



IMPACT OF PHYTASE SUPPLEMENTATION IN RESTRICTED CALCIUM AND PHOSPHORUS BROILER DIETS ON PERFORMANCE, BLOOD PARAMETERS AND BONE CHARACTERISTICS

[49]

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ABSTRACT

Six weeks feeding trial using 210 one-day old unsexed Cobb 500 chicks was carried out to study the effects of adding phytase enzyme on growth performance, blood parameters and bone characteristics in broiler chicks. Three starter diets were used from 1 to 21 days; T100 {100% of Ca & non-phytate phosphorus NPP requirements (1.00% Ca and 0.50% NPP)}; T75 {75% of Ca & NPP requirements (0.75% Ca and 0.38% NPP)} and T50 {50% of Ca & NPP requirements (0.51% Ca and 0.25% NPP)}. Three grower diets were used from 22 to 42 days; T100 {100% of Ca & NPP requirements (0.91% Ca and 0.46% NPP)}; T75 {75% of Ca & NPP requirements (0.68% Ca and 0.34% NPP)} and T50 {50% of Ca & NPP requirements (0.45% Ca and 0.23% NPP)}. Seven dietary treatments were distributed according to diets fed consecutively during starter and grower phases as; (100/100) fed starter T100 then grower T100; (100/75) fed starter T100 then grower T75; (100/50) fed starter T100 then grower T50; (75/75) fed starter T75 then grower T75; (75/50) fed starter T75 then grower T50 and (50/50) fed starter T50 then grower T50. All diets added phytase enzyme (FTU 10000/Ton). Each treatment comprised of 30 chicks in 3 replicates of 10 chicks each. Results showed that live body weight and weight gain did not significantly affected by restricted Ca and NPP diets at starter period, where weight gain was significantly affected at grower period. Birds group received diet containing 100/75 with phytase (T3) gave the highest significant values for LBW and BWG, then 50/50 with phytase (T7) as compared with the other experimental groups during the grower and overall periods. Feed

consumption and conversion ratio values were no significant differences among groups fed different dietary treatments during starter and grower phases. Plasma P concentrations was significantly affected however plasma Ca concentrations, GOT and GPT values were not significantly affected by the reduction of dietary Ca and NPP with adding phytase. Percentages of tibia ash, Ca and P were significantly affected by dietary treatments. Supplementation of phytase has a significant effect on tibia breaking strength, while supplementation of phytase hasn't any effect on tibia Seed or index. It is obvious that, the best performance was seen with (100/75) diet without any adverse effect on productive performance, blood parameters and most of tibia measurements and chemical composition.

Keywords: *calcium, phosphorus, phytate, NPP, broilers, performance, blood, tibia*

INTRODUCTION

The general efficiency of dietary P utilization (plant origin) is relatively low (20–27%), and a significant amount of P is contained in litter and manure (Ferket et al 2002).

Phosphorus is an essential mineral for poultry because it plays an important role in metabolic and structural processes, and is an essential mineral to achieve maximal potential in growth performance. However, plant phytate binds to minerals and other nutrients to strictly decrease nutrient availability and harmfully affect digestion and absorption processes. To solve this problem, phytase, an exogenous enzyme, is commonly used as a feed additive

to release phytate-bound P. Dietary supplementation with exogenous phytase is an effective method of improving P digestibility (Walk et al 2013; Selle and Ravindran, 2007; Adeola and Cowieson, 2011).

Exogenous phytase enzyme is incorporated recently in poultry diets not only to reduce phosphorus supplementation, but also to liberate minerals, particularly calcium, amino acids and carbohydrates by the hydrolysis of phytate complex (Oluyinka et al 2007; Slominski, 2011). However, nutrient utilization can also be affected by extra factors, such as dietary calcium, phosphorus, protein, and energy levels, intestinal pH, environmental temperature, etc. Consequently, inorganic phosphorus is added to cover bird's requirements of phosphorus.

The current study aimed to investigate the effect of Ca and non-phytate phosphorus (NPP) restriction in broiler diets supplemented with phytase enzyme on performance, some blood components and bone measurements.

MATERIALS AND METHODS

Experimental diets and birds

The current study was carried out at the Poultry Nutrition Unit, Poultry Production Department, Faculty of Agriculture, Ain Shams University. Two hundred and ten chicks were randomly assigned to seven dietary experimental treatments (7 treatments x 3 replicates x 10 chicks in each) and were housed in three-tiered batteries equipped with feeders and drinking nipples. Feed and water were offered *ad-libitum* and chicks were kept under similar environmental and managerial conditions during the period 1-42 days of age. Three different starter diets (fed from 1 to 21 days); diet (100) contained standard requirements suggested by the guidebook of Cobb500 broilers of Ca and NPP during starter period; diet (75) contained 75% of Ca and NPP standard requirements and diet (50) contained 50% of Ca and NPP standard requirements. Three different grower diets (fed from 22 to 42 days); diet (100) contained standard requirements suggested by the guidebook of Cobb 500 broilers of Ca and NPP during grower period; diet (75) contained 75% of Ca and NPP standard requirements and diet (50) contained 50% of Ca and NPP standard requirements. Seven dietary treatments were distributed according to diets fed consecutively during starter and grower phases as; (100/100, T1); (100/100, T2) with phytase; (100/75, T3) with phytase; (100/50, T4) with phytase; (75/75, T5) with phytase; (75/50, T6)

with phytase and (50/50, T7) with phytase. Diets listed in Table (1), were formulated ensuring enough supply of nutrients suggested by the guidebook of Cobb500 broilers to be isocaloric and isonitrogenous according to NRC (1994) and were offered in mash form.

Growth performance

Live body weight (LBW) of each replicate was recorded in the early morning. The average body weight gain (BWG) was calculated per replicate by subtracting the initial body weight of a bird in a certain stage from the final one in the same stage. Average of daily feed consumption (DFC) was calculated from the difference between the weekly amount of feed provided for each replicate within treatments and the residual quantity for the same replicate. Feed conversion ratio (FCR) was calculated in different stages as the amount of feed consumed, in grams, in a certain stage which is required to produce out one gram of weight gain in the same stage.

Blood plasma

At 42 days of age, six birds from each treatment having body weight around the average of their treatment were chosen and sacrificed by severing the carotid artery and the jugular vein. Blood samples were collected simultaneously with slaughtering in heparinized tubes. Blood samples were immediately centrifuged at 3000 rpm for 10 minutes to separate plasma. Plasma calcium, phosphorus, (Tietz, 1995) Aspartate transaminase (AST) and Alanine transaminase (ALT) were carried out. Plasma GOT and GPT (AST and plasma ALT) were determined using a colorimetric method according to Reitman and Frankel (1957).

Bone measurements

Tibia of both legs were removed, cleaned of flesh and all soft tissue, cartilage caps were removed, oven-dried and dry tibia weight was recorded according to the method described by Samejima (1990). The Seedor index (SI) was obtained when a tibia dry weight (in grams) is divided by its length (in centimeters), as proposed by Seedor et al (1991). It represents an indication of bone density: the higher the value, the denser the bone. The tibia samples were ground for procedure of the chemical analysis; bone ash was determined colorimetrically.

Table 1. Feed ingredients and chemical composition of experimental diets

Ingredients%	Starter			Grower		
	100%	75%	50%	100%	75%	50%
Yellow corn	56.68	57.14	58.52	63.95	64.31	65.10
Soybean meal (44 % CP)	31.15	33.35	34.00	25.18	27.70	29.15
Corn gluten meal (60%CP)	5.60	4.00	3.35	4.10	2.20	1.05
Vegetable Oil	2.00	2.00	1.65	2.50	2.50	2.35
Ca Carbonate	1.60	1.17	0.77	1.47	1.08	0.70
MCP#	1.85	1.25	0.64	1.65	1.10	0.56
NaCl	0.30	0.30	0.30	0.30	0.30	0.30
Premix**	0.30	0.30	0.30	0.30	0.30	0.30
HCl Lysine	0.28	0.24	0.22	0.31	0.26	0.23
DL- Methionine	0.24	0.25	0.25	0.24	0.25	0.26
Total	100	100	100	100	100	100
Calculated analysis						
CP %	22	22.01	22.01	19.06	19.02	19.01
ME (Kcal/Kg)	2999	3004	3006	3103	3101	3102
Calcium %	1.01	0.754	0.51	0.91	0.68	0.45
NPP*	0.51	0.382	0.25	0.46	0.34	0.23
Lysine%	1.32	1.327	1.32	1.2	1.19	1.19
Methionine %	0.62	0.617	0.61	0.57	0.57	0.57
Meth. + Cyst. %	0.98	0.98	0.98	0.89	0.89	0.89

#MCP: mono-calcium phosphate, * NPP: non-phytate phosphorus.

**The premix contains: Vitamins: A: 12000000 IU; Vit. D3 2000000 IU; E: 10000 mg; K₃: 2000 mg; B₁:1000 mg; B₂: 5000 mg; B₆:1500 mg; B₁₂: 10 mg; Biotin: 50 mg;Choline chloride: 250000 mg; Pantothenic acid: 10000 mg; Nicotinic acid: 30000 mg; Folic acid: 1000 mg; Minerals: Mn: 60000 mg; Zn: 50000 mg; Fe: 30000 mg; Cu: 10000 mg; I: 1000 mg; Se: 100 mg and Co: 100 mg.

Tibia breaking strength was determined using the method prescribed by **Flemming et al (1998)** by using an Instron Universal material testing machine.

Calcium and phosphorus retention

At the end of the experimental period, six samples of excreta were weighed and ground prior to analysis for calcium and phosphorus as described by **AOAC (1990)**.

Retention was calculated as follows:

$$\% \text{ Retention} = (\text{consumed} - \text{excreted}) \times 100 / \text{consumed}, \text{Pintar et al (2005)}$$

Statistical Analysis

Data were subjected to one-way ANOVA analysis of variance General Linear Model (GLM) procedure of SAS software **SAS (1998)** user's guide according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij} \text{ Where; } \mu = \text{overall mean, } T_i = \text{dietary treatment, } e_{ij} = \text{experimental error. Individual effects of dietary treatments were compared using } \text{Duncan (1955)} \text{ multiple range tests at } \alpha \text{ level equal to } 0.05.$$

RESULTS AND DISCUSSION

Growth performance

Results presented in **Table (2)** showed no significant ($P>0.05$) differences among groups fed different dietary treatments in live body weight (LBW) and weight gain (WG) values during starter phase. However, values of overall LBW and WG showed significant ($P>0.01$) differences among groups fed different diets. On the other hand, birds group received diet containing 100/75 with phytase (T3) gave the highest significant values for LBW and BWG as compared with the other experimental groups during the grower and overall periods. Feed consumption (FC) values presented in **Table (2)** showed no significant ($P>0.05$) differences among groups fed different dietary treatments during starter and grower phases. Feed conversion ratio (FCR) values shown in **Table (2)** demonstrate no significant ($P>0.05$) differences among groups fed different dietary treatments during starter and grower phases. As shown in **Table (2)**, when comparing (100/100) with (75/75) or (50/50), it is comprehensible that no adverse effects were observed on FC and FCR when calcium and phosphorus levels were reduced in gentle approach between starter and grower phases (**Abdelaziz, 2011**). In addition, as the degree of diminishing Ca and NPP levels is less steep approach, the negative effects of feeding diets based on these treatments are relatively declined.

Our findings are in harmony with **Thacker et al (2013)** and **Jiang et al (2013)** where feed efficiency in broiler chicks was similar despite differences in the dietary available P.

Data of productive performance are commonly, in agreement with the results of **Abdelaziz (2011)** who stated that using low levels of both Ca and NPP diets gave results nearly matching those of control group. Current results are also in conformity with those of **Thabet (2010)**; **Dhandu and Angel (2003)** and **Angel et al (2000)**.

The fact that birds fed (100/75) diet presented performance similar to those fed (100/100) diet could be explained by the fact that broilers fed the diet with low NPP and Ca demonstrated a certain ability to adapt to these minerals deficiency (**Yan et al 2005**).

Blood parameters

Plasma Ca and P concentrations and GOT and GPT activity are presented in **Table (3)**. Results showed no significant ($P>0.05$) differences among

groups fed different dietary treatments in plasma Ca concentrations and GOT and GPT activity values. Plasma P values showed that deviations between different treatments have been come out at the end of grower period. Data indicated that birds fed (100/100) without phytase diet have shown significantly higher ($P\leq 0.05$) values of plasma P concentration. During the grower phase, birds fed (75/50, T6) diet have significantly ($P\leq 0.05$) the lowest P value as compared to among all treatments, while those fed (100/100) diet with or without phytase, whereas birds fed (100/75), (100/50), (75/75) diet have shown significantly similar values. Plasma GOT and GPT values obtained at the end of grower period indicated no significant differences between all seven groups. Data of plasma calcium, phosphorus, GOT and GPT agreed with those obtained by **Abdelaziz (2011)** and **Bolu et al. (2006)**. When the blood Ca decreases, parathyroid hormone (PTH) motivates the transfer of Ca and P from body skeleton to blood and impresses kidneys to produce endogenous cholecalciferol which affects small intestine and increases Ca and P absorption (**Kheiri and Rahmani, 2006**).

Bone composition and measurements

Data of tibia ash percentage are presented in **Table (4)**. The data showed that birds of T7 had recorded significantly ($P\leq 0.05$) lowest values of tibia ash percentage when compared to those others. In addition, it observed that reducing phosphorus intake of bird's diets resulting in linearly decreased ($P<0.05$) of tibia ash percentage. These results are in agreement with **Thabet (2010)** who found that tibia ash of broilers fed low Calcium and Available Phosphorus diets was lower than those of broilers fed control diet. On the other hand, broilers fed diet containing low level of phytase showed higher P and Ca content within the tibia than broilers fed diet containing high level of phytase.

Data of tibia Ca percentage showed that birds fed (100/75, T3) diet had significantly ($P\leq 0.05$) higher values compared to those fed (100/100, T1) without phytase or (100/100, T2) with phytase.

Data of tibia P percentage showed no significant ($P>0.05$) differences between groups except T1 and T3. Tibia P% of broiler had diet (100/75) was lower significantly than that (100/100) without phytase. In addition, it is noticed that birds fed (100/75) diet appeared appreciably equivalent to those fed T2 (100/100) or T5 (75/75) and T6 (75/50) diets. These results agree with **Thabet et al (2014)**. Results were generally in harmony with **Abdelaziz (2011)** and **Yan et al (2005)**, bone mineral content of birds at

Impact of Phytase Supplementation in Restricted Calcium and Phosphorus Broiler Diets on Performance, Blood Parameters and Bone Characteristics 667

Table 2. Effect of different dietary treatments on productive performance of broiler chicks

Items	Dietary Treatments (0-42 days)								SEM	P-value
	T1	T2	T3	T4	T5	T6	T7			
Live body weight (g)										
3 weeks	654.9	654.07			669.66		696.03		17.47	0.5279
6 weeks	1816.13 ^e	1879.03 ^d	2032.0 ^a	1941.8 ^c	1941.1 ^c	1799.6 ^e	1984.04 ^b		9.74	0.0001
Body weight gain (g/n)										
0-3 weeks	609.3	609.51			624.19		652		18.13	0.4760
3-6 weeks	1161.23 ^d	1232.33 ^c	1385.63 ^a	1272.70 ^{bc}	1267.30 ^{bc}	1134.13 ^d	1288.00 ^b		14.35	0.0001
0-6 weeks	1770.53 ^e	1832.83 ^d	1988.23 ^a	1898.13 ^c	1896.93 ^c	1752.90 ^e	1940.00 ^b		15.43	0.0001
Feed consumption (g)										
0-3 weeks	868.33	811.1			834.66		864		18.33	0.5285
3-6 weeks	2488.85	2550	2651.33	2511.78	2523.5	2444.68	2615.64	31.98	0.8878	
0-6 weeks	3357.19	3367.67	3464.67	3314.11	3363.52	3274.01	3479.64	48.1	0.8878	
Feed conversion ratio (g feed/ g gain)										
0-3 weeks	1.42	1.33			1.33		1.33	0.04	0.6324	
3-6 weeks	2.14	2	1.91	1.97	1.99	2.15	2.03	0.07	0.5425	
0-6 weeks	1.89	1.83	1.74	1.74	1.77	1.87	1.79	0.05	0.5753	

^{a, b, c, d, e} Means within the same row with different superscripts are significantly different. SEM = standard error of means.

Table 3. Effect of different dietary treatments on some blood plasma parameters at 42 days of age

Items	Dietary Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
Calcium (mg/dl)	8.9	8.92	8.9	8.79	8.54	8.76	8.8	0.21	0.8941
Phosphorus (mg/dl)	7.64 ^a	7.53 ^{ab}	7.38 ^b	7.46 ^b	7.47 ^b	6.88 ^d	7.18 ^c	0.05	0.0001
GOT (U/dl)	227.53	227.2	226.93	227.23	226.81	226.86	225.22	1.14	0.8424
GPT (U/dl)	14.66	14.69	14.68	14.67	14.67	14.62	14.64	0.04	0.9467

^{a, b, c, d} Means within the same row with different superscripts are significantly different. SEM = standard error of means.

Table 4. Effect of different dietary treatments on some aspects of bone composition and measurements at 42 days of age

Items	Dietary Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
Bone Composition									
Tibia Ash %	40.00 ^a	36.33 ^{ab}	34.67 ^{ab}	33.35 ^b	33.33 ^b	33.31 ^b	30.23 ^c	0.22	0.0002
Tibia Ca %	19.99 ^c	20.13 ^{bc}	22.53 ^a	20.95 ^{cb}	19.70 ^c	20.11 ^{cb}	21.32 ^{ab}	0.38	0.0015
Tibia P %	13.92 ^a	11.15 ^{bc}	10.05 ^c	11.85 ^b	11.25 ^{bc}	11.26 ^{bc}	11.53 ^b	0.34	0.0005
Bone Measurements									
TBS(Kg/cm ²)	31.63 ^a	31.10 ^a	30.07 ^b	29.80 ^b	30.13 ^b	29.50 ^b	29.97 ^b	0.23	0.0004
Tibia Seedor Index (SI)	1.09	1.03	1.17	1.01	1.02	1.11	1.18	0.08	0.6562

a, b, c, d, e Means within the same row with different superscripts are significantly different. Seedor et al (1991). SEM = standard error of means. TBS= Tibia breaking strength

(100/100) treatment had the highest values. The authors indicated that when expressing tibia ash (g) in relation to consumed NPP or Ca (g), birds fed (100/75) had higher ash weight per gram of NPP or Ca consumed at all ages as compared with that of birds fed the (100/100) treatments. This could be attributed to considerable adaptation to P and Ca restriction in (100/75) birds. These observations agreed with Coto et al (2008a and b), Fritts and Waldroup (2003).

Tibia breaking strength (TBS) was higher significantly ($P > 0.05$) values for broilers fed (100/100) diets with or without phytase. Data were agreement with Xian et al (2013) who found that supplementation of phytase significantly improved ash percentage and P content of tibia at 42 days of age and tended to increase breaking strength of chickens at 42 days of age.

Tibia breaking strength reflects the rigidity of bones. The low breaking strength values in medium NPP treatment on day 42 meant that the tibia was more fragile, thus likely indicating the diet was deficient in P. Tibia Seedor index (SI) values showed that birds have non-significant ($P \geq 0.05$) differences among all treatments. Data of bone measurements were generally in harmony with Abdelaziz (2011).

Calcium and phosphorus retention

Calcium and phosphorus excretion and retention of broiler chicks fed different dietary treatments are summarized in Table (5). Broilers fed diet of T1 (100/100; negative control; without phytase) had the highest significant records of P and Ca excretion

then broilers of T2 (100/100; positive control; with phytase) than those of other groups. Decreasing dietary P showed decreasing of P and Ca excretion which reflect on enhancement retention of both P and Ca retention. These results mean that the unused portions of the phosphorus as well as the indigestible phytate are excreted resulting in high percent of P in manure in broilers fed T1 while adding phytase for others groups resulting in decreasing P excretion which reflected on Ca and P retention percentage. The results are confirmed by results of El-Sherbiny et al (2010) that found decreasing dietary dicalcium phosphate (DCP) showed significant decrease in the excreted Ca and P, using enzymes in poultry diets improves availability of certain nutrients, mainly phosphorus and calcium, diminishing its presence on excreta. In addition, Cowieson et al (2004) reported that high percent of phytate consumption in birds' diets is recovered in the excreta which reflect on increasing cost of the diets and contributes to environmental pollution (Pallauf et al 1994; Musapuor et al 2006). So, phosphorus is one of the most effective factors in environment contamination. Hence, when feeding broiler chickens P deficient diets, a reduction in the dietary Ca content lead to an improvement in P digestibility, bone P and performance (Walk et al 2012). The increased broiler chick's performance could also attribute to increased activity of endogenous phytase at lower Ca level. Similarly, Bradbury et al (2014) noted that birds given a ration with Ca: NPP ratio of 4:1 had the lowest tibia ash and standing ability in comparison to lower ratios, resulting in impaired mobility.

Table 5. Effect of different dietary treatments on calcium and phosphorus retention at 42 days of age

Items	Dietary Treatments								
	T1	T2	T3	T4	T5	T6	T7	SEM	P-value
P Excretion %	47.12 ^a	44.32 ^b	40.37 ^c	26.29 ^d	27.04 ^d	23.35 ^e	24.60 ^{ed}	1.48	0.0001
P Retention %	52.88 ^e	55.68 ^d	59.63 ^c	73.71 ^b	72.96 ^b	76.65 ^a	75.40 ^{ab}	1.48	0.0001
Ca Excretion %	50.09 ^a	54.35 ^a	35.42 ^b	24.01 ^{cd}	21.85 ^d	29.92 ^{cd}	22.96 ^{cd}	2.36	0.0001
CaRetention %	49.90 ^d	54.65 ^d	64.58 ^c	75.99 ^{ab}	78.15 ^a	70.08 ^{bc}	77.04 ^{ab}	2.36	0.0003

^{a, b, c, d, e}Means within the same row with different superscripts are significantly different. SEM = standard error of means.

CONCLUSION

We can conclude that reduced requirements of both Ca and NPP in broiler diets by about 75% with adding phytase as in (100/75) treatment, to save cost of broiler feed decreasing as well as without any adverse effects on performance, blood and bone features.

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تأثير إضافة إنزيم الفيتيز لعلائق بداري التسمين المنخفضة في الكالسيوم والفوسفور على الأداء الإنتاجي وصفات العظام

[49]

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الموجز

(75/75) مغذاه على بادىء T75 ثم نامى T75، (50/75) مغذاه على بادىء T75 ثم نامى T50 و (50/50) مغذاه على بادىء T50 ثم نامى T50. احتوت كل معاملة على 30 طائر مقسمة الى 3 تكرارات بكل منهم 10 طيور. أوضحت النتائج أن وزن الجسم والوزن المكتسب اليومي للطيور لم يتأثر معنويا بالتغذية على علائق منخفضة في الكالسيوم والفوسفور حتى مستوى (75/100)، بينما لم تتأثر كل من الاستهلاك الغذائي اليومي ومعامل التحويل الغذائي معنويا. لم يتأثر تركيز الكالسيوم وتأثر الفوسفور معنويا، بعكس نشاط إنزيم GOT، GPT في بلازما الدم الذي لم يتأثر معنويا بخفض الكالسيوم والفوسفور مع إضافة إنزيم الفيتيز. تأثرت نسبة الرماد لعظمة الساق وكذلك نسبة الكالسيوم والفوسفور معنويا بخفض الكالسيوم والفوسفور. بنفس الاتجاه، فقد تأثرت قوة كسر عظمة الساق بتأثير المعاملات الغذائية.

ويستنتج من هذه الدراسة أن التحديد الغذائي المبكر لعنصري الكالسيوم والفوسفور مع إضافة إنزيم الفيتيز يمكن أن يحافظ على الأداء الإنتاجي ومقاييس جودة العظام وذلك حتى مستوى انخفاض 75% من الاحتياجات من العنصرين خلال فترة النامي.

الكلمات المفتاحية: إنزيم الفيتيز، كالسيوم، فوسفور، بداري التسمين، الأداء الإنتاجي للدم، عظمة الساق

تم استخدام 210 كتكوت تسمين غير مجنس عمر يوم لمدة 6 أسابيع لدراسة تأثير التحديد الغذائي لعنصري الكالسيوم والفوسفور مع إضافة إنزيم الفيتيز على الأداء الإنتاجي ومقاييس العظام ونسبة العنصرين في الزرق لبداري التسمين. استخدمت ثلاث علائق بادىء في الفترة من 1 وحتى 21 يوم: T100 (100% من احتياجات الكالسيوم والفوسفور متاح) 1.00% كالسيوم و 0.50% فوسفور متاح؛ T75 (75% من الاحتياجات) 0.75% كالسيوم و 0.38% فوسفور متاح و T50 (50% من الاحتياجات) 0.51% كالسيوم، 0.25% فوسفور متاح. كذلك تم استخدام ثلاث علائق نامي في الفترة من 21 وحتى 42 يوم: T100 (100% من الاحتياجات) 0.91% كالسيوم، 0.46% فوسفور متاح؛ T75 (75% من الاحتياجات) 0.68% كالسيوم، 0.34% فوسفور متاح و T50 (50% من الاحتياجات) 0.45% كالسيوم، 23% فوسفور متاح مع إضافة إنزيم الفيتيز 10000 وحدة دولية لكل كيلو جرام. تم توزيع الطيور على سبع معاملات غذائية على أساس ما يغذى عليه الطيور في مرحلتي البادىء والنامي بالتتابع لتكون: (100/100) مغذاه على بادىء T100 ثم نامى T100، (75/100) مغذاه على بادىء T100 ثم نامى T75، (50/100) مغذاه على بادىء T100 ثم نامى T50،

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