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## Effect of Fiber Degrading Enzyme (Extracted from *Chatomium thermophyle*) on Egg Quality, Dry Matter Retention and Economic Appraisal in Old Age Layers Using High Fiber Rations

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**Abstract:** Present study was conducted to evaluate the effects of fiber degrading enzyme on egg quality parameters in old age layer (80 weeks old). For this purpose, 120 white leghorn layers of uniform body weight were randomly selected and divided into 15 experimental units of 8 birds each. Five treatments (each having three replicates) i.e., control T1 (commercial layer mash), T2 (layer mash having 6% fiber + 1X enzyme), T3 (layer mash having 8% fiber + 1X enzyme), T4 (layer mash having 6% fiber + 2X enzyme) and T5 (layer mash having 8% fiber + 2X enzyme) were randomly allotted to experimental units. Egg quality parameters including egg weight, egg shell thickness, yolk index, haugh-unit value, blood and meat spot and dry matter retentions were measured. Data was analyzed statistically using analysis of variance technique under completely randomized design. The effect of treatments on egg weight was found to be significant ( $p < 0.01$ ). The birds under treatment T4 (64.80 g) showed significantly higher egg weight compared with the control group (62.75 g). Enzyme supplementation also have significant effect on eggshell thickness and albumin height. The birds under control treatment showed maximum value (0.3725 mm) of eggshell thickness but the difference was non-significant compared with T4 (0.3550 mm) and T5 (0.3587 mm) group. Similarly the maximum values for albumin height was observed in the control group (7.159 mm); however, the difference was non-significant with T3 (6.645 mm) and T4 (6.558 mm). The effect of treatment on yolk height, yolk diameter, yolk index, haugh unit and dry matter retention was found to be non significant. Maximum profit/bird was observed in T2 followed by T4, T5, T3 and T1, respectively.

**Key words:** Fiber degrading enzyme, aged layers, egg quality, dry matter retention

### INTRODUCTION

Enzymes are the biological catalyst, which speed up a chemical reaction without being utilized. Usually enzymes are added into the poultry ration to facilitate the breakdown of larger molecular structure of feed ingredients into smaller one by specific action and making these nutrients readily available to the active system for the better assimilation and absorption (Esonu *et al.*, 2005; Elmenawey *et al.*, 2010; Yusrizal *et al.*, 2013).

However, some polysaccharide such as cellulose and B-glucan, are not hydrolyzed by enzymes produced within gastrointestinal tract of poultry. Addition of food enzymes reduce the negative impacts of the undigested residues on digesta viscosity. Normal digesta require unimpeded movement of enzyme, substrate and digestion products throughout the digesta and especially close to absorptive gut wall. As the viscosity of digesta increases, the rate of diffusion increases and this cause reduced digestibility of all substrates (Acamovic, 2001; Chesson, 2001; Brenes *et al.*, 2002).

Normally feedstuffs comprise of protein, starch, fat and fiber. In monogastric animals the fiber component has been considered to be wasted and in some instances, Non-starch polysaccharides (NSP) can exert anti-nutritive activity on the animal. The Non-starch Polysaccharides (NSP) of barley, wheat and rye have been the most intensively investigated (Chesson, 2001; Brenes *et al.*, 2002; Hetland *et al.*, 2002; Hetland *et al.*, 2003). Supplementation of Beta-glucan in poultry feed in concentrations ranging from 30-60 g/kg dry matter has been shown to depress production in broilers and layers and cause sticky droppings (pasted vents) and reduced egg production. Wheat and rye contain relatively high levels of arabinoxylans or pentosans (50-80 g/kg dry matter for wheat; 100 g/kg dry matter for rye) which can also have a negative effect on bird performance (Choct and Annison, 1990; Pan *et al.*, 1998). Ingestion of NSP by monogastric results in increased viscosity of the digesta (Burnett, 1966; Antoniou and Marquardt, 1983). This increased viscosity reduces the passage rate of the feed leading to overall reductions in consumption and decreased performance, sticky droppings and dirty eggs (Igbasan and Guenter, 1997; Cowan and Korsbak,

1999). The addition of enzymes to the poultry diet, to address NSP viscosity, can improve feed efficiency, manure quality, health and welfare of the birds and increase the use of lower cost feed ingredients.

Some ingredients present in feed bind other feed components such as phosphorus, calcium and trace minerals. Therefore, it is speculated that the use of appropriate enzymes will increase the availability of these feed components, many of which can influence eggshell quality (Jaroni *et al.*, 1999a; Zhang *et al.*, 2000). Concern has been expressed about reduced eggshell quality resulting from the use of enzymes (Elmenawey *et al.*, 2010).

The benefits of adding commercial enzyme preparations to poultry feed have been researched extensively for broilers (Pan *et al.*, 1998; Cowan and Korsbak, 1999; Boguslaw *et al.*, 2010). However, there is little information available about the benefits of adding enzymes to layer diets (Zhang *et al.*, 2000). Layers and broilers differ in a number of ways including the fact that broilers are immature birds, whereas layers are mature birds. There is evidence that bird age, even within broilers, can influence digestive function (Petersen *et al.*, 1999).

A cellulose enzyme extracted from the fungus *Chaetomium thermophile* at National Institute of Biotechnology and Genetic Engineering (NIBGE) Faisalabad was produced through submerged fermentation in 20 liter bioreactors using oven-dried ground wheat straw (1 mm size) as a substrate. The enzyme was harvested after centrifugation at 10,000 rpm for 15 min at 10°C and stored for addition to feed mix. This enzyme contains Xylanase (30U/mL), Endoglucanase (2.5 U/mL) and B glucosidase (1.5 U/mL) and is under trial for final recommendation. The present project was planned with collaboration of National Institute of Biotechnology and Genetic Engineering (NIBGE) with the objective to evaluate its effect on egg quality parameters and economics of aged layers.

## MATERIALS AND METHODS

In order to evaluate the effect of fiber degrading enzyme different levels of fiber were used in the diets of aged layer (80 week old). This study was carried out at Poultry Research center, University of Agriculture, Faisalabad, Pakistan.

Birds were kept in thoroughly cleaned and disinfected cages in an environmentally control house. Each cage had four units with specification of 2 birds/unit having length, width and height of 16.5, 15.5 and 14.5 inches, respectively. The birds were given broad-spectrum antibiotics i.e., oxytetracycline at 125 g/bag of feed for one week prior to start of experiment to reduce the chances of disease outbreak. Birds were also vaccinated against Newcastle disease (ND) according

to the recommended vaccination schedule. Diets supplemented with enzyme and water were offered ad-libitum. Seventeen hours light with the intensity of 3/4 foot candle was provided to the birds through out the experimental period.

One hundred and twenty White Leghorn Layers of same age (80 weeks) and weight (1700+100 g) were randomly distributed into five groups (each group was comprised of 3 replicates with a density of 8 birds in each replicate). These birds in each group were fed different experimental rations (Table 1) from 80 to 88 weeks of age. The ingredient composition of diets is given in Table 2.

**Egg quality parameters:** At the start and end of each experimental week data were collected for the following parameters.

**Egg weight:** Each egg was weighed on a weighing balance and weight was recorded in grams. Then it was broken and contents were poured out in a petri dish for further evaluation.

**Egg shell thickness:** Shell thickness (in mm) was recorded using a micrometer screw gauge. Shell membrane was removed manually. One reading was taken at one end and other reading was taken from the girth of the shell. Average of the two was taken as the final reading.

**Yolk index:** Yolk index was calculated by dividing the yolk height by the yolk diameter. Diameter of the yolk was carefully recorded using a vernier caliper and the height of the yolk was determined by the egg meter.

**Haugh-unit value:** Albumin height was noted at two different places using a spherometer. The readings thus obtained were used to calculate Haugh Unit (HU) value by the following formula:

$$\text{Haugh unit} = 100 \log [H+7.57-1.7 W^{0.37}]$$

Where:

H = Observed Albumin height (mm)  
W = Observed Weight of the egg (g)

**Blood and meat spots:** Eggs were carefully observed for presence of any blood and meat spot.

**Dry matter retentions:** During the last three days of the experiment, feed and excreta samples of each replicate were collected for the determination of dry matter retention (DMR). DMR was determined according to the method proposed by Marquardt *et al.* (1979). Dry matter of feed and excreta was determined by putting 10 gm of

Table 1: Ingredient composition of rations used

Ingredient	Ration T1 (Control)	Ration T2 Enzyme @ 1X Enzyme	Ration T3 Enzyme @ 1X Enzyme	Ration T4 Enzyme@ 2X Enzyme	Ration T5 Enzyme @ 2X Enzyme
Maize grain	39.50	23.50	25.50	23.50	25.50
Wheat corn	00.00	20.00	10.00	20.00	10.00
Rice Tips	20.00	0.00	0.00	0.00	0.00
Rice Polishing	5.00	13.00	16.00	13.00	16.00
Canola meal	6.00	10.00	2.00	10.00	2.00
Soybean meal	8.00	0.00	0.00	0.00	0.00
Sunflower meal	0.00	10.00	24.00	10.00	24.00
Corn gluten meal 30%	2.00	0.00	0.00	0.00	0.00
Corn gluten meal 60%	1.00	2.50	0.00	2.50	0.00
Soy bean oil	0.00	2.50	4.00	2.50	4.00
Fish meal	4.50	5.00	5.00	5.00	5.00
Molasses	4.00	3.00	3.00	3.00	3.00
Premix	0.50	0.50	0.50	0.50	0.50
Limestones	7.00	9.00	9.00	9.00	9.00
DCP	2.50	1.00	1.00	1.00	1.00
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>
<b>Nutrients</b>					
Crude protein (%)	17.00	17.00	17.00	17.00	17.00
Metabolizable energy kcal/kg	2732	2618	2615	2618	2615
Crude fiber (%)	4.00	6.00	8.00	6.00	8.00

Table 2: Allocation of various treatments to the experimental diets

Treatments	Rations	Protein (%)	Energy Kcal/kg
T1 (Control)	Commercial Layer mash (Ration No. 3)	17	2732
T2	Layer mash +1X Enzyme + 6% fiber	17	2618
T3	Layer mash +1X Enzyme + 8% fiber	17	2615
T4	Layer mash +2X Enzyme + 6% fiber	17	2618
T5	Layer mash +2X Enzyme + 8% fiber	17	2615

each feed and excreta samples in the separate Petri dishes and then taken these in the oven at temperature of 105 F for a period of 24 h. First reading was taken after 24 h of placing in the oven whereas; further two readings were taken at an interval of one h. Average value of feed and excreta DM of each replicate sample was used to calculate dry matter retention by using the following formula:

$$DMR = \frac{\text{Intake of DM} - \text{Out go of DM}}{\text{Intake of DM}}$$

**Statistical analysis:** Data collected on laying performance was analyzed statistically using analysis of variance technique under completely randomized design. The significance of differences among the treatments means was evaluated by least significance difference test (Steel *et al.*, 1996).

## RESULTS AND DISCUSSION

The results for the egg quality parameters including egg weight, egg shell thickness, albumin height, yolk height, yolk diameter, yolk index and haugh-unit values observed during the trial period are shown in Table 3. It was observed that there was significant effect of treatments on egg weight. Treatment T4 having 2X enzyme concentration with 6% fiber showed significantly

higher egg weight compared with the control group. However, this difference in egg weight was non significant compared with T3 and T5 groups. These results were in line with the finding of Pan *et al.* (1998) who studied two sources of grains (wheat and rye) with two levels of crude enzyme preparations (0.0 and 0.1%) with (0.105%) or without supplementation of phosphorous and reported significant improvement in egg weight. The result of the present study was also in agreement with the finding of Jaroni *et al.* (1999b) who studied two levels of wheat middlings (8 and 16%) in corn soyabean based control diet with addition of increasing level of xylanase enzyme (0.1, 0.2%) and reported significantly improved effect on egg weight. The results of the present study showed that enzyme supplementation significantly improved egg weight. This might be due to the reason that enzyme addition reduced the viscosity of the digesta which in turn improved the digestion and absorption of the nutrients. These absorbed nutrients provided energy for improving the egg weight. The controversial results were reported by Roberts *et al.* (1999) who reported significant reduction in egg weight with the supplementation of enzyme in the diets. The results of the present study were not in line with the findings of Roberts *et al.* (1999) and Esonu *et al.* (2005). They found non-significant effect of enzyme supplementation on egg weight.

Table 3: Effect of enzyme supplementation (*chaetomium thermophile*) on layer egg quality parameters during the experimental period (80-88) weeks

Treatment	Egg weight (gm)	Egg shell thickness (mm)	Albumin height (mm)	Yolk height (mm)	Yolk diameter (cm)	Yolk index	Haugh units
T1 (Control)	62.75 <sup>b</sup>	0.3725 <sup>a</sup>	7.159 <sup>a</sup>	16.040	3.948	4.115	77.827
T2	60.37 <sup>c</sup>	0.3343 <sup>c</sup>	6.092 <sup>b</sup>	17.314	3.894	3.998	72.600
T3	63.39 <sup>ab</sup>	0.3429 <sup>bc</sup>	6.645 <sup>ab</sup>	15.851	3.908	4.079	73.601
T4	64.80 <sup>a</sup>	0.3550 <sup>ab</sup>	6.558 <sup>ab</sup>	15.492	4.000	3.914	70.889
T5	63.16 <sup>ab</sup>	0.3587 <sup>ab</sup>	6.348 <sup>b</sup>	15.359	3.954	4.022	71.059

Same superscripts on means in columns show non significant difference

The layers under control treatment showed maximum value of eggshell thickness and the difference was non-significant with T4 and T5 groups. The results of the present study were in accordance with the findings of Pan *et al.* (1998), Roberts *et al.* (1999) and Esonu *et al.* (2005). They reported nonsignificant effect of enzyme supplementation on egg shell thickness.

The results of the present study showed that enzyme supplementation did not improve the egg shell thickness which may be due to unavailability of Ca level which is present in the bind form in the high fiber diets. However, controversial results were reported by the Robert (2003); Arturo *et al.* (2004) who reported that enzyme supplementation significantly improved egg shell thickness.

The layers under control treatment showed maximum value of albumin height and but the difference was nonsignificant with treatment T3 and T4 having 1X and 2X enzyme concentration with 8 and 6% fiber, respectively. The results of the present study were in accordance with the finding of Roberts *et al.* (1999). They reported non significant effect of enzyme supplementation on albumin height. The reasoning for lower albumin height in the groups fed diets with enzyme may be due to more egg production in these groups which reduced albumin thickness. But controversial results were reported by the Pan *et al.* (1998), Jaroni *et al.* (1999a) and Esonu *et al.* (2005) who reported that enzyme supplementation significantly improved albumin height.

Yolk height and yolk diameter was used to calculate the yolk index. The results of the present study showed that enzyme supplementation have non significant effect on yolk height as well as yolk diameter, so subsequently the effect on yolk index was also non-significant.

The results of the present study was not in accordance with the finding of the Esonu *et al.* (2005) who reported that enzyme supplementation showed significant improvement in the yolk index.

Enzyme supplementation had non-significant effect on haugh units. These results in agreement with the findings of Roberts (2003), Elmenawey *et al.* (2010) and Yusrizal *et al.* (2013). They reported that enzyme supplementation have non significant effect on haugh

unit. The results of the present study showed that enzyme addition had not any affect on the internal quality of the egg; However, other reason might be the age of the hen. The result of present study was not in accordance with the findings of (Elmenawey *et al.*, 2010; Yusrizal *et al.*, 2013). They reported that fiber degrading enzymes have significant reduction in haugh units in layers at different ages.

Dry matter of feed and feces were determined to calculate the dry matter retention in the birds. The results of the present study showed that the enzyme supplementation have not significant effect on dry matter retention in the bird. The results of the present study showed that enzyme supplementation increase the digestibility of organic matter that was used to increase the production performance of the bird and less is retained in the body of the birds. The results of present study was not in line with the findings of Brenes *et al.* (2002) reported that enzyme supplementation of diet containing lupen seed significantly ( $p < 0.05$ ) improved the dry matter retention of layers (7-21 days). This may be due to age difference of the bird.

It was found that maximum profit per bird was in treatment T2 (20.91) followed by T4 (19.50), T5 (15.94), T3 (11.78) and control group(6.17). Profit per bird was increased from the 6.17 to 20.91 by the enzyme supplementation in layer diets.

**Conclusion:** It is concluded that enzyme supplementation with 2X Enzyme and 6% fiber has significant effect on egg weight, egg shell thickness and albumin height in old age layers.

**Author's declaration:** The authors declare that they have no conflict of interest

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