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Comparative study between different treatments of Guar meal in broiler diets on productive performance and immunity.

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Abstract

Diminishing the cost of broiler chicken diet is a critical issue in the poultry industry. Numerous studies were performed to achieve this pivotal objective by diet supplementation with alternative feed additives. Guar meal (GM) is a relative inexpensive high protein meal and sold at about half the price of soyabean meal (SM), making it an appealing potential source of protein in poultry feeds. This study was conducted to compare the effect of different treatment of GM on the performance, blood biochemistry, mortality rate (MR) and health condition, histomorphological examination of intestine, digestive tract measurements , and economic efficiency of broilers fed guar meal. A total 180 one day old unsexed broiler chicks (Hubberd) were used. The chicks were individually weighed and randomly assigned (30 birds/group) to one of the following treatment groups Control negative: Co (-ve), birds fed corn-soyabean diets as a control basal diets, Control positive: Co (+ve) replacing 5% soyabean meal by (GM) without treatment. And another four treatments replacing 5% soyabean meal by (GM) treated as the following; T1: treated with heat (120C for 30 minute), **T2**: fermented with fungi (Trichoderma reesei)) for 21 day; **T3** added with b-manannase enzyme 30/100kg; **T4** added (NDF) sodium diformate 20g/100kg. Results indicated that no significant defference between (T1, T2, T3 and T4) and control negative (-ve) but control positive (+ve) group has low significant defference with other groups in performance, the plasma total protein showed significant differences among groups and in T2 (5% GM +fungi) diet was highest significant decrease in plasma albumin, A/G ratio and cholesterol in T1 compared to other groups. Plasma AST and ALT activity were significantly differences affected by different dietary treatments. Histopathological examination showed the intestinal mucosa and submucosa (villus height, villus width, crypt depth and muscle thickness) is apparently normal in control negative (-ve), T2, T3, T4 groups and there was a mucosal degeneration and hemorrhage in intestinal villi in control positive (+ve) and T1 groups. In conclusion, using treated GM as a replacement for SM at 5% level in broiler diet can reduce the diet cost.

Keywords: *Broiler, guar, perfprmance, immunity, histomorphology, intestine.*

Introduction

Broiler chickens are a great source of protein for human. Therefore, numerous studies focuson broiler nutrition, to maintain sustainable broiler production to meet the human demand for protein. A balanced ration formulation is hence of great importance in poultry production **(Ravindran. 2005 and Field CJ *et al.,* 2000).** The increase in feed prices is due to the higher price of raw materials of soybean meal which has limited production in Egypt. The protein sources generally is considered the most expensive component of feeds for broiler chickens **(Wilson and Bayer, 2000 and Saleh *et al.* 2004).** One way to reduce the dependency on soybean meal is to find out alternative sources such as guar meal and any feedstuffs must be able to substitute for (SM) totally or partially and not have a negative impact on the efficiency or quality of poultry production **(Ojewola *et al.,* 2006).** Guar meal (GM) is a relative inexpensive high protein meal and sold

at about half the price of (SM), making it an appealing potential source of protein in poultry feeds **(Hussein, 2012).** Guar meal is the main by-product of guar gum production and It is a mixture of germs and hulls at a ratio of 25% germs to 75% hulls. It rich in protein containing about 40% protein in the dry matter, it is used as a feed ingredient, but may require processing to improve palatability and remove anti -nutritional factors. **(Lee *et al*. 2004).** The negative effects of adding guar meal on body weight and feed conversion ratio was repoted in previous studies which may be attribute to the presence of inhibitors in guar meal such as Guar gum, trypsin inhibitor, saponin, polyphenols and hem agglutinin or some other unknown toxic substances. **Hassan *et al.* (2007)** found that guar meal contains 5-13% of dry matter triterpenoids guar saponin and **Lee *et al.,* (2004)** stated that 13-18% gaur gum, residual galactomannans gum. **Yama moto *et al.* (2000); Maisonnier *et al.,* (2001)** found that some beneficial physiological functions of galactomannans such as, decreasing plasma cholesterol, inhibition colonization of pathogenic gastrointestinal bacteria, improve the intestinal histomorphology (villi length, villi width and crypt depth) **(Bengmark, 1998)** and enhance of macrophage activation thus exhibiting immunostimulatory activity **(Duncan *et al.,* 2002).** There are a limited number of studies focused on the effect of uses Guar meal in the broiler chicken diet. Therefore, the current study was conducted to evaluate the possibility of partial replacing soybean meal with guar meal in traditional corn-soy diets and measuring growth performance, some blood parameters, digestive tract measurements, histomorphological changes of intestine and economic efficiency.

Materials and Methods

Ethics statement

This study was carried out in strict accordance with the recommendations of the Committee on the Ethics of Animal Experiments of Agricultural Experiment and Research Station at Shalakan, Faculty of Agriculture, Ain Shams University. The aim of this present study was to investigate the productive performance, some blood parameters and economical evaluation of broiler chicks (Hubbard) as affected by using guar meal (GM) as a partial replacer of soybean meal (SM) in the diets. Chemical composition of SM and GM used in present study (on air dried basis) are shown in Table (1).

Guar Scientific classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: Leguminosae
Tribe: Indigofereae
Genus: Cyamopsis
Species: C.tetragonoloba
Synonyms: Cyamopsis
Vernacular Name: Guar

Table (1). Chemical composition of soybean meal and guar meal

Item %	Soybean meal	Guar meal
Dry matter	88.89	89.49
Organic matter	94.11	92.07
Crude protein	43.1	48.9
ME kcal / Kg	2230	2520
Crude fiber	6.11	7.66
Ether extract	2.64	7.04
Nitrogen free extract	41.49	35.48
Ash	5.89	6.13
Amino acids		
Lysine	2.37	2.88
Methionine	1.25	0.75
Cystine	1.06	0.76
Arginine	2.64	3.50
Tryptophan	1.42	0.65
Valine	2.47	2.31
Concentration of anti-nutritional compounds:		
Gum %	Not found	Found
Saponin %	-	8.84
Tannins %	-	1.76
Trypsin Inhibitor Activity TIU/g	-	3520
Phytic acid %	-	0.535

Experimental materials

Six experimental diets were formulated in which control (-ve)was containing 0.0% GM in starter, grower, and finisher in the other five experimental diets GM were incorporated at level of 5% in starter, grower, and finisher, control (+ve) without treatment and the other four treatments treated by three different ways:

Physical treatments

T1 replacing 5.0 % soybean meal of the basal diet by Guar meal (GM) treated with heat (121°C for 30 minute) and then incorporated in starter, grower and finisher diets,

Biological treatments

Tricoderma reesei (TR) was obtained from the Microbiological Chemistry Center (MIRCEN), Faculty of Agriculture, Ain Shams University. The organism was propagated and maintained on potato dextrose agar.

Additives:

T3 diet contained 5.0 % guar meal (GM) mixed with Hemicell HI enzyme produced by Bacillus lentus. The active ingredient is Endo-1, 4-B-D-mannanase β -mannanase –Hemicell 300 g / ton which contain 160 million units β - manannase activity / Kg, **Vohra, P., and F.H. Kratzer., (1964)a; Patel, M.B.; and McGinnis, J., (1985); Lee *et al.,* (2003); Lee *et al.,* (2004), Lee *et al.,* (2005); Lee *et al.,* (2009)**. T4 diet was formulated by replacing 5.0 % soybean meal of the basal diet by Guar meal (GM) mixed with (NaDF) sodium diformate 200 g / ton.

Experimental birds, management and design experiment:

One hundred and eighty one-day old unsexed broiler chicks (Hubbard) were randomly allocated to six treatments of 30 birds in 6 groups (5 replicate per group). Chicks were reared under similar conditions of management during the experimental period, up to 35 days of age. The incubation temperature of 32ÊC was gradually decreased to 26ÊC by the 3rd week of age, and the chicks were exposed to 23 h of light. All birds were vaccinated by drinking-water-based vaccination against Newcastle disease by Hitchner B1 at the age of 7 days, against Gumboro at 14 days and Lasota twice at 18 and at 28 days of age.

The experimental diets:

Diets were formulated according to the recommended nutrient by Hubbard manual for broiler chicks and were offered in mash form. During the experimental period, which lasted 35 days, chicks were fed on the experimental diets. Feed was offered for ad libitum in mash form in stainless steel feeders for each pen. Three periodical diets were formulated in the experiments includes, starter from 1 to 11 days of age, grower from 12 to 22 days of age and finisher from 23 to 35 days of age. Diets were formulated to contain 3029, 3076 and 3171 Kcal/Kg metabolizable energy with 23, 21 and 20 and crude protein for starter, grower and finisher diets, respectively. In all experimental diets (starter, grower, finisher) crude protein, metabolizable energy, minerals and vitamins mixture, were fitted to cover the optimal requirements of Hubbard broiler chicks according to the breed manual. Fresh water was accessible all the time by automatic nipple drinkers. Corn -soybean basal diets were formulated to satisfy the recommended requirements of chicks were fed to the birds in the control groups of the experiment.

Experimental design:

In this experiment, the chicks were assigned to six experimental diets during the period from 1 to 35 days as follows and shown in Table (2).

Co (-ve): Birds fed corn-soybean diets as control basal diets in starter, grower and finisher diets.

Co (+ve): Replacing 5.0 % soybean meal of the basal diet by Guar meal (GM) without treatment in starter, grower and finisher diets.

T1: Replacing 5.0 % soybean meal of the basal diet by Guar meal (GM) treatment with heat (121°C for 30 minute) in starter, grower and finisher diets.

T2: Replacing 5.0 % soybean meal of the basal diet by Guar meal (GM) fermentation with fungi (Trichoderma reesei) for 21 days in starter, grower and finisher diets.

T3: Replacing 5.0 % soybean meal of the basal diet by Guar meal (GM) added with β - manannase enzyme 30g / 100kg in starter, grower and finisher diets.

T4: Replacing 5.0 % soybean meal of the basal diet by Guar meal (GM) added with (NDF) sodium diformate 20g / 100kg in starter, grower and finisher diets.

Table (2). Experimental design

Items		Treatments				
	Control (-ve)	Control (+ve)	T1	T2	T3	T4
Starter	SM	GM 5%	GM 5% + heat	GM 5% + fungi	GM 5% + β-mannanase	GM 5% + NDF
Grower	SM	GM 5%	GM 5% + heat	GM 5% + fungi	GM 5% + β –mannanase	GM 5% + NDF
Finisher	SM	GM 5%	GM 5% + heat	GM 5% + fungi	GM 5% + β –mannanase	GM 5% + NDF

SM=Soybean meal, GM= Guar meal
Control (-ve) = SM, Control (+ve) = GM 5% without treatment, T1 = GM 5% treatment with heat, T2 = GM 5% treatment with Fungi (trichoderma reise), T3 = GM 5% treatment with β -mannanase 30g / 100kg, T4 = GM 5% treatment with NDF 20g / 100kg.

Table (3). Composition of the experimental grower diets and their chemical and calculated analysis

Ingredient (%)	Starter (1-11)		Grower (12-22)			
	SM	GM	SM	GM	SM	GM
Yellow corn	52.05	55.64	55.91	58.32	56.8	59.20
Soybean meal (44%)	31.50	26.50	30.00	25.00	28.25	23.25
Guar meal (50%)	0	5.00	0	5.00	0	5.00
Corn gluten meal (60%)	7.20	6.47	4.86	4.15	4.40	3.67
Wheat bran	2.00	2.00	1.50	2.00	2.00	2.00
Soy bean oil	3.0	3.4	3.65	2.00	5.00	3.35
Di Calcium Phosphate	1.85	1.85	1.60	1.60	1.34	1.34
Limestone	1.30	1.33	1.50	1.50	1.35	1.37
Common Salt	0.3	0.3	0.30	0.3	0.30	0.3
Premix *	0.3	0.3	0.30	0.3	0.30	0.3
Dl-Methionine	0.29	0.28	0.28	0.27	0.21	0.20
L-Lysine	0.21	0.18	0.10	0.06	0.05	0.02
Total	100	100	100	100	100	100
Calculated analysis ***						
Crude protein %	23.00	23.00	21.00	21.00	20.00	20.00
ME,Kcal/kg	3029	3031	3076	3079	3171	3173
C/P ratio	131.7	131.8	146.5	146.6	158.5	158.6
Calcium %	1.00	1.01	1.01	1.01	0.90	0.90
Av. Phosphorus %	0.50	0.50	0.45	0.45	0.40	0.40
DL-Methionine %	0.64	0.64	0.61	0.61	0.53	0.53
Meth. + Cyst. %	0.97	0.97	0.97	0.97	0.84	0.84
L-Lysine %	1.3	1.3	1.15	1.15	1.06	1.07
Crude Fiber %	3.88	3.96	3.75	3.83	3.70	3.77

*Premix, vitamin and mineral mixture supplied each kg diet: Vit A 12000 IU, Vit D3 2500 IU, Vit E 12mg, Vit k3 3mg, Vit B1 1mg, Vit B2 6mg, Vit B6 3mg, Vit B12 13mg, Niacin 30mg, P antothenic acid 12mg, Folic acid 1mg, Biotin 75mg, choline chloride 600mg, copper 5mg, Manganese 70mg, Zinc 50mg, Iron 60 mg, Selenium 0.1mg and cobalt 0.1mg. Carrier (CaCo3) add to 3 Kg.
**Calculated according to feed composition tables for animal and poultry feedstuffs used in Egypt (2001).

Growth Performance Parameters:
Body weight and body weight gain of bird were determined according to **(Brady, 1968)** Body weight gain =final weight-initial weight. The feed consumption was calculated per group. Feed conversion ratio (FCR) was calcu- lated according to **(Sainsbury, 1984)** FCR=

$$\frac{\text{Total feed intake}}{\text{Body weight gain}}$$

Blood plasma constituents:
Blood samples were collected from slaughtered birds which were randomly chosen to represent each treatment at the end of the experiment. Blood samples were collected in heparinized glass tubes, centrifuged at 3000 rpm for 15 minutes. Plasma was stored at – 20°C until performing the biochemical analyses. Analyses of plasma were carried out for quantitative determination of blood parameters by spectrophotometer (plasma total protein, albumin, cholesterol, ALT and AST) using suitable commercial kits. Globulin was calculated by subtracting albumin content from total protein. Then A/G ratio (albumin / globulin ratio) was calculated.

Pathological examination:

1. Mortality rate (MR) and health condition :
The accumulative mortality rate was calculated by subtracting the number of the live birds at the end of the experiment from the total number of birds at the beginning of the same experimental group. The values were calculated as percentage of the initial number of chicks.

2. Digestive tract measurements:
Digestive tract Length (cm)/ 100 g body weight, Small intestine Length (cm) / 100 g body weight, Small intestine weight (g) and Intestine thickness was determined by the formula described by **(Stutz *et al.*, 2000)** as small intestine weight (g) / small intestine length (cm).

3. Histomorphological examination of intestinal sections:
At the end of the experiment, representative tissue samples from the ileum of approximately midway between Meckel´s diverticulum and the ileocecal junction. Segments were flushed with saline solutions (0.9% NaCl) to remove contents and were fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (HE) dyes and then examined microscopically according to **(Bancroft and Layton, 2013)**
Morphometric analyses of digital photos of light microscop were performed by image analysis program (Image J software) according to **(Abràmoff *et al.*, 2004).**Villus height was measured from the villus tip to the bottom, excluding the crypt. A total of 3 villi were examined from different transverse sections in each bird **(Lillie 1999)**.
Villus Width, Crypt Depth and Muscular Layer Thickness (muscularis mucosa + submucosa + muscle) were determined and calculated under electric microscope provided with computerized camera. The values were measured with an oculometer at a magnification of 5x under a light microscope fitted with the stage micrometer and using an image analyzer **(Leica Microsystems Co., Ltd., Germany)**. Four microscopic fields per bird were measured, and the average value was expressed as the morphological value for each bird.

Data and Statistical analysis:
Data collected in both experimental in this study, was statistically analyzed using the general linear models (GLM) of SAS, (2004) using one-way analysis of variance to test the effect of different dietary treatment according to the control group. Duncan's Multiple Range Test **(Duncan, 1955)** was used to separate means when separation was relevant. Statistical significance was accepted at a probability level of 0.05.

Results

Effect of feeding different dietary treatments on productive performance
Results presented in table (4) showed that initial LBW values did not significantly ($P > 0.05$) different between all tested groups. Chicks fed control (-ve), T1 (5% GM + heat), T2 (5% GM + fungi) and T3 (5% GM + B. mannanase) and T4 (5% GM + NDF) were significantly ($P < 0.05$) higher in LBW than those fed control(+ve), which was the lowest in starter period (1 -11 days) and significant by higher ($P < 0.01$) in period (12-22 days) grower and (23-35 days) finisher period.There were significant ($P < 0.05$) differences among groups fed

different dietary treatments in BWG. Values during (1-11 days) of age indicated that chicks fed T1, T2, T3 and T4 diet were the highest in BWG compared to those fed diets containing control (+ve) (5% GM) was the lowest in BWG. Also, period from 1-35 days of age, BWG values indicate that there were significant higher ($P < 0.01$) differences observed in BWG values between treatments throughout the experimental Period, whereas, chicks fed control (-ve) diet, T2,T3 and T4 diet, were the higher ($P < 0.01$) than all other treatments. In addition to, there were significant ($P < 0.05$) differences among groups fed different dietary treatments in FC values during the period finisher from (23-35 days) of age. Chicks fed control (-ve) (0.0% GM), T2, T3and T4 diets consumed significant ($P < 0.05$) more feed than all treatments. In the same trend, the period (1-35 days) of age values of FC indicated that chicks fed control (-ve) (0.0% GM) diet consumed significantly ($P < 0.01$) more than all treatments in the period from (1-35 days) of age. In the same order, the FCR indicated significant differences between chicks fed diets containing 5% GM with different treatments (T1-4) compared with those fed 5% GM without treatments control (+ve). The best FCR was detected for chicks fed T4 diets (1.52), T3 diets (1.54) or T2 diets (1.53). On the other hand, the worst FCR were found with birds fed diets control (+ve).

Table (4). Effect of feeding different dietary treatments on productive performance

Items	Treatments							
	Control (-ve)	**Control (+ve)**	**T1**	**T2**	**T3**	**T4**	**SE**	**Sig**
Live body weight (g)								
1 day	41.80	41.86	41.27	41.46	41.53	41.67	±0.11	N.S
11 days	196.25^{a}	170.22^{b}	199.97^{a}	210.17^{a}	201.88^{a}	204.55^{a}	±6.55	*
22 days	859.87^{a}	778.07^{b}	852.95^{a}	935.48^{a}	893.30^{a}	918.20^{a}	±18.33	**
35 days	1844.75^{a}	1535.67^{b}	1735.00^{a}	1835.25^{a}	1829.00^{a}	1847.00^{a}	±33.17	**
Body weight gain (g)								
1-11 days	154.38^{ab}	128.42^{b}	158.70^{a}	168.70^{a}	160.35^{a}	162.88^{a}	±9.51	*
12-22 days	663.62^{abc}	607.85^{c}	657.70^{bc}	659.27^{a}	659.27^{ab}	622.15^{ab}	±18.76	**
23-35 days	984.88^{a}	757.60^{c}	882.05^{b}	899.77^{a}	935.70^{a}	928.80^{a}	±46.27	*
1-35 days	1802.88^{a}	1491.86^{b}	1693.73^{ab}	1793.78^{a}	1787.47^{a}	1805.13^{a}	±48.75	**
Feed consumption (g)								
1-11 days	208.00^{a}	185.00^{b}	211.60^{a}	201.13^{a}	200.53^{a}	201.00^{a}	±3.15	**
12-22 days	949.86^{a}	890.52^{b}	1000.00^{a}	964.62^{a}	968.10^{a}	955.05^{a}	±15.53	*
23-35 days	1676.42^{a}	1457.00^{b}	1470.00^{b}	1570.33^{a}	1598.00^{a}	1593.67^{a}	±26.16	*
1-35 days	2834.28^{a}	2532.52^{c}	2681.60^{b}	2736.09^{ab}	2766.63^{ab}	2749.72^{ab}	±38.74	**
Feed conversion ratio								
1-11 days	1.35^{ab}	1.44^{a}	1.33^{ab}	1.19^{b}	1.25^{ab}	1.23^{ab}	±0.08	*
12-22 days	1.44	1.47	1.52	1.46	1.46	1.53	±0.05	N.S
23-35 days	1.70^{b}	1.92^{a}	1.80^{ab}	1.75^{b}	1.71^{b}	1.72^{b}	±0.06	*
1-35 days	1.57^{b}	1.70^{a}	1.58^{b}	1.53^{b}	1.54^{b}	1.52^{b}	±0.08	*

a, b and c means the same row with different superscripts are significantly different sig. = significance, ***($P \leq 0.001$), **($P \leq 0.01$), * ($P \leq 0.05$), N.S = Non significant

SM=Soybean meal, GM= Guar meal

Control (-ve) = SM, Control (+ve) = GM without treatment, T1 = GM treatment with heat, T2 = GM treatment with Fungi (trichoderma reise), T3 = GM treatment with b-mannanase 30g / 100kg, T4 = GM treatment with NDF 20g / 100kg.

Effect of feeding different dietary treatments on some plasma constituents of broiler chicks. Total Protein (TP), albumin, globulin and A/G ratio g/dl values are present in Table (5). The plasma values of total protein show there were significant ($P < 0.05$) differences among groups, whereas, chicks fed T2 was the highest in (TP %).In addition, the plasma values of albumin showed that there were significant ($P < 0.05$) differences among groups. Whereas, chicks fed control (-ve) similar to control (+ve), T2and T3 were the highest in albumin values and chicks fed T1 was the lowest. Globulin values were significant ($P < 0.05$) differences between treatments, chicks fed T1nd T2 diet showed significant higher ($P < 0.05$) values f globulin (mg/dl) when compared to other treatments. Also, T1 showed the lowest significant ($P > 0.05$) differences in A/G ratio. Values of different treatments ranged between 0.68 and 1.43 for T1 and control (+ve) respectively. Plasma AST and ALT values were significantly ($P > 0.05$) differences affected by different dietary treatments. Concerning hepatic enzymes, it is well-known that, AST and ALT usually appear in plasma when there is damage on the liver and muscle tissues caused be excessive stress. T2 had the lowest value of AST 97.57 (IU/L) compared with control (-ve) and other treatments. Also, T2 only the lowest value for ALT 17.00 (IU/L), the differences were significant and this due to treatments have adverse effect on liver. Plasma cholesterol showed that there were significant ($P < 0.01$) differences between all experimental treatments. Results showed that feeding chicks on GM lowered ($P \leq 0.01$) their blood content of cholesterol to treatments T1 compared with this given control (-ve) diet.

Table (5). Effect of feeding different dietary treatments on blood plasma constituents

Items			Treatments				
	Control(-ve)	Control (+ve)	T1	T2	T3	T4	Sig
Total protein (mg/ dL)	4.05±0.16^{ab}	3.82±0.09^{b}	3.86±0.05^{b}	4.56±0.32^{a}	4.14±0.09^{ab}	3.58±0.23^{b}	*
Albumin (mg/ dL)	2.31±0.04^{a}	2.25±0.07^{a}	1.57±0.33^{b}	2.18±0.37^{a}	2.29±0.06^{a}	1.92±0.10^{ab}	*
Globulin (mg/ dL)	1.74±0.36^{ab}	1.57±0.17^{b}	2.29±0.38^{a}	2.38±0.32^{a}	1.85±0.04^{ab}	1.66±0.13^{b}	*
A / G ratio	1.32±0.05^{ab}	1.43±0.24^{a}	0.68±0.33^{b}	0.91±0.15^{ab}	1.23±0.01^{ab}	1.15±0.03^{ab}	*
AST (IU/L)	128.00±8.00^{ab}	110.00±13.00^{ab}	149.00±26.00^{a}	97.57±10.00^{b}	117.00±12.00^{ab}	115.00±13.00^{ab}	*
ALT (IU/L)	21.00±1.00^{b}	31.33±1.66^{a}	22.66±0.33^{b}	17.00±1.00^{c}	22.00±0.27^{b}	19.00±3.00^{bc}	**
Cholesterol (mg/ dL)	135.14±5.4^{b}	151.35±16.21^{ab}	106.31±9.01^{c}	167.57±5.67^{a}	127.93±1.80^{bc}	138.74±9.01^{b}	**

a, b and c means the same row with different superscripts are significantly different sig. = significance, ***($P \leq 0.001$), **($P \leq 0.01$), * ($P \leq 0.05$), N.S = Non significant
SM=Soybean meal, GM= Guar meal
Control (-ve) = SM, Control (+ve) = GM without treatment, T1 = GM treatment with heat, T2 = GM treatment with Fungi (trichoderma reise), T3 = GM treatment with b-mannanase 30g / 100kg, T4 = GM treatment with NDF 20g / 100kg.

Pathological examination:

1-Effect of feeding different dietary treatments on Morality rate and health condition:

Under the condition of this experiment all chicks appeared healthy and mortality rate was different among treatments during the period (1-35 days) of age. Chicks fed control negative diets (0.0% GM) gave slightly higher mortality rate (8.37 %) compared to all treatments, while T2 (5% GM + fungi) and T4 (5% GM + NDF) diets recorded the lowest value being the same figure (2.77%).

2-Effect of feeding different dietary treatments on digestive tract measurements :

Results in Table (6) show the relationship between different dietary treatments and digestive tract measurements.

A- Digestive tract length and small intestine length:

The digestive tract and small intestine length (cm/100 g body weight), was increased by feeding T2-T3-T4 diets compared with those fed control or T1diets. Higher digestive tract length was recorded by T2 and T3 (16.40 and 13.41 cm/100 g BW), respectively. While, lower figures were found in chickens fed control or T4 diets (12.12 and 11.95 cm/100 g BW), respectively, without any significant differences.

Small intestine length showed the same trend since chickens fed T1 or T2 gave significantly higher figures being (11.23 and 11.65 cm/100 g BW), respectively.

On the other hand, chickens fed control or T5 diets showed significantly lower figures being (10.40 and 10.09 cm/100 g BW), respectively.

B- Small intestine weight and thickness:

Data presented in Table (6) indicated that small intestine weight and thickness were significantly decreased by feeding control diets compared with those fed other dietary treatments. Higher small intestine weight and thickness were detected for the chickens fed T3 diets compared with those fed control diets. The corresponding figures were (79.18 vs. 49.76 g) and (0.446 vs. 0.296), respectively. And differences between these two treatments were significant.

Table (6). Effect of feeding different dietary treatments on digestive tract measurements.

Items	Treatments						
	Control (-ve)	Control (+ve)	T1	T2	T3	T4	Sig
Digestive tract Length (cm)/ 100 g body weight	12,12±0.48	16.40±0.39	13.41±0.63	12.57±0.37	12.95±0.50	11.95±0.68	N.S
Small intestine Length (cm) / 100 g body weight	10.40±0.61^{b}	11.23±0.57^{a}	11.65±0.78^{a}	10.97±0.83^{ab}	11.12±0.48^{a}	10.09±0.73^{b}	*
Small intestine weight (g)	49.76±1.64^{c}	54.86±3.89^{bc}	77.43±4.85^{a}	79.18±3.35^{a}	63.03±3.15^{b}	58.88±5.14^{b}	*
Small intestine thickness*	0.296±0.020^{c}	0.343±0.023^{bc}	0.403±0.014 $_{ab}$	0.446±0.034^{a}	0.336±0.023^{bc}	0.357±0.041^{bc}	*

a, b and c means in the same row with different superscripts are significantly different, sig.= significance, * (P≤0.05), N.S= Non significant *thickness= small intestine weight / small intestine length.

Control negative = SM, Control positive = GM without treatment, T1= GM treatment with heat, T2= GM treatment with Fungi (trichoderma reesei), T3= GM treatment with b-mannanase 300g / ton, T4= GM treatment with NaDF 200g / ton.

3-Effect of feeding different dietary treatments on histomorphological examination of intestinal section:

In control (-ve) (0.0% GM) showing normal mucosa, submucosa, and musculosa (photo.1),while in Control (+ve) (5% GM) showing shortened villi with focal sloughing in the mucosal epithelium at villus tips (photo.2), sloughed villi with mononuclear cells infiltrations (photo.3), shortened villi with interglandular hemorrhage with presence of free RBCs (photo.4).In T1(5% GM + heat)showing congestion of mesenteric blood vessels and necrosis and hemorrhage in the tips of villi (photo.5) and hemorrhage among destructed villi (photo.6).In T2 (5% GM + fungi) showing apparently normal mucosa, submucosa, and musculosa (photo.7and 8).In T3 (5% GM + β-mannanase) showing apparently normal mucosa, submucosa, and musculosa with slight increase in villi length and thickness (photo 9 and 10). InT4 (5% GM + NaDF) showing apparently normal mucosa, submucosa, and musculosa (photo 11 and 12).

As shown in Table (7) and histopathological examination, data of histomorphological examination of the intestine section of villus height, villus width and crypt depth showed apparently normal mucosa and submucosa in T2, T3, T4 and control (-ve) groups with significant ($P < 0.05$) differences within treatments. Values of different treatments ranged from 322.00 to 425.00, 4.37 to 9.07 and 3.27 to 4.27 for villus height, villus width and crypt depth, respectively. Conversely, no significant differences in muscle thickness among treatments, however, chicks fed T1 recorded muscle thickness (7.86) while control diets recorded the lowest value (6.80).

It is clear from the previous observation that chicks fed T2, T3 and T4 diet presented an improvement in histological appearance of small intestine as observed by developed morphological changes in villus height, width, muscle thickness and size of crypt depth which imply a greater efficiency of feed absorption.

Table (7). Effect of feeding different dietary treatments on histomorphological examination of the intestinal section

Items			Treatments				
	Control -ve)	Control (+ve)	T1	T2	T3	T4	Sig
Villus height (µm)	322.00±2.88^{c}	359.00±4.62^{bc}	392.00±36.37^{ab}	369.00±25.98^{abc}	429.33±4.91^{a}	425.00±16.17^{a}	**
Villus width (µm)	4.90±2.51^{b}	5.20±2.42^{b}	4.37±1.87^{b}	9.07±0.09^{a}	5.20±2.42^{b}	9.07±0.03^{a}	*
Muscle thickness (µm)	6.80±0.58	7.86±0.15	7.83±0.37	7.70±0.12	7.83±0.26	7.63±0.43	N.S
Crypt depth (µm)	4.17±0.49^{ab}	3.27±0.03^{b}	3.60±0.01^{ab}	3.50±0.40^{ab}	4.07±0.20^{ab}	4.27±0.15^{a}	*

a, b and c means in the same row with different superscripts are significantly different, sig.= significance, **($P \leq 0.01$), * ($P \leq 0.05$), N.S= Non significant

Control+ve= SM, Control-ve= GM without treatment, T1= GM treatment with heat, T2= GM treatment with Fungi (trichoderma reesei), T3= GM treatment with b-mannanase 300 g / ton, T4= GM treatment with NaDF 200 g / ton.

Effect of feeding different dietary treatments on histomorphological examination of intestinal section.

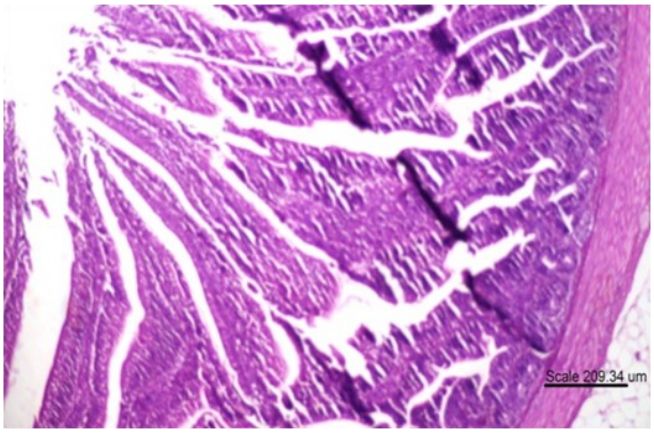

Photo. (1): Control (-ve) (0.0% GM) showing normal mucosa, submucosa, and musculosa. H&E. X200.

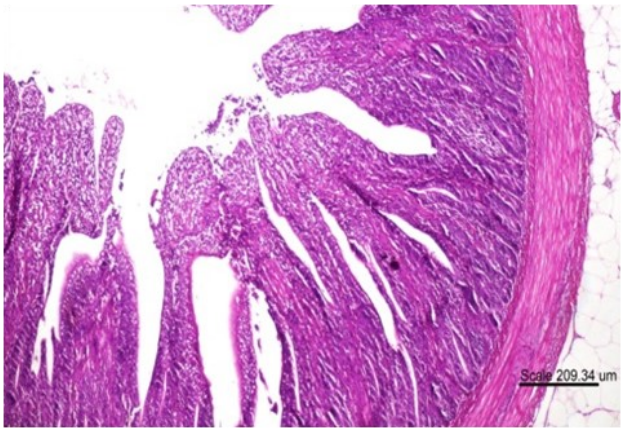

Photo. (2): Control (+ve) (5% GM) showing shortened villi with focal sloughing in the mucosal epithelium at villus tips.H&E.X200.

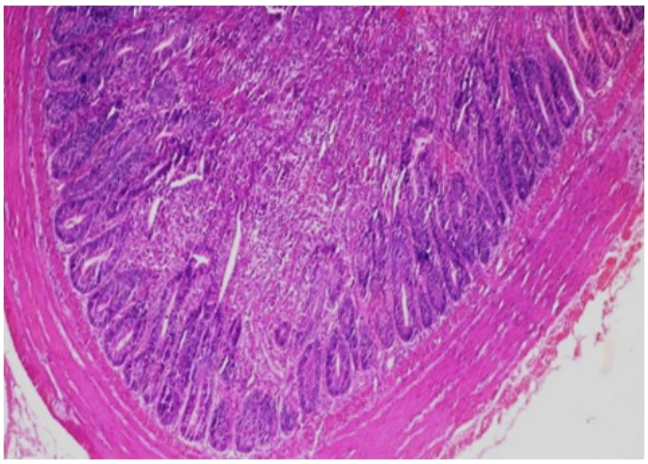

Photo. (3): Control (+ve) (5% GM) showing sloughed villi with mononuclear cells infiltrations.H&E.X100.

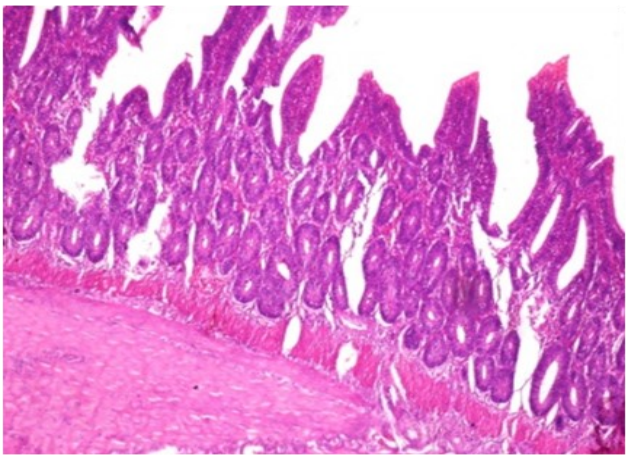

Photo. (4): Control (+ve) (5% GM) showing shortened villi with interglandular hemorrhage with presence of free RBCs.H&E.X200.

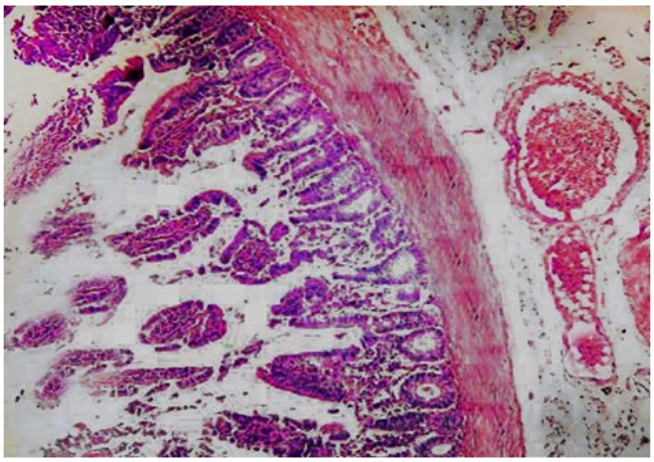

Photo. (5): T1(5% GM + heat)showing congestion of mesenteric blood vessels and necrosis and hemorrhage in the tips of villi (arrow). H&E x 150.

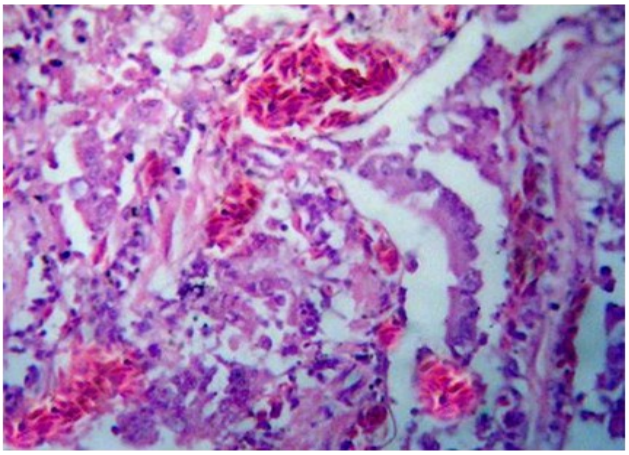

Photo. (6): T1(5% GM + heat) showing hemorrhage among destructed villi .H&E.X400.

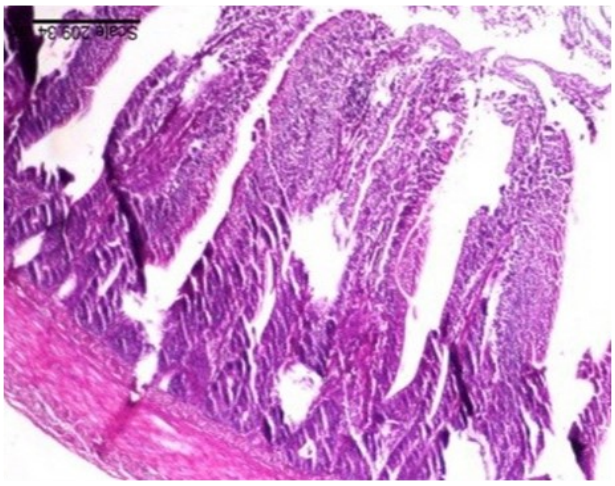

Photo. (7): T2 (5% GM + fungi) showing apparently normal mucosa, submucosa, and musculosa.H&E.X200

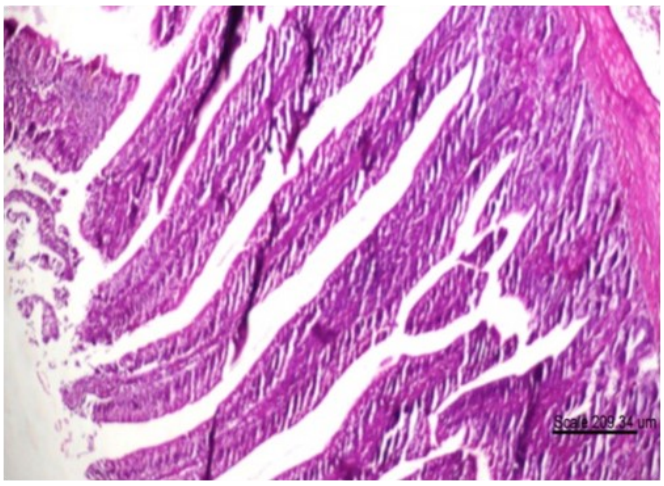

Photo. (8): T2 (5% GM + fungi) showing apparently normal mucosa, submucosa, and musculosa.H&E.X200

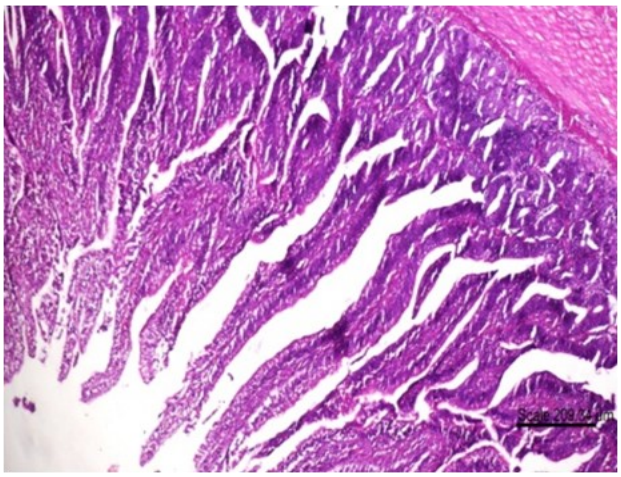

Photo. (9): T3 (5% GM + β-mannanase) showing apparently normal mucosa, submucosa, and musculosa.H&E.X200.

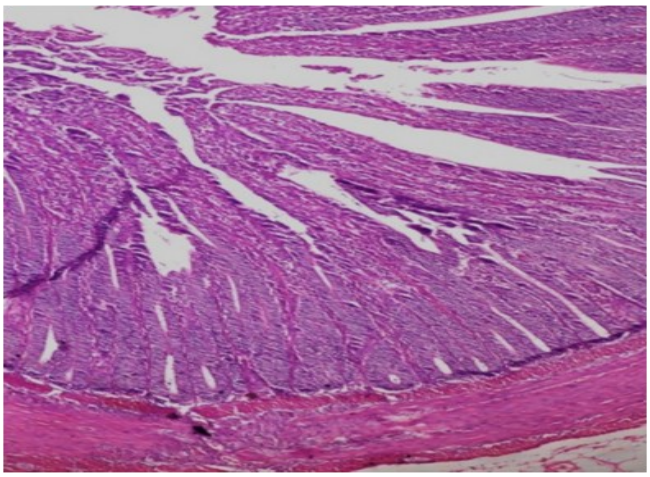

Photo. (10): T3 (5% GM + β-mannanase) showing apparently normal mucosa and submucosa, and musculosa with slight increase in villi length and thickness. H & E.X200.

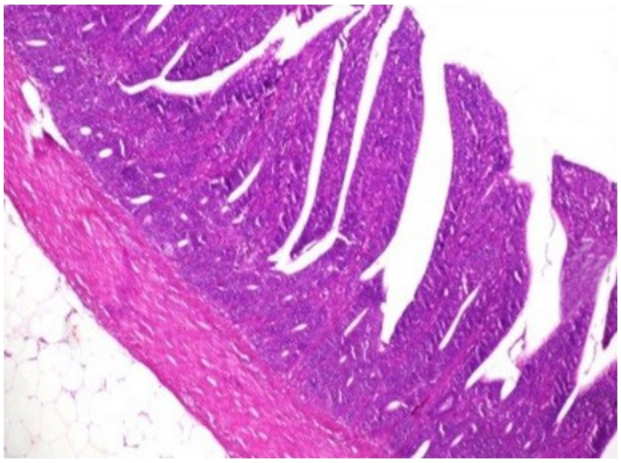

Photo. (11): T4 (5% GM + NaDF) showing apparently normal mucosa, submucosa, and musculosa.H&E.X200.

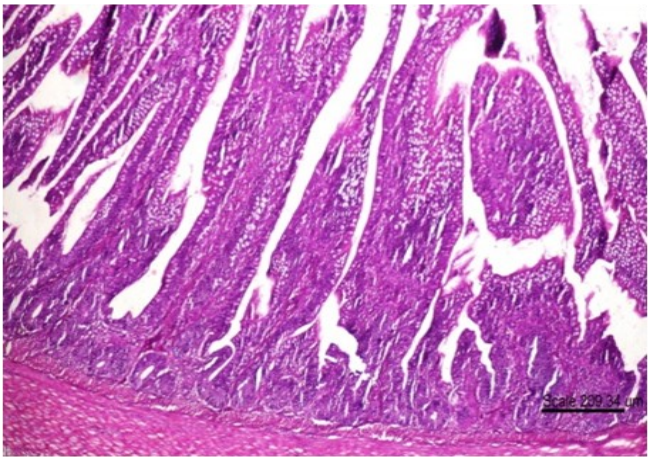

Photo. (12): T4 (5% GM + NaDF) showing apparently normal mucosa, submucosa, and musculosa.H&E.X200.

Discussion

Growth performance:

Using GM as a partial replacement (<10%) of soybean in poultry diets may be a useful economic strategy for decreasing feed costs without any negative effects on production. the negative effects of adding guar meal on body weight and feed conversion ratio may be attribute to the presence of inhibitors in guar meal such as Guar gum, trypsin inhibitor, saponin, polyphenols and hem agglutinin or some other unknown toxic substances. **Hassan *et al.* (2007) found** that guar meal contains 5-13% of dry matter triterpenoids guar saponin and **Lee *et al.,* (2004)** stated that 13-18% gaur gum, residual galactomannans gum**. Maisonnier *et al.* (2001)** indicated that addition guar gum in broiler chicken diets increase digesta viscosity and decrease nutrients digestibilities, with most pronounced effects being observed for lipids, then for proteins and lowest for starch. Increased intestinal viscosity reduces the ability of the gut to vigorously mix intestinal contents and decreases the rate of passage of digesta through the intestine (**Salih *et al.,* 1991**). **(Vohra and Kratzer, 1964a)** showed that guar gum decrease growth and performance of broiler chickens even when guar gum contain in *meal and fed at low concentrations.* ***(Duncan et al.,* 2002; Zhang and Tizard, 1996) r**eported that number of experiments have demonstrated that b-mannans crossing the intestinal mucosa are potent stimulators of innate immune system, resulting in increased proliferation of macrophages and monocytes and resultant cytokine production. So the positives results in T2 and T3 may be attributed to the effect of β –mannanase addition which increased villus height and crypt depth and decreased goblet cell number, epithelial thickness and ratio of crypt depth to villus height in different sections of small intestine, suggesting that B-mannanase improves gut morphology in broiler chickens (**M. Mehri *et al.,* 2010).** On the hand, the positive effect of group which treated by using sodium diformate (NDF) is in harmony with **(Vogt *et al.,* 1981)** who stated that, the improved broiler performance occured when diets were supplemented with ingle acids as formic acid**. Izat *et al.* (1990a)** found significantly reduced levels of Salmonella spp. in carcass and caecal samples after including calcium formate in broiler diets. An important limitation, however, is that organic acids are rapidly metabolised in the fore-gut (crop to gizzard) of birds, which will reduce their impact on growth performance. A new molecule (sodium diformate) has been proven to be effective against pathogenic bacteria, including salmonella, along the whole gastro-intestinal tract **(Lückstädt *et al.,* 2009).**

Biochemical analysis:

It is well known that concentration of total protein is of importance, since any abnormalities in blood plasma proteins may indicate that some pathologic or other induced factors (i.e., water balance and nutritional state), is responsible. Moreover, alteration in blood plasma protein values many associate with either kidney or liver dysfunction. There is no significant difference between control (-ve) and control (+ve) groups in total protein, albumin, globulin and A/G ratio, this is may be due to the 13-18% gaur gum, residual galactomannans gum (**Lee *et al.,* 2004). Bengmark, (1988)** showed that galactomannan inhibits colonization of pathogenic gastrointestinal bacteria and **Duncan *et al.,* (2002)** stated that galactomannan enhances macrophage activation thus exhibiting immunostimulatory activity. In addition, there is no significant difference between T2 and T3 proups copaire with control (-ve) in total protein, albumin, globulin and A/G ratio, this is due to Saponin-rich guar meal extract exhibited antibacterial activity. Thus, guar products may have potential as an antibiotic alternative in poultry though it is still unclear if this effect is due to residual guar gum, saponin, or some unknown component of guar meal **(Hassan *et al.,* 2008). Yamamoto *et al.* (2000); Maisonnier *et al.,* (2001)** have shown some beneficial physiological functions of galactomannans. Such as, decreasing plasma cholesterol, this is explain that no significant difference between all groups.

Pathological Examination:

Morality rate and health condition:

Chicks fed control diets (0.0% GM) gave slightly higher mortality rate (8.37 %) compared to all treatments, while T3 (5% GM + fungi) and T4 (5% GM + NDF) diets recorded the lowest value being the same figure (2.77%).**Zhang and Tizzard (1996) and Ross**

***et al.* (2002)** showed that of various configurations are components of the surface of numerous pathogens such as fungi, bacteria, and viruses. There is an innate immune system in animals that is highly attuned to quickly recognize antigens on pathogens, especially comprehensive β-mannanas also, shown that β-mannan is capable of stimulating the innate immune system and so potentially stimulating a nonproductive energy exhausting innate immune response.

Effect of feeding different dietary treatments on digestive tract measurements:

Higher digestive tract length was recorded by T2, while, lower figures were found in chickens fed control or T4 diets, respectively, without any significant differences. Small intestine weight and thickness were significantly decreased by feeding control diets compared with those fed other dietary treatments.

In this trend, **Baurhoo *et al.* (2007)** showed that fructo oligosaccharides and mannanase oligosaccharides are classified prebiotics that beneficially affect gut health via different modes of action. Guar meal has high level of protein and it is used in poultry diets as protein feedstuff. However, guar meal may have adverse effects on poultry performance in high levels, which may have beneficial effects on gut health and immune response in low levels, because of presence guar gum which is not removed completely of the guar meal.

It is clear from the previous observation that chicks fed T3 or T4 diet presented an improvement in histological appearance of small intestine as observed by developed morphological changes in villus height, width, muscle thickness and size of crypt depth which imply a greater efficiency of feed absorption.

Effect of feeding different dietary treatments on histomorphological examination of intestinal section:

From the previous observation that chicks fed T2, T3 and T4 diet presented an improvement in histological appearance of small intestine, villus height, width, muscle thickness and size of crypt depth which imply a greater efficiency of feed absorption. **Mehri *et al.* (2010)** supplied diet which contains 0, 500, 700 or 900 g/ ton β-mannanase. 900 g / ton β-mannanase supplementation significantly reduced feed intake but did not influenced body weight gain and feed conversion ratio in both finisher and total period. This level of supplementation increased villus height and crypt depth and decreased goblet cell number, epithelial thickness and ratio of crypt depth to villus height in different sections of small intestine, suggesting that β-mannanase improves gut morphology in broiler chickens. The addition of β-mannanase at 700 and 900 g/ton significantly reduced jejunal viscosity compared with the control group. β-mannanase did not influence the blood serum proteins (albumin and globulins).

Hodges (1974) found that increased number and size of crypt was known to enhance nutrients digestibility via pH regulation in the digestive tract. It is well known that crypt secrets fluids containing different vital substances essential for the internal micro-environment of the small intestine segments. These fluids are rapidly absorbed from lumen making a circulation from crypt to villi, which results in a watery vehicle supply for improving absorption of nutrients, elaboration and production of antibodies and lymphocytes along with an increase in goblet cells which secrete and release substances responsible for reduction the pH value in the intestinal lumen.

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