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The impact of adding phytase to plant protein concentration in broiler diets

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Abstract
The objective of this study was to evaluate the nutritional value of phytase supplementation to plant protein diet on broiler performances. A total number of 200 un-sexed one-day old Ross commercial broilers were distributed randomly and divided equally into four treatment groups nearly equal in average live body weight. Each treatment was represented by 50 chicks in five replicates of 10 chicks each and kept under similar hygienic, environmental and management conditions in flour pens. Two different basal diets were formulated, the first was containing fish and meat meal (animal diet, AD) and fed to the first treatment (D1), another one was containing soybean and corn gluten meal as a source of protein (plant diet, PD) and fed to the second treatment group (D2). The third and fourth treatment groups (D3 and D4) were fed the second diet (plant diet) with phytase (*Citrobacter phytase*) addition (500 and 1000 FTU/kg, for each treatment, PD+Ph1 and PD+Ph2, respectively). The experimental period lasted forty two days. All diets were formulated to be isocaloric and isonitrogenous in each of the experimental periods (starting, growing and finishing periods) according to the strain catalog recommendation.
The Results of this study clearly indicated that, at the end of starting period (21 d of age), both levels of phytase addition to PD increased ($P \leq 0.01$) broiler (BW) and (BWG) for both (D3 and D4 than those fed (D2) or fed (D1). However, at the end of the experiment periods (42 d of age), chicks fed (D4) recorded the highest BW and BWG (2123.89 and 2070.04 g, respectively) comparing with the other treatments. Supplementation broiler plant protein diets (D3 and D4) with phytase during growing and finishing periods (22-42 d of age) and for all excremental periods (0 - 42 d of age), improved ($P \leq 0.01$) (FCR) comparing with those fed PD.
The addition of both levels of photos to PD ($P \leq 0.01$) increase relative weights of eviscerated carcass, bursa of Fabricius, spleen and pancreas. Relative weights of abdominal fat dissected were ($P \leq 0.01$) decreased for the broiler fed (D3 and D4) comparing with those fed (D1 and D2). Chemical composition of chicks meat indicated that phytase supplementation to PD (D3 and D4) ($P \leq 0.01$) decreased ether extract percentages comparing with those fed AD or PD while, the crude protein percentages had the opposite trend. Addition of phytase to broiler PD ($P \leq 0.01$) decreases plasma total cholesterol, total lipid concentrations and broiler meat total cholesterol. Supplementation of phytase to PD ($P \leq 0.01$) increased digestibility coefficient values of crude protein, ether extract, crude fiber and nitrogen free extract compared with the digestibility values for the broiler fed PD.
In conclusion, the investigation clearly indicated that phytase supplementation to broiler plant diets has important role in improving broiler performances, nutrient digestibility and blood lipid profiles.

Keywords: Phytase, broiler performance, carcass traits, blood lipid profiles and economical efficiency

Introductions

Enzymes which are biological catalysts composed of amino acids with minerals and vitamins. The use of enzymes has many benefits in poultry diets include not only enhanced feed conversion and bird performance, but also reduced output of excreta problems of the environment.

No doubt that the supplementation of phytase enzyme has become an effective tool to bring improvements in the bioavailability of phosphorus present in feed stuffs and also to minimize the environmental pollution of phosphorus which animals excrete to the environment. Soybean meal (Glycine max) acts as a major food item for humans and animals because of its high beneficial health and nutritional values. It is an important dietary source of protein, fat, vitamins, minerals and fiber. Soybean also is a source of many other valuable biological active compounds such as Phyto estrogens, which has potentially high benefits for the health of human beings (Messina, 1999) [41]. Apart from this, there are other compounds which are present in soybean like phytate (anti nutritional factor) and inhibitors of trypsin that can act as negative nutritional factors and produces hindrance in protein digestibility and these chelated with other essential nutritional elements, including Ca, Fe, and Zn hence reducing their availability in gut (Liener, 1994; Hurrell, 2003) [37, 30]. There was a linear significant decrease in (LBW) at 38 days of age, (LBWG), (FI), crude protein conversion (CPC), performance index (PI), carcass weight after evisceration and dressing percentage with decreasing available phosphorus, (Ragab, *et al*. 2013) [48].

Phytases are the phosphor hydrolytic enzymes that are capable to start the stepwise removal of phosphate from the phytate. Due to soyabean high protein quality it is extensively used as a protein supplement in poultry feeds (Stahl *et al*., 2003; Lei *et al*., 1993; Boling *et al*., 2000) [58, 35, 11]. Different techniques and strategies have been employed to bring useful improvements in its nutritional value which normally includes mixing with corn, and supplementing it with limited amino acids (Liu *et al*., 1997) [38], supplementing with some enzymes or treating with some organic acids (Ravindram and Kornegay, 1993) [49]. Supplementations of exogenous phytases and carbohydrates increases the dietary utilization of all essential nutrients in the body which on other way would be lost to the animals and wasted to the environment. Furthermore, it is also well documented that benefits of phytase action are not restricted only to Ca and phosphorus release, but also includes a better absorption of trace minerals also.

The aim of this investigation is to evaluate the nutritional value of phytase supplementation to broiler plant diets on broiler performances. Since nutritionist nowadays minimize or eliminate the need for animal or fish meals in poultry diets.

Materials and Methods

Birds and management

The present study was carried out at Borg El-Arab of Animal production, research station belonging to Animal production, research institution (Doki), Egypt during the period from December 2021 to January 2022. This study was carried out to investigate the effect of phytase supplementation to plant protein diet on broiler performances. Two hundred un-sexed one-day old Ross commercial broilers were wing-banded, weighed and randomly distributed into four experimental groups. Each treatment was represented by 50 chicks in five replicates of 10 chicks each All chicks were reared under similar hygienic, environmental and management conditions in flour pens (1.5 m X 2 m) during starting, growing and finishing periods (0-21, 22-35 and 36-42 days of age, respectively).

Experimental diets

Two different basal diets were formulated, the first was containing fish and meat meal (animal diet, AD) and fed to the first treatment (D1), the other one was containing soybean and corn gluten meal as a source of protein (plant diet, PD) and fed to the second treatment group (D2). The third and fourth treatment groups (D3 and D4) were fed the second diet (PD) with Phytase addition (500 and 1000 FTU/kg diet, PD+Ph1 and PD+Ph2, for each treatment, respectively). All diets were formulated to be isocaloric and isonitrogenous in each of the experimental periods, starting (0-21 days of age), growing (22-35 d of age) and finishing (36-42 d of age), according to the strain catalog recommendation Table (1).

Table 1: Composition and the nutritive value of the experimental diets (kg / ton).

Ingredients	Starter Diets 0-21 d		Grower Diets 21-35 d		Finisher Diets 35-42 d	
	AD	PD	AD	PD	AD	PD
Yellow corn	633.00	570.20	691.00	626.40	762.00	674.20
Wheat bran	22.00	0.00	23.00	8.00	0.00	22.00
Soybean meal (48%)	152.00	313.00	100.00	261.00	60.00	150.00
Corn gluten (60%)	130.00	46.00	120.00	34.00	113.50	80.00
Fish meal (72%)	8.00	0.00	8.00	0.00	8.00	0.00
Meat meal (50%)	25.00	0.00	25.00	0.00	25.00	0.00
Calcium Carbonate	11.00	15.00	13.00	14.00	12.00	17.00
Dicalcium Phosphate	10.00	17.00	10.00	17.00	9.00	16.00
Premix*	3.00	3.00	3.00	3.00	3.00	3.00
Soy oil	0.00	30.00	0.00	30.00	0.00	30.00
Table Salt (Na Cl)	2.30	3.00	2.40	3.00	2.50	3.00
DL. Methionine	0.10	1.20	0.40	1.5	0.40	1.00
L. Lysine	3.00	0.50	3.10	1.00	3.00	2.70
Tetracycline	0.10	0.10	0.10	0.10	0.10	0.10
Coxistate	1.00	1.00	1.00	1.00	1.00	1.00
Total (Kg)	1005.00	1000	1000.00	1000.00	999.5	1000.00
Chemical analysis						
Crude protein %	22.65	22.61	20.00	20.00	18.00	18.00
ME k cal / kg	3060	3060	3100	3100	3200	3200
Ether Extract %	3.41	5.64	3.57	5.81	3.69	6.09
Crude fiber %	2.74	3.11	2.53	2.97	2.19	2.63
Calcium %	1.00	1.00	1.00	1.00	0.96	0.96
P. (available) %	0.46	0.46	0.45	0.45	0.42	0.42
Lysine %	1.01	1.05	0.98	0.98	0.92	0.92
Methionine + cysteine %	0.81	0.81	0.77	0.77	0.72	0.72

*Provided the following per kg of diet: Vit. A, 1200 IU; Vit. D. 3000 IU; Vit. E, 100 IU; Vit. C, 3 mg; Vit. K, 4 mg; VitB1, 3 mg; Vit B2, 3 mg; Vit B6, 5 mg; Vit B12, 0.03 mg; Bantothinic acid, 15 mg; Folic acid, 2 mg; Biotin, 0.20 mg; Cobalt, 0.05 mg; Copper, 10 mg; Iodin, 50 mg; Manganese, 90 mg; Selenium, 0.20 mg and Zinc, 70 mg.

The four experimental treatments and diets were as follows:
D1:- fed animal diet (AD) (without phytase supplementation).
D2:- fed plant diet, (PD) (without phytase supplementation).
D3:- fed PD+500 FTU phytase/kg diet, (PD+Ph1).
D4:- fed PD+1000 FTU phytase/kg diet, (PD+Ph2).
Feed and water were provided *ad-libitum*, meanwhile birds were allotted with 24 hr light during the experimental periods. Vaccination and medical program were done under supervision of a veterinarian.

Studied traits:
Body weight (BW) and feed intake (FI) were recorded weekly and at the end of the experiment (42 day of age). Body weight gain (BWG) and feed conversion ratio (FCR) were calculated for each period. At the end of the experiment, economic efficiency (EE) and relative economic efficiency (REE) were calculated according to input-output analysis data.

Slaughter test
At the end of the experimental period (42 d of age), ten chicks from each treatment (one male and one female from each replicate) were randomly selected and slaughtered. Data of carcass traits were calculated as g/100 g of live body weight. Skinless-boneless pooled samples from breast and thigh muscles were chemically analyzed for crude protein (CP), ether extract (EE), and ash, according to AOAC (2012) and the values were expressed on a DM basis.

Blood constituents
At the time of slaughter, two blood samples from each chick were collected, one of heparinized test tubes and another without heparin. Samples were centrifuged at 3500 rpm for 15 minutes to obtain plasma or serum. Plasma samples were assigned for determination of total protein (Peters, 1968) [46], total cholesterol (Ellefson and Caraway, 1976) [19], total lipids (Bucolo and David, 1973) [13], createnine (Husdan and Rapaport, 1968) [31], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity (Reitman and Frankel, 1957) [53]. Cholesterol level in skinless-boneless pooled samples from breast and thigh muscles was extracted and determined according to Folch *et al*. (1957) [22] and Charles and Richmond (1974) [14]. Serum samples were used for determination of malonaldhyde (MDA) (Yagi, 1984) [60]. The procedures were similar as described by available commercial kits (Bio-Diagonosis Co., Cairo, Egypt).

Nutrient digestibility
At the end of the experiment, five chicks per treatment group (one diet from each replicate) were randomly taken to evaluate the digestibility percentages of nutrients in the experimental diets. The procedure described by Jakobsen *et al*. (1960) [32] was used for determination fecal protein excreta samples. Digestion coefficients of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE) were calculated (Fraps, 1946) [23].

Statistical analysis
Data obtained were statistically analyzed using the General linear model of SAS (2004) [56]. Differences among treatment means were estimated by Duncan's multiple range test (Duncan, 1955) [16]. One way analysis model:

$$X_{ij} = \mu + T_i + e_{ij}$$

Where: X_{ij} = the individual observation.
μ = the overall mean.
T_i = Treatments (i = 1, 2, 3 and 4).
e_{ij} = Experimental error.

Results and Discussion
Growth performance
Results Table (2 and 3) showed (P<0.01) effect on (BW) between different treatments at the end of experimental periods, also, (BWG) was (P<0.01) effect during the starting period (0-21d of age) and for the overall mean of the experiment (0-42 d of age). At the end of starting period (21 d of age), the results clearly indicated that BW and BWG for the chicks fed (D3 and D4) were surpassed than those fed (D2) or fed (D1).

Table 2: Effect of Phytase supplementation on broiler body weight (BW), (Means (SE)

Treatments	Initial BW (g/chick)	BW (g/chick) 21d	BW (g/chick) 42d
D1	55.18±4.60	733.62±15.44^{a}	2082.22±48.09^{ab}
D2	55.90±4.43	691.61±11.36^{b}	2030.49±67.43^{b}
D3	56.26±3.81	747.92±9.32^{a}	2069.33±26.63^{ab}
D4	53.85±1.40	750.96±7.01^{a}	2123.89±22.18^{a}
Significant	NS	**	**

Means within the same column with different superscript are significantly different.
** Significantly, at 0.01 NS: No significant

Table 3: Effect of Phytase supplementation on broiler body weight gain (BWG), (Means±SE)

Treatments	BWG (g/chick) 0-21	BWG (g/chick) 22-42wk	BWG (g/chick) 0-42d
D1	678.44±15.17^{b}	1348.60±44.19	2027.04±49.94^{ab}
D2	635.71±7.58^{c}	1338.83±71.13	1974.54±70.14^{b}
D3	691.66±8.91^{ab}	1321.11±30.41	2013.07±27.13^{ab}
D4	697.11±7.55^{a}	1372.93±821.58	2070.04±22.91^{a}
Significant	**	NS	**

Means within the same column with different superscript are significantly different.
** Significantly at 0.01 NS: No significant

The lowest BW and BWG (691.61 and 635.71 g) were recorded for chicks fed (D2), at the aforementioned period (21 d of age). However, the BW and BWG for broiler fed (D1) was significantly increased as compeer to those fed

(D2) at the same period (21 d of age). On the other hand, BWG for all treatments, during growing and finishing period (22-42 d of age), were almost equally and insignificantly affected by phytase supplementation. However, at the end of the experiment (42 d of age), chicks fed (D4) recorded the highest BW and BWG (2123.89 and 2070.04 g, respectively) comparing with the other treatments and the BW and BWG for the chicks fed (D2) which recorded the lowest value (2030.49 and 1974.54 g, respectively), while broiler fed (D3) was almost equal with those fed (D1). The insignificant differences between final BW and BWG for the broiler fed AD or PD at the end of the experimental period (42 d of age) were agreements with the finding of Khaled (2001) [33], Arafa *et al*. (2001) [7] and Abou El-Wava (2003) [5] that broiler fed on plant protein diets showed no significant differences in growth performance compared to those fed animal protein diets. Also, increasing BW and BWG for the broiler fed AD comparing to those fed AD during the growing period (0-21 d of age) was agreement with that reported by Herstad (1981) [28] and Abdel-Salam *et al*. (1985) [3] who reported that including fish meal in growing chick diets significantly improved body weight compared with those obtained from plant protein diets. The positive effects of phytase supplementation on growth performances clearly indicated that it had an important role in the early period of broiler growth. Our results were supported by Alshamiri *et al* (2021) broiler fed basal diet supplemented with 1500 FTU/Kg caused a significant ($P \leq 0.05$) improvement in (BW) and (BWG) as compared with broiler fed on vassal diet. Besides, Baloch *et al* (2021) [10] showed that the maximum chicken body weight was noted in group of broiler fed basal diet plus phytase 0.075 g/kg compared to broiler fed basal diet without phytase or with 0.05 g/kg and 0.025 g/kg. Also, When the inclusion of 2,500 FYT/kg of feed increased WG from 0 to 21 d ($P \leq 0.05$) by 3.11% compared to broiler chickens fed diets containing 1,000 FYT/kg, (Sens *et al* 2021). Similar results were observed by Hao *et al* (2017) [25] who used two doses of phytase (300 and 500 IU) in broiler diet. Also, Soliman and Al- Youssef (2020) [44] found that the supplementation of microbial phytase (500 FTU/Kg) with 1 or 2% citric acid into the control diet ($P \leq 0.05$) improved (BWG) of chicks. As well as, Khan *et al* (2019) [34] who observed significantly higher differences ($P<0.05$) in FCR, live body weight in experimental groups treated with phytase enzyme compared to the control group. In our study gradually increase in the body weight gain was observed in D3 and D4 as compared to control. Hassan *et al.* (2011) [27] showed that addition of phytase 375 U/Kg to the basal diet (deficient in lysine (90% of the requirements)) significantly improved BWG. Narasimha *et al*. (2013) [45] and they reported that the body weight gain in broiler chickens fed with basal diet added with phytase was significantly ($P<0.01$) higher. The mode of action of phytase in improving body weight gain may be due to release of phosphorus, (Ebrahimnezhad *et al*. 2008) [17]. Compared with the broiler fed diet without phytase Wang *et al* (2013) [59] the diet supplemented phytase improved the weight and weight gain of broilers ($P<0.05$), from 4-weeks to 6-weeks, the weight of broiler-diet 0.02 and 0.03% phytase were higher than that of 0.01% phytase broilers-diet ($P<0.05$). Moreover, the significant ($P<0.01$) increase on BW and BWG were compatible with the improvement of nutrient digestibility coefficient percentages (Table 10) and associated with significant ($P<0.01$) increase of CP and EE percentages in broiler meat (Table 6). Nagata *et al*. (2011) [43] reported that the protein and energy levels in diets containing phytase influenced feed intake, weight gain and feed conversion rate of the broilers. It has been shown that phytase could improve the availability of phytate P, total P, some other minerals and amino acids. Rutherford *et al*. (2012) [54], using a low-phosphorus corn-soybean meal diet, reported that the inclusion of a novel microbial phytase into the diet greatly increased ileal phytase P and total P absorption and ileal threonine, tyrosine, and histidine. Lelis *et al*. (2012) [36] reported that the diets containing phytase could release protein, energy, amino acid and digestive enzymes which were chelated with phytic acid. These reasons might improve the growth performance of broilers. According to the above results, the best addition of phytase supplementation in the diets was 0.02%. El-Nagmy *et al*. (2004) [20] found that phytase supplementation (0, 400, 800 U/kg) improved BWG of broiler chickens fed diets containing two levels of protein (23/21% and 20/18% CP) during the growing/finishing period.

Feed intake and fed conversion

The amount of (FI) was insignificantly different between broiler treatment groups fed animal or plant diets with or without phytase supplementation, during the starting period (0-21 d of age), Table 4. Birds fed the supplemented control diet with phytase 1500 FTU had higher feed consumption than those control groups, (Metwally *et al* 2020) [40].

Table 4: Effect of Phytase supplementation on broiler feed intake (FI), (Means±SE)

Treatments	FI (g/chick)		
	0-21d	**22-42d**	**0-42d**
D1	907.34±39.57	2721.03±56.92[ab]	3628.57±79.23[ab]
D2	905.00±39.21	2751.22±66.03[a]	3656.03±62.15[a]
D3	880.68±22.24	2686.82±35.14[b]	3567.50±42.36[b]
D4	893.88±16.81	2686.54±25.25[b]	3580.42±24.57[b]
Significant	NS	**	**

Means within the same column with different superscript are significantly different.
** Significantly at 0.01 NS: No significant

However, during the growing periods (0–21 d of age) and finishing periods (22-42 d of age) and for all excremental periods (0-42 d of age), broiler (D3 and D4) were ($P<0.01$) consumed the lowest amount of feed without insignificant differences with those fed (D1). On the other hand, the broiler groups fed (D2) consumed the highest amount of feed comparing with the other treatments, but it's statistically equal with those fed (D1), Table 4. Motawe *et al*., (2012) [42] showed that phytase supplementation FI was not affected by phytase supplementation at starter/grower period. Edwin *et al*., (2004) [18], Hanna *et al*., (2008) [26] and Cowieson and Ravindran (2008) [15] a reported no effect of exogenous enzyme supplementation on feed intake in broilers. The improvement in feed conversion can be related to increase protein digestibility and retention reported in the current study (Table 10). In this connect Baloch *et al* (2021) [10] said that the feed conversion ratio was ($P<0.05$) higher in broiler fed basal diet provided with phytase 0.075 g/Kg, followed by those added with 0.025 and 0.05 g/Kg phytase, respectively when compared with the control group. While, feed intake was minimum in broiler fed basal diet provided with phytase 0.075 g/Kg. Feed intake and feed conversion ratio significantly increased due to adding 1% citric acid and microbial phytase levels into control diet, (Soliman and Al-Youssef 2020) [44]. Supplementation of NSP enzymes, synbiotics and phytase alone or in combination had a significant effect on feed intake, (Narasimha *et al*. 2013) [45]. Supplementation broiler plant protein diet (D3 and D4) with phytase during starting (0-21 d of age), growing and finishing periods (22-42 d of age) and for all excremental periods (0-42 d of age), improved significantly FCR comparing with those fed D2 and had the best FCR Table (5).

Table 5: Effect of Phytase supplementation on feed conversion ratio (FCR), (Means±SE)

Treatments	FCR (g feed/g Gain)		
	0-21 d	22-42 d	0-42 d
D1	1.34±0.07[ab]	2.03±0.04[ab]	1.80±0.07[ab]
D2	1.38±0.07[a]	2.08±0.12[a]	1.85±0.08[a]
D3	1.27±0.03[b]	2.04±0.06[ab]	1.77±0.03[ab]
D4	1.28±0.02[b]	1.96±0.04[b]	1.73±0.02[b]
Significant	**	*	**

Means within the same column with different superscript are significantly different.
** Significantly at 0.01

However, results indicated that FCR for broiler fed (D3 and D4) statistically equal with those (D1) during the aforementioned periods. During the growing and finishing periods (22-42 d of age) broiler fed the highest level of phytase (D4) recorded the best FCR (2.12) than the other treatment groups, while those fed (D3) was statistically equal with those fed (D1). Generally, the worst FCR was recorded for the broiler fed (D2) during the all experimental periods Table (5). However, Broch *et al* (2021) [12] indicated that dietary 500, 1000, or 1500 FTU kg^{-1} the phytase did not affect (P>0.05) on vroiler FI and FCR during the tested period (1 to 42 days old). The insignificant differences between of FI and FCR for the broiler fed AD or PD were agreements with the results of Khaled (2001) [23] who found that feeding broiler chicks on diets containing animal protein sources has no superiority over diets containing vegetable protein sources in terms of feed conversion. However, those results disagreed with that reported by Herstad (1981) [28] and Abdel-Salam *et al*. (1985) [3] who reported that including fish meal in growing chick diets significantly improved feed conversion compared with those obtained from plant protein diets.

Carcass traits

Result Tables (6 and 7) show the effect of phytase supplementation to PD on some relative broiler carcass traits (expressed as g/100 g of LBW) at the end of the experiment (42 d of age). Generally, eviscerated carcass relative weights were (P≤0.01) increase for the broiler fed PD with or without phytase supplementation as compared to those fed (D1), Table (6). On the other hand, addition, both levels of phytase to PD (P≤0.01) increase relative eviscerated carcass weights from 69.68 g/100g LBW (D2) to 72.19 and 72.47 g/100g LBW (D3 and D4, respectively). The highest relative eviscerated carcass weight (72.47 g/100g LBW) was recorded for the broiler-diet (D4) and the lowest (67.73 g/100g LBW) was recorded for the broiler fed (D1) Table (6).

Table 6: Effect of Phytase supplementation on relative weights of carcass, giblets, abdominal fat and chick's meat analysis for crude protein and ether extract percentages (Means±SE).

Treatments	Carcass g/100g LBW	Giblets g/100g LBW	Abdominal Fat g/100g LBW	Chicks meat analysis	
				Crude protein %	Ether extract %
D1	67.73±2.45[c]	5.53±0.19	29.78±5.21[a]	21.85±0.35[c]	3.89±0.19[a]
D2	69.68±1.18[b]	5.12±0.13	27.60±7.77[a]	21.90±0.25[c]	3.40±0.03[b]
D3	72.19±0.73[a]	5.46±0.31	17.15±2.79[b]	22.19±0.24[b]	3.19±0.06[c]
D4	72.47±0.43[a]	5.64±0.11	18.88±4.72[b]	22.95±0.28[a]	3.09±0.02[c]
Significant	**	NS	**	**	**

Means within the same column with different superscript are significantly different.
** Significantly at 0.01 NS: No significant

Total giblets (heart gizzard and liver) relative weights were not significantly different between treatments. Relative weights of abdominal fat dissected were (P≤0.01) decreased for the broiler fed (D3 and D4), 17.15 and 18.88 g/100 g LBW, respectively, comparing with those fed (D1 and D2, 29.78 and 27.60 g/100g LBW, respectively) Table (6).
Chemical composition of chicks meat indicated that phytase supplementation to (D3 and D4) (P≤0.01) increased crude protein percentages comparing with those fed (D1 and D2). The highest crude protein meat percentage (22.95 %) was recorded for broiler fed (D4) and the lowest percentages were recorded for those fed (D1 and D2) 21.85 and 21.95 %, respectively. Generally, ether extract meat percentages were (P≤0.01) decreased for broiler fed (D2 and D3, or D4) comparing with those fed (D1). On the other hand, addition, both levels of phytase (500 and 1000 FTU/kg of feed) to plant diet (D3 and D4) (P≤0.01) decreased broiler meat ether extract percentages comparing with those fed (D2) or broiler fed (D1) Table (6). Our results associated with Alshamiri *et al* (2021) showed that there were significant (P≤0.05) differences in live body weight, hot and cold carcass. The weights of the different parts (breast, whole leg, thigh, drumstick and wing) and dressing percentage, showed significant (P≤0.05) differences between the broiler fed basal diet supplemented with 1500 FTU/Kg. These findings are in accordance with that of Abo Omar, and Sabha (2009) [4], who showed that phytase supplementation significantly (P≤0.05), increased the percentages of most carcass values of diets deficient in phosphorus. However, Baloch *et al* (2021) [10] proved that there were no visible differentiation between carcass of broiler fed diets containing (0, 0.05, 0.075 and 0.025 g/kg) phytase enzyme provided in broiler feed except for spleen, lung, gizzard their weight was higher significantly in chicks provided phytase enzyme compared with broiler fed basal diet where the broilers were not provided phyase enzyme.
No effect was detected on breast muscle color, relative weight of the liver, spleen, bursa, breast muscle, abdominal

fat, and gizzard associated to phytase supplementation, Hao *et al* 2017) [25]. Khan *et al* (2019) [34] revealed that dietary supplementation of phytase enzyme has (P<0.05) effect of weights of small intestine without digesta and increased weight of liver, spleen in group of broiler fed basal diets containing 1500FTU phytase per kg of feed as compared to control group. However, no significant effect on weight of gizzard and relative live weights was observed in groups treated with phytase enzyme at the levels of 500FTU, 1000FTU, and 1500FTU per kg of feed respectively when compared to control. Relative weights of bursa of Fabricius, spleen and pancreas were (P≤0.01) increase due to adding the both levels of phytase to broiler PD comparing with the relative weights recorded for broiler fed AD or PD Table (7). Salem *et al*. (2003) [55] found that phytase at 600 U/kg in broiler diets containing different levels of P had no effect on carcass yield at 4 wk of age, however it increased carcass yield at 7 wk of age. Also, Abd El-Samee (2002) [2] reported that phytase supplementation at 750 U/kg to broiler diets did not significantly affect carcass characteristics and meat analysis of 7 wk of age broiler chicks. Qota *et al*. (2002) [47] found that the phytase addition of 500 U/kg to broiler diets contained 10% linseed cake did not significantly affect dressing, liver, gizzard, heart, giblets, and pancreas percentages, as well as meat physical characteristics such as pH value; tenderness, WHC and color intensity. Phytase increased percentage CP of meat, while it decreased fat and DM content of meat.

Table 7: Effect of Phytase supplementation on relative weights of bursa, spleen and pancreas (Means±SE)

Treatments	Bursa g/100g LBW	Spleen g/100g LBW	Pancreas g/100g LBW
D1	0.086±0.02^{b}	0.192±0.02^{bc}	0.204±0.02^{b}
D2	0.085±0.02^{b}	0.175±0.05^{c}	0.209±0.01^{b}
D3	0.094±0.01^{a}	0.206±0.04^{a}	0.241±0.01^{a}
D4	0.096±0.01^{a}	0.208±0.01^{a}	0.251±0.01^{a}
Significant	**	**	**

Means within the same column with different superscript are significantly different.
** Significantly at 0.01 NS: No significant

The stimulatory effect of the phytase treatment on growth and development of the lymphoid organ, bursa and spleen, may be enhancing the immune system. The bursa of Fabricius is a key lymphoid organ that is responsible for the development and maturation of B-lymphocytes.

Blood constituents

Results presented in Tables (8 and 9) shown that addition of phytase to broiler PD (P≤0.01) decrease total cholesterol and lipid concentration. Also, the concentration of total broiler meat cholesterol was (P≤0.01) decreased for the broiler fed PD with or without phytase supplementation compared with those fed (D1). Total protein and transaminase enzyme activities ALT and AST concentrations were insignificantly different between treatments. The value of serum (MDA), a lipid peroxidation product, is a marker for oxidative stress and is related to all oxidative factors was (P≤0.01) decreased by increasing phytase supplementation to PD. The highest (P≤0.01) serum MDA concentration was recorded for broiler fed AD while the lowest (P≤0.01) concentration was recorded for the broiler fed the highest level (D4). Similler Rawia *et al* (2020) [52] recorded that phytase supplementation had a significant decreasing effect on broiler chicken's cholesterol, triglyceride and low density lipoprotein values. Attia *et al* (2020) compared between *Aspergillus niger* phytase and an Escherichia coli phytase feed additive in the plant broiler diet and reported that the use of bacterial phytase have (P<0.01) increased in total protein and globulin but decreased (P<0.05) the plasma cholesterol in comparison to fungal phytase.

Table 8: Effect of Phytase supplementation on plasma total cholesterol (T.Ch), lipids (T. Lip), protein (T. Protein) and serum malonaldhyde (MDA) concentration (Means±SE)

Treatments	T. Ch. (mg/dl)	T. Lip (mg/dl)	T. Protein (g/dl)	MDA (mg/ml)
D1	86.41±0.79^{a}	4.322±0.13^{a}	5.46±0.40	5.29±0.04^{a}
D2	83.39±1.57^{b}	3.965±0.09^{b}	5.24±0.46	4.14±0.06^{b}
D3	81.99±0.27^{c}	3.748±0.15^{c}	5.20±0.09	3.91±0.07^{c}
D4	80.08±0.22^{c}	3.701±0.03^{c}	5.23±0.05	3.77±0.03^{d}
Significant	**	**	NS	**

Means within the same column with different superscript are significantly different.
** Significantly at 0.01 NS: No significant

Table 9: Effect of Phytase supplementation on plasma transaminase enzyme activities (AST and ALT) concentration and the total cholesterol content of broiler meat (Means±SE)

Treatments	ALT U/L	AST U/L	T. Ch. Of meat (mg/100g)
D1	16.75±0.19	128.63±0.41	1.48±0.05^{a}
D2	16.67±0.29	129.25±0.37	1.39±0.05^{b}
D3	16.52±0.21	128.71±0.63	1.40±0.01^{b}
D4	17.09±0.08	129.18±0.16	1.40±0.01^{b}
Significant	NS	NS	**

Means within the same column with different superscript are significantly different.
** Significantly at 0.01 NS: No significant

While, Huff *et al*., (1998) [29] revealed that diet of broiler chicks supplemented with 500 FTU/kg of phytase had not significant effects on triglyceride levels. Cholesterol of birds fed Phytase enzyme (1500 FTU) were higher than non-fed Phytase enzyme levels (0 FTU), (Metwally *et al* 2020) [40]. However, Phytase supplementation tends to decrease significantly the activity of the plasma content of alkaline phosphatase, (ALP) but the plasma content of Ca and P was not significantly affected by phytase supplementation (Ghazalah and Alsaady, 2008) [24].

Nutrient digestibility

The effects of feeding broiler of different diets on nutrient digestibility percentages are presented in Table (10). It is worthy noted that supplementation of phytase to PD ($P\leq0.01$) increased digestibility percentages of (CP), (EE), (CF) and (NFE) compared with the digestibility values for the broiler fed (D2). Digestibility percentages of DM for the broiler fed PD supplemented with both levels (D3 and D4) were ($P\leq0.01$) increased than the DM digestibility for the broiler fed (D2) and its almost equal with the DM digestibility for the broiler fed (D1). Also, digestibility percentages of CP for the broiler fed (D3 and D4) ($P\leq0.01$) increased than the CP digestibility for the broiler fed (D1) or (D2).

Table 10: Effect of Phytase supplementation on broiler digestibility coefficients percentages of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen free extract (NFE) (Means±SE)

Treatments	DM %	CP %	EE %	CF %	NFE %
D1	84.48±0.77[a]	86.91±0.98[b]	72.39±0.38[c]	42.76±0.26[c]	73.46±0.81[a]
D2	81.39±0.93[b]	82.15±0.83[c]	70.07±0.52[d]	40.44±0.40[d]	69.69±0.52[c]
D3	83.39±0.51[a]	90.23±0.55[a]	73.63±0.81[b]	44.21±0.24[b]	72.83±0.54[b]
D4	82.41±0.46[ab]	90.88±0.30[a]	74.85±0.24[a]	48.03±0.49[a]	73.94±0.21[a]
Significant	**	**	**	**	**

Means within the same column with different superscript are significantly different.
** Significantly at 0.01

Digestibility percentages of EE, CF and NFE were ($P<0.01$) increased for the broiler fed (D4) and the lowest percentages were observed for those fed (D2). The improvement of digestibility percentages for all nutrients associated with the increasing of BW and BWG results (Tables 2 and 3) and the improvement of feed utilization which improves the FCR (Tables 4) for the broilers fed PD+Phytase at both levels (500 and 1000 FTU. Kg diet). Effects of phytase enzyme supplementation on feed nutrients digestibility were investigated by some experiments. Sebastian, *et al*., (1996) [57] reported that supplementation of phytase significantly improved of ileal digestibility of crude fat. Phytase may also improve the utilization of protein, amino acid and apparent metabolic energy of the broiler diets supplemented with phytase enzyme (Ravindran, *et al*., 2000) [51]. As stated by Beeson *et al*. (2017), greater concentrations of phytase in the diet can intensify the dephosphorylation of phytate, contributing to the elimination of IP6 and lesser phytate esters (IP5-IP1) from the tract and therefore increasing the availability of the previously phytate-bound nutrients, in addition to improving the overall nutrient solubility as the antinutritional effects caused by the presence of phytate are hindered. Also, Zobac *et al*. (2004) [62] observed an improvement in digestibility of nitrogen by adding lactic acid and phytase into low available phosphorus broiler diets. The improvement in protein utilization and retention by adding phytase may be related to its effect on reducing nitrogen-phytate complex, which inhibits protein digestion (Abd El-Hack *et al*., 2018) [1]. However, Hao *et al* (2017) [25] reported that the phytase supplementation did not effect on nutrient digestibility (N, Ca, and P) during the experiment. A further confirmation is that the use of phytase has a positive impact on CP digestion. As known, phytase has a positive effect on protein digestibility as phytate can bind an amount of free amino acids, such as lysine, (Ravindran *et al* (1999) [50]. The inclusion of phytase to proiler diet at (1,734; 788; 1,204; and 1,255 FTU) produced the highest values for digestibility, A quadratic effect for Ether extract digestibility coefficient, mineral matter digestibility coefficient, Apparent metabolizable energy, and Nitrogen-corrected apparent metabolizable energy) was observed, (Fernandes, *et al* 2019) [21]. Phytase supplementation may improve the utilization of other nutrients. (DM) digestibility of broiler chicks increased when phytase was increasingly added from 250 to 2500 FTU/kg diet compared with basal diet containing 0.19% NPP without phytase (Zhang *et al*., 2000) [61]. Attia *et al*. (2002) [8] reported that phytase supplementation at 1000 FTU/kg in broiler diets increased the digestibility of CF, which may provide additional advantageous effective from hydrolysis of cell walls. Thus, an increase in apparent metabolizable energy value of foodstuffs may be noted, owing to the slightly higher apparent digestibility of nitrogen free extract and ether extract (EE). Abd-Elsamee (2002) reported that the digestibility of DM, crude protein (CP), EE and NFE as well as nitrogen retention were improved with the addition of microbial phytase (750 FTU/kg of diet) in broiler diets. Also, supplementation of microbial phytase to the diets fed to laying hens substantially improved the digestibility of nutrients particularly CP and EE. Phytase supplementation increased the digestibility of amino acids in layer diets (Liu *et al*., 2007) [39]. Increased protein utilization and amino acid digestibility by phytase may partly be mediated through reduced endogenous loss of amino acids (Cowieson and Ravindran, 2008) [15]. In conclusion, phytase is very effective in improving the digestibility of different feed ingredients plant protein rather than animal protein.

Conclusion

The investigation clearly indicated that phytase supplementation has an important role in improving broiler performances, blood lipid profiles and increasing the digestibility for broiler fed plant protein diets.

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