Vitamin B1, mg/kg | 2.40 | 2.00 | 2.00 |
| Vitamin B2, mg/kg | 9.60 | 8.00 | 8.00 |
| Vitamin B3, mg/kg | 36.00 | 30.00 | 30.00 |
| Vitamin B4, mg/kg | 600.00 | 500.00 | 500.00 |
| Vitamin B5, mg/kg | 12.00 | 10.00 | 10.00 |
| Vitamin B6, mg/kg | 3.60 | 3.00 | 3.00 |
| Vitamin B12, mg/kg | 0.03 | 0.025 | 0.025 |
| Vitamin Bc, mg/kg | 0.60 | 0.50 | 0.50 |
| Vitamin H, mg/kg | 0.12 | 0.10 | 0.10 |

| Ingredients | Age | | |
|---|---|---|---|
| | 0 to 10 days | 11 to 21 days | 22 to 35 days |
| Iron, mg/kg | 30.00 | 25.00 | 25.00 |
| Copper, mg/kg | 12.00 | 10.00 | 10.00 |

Digestibility, Absorption Of Nutrients And Productivity Of Poultry

Poultry growth was assessed by monitoring weight gain. Broilers were weighed on days 1, 8, 15, 22, 28 and 35. The safety of poultry feed supplementation was determined by monitoring daily mortality in each group of birds, taking into account the reason.

To study the effect of the phytase supplementation on the digestibility and absorption of nutrients, a balance experiment was conducted. This involved a comparative quantitative and chemical analysis of the feed and fecal matter of birds using methods described in Russian State Standard 31640 – 2012, 32933 – 2014 and 31675 – 2012 (GOST 31640 – 2012 «Feeds. Methods for determination of dry matter content». GOST 32933 – 2014 «Feeds, compound feeds. Method for determination of crude ash», GOST 31675 – 2012 «Feeds. Methods for determination of crude fiber content with intermediate filtration», Kjeldahl method of nitrogen determination, Soxhlet extraction method of fat determination). Digestibility coefficient was calculated as the ratio of digested to ingested nutrients, expressed as a percentage.

Analysis Of Biochemical Parameters Of Serum

On days 5, 20 and 35 post-hatch, 5 chickens were randomly selected from each group for venous blood sampling. Heparinized whole blood, stabilized with Trilon B was used. Levels of total protein, urea, creatinine, total calcium, inorganic phosphorus, aspartate aminotransferase and alanine aminotransferase in sera were measured using BioChem SA analyzer (USA).

Veterinary-sanitary assessment of meat and studying the structure of the internal organs of poultry

For veterinary-sanitary assessment of broiler meat, poultry were humanely sacrificed by chloroform asphyxiation on 36 day of the experiment. To study the effect of phytase supplementation on the veterinary and sanitary indicators of broiler meat quality, a complex of organoleptic and laboratory studies was conducted. An organoleptic study was performed according to Russian State Standard 7702.0–74 (GOST 7702.0–74 «Poultry meat. Sampling methods. Organoleptic quality assessment methods»). Such factors as the appearance, smell, color, consistency of muscle tissue and fat, the state of incised muscles, the transparency and aroma of chicken broth were determined. Bacteriological examination of muscle tissue was performed according to Russian State Standard 7702.2–74 (GOST 7702.2–74 «Poultry meat. Methods of bacteriological analysis»). Microscopic analysis of smears collected from carcasses and physico-chemical studies were performed in accordance with Russian State Standard 7702.1–74 (GOST 7702.2–74 «Poultry meat. Methods of chemical and microscopic analysis of meat freshness»). This analysis included Gram staining of the smears, pH, amino-ammoniac nitrogen,

peroxidase, benzidine measurements, reaction with copper sulfate, reaction to ammonia and ammonium salts, acid and peroxide value of fat were determined. The biological value and meat safety were determined using the ciliate Tetrahymena pyriformis in accordance with the Methodological Guidelines for the Toxic-Biological Evaluation of Meat, Meat Products and Milk using Tetrahymena pyriformis (1997).

Statistical analysis

All data were analyzed using Microsoft Excel. Values are expressed as mean ± SEM of results from three independent experiments. Statistical significance was determined using a Student t test with significance set at a P value of $< 0.05$.

# Results And Discussion

In the current poultry industry, phytases are commonly used as a feed supplement to increase the absorption of phosphorus, reduce the cost of feed and, consequently, to reduce phytate excretion into the environment [22]. In addition, there is evidence that the use of phytases as a feed additive has a positive effect on the productive characteristics of poultry [3]. This could be due to improved phytase-mediated utilization of phosphorus, $Ca^{2+}$ and amino acids [23]. The AgpP Pantoea sp. 3.5.1 phytase used in our study belongs to the family of histidine acid 3-phytases [24], which includes enzymes successfully used as feed additives [18]. Our previous study showed that Pantoea sp. 3.5.1 phytase expressed in yeast Pichia pastoris (termed AgpP-P) has a high thermostability, a wide range of pH values and temperatures at which the activity and stability of the enzyme is maintained (Suleimanova, In Press). These properties make AgpP-P phytase a good candidate for the use as a feed additive. In this study we investigated the effect of AgpP-P phytase supplementation on productivity, nutrient availability, veterinary-sanitary assessment of meat and serum biochemical parameters of Hubboard broilers.

Effect of AgpP-P phytase supplementation on digestibility and absorption of nutrients and productivity of broilers

It is known that phytate can have an inhibitory effect on the activity of many endogenous digestive enzymes secreted in the gastrointestinal tract of animals by chelating the co-factors necessary for the activity of the enzymes [19]. Phytase addition can potentially improve utilization of nutrients in the feed. Our results showed that AgpP-P supplementation resulted in overall increase in digestibility coefficient of protein, dry matter, fiber and fat in phytase-fed broilers. It was particularly effective in the increase of digestibility of fiber (by 26.2%, $P < 0.05$, Table 2). Previously, Zhang et al. (2000) showed that phytase supplementation of broilers feed in concentration 250–2500 FTU/kg improves the digestibility of dry matter (DM) [25]. However, Lan et al. (2002) reported that phytase has to be added to the feed in a concentration range of at least 500–1000 U/kg to significantly increase DM digestibility. Improvements in digestibility of crude fiber and crude protein in broilers receiving phytase was showed by Attia et al. (2002) and Abd-Elsamee (2002) [27, 28]. In contrast, Ptak et al. (2013) reported that adding phytase did not affect fat digestibility [29].

Table 2
Effect of phytase supplementation on nutrient digestibility

| Digestibility coefficient | Control group | Phytase-fed group |
|---|---|---|
| Protein | 74 ± 0.6 | 74 ± 0.6 |
| Dry matter | 72.6 ± 0.9 | 72.6 ± 0.9 |
| Fiber | 10.3 ± 0.4 | 10.3 ± 0.4 |
| Fat | 58.5 ± 0.9 | 58.5 ± 0.9 |

Phytate-bound phosphorus has the ability to bind and form insoluble complexes with minerals necessary for metabolism, and thus such complexes cannot be properly used by the organism [30]. We determined the effect of phytase on the balance and the absorption coefficients of Ca, P and N (Table 3). Supplementation of AgP-P increased P and Ca digestibility in broiler chickens by 38.2 and 4.4%, respectively ($P < 0.05$). Our results were similar to those obtained earlier by Selle et al. (2009) and Chung et al. (2013) [31, 32]. This increase in Ca and P availability is due to the fact that phytase liberates these minerals from the Ca-phytate complex, rendering them available for absorption [33]. In turn, phytase supplementation had no effect on absorption and digestibility of N, which was also shown by Ptak et al. (2013) and Hao et al. (2018) [29, 34].

Table 3
Effect of phytase supplementation on absorption of calcium, phosphorus and nitrogen

| Indicator | Control group | Phytase-fed group |
|---|---|---|
| | Ca | |
| Ingested, g | 1.20 ± 0.01 | 1.20 ± 0.006 |
| Excreted, g | 0.52 ± 0.007 | 0.49 ± 0.003 |
| Digested, g | 0.68 ± 0.005 | 0.71 ± 0.008 |
| Digestibility coefficient, % | 56.6 | 59.1 |
| | P | |
| Ingested, g | 0.75 ± 0.003 | 0.74 ± 0.003 |
| Excreted, g | 0.53 ± 0.002 | 0.44 ± 0.002 |
| Digested, g | 0.22 ± 0.006 | 0.30 ± 0.004 |
| | N | |
| Ingested, g | 3.17 ± 0.04 | 3.12 ± 0.03 |
| Excreted, g | 1.48 ± 0.005 | 1.44 ± 0.005 |
| Digested, g | 1.69 ± 0.008 | 1.68 ± 0.006 |
| Digestibility coefficient, % | 53.3 | 53.8 |

Reduction of P excretion is particularly important in the reduction of P pollution by poultry manure. In the present study, the increase of P retention resulted in a significant decrease in P excretion. Compared to the control group, P excretion in broilers receiving phytase was reduced by 16.9% ($P < 0.05$). Earlier, Lan et al. (2002) reported that addition of phytase at a concentration of 500 U/ kg of feed reduced the excretion of phosphorus by 56.8% and 59.1% for days 11–13 and 18–20, respectively [26]. Therefore, we have shown that AgpP-P supplementation improves the digestibility and absorption of feed ingredients and reduces the excretion of phosphorus in broiler chickens.

The results of phytase supplementation on growth performance of poultry are presented in Tables 4 and 5. Results showed that addition of AgpP-P during the first 2 weeks of chicken life did not affect the weight gain. This could be linked to the low feed intake by chickens during the said period of growth. Starting from week 3, however, phytase supplement-fed chickens gained on average 18 grams more compared to the control group. By the end of week 5 the difference in weight gain between two groups reached 59.5 grams which correspond to 4% increase ($P < 0.05$). However, it is interesting that, along with such a small increase in broiler body weight gain, the amount of total feed consumed by broilers when AgpP-P was added to the diet of was reduced compared to the control groups (Table 5). We suppose that

the phytase hydrolyses the phytate and reduces its anti-nutritional effects, therefore enhancing the efficient utilization of feed, which is very important from an economic point of view. The increase in poultry weight gain following the addition of phytase has been shown by several researchers. Shirley and Edward (2003) found that supplementing phytase from 0 to 12000 U/kg significantly increased body weight gain from 287 to 515 g/chick [35]. De Sousa et al. (2015) reported that broilers fed on the phytase supplemented diet showed 4.40, 11.04 and 7.14% ($P < 0.05$) improvement in feed intake, weight gain and feed conversion ratio, respectively [36]. Moreover, Lim et al. (2001) in a study demonstrated that inclusion of phytase in feed increased weight gain and reduced mortality [37].

Table 4
Change of broilers weight (g)

| Indicator | Control group | Phytase-fed group |
|---|---|---|
| 0 w | 22.1 ± 0.21 | 22.5 ± 0.20 |
| 1 w | 297.6 ± 0.35 | 306.8 ± 0.36 |
| 2 w | 370.8 ± 1.14 | 373.6 ± 1.02 |
| 3 w | 572.3 ± 1.80 | 591.1 ± 2.20 |
| 4 w | 926.0 ± 4.55 | 978.5 ± 3.44 |
| 5 w | 1488.3 ± 8.67 | 1547.8 ± 7.98 |

Table 5
Growth performance of broilers

| Indicator | Control group | Phytase-fed group |
|---|---|---|
| Initial weight (g) | 22.1 ± 0.21 | 22.5 ± 0.20 |
| Final weight (g) | 1488.3 ± 8.67 | 1547.8 ± 7.98 |
| Absolute gain (g) | 1466.2 ± 3.46 | 1525.3 ± 3.98 |
| Feed intake (g) | 2521 ± 7.48 | 2481 ± 8.01 |

Effect of AgpP-P phytase supplementation on biochemical parameters of chicken serum

Results of biochemical analysis of blood serum are shown in Fig. 1.The concentration of total serum calcium (Ca) in AgpP-P supplemented group was consistently higher compared to control group (Fig. 1A). In contrast, other authors have reported that phytase supplementation had no effect on Ca level in the serum [26]. AgpP-P supplementation in the experimental group also increased levels of inorganic phosphorus (P) in the serum. The level of P was significantly elevated in the serum of 20-old-days chickens feeding on AgpP-P diet and by day 35 post-hatch this indicator was higher by 12.2% ($P < 0.05$) in the serum compared to the age-matched chickens from the control group (Fig. 1B). In agreement with our

data, multiple studies have also highlighted elevations of P concentrations in serum when chicken diet was supplemented with phytase [9, 26, 38].

Phytate supplementation of chicken feed increased the total serum protein content from days by the end of the experiment. In the experimental group, this indicator was higher by 5.6% ($P < 0.05$) in comparison with the control group (Fig. 1D). With the accumulation of protein in the blood, there was a decrease in the concentration of urea. From days 5 to 20, the urea content in the control and experimental groups decreased by 7.5% and 11.6% ($P < 0.05$), respectively. A similar observation was made when analyzing data for the 35th day (Fig. 1C). Urea is an indicator of the cost of the entire protein stock of the body. A decrease in the rate of urea synthesis thus reflects a higher accumulation of protein in the blood. In contrast with these findings, Wang et al. (2013) reported no effect of phytase on serum total protein and urea nitrogen [38].

Dietary inclusion of AgpP-P decreased serum aspartate aminotransferase (AST) activity by 2.9% at 35 d of age ($P < 0.05$) (Fig. 1E). However, in contrast to mammals, activity of AST is not liver-specific in birds [39]. Elevated activities usually indicate liver or muscle damage, but no particular significance is associated with low AST activity. The phytase supplementation caused an increase in serum alanine aminotransferase (ALT) activity by 3.7% ($P < 0.05$) (Fig. 1F). Plasma ALT activity has been reported to be low in all tissues of chickens [40], but ALT activities often increase due to damage in many tissues [41]. Therefore, specific diagnostic value of these enzymes in birds is poor. The phytase supplementation decreased serum alkaline phosphatase activity (Fig. 1G). Similar results have been reported by Atia et al. (2000) in turkeys and Viveros et al. (2002) in chickens [9, 42]. The decrease in serum alkaline phosphatase activity is associated with diets supplemented with phytase might reflect the downregulation of this enzyme resulting from the increased availability of phosphorus. However, Wang et al. (2013) showed that plasma alkaline phosphatase activity was not affected by phytase in broiler chicks [38].

Effect of AgpP-P phytase supplementation on veterinary-sanitary assessment of broiler meat

Veterinary and sanitary examination of broiler chicken meat was conducted according to Russian State Standards 7702.0–74, 7702.1–74 and 7702.2–74. (Tables 6, 7). Carcasses of chickens from the control and AgpP-P supplemented groups inspected 24 hours post sacrifice were found to have a similar appearance: dry whitish-yellow skin with a pink tint, the muscles were dense, elastic in texture, slightly moist on the site of cut. Chicken breast muscles and dark meat had a normal appearance. The odor from the surface and from the incision of muscles corresponded to that of a standard fresh meat. Chicken fat appeared to be pale yellow, elastic without any extrinsic odors. The broth, made from the meat of both groups, was transparent, fragrant, with a pleasant smell, drops of fat were observed on the surface of the broth. Analysis of fat tissue parameters (peroxide and acid number) from both groups met Russian State Standards for human consumption without any restrictions.

Interestingly, the pH value of the dark meet collected from the AgpP-fed chickens was slightly higher in comparison with pH of the dark meet collected from the control group. Previously it was notices that the

meat of broilers fed on phytase supplements has decreased pH values Hao et al. (2018) (Table 6) [34]. It is known that the activity of muscle tissue peroxidase is manifested in weak acidic medium, which is seen in fresh meat. Therefore, the reaction to peroxidase is one of the important sanitary assessment indicators of meat quality. Reaction to peroxidase of the meat samples collected from both groups was positive. In a contrary, the reaction to the products of the primary breakdown of proteins, ammonia and ammonium salts was negative for both chicken groups (Table 6).

Table 6
Physico-chemical parameters of broiler chicken meat

| Indicator | Control group | | Phytase-fed group | |
|---|---|---|---|---|
| | Leg muscle | Breast muscle | Leg muscle | Breast muscle |
| pH value | 5.70 ± 0.04 | 5.71 ± 0.09 | 5.83 ± 0.03 | 5.65 ± 0.17 |
| Peroxidase reaction | + | + | + | + |
| Reaction to products of primary protein breakdown | - | - | - | - |
| Reaction to ammonia and ammonium salts | - | - | - | - |
| «+» positive reaction; «-» negative reaction | | | | |

The content of volatile fatty acids is another important indicator of the sanitary assessment of meat quality. According to Russian State Standard GOST 7702.1–74 the content of volatile fatty acids for fresh, high-quality poultry meat, should not exceed 4.5 mg KOH/g (GOST 7702.1–74 «Poultry meat. Methods for chemical and microscopic analysis of meat freshness»). The analysis showed that for the meat samples collected from both groups, this indicator was within 1.92–2.08 mg KOH/g. The acid content of fat collected from chilled and frozen carcasses of all types of poultry should not exceed 1 mg KOH/g according to the same Russian State Standard (GOST 7702.1–74 «Poultry meat. Methods for chemical and microscopic analysis of meat freshness»). In agreement, the analysis showed that for the fat of the chickens from the control and phytase-fed groups, the acid numbers were 0.55 ± 0.02 KOH/g, and 0.59 ± 0.02 KOH/g, respectively. Microscopic analysis of the muscle surface in the samples collected from both groups showed the presence of isolated cocci and Gram-positive rod-shaped bacteria in the microscope field, which is an indicator of the freshness of the meat (SanPin 2.3.2.1078-01, Hygienic requirements for safety and nutrition value of food products, Health and hygiene rules and standards, 2002).

As shown in Table 7, indicators of the relative biological value of the meat of the experimental and control groups did not have any significant differences. The fluctuation of indicators occurred within a 99.0-99.2% range. The analysis showed that meat from any of the groups was not toxic for ciliate

Tetrahymena pyriformis (Table 7). Normally, the number of altered cell forms ranges between 0.1 and 1.0% (Methodological Guidelines for the Toxic-Biological Evaluation of Meat, Meat Products and Milk using Tetrahymena pyriformis (1997)).

Table 7
The biological value of broiler chicken meat

| Indicator | Control group | Phytase-fed group |
|---|---|---|
| Relative biological value,% | 99.0 ± 0.2 | 99.2 ± 0.3 |
| Toxicity to Tetrahymena pyriformis,% | 0.2 ± 0.02 | 0.1 ± 0.02 |

The analysis for heavy metal contamination (lead, cadmium, mercury, and arsenic) came back negative for samples collected from both groups of birds.

Thus, the meat of broiler chickens, which additionally received AgpP-P preparation to the ration meets the standards for human consumption and corresponds to Russia State Standards (GOST) for fresh, good-quality meat according to organoleptic, physicochemical and bacterioscopic characteristics.

# Conclusions

The results of present study demonstrated that addition of AgpP-P phytase to the diet of broiler chickens has a positive effect on the nutrient availability and productivity of birds. Biochemical parameters in serum remained within the physiological norm. The meat of phytase-fed broilers met the Russian State Standards (GOST) for fresh good-quality meat based on the organoleptic and physicochemical characteristics. Thus, it is shown that AgpP-P phytase has the potential for use as a feed additive in poultry farming.

# Declarations

### Ethics approval and consent to participate

All experimental procedures were carried out in accordance with the Institutional Animal Care and Use Protocol.

### Consent for publication

Not applicable.

### Availability of data and material

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

DB and AD obtained phytase in a bioreactor.

SS, AD, AM and MS selected of materials and methods and organized an experimental plan. DB, SS, GH and ML conducted experiments and analyzed of experimental data. DB prepared a manuscript. AM and MS edited and approved submitted manuscript.

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# Figures

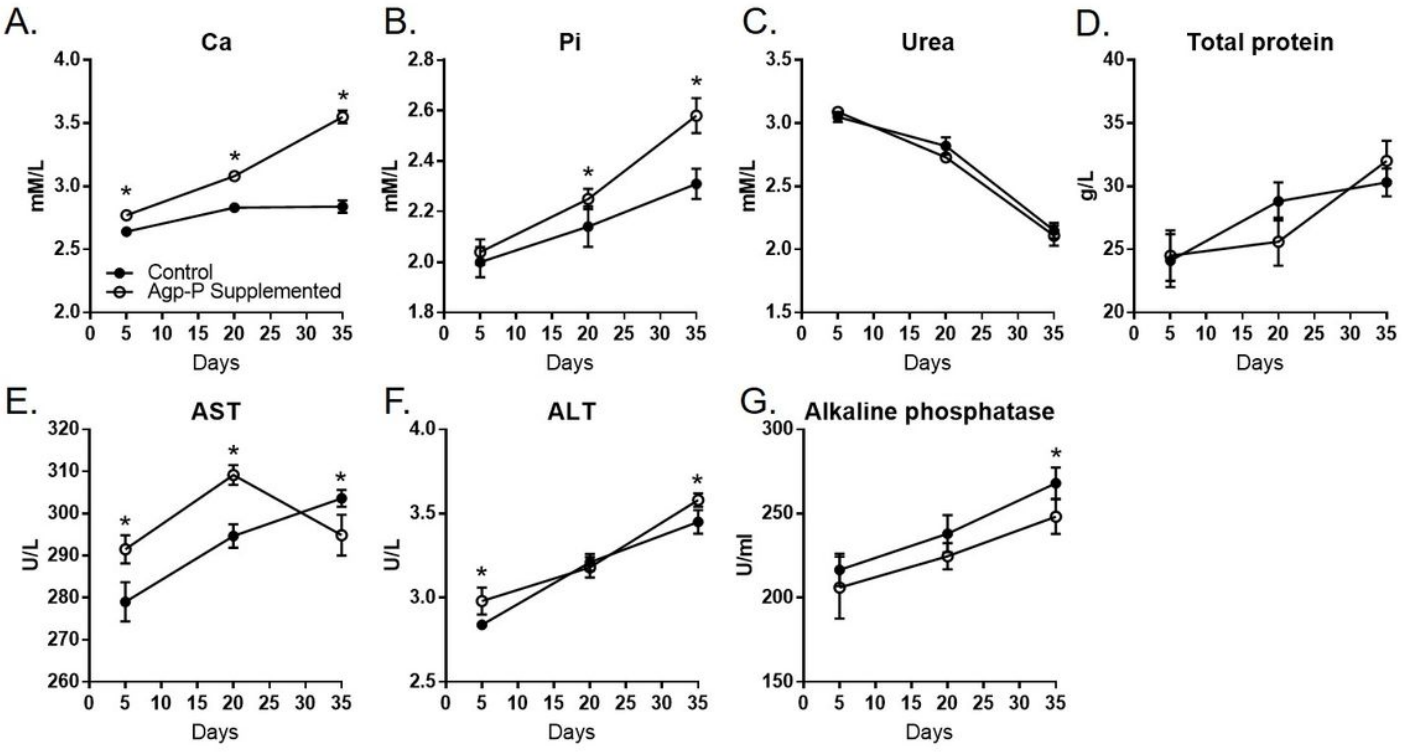


**Figure 1**

Effect of phytase on serum Ca (A), P (B), urea (C), protein (D), aspartate aminotransferase, AST (E), alanine aminotransferase, ALT (F), and alkaline phosphatase (G) activities in broilers