Effect of Locally Extracted Phytase on Blood Hematological and Serum Biochemistry of Broiler Chickens

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(received 19-10-2023; revised 18-09-2025; accepted 18-09-2025)

ABSTRAK

Lemma M, Girma M, Ameha N, Beker A, Zeryehun T, Yirgalem M. 2025. Pengaruh fitase yang diekstrak secara lokal dalam hematologi darah dan biokimia serum ayam broiler. JITV 30(3):140-149. DOI:http://dx.doi.org/jitv.v30i3.3409

Penelitian dilakukan untuk menguji pengaruh fitase yang diekstraksi secara lokal pada performa ayam pedaging dengan atau tanpa suplementasi fitase pada hematologi darah dan biokimia serum ayam pedaging. Sejumlah ayam pedaging Cobb 500 umur 180 hari secara acak dikelompokkan ke dalam empat perlakuan pakan menggunakan rancangan acak lengkap (RAL) yang direplikasi tiga kali dengan masing-masing sebanyak 15 ekor anak ayam. Rancangan percobaan adalah rancangan acak lengkap (RAL) dan ayam diberi pakan yang mengandung fitase pada laju dosis 0, 300, 600, dan 1200 FTU/kg selama 42 hari. Terdapat perbedaan yang signifikan (P<0,05) di antara perlakuan terhadap persentase volume darah yang terdiri dari sel darah merah (PCV), Hb, dan WBC. Namun, tidak ada perbedaan yang signifikan (P>0,05) dalam jumlah RBC, MCV, MCH, atau MCHC pada ayam dengan pakan yang disuplementasi dengan enzim. Nilai total protein, albumin, dan globulin tidak meningkat secara signifikan (P>0,05) pada kelompok yang diberi suplemen dibandingkan dengan kelompok kontrol T1 selama seluruh periode pertumbuhan. Konsentrasi kalsium berkisar antara 1-1,49% dan 0,75-0,89%, sedangkan fosfor berkisar antara 0,37-0,52% dan 0,28-0,44% pada pakan starter dan finisher, masing-masing, dan berada dalam kisaran yang direkomendasikan dalam ransum broiler. Berdasarkan hasil analisis anggaran parsial ini, ayam broiler T4 (1200 FTU/kg) mencapai keuntungan yang lebih tinggi dibandingkan ayam yang diklasifikasikan sebagai T2, T3, dan T1. Penggunaan fitase mungkin lebih menguntungkan daripada pemberian pakan kontrol. Tidak terdapat perbedaan mortalitas yang signifikan antar perlakuan (P>0,05).

Kata Kunci: Broiler, Hematologi, Mortalitas, Serum, Fitase, Fitat

ABSTRACT

Lemma M, Girma M, Ameha N, Beker A, Zeryehun T, Yirgalem M. 2025. Effect of locally extracted phytase on blood hematological and serum biochemistry of broiler chickens. JITV 30(3): 140-149. DOI:http://dx.doi.org/jitv.v30i3.3409

Experiments were conducted to examine the effect of locally extracted phytase on the performance of broilers with or without phytase supplementation on the blood hematological and serum biochemistry of broiler chickens. A total of 180-day-old Cobb 500 broilers were randomly assigned to four treatment diets in a completely randomized design (CRD), with 15 chicks per replicate and three replications. The experiment used a completely randomized design (CRD), and the birds were fed experimental feed containing phytase at 0, 300, 600, and 1200 FTU/kg for 42 days. There were significant differences (P<0.05) among treatments for Packed cell volume (PCV), Hb, and WBC. However, there were no significant (P>0.05) differences in RBC count, MCV, MCH, or MCHC of broilers fed the diet supplemented with enzymes. Total protein, albumin, and globulin values did not increase significantly (P>0.05) in the supplemented groups compared with the T1 control group throughout the growth period. The calcium concentration ranged from 1-1.49% and 0.75-0.89% in starter and finisher diets, respectively, whereas phosphorus ranged from 0.37-0.52% and 0.28-0.44% in starter and finisher diets, respectively, and it is within the range recommended in the broiler ration. According to the results of this partial budget analysis, T4 (1200 FTU/kg) broiler chickensachieved higher profits than chickens classified as T2, T3, and T1, respectively. Using phytase may be more profitable than feeding control. There was no significant difference in mortality between treatments (P>0.05).

Key Words: Broiler, Hematology, Mortality, Serum, Phytase, Phytate

INTRODUCTION

The poultry industry in developing countries faces challenges due to the high costs of conventional feed ingredients, such as yellow corn and soybean meal, which are primarily used in poultry rations (Alagawany & Attia 2015). Thus, there is an urgent need for nutritious and affordable feeds. Nowadays, feed accounts for about 75% of total animal production costs. Most poultry feed ingredients have historically been of plant origin, with the anti-nutritional factor phytate present as mixed phytate salts (AOAC 2006).

Feedstuffs such as corn and soybean meal have low phosphorus bioavailability due to phytate (Adeola 2004). Phytate has low solubility in the small intestine, poultry poorly absorb it, and its negative charge makes it a potent mineral chelate that forms insoluble salts with minerals. Birds do not produce the enzymes needed to digest NSP. Supplementing with NSP-degrading enzymes not only reduces the anti-nutrient effect of NSP but can also release nutrients available to birds from NSP (Balamurugan & Chandrasekaran, 2009).

Phytase is the only known enzyme that can initiate the phosphate hydrolysis at carbon 1, 3, or 6 in the inositol ring of phytate. The removal of the phosphate group by phytase results in the release of calcium, iron, zinc, and other metals (Konietzny & Greiner 2006). Phytase increased feed intake and body weight in poultry (Murugesan et al. 2005). The inclusion of phytase in broilers' diets with a low concentration of non-phytate phosphorus increased phosphorus retention with concomitant reduced excretion (Juanpere et al. 2004). As more phosphorus is released from phytate, leading to more breakdown of intact IP-6, the less able it is to bind or chelate minerals, starch, or proteins directly or via ionic bridges (Selle 2007).

However, the effects of extrinsic enzymes vary and depend on a variety of factors, including the age of the bird and the quality and type of the diet (Bedford 2000; Acamovic 2001). To solve this problem, phytase is commonly used as a feed additive to release P bound to phytic acid. Extrinsic phytase dietary supplements are effective in improving P digestibility (Walk et al. 2013; Selle & Ravindran 2007; Adeola & Cowieson 2011). Many commercially available multienzyme complexes can improve the nutritional value of protein-rich plant-based feed ingredients. However, imported commercial phytase is expensive and unavailable in Ethiopia. Therefore, to replace the commercial phytase enzyme, this research is locally extracting a phytase enzyme from available material (rye). The immune system in poultry can be identified from hematological profiles of the animal's blood. Hematological testing is used not only for diagnosis and therapeutic purposes but also for monitoring treatment responses (Oloche 2015). Therefore, this study aimed to determine the effects of phytase supplementation on hematology profiles in broilers.

MATERIALS AND METHODS

Description of the study area

The study was carried out at the Poultry farm of Haramaya University of Agriculture, which is located at a distance of 510 km east of Addis Ababa, at 42°3' east of longitude, 9°26'north latitude, and an altitude of 1980 meters above sea level. The mean annual rainfall of the area is 780 mm, and the average minimum and maximum temperatures are 8.5 and 24.4°C, respectively (Fedis Agricultural Research Centre)). Experimental work was conducted at Haramaya University's poultry farm for 42 days, from September to October 2021.

Ethical approval

The protocols for this experiment, use, and care of broilers were carried out in accordance with the guidelines of the Animal Care and Use Committee of Haramaya University, Ethiopia. Name of the approving committee: 1. Dr. Mulatu Wagari (chair) 2. Mr. Bacha Daba (Secretary) 3. Dr. Sisay Girma 4. Mr. Nega Baraki 5. Dr. Anteneh Belayneh 6. Dr. Dereje Tadese 7. Dr. Hirut Yirga 8. Dr. Teshome Seyum

Extraction procedure of phytase from rye

Rye grains were bought from a nearby market in Harar town, Ethiopia. The average phytase activity in rye kernels will be around 3.7 U/g. Based on this, 81 grams of rye were used to extract 300 U/g, 162 grams of rye were used to extract 600 U/g, and 324 grams of rye were used to extract 1200 U/g. In total, 568 g of rye yielded 2100 U/g of phytase. Small amounts of rye germ were ground in an Ultra-Turrax (5-10 min) at 4 °C in ice-cold 100 mM sodium acetate buffer, pH 5.0, whereas larger amounts were ground in a kitchen blender in the same buffer. Afterward, the soluble compounds were extracted by shaking for 2 h at 40°C. The cell debris was removed by centrifugation at 20,000 g for 30 min. Samples were weighed and placed in 250 ml Erlenmeyer flasks, then extracted using the procedures described by Harland and Oberleas (1977). The enzyme extraction was conducted at the Biotechnology Laboratory of Haramaya University.

The effect of pH on enzyme stability was tested over the pH range 1.0-9.0 at 4°C. In the pH range from 4.0 to 7.5, the phytase was relatively stable, while below pH 3.0 and above pH 7.5, a rapid decline in activity was observed. Within 10 days, more than 90% residual activity was measured at pH 4.0. At pH 2.5 and 8.0, 70% and 46% of the original activity were lost over 24 h of incubation, respectively.

Purification of the phytase

The cell-free culture supernatant was dialyzed and concentrated using the Lab scale TFF filtration system (Millipore, Bedford). The dialyzed and concentrated culture was then applied to an anion-exchange chromatographic column (diethylaminoethyl, HiPrep 16/10 DEAE-Sepharose FF, Pharmacia, Sweden)

equilibrated with 20 mM Tris-HCl buffer, pH 8. After washing the column, the bound enzyme was eluted at a flow rate of 1 ml/min using a linear gradient from 0 to 100% of 1 M NaCl in 20 mM Tris-HCl buffer, pH 8. Fractions were collected in volumes of 5 ml. Fractions containing phytase activity were pooled, dialyzed, and concentrated using the Labscale TFF filtration system (Millipore, Bedford). The concentrated enzyme was applied to a gel filtration column (HiPrep 16/60 Sephacryl S-100 HR, Pharmacia, Sweden) pre-equilibrated with 50 mM phosphate buffer and 200 mM NaCl at pH 8, and eluted using the same buffer at a flow rate of 1 ml/min. Fractions were collected every 5 min, and those with high phytase activity were pooled. The pooled fractions were dialyzed using ultrafiltration tubes at 5000 rpm for 20 min at 4°C in Centricon 10 (Amicon, USA) ultrafiltration concentrators (membrane cutoff of 10 kDa). During all the purification procedures, all collected and pooled fractions were tested for absorption (wavelength 280 nm), total protein (wavelength 595 nm), and phytase activity (wavelength 355 nm).

Seed germination

Rye grains were soaked in the following solutions: (1) 0.1% Tween-80 for 5 minutes, (2) 0.5% NaOCl for 2 minutes, (3) 0.75% H2O2 for 1 minute. After soaking, the rye grains were thoroughly rinsed in sterile water. The seeds were then allowed to germinate in a sterile box in a dark place at 20°C. Once a day, the seeds are washed with sterile water; after rinsing, the water is completely removed (Gibson et al., 1988).

The seeds were culled for broken seeds, then weighed out for sprouting. For each time segment, samples of each seed were placed in quart glass jars. The mouths of the jars were covered with flexible plastic screening held in place with a string. The grains had been soaked in deionized water for 12 hours before sprouting was considered underway. After 12 hours, the deionized soaking water is drained, and half a liter of deionized water is dispensed into the jar. Seeds were rinsed with sterile water once a day; the water was removed entirely after each rinse.

Excess water is shaken out of the bulb before placing the bulb, mouth facing down, to empty the seeds. The seeds were then allowed to germinate in sterile trays indoors in the dark at 20°C. Every afternoon, the grains are rinsed with sterile water, which is then removed. One hundred milliliters of 1.2% HCl is delivered to every flask. The flask becomes sealed with plastic wrap. The flasks were shaken at 2 hundred rpm for 2 hours at 26°C. The samples had been vacuum filtered with #1 Whatman Filter paper. The filtrate was stored for no more than 1 week at 1 °C in the refrigerator. During the first 7-10 days, phytase activity started.

Experimental animals and management

The experiment was conducted at Haramaya University poultry farm for 42 days. Before starting the actual experiment, the experimental house was cleaned and washed, and the floor was covered with a 7cm layer of wood shavings, which was thoroughly disinfected before placement of the experimental birds. Two infrared lamps, each providing 250 watts, were fitted for each pen as a source of heat and light. A circular plastic feeder and waterer were placed in each pen a day before the birds were placed.

A total of one hundred eighty (180) day-old unsexed broiler chicks were purchased from available farms around Debre Zeit's private farm. Water was available at all times, and the experimental ratio was measured on an ad libitum basis twice a day at 8:00 and 16:00 hrs. The refusals were recorded every morning to determine feed and nutrient intakes. Broilers were weighed by pen at 0, 7, 14, 21, 28, 35, and 42 days of age using a sensitive balance. There are three phases: 0 to 10, 11 to 24, and 25 to 42 days of age. Standard biosecurity protocols were employed throughout the experimental period, and the chicks were vaccinated against viral diseases.

Design of the Experiment and Ingredients

A total of 180-day-old Cobb 500 broilers were randomly assigned to four treatment diets in a completely randomized design (CRD), with 15 chicks per replicate and three replications. The treatment rations were formulated using FeedWin Interactive software to be isocaloric and isonitrogenous, meeting the nutrient requirement standards for broilers (NRC, 1994), as shown in Table 1 below. Accordingly, each of the starter treatment rations contained about 3100 kcal ME/kg of energy and 22% crude protein, while each of the finisher's treatment rations contained 3200 kcal ME/kg of energy and 20% crude protein. The starter phase lasted until 3 weeks of age (days 1st to 21st), and the finisher phase lasted from the beginning of the third week until slaughter (days 22nd to 42nd). The starter and finisher diets were formulated separately. The treatments consisted of a control diet for each phase, and three other diets were formulated, each with increasing levels of the phytase enzyme added to the feed at 0, 300, 600, and 1200 FTU/kg for T1, T2, T3, and T4, respectively.

Hematological parameter

Blood samples were collected from the wing vein of 2 birds per replication into a 5 ml sterile syringe using a 23-gauge needle for hematological and bioche-

Table 1. Composition of ingredients fed to broilers from 0-3 weeks and 4-6 weeks of age

Ingredients (%)	0-3 weeks (Starter Phase)	4-6 weeks (Finisher Phase)
Maize	56.76	61.39
Soyabean	18.45	13.65
Wheat	2.92	8.2
Methionin	0.5	0.1
Dicalcium	0.5	0.5
Nougseed cake	17.46	13.66
Salt	0.97	0.5
Premix	0.5	0.2
Limestone	0.97	1.3
Lysine	0.97	0.5
Total	100	100

mical parameters at the finisher phase (42 days of age). From each chicken, 1-2 mL of blood was collected aseptically through the brachial (wing) vein as described by Kelly (2013).

Each blood sample was immediately transferred into a tube containing ethylenediaminetetraacetic acid as an anticoagulant. The red blood cell (RBC) and white blood cell (WBC) counts were determined using a hemocytometer (Irizaary-Rovira 2004). The hemoglobin (Hb) concentration was evaluated by matching the acid hematin solution to a standard colored solution in the Sahlis hemoglobinometer. Packed cell volume (PCV) was measured by the microhaematocrit method. Mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), and mean corpuscular hemoglobin (MCH) were computed as = (Ross et al. 1976; Irizaary-Rovira, 2004). These parameters were determined using blood harvested into the tube containing EDTA.

Serum biochemical parameters

Serum was separated after centrifugation at 3,000 (rpm x g) for 15 min and stored at -20° C until used. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, cholesterol, glucose, and triglycerides concentrations were measured by using enzyme/ buffer and substrate kits, such as AP (Alkaline Phosphatase), at Haramaya University Health Center Laboratory. Total serum protein was determined by refractometer (George 2001). Total serum immunoglobulin concentration was determined by the serum zinc sulfate turbidity test, measuring the optical density of the test and the control separately at 545nm using a spectrophotometer (Kassaye et al. 2016).

Economic efficiency analyses

The net profits from broilers were calculated based on the cost of broiler birds and the feed each bird consumed in the respective treatments, along with other costs. To estimate the net benefit of phytase feeding, a partial budget was prepared, accounting for full feed costs according to the principles developed by Upton (1979). The partial budget analysis involves calculating variable costs and benefits. Partial budget measures the cost of the chicken, feed, and other inputs (if any), and the profit after the experiment—the difference between gains and losses from the proposed change.

The costs of experimental feed ingredients and basal feeds were used to calculate the feed cost for each treatment. Total variable cost includes feed and other costs. The total rate of return (TRR) was calculated as the difference between the sale and purchase prices in the partial budget analysis. The net income (NI) was expressed by subtracting the total variable cost (TVC) from the total return between the changes in the total rate of return (ΔTRR) and total variable cost (ΔTVC).

The marginal rate of return (MRR) measures the increase in net income (ΔNI) per additional unit of expenditure (ΔTVC), as shown below.

$$MRR = \frac{\Delta \text{NI}}{\Delta \text{TVC}}$$

The chick sale price-to-feed cost ratio was also calculated as an additional parameter to evaluate the efficiency of the change in feed ingredients.

Statistical analysis

All data collected in this study were analyzed using SAS (2016). Differences between treatment means were separated using Duncan's Multiple Range Test. The following model was used for data analysis. Yij= μ + Ti + eij, where Yij= represents the jth observation in the ith treatment level, μ= overall mean, Ti= treatment effect, and eij= random error.

RESULTS AND DISCUSSION

The chemical composition of the experimental feed ingredients was presented in Table 2 and determined by proximate analysis, which analyzed the dry matter (DM), ash, crude protein (CP), ether extract (EE), and crude fiber (CF). Two experiential diets used in the experiment are chick starter and finisher diets. They were made from local feed ingredients commonly used in poultry feeding in Ethiopia. The diets were formulated to meet the NRC (1994). The metabolizable energy of the experimental diets was determined by the indirect method using the formula given by Wiseman (1987): ME (Kcal/kg DM) = 3951 + 54.4 EE - 88.7 CF - 40.8 Ash. The formulation, calculated, and determined analyses of the diet are shown in Tables 2 and 3 below, respectively.

The experimental birds were fed on the starter diet for the first three weeks (1-21 days) of the experiment. Thereafter, they were fed on the finisher diet (22-42 days) supplemented with four levels of phytase enzyme having 0.0 FTU/Kg, 300 FTU/Kg, 600FTU/Kg, and 1200FTU/Kg, for the last four weeks of the experiment (22-42 days). The phytase has been added to the feed in powder form, and it should be added from the starter to the finisher phase. The feed was formulated twice, once at the starter phase and once at the finisher phase. So the chickens were fed with phyase for 42 days. The calcium concentration ranged from 1-1.49% and 0.75-0.89% in starter and finisher diets, respectively, whereas phosphorus ranged from 0.37-0.52% and 0.28-0.44% in starter and finisher diets, respectively, and are within the range recommended in the broiler ration (NRC 1994).

Haematological profiles

The effects of phytase supplementation on the blood hematological parameters of the broiler chickens are listed in Table 4. Packed cell volume (PCV), Hb, and WBC values were significantly ($P<0.05$) influenced by the dietary treatments, while all other parameters were not significantly ($P>0.05$) different. The values for PCV ranged from 27.783 – 33.053%, Hb from 9.11 – 11.05g/dl, and WBC from 4.8 – 6.09×109/L, respectively. It was observed that PCV, Hb, and WBC values were reduced across dietary treatments. PCV, Hb, and WBC were significantly ($P<0.05$) influenced by the dietary treatment; the values obtained were within the normal range for broiler chicken reported by (Oloche et al. 2015).

Table 4 shows that white blood cell counts in the control and treated groups were 6.31, 6.17, 5.92, and 5.62 × 10^9/l at T0, T1, T2, T3, and T4, respectively. The experimental birds were fed on different groups that were significantly different from one another. This indicates a potential effect of diet on white blood cell levels, as the treated groups showed lower average counts than the control group, and there were statistically significant differences between the diet groups.

The present results were in disagreement with the findings of Shareef and Al-Dabbagh (2009), and Alkhalf et al. (2010) observed a non-significant ($P>0.05$) difference in PCV of broilers fed the diet supplemented with enzymes. In contrast, Cowieson et al. (2006), Rahman et al. (2013), and Kaushal et al.

Table 2. Chemical composition of ingredients and treatment diets (percentage on DM basis)

Ingredient	(%)	Chemical Composition							
		DM	ash	EE	CF	CP	Ca	P	ME
Maize	46	87.74	5.35	3.4	3.85	9.1	0.055	0.375	3576.2
Soyabean	13.39	92.22	6.2	9.6	10.26	31.08	0.37	0.885	3310.218
Wheat Short	15.5	88.6	8.25	2.6	9.9	14.01	0.24	0.705	2877.71
Nougseed cake	15	93.56	11.9	7.25	15.7	28.24	0.6	0.8	2467.29
Phytase	-	94.52	1.6	0.95	1.5	13	1.2	0.6	3804.35

ME= Metabolizable energy, DM= total dry matter, CP= Crude protein, EE= Ether extract, Kcal= Kilocalorie, Kg= kilogram

Table 3. Chemical composition of ingredients and treatment diets (% on DM basis)

Ingredients	Item							Chemical composition
	%DM	CP	CF	EE	ash	Ca	P	ME(Kcal/Kg)
Maize	89.88	9.06	3.54	3.86	4.42	0.075	0.50	3666.65
Wheat short	90.46	14.90	8.85	3.58	5.95	0.18	0.59	3117.997
Soybean meal	92.64	35.85	9.6	6.51	6.22	0.34	0.74	3199.848
Nougseed cake	92.95	29.61	16.46	7.43	11.26	0.41	0.57	2435.782
Phytase	94.52	35.32	0.5	4.00	4.53	1.2	0.6	3939.426
Starter phase	%DM	CP	CF	EE	ash	Ca	P	ME(Kcal/kg)
T1	92.27	22.82	9.3	7.4	10.7	1.0	0.51	3092.09
T2	91.63	22.92	9.14	8.35	11.65	1.0	0.52	3119.202
T3	91.07	22.45	9.21	7.2	10.2	1.49	0.51	3109.593
T4	92.45	22.42	8.7	7.03	10.3	1.37	0.37	3141.502
Finisher phase	%DM	CP	CF	EE	Ash	Ca	P	ME(Kcal/kg)
T1	92.88	21.1	8.37	8.17	9.83	0.89	0.32	3251.965
T2	92.62	20.76	7.8	7.45	9.6	0.89	0.32	3272.74
T3	91.52	20.31	8.87	8.1	10.3	0.87	0.44	3184.631
T4	93.62	20.12	9.62	7.01	11	0.75	0.28	3030.25

T1, T2, T3, and T4 0, 300, 600, and 1200 FTU/Kg, respectively, DM= dry matter, CP= crude protein, EE= ether extract, CF= crude fiber, Ca= calcium, P= phosphorus, ME= Metabolisable Energy, Kcal= kilocalorie, Kg= kilogram

(2015) found a significant ($P<0.05$) increase in PCV and Hb content of broilers fed the diet supplemented with enzymes. Ferdous et al. (2018) noted a significant ($P<0.01$) increase in PCV and Hb in broilers fed a diet supplemented with enzymes and multivitamins. Hosseini (2011) noted a significant ($P<0.05$) increase in Hb of broilers fed the diet supplemented with yeast (*Saccharomyces cerevisiae*). Ahmed et al. (2007) found a significant ($P<0.05$) increase in PCV and Hb values in broilers fed a diet supplemented with enzymes (Alquerzim). Milanovic et al. (2008) noted an increase in PCV and Hb of broilers fed the diet supplemented with organic iron. The analyzed data show that there were no significant differences ($P>0.05$) in MCV, MCH, and MCHC among broilers fed the enzyme-supplemented diet. The present results were consistent with those of Shehab et al. (2012), who observed non-significant ($P>0.05$) differences in MCV in broilers fed a diet supplemented with enzymes. Chuka (2014) found no significant differences ($P>0.05$) in MCV, MCH, and MCHC in broilers fed a diet supplemented with enzymes and probiotics. The results of hematological variables in this study suggest that the test diets did not pose any severe effects on the health status of the experimental birds.

Serum biochemistry

The effects of phytase supplementation on the blood serum biochemistry parameters of the broiler chickens are listed in Table 5 below. Total protein, albumin, and globulin values did not increase significantly ($P>0.05$) in the supplemented groups compared with the T1 control group throughout the growth period. The results of the present experiment were in accordance with the findings of Chuka (2014) noted non-significant ($P>0.05$) differences in serum globulin, and serum albumin of broilers fed the diet supplemented with enzymes and in contrast with the findings of Hassen & Chauhan (2003) who found a significant ($P<0.05$) increase in serum globulin levels in broilers fed a diet supplemented with enzymes. Paryad and Mahmoudi (2008) and Yazhini et al. (2018) noted a significant ($P<0.05$) increase in total protein, albumin, and globulin concentrations in broilers fed the diet supplemented with probiotic (S. cerevisiae). Shareef and Al-Dabbagh (2009) and Chaudhary et al. (2017) observed a significant ($P<0.05$) increase in serum total protein of broilers fed the diet supplemented with probiotics. Chuka (2014) and Kaushal et al. (2019) found a significant ($P<0.05$) increase in total protein in broilers

fed a diet supplemented with enzymes. The dietary treatment did not influence all measured serum biochemical parameters, indicating that the animals well tolerated the diets.

Partial budget analysis

The economic returns associated with subbudgets are shown in Table 6. A producer's goal in poultry production is to achieve the highest growth rate at the lowest feed cost per unit of live weight gain. According to this partial budget analysis, T4 broiler chickens achieved higher profits than those classified as T2, T3, or T1. T4 birds had the highest cost of selling chicks for feed compared to other treatments. Therefore, the results of this study suggest that using phytase may be more beneficial than feeding control. T4 was the most cost-effective diet per chick reared in this study.

Mortality

The death is recorded in Table 7 below. According to the present study, there was no significant difference in mortality rate between the treatments (P>0.05). The cause of chicken deaths during this period was transportation stress and the inability of individuals to adapt to the environment. Examination in all phases of dead chicken showed the presence of watery fluid in the abdominal cavity and pericardial sac and sudden death; this may be due to the high growth rate of broiler chicken and rapid growth, which causes the heart and lungs not to develop well enough to support the remainder of the body, resulting in congestive heart failure and tremendous death losses (Martin 1997).

Table 4. Effects of phytase supplementation on the blood hematological parameters of broiler finishers from (0-42 days)

Parameters	Treatments					
	T1	T2	T3	T4	SEM	P.value
PCV (%)	33.053^{a}	32.82^{a}	30.503^{ab}	27.783^{b}	0.95	0.014
Hb(g/dl)	11.05^{a}	10.12^{ab}	9.94^{ab}	9.11^{b}	0.3536	0.03
RBC($\times10^{12}$/L)	2.76	2.78	2.76	2.95	0.01	0.21
WBC($\times10^{9}$/L)	6.09^{a}	5.95^{a}	5.73^{ab}	4.8^{b}	0.29	0.03
MCV(fl)	138.60	138.33	139.07	139.09	0.86	0.90
MCH(pg)	31.81	31.23	30.85	29.63	0.01	0.66
MCHC (%)	23.45	23.19	22.23	21.25	35.61	0.31

PCV= Packed cell volume; Hb= Hemoglobin; RBC= Red blood cell; WBC= White blood cell; MCV= Mean Corpuscular Volume; MCHC= Mean Corpuscular Hemoglobin Concentration; fL= femtoliters, pg= picograms, dl= deciliter; T1, T2, T3, and T4= 0, 300, 600, and 1200 FTU/Kg, respectively

Table 5. Effects of phytase supplementation on the blood serum biochemistry parameters

Parameters	Treatments					
	T1	T2	T3	T4	SEM	P.value
Total protein(g/dl)	3.65	3.65	3.79	3.65	0.48	0.99
Albumin (g/dl)	1.90	2.31	2.04	1.95	0.51	0.89
Globulin (g/dl)	1.75	1.67	1.75	1.69	0.52	0.99
Total immunoglobulin(g/dl)	0.61	0.91	0.83	1.06	0.03	0.08

T1, T2, T3, and T4 = 0, 300, 600, and 1200 FTU/Kg, respectively, SEM = Standard Error of Mean; dl = deciliter

Table 6. Economics of phytase supplementation in broiler chicken

Particulars	Treatments			
	T1	T2	T3	T4
Purchase price/bird (birr)	40.00	40.00	40.00	40.00
Total feed consumed(kg)/chick	3.89	3.93	3.89	3.93

Particulars	Treatments			
	T1	T2	T3	T4
Selling price/bird(chick)	207.9	238.2	221.25	256.35
Feed cost/bird (birr)	56.40	56.98	56.40	56.98
Other cost /bird (birr)	22.22	22.22	22.22	22.22
TVC/bird (birr)	78.62	79.2	78.62	79.2
TR (birr)	167.9	198.2	181.25	216.35
NR (birr)	89.28	119	102.63	137.15
ΔTR(birr)	0.00	30.3	13.35	48.85
ΔNR(birr)	0.00	29.72	-16.37	34.52
ΔTVC	0.00	0.87	-0.72	0.58
MRR	-	34.13	22.73	59.51
Chicks sale/feed cost	3.68	4.18	3.92	4.49
Feed cost/Chick sale ratio	0.27	0.24	0.25	0.22

Meat price @ Rs 150 per kg of live weight, Feed price @ Rs 14.5 per kg; Net Return, ΔTVC = Change in total Variable Cost, ΔNR = Change in Net Return; MRR = Marginal Rate of Return; T1, T2, T3 and T4 = 0, 300, 600 and 1200 FTU/Kg, respectively, TR= Total Return, NR = net return

Table 7. Effect of adding phytase on the mortality percentage of broilers

Parameters (%)	Treatment					
	T1	T2	T3	T4	SEM	P value
Mortality of the starter	2.95	2.93	0.73	0.3333	0.73333	0.09
Mortality of finisher	0.733	2.22	0.733	0.000	0.2357	0.08
Mortality of the entire	3.63	5.12	1.42	0.31	0.01	0.6775

Meat price @ Rs 150 per kg of live weight, Feed price @ Rs 14.5 per kg.T1, T2, T3, and T4 = 0, 300, 600, and 1200 FTU/Kg, respectively, SEM= Standard error of the mean

CONCLUSION

There were significant differences ($P<0.05$) among treatments for Packed cell volume (PCV), Hb, and WBC. However, there were no significant ($P>0.05$) differences in RBC count, MCV, MCH, or MCHC of broilers fed the diet supplemented with enzymes. Total protein, albumin, and globulin values did not increase significantly ($P>0.05$) in the supplemented groups compared with the T1 control group throughout the growth period. The calcium concentration ranged from 1-1.49% and 0.75-0.89% in starter and finisher diets, respectively, whereas phosphorus ranged from 0.37-0.52% and 0.28-0.44% in starter and finisher diets, respectively, and it is within the range recommended in the broiler ration (NRC, 1994). According to the present partial budget analysis, broiler chickens in T4 yielded higher profits than those in T2, T3, and T1, respectively. Using phytase may be more profitable than feeding control. No significant difference ($P>0.05$) in mortality percentage between the treatments in all phases.

AUTHOR CONTRIBUTION STATEMENT

Mengistu Lemma came up with the idea and wrote the paper. Negasi Ameha, Meseret Girma, and Ali Beker, who also authorized the final version for publication, critically edited the manuscript for key intellectual content.

CONFLICT OF INTERESTS

The authors state that they have no conflicts of interest.

ACKNOWLEDGMENTS

The authors thank the Ethiopian Ministry of Education, Wachemo University, and Haramaya University Research Affairs Office for the funds allocated to implement the research project. A memorable impression is of the Haramaya University biotechnology laboratory for phytase extraction, and of the poultry farm workers for their kind support with critical research inputs and for their help on the farm and in the laboratory.

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