

EFFECT OF PHYTASE ENZYME AND CITRIC ACID ON PRODUCTIVE PERFORMANCE, NUTRIENT RETENTION AND TIBIA BONE OF BROILER CHICKS FED LOW AVAILABLE PHOSPHORUS DIET

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SUMMARY

The study investigate the effect of feeding low available phosphorus (AP) diet supplemented with three levels of microbial phytase (MP) and /or three levels of citric acid (CA) on broiler performance, tibia bone and nutrient retention. Four hundred day- old Ross broiler chicks were randomly allocated into ten treated groups, with four replicates of 10 birds each. The chicks were fed ten different diets as normal phosphorus 0.45% AP (positive control, PC) and nine groups with phosphorus deficient diets 0.25% AP (negative control, NC) supplemented with three CA levels as 0%, 1% or 2% and / or MP levels 0, 500 and 1000 FTU/Kg. The results indicated that supplementation of MP and /or CA into NC diet significantly improved body gain of chicks. Feed intake significantly increased due to adding 1% CA and MP levels into NC diet. Feed conversion ratio improved due to supplementing MP or CA into NC diet. There is no significant effect on tibia width and breaking strength by adding MP and/or CA into NC diet. Adding MP into NC diet significantly improved tibia length, weight, and ash content while, citric acid affected significantly at the first stage of growth only. Blood phosphorus increased significantly by supplementing MP into NC diet at the 5th week of age, while blood calcium was not affected significantly by MP and/or CA. Tibia calcium of chicks fed NC diet was higher than those fed PC diet or NC diet enriched with MP and/or CA. Tibia phosphorus did not response clearly for adding MP or CA into NC diet. Nitrogen and calcium retention of chicks fed NC were significantly lower than those fed PC or NC diet supplemented with 1000 FTU/kg MP and CA. Phosphorus retention of chicks fed NC diet was significantly similar for those fed PC or NC diets supplemented with MP and/or CA. It can be concluded that adding MP at 500 FTU/kg level with 1% CA into NC diet was effectiveness to improve body weight gain and tibia bone characteristics as like as those fed PC or NC diet enriched with 1000 FTU/kg MP.

Keywords: *Broiler performance, phosphorus level, blood analysis, bone measurements and nutrient utilization.*

INTRODUCTION

Corn and soy are commonly used in broiler diets where two thirds of phosphorus content is in the form of phytate which is unavailable for poultry due to insufficient endogenous phytase enzyme (Woyengo *et al.* 2010; Deepa *et al.*, 2011). Phosphorus represents an essential element for growth and bone formation of broiler chicks. As well, phosphorus is considered one of the most expensive feed ingredients in broiler diets (Ismail *et al.*, 2011). Therefore, high percentage of unavailable plant phosphorus represents an economic problem due to addition of inorganic expensive phosphorus sources which increase the diets cost. Furthermore, excretion of unavailable phosphorus can result in environmental pollution Vieira *et al.*, (2018).

Supplementation of phytase into broiler diets established as a mean to increase plant phosphorus utilization, retention and to reduce the addition of expensive inorganic phosphorus in broiler diets (Cowieson *et al.*, 2006). As well, addition of phytase improves the efficiency of dietary protein and energy utilization and increases the performance characteristics of broiler chicks (Deepa *et al.*, 2011).

Intestinal pH is one of the several factors affecting the efficacy of exogenous phytase enzyme, which is most active at 2.5 to 5.5 pH (Abd El-Hack *et al.*, 2018). Therefore, the effectiveness of phytase may be enhanced by reducing intestinal pH with supplementation of organic acids (Nourmohammadi *et al.*, 2010) such as citric acid (El-Afifi *et al.*, 2001) which has been reported to improve the efficacy of phytase (Redcliffe *et al.*, 1998). Several studies indicated that citric acid has been used successfully as

feed additives into broiler diets to improve the digestibility and retention of dietary phosphorus, calcium and protein, due to its role in increasing the solubility of minerals and intestinal villi length, (El-Afifi *et al.*, 2001, Aydin *et al.*, 2010 and Soliman *et al.*, 2012). Furthermore, addition of citric acid into phosphorus deficient diets increase phytate-phosphorus utilization (Boling *et al.*, 2000) and enhances the efficiency of phytase (Redcliffe *et al.*, 1998). Previous studies (Brenes *et al.*, 2003; Hariharan & Gangadevi 2015) noted an interaction between citric acid and phytase enzyme in improving performance and mineral retention of broiler chicks. Therefore, citric acid may be useful to improve the efficacy of phytase enzyme and reduce the level of its supplementation into broiler diets. The current study aimed to determine the effect of adding citric acid and/or phytase enzyme into low available phosphorus (AP) diet on performance of broiler chicks. As well, the complimentary effect on tibia bone, minerals and nitrogen retention were included.

MATERIALS AND METHODS

Experimental Procedure:

The experimental procedure was approved by the Institutional Animal and Poultry care and use committee at College of Agriculture Sciences and Food King Faisal University Saudi Arabia Kingdom. Four-hundred-day-old Ross 308 broiler chicks were allocated randomly into 10 treated groups with four replicates of 10 birds each. Two diets (Table 1) were formulated according to NRC (1994) as normal phosphorus diets 0.45% AP (positive control PC) and phosphorus deficient diets 0.25% AP (negative control NC) which was supplemented with three levels of microbial phytase (MP), three levels of citric acid (CA) and three levels of their combination. The experimental diets were T1, normal phosphorus diet (PC); T2, phosphorus deficient diet (NC); T3, NC diet + 1% CA; T4, NC diet + 2% CA; T5, NC diet + 500 FTU MP; T6, NC diet +500 FTU MP+ 1% CA; T7, NC diet + 500 FTU MP + 2% CA; T8, NC diet+ 1000 FTU MP; T9, NC diet + 1000 FTU MP + 1% CA; T10, NC diet +1000 FTU MP + 2% CA.

The 35 days experimental period was divided into two stages, from 0 to 3 weeks and from 3 to 5 weeks of age. Water and mash feed were provided *ad lib*. The chicks were placed in floor pens with wood-shavings litter. Fan and air conditions were used for keeping suitable temperature. Artificial lighting was provided constantly.

Body weights were recorded weekly for each chick and average body weight gain was calculated for each replicate and treatment group. Feed intakes were obtained weekly in gram, and feed conversion ratio was calculated as gram feed /gram gain.

At the end of the two experimental stages 8 chicks per treatment representing the four replicate groups were slaughtered. For separating serum blood samples were collected and centrifuged at 1500 rpm for 10 minutes. Serum was decanted and frozen till chemical analysis. Plasma phosphorus and calcium concentrations were determined using special chemical kits from United Diagnostic Industry, Dammam, Saudi Arabia Kingdom.

Slaughtered birds were defeathered, eviscerated and the internal organs were separated. Right and left tibia were excised, defleshed and dried at 105°C. Right tibia was weighted in grams and its width, length was measured by caliper in mm (Zhang & Coon 1997). Tibia bone was ashed in muffle furnace at 600°C and ash weight was calculated as percentage of dry tibia (Chung & Baker 1990). Tibia content of calcium and phosphorus were determined according to (AOAC 1990). Left tibia was used to measure tibia breaking strength (TBS) in kg/ cm². TBS was determined by applying the simple three-point bending concept using Instron Universal Testing Machine. Determination process was conducted at the Research Center of Properties and Testing of Materials, Faculty of Engineering, Ain Shams University. TBS was defined as the upper limit force that the tibia bone can afford when applied perpendicularly on the longitudinal axis of this bone and before the bone itself is broken (Crenshaw *et al.*, 1981).

Digestibility Trail and Chemical Assays:

Digestibility trail was conducted at the end of experiment using four chicks from each treatment represented the four replicates group. The chicks were housed in individual cages with trays for collecting excreta and calculating feed intake for three days. Excreta was homogenized, weighed, and dried in an oven at 60 C° then representative samples were taken for determining nitrogen, calcium and phosphorus content. Nitrogen was determined using Kjeldahl method AOAC (1990), and apparent nitrogen retention was calculated as: $N \text{ intake} - N \text{ output} / N \text{ intake} \times 100$. For calculating phosphorus and calcium retention, excreta samples were ashed in muffle furnace at 600°C then digested with nitric

perchloric. Calcium and phosphorus content of excreta were determined using spectrophotometer and special chemical kits (United Diagnostic industry, Dammam, Saudi Arabia kingdom). Mineral retention percentage was calculated as: $\text{Ca or P intake} - \text{Ca or P output} / \text{Ca or P intake} \times 100$.

Table (1): Composition and calculated analysis of experimental basal control diets.

Item	Positive control diet 0.45% A.P	Negative control diet 0.25% A.P
Ingredients %:		
Yellow corn	55.15	57.19
Soybean meal (48%)	37.3	37
Vegetable oil	3.7	3
Dicalcium phosphate	1.7	0.66
Limestone	1.5	1.5
Common salt	0.25	0.25
Vit. & min. premix*	0.2	0.2
DL. Methionine	0.2	0.2
Total	100	100
Calculated composition:		
Crude protein%	22.6	22.6
M.E. Kcal/kg	3107	3107
% Calcium	1.05	0.825
% Total phosphorus	0.703	0.511
% Available phosphorus	0.443	0.25
% Methionine + cysteine	0.916	0.92
% Lysine	1.24	1.24

*Composition of vitamin and mineral premix. Each 2 kg of vitamin and mineral mixture contains: 12000000 IU vitamin A; 2000000 IU D3; 10g E ; 1g K ; 1 g B1 ; 5g B2; 1500mg B6 ; 10mg B12 ;10g Pantothenic acid ; 20g Nicotinic acid ; 1g Folic acid ; 50mg Biotin ; 500 g choline chloride ;4 g copper ; 300 mg iodine ; 30g iron, 60 g Manganese; 50g Zinc; and 100mg selenium

Statistical Analysis:

Data were subjected to analysis of variance using general linear model (GLM) procedure described in SAS User's Guide 1998. Differences among means were tested using Duncan's multiple range tests. The following two models were used for data analysis: Model 1, to compare among the ten treated groups including positive control while model 2 was used to determine the main effects (phytase, citric) and their interaction.

1. $Y_{ij} = \mu + MPCA_i + e_{ij}$
2. $Y_{ijk} = \mu + MP_i + CA_j + (MP \times CA)_{ij} + e_{ijk}$

Where Y_{ijk} , Y_{ij} = individual observation, μ = experimental mean, MP_i = microbial phytase effect, CA_j = citric effect, $(MP \times CA)_{ij}$ = the interaction between phytase and citric, $MPCA_i$ = effect of phytase and citric, e_{ijk} , e_{ij} = experimental error.

RESULTS AND DISCUSSION

Performance Measurements:

Performance aspects of chicks are shown in Table 2. It is clear that the chicks fed NC diet recorded the lowest body weight gain value. However, the overall mean of mortality (data not shown) was less than 2% and not related to feeding phosphorus deficient diets. Supplementation of MP or/ and CA into NC diet significantly ($P \leq 0.05$) improved body gain of chicks. Several studies proved that, addition of phytase into low AP diets significantly improved body weight of broiler chicks (Brenes *et al.*, 2003; Ahmad *et al.* 2000). The mode of action of phytase in improving body weight gain may be due to release of P from phytate, which enhanced the digestibility of starch and energy production

(Ebrahimnezhad *et al.* 2008). The improvement effect of CA on body weight gain have been reported previously and related to its effect in reducing digestive tract pH. Low pH value is responsible for enhancing nutrients utilization and hence improves body weight gain (El-Afifi 2003; Talebi *et al.*, 2010). Body gains of birds fed 500 FTU/Kg MP with 1 or 2% of CA (T6 & T7) were significantly higher than those fed PC (T1) diet at 5 weeks of age and similar at 3 weeks of age. As well as the interaction between phytase and citric acid for improving body gain was significant ($P \leq 0.05$) at the second stage of growth. The current result indicate to complimentary effect between MP and CA in improving body weight gain and the level of 500 FTU/Kg MP with 1 or 2% CA is effectiveness

Table (2): Effect of phytase and/or citric acid supplementation on performance of broiler chicks.

Treatments			Body weight gain (g)		Feed intake (g)		Feed	conversion ratio
A.P %	MPFTU /kg	CA %	0 -3 wk	0 -5 wk	0 -3 wk	0 -5 wk	0 -3 wk	0 -5 wk
T1, 0.45(PC)	-	-	594.6 ^{ab}	1784.0 ^{bcd}	829.1 ^{bc}	3202.9 ^{cde}	1.39 ^b	1.79 ^{abc}
T2, 0.25(NC)	0	0	344.0 ^e	1106.4 ^e	573.7 ^d	2139.9 ^f	1.68 ^{ab}	1.95 ^{ab}
T3, 0.25	0	1%	490.2 ^{cd}	1578.8 ^d	866.9 ^{abc}	3035.3 ^{de}	1.83 ^a	1.96 ^a
T4, 0.25	0	2%	455.8 ^d	1744.7 ^{cd}	758.3 ^c	2952.0 ^e	1.66 ^{ab}	1.69 ^c
T5, 0.25	500	0	563.7 ^{bc}	1757.0 ^{cd}	827.5 ^{bc}	3273.5 ^{bcd}	1.47 ^b	1.86 ^{abc}
T6, 0.25	500	1%	588.6 ^{abc}	2083.7 ^a	901.4 ^{ab}	3669.4 ^a	1.54 ^{ab}	1.76 ^{abc}
T7, 0.25	500	2%	574.0 ^{bc}	1906.9 ^{abc}	872.0 ^{abc}	3289.2 ^{bcd}	1.52 ^{ab}	1.73 ^{bc}
T8, 0.25	1000	0	607.3 ^{ab}	2076.3 ^a	907.2 ^{ab}	3587.4 ^{ab}	1.50 ^{ab}	1.73 ^{bc}
T9, 0.25	1000	1%	5 ^a 675	2036.0 ^{ab}	977.4 ^a	3699.1 ^a	1.45 ^b	1.82 ^{abc}
T10, 0.25	1000	2%	534.0 ^{bcd}	1977.2 ^{abc}	847.6 ^{bc}	3411.0 ^{abc}	1.59 ^{ab}	1.72 ^{bc}
		SEM	31.1	80.5	34.6	102.9	0.10	0.069
Overall Means Phytase								
0.25	0	-	430.0 ^b	1476.6 ^b	733.0 ^b	2709.1 ^b	1.73 ^a	1.87
0.25	500	-	575.4 ^a	1915.9 ^a	867.0 ^a	3410.7 ^a	1.51 ^b	1.79
0.25	1000	-	605.6 ^a	2029.8 ^a	910.7 ^a	3565.8 ^a	1.51 ^b	1.76
Overall Means Citric								
0.25	-	0	505.0 ^b	1646.6 ^b	769.5 ^b	3000.2 ^c	1.55	1.85 ^a
0.25	-	1%	584.8 ^a	1899.5 ^a	915.2 ^a	3468.0 ^a	1.61	1.85 ^a
0.25	-	2%	521.3 ^b	1876.3 ^a	826.0 ^b	3217.4 ^b	1.59	1.71 ^b
Interaction MP x CA			NS	0.004	0.01	0.002	NS	NS

A.V, available phosphorus; MP, microbial phytase; CA, citric acid; PC, positive control (0.45% phosphorus); NC, negative control (0.25% phosphorus)

^{a, b, c, d} Mean values within a column with no common superscript differ significantly from each other ($P < 0.05$).

SEM, standard error of means; NS, not significant

The value \pm stander error

to improve body gain of chicks fed phosphorus deficient diet as like as those fed PC diet (T1) or 1000 FTU/Kg MP (T8). The present finding confirms the results of Nourmohammadi *et al.* (2012) noted extra increment in body weight gain of broiler chicks fed diets enriched with phytase and citric acid. This increment is related to increase phytase efficiency as results of low intestinal pH induced by citric acid (Brenes *et al.*, 2003 & Vieira *et al.*, 2018).

The overall values of feed intake significantly increased ($P \leq 0.05$) due to adding 1% CA and MP into diets compared with 2% CA or un-supplemented groups. As well, the interaction between CA and MP in increasing feed intake was significant at both stages of growth. The reduction in feed consumption of chicks fed low AP diets was observed by Sacakli *et al.* (2006). As well, inclusion CA with 2% may reduce feed intake due to its effect in lowering palatability and increasing the transmission time of feed in intestinal tract (Nourmohammadi *et al.*, 2010). Feed conversion ratio improved due to adding MP or CA into NC diet, the improvement was significant ($P \leq 0.05$) at the 3rd and 5th week for MP and CA respectively.

The improvement in feed conversion can be related to increase protein digestibility and retention reported in the current study (Table 5). The results are in harmony with those of Zobac *et al.* (2004) and El-Afifi (2003) who observed an improvement in feed conversion ratio due to adding phytase or CA respectively into broiler diets.

Tibia Ash and Morphometry:

The values of tibia width and breaking strength (Table 3) were not differed significantly due to adding MP and/or CA into NC diet as well, the interaction lacked significance. These results are consistence with Sreeja *et al.* (2018) who did not observe any differences in tibia width by adding phytase into low AP diets.

Table (3): Effect of phytase and/or citric acid supplementation on tibia morphometry and ash content.

Treatments			Tibia width (mm)		Tibia length (mm)		Tibia weight (g)		Tibia ash (g)		Tibia Ash (%)		Tibia strength Kg/cm ²	
A.P %	MP FTU/kg	CA %	0 -3 Wk	0 -5 Wk	0 -3 Wk	0 -5 wk	0 -3 wk	0 -5 Wk	0 -3 wk	0 -5 wk	0 -3 wk	0 -5 wk	0 -3 wk	0 -5 wk
T1, 0.45 (PC)	-	-	4.88	7.34 ^a	63.69 ^{abc}	84.83 ^{bc}	2.00 ^{bc}	6.20 ^{ab}	0.68 ^{bc}	2.02 ^{ab}	13.90 ^{bc}	32.66 ^{ab}	14.67	23.50
T2, 0.25 (NC)	0	0	4.59	6.30 ^b	59.83 ^e	86.13 ^{abc}	1.9b ^c	5.54 ^{ab}	0.57 ^c	1.81 ^{abc}	12.26 ^{cd}	32.73 ^{ab}	13.33	18.50
T3, 0.25	0	1%	4.63	5.91 ^b	63.86 ^{abc}	82.34 ^c	2.14 ^{abc}	4.85 ^b	0.64 ^{bc}	1.50 ^c	13.79 ^{bc}	30.98 ^b	14.33	16.75
T4, 0.25	0	2%	5.31	6.43 ^b	60.09 ^{de}	86.29 ^{abc}	1.80 ^c	5.09 ^{ab}	0.56 ^c	1.80 ^{abc}	10.52 ^d	35.05 ^a	12.67	15.50
T5, 0.25	500	0	4.86	6.46 ^b	62.11 ^{bcde}	90.52 ^{ab}	2.13 ^{abc}	6.28 ^{ab}	0.75 ^{abc}	1.94 ^{abc}	15.48 ^{ab}	30.94 ^b	12.00	22.25
T6, 0.25	500	1%	5.18	6.05 ^b	63.09 ^{abcd}	89.85 ^{abc}	2.43 ^{ab}	5.84 ^{ab}	0.83 ^{ab}	2.01 ^{ab}	15.95 ^{ab}	34.42 ^{ab}	16.00	17.75
T7, 0.25	500	2%	5.07	5.98 ^b	64.8 ^{3ab}	84.63 ^{bc}	2.42 ^{ab}	4.92 ^{ab}	0.85 ^{ab}	1.62 ^{bc}	16.66 ^{ab}	32.94 ^{ab}	15.33	17.25
T8, 0.25	1000	0	4.65	5.90 ^b	60.82 ^{cde}	86.55 ^{abc}	1.95 ^{bc}	5.26 ^{ab}	0.64 ^{bc}	1.88 ^{abc}	13.68 ^{bc}	35.40 ^a	13.00	19.25
T9, 0.25	1000	1%	5.43	6.56 ^a	65.92 ^a	89.81 ^{abc}	2.65 ^a	6.31 ^{ab}	0.93 ^a	2.20 ^a	17.17 ^a	34.98 ^a	17.33	21.25
T10, 0.25	1000	2%	4.64	6.30 ^b	63.48 ^{abcd}	93.46 ^a	1.95 ^{bc}	6.44 ^a	0.68 ^{bc}	2.11 ^{ab}	14.68 ^{abc}	33.06 ^{ab}	13.33	19.50
		SEM	2.66	0.29	1.09	2.38	0.18	0.44	0.07	0.15	0.97	1.20	1.79	2.39
Overall Means Phytase														
0.25	0	-	4.84	6.21	61.26 ^b	84.92 ^b	1.95 ^b	5.16 ^b	0.59 ^b	1.70 ^b	12.19 ^b	32.92	13.44	16.92
0.25	500	-	5.04	6.16	63.34 ^a	88.33 ^{ab}	2.33 ^a	5.68 ^{ab}	0.81 ^a	1.85 ^{ab}	16.03 ^a	32.76	14.44	19.08
0.25	1000	-	4.91	6.25	63.41 ^a	89.94 ^a	2.18 ^{ab}	6.00 ^a	0.75 ^a	2.06 ^a	15.17 ^a	34.48	14.56	20.00
Overall Means Citric														
0.25	-	0	4.70	6.22	60.92 ^a	87.73	1.99 ^b	5.69	0.65 ^b	1.88	13.80 ^b	33.02	12.78	20.00
0.25	-	1%	5.08	6.17	64.29 ^{ab}	87.33	2.41 ^a	5.67	0.80 ^a	1.90	15.64 ^a	33.46	15.89	18.58
0.25	-	2%	5.01	6.24	62.80 ^b	88.12	2.06 ^b	5.48	0.70 ^{ab}	1.84	13.95 ^{ab}	33.68	13.78	17.42
Interaction MP x CA			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

A.V, available phosphorus; MP, microbial phytase; CA, citric acid; PC, positive control (0.45% phosphorus); NC, negative control (0.25% phosphorus)

^{a, b, c, d} Mean values within a column with no common superscript differ significantly from each other (P<0.05).

SEM, standard error of means; NS, not significant

The value ± stander error

However, Zivkov *et al.* (2012) reported an improvement in tibia breaking strength due to supplement phytase into low AP broiler diets. This dissimilarity may be related to the differences in the experimental conditions, especially, level of phytase and method of measuring tibia strength. Tibia length, weight and ash content (g) of chicks significantly (P≤0.05) improved due to adding MP into NC diet at both stages of growth, while citric acid affected significantly (P≤0.05) at the first stage of growth only. Brenes *et al.*(2003) and Sacakli *et al.*(2006) reported that tibia weight, length and ash content enhanced by supplying low AP broiler diets with citric acid or phytase due to its effect in increasing the retention rate of calcium and phosphorus.

Phosphorus and Calcium Values:

Blood calcium levels were not affected significantly by adding MP and/or CA into NC diet at the two stages of growth (Table 4). The current finding agrees with Hariharan & Gangadevi (2015) who did not observe any significant effect on plasma concentration of calcium due to adding phytase or citric acid into low phosphorus diets. On the other hand, Sheikh *et al.* (2010) stated that organic acid supplementation increased blood concentration of calcium. This disagreement may be related to use different levels and types of organic acids, butyric, fumaric and lactic in their experiment against citric acid in the current work. Blood phosphorus significantly ($P \leq 0.05$) increased due to adding MP into low AP diet at the 5th week of age, while CA supplementation has no effect. The present results are in harmony with the finding of Luciana *et al.* (2016) that, phytase supplementation can increase blood concentration of phosphorus. In contrary Hariharan & Gangadevi (2015) reported that blood phosphorus level did not response for adding phytase, while it increased due to citric acid supplementation. This variance may be related to the differences in supplementation levels of MP and experimental conditions.

Table (4): Effect of phytase and/or citric acid supplementation on plasma and tibia content of calcium and phosphorus.

Treatments			Blood calcium (mg/dl)		Blood phosphorus (mg/dl)		Tibia calcium %		Tibia phosphorus %	
A.P %	MP FTU/kg	CA %	0 -3 Wk	0 -5 wk	0 -3 wk	0 -5 Wk	0 -3 Wk	0 -5 wk	0 -3 wk	0 -5 wk
T1, 0.45 (PC)	-	-	11.31	9.54	5.72 ^c	5.54 ^{ab}	11.79 ^{bc}	11.81 ^{ab}	9.17 ^b	6.01 ^{cd}
T2, 0.25 (NC)	0	0	10.31	10.03	5.32 ^{cd}	5.33 ^{bc}	15.97 ^a	12.68 ^{ab}	8.77 ^{bc}	6.71 ^{ab}
T3, 0.25	0	1%	10.72	9.67	5.61 ^{bc}	5.44 ^{bc}	12.91 ^{bc}	12.21 ^{ab}	9.11 ^b	6.28 ^{bc}
T4, 0.25	0	2%	11.67	9.61	6.11 ^a	5.03 ^c	11.58 ^{bc}	12.60 ^{ab}	8.72 ^{bc}	6.03 ^{cd}
T5, 0.25	500	0	11.17	9.74	5.41 ^{bcd}	5.48 ^{abc}	12.40 ^{bc}	12.27 ^{ab}	9.09 ^b	6.87 ^a
T6, 0.25	500	1%	11.08	9.45	5.10 ^d	5.50 ^{abc}	12.15 ^{bc}	13.17 ^a	8.99 ^b	6.28 ^{bc}
T7, 0.25	500	2%	9.74	9.67	5.24 ^d	5.46 ^{abc}	12.20 ^{bc}	12.02 ^{ab}	9.08 ^b	5.72 ^d
T8, 0.25	1000	0	11.52	9.67	5.72 ^b	5.64 ^{ab}	10.76 ^c	12.21 ^{ab}	8.21 ^c	6.11 ^{cd}
T9, 0.25	1000	1%	10.95	9.93	5.28 ^{dc}	5.94 ^a	13.17 ^b	11.25 ^b	10.20 ^a	6.03 ^{cd}
T10, 0.25	1000	2%	10.44	9.68	5.22 ^d	5.62 ^{ab}	12.66 ^{bc}	12.35 ^{ab}	9.01 ^b	6.21 ^c
SEM			0.65	0.217	0.116	0.153	0.68	0.43	0.207	0.15
Overall Means Phytase										
0.25	0	-	10.90	9.77	5.68 ^a	5.26 ^b	13.49 ^a	12.49	8.87	6.34
0.25	500	-	10.66	9.62	5.25 ^b	5.48 ^b	12.25 ^b	12.91	9.06	6.29
0.25	1000	-	10.97	9.76	5.41 ^b	5.74 ^a	12.20 ^b	11.9	9.14	6.12
Overall Means Citric										
0.25	-	0	11.04	9.81	5.49	5.48	13.04	12.38	8.69 ^b	6.56 ^a
0.25	-	1%	10.91	9.68	5.33	5.62	12.74	12.32	9.43 ^a	6.2 ^b
0.25	-	2%	10.61	9.65	5.52	5.37	12.15	12.21	8.94 ^b	5.99 ^b
Interaction MP x CA			NS	NS	0.01	NS	0.006	NS	0.02	0.004

A.V, available phosphorus; MP, microbial phytase; CA, citric acid; PC, positive control (0.45% phosphorus); NC, negative control (0.25% phosphorus)

^{a, b, c, d} Mean values within a column with no common superscript differ significantly from each other ($P < 0.05$).

SEM, standard error of means; NS, not significant

The value \pm standard error

Tibia calcium of chicks fed NC diet was higher than those fed PC diet or NC diet enriched with MP and/or CA; however, the differences lacked significance at the 5th week of age. It can be suggested that low AP in NC diet stimulate releasing more phosphorus molecules from bone in a physiological attempt to keep blood calcium to phosphorus ratio constant. Releasing phosphorus molecule may relatively increase the ratio of calcium because of bone composed of tricalcium phosphate, in this concern the references are still absence. Brenes *et al.* (2003) noticed a significant increase in tibia calcium of broiler chicks due to feeding low AP diet (2.5g/kg diet) illustrating that reduced dietary AP level increased plasma calcium which reflects on increasing calcium deposition in tibia.

Tibia content of phosphorus did not response clearly for adding MP or CA into NC diet. Hariharan & Gangadevi (2015) did not find any significant differences in tibia content of phosphorus due to reducing dietary AP by 40% or by adding phytase or CA into low P diet. In contrary several studies indicated that, addition of MP and/or CA into low AP diet enhance phosphorus releasing from phytate complex which can reflect in enhancing phosphorus deposition in tibia bone (Brenes *et al.*, 2003; Zobac *et al.*, 2004; Sheikh *et al.*, 2010).

Nutrient Retention:

Nitrogen and calcium retention of chicks fed NC were significantly ($P \leq 0.05$) lower than those fed PC diet or NC diets supplemented with 1000 FTU/kg MP and CA (Table 5). Zobac *et al.* (2004) observed an improvement in digestibility of nitrogen by adding lactic acid and phytase into low AP 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). Furthermore, dietary organic acid reduces gastric pH and increases the rate of pepsinogen transformation to pepsin which promotes protein digestion and retention (Khodambashi Emami *et al.*, 2013). Similarly, Manobhavan *et al.* (2016) noted an increase in calcium retention by adding phytase into low AP diet. They related this improvement in calcium retention to release more calcium molecules from phytate bonds by the action of phytase. Deepa *et al.* (2011) suggested that, low intestinal pH resulting from citric acid supplementation may increase solubility and absorption of calcium, as well, make phytic acid more protonized preventing formation of calcium phytate complex and increasing calcium retention. Phosphorus retention of chicks fed NC diet was significantly similar for those fed PC or NC diets supplemented with MP and/or CA (Table 5). It can be suggested that the birds have a greater ability to retain more phosphorus from NC diet, in a response for rising plasma phosphorus level. Therefore, the phosphorus retention % was not affected clearly because it calculated as percentage of total intake which is low in NC diet. Brenes *et al.* (2003) noted an enhancement in phosphorus retention of chicks fed low AP diets. In contrary, Deepa *et al.* (2011) reported that, phytase and citric acid supplementation improved phosphorus retention due to their beneficial effect on releasing more phosphorus molecules from phosphorus-phytate-complex.

CONCLUSION AND APPLICATIONS

1. Adding phytase enzyme at 500 FTU/kg level with 1% citric acid into NC diet was effectiveness to improve body weight and tibia bone characteristics as like as those fed PC diet or NC enriched with 1000 FTU/kg phytase enzyme.
2. This finding can be applied successfully in poultry formulation for increasing the utilization of phytate phosphorus, reducing the ratio of inorganic phosphorus and phytase supplementation units as well, controlling the pollution resulting from extra phosphorus excretion.
3. There is no clear effect of citric acid or phytase enzyme supplementation on plasma or tibia content of phosphorus.

Table (5): Effect of phytase and/or citric acid supplementation on Calcium and phosphorus retention.

Treatments			Nitrogen Retention %	Calcium Retention %	Phosphorus Retention %
A.P %	MP FTU/kg	CA%			
T1, 0.45 (PC)	-	-	65.53 ^a	55.33 ^a	53.30 ^{ab}
T2, 0.25 (NC)	0	0	58.60 ^c	47.93 ^d	54.30 ^a
T3, 0.25	0	1%	59.70 ^{bc}	50.57 ^{bcd}	50.43 ^{ab}
T4, 0.25	0	2%	58.03 ^c	51.30 ^{bcd}	48.10 ^b
T5, 0.25	500	0	60.13 ^{bc}	48.80 ^{cd}	54.83 ^a
T6, 0.25	500	1%	59.87 ^{bc}	53.80 ^{ab}	53.63 ^{ab}
T7, 0.25	500	2%	59.93 ^{bc}	48.73 ^{cd}	51.63 ^{ab}
T8, 0.25	1000	0	63.53 ^{ab}	53.00 ^{ab}	49.33 ^{ab}
T9, 0.25	1000	1%	64.87 ^a	55.47 ^a	52.50 ^{ab}
T10, 0.25	1000	2%	63.67 ^{ab}	52.27 ^{abc}	50.63 ^{ab}
SEM			1.23	1.17	1.70
Overall Means Phytase					
0.25	0	-	58.78 ^b	49.94 ^b	50.94
0.25	500	-	59.98 ^b	50.44 ^b	53.37
0.25	1000	-	64.02 ^a	53.58 ^a	50.82
Overall Means Citric					
0.25	-	0	60.76	49.91 ^b	52.82
0.25	-	1%	61.48	53.28 ^a	52.19
0.25	-	2%	60.54	50.77 ^b	50.12
Interaction MP x CA			NS	NS	NS

A.V, available phosphorus; MP, microbial phytase; CA, citric acid; PC, positive control (0.45% phosphorus); NC, negative control (0.25% phosphorus).

^{a, b, c, d} Mean values within a column with no common superscript differ significantly from each other ($P < 0.05$).

SEM, standard error of means; NS, not significant.

The value \pm standard error.

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تأثير أنزيم الفيتيز وحامض الستريك على الأداء الإنتاجي والاستفادة من العناصر الغذائية وعظمة الفخذ لكتاكيت التسمين المغذاة على علائق منخفضة الفوسفور

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هدفت الدراسة لمعرفة تأثير تغذية كتاكيت اللحم على علائق منخفضة الفوسفور مضاف إليها ثلاثة مستويات من الفيتيز الميكروبي على حدة أو مع ثلاثة مستويات من حامض الستريك على الأداء الإنتاجي وخصائص عظمة الفخذ ومعدل الاستفادة من بعض العناصر الغذائية. استخدمت الدراسة عدد أربع مائة كتكوت تسمين روس عمر يوم تم تقسيمهم عشوائياً إلى عشر مجموعات تم تغذيتها على عشر علائق مختلفة الأولى تحوي 0.45% فوسفور متاح (كونترول موجب) وتسع علائق تحوي 0.25% (كونترول سالب) مضاف لها ثلاث مستويات من حامض الستريك 0%، 1% أو 2% على حدة أو مع ثلاث مستويات من الفيتيز 0 و 500 و 1000 FTU / Kg. وقد أظهرت النتائج أن إضافة حامض الستريك بمفرده أو مع الفيتيز إلى العلائق منخفضة الفوسفور أدى إلى تحسن معنوي في وزن الجسم المكتسب. ازداد استهلاك العلف معنوياً نتيجة إضافة 1% حامض ستريك مع مستوي الفيتيز إلى علائق الكونترول السالب. كذلك تحسن معدل التحويل الغذائي نتيجة لإضافته حامض الستريك وأنزيم الفيتيز إلى الكونترول السالب. لم يكن هناك تأثير معنوي لإضافته حامض الستريك أو الفيتيز على سمك عظمة الفخذ أو صلابتها. طول ووزن العظم ومحتواه من الرماد تحسن معنوياً نتيجة لإضافته أنزيم الفيتيز إلى علائق الكونترول السالب بينما أثر حامض الستريك معنوياً على عمر 3 أسابيع فقط. محتوى الدم من الفوسفور ارتفع معنوياً بإضافة الفيتيز إلى العلائق عند الأسبوع الخامس من العمر بينما لم يتأثر الكالسيوم في الدم بشكل كبير بإضافة الفيتيز أو حامض الستريك. محتوى عظم الفخذ من الكالسيوم ارتفع نتيجة للتغذية على العليقة منخفضة الفوسفور (كونترول سالب) مقارنة بتلك المغذاة على علائق الكونترول الموجب أو السالب المدعم بإضافة الفيتيز أو الستريك بينما لم يختلف محتوى العظام من الفوسفور معنوياً نتيجة للمعاملات. معدل احتجاز النتروجين والكالسيوم تحسن معنوياً نتيجة لإضافته حامض الستريك والفيتيز إلى العلائق بينما لم يتأثر نسبة الفوسفور المحتجز نتيجة للإضافة. استخلصت الدراسة أن إضافة أنزيم الفيتيز بمستوى 500 FTU مقترناً بمستوى 1% حمض الستريك أدى لتحسن وزن الجسم وخصائص عظمة الفخذ بصورة مماثلة لتلك المغذاة على علائق مضاف لها 1000 FTU أنزيم الفيتيز أو المحتوية على مستوى كاف من الفوسفور.