Egypt. Poult. Sci. Vol (37)(IV): (1105-1117)(2017) **(1763)**

Egyptian Poultry Science Journal

http://www.epsaegypt.com

ISSN: 1110-5623 (Print) – 2090-0570 (Online)

INFLUENCE OF Escherichia coli 6-PHYTASE SUPPLEMENTATION ON PERFORMANCE AND EGG QUALITY IN HI-SEX LAYING HENS FED PHOSPHORUS DEFICIENT DIETS

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Received: 08 / 10 /2017 Accepted: 23 / 10 /2017

ABSTRACT: The purpose of this study was to examine the Escherichia coli 6-phytase on performance and egg quality in Hi-Sex laying hens fed phosphorus-deficient diets. A total of 21600 Hi-Sex laying hens (48 week old) were divided in to three treatment groups and each treatment has 6 replicates (n= 1200 birds). The first group fed a basal diet (0.39% AP) as positive control, the second group fed phosphorus-deficient diet (0.26% available phosphorus) as negative control and the third group fed phosphorus-deficient diet (0.26% available phosphorus) plus 60 g/ ton feed Escherichia coli 6-phytase (500 FTU/Kg) for 30 days. All hens were fed the same amount of diets being, 112 gram / day during all the experiment period. Egg production, egg weight and egg quality characteristics were measured three times during the experiment at 0 day, 14 days and 30 days. Egg yolk and albumin weights were not influenced by reducing either available phosphorus or phytase supplementation. While; shell weight and shell thickness were decreased by reducing available phosphorus while improved by added phytase to the negative control. Furthermore, egg production, shell quality and feed conversion ratio were improved at the termination of the trial by added E. coli 6-phytase to the negative control. Interestingly, egg yolk cholesterol was decreased by phytase supplementation on the other hand; calcium, phosphorus and fat contents in yolk were increased. In conclusion, the results indicated that Escherichia coli 6-phytase was efficacious in corn-soybean meal-phosphorus deficient diets fed to Hi-sex laying hens and can be used to reduce diet supplementation with inorganic phosphorus and to improve egg shell quality

Key words: Escherichia coli 6-phytase - Hens - Egg quality - Yolk cholesterol.

INTRODUCTION

Phosphorus is a needful mineral, pivot to growth and development both structurally and metabolically in poultry (Boiling et al., 2000). The phytate phosphorus in diets is poorly utilized by poultry in spite of the presence of phytase activity in the brush border membrane of their digestive tracts (Maenz and Classen, 1998). As an outcome, inorganic phosphorus is added to poultry diets to favor optimal growth and production. This pursuit ultimately leads to a large part of dietary phosphorus not being utilized by the birds and being excreted in feces.

A lot of the total phosphorus found in domestic animal diets is existing in the form of phytate. The phytate-bound phosphorus is broadly unavailable to poultry as they do not surely have the enzymes needed to break it down. The ability of poultry to utilize phytate phosphorus is poor (Ravindran et al., 1995) due to either scanty quantity or a lack of intestinal phytase excretion. This weakness of poultry to utilize phytate phosphorus results in the excretion of large amounts of phosphorus in the feces, posing an environmental apprehension mostly in areas of bushy poultry production (Kornegay, 1996). The laying hens required phosphorus for production of eggs, surrogate of tissue metabolites such as nucleotides and phospholipids and to preserve skeletal integrity. Phytate was observed to reduce mineral bioavailability as well as nutrient digestibility, it complexes with protein and inhibits μ-amylases, trypsin, tyrosinase and pepsin (Caldwell, 1992).

An exogenous phytase can be added to laying hens diets to hydrolyze phytate within the digestive tract, making more phytate phosphorus obtainable for use by the poultry and reducing the necessity for dietary inorganic phosphorus supplementation (Maenz, 2001). Microbial phytase supplementation has the capability to significantly improve phytate P utilization in laying hens diets and the results have been well documented by several studies (Snow et al., 2004; Hughes et al., 2009). The phytase enzymes diverge in the source from which they are derived (fungi or bectraia). They may diverge in quality such as optimum pH, calorific stability, and capacity to fight hydrolysis within the digestive tract of the birds. Any difference in these merits will affect the ability of the phytase enzyme to function effectively and systematically within the digestive tract (Onyango and Adeola, 2009). Thus, all phytase enzymes produced must be examined in vivo to ensure efficacy before they are introduced to the poultry feed market. There have been a few studies carried out on the effect of an E.coli-derived 6-phytase supplementation in laying hen diets. So, the present study was, therefore, conducted to assess the efficacy of Escherichia coli-derived 6-phytase on performance and egg quality in Hi-sex laying hens fed phosphorus-deficient diets

MATERIALS AND METHODS

Animals and experimental design

The present study was done in the research unit of Al-Sabeel Al-Gadidah Company for poultry production, Tanta, Al-Gharbia, Egypt and affirmed by the Committee on the Ethics of Animal Experiments of Kaferelsheikh University,

Egypt. All transactions and trials arraigned with the guidelines and were advisable by the local moral commission of the bird experiments of the Department of Poultry Production, Faculty of Agriculture, Kaferelsheikh University, with respect to animal experimentation and care of animals under study. Twenty-one thousand and six hundred Hi-Sex laying hens (48 week old) were divided in to three groups and each treatment has six replicates (r= 1200 birds). The first group fed a basal diet (positive control with 0.39% AP), the second group fed phosphorus-deficient diet (0.26% available phosphorus as a negative control) and the third treatment fed phosphorus-insufficient diet (0.26% available phosphorus) plus 60 g/ ton feed Escherichia coli 6-phytase (500 FTU/Kg) for 30 days. All birds were fed the same feed amount 112 gram / day during all the experiment period. The composition and calculated analysis of the diets are shown in Table 1. The phytase used in this trial was a bacterial phytase source which developed E. coli 6-phytase (Quantum phytase, AB Vista Feed ingredients provided by Egavet Company, 106, King Faisal Street, Giza, Egypt). The optimal dose of phytase is 60 g/ton feed in laying hens diets. The experiment was conducted in close system farm with 15 h light: 9 h dark cycle. Room temperature was preserved as 24°C with prorated humidity from 50 to 70 % during the experiment.

Production and Quality of Eggs and yolk minerals

Egg production ratio and egg weight were measured every day during the experimental time. Egg quality was analyzed three times during the experiment at beginning and after two and four weeks by measured egg, yolk, albumin, shell weights and shell thickens for 30 eggs per group. The egg weight and egg quality estimate was completed on individual eggs. Egg yolk minerals content (Ca and P) were measured by the assimilation method. At the termination of the trial, 30 egg yolks per group were used to analyze calcium, phosphorus and total cholesterol contents (Saleh et al., 2017). Egg yolk samples were analyzed for dry matter (DM) by drying samples at 105 ◦C for 24 h in forcible air oven. The crude protein was specified by using Kjeldahl method and crude fat was specified by Soxhlet using AOAC methods (AOAC, 1995). Total cholesterol was determined in the fat via extraction from the egg yolk with a chloroform and methanol admixture (2:1 vol: vol). Total cholesterol was detached from total yolk fat after saponification with KOH and extraction with ethyl Ether by the modified method of the (Folch et al., 1957; Saleh, 2013). The sample was subjected to chromatographic analysis in a PU-4600 (PyeUnican, Cambridge, UK) chromatograph with a flame ionization detector, under the following conditions: the length of a glass column, 1 m; internal diameter, 4 mm; temperature: detector, 300°C; injector, 290°C; column, 260°C; carrier gas, argon; flow rate, 50 cm^3 /min; and internal criterion Dotriacontane (Sigma, St. Louis, MO).

Statistical Analysis

The differences between the treatments and the control were analyzed with a General Liner model using program SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released at 23 August 2008). Tukey's multiple comparison test

was used to identify which treatments conditions were significantly different from each other at a significance level of $p<0.05$.

RESULTS

Laying hens' production and egg quality at beginning of experiment are summarized in Table 2. There no difference in egg production, egg weights, FCR and all egg quality characterization between the two experimental groups and control group. The effect of 6-phytase on egg quality in laying hens fed phosphorus-deficient diets after two weeks of the experiment is presented in Table 3. Egg yolk and albumin weights were not affected by reduced phosphorus or added phytase. However, shell weight and shell thickness were significantly decreased in the negative control but they were improved in the negative control plus phytase compared to control group. The effects of 6-phytase on performance and egg quality in laying hens fed phosphorus-deficient diets after four weeks of the experiment and mortality ratio are abridged in Table 4. Egg production and feed conversion ratio were improved in phytase group compared with non phytase groups. Egg, yolk and albumin weights were not affected by reduced phosphors or phytase groups but shell weight and shell thickness and mortality rate were changed. Reducing phosphors caused an increase in mortality rate and decrease in shell weight and shell thickness. However, by added phytase in the negative control, shell weight and shell thickness were improved and the mortality rate was deceased.

Figure 1. showed the effect of 6-phytase on egg yolk calcium (A), phosphorus (B) and total cholesterol (C) content in laying hens. Calcium and phosphorus contents in egg yolk at the end of the experiment were decreased by decreased the phosphorus in the diets and they improved after added phytase. Moreover, egg yolk total cholesterol was decreased by feeding phytase as compared to non phytase groups. Crude protein content in egg yolk was not affected by reduced phosphorus or phytase while, fat content was increased by phytase group compared to the non-phytase groups (figure 2).

DISCUSSION

Egg production and feed conversion ratio were improved and mortality rate was decreased in this study by added phytase to the laying hens fed phosphorus-deficient diets as observed in Table (4). These results are in agreement with Punna and Roland (1999) who reported that incorporating 300 U/kg of phytase into a laying hen diet containing 0.1% nonphytate phosphorus performed an improvement in egg production and feed conversion over with a decrease in mortality rate. Lim et al. (2003) found that supplementing a low phytate laying hens diet with phytase increased egg production. Moreover, Van Der Klis et al. (1997) found that performance and egg production were significantly improved by dietary supplementation with phytase, whilst Jalal and Scheideler (2001) found that phytase supplementation in laying hens diets caused an improve in feed conversion and egg mass. Also, Francesch et al. (2005) reported that laying hens consuming a diet low in nonphytate phosphorus with supplementary phytase perfected as well as hens that were fed diets containing

higher levels of nonphytate phosphorus without supplementary phytase in the laying hen's diets.

Next to improvements in production, calcium, phosphorus and fat contents in egg yolk were increased by added phytase to the laying hens fed phosphorus-deficient diets (0.26% A.P). These results may be due to the improve of nutrients digestibility and availability by phytase addition in hen's diets. It is well familiar that phytate reduces the availability of phosphorus and other minerals, such as Ca, Zn, and Cu. In like manner, it is guessed that phytate may also affect the availability of nutrients such as protein, amino acids, and energy in laying hens diets ingredients. Due to its polyanionic nature, the phytic acid molecule can chelate di- or trivalent cations and react with nutrients (carbohydrates, fat and proteins); this caused reducing in their availability to laying hens reported by Selle et al. (2000). The improvements in protein and energy digestibility due to phytase supplementation would also be expectant to result in improved energy utilization in laying hens. Moreover, Jalal and Scheideler (2001) reported that supplementation of phytase in corn-soybean diets caused an improvement in Ca and P digestibility which might improve shell quality by 10%.

Shell weight and shell thickness were decreased by reducing nonphytate phosphorus (0.26%) but, they improved by added phytase. However, yolk and albumin weights were not influenced in this study. The improvement in shell quality may be due to the increased in P digestibility by phytase addition. These results are congruent with Casartelli et al. (2005) who studied the effect of phytase supplementation at levels (0, 1000 FTU/ Kg) on shell quality parameters from 32-48 weeks of hens age, they found an improvement in shell weight and shell thickness by phytase groups. Also, Plumstead (2007) reported that the increase of shell weight and the improving of shell thickness as a result of phytase supplementation in hens diets which let a good utilization of phosphorus by hens.

Total cholesterol content in egg yolk was decreased in this study. This is in agreement with Żyła et al. (2012) who found that yolk cholesterol was decreased by phytase supplementation in laying hens diets and they suggested that the decreasing of total cholesterol might be due to the increased of unsaturated fatty acids content in egg yolk by phytase supplementation in the Hi-sex laying hens diets.

CONCLUSION

Using Escherichia coli-derived 6-phytase can increase egg production and improve shell quality in Hi-sex laying hens fed phosphorus-deficient diets.

ACKNOWLEDGEMENT

The author is grateful to Al-Sabeel Al-Gadidah Company for poultry production, Egypt for supplying laying hens and experimental diets. Also, the author is thanking Egavet Company for providing the enzyme used in the experiment.

Table (1): The ingredient and nutrient composition of experimental diets

Ingredient (%)	**Positive control**	**Negative control**	**Negative control + phytase**
	---------- g/kg diet ----------		
Yellow Corn	615	617	617
Corn gluten, 62%	63	62	62
Soybean meal, 46%	143	143	143
Soy oil	3	3	3
Sunflower meal, 36%	43	43	43
Dicalcium phosphate	14.5	7	7
Wheat bran	7.5	9.5	9.5
Lysine	2	2	2
DL-Methionine	0.7	0.7	0.7
Limestone	95	99.5	99.44
Vitamins and minerals mixture[1]	3	3	3
NaCl	2.6	2.6	2.6
Sodium bicarbonate	2	2	2
Potassium carbonate	5.7	5.7	5.7
Phytase	-	-	0.06
Nutrient composition (calculated values)			
Metabolizable energy, Mcal/kg	2.751	2.753	2.755
Crude protein, %	17.01	17.01	17.01
Calcium, %	4.01	4.02	4.01
Total Phosphorus, %	0.62	0.48	0.48
Available Phosphorus, %	0.39	0.26	0.26
Sodium, %	0.183	0.183	0.183
Chloride, %	0.192	0.192	0.192
Lysine %	0.872	0.871	0.871
Methionine %	0.431	0.430	0.431

[1]Vitamin mineral premix (units per kilogram of feed): vitamin A, 10,000 IU; vitamin D3, 3,500 IU; vitamin E, 35 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B12, 0.012 mg; pyridoxine, 1.5 mg; thiamin, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; and zinc, 80 mg.

Table (2): Performance characteristics and egg quality parameters before starting the experiment

Ingredient	Positive control	Negative control	Negative control + phytase
Feed intake/hen/day(gram)	112 ± 0.04	112 ± 0.06	112 ± 0.03
Egg production (%)	88 ± 1.2	85 ± 2.3	86 ± 1.8
Egg weight (gram)	58.32 ± 2.4	56.81 ± 1.7	56.95 ± 1.4
FCR	2.20 ± 0.2	2.31 ± 0.3	2.28 ± 0.1
Yolk weight (gram)	15.76 ± 1.8	15.87 ± 1.4	15.91 ± 1.6
Albumin weight (gram)	36.55 ± 2.6	34.78 ± 2.8	34.92 ± 3.5
Shell weight (gram)	6.01 ± 0.9	6.15 ± 0.8	6.12 ± 0.5
Shell thickness (μm)	406.32 ± 25.9	413.00 ± 34.7	410.48 ± 37.4

Egg quality results are presented as means ± SEM (n=30).

Table (3): Effect of 6-phytase on egg quality characteristics in laying hens fed phosphorus-deficient diets after two weeks

Ingredient	Positive control	Negative control	Negative control + phytase
Egg weight (gram)	57.412 ± 2.2	57.90 ± 1.9	57.22 ± 2.4
Yolk weight (gram)	15.869 ± 1.2	15.57 ± 1.1	15.17 ± 1.4
Albumin weight (gram)	35.579 ± 2. 1	36.56 ± 2.2	36.03 ± 2.8
Shell weight (gram)	5.963 ± 0.5^{ab}	5.77 ± 0.5^{b}	6.20 ± 0.7^{a}
Shell thickness (μm)	408.00 ± 29.8^{a}	394.14 ± 24.8^{b}	408.67 ± 31.3^{a}

[a-c] Means within the same row with different superscripts differ ($p<0.05$). Egg quality results are presented as means ± SEM (n=30).

Table (4): Effect of 6-phytase on performance, egg quality characteristics and mortality in laying hens fed phosphorus-deficient diets after four weeks

Ingredient	**Positive control**	**Negative control**	**Negative control + phytase**
Egg production (%)	81 ± 1.5^{b}	84 ± 2.1^{ab}	87 ± 1.3^{a}
Egg weight (gram)	59.11± 1.2	58.35± 1.7	59.42± 2.1
FCR	2.33 ± 0.3^{a}	2.28 ± 0.3^{a}	2.16 ± 0.2^{b}
Yolk weight (gram)	15.15± 1.1	15.71± 1.5	15.91± 1.3
Albumin weight (gram)	37.99± 2.5	37.02± 2.0	37.46± 2.4
Shell weight (gram)	5.97± 0.4^{ab}	5.61± 0.4^{b}	6.05± 0.6^{a}
Shell thickness (μm)	401.67± 39.1^{a}	374.25± 34.8^{b}	402.86± 30.9^{a}
Mortality rate (%)	0.57 ± 0.08^{ab}	1.24 ± 0.07^{a}	0.47 ± 0.05^{b}

$^{a-c}$ Means within the same row with different superscripts differ ($p<0.05$). Egg quality results are presented as means ± SEM (n=30).

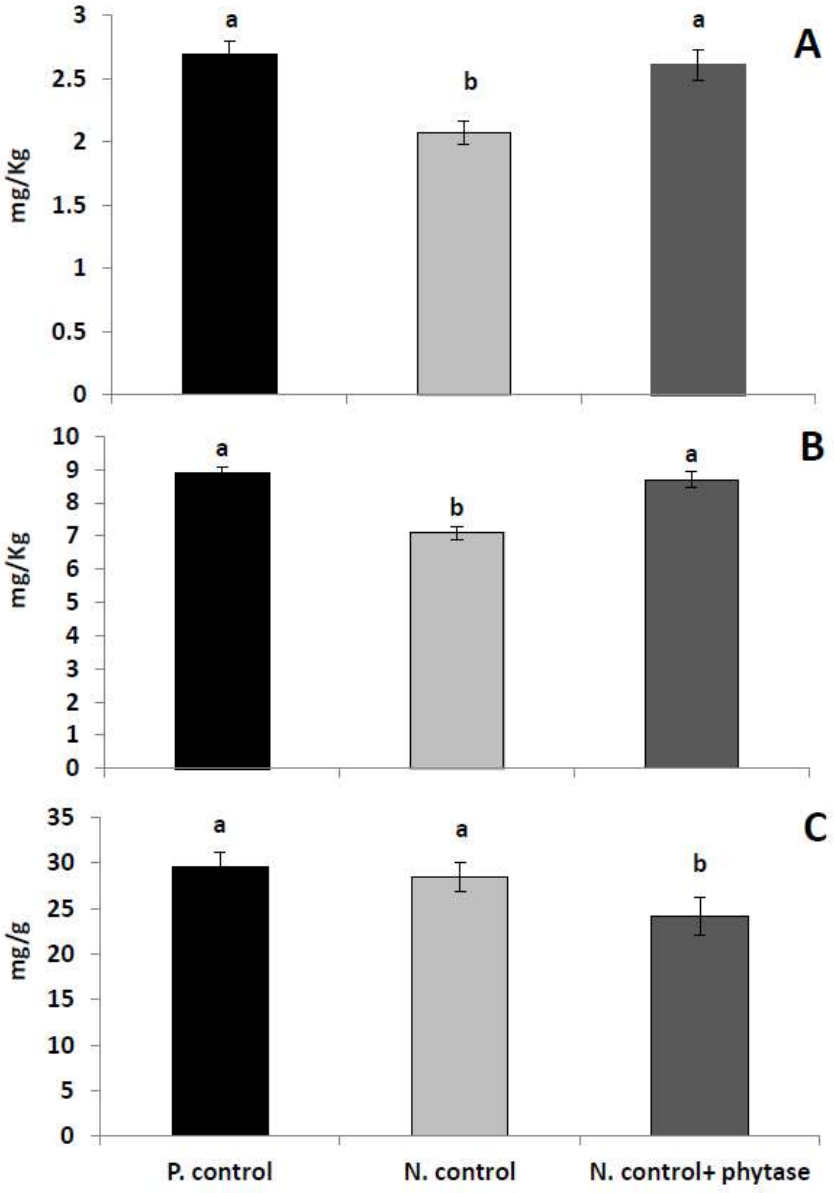

Figure (1): Effect of 6-phytase on egg yolk calcium (A), phosphorus (B) and total cholesterol (C) content in laying hens. [a-c] Means within the same row with different superscripts differ ($p<0.05$). The results are presented as means ± SEM (n=30).

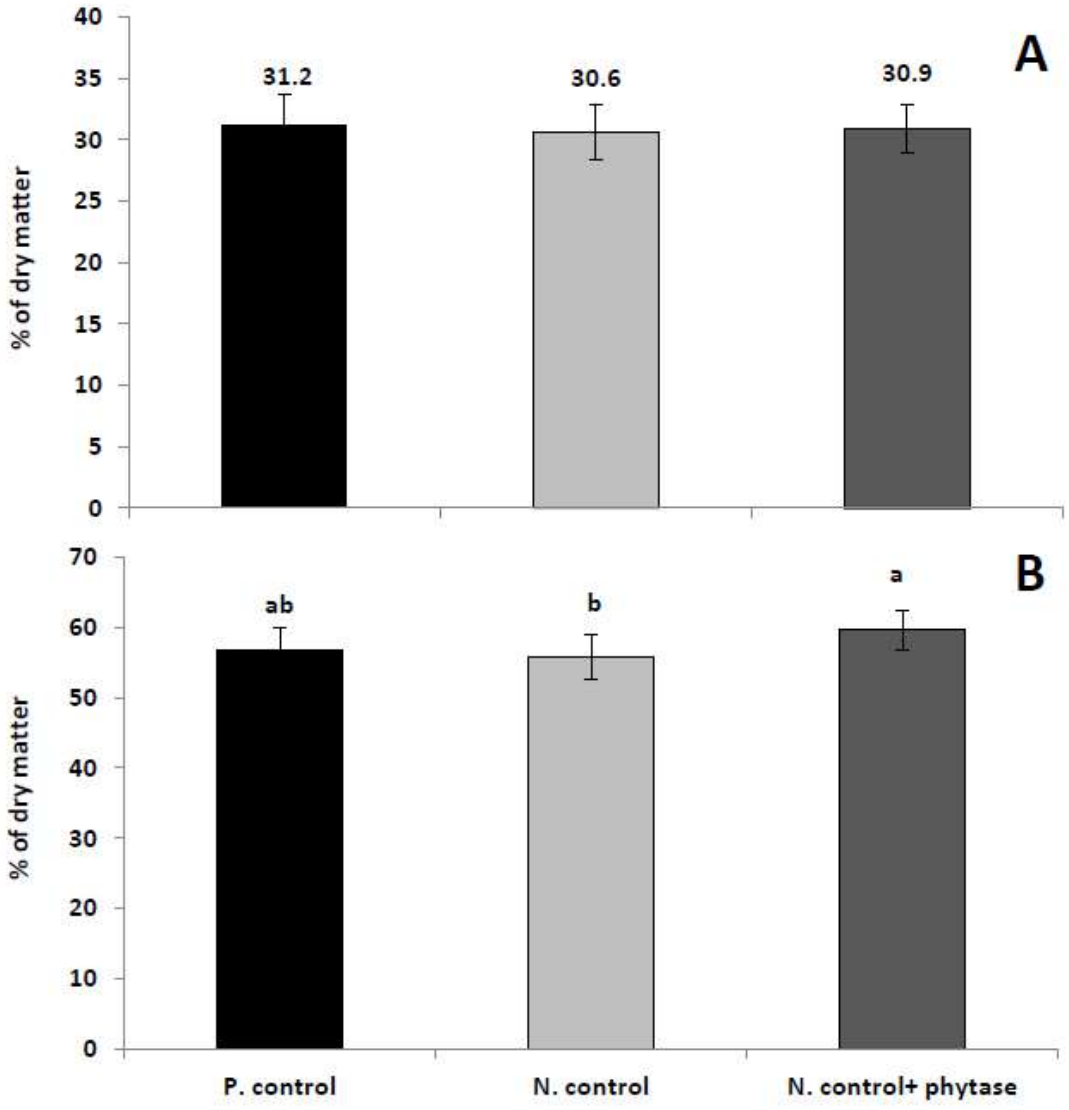

Figure (2): Effect of 6-phytase on egg yolk crude protein (A) and fat (B) contents in laying hens. [a-c] Means within the same row with different superscripts differ ($p<0.05$). The results are presented as means ± SEM (n=30).

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Escherichia coli 6-phytase - Hens - Egg quality - Yolk cholesterol.

الملخص العربي

تأثير إضافة انزيم الفيتيز-6 الناتج من الإيشريشيا كولاي علي الأداء الإنتاجي وجودة البيض للدجاج البياض (هاي سكس) المغذي علي علائق منخفضة في محتواها من الفوسفور

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تمت هذة الدراسة لإختبار تأثير إضافة انزيم الفيتيز-6 (500 FTU/Kg) الناتج من بكتيريا الإيشريشيا كولاي علي الأداء الإنتاجي وجودة البيض في الدجاج البياض المغذي علي عليقة منخفضة في محتواها من الفوسفور. تم استخدام 21600 دجاجة بياضة (هاي سكس) عند عمر 48 أسبوع وتم تقسيمهم إلى ثلاثة معاملات (3 عنابر) وكل معاملة تنقسم إلى 6 مكررات وكل مكررة تتكون من 1200 طائر. المجموعة الأولي تتغذي علي العليقة الأساسية (كنترول موجب يحتوى 0,39% فوسفور متاح) والمجموعة الثانية تتغذي علي عليقة منخفضة في محتواها من الفوسفورالمتاح (0,26%) (كنترول سالب) والمجموعة الثالثة تتغذي علي عليقة الكنترول السالب مضاف اليها 60 جرام انزيم الفيتيز-6/ طن علف واستمرت التجربة حتي 30 يوم وخلال هذه الفترة كانت جميع الطيور تتغذي علي مقدار ثابت من العليقة 112(جرام/يوم/ طائر). تم قياس إنتاج البيض ووزن البيض يومياً مع قياس جودة البيض علي ثلاثة فترات في بداية التجربة وبعد اسبوعين وفي نهاية التجربة. تشير النتائج إلى عدم تأثر كلاً من وزن البيضة ونسبة البياض والصفار بإنخفاض نسبة الفوسفور أو بالإنزيم بينما إنخفض سمك القشرة ووزن القشرة بخفض نسبة الفوسفور في العليقة (معاملة 2) بينما تحسنت عند إضافة الإنزيم (معاملة 3). تحسن معدل إنتاج البيض وكذلك نسبة الاستفادة من العليقة في نهاية التجربة بإضافة انزيم الفيتيز إلى العليقة المنخفضة في نسبة الفوسفور. ومن المثير للإهتمام إنخفاض نسبة الكوليسترول الكلي في صفار البيض، علي الجانب الآخر ارتفع محتوي الصفار من الكالسيوم والفوسفور والدهون الكلية . تلخص هذة النتائج أن إستخدام انزيم الفيتيز-6 الناتج من بكتريا الإيشريشيا كولاي في علائق تتكون من ذرة صفراء وكسب فول صويا ومنخفضة في نسبة الفوسفور يحسن من الإستفادة من الفوسفور، ويقلل من إستخدام الفوسفور غير العضوي في العلف وكذلك يحسن من جودة قشرة البيض.