

Effect of Chito-oligosaccharide as Feed Additives on Egg Production and Performance of Laying Hens

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Abstract

This study was carried out to evaluate the effect of Chitozinc and Chitonal on performance of laying hens. The studied parameters were feed intake, egg production %, egg weight, egg mass and feed conversion ratio as well as lipid profile in egg yolk. Sixty Lohmann Brown laying hens (34 weeks age) were assigned to three groups, each of 20 hens. The 1st group (control) was fed on basal diet only, the 2nd group was fed on basal diet +1ml of Chitonal /liter drinking water and the 3rd group was fed on basal diet + 200 gm. Chitozinc /ton. All groups were kept under observation for 45 days and egg production (EP) % , Feed intake FI (g/hen), average egg weight (EWT) in (g) , egg mass production (g/hen/day) and feed conversion ratio FCR (Kg feed/Kg egg) were calculated along the experiment. Eggs were collected from each group three days before the end of the experiment for biochemical analysis of fat content in egg yolk.

The results showed that addition of 1ml of Chitonal /liter to drinking water improved egg production (EP) % , Feed intake FI (g/hen), average egg weight (EWT), egg mass production (g/hen/day) and feed conversion ratio FCR (Kg feed/Kg egg) , and a significant decrease in total lipid, total cholesterol, low density lipoprotein (LDL) in egg yolk. A significant decrease in very low density lipoprotein (VLDL), triglyceride, and atherogenic index (AI) and a significant increase in high density lipoprotein (HDL) in egg yolk in both 2nd and 3rd groups was reported. The results clarified that, the use of Chitozinc and Chitonal improved the performance and lipid profile in egg yolk of laying hens.

Keyword: Chito-oligosaccharide, Chitosan, layers, egg, lipids profile.

Introduction

Chitosan is a nontoxic polyglucosamin widely spread in nature. It is deacetylated to varying degree from a chitin, a component of exoskeleton of shrimps, crabs and insects. (Choi et al., 2006 and Xia et al., 2011). Since Chitosan contains reactive functional groups as amino acids and hydroxyl groups it is characterized by antimicrobial, anti-inflammatory, antioxidative, antitumor, immunostimulatory and hypocholesterolemic

properties. In modern poultry production, many different feed additives are used to improve the performance. The most important and widely used are organic acids, enzymes, probiotics and prebiotics, and herb extracts. One relatively new and less widely used feed additive is Chitosan (COS), which is nontoxic polyglucosamin, seldom found in nature in some mushrooms consisting of β (1 \rightarrow 4)-2-acetamido-D-glucose and β (1 \rightarrow 4)-2-amino-D-glucose units. It is a deacetylated to varying degrees form of chitin, widespread in nature component of exoskeleton of shrimps, crabs and insects (**Koide, 1998 and Singla and Chawla, 2001**). Unlike chitin, COS is soluble in acidic solutions (**Shahidi et al., 1999**) and it is partially digested in the gastrointestinal tract of monogastric animals (**Hirano et al., 1990 and Okamoto et al., 2001**). Therefore, various oligosaccharides are now being added to livestock feed as prebiotics to improve animal health and production (**white et al., 2002 and Lemieux et al., 2003**).

In this experiment, Chitozinc and Chitonal were used at a concentration of 200gm /ton and 1ml /liter drinking water respectively; as a new physiological material because it is known to be nontoxic, bio compatible and biodegradable (**Choi et al., 2006**) and have anticancer (**Jeon and Kim,2002**), antibacterial (**Jeon et al.,2001**) and antifungal (**Zhang et al.,2003**) activities and reduce serum cholesterol level (**Muzzarelli et al., 1990 ; Kochkina and Chirkove, 2000 and Huang et al.,2005**). It was suggested that dietary Chito-oligosaccharide can increase nutrient digestibility and weight gain in broilers due to its antifungal and antimicrobial activities (**Joen et al., 2000**). Therefore, it can be postulated that, its supplementation can improve performance of laying hens. There are only few reports on the effect of dietary COS on growth performances on layers; Therefor the goal of this study was to investigate the effects of both Chitozinc and Chitonal as a COS supplements on growth performance and lipid profiles of egg yolk.

Materials and Methods

Materials:

Chitozinc and Chitonal soul made in Korea, Samyang Anipharm and distributed by Soggy Pharm Egypt.

Experimental design:

Sixty Lohman brown laying hens aged 34 weeks were assigned into three equal groups. Hens were provided daily with equal quantities of pre-weighed ration given in mash form according to (**NRC, 1994**). Groups treatments were as follows: the 1st group was fed on basal diet, the 2nd group was fed on basal diet +1ml of Chitonal /liter drinking

water and the 3rd group was fed on basal diet + 200 gm. Chitozinc /ton. Hens were reared on floor with 16 hr. light /day. During the experimental period, eggs were collected daily three times a day at 9h, 13h, and 16h. Daily egg production (EP) %, Feed intake FI (g/hen) the differences between feed given and left over, average egg weight (EWT) in (g), egg mass production (g/hen/day) which is a factor of egg weight and egg production, and feed conversion ratio FCR (Kg feed/Kg egg) the ratio between feed intake / hen in Kg and egg mass / hen in Kg were calculated in each dietary treatment.

The eggs were collected from each group three days before the end of the experiment to carry out chemical analysis of egg yolk fat content (total lipid, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride, very low density lipoprotein (VLDL), and atherogenic index (AI) in egg yolk).

Yolks were carefully separated into a container for lipid extraction, one gram of yolk was placed into a centrifuge tube, homogenized with 15ml of polar solvents chloroform: methanol mixture, 2:1 (v/v), vortexed, filtered and evaporated according to **Folch et al. (1957)** and then lipid extract was stored at – 20 °C until biochemical analysis. Total lipids were estimated according to **Frings et al.(1972)**, triglycerides by method described by **Fossati and Prencips (1982)**, total cholesterol concentration was determined according to **Artiss and Zak (1997)**. Low density lipoprotein (LDL) cholesterol was determined according to **Wieland and Seidal (1983)**. High density lipoprotein (HDL) cholesterol was determined enzymatically as described by **Dernacker and Hifrnans (1980)**. Very low density lipoprotein (VLDL) was determined by formula: $VLDL = \text{Triglycerides} / 5$ and the atherogenic index (AI) was calculated by formula: $AI = (\text{Total cholesterol} - HDL) / HDL$ (**Norbert, 1955**).

Statistical analysis:

The influence of dietary treatments were examined using analysis of variance (ANOVA) while differences among means were evaluated using Duncan's multiple range test at 5% probability with the aid of software **SPSS (2006)**.

Results and Discussion

Results of the experiment are shown in table (1) and (2). **Table (1)** revealed that, there were significant differences among treatments in layers fed on basal diet +1ml of Chitonal /liter drinking water and other two groups. There were an improvement in egg production which was emphasized by a significant increase ($P < 0.05$) in egg production (EP) %, feed intake FI (g/hen), average egg weight (EWT) in (g), egg mass production (g/hen/day) and feed conversion ratio FCR.

Some studies in broilers indicated that dietary Chitosan treatment could gain superior performance and feed conversion ratio than the control group (**Suk, 2004 and Khambualai et al., 2008 and 2009**). In addition, **Tang et al. (2005)** reported that Chitosan could improve the growth performance and feed efficiency of piglets.

Some authors as **Huang et al. (2005), Shi et al. (2005) and Li et al. (2007)** reported that diet supplementation with COS (0.005 or 0.01%) significantly improved the growth performance in broilers. Similar positive effects of COS on BWG and FCR were reported by **Suk (2004), Zhang et al. (2008) and Zhou et al. (2009)**. These improvements may be due oligosaccharides which acts as prebiotics and has beneficial effects on gut microflora which improves health condition and product performance **Stanley et al. (1999) and Berry and Lui (2000)**. **Hirano and Nagao (1989) and Zhange et al. (2003)** suggested that COS have antifungal effect while **Joen et al. (2000)** had another opinion that, it has antimicrobial activities that improved gut health which may promote nutrient digestibility and improve growth performances and activate the intestinal villi and epithelial cells **Khambualai et al. (2009)**. On the other hand, **Shi et al. (2005)** found that, low concentration of dietary Chitosan could elevate nitrogen utilization and amino acid digestibility. **Yan et al. (2010)** found that dietary inclusion of COS (0.01, 0.02%) improved egg weight but did not affect egg production. **Chen et al. (2006) and Meng et al. (2010)** also found that COS improved growth performances and egg production. Moreover, the reason of increased egg weight may be due to increase nutrient digestibility as a result of antimicrobial and antifungal activities of COS (**Li et al., 2007**).

Results presented in **Table (2)** showed that the egg yolk lipid profile indicated a significant reduction ($P < 0.05$) in total lipid, total cholesterol, triglyceride, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and atherogenic index (AI), while induced a significant increase in high density lipoprotein (HDL) in egg yolk in both two groups (basal diet +1ml of Chitonal/liter drinking water), (basal diet + 200 gm. chitozinc /ton). In laying hens, egg cholesterol is synthesized in the liver and secreted into the blood stream as very low density lipoprotein (VLDL) particles, the main yolk cholesterol carrying macromolecules. Plasma VLDL particles are then internalized by the oocyte vitellogenin receptor in the rapidly growing follicles and deposited to yolk. Thus, cholesterol is mainly excreted through the egg from the hen. Faecal neutral and acidic sterol represents a second major pathway for elimination of cholesterol (**Hall and McKay, 1993**). It has been suggested that either selective inhibition of liver cholesterol biosynthesis or increased excretion of cholesterol from the body would result in reduction of egg cholesterol. Diets supplemented with Chitosan significantly reduced the

apparent digestibility of fat and the lipase activity of distal jejunum. This observation is supported by previous study (**Walsh et al., 2013**). To date, the mechanism may be postulated as follows: **firstly**, Chitosan might interrupt enterohepatic bile acid circulation. Bile salt is mixed with dietary lipids and emulsifies the lipid particle, and the lipid particle size was reduced. The smaller particle size allowed for greater surface exposure to pancreatic and intestinal lipase which is adsorbed on to the particle surface, and lipase activity is stimulated (**Kobayashi et al., 2002**). Chitosan which differs from other dietary fibers because of its cationic characteristics can reduce fat absorption and interrupt enterohepatic bile acid circulation by electrostatic and hydrophobic forces (**Sumiyoshi and Kimura, 2006**) and (**Aranaz et al., 2009**). **Secondly**, Chitosan might increase the viscosity of the intestinal contents. It has been generally accepted that the anti-fatty effects of Chitosan originate from its unique fat-binding properties. Dietary Chitosan dissolves in the stomach, emulsifying fat and forming a gel, which binds with the fat in the intestine **Gades and Stern (2003)**, **Zeng et al. (2008)** and **Zhang et al. (2008)**, increasing the viscosity of the intestinal contents and the unstirred layer in the intestine and slowing nutrient diffusion, which resulted in a highly effective increase in the excretion of fat (**Santas et al., 2012**). Therefore, the inhibitory effect of Chitosan on lipase activity may be attributed to the viscosity of Chitosan which restricted the access of the pancreatic lipase to the lipids within the droplets and the reduction of bile acid concentration in the intestinal tract. **Thirdly**, Chitosan has profound impact on circulating adipocytokines (e.g. leptin, resistin, IL-6, and C-reactive protein, etc.), which significantly suppress appetite, regulate energy metabolism, and prevent lipid accumulation in peripheral tissues (**Unger, 2003 a&b**) and therefore chitosan can lower fat mass, regulate the level of circulating triglycerides and reduce the accumulation of lipids both in the liver and in the muscle tissue (**Liu et al., 2012** and **Walsh et al., 2013**).

Chitosan decreased absorption of lipids in intestine by binding to lipids and fatty acids directly (**Tanaka et al., 1997**) or binding bile acids and fatty acids (**Ventura, 1996**) and lower plasma cholesterol (**Bokura and Kobayashi, 2003**) without adverse effects. It acts by inhibiting the absorption of cholesterol and bile acids (**Ventura, 1996**) due to stronger electrostatic attraction between the cationic polysaccharide and anionic substances like bile acids and fatty acids. COS inhibit micelle formation during the lipid digestion in tract by forming ionic bond with the bile salts and acids (**Rumunan-Lopez et al., 1998**). **Li et al. (2007)** reported that there was no effect on total cholesterol, triglyceride and HDL- cholesterol. **Du et al. (2001)** reported that COS with degree of polymerization between 3 and 8 enhanced immunity and growth performance in

livestock; so it can be said that laying hens performance may be improved by COS supplementation.

Table (1): Effects of Chitozinc and Chitonal for 45 days on performance of laying hens (n= 20 for each group).

Treatments Parameters	1 st group Control	2 nd group 1 ml Chitonal/L	3 rd group 200 gm Chitozinc/ton	P value
Daily feed intake (gm.)	107.38 ± 4.99	113.67 ± 7.19	113.90 ± 5.42	0.668
Daily egg production (%)	76.67 ± 2.50 ^a	90.35 ± 2.67 ^b	80.95 ± 2.83 ^a	0.008*
Daily egg weight in gm.	45.00 ± 1.54 ^a	52.78 ± 1.71 ^b	45.88 ± 1.54 ^a	0.007*
Daily egg mass production	34.68 ± 2.17 ^a	47.90 ± 2.83 ^b	37.25 ± 2.57 ^a	0.005*
Feed conversion ratio	4.45 ± 0.14 ^a	3.38 ± 0.17 ^b	4.38 ± 0.18 ^a	0.001*

Data were represented as means ± SE.* significantly difference using ANOVA test at P<0.05.

Mean in the same row with different letters are significantly different (Duncan multiple range test P<0.05).

Table (2): Effect of Chitozinc and Chitonal on lipid profile of egg yolk of laying hens for 45 days (n= 20 for each group).

Treatments Parameters	1 st group Control	2 nd group 1 ml Chitonal/L	3 rd group 200 gm Chitozinc/ton	P value
Total lipid %	13.06 ± 0.51 ^a	10.5 ± 0.50 ^b	12.36 ± 0.26 ^a	0.004*
Total cholesterol (mg/g)	73.2 ± 2.92 ^a	64.2 ± 1.62 ^b	68.6 ± 1.50 ^{ab}	0.034*
LDL (mg/g)	39.8 ± 0.66 ^a	35.6 ± 1.08 ^b	37.2 ± 0.73 ^{ab}	0.013*
HDL (mg/g)	28 ± 1.73 ^a	35.8 ± 0.80 ^b	33.4 ± 1.75 ^b	0.009*
Triglyceride (mg/g)	122 ± 2.59 ^a	109.8 ± 1.28 ^b	114.8 ± 1.39 ^b	0.002*
VLDL (mg/g)	24.4 ± 0.52 ^a	21.96 ± 0.26 ^b	22.9 ± 0.28 ^b	0.002*
AI	1.66 ± 0.19 ^a	0.78 ± 0.05 ^b	0.89 ± 0.11 ^b	0.001*

Data were represented as means ± SE.* significantly difference using ANOVA test at P<0.05.

Mean in the same row with different letters are significantly different (Duncan multiple range test P<0.05)

Conclusion and Recommendations

It can be concluded that, the addition of Chitonal and Chitozinc to laying hens can help improving the productive performance and cause significant decreases in total lipid, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and atherogenic index (AI), while induced a significant increase in high density lipoprotein (HDL) in egg yolk. These results suggested that, the use of such products for layers had superior influence on performance and lipid profile of egg yolk. It is recommended that the use of such Chitonal and Chitozinc as natural products in layers can produce a more healthy eggs especially for elderly and ill people.

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