EFFECT OF PHYTASE SUPPLEMENTATION ON EGG PROUDUCTION AND EGG SHELL QUALITY OF AGED LAYING HENS

By

ZEINAB HAMED EL-SAID MAHMOUD

B. Sc. Agric. Sc. (Poultry Production), Cairo University, 2007

A thesis submitted in partial fulfillment

of

the requirements for the degree of

In
Agricultural Science
(Poultry Nutrition)

Department of Poultry Production Faculty of Agriculture, Ain Shams University

Approval Sheet

EFFECT OF PHYTASE SUPPLEMENTATION ON EGG PROUDUCTION AND EGG SHELL QUALITY OF AGED LAYING HENS

By

ZEINAB HAMED EL-SAID MAHMOUD

B. Sc. Agric. Sc. (Poultry Production), CairoUniversity, 2007

This thesis for M.Sc. degree has been approved by: Dr. Ahmed Hussein Abd El Maged Head of Research, Animal Production research institute, Agricultural research center Dr. Nabil Mohamed Hassan El-medany Prof. of Poultry Nutrition, Faculty of Agriculture, Ain Shams University Dr. Mahmoud Yousef Mahrous Associated of Poultry Breeding, Poultry Production Department, Faculty of Agric., Ain Shams University Dr. Alaa El-Dien Abdel-Salam Hemid Prof. of Poultry Nutrition, Faculty of Agriculture, AinS hams University

Date of Examination: 28 / 6 / 2015

EFFECT OF PHYTASE SUPPLEMENTATION ON EGG PROUDUCTION AND EGG SHELL QUALITY OF AGED LAYING HENS

By

ZEINAB HAMED EL-SAID MAHMOUD

B. Sc. Agric. Sc. (Poultry Production), Ain Shams University, 2007

Under the supervision of:

Dr. Alaa El-Dien Abdel-Salam Hemid

Prof. of Poultry Nutrition, Department of Poultry Production, Faculty of Agriculture, Ain Shams University(Principal supervisor)

Dr. Mahmoud Yousef Mahrous

Associated of Poultry Breeding, Department of Poultry Production, Faculty of Agriculture, Ain Shams University

Dr. Hany Ali Thabet

Lecture of Poultry Nutrition, Poultry Production Department Faculty Faculty of Agriculture, Ain Shams University

ABSTRACT

Zeinab Hamed El-Said Mahmoud: Effect of Phytase Supplementation on Egg Production and Egg Shell Quality of Aged Laying Hens. Unpublished M. Sc. thesis, Department of Poultry Production, Faculty of Agriculture, Ain Shams University, 2015.

This study was carried out at the poultry breeding Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University. The study aimed to investigate the effect of different levels of phytase on egg production and eggshell quality measurements in laying hen of(Hyline;W36 and Brown) layers during the last period of production. Feed was offered *ad lipitum* in metal feeders; water was supplied through automatic nipples. The layers were directly fed a commercial diet according to the producer recommended manual guide of Hy-Line layers 2011 from 52 week of age to 64 week of age as control treatment and supplied 2 levels of phytase (300FTU- 450FTU).

Data presented in this study showed an improvement in egg mass of bird fed phytase 450 FTU compared with control group but phytase 300 FTU was intermediate. This was the trend of egg number for white Hyline hens. Increasing egg production as a results of Phytase supplementation may be due to that phytate presented in the most ingredients of poultry diet is capable of forming complexes with essential nutrients such as protein and some in organic captions. The main effects data indicated that, egg mass at 52 and 64 weeks of ages weren't significantly different, it can be observed that, the addition of phytase enzyme at a levels of 300 and 450 (FTU) kg⁻¹ of feed led to an increase in the number of eggs compared to control.

Kye Words:

Phytase, egg production, egg quality and feed consumption.

ACKNOWLEDGMENTS

Firstly, I wish to express my prayerful thanks to **ALLAH** for every thing. My deepest gratitude and sincere thanks are extended to **Prof. Dr. Alaa El-Dien Abdel-Salam Hemid,** professors of poultry nutrition, Department of poultry production, Faculty of Agriculture, Ain Shams University, in favor of his profitable support and precious advice throughout the practical study. I wish to express my sincere gratitude to **Dr.Mahmoud Yossef**, Associate Professor of Poultry Breeding, Poultry Production Department, Faculty of Agriculture, Ain Shams University for his supervision, encouragement and interest and I wish to express my sincere gratitude to **Dr. Hany Ali Thabet,** Lecture of Poultry Nutrition, Poultry Production Department Faculty of Agric., Ain Shams University for his supervision, encouragement and interest. And My sincere gratitude also is due to Mr. **Abdelmoniem Mohamed Abdelmoniem** about his assistant.

Finally all thanks to father ask my Allah peace him, mother, my husband and my sons for their encouragement, brother, and sisters for they love.

CONTENTS

LIST OF TABLES	ii
LIST OF FIGURES	iii
I- INTRODUCTION	1
2. REVIEW OF LITERATURE	3
2.1. Overview of phytase	7
2.2. Effect of phytase on performance	12
2.2.2.Feed intake	12
2.1. Body weight and body weight gain	13
2.2.3. Feed efficiency	14
2.2.4. Egg production characteristics and eggshell quality	17
3. MATERIALS AND METHODS	17
3.1. experimental design	18
3.1.2.Experimental diets	19
3.2.Lighting Program	19
3.3. Vaccination	19
3.4. Measurements and observations	19
3.4.1. Body weight and weight gain	19
3.4.2. Feed consumption and feed conversion	19
3.4.3. Egg production measurements	19
3.4.4 Egg quality measurements	20
3.4.5.Hugh units	20
3.4.6. Strength of eggshell	20
3.6. Statistical analysis	21
4-RESULTS AND DISCUSSION	23
4.1. Body weight and body weight gain	23
4.2. feed consumption and feed conversion ratio.	25
4.3. Egg production	31
4.4.internal egg quality	37
4.6.Shell parameter.	52
5. SUMMARY AND CONCLUSION.	56
6. REFERENCES	58
7.ARABIC SUMMARY	

LIST OF TABLES

No.		Page
1	Composition and calculated analysis of experimental	17
	diets	
2	Number of layers for each treatment in the 2 strains.	18
3	Effect of different treatments on bodyweight g/h of	24
	layers hens overall period.	
4	Effect of different treatments on Feed consumption	27
	and Feed conversion of layers hens (52-56) week	
5	Effect of different treatments on Feed consumption	28
	and Feed conversion of layers hens (57-60)week	
6	Effect of different treatments on Feed consumption	30
	and Feed conversion of layers hens (61-64)week	
7	Effect of different treatments on Egg mass, Egg	33
	production and Egg weight of layers hens (52-	
	56)week	
8	Effect of different treatments on Egg mass, Egg	34
	production and Egg weight of layers hens (61-64)	
9	Effect of different treatments on Egg mass, Egg	35
	production and Egg weight of layers hens (57-60)week	
10	Effect of different treatments on internal egg quality of	39
	layers hens(53-56)week	
11	Effect of different treatments on Internal egg quality of	40
	layers hens (57-60)week	
12	Effect of different treatments on Internal egg quality of	41
	layers hens (61-64)week	
13	Effect of different treatments on external egg quality	46
	(53-56)week	
14	Effect of 57-60different treatments on external egg	47
	quality(53-56)week	

15	Effect of different treatments on external egg	48
	quality(57-60)week	
16	Effect of different treatments on Shell parameter of	52
	layers hens (53-56)week	
17	Effect of different treatments on Shell parameter of	53
	layers hens (57-60)week	
18	Effect of different treatments on Shell parameter of	54
	layers hens (61-64)week	

LIST OF FIGUR

No.		Page
1	Effect of different treatments on bodyweight g/h of layers hens overall period	25
2	Effect of different treatments on Feed Consumption (g/h)	29
3	Effect of different treatments on Feed conversion (g feed/g egg)	29
4	Effect of different treatments on Egg Mass (g)	36
5	Effect of different treatments on Egg Production (%)	36
6	Effect of different treatments on Egg Weight (g)	37
7	Effect of different treatments on Internal egg	42
8	quality(Yolk weight) Effect of different treatments on Internal egg quality(Albumin weight).	42
9	Effect of different treatments on Internal egg quality(Yolk%).	43
10	Effect of different treatments on Internal egg quality(Hough unit).	43
11	Effect of different treatments on Internal egg quality(Yolk Index).	44
12	Effect of different treatments on Internal egg quality(Albumen %).	44
13	Effect of different treatments on External egg quality (Shape index)	49
14	Effect of different treatments on External egg quality (Shell strength)	49
15	Effect of different treatments on External egg quality (Shell weight dry)	50
16	Effect of different treatments on External egg quality (Shell weight dry%)	50
17	Effect of different treatments on External egg quality (Shell thickness)	51
18	Effect of different treatments on Shell Calcium %	55
19	Effect of different treatments on Ash in the shell %.	55

1. INTRODUCTION

Phosphorus (P) is an essential mineral in all feeds for poultry. Phosphorus is one of the mineral essential for development structurally metabolically growth and production. Moreover, P is considered as an expensive nutrient that commonly supplemented in poultry diets. It represents the third most expensive nutrients following protein and energy. Birds diets are generally formulated based on corn, soyabean meal, approximately two-thirds of the total P in plants, which are the major constituents of poultry diets, is in the form of phytate. A number of studies have demonstrated that use of microbial phytase supplementation in feeding poultry has the ability to hydrolysis, releasing phytic acid in phosphate form NRC (1994) and adding microbial phytase in laying hen diet improves phytate P utilization and productive performance (Boling et al., 2000a, b; Jalal and Scheideler, 2001; Narahari and Jayaprasad, 2001; Keshavarz, 2003; Lim et al., 2003; Plumstead, 2007). Also, Francesch et al. (2005) and Jalal and Scheideler (2001) reported an improvement in egg production, hen weight gain, feed conversion, egg mass and feed consumption in hens that fed a diet low in NPP with supplementary phytase when compared to hens fed a low NPP diet without supplemental phytase.

Wu etal. (2006) reported that, Phyzyme or Natuphos supplementation into diets containing 0.11% nonphytate phosphorus significantly reduced excreta P (approximately 58 and 54%, respectively) with no adverse effect on egg production and egg mass. Plumstead (2007) studied the effects of varying dietary nonphytate phosphorus level with or without added phytase enzyme on performance of broiler breeders from 29 to 64 week of age. and found that, eggs per hen housed, hen day egg production (%), fertility (%) and feed per dozen eggs were increased when phytase was added by 500 FTU. Addition of phytase to the 0.15% nonphytate phosphorus diet improved total hen housed egg production to

the levels equal or better than the hens fed the 0.35% nonphytate phosphorus diet(**Hughes** *et al.*, **2008**). This study aimed to investigate the effect of different levels of phytase on egg production and eggshell quality measurements in laying hen of (Hy-lineW36 and Brown) layers during the last period of production.

2. REVIEW OF LETERATURE

2.1. Overview of phytase

Finding ways to reduce feed costs of production is not a new concept. The animal feed industry as a whole has been working toward solutions to lower costs up front and as a result have a lower cost at the end product for the consumer. However, recently with grain prices on the rise, producers are examining alternative ways to reduce feed costs.

Although there are a number of methods, the addition of exogenous enzymes has proven to be an effective cost saving mechanism, not only are previously unutilized portions of feedstuffs now accessible to the animal, they also allow the nutritionist to alter the content of the diet.

Exogenous enzymes have been available for a number of years; and recently their use has increased exponentially. This is mainly due to increased feed costs, for example, in 2008 corn rose from \$2 to approximately \$8 a bushel (Clark, 2009). Along with this trend, dicalcium phosphorous increased from \$200 per metric ton to \$1,000 per metric ton, and then stabilized near \$700 in 2008 (Clark, 2009). These are two examples of the high costs of diet components for laying hen rations

There are two different methods for incorporating exogenous enzymes into the formulated diet. The first of which is called the "over the top" method which improves performance economically, and consists of supplementing standard diets with enzymes without altering nutrient levels (Costa et al, 2008).

The second approach is to adjust the diet formulation by reducing nutrients and adding exogenous enzymes in order to restore nutritional value of the standard diet (Costa et al, 2008). Both of these methods lead to a reduction in costs, the second having the most dramatic drop in costs. The addition of exogenous enzymes to poultry diets has proven to be a substantial cost saver, on average the addition allows the producer to save approximately \$3 per metric ton (Clark, 2009).

In addition to the tangible cost savings that exogenous enzymes offer, there are also nutritional benefits. Exogenous enzyme supplementation in the diet improves production efficiency of poultry by increasing digestion of low quality products and reducing nutrient loss through excreta (Costa et al 2008).

Phytase is used to hydrolyze phytate, which is found in every vegetative ingredient. Phytate not only binds phosphorous but also binds other positively charged ions such as calcium, sodium, magnesium, potassium and zinc (Leeson and Summers, 2001; Selle and Ravindran, 2007; Costa et al 2008). Therefore when phytate is effectively hydrolyzed, it not only makes phosphorous more available to the animal but also any other positively charged cation that was bound to phytate. Due to the fact that enzymes have an effective cost saving effect coupled with an improvement in nutrient availability to the bird, enzymes are a viable cost saving solution in today's market

Inorganic phosphorous is an expensive ingredient in the poultry industry, as stated earlier, phosphorous in recent years has been priced as high as \$1,000 per metric ton (Clark, 2009). In addition to the high cost, the amount of phosphorous excreted by the animal is also an environmental concern. The environmental concern for phosphorous stems from the fact that poultry manure is used as fertilizer, and with excess phosphorous in the manure, it may not be properly utilized by plants, accumulating in the root zone, leaching, run off, and erosion can lead to pollution of surface water (Panda et al., 2005). This problem stems from the fact that a large portion of phosphorous in plant sources is in the form of phytic acid, which is unavailable to the bird. As a result, the producer has had to overcompensate for the lack of available phosphorous with the addition of inorganic phosphorous supplementation. However, with the use of phytase, the bird is able to break down the phytic acid molecule and for release phosphorous; as a result this reduces the amount of the potential pollutant in manure (Selle and Ravindran, 2007; Costa et al, 2008).

Phytase was first detected in 1907 in rice bran; however, attempts to develop it into a feed enzyme did not occur until 1962 (Selle and Ravindran, 2007). This interest became apparent in the late 1960's as numerous research articles were published, due to the concern with the negative effects of phytate on both calcium and phosphorous availability in broiler chicks (Selle and Ravindran, 2007). The first phytase feed enzymes became available in 1991, mainly due to legislation in the Netherlands demanding a decrease in phosphorous pollution in the environment (Selle and Ravindran, 2007). Phytase is effective as it allows previously unavailable phosphorous to become available as it breaks down phytate phosphorous compounds. Phytate phosphorous is commonly found in plant ingredient sources, as most of the phosphorous in plants is bound with phytic acid (Leeson and Summers, 2001).

In poultry specifically, there is no endogenous phytase produced, so as a result there is little to no phytic acid breakdown (Leeson and Summers, 2001). The phytic acid molecule has six phytic acid residues and these residues have a high affinity for several cations; and one mole of phytic acid can bind 3 to 6 moles of calcium (Scott et al., 2001). Along with calcium, other minerals that can bind with phytic acid are: sodium, magnesium, potassium, zinc and copper; phytic acid also as the ability to bind various amino acids forming insoluble phytate protein complexes (Costa et al 2008; Leeson and Summers, 2001). Thus, phytase catalyzes the hydrolytic cleavage of the phosphorus acid esters of inositol, which liberates phosphorous and allows it to be absorbed (Leeson and Summers, 2001). Along with phosphorous, calcium or any other cation or amino acid that was once bound to the phytic acid is now liberated and ready to be absorbed.

Phytase activity is defined as fytase units (FTU), where one FTU is the amount of enzyme that liberates 1 µmol inorganic orthophosphate/min from 0.00512 mol L -1 sodiumphytate at pH 5.5 and a temperature of 37°C (Selle and Ravindran, 2007). In addition to FTU, several other abbreviations such as FYT, U and PU have all been used to denote

phytase activity of different commercial microbial phytases (Selle and Ravindran, 2007).

Phytase feed enzymes can fall into two different categories depending on the site in which hydrolysis on the phytate molecule takes place (Selle and Ravindran, 2007). The two different forms are: 3-phytase (EC 3.1.3.8), which liberates the P moiety at the third carbon (C3), whereas 6-phytase (EC 3.1.3.26) commences at position C6 of the myo-inositol hexaphosphate ring (Selle and Ravindran, 2007).

There are several distinct microbial phytase currently available for poultry feed markets. The three most commonly used phytase feed enzymes are derived from A. niger, which is a 3-phytase, and Peniophoralyciiand Escherichia coli which are 6- phytases (Selle and **Ravindran**, 2007). Phytase feed enzymes are included in poultry rations as granulates or liquids in post-pelleting application systems, to avoid thermostability problems at high pelleting temperatures (>80°C) (Selle and Ravindran, 2007). The site of phytase activity in the gastrointestinal tract has received little attention. However based on previous research, it is likely that phytate hydrolysis mainly takes place in the fore-stomach (crop, proventriculus, gizzard) where the pH is more conducive to phytate activity (Selle and Ravindran, 2007). Based on numerous studies, the crop is the most likely primary site of degradation by exogenous phytase(Liebertet al., 1993; Takemasa et al., 1996; Kerr et al., 2000; Selle and Ravindran, 2007). Another aspect to consider when examining where phytase is the most active is to consider the type of bacteria the phytase is derived from. For example, E.coli derived phytase is more active in the small intestine than phytase derived from *P.lycii* (Onyango et al., 2005b, Selle and Ravindran, 2007). This may be the reason why E.coli derived phytase has a greater resistance to endogenous, proteolytic enzymes (Igbasanet al.; 2000; Selle and Ravindran, 2007).