



ISSN (E): 2277- 7695  
ISSN (P): 2349-8242  
NAAS Rating 2017: 5.03  
TPI 2017; 6(7): 1021-1023  
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www.thepharmajournal.com  
Received: 15-05-2017  
Accepted: 16-06-2017

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## Effect of $\alpha$ -Galactosidase supplementation to toasted guar (*Cyamopsis tetragonoloba*) meal based diets on body weight and serum biochemical parameters in commercial layers

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### Abstract

An experiment was conducted on 5280 birds of 37 weeks old (WL-BV300) for 112 days (4 periods) to determine the effect of  $\alpha$ -galactosidase supplementation to guar (*Cyamopsis tetragonoloba*) meal (GM) based diets on Body weight (BW) and Serum biochemical parameters like total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL- cholesterol and triglycerides. Six experimental diets (Each treatment has 880 birds at 10 replicates of 88 birds in each) were prepared having 3% GM (3GM), 3% GM with  $\alpha$ -galactosidase (3GME), 3% GM low (100 kcal/kg) energy (3GML), 3GML with  $\alpha$ -galactosidase enzyme (3GMLE), 6% GM (6GMH) and 6% GM with  $\alpha$ -galactosidase (6GMHE). Results of the experiment did not shown the effect ( $P>0.05$ ) on BW and serum biochemical parameters on adding  $\alpha$ -galactosidase enzyme at 2 IU/kg diet to toasted GM based diets in commercial layers.

**Keywords:**  $\alpha$ -galactosidase, Guar meal, Total cholesterol, HDL-cholesterol LDL- cholesterol, VLDL-cholesterol and triglycerides

### 1. Introduction

Guar (*Cyamopsis tetragonoloba*) meal (GM) is the potent feed ingredient for the poultry as well as livestock since cultivating many parts of India and relatively inexpensive compared to soya bean meal. It was mainly used as vegetable for human and used as thickening agent in pharmaceuticals industry, cheese and ice cream preparations because due to the presence of galactomannan gum present in their seeds. GM is the by-product of guar gum industry and total annual production of GM in India is 1.97 million tons (NRRRA, 2014) [5]. Most of GM is used as protein source in the rations of livestock and poultry since GM contains high protein (48.6%) and contain an anti-nutritional factor "Galactomannan" and gum content in the GM was 46g/kg in which mannans is 20 and galactans 22g/kg (Rama Rao *et al.*, 2015) [6] which is causing negative effects on bird performance, by increasing the viscosity of digesta. Enzyme added in the diet breaks the polymeric chain of fibrous material more effectively, thereby reducing the gut viscosity and improve their nutritive value of the meal (Smith and Annison 1996) [9]. A-Gal Pro is a product produced by a genetically modified strain *saccharomyces cerevisiae*. Its active principle is  $\alpha$ -galactosidase, which can hydrolyze  $\alpha$ -galactose in feed. High viscosity is generally connected with delayed gastric emptying and increased small intestinal transit time, hence inhibiting the absorption of nutrients (Blackburn and jhonson, 1981) [1]. This experiment was conducted to evaluate effects of dietary inclusion of GM supplemented with  $\alpha$ -galactosidase enzyme on Body weight and serum blood biochemical parameters of laying hens.

### Materials and Methods

A total number of 5280 birds of 37 week old having uniform weight and egg production were randomly distributed in to six treatments with 10 replicates (88 birds in each) per group. Diets were prepared having 3% GM (3GM), 3% GM with  $\alpha$ -galactosidase (3GME), 3% GM low (100 kcal/kg) energy (3GML), 3GMB with  $\alpha$ -galactosidase enzyme (3GMLE), 6% GM (6GMH) and 6% GM with  $\alpha$ -galactosidase (6GMHE). Enzyme AGal-Pro 180P ( $\alpha$ -galactosidase) was procured from the M/S Kerry Food Ingredients (cork) limited, and added at the rate of 2U/Kg diet. The diets were fed to respective group for four periods. The data on bodyweight was recorded at the beginning and at the end of experiment.

Body weight of sixteen (four cages) birds per each replicate was recorded to calculate body weight. Body weight was recorded on the same cage at beginning and at the end of the experiment was calculated to the nearest one gram accuracy.

**Blood Biochemical parameters**

About 3ml blood sample was collected from one bird from each replicate at the end of the experiment. Blood samples were collected aseptically from wing vein with the help of sterilized needles and placed in a clean sterilized vacutainers and kept in incubator at room temperature for serum collection. The serum samples were centrifuged at 4000 RPM for 10 minutes and transferred to 1.0 ml eppendorf tubes which were stored at -20 °C for estimation of various biochemical constituents.

**Total cholesterol** was estimated by using kit number RCHO 1031 (Coral clinical systems Diagnostic Pvt. Ltd.). The cholesterol reagent of 1.0 ml was taken in a cuvette, to it, 0.01 ml of sample was added and mixed well then incubated for 10 minutes at room temperature and OD of colour developed was read at 505 nm. The serum cholesterol count was estimated as follows.

$$\text{Cholesterol (mg/dl)} = \frac{\text{Sample optical density}}{\text{Standard optical density}} \times 200$$

**HDL-cholesterol estimation** was estimated in serum using kit number B121585 (Erba Diagnostics Pvt. Ltd.)

1. Precipitation of LDL, VLDL and Chylomicrons by adding serum of 0.25 ml and precipitating reagent of 0.5ml and mix well and allowed the mixture to stand for 10 minutes at room temperature (15 -30 °C), centrifuged at 4000 R.P.M for 10 minutes to obtain clear supernatant. Used the supernatant to determine the concentration of HDL cholesterol in the serum sample.
2. The reagent of 1ml was taken in cuvette; to it 0.5 ml of supernatant is added and mixed well, then incubated for 10 minutes at room temperature and read the OD at 500nm. The concentration of HDL cholesterol was calculated with the following formulae.

$$\text{HDL- Cholesterol (mg/dl)} = \frac{\text{Sample optical density}}{\text{Standard optical density}} \times 75$$

**Triglycerides** serum using kit number PBTGL (2)-51219 (Proton Biologicals India Pvt. Ltd). The reagent of 1ml was taken in a cuvette, to it, 0.1ml of serum sample was added and mixed well then incubated for 10 minutes at room temperature and the optical density was read at 500 nm. The concentration of triglycerides in serum was estimated with the following formulae

$$\text{Triglycerides (mg/dl)} = \frac{\text{Sample optical density}}{\text{Standard optical density}} \times 200$$

**VLDL cholesterol** was calculated by employing the Friedwald formula (1972). The results are expressed as mg/dl of serum.

$$\text{VLDL- cholesterol (mg/dl)} = \frac{\text{Triglycerides (mg/dl)}}{5}$$

**LDL Cholesterol** calculated by employing Friedwald formula

(1972). The results are expressed as mg/dl of serum

$$\text{LDL-cholesterol} = \text{Total cholesterol} - (\text{VLDLC} + \text{HDL C})$$

**Statistical Methodology**

The data were analyzed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 15<sup>th</sup> version and comparison of means was done using Duncans multiple range test and significance was considered at  $P < 0.05$ .

**Results and Discussion**

The average body weight of the birds fed with the  $\alpha$ -galactosidase enzyme did not shown any significant ( $P > 0.05$ ) difference in the experimental period. A positive weight gain was observed among layers fed control and low energy diets supplemented with or without  $\alpha$ -galactosidase to GM based diets (Table-1). Similarly Scheideler *et al.* (2005) [7] and Sohail *et al.* (2003) [10] observed no effect of dietary energy variation and NSP enzymes supplementation on body weight gain. However, Gunawardhana *et al.* (2009) [3] reported that hens fed diets supplemented with enzyme (cocktail of multi carbohydrase enzyme) with various dietary energy levels (2791 and 2857 kcal of ME/kg) had significantly higher body weight than the hens fed diets without enzyme. Dietary supplementation of  $\alpha$ -galactosidase to the GM based diets with or without ME reduction in commercial layers did not influence the concentrations of total cholesterol, HDL-cholesterol, triglycerides, LDL-cholesterol or VLDL-cholesterol (Table-2).

**Table 1:** Body weight (g) in layers (37-52 week of age) fed with and without  $\alpha$ - galactosidase enzyme in guar meal based diets.

Diet	Initial (36 week)	Final (52 week)
3GM	1441	1457
3GME	1431	1451
3GML	1412	1420
3GMLE	1437	1441
6GMH	1420	1445
6GMHE	1442	1456

**Table 2:** Serum biochemical parameters in layers fed with and without  $\alpha$ - galactosidase enzyme in guar meal based diets

Diet	Total cholesterol	HDL	Triglycerides	LDL	VLDL
	(mg/dl)				
3GM	180.2	56.77	257	71.91	51.52
3GME	195.0	78.44	253	65.83	50.73
3GML	160.6	65.80	195	55.78	39.02
3GMLE	190.4	80.53	237	62.38	47.49
6GMH	185.6	78.97	250	56.44	50.19
6GMHE	162.4	69.40	197	53.48	39.52

Wang *et al.* (2005) [11] reported significantly ( $P < 0.05$ ) higher total cholesterol in broilers fed corn soy bean diet with supplementation of  $\alpha$ -galactosidase (250mg/kg diet). Contradicting results might be due to difference in diets and birds. Shahbazi (2012) [8] reported layers fed with  $\beta$ -mannanase to the GM based diets did not affect total cholesterol, HDL-cholesterol, triglycerides, LDL-cholesterol. Mohammad and Kazem (2012) [4] reported that broilers fed with  $\beta$ -mannanase to the different levels of GM did not influence the concentration of total cholesterol, HDL-cholesterol, triglycerides, LDL-cholesterol.

Based on the results of the present study, it is concluded that,

bodyweight and serum biochemical parameters like triglycerides, total cholesterol, HDL, VLDL, LDL cholesterol on supplementation of  $\alpha$ -galactosidase (2 IU/kg diet) to the toasted GM based diets did not affect significantly to the in layers.

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