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IMPROVING PHYTATE PHOSPHORUS AVAILABILITY BY ADDING MICROBIAL PHYTASE TO BROILER DIET

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ABSTRACT

A 42 days experiment was conducted with one day-old broiler chicks (n=750) to evaluate the effectiveness of supplemental microbial phytase for improving the availability of Phytate-P (pp) in corn-soybean diet when different levels of nonphytate phosphorus (NPP) as a Dicalcium Phosphate (DCP) were fed. The broiler chicks were distributed to five treatments with three replicates. Each replicate contains 50 broiler chicks. The dietary treatments were formulated by reducing DCP level and assuming an increase of PP availability as a result of adding phytase (UP). The dietary treatments were as follow: C, control group corn-soybean diet (0% pp availability); T_1 control diets + 150 UP/ton; T_2 control diet + 300 UP/ton; T_3, (60% of PP available) + 150 UP/ ton and T_4, (100% PP availability) + 150 UP/ ton. The final live body weights of broiler from T_3 and T_4 groups weren't significantly differ ($P>0.05$) from T_1 and T_2, broilers but were significantly lower ($P<0.05$) than that of control group. The Gain: feed ratio of broilers from T_2, T_3 and T_4 were significantly higher ($P<0.05$) compared with T_1 and the control groups. No significant differences lower ($P>0.05$) were found between all groups in the accumulative feed intake. Calcium concentration in blood serum of chicks from T_4 was significantly lower ($P<0.05$) compared with other treatments, but P concentration was significantly higher ($P<0.05$) in serum of chicks from T2 compared with other treatments. Moreover, the result of economical evaluation indicated that feeding chicks in groups T_2, T_3 and T_4 resulted in higher cost of live body weight and lower economical efficiency compared with other groups. In conclusion, the results of this experiment show that addition of microbial phytase increase availability of phytate-P in corn-soybean meal diet for broilers. Further research is needed to determine appropriate levels of Phytase to be added to broiler diets to maximize release of phytate-P.

Key words: Broiler, Phytate, Phytase, Cost and Body weight

INTRODUCTION

Much of the world's human populations suffer from malnutrition. In Jordan and many other third world countries, the profit of raising broiler is very marginal because of the high cost of feed, poor growth rate, poor feed conversion and

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high mortality rate. Jordan is one of the countries in the Middle East raise at least 8-10 million broiler chicks/ month. This number is very high because of the competitive price of unit of white meat compared with red meat. Increasing the availability of some feed's nutrients such as phytate Phosphorus can reduce the high cost of broiler feed.

Dicalcium phosphoate (DCP) is one of the main components added to broiler soybeab-corn ration as a source of inorganic phosphorus to optimize growth rate and skeletal development. This addition of DCP is very crucial because of the low availability of phosphorus in dietary plant rations. About 70% of phosphorus in plant rations present in the form phytate P, which is only 10-53% can be utilized (**Edward & Veltmann, 1983 and Ballam *et al* 1984**). Because of that, considerable amount of P excreted by broiler in feces to the environment, which lead to environmental pollution. Moreover, phytate P caused a negative effect on the solubility of protein and function of pepsin because of their ionic binding with proteinized amino acids (**DeRham and Jost, 1979**). Phytate P present in plants as a mixed with calcium, magnesium and potassium salt of phytic acid (**Anderson, 1915**).

One approach to reduce environmental pollution, feed cost and increases the availability of phytate P to broiler, is by using microbial phytase enzyme. Microbial phytase enzyme is produced by *Asprigillus ficuum* fermentation, which found to be effective in hydrolyzing phytate P when added to the corn-soybean (SBM) diet (**Nelson *et al* 1971**) and increases the availability of phytate P to more than 65% (**Simons *et al* 1990**).

Therefore, this experiment was conducted to determine the effect of adding microbial phytase to the diet of broiler fed corn-SBM ration with different levels of dietary non-phytate phosphorus (NPP).

MATERIAL AND METHODS

Seven hundred and fifty one day old unsexed Hubbared broiler chicks were used in this study for 42 days to investigate the effect of using microbial phytase with corn-soybean broiler diet for improving the utilization of phytate P. Chicks were divided to five treatments of three replicates, fifty chicks in each replicate and separate pen. Chicks reared in concrete floor pens, which were covered with approximately 7-cm wood shaving. Diets and water provided for ad-libitum consumption. Three dietary rations were formulated according to **NRC (1994)** to have the same nutritive values, isocaloric and isonitrogenous, except for available phosphorus P (**Table 1**). Different levels of non-phytate phosphorus (NPP) were achieved by adding different levels of Dicalcium Phosphate (DCP) as shown in **Table (1)**. The treatments are as follows: C, control group corn-soybean diet (1.54% and 1.09% DCP for starter and finisher diets, respectively); T_1, control diet + 150 unit phytase (UP)/ ton; T_2, control diet + 300 UP/ ton; T_3, (1.06 and 0.61% DCP for starter and finished diets, respectively) + 150 UP/ ton and T_4 (0.26 and 0% DCP for starer and finisher diets, respectively) + 150 UP/ ton. The different levels of DCP were used assuming the phytate P availability will increase and cover the chick's requirements as a result of adding phytase enzyme. The phytase enzyme was NOVO® CT from Novo

Table 1. Composition of starter and finisher diets

Ingredient	Starter control	Starter 60% Available P	Starter 100% Available P	Finisher control	Finisher 60% Available P	Finisher 100% Available P
Corn	613	618	627	673	674	680
Soya	345	343	342	291	290	289
Palm oil	6.0	4.0	-	9.0	7.1	4.8
DCP*	15.4	10.6	2.6	10.9	6.1	-
$CaCO_3$	15.1	17.6	21.9	15.2	17.7	20.9
Methionine	2.0	2.0	2.0	0.8	0.8	0.8
Lysine	0.1	0.1	0.1	0.8	0.8	0.8
Vit. & Min.[b]	1.0	1.0	1.0	1.0	1.0	1.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0
Cost JDs/T	184	182	179	174	172	171
Calculated chemical analysis (as fed):						
ME Kcal/ kg	2947	2947	2940	3054	3038	3035
CP%	20.4	20.4	20.4	19.9	19.9	19.9
ME/CP%	144.5	144.5	144.1	153.0	152.7	152.3
Total Ca%	1.01	1.00	0.994	0.853	0.860	0.888
Total P %	0.662	0.578	0.434	0.608	0.506	0.378

* P<0.05

Norddisk, Denmark. The chicks fed the starter diets for 28 days and the finisher diets for 14 days. Chicks were kept under the same environmental conditions and vaccinated against common disease. Body weight (g) and feed intake (g) were determined weekly for 6 weeks. Average body weight gain (g) and feed conversion (g feed/g body gain) were calculated. At slaughter, blood and thigh bone samples were collected from 3 chicks/ each replicate. Thigh bones were dried in oven at 105°C over night and burned in the muffle furnace (600°C/ 6 hrs) (AOVA, 1990). Blood samples were centrifuged at 3000 x g/15 minutes and serum samples collected and analyzed for P calorimetrically and Ca by Atomic Absorption Spectrophotometry (AOAV, 1990).

Data were analyzed using the SPSS® (version 10) program (2000) for a Completely Randomized Design (CRD) with repeated measurements. Least significant differences (LSD) were used to compare between means.

RESULTS

The performance parameters of the broiler chicks of this experiment in terms of live body weight (LBW, g), accumulative feed intake (ACFI, g) and feed conversion (FC) for different ages, 1-4 weeks, 1-5 weeks and 1-6 weeks are presented in **Table (2)**. At weeks of age, the live body weight (LBW) of the birds from C, T_1 and T_2 were not significantly differ (P>0.05) from each other, but significantly differ (P<0.05) than birds from T_3 and T_4 groups which showed lower values. Moreover, the LBW of birds from T_3 was significantly lower (P<0.05) than birds from T_4.

Accumulative feed intake (ACFI, g) of birds from T_3 was significantly lower (P<0.05) than birds from all other groups. On the other hand, FC was significantly higher in T_3.

At 6 weeks old, it could be seen that groups were significantly lower (P<0.05) compared with birds from the control, but not differ significant when compared with birds from T_1 and T_3 groups. Moreover, no significant differences (P>0.05) between all groups in term of ACFI, but the FC was significantly different (P>0.05) between all groups in term of ACFI, but the FC was significantly higher (P<0.05) for birds from T_2, T_3 and T_3 and T_4 compared with the control and T_1.

Table (3) shows the thigh bone ash percentage, concentrations of calcium (Ca) and phosphorus (P) in blood serum of chicks at slaughter. Calcium concentrations in blood serum of chicks from T_4 were significantly lower (P<0.05) compared with the control, T_1, T_2 and T_3 (9.2 vs 10.9, 10.8, 11.2 and 9.8 mg/dl, respectively).

Phosphorus concentrations in serum of chicks from T_2 were significantly higher (P<0.05) compare with the control, T_1, T_3 and T_4 (7.1 vs 6.1, 6.85, 6.9 and 6.72 mg/ dl, respectively).

Table 2. The effect of supplemental Phytase on the performance of broiler chicks

Criteria	Ag (wk)	C	T1	T2	T3	T4	SE
Live bod weight (g)	1-4	876c	825bc	915c	515a	696b	72.1
	1-5	1286bc	1075ab	1330c	840a	1003a	105
	1-6	1753b	1650ab	1470ab	1350a	1400a	131.5
Accumulative feed intake (g)	1-4	1275b	1242b	1233b	1087a	1191b	41.04
	1-5	2136b	2111b	2091b	2012b	1719a	87.77
	1-6	3031	2816	2982	2619	2933	171.3
Feed conversion	1-4	1.54ab	1.58ab	1.41a	2.29c	1.89bc	0.196
	1-5	1.72ab	2.05bc	1.62a	2.15c	2.16c	0.178
	1-6	1.78a	1.75a	2.09b	2.00b	2.27b	0.269

Treatments* (columns C–SE)

* P<0.05

Table 3. The effect of supplemental Phytase on the calcium and phosphorus concentration in blood serum and ash% (dry weight) of high bones.

Criteria	Treatments*					
	Control	T1	T2	T3	T4	SE
Calcium mg/dl	10.9a	10.8a	11.2a	9.8a	9.2b	0.73
Phosphorus mg/dl	6.10a	6.85a	7.10b	6.90a	6.72a	0.3
Ash %	41.74	42.6	45.2	43.6	39.3	4.13

* $P<0.05$

Moreover, the thigh bone ash percentage of birds from all groups weren't significantly differ ($P>0.05$) from each other which gives a good indication of the efficiency of phytase enzyme in releasing part or all phytate-P from plant feed.

Economic evaluation

The economic efficiency percentage depends mainly on the final body weight and the amount and cost of feeds to produce one Kg body weight. **Table (4)** shows total feed consumed, cost of starter and finisher rations and the economical efficiency of the experimental diets for this study. The results of economical evaluation showed a lower cost per Kg. LBW was detected in birds from the control and T_1 groups (0.308 and 0.305 JDs, respectively) compared with T_2, T_3 and T_4 (0.368, 0.342 and 0.366 JDs, respectively). Thus, birds from T_2, T_3 and T_4 showed the lowest economic efficiency % compared with birds from other groups (control and T_1).

Table 4. The economical efficiency of broiler chicks fed different levels of Dicalcium phosphate supplemented with phytase*.

Item	Control	T_1	T_2	T_3	T_4
Feed consumed/kg/bird					
1- 28 days	1.275	1.242	1.233	1.087	1.191
28-42	1.765	1.574	1.749	1.532	1.742
Feed price/JD's/Ton:					
1-28 days	184	184.45	184.9	182.45	179.45
28-42 days	174	174.45	174.9	172.45	171.45
Feed cost per bird (JD's)					
1-28 days	0.2346	0.2291	0.2280	0.1983	0.2137
28-42 days	0.3055	0.2746	0.3131	0.2642	0.2987
Total cost JD's	0.5401	0.5037	0.5411	0.4625	0.5124
LBW/Kg	1.753	1.650	1.470	1.350	1.400
Cost of 1 kg LBW (JD's)	0.308	0.305	0.368	0.3425	0.366
Economic efficiency %**	133.76	136.1	95.6	110.22	96.72

* The price of phytase enzyme is 3.00 JD's/1 KG

$$**Economic\ efficiency\% = \frac{Market\ price/kg\ LBW^{1}}{(Cost\ of\ 1\ kg\ LBW)} \times 100$$

[1] The market price is 0.72 JD's / kg LBW

DISCUSSION

The results of this study indicate that supplemental of poultry diets with DCP can be reduced by adding microbial phytase enzyme, but the level of reduction depends mainly on many factors. In this study, 13 and 34% of total phosphorus were achieved by reducing the percentage of DCP (treatment 3 and 4, respectively) and assuming that phytate P will be available (60 or 100%) as a result of adding microbial phytase. The ABW of birds from T_3 and T_4 didn't differ even though the level of total P differ (0.476 and 0.369, respectively). Moreover, the LBW of birds from T_3 and T_4 were significantly lower ($P<0.05$) compared with the birds from the control group. Many factors may influence the effect of microbial phytase enzyme activities, which may explain the lower LBW of birds from groups T_3 and T_4. The main factors is the dose of the enzyme used which might not be enough to release all the phytate P. **Jongbloed *et al*** (1993) studied the effect of microbial phytase on P digestibility in a dose response experiments. The efficiency of microbial phytase appeared to be related to its dose and type of diet. **Simons and Versteegh** (1993) identified the minimal equivalency of inorganic P per amount of phytase, which was based on performance. Some dietary factors may also affect the phytase activities. It is commonly known that the higher dietary calcium (Ca) decreases absorption of P (**Jongbloed, 1987**). **Kornegay (1995)** in broiler and **Klis *et al*** (1992) in layer reported that the apparent digestibility of P linearly decreases with higher Ca, but no interaction with microbial phytsae could be demonstrated.

The productivity performance of birds from T_1 and T_2, positive control with phytase enzyme, were not significantly differ from the control (C) in term of LBW and general performance. This may imply that the P requirement was met from control diets and no significant effect of adding phytase with high DCP as NPP. This finding agreed with **Denbow *et al*** (1995) and **Qian *et al*** (1996) and disagreed with **Simons & Versteegh (1993)** and **Yi *et al*** (1995) who detected an improvement in broiler performance by adding phytase even though their diets cover the P requirements.

The data of the feed conversion is consistent with previous reports of several investigators (**Sohail & Roland, 1999; Swick & Ivey, 1990 and Denbow *et al* 1995**). Who reported a lower feed conversion in broiler fed high non-phytate P compare with broiler fed lower NPP as a DCP.

The bone ash values obtained in this study indicated that birds fed low non-phytate P with phytase probably wouldn't have P deficiency (**Table 3**). This result agreed with those of **Carlos and Edward** (1998) who reported the same trend with laying hens. Moreover, **Sohail and Ronald** (1997) reported that birds fed different levels of non-phytate P, response differently to supplemental phytase. The response to adding phytase was more pronounced on the lower dietary NPP treatments than the higher NPP treatments. In other studies done by **Denbow *et al*** (1995); **Mitchell & Edward** (1996a &b) and **Qian *et al*** (1996 & 1997) that supplemental phytase improves the quality of bone and its effect on bone strength was greater at the lower of NPP. Moreover, **Ahmed *et al*** (2000) and **Broz *et al*** (1994) revealed that phytase supplementation of a corn and soybean meal of poultry diet increased the tibia ash of broiler chicks, which may be due to liberation of inorganic P and Ca from the phytate molecular by supplementing phytase.

The normal level of Ca and P concentration in blood serum of broiler chicks are 8.9-13.0 and 4.5-7 mg/dl respectively (**Puls, 1988**). In this study, all the values for Ca and P were within the normal levels, which mean that microbial phytase releases phytate-P and increases the availability P to cover the reduction of DCP. **Rama Rao *et al* (1999)** reported an increase in P retention when phytase was supplemented to basal diet of broiler. This trend of P retention as a result of phytase supplementation was also reported by many investigators **Broz *et al* (1994); Kornegay & Qain (1994) and Yi *et al* (1994)**. Moreover, improvements in utilization of dietary Ca by supplemental phytase have been reported by **Mitchel & Edward (1996 a & b)**. This may explain that all the values of Ca and P concentrations in blood serum of chicks from all groups were within the normal levels.

CONCLUSION

In conclusion, it is very clear from these results that adding Phytase enzyme to broiler ration with lower DCP (non-phytate phosphorus), can give relatively similar or close performance to chicks fed regular ration with high DCP. This can be considered as a good indication of the efficiency of phytase enzyme in releasing part or all phytate-P from plant feed. On the other hand, more studies are needed to identify the accurate levels of phytase enzyme that must be used to release phytate-P and consequently the amount of DCP in broiler diets to reduce the environmental pollution.

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تحسين إتاحة الفسفور من الفايتيت نتيجة إضافة إنزيم الفايتيز الميكروبي لعلف الدجاج اللاحم

[٤]

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تم إجراء تجربة لمدة ٤٢ يوما على صيصان دجاج لاحم (لوهمان) عمر يوم (ن= ٧٥٠ صوص) لدراسة أثر إضافة أنزيم الفايتيز الميكروبي على إتاحة الفسفور من الفايتيت (pp) في خلطات الدجاج اللاحم (ذرة صفراء+ صويا) باستخدام مستويات مختلفة من ثنائي فوسفات الكالسيوم (DCP) كفسفور متاح (NPP). تم توزيع الصيصان على ٥ معاملات/ ٣ مكررات وبكل مكرر ٥٠ صوص. تم تجهيز الخلطات العلفية على

أساس توفير الـ (pp) بنسب مختلفة أثر إضافة أنزيم الفايتيز.

كانت المعاملات كما يلي

شاهد (صفر% PP متاح):

T1- شاهد + ١٥٠ وحدة فايتيز/ طن علف.

T2 – شاهد + ٣٠٠ وحدة فايتيز/ بطن علف.

T3- (افتراض ٦٠% من PP متاح) + ١٥٠ وحدة فايتيز/ طن علفك

T4- (افتراض ١٠٠% من PP متاح) + ١٥٠ وحدة فايتيز/ طن علف.

أوضحت النتائج عدم وجود فروق معنوية (P<0.05) في الوزن النهائي للطيور من المعاملات ٣، ٤ المعاملات مقارنة بمعاملات ١-٢، ولكنها كانت منخفضة معنوياً (P<0.05) مقارنة بالشاهد. أما معامل التحويل الغذائي للطيور من المعاملات ٢، ٣، ٤ فكانت أعلى معنويا (P<0.05) مقارنة بالطيور من المعاملات (١) والشاهد. ولا توجد أى فروق معنوية (P>0.05) بين المعاملات المختلفة من حيث كمية العلف النهائي المستهلك لكل طائر.

كما أوضح التحليل الاقتصادي ارتفاع تكلفة إنتاج ١ كم/ وزن الحى للمعاملات ٢، ٣، ٤ ولكن كانت منخفضة من ناحية الكفاءة الاقتصادية مقارنتا بالطيور من المعاملات والشاهد.

في الخاتمة، الواضح الاستجابة العالية لإتاحة الـ PP في خلطات الدجاج اللاحم (ذرة صفراء+ صويا ذات تراكيز منخفضة من NPP) أثر إضافة أنزيم الفايتيز الميكروبي. ولكن يحتاج هذا الموضوع لدراسات أخرى لتحديد المستويات المثلى لأنزيم الفايتيز التى يجب أن تضاف لخلطات الدجاج اللاحم لإتاحة الـ P الموجود في الـ PP.

الكلمات المفتاحية: دجاج لاحم، فايتيت، فايتيز، التكاليف، وزن الجسم

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