Effect of *β*-Mannanase (Hemicell®) on Growth Performance and Immunity of Japanese quail

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A B S T R A C T

The objective of the current study was to evaluate the effects of a commercial prebiotic (Hemicell®) as a growth promoter on growth performance, intestinal morphometry and IL-1 and IL-2 gene expression in spleen of Japanese quail. A total number of 360 one-day-old Japanese quail chicks were randomly allocated into 4 equal groups. Group 1(control group) fed on the basal diet that not supplemented with prebiotic, group 2 fed on basal diet supplemented with 0.5 g Hemicell/kg diet, group 3 fed on basal diet supplemented with 1.0 g Hemicell/kg diet and group 4 fed on basal diet supplemented with 1.5 g Hemicell/kg diet. To evaluate growth parameters, 30 birds from each group were weighed weekly for six successive weeks. Spleen tissues were collected at 21 and 42 days after supplementation from all quail groups (control and experimental groups). At the end of the experiment, the results of the current study revealed that, feeding Japanese quails on diet supplemented with Hemicell resulted in improved growth and growth performance as indicated by increased body weight and weight gain. Also, intestinal morphometry revealed an increase in height and base width of intestinal villi in a dose dependent manner. Moreover, Real time PCR indicated upregulation of the *IL-1* and *IL-2* genes expression in a dose and time dependent manner. In conclusion, addition of β-mannanase in Japanese quail diets would have beneficial effects on performance and immunity in birds.

Keywords: Japanese quail, Prebiotic, (Hemicell®), Gene expression.

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1. INTRODUCTION

Soluble non-starch polysaccharides (SNSP) are present in the hemicellulose fraction of fiber as primary cell wall constituents which are rich in corn and soybean meal, the main poultry diets (Dhawan and Kaur, 2007). This carbohydrate class is one of the main anti-nutritional factors in broiler diets. Non Starch Polysaccharides (NSPs) are polymeric carbohydrates which differ in composition and structure from starch (Morgan *et al.*, 1995) and possess chemical cross linking among them therefore, are not well digested by poultry (Annison, 1993).

The anti-nutritional properties of SNSP are caused by their solubility in liquid medium and increased viscosity, which interferes in the mixing of digestive enzymes with nutrients and prevents adequate digesta movement and product transport from the hydrolysis of molecules in the intestinal

mucosa, leading to impaired digestion and absorption of nutrients (Slominski, 2011). Gao *et al*. (2008) demonstrated that adding a carbohydrase (xylanase) to broiler feed can reduce the relative weights of the duodenum, jejunum, colon, and pancreas. This finding shows that the anti-nutritional effect is not only restricted to altering the digesta flow but also causes disturbances in the normal functioning of the digestive organs, with increased endogenous secretions such as water, proteins, electrolytes, and lipids (Angkanaporn *et al*., 1994). Monogastric animals including poultry cannot produce or produce insufficient amounts of the specific enzymes necessary to hydrolyze and digest Non-Starch Polysaccharide present in the cell wall of the grains and they remain un-hydrolyzed (Opalinski *et al.*, 2010). In poultry, only the amylase enzyme produced by the pancreas can hydrolyze starch into smaller units that can be absorbed; therefore, the presence of exogenous enzymes is needed (O'NEILL *et al.*, 2014).

Enzymes break down the NSPs, decreases intestinal viscosity and eventually improve the digestibility of nutrients by improving gut performance. The endocarbohydrase *β*-1,4-mannanase supplementation in monogastric animal feed improves performance, increases weight gain (Daskiran *et al*., 2004; Kong *et al*., 2011), and reduces feed conversion (Lee *et al.*, 2003; Zou *et al.*, 2006) because of the increased apparent metabolizable energy values (Daskiran *et al*., 2004; Kong *et al*., 2011; Mussini *et al*., 2011). Based on the earlier observed benefits, endocarbohydrase *β*-1,4-mannanase may be employed as a strategy to reduce production costs because it can be used in lower energy broiler diets which leads to improved feed energy use, and the animals exhibit similar growth performance to broilers receiving adequate energy levels (Wu *et al*., 2005; Li *et al*., 2010). The use of endocarbohydrase *β*-1,4-mannanase in broiler diets is beneficial because it hydrolyzes a certain SNSP class, which reduces viscosity in the intestinal environment (Lee *et al*., 2003; Mehri *et al*., 2010); lower water retention from carbohydrate disaggregation into smaller molecules; and increases the availability of carbohydrates, which are absorbed and utilized by the animals (Saki *et al*., 2005). The endocarbohydrase *β*-1,4-mannanase (Hemicell®) randomly cleaves the 1,4-*β* glycosidic bonds of the main mannan, galactomannan, glucomannan, and galactoglucomannan chains as well as the bonds of the mannan chain itself to yield mannobiose, mannotriose, and mannose as products during hydrolysis (Dhawan and Kaur, 2007).

Hemicell is a fermentation product of *Bacillus lentus*; its active ingredient is *β*-mannanase, which is responsible for the hydrolysis of β-mannans, thus reducing intestinal viscosity, promoting better nutrient digestibility, and acting on pathogens after hydrolysis. Li *et al.*, (2010) indicated that β-mannanase supplementation could increase the coefficient of total tract apparent digestibility (CTTAD) of diet components, while the decrease in immunoglobulin levels is suggestive of a down-regulated immune system that might have allowed nutrients to be redirected towards optimum performance. Moreover, endocarbohydrase *β*-1,4-mannanase supplementation can attenuate the immune response of broilers caused by the reduced production of immunoglobulins (Ig) (Li *et al*., 2010) and leukocytes (Mehri *et al*., 2010) because of the mannans present in the feed (corn and soya bean meal) stimulate the immune system.

Therefore, the aim of the present work was to investigate the mechanism by which how the commercial endo-β-D-mannanase (Hemicell®) induces its growth promoting effect through measuring its effect on overall

growth performance (live body weight, weight gain, feed intake, and feed conversion ratio) and on histologic and morphometric study of intestinal villi in Japanese quail. In addition to that, investigate how the commercial endo-β-D- mannanase (Hemicell®) improves the immunity through measuring the splenic gene expression of Interleukin-1 (*IL-1*) and Interleukin-2 (*IL-2*).

2. Materials and methods

2.1. Birds and housing:

A total number of 360 healthy unsexed one day old Japanese quail chicks were used in this experiment. Birds were randomly assigned into four equal groups. Chicks of each group were housed in brooding batteries for the first 3 weeks then birds were transferred into batteries with individual cages, each cage contains one male and three females**.** The birds were reared under 23 hr light: 1 hr dark then after 5 weeks of age, birds were given 16 hr of light: 8 hr dark. The ambient temperature was 37^{o}C for the first 2 days and then decreased stepwise by 3^{o}C at 4 days intervals to reach 21^{o}C. Birds were allowed *ad libitum* access to feed and fresh water. Diets were formulated as starter (the 1st 2 weeks of age) and breeder diets (table 1).

Table 1: Composition of the ration was as the following:

Ingredients	***Ration***	
	Starter	***Breeder***
Maize (%)	54	62
Soya bean meal, (CP 44 %)	33	22
Concentrate (%)	10	10
Wheat bran (%)	3	-
Limestone (%)	-	5.7
Sodium chloride (%)	-	0.3
Calculated nutrient content		
Metabolizable energy (kcal/Kg)	2825	2809
Crude protein (%)	24.8	20.2
Methionine (%)	0.46	0.648
Cysteine (%)	0.325	0.257
Methionine + Cysteine (%)	0.211	0.211
Crude fiber (%)	3.99	3.061
Crude fat (%)	3.02	3.15
Linoleic acid (%)	1.37	1.45
Calcium (%)	0.84	2.95
Available phosphorus (%)	0.49	0.46

Concentrates providing the following per kilogram of diet: crude protein 520g; vitamin A 120000 IU; vitamin E 100 mg; vitamin K_3 21 mg; vitamin $B_1$10 mg; vitamin B_2 40 mg; vitamin B_6 15 mg; pantothenic acid 100 mg; vitamin B_{12} 0.1 mg; Fe 0.3 mg; Mn 600 mg; Cu 50 mg; Co 2 mg; Se1 mg and Zn 450 mg.

2.2. Feeding and watering:-

Birds were allowed ad libitum access to feed and fresh water. A commercial balanced broiler starter ration containing 24.8 % crude protein and metabolizable energy of about 2825 Kcal/Kg was used for feeding of the young birds (1st 2 weeks of age). While adult quails was fed diet containing 20.2 % crude protein and 2809 kcal/Kg metabolizable energy (Hassan et al., 2003a).

Experimental design and feeding program:

Healthy one -day old Japanese quail chicks (Coturnix coturnix japonica) of both sexes were used in this experiment. They were divided randomly into a control group and 3 treatment groups each containing 90 chicks. The length of the experiment was 6 weeks (from day old chick till the 6th week of age). Each group was divided into three replicates with 30 chicks in each. The first group was fed on the basal diet that did not supplemented with prebiotic, group 2, fed on basal diet with 0.5 g Endo-β-D-mannanase (Hemicell®)/kg diet, group 3 fed on basal diet with 1.0 g Hemicell®/kg diet and group 4 fed on basal diet with 1.5 g Hemicell®/kg diet.

Growth performance parameters:

The Japanese quail chicks were weighed individually at the start of experiment, then every week for recording of live body weights and body weight gains (differences between each two successive weights).

Feed intake:

The experimental diets were provided regularly at morning and the daily feed intake was calculated by difference between the weight of offered feed and remained portion, then divided by the number of the birds in

each group per day and totalized to be per week.

Feed Conversion Ratio (FCR):

Feed Conversion Ratio was calculated by dividing the amount of feed consumed (g) during the week by gain in weight (g) during the same week according to (lambert et al., 1936).

$$FCR = \frac{\text{Feed intake (g) /bird/week}}{\text{Body weight gain (g) /bird/week}}$$

Q*uantitative morphometrical analysis of intestinal villi / surface area*:

After 42 days of treatment, immediately after slaughter in the evisceration stage, the duodenum was carefully removed from the abdominal cavity and specimens of duodenum were taken by cutting in a size of 1 cm^2 each to examine the height, base width and crypt depth of intestinal villi of all groups. One-piece duodenum from each quail of the same age and diet (treated and control) was cut and subjected to histological and morphological examination (Hofmann and Schnorr, 1982).

2.3. RNA extraction from spleen:

Materials

RNA extraction kit (easy-REDTM, iNtRON Biotechnology, #17063, South Korea) which includes Easy-RED solution (phenol and guanidinium isothiocyanate).

Principle

Pure RNA was extracted from spleen of Japanese quail fed on Hemicell supplemented diet using total RNA Purification Kit following the manufacturer protocol (iNtRON Biotechnology, easy-RED^{TM} Total RNA Extraction Kit). According to livak and schmittgen, (2001)

2.4. Statistical analysis:

All the data were expressed as means ±S.E. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS, 18.0 software, 2011 and the individual comparisons were obtained by Duncan's multiple range test (DMRT). Values were considered statistically significant when $p<0.05$.

3. RESULTS

Growth parameters

1- Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on live body weight (g) of Japanese quail:

Statistical analysis of the obtained results showed that feeding Japanese quails on diet supplemented with different levels of Hemicell® (0.5, 1.0 and 1.5 g Hemicell® / kg diet) had a significant ($p<0.05$) increase in live body weight during all experimental weeks (from the first week to the 6th week) in a dose dependent manner, in comparison with control group (feed basal diet without Hemicell® supplementation) as shown in table (3).

2- Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on body weight gain (g) f Japanese quail:

Statistical analysis of the obtained results showed that feeding Japanese quails on diet supplemented with different levels of Hemicell® (0.5, 1.0 and 1.5 g Hemicell® / kg diet) had a significant ($p<0.05$) increase in body weight gain from the 1st week and attain the maximum values during the 3rd and 4th week then decline during the 5th week then increase again during the 6th week in comparison with control group as shown in table (3).

3- Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on daily feed intake (g) of Japanese quail:

Statistical analysis of the obtained results showed that feeding Japanese quails on diet supplemented with various levels of Hemicell® (0.5, 1.0 and 1.5 g Hemicell® / kg diet) had no significant change in daily feed intake along the period of the experiment (from the 1st week and till the 4th week) in

comparison with the control group as shown in table (4). But there is a significant ($p<0.05$) decrease in the feed intake at the 5th and 6th week of age in Japanese quails fed on diets supplemented with high level of Hemicell (1.5 g/kg diet) in comparison with either control group or groups supplemented with low level of Hemicell (0.5 and 1.0 g/kg diet).

4- Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on feed conversion ratio (g) of Japanese quail:

Statistical analysis of the obtained results showed that feeding Japanese quails on diet supplemented with various levels of Hemicell® (0.5 and 1.0 g Hemicell® / kg diet) had no significant change in feed conversion ratio along the period of the experiment (from the 1st week and till the 6th week) in comparison with the control group as shown in table (5). However, the birds supplemented with 1.5 g Hemicell® / kg diet showed non-significant decrease in feed conversion ratio along the period of the experiment but the decrease was significant only at the end of the experiment (during the 6th week) as shown in table (5).

Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on intestinal morphometry (µm) of Japanese quail:

Data presented in table (6) show the effect of Hemicell® supplementation to Japanese quail diet on the duodenal morphometry. Statistical analysis of the obtained results showed that there was a significant ($p<0.05$) change between groups supplemented with Hemicell® and control group in a dose response manner.

Results obtained from Japanese quails that supplemented with 0.5 g Hemicell® showed no statistical significant increase in the height of intestinal villi and also showed no statistical significant decrease either in the base width or crypt depth of the intestinal villi in comparison with the control group as shown in table (6).

There was a statistical significant ($p<0.05$) increase in the height of the intestinal villi among the birds feed diets supplemented with Hemicell® at doses (1.0 and 1.5 g/ kg diet) in comparison with the control birds not supplemented with Hemicell® and in a dose response manner as shown in table (6).

Regarding to the base width and crypt depth of the villi, the results showed that there is a statistical significant ($p<0.05$) increase in the base width and crypt depth of the intestinal villi in the birds feed diets supplemented with Hemicell® at a dose of (1.5 g/ kg diet) in comparison with the control birds as shown in table (6).

Moreover, the results revealed that there is a marked difference in the height of intestinal villi between the males and the females of Coturnix coturnix Japonica where intestinal villi of females were longer than those of males (data not shown).

Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on histological structure of duodenal villi:

Histological examination of group 1 (control) showed normal structure of the duodenum where the villi were covered with simple columnar epithelium in addition to goblet cells. The core of the villi was filled with C.T. The duodenal villi of Hemicell® supplemented groups (0.5, 1.0, 1.5g) showed a gradual increase in the number of goblet cells respectively in comparison with that observed in the control group.

Molecular analysis

Real time PCR was used to detect the relative expression of *IL-1*, and *IL-2* genes that reflects the changes in transcription levels of these genes in spleen of quail after supplementation of Endo-*β*-D-mannanase (Hemicell®) at three different doses (0.5, 1,

1.5 g / kg diet) for two time points (21 Day and 42 Day).

Effect of Endo-β-D-mannanase (Hemicell®) on the relative expression of IL-1 gene in quail spleen:

Our results revealed a significant (P≤0.05) up regulation of *IL-1* gene expression level in quail spleen following supplementation of Endo-*β*-D-mannanase (Hemicell®) (G2-4) for 2 different time periods (21 Days and 42 Days) as compared to control groups (G1) (Table 8). This up regulation was dose dependent, i.e. higher Endo-*β*-D-mannanase (Hemicell®) dose led to higher decreased *IL-1* gene expression. This up regulation was also time dependent, i.e. supplementation for 42 Days resulted in higher expression than supplementation for 21 Day.

Effect of Endo-β-D-mannanase (Hemicell®) on the relative expression of IL-2 gene in quail spleen:

Our results revealed a significant (P≤0.05) up regulation of *IL-2* gene expression level in quail spleen following supplementation of Endo-*β*-D-mannanase (Hemicell®) (G2-4) for 2 different time periods (21 Days and 42 Days) as compared to control groups (G1) (Table 8). This up regulation was dose dependent, i.e. higher Endo-*β*-D-mannanase (Hemicell®) dose led to higher decreased *IL-2* gene expression. This up regulation was also time dependent, i.e. treatment for 42 Day resulted in higher expression than treatment for 21 Days.

Table 2: Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on live body weight (g) of Japanese quail.

Body weight / Bird groups	Initial body weight	1st week	2nd week	3rd week	4th week	5th week	6th week
G1 (Control)	10.37[a] ± 0.31	29.20[c] ± 0.56	62.81[b] ± 0.69	102.74[c] ± 0.86	149.7[b] ± 1.78	181.29[c] ± 1.20	203.54[c] ± 1.07
G2 (0.5 g Hemicell®) /kg diet	10.38[a] ± 0.28	31.93[ab] ± 0.71	70.90[a] ± 0.96	116.44[b] ± 1.30	166.32[a] ± 1.32	196.83[b] ± 1.36	232.98[b] ± 1.33
G3 (1.0 g Hemicell®) /kg diet	10.08[a] ± 0.25	31.56[b] ± 0.98	72.51[a] ± 1.09	120.52[a] ± 0.96	167.16[a] ± 1.19	203.58[a] ± 1.55	245.92[a] ± 1.59
G4 (1.5 g Hemicell®) /kg diet	10.07[a] ± 0.29	34.03[a] ± 0.88	72.79[a] ± 0.94	120.82[a] ± 1.46	167.67[a] ± 2.46	202.08[a] ± 1.72	244.47[a] ± 1.75

Means with different letters at the same column differ significantly at (p≤0.05).

Table 3: Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on body weight gain (g) of Japanese quail.

Weight gain / Bird groups	1st week	2nd week	3rd week	4th week	5th week	6th week
G1 (Control)	18.83[b] ± 0.93	33.61[b] ± 1.24	39.99[b] ± 1.20	46.97[ab] ± 1.26	31.58[b] ± 1.33	22.25[c] ± 1.30
G2 (0.5g Hemicell®) /kg diet	21.55[a] ± 0.58	38.97[a] ± 0.84	45.54[a] ± 1.01	49.88[a] ± 0.90	30.51[b] ± 1.03	36.15[b] ± 1.23
G3 (1.0 g Hemicell®) /kg diet	21.64[a] ± 0.89	40.95[a] ± 1.05	48.01[a] ± 1.41	46.64[ab] ± 1.09	36.42[a] ± 1.35	42.34[a] ± 1.13
G4 (1.5 g Hemicell®) /kg diet	23.96[a] ± 0.86	38.66[a] ± 1.18	48.03[a] ± 1.18	46.85[ab] ± 1.09	34.41[a] ± 1.06	42.39[a] ± 1.30

Table 4: Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on daily feed intake (g) of Japanese quail.

Feed intake / Bird groups	1st week	2nd week	3rd week	4th week	5th week	6th week
G1 (Control)	6.16^{a} ± 0.93	12.01^{a} ± 1.24	24.23^{a}± 1.20	30.05^{a} ± 1.26	30.82^{a}± 1.33	33.29^{a} ± 1.30
G2 (0.5g Hemicell®)/ kg diet	6.60^{a} ± 0.58	12.21^{a} ± 0.84	25.23^{a} ± 1.01	28.04^{a} ± 0.90	30.19^{a} ± 1.03	33.25^{a} ± 1.23
G3 (1.0 g Hemicell®)/ kg diet	7.03^{a} ± 0.89	13.01^{a} ± 0.05	22.35^{b} ± 0.41	36. 45^{a} ± 1.09	28.97^{ab} ± 1.35	31.03^{a} ± 1.13
G4 (1.5 g Hemicell®)/ kg diet	7.21^{a} ± 0.86	13.25^{a} ± 0.18	18.18^{c} ± 0.18	34.80^{a} ± 1.09	26.30^{b} ± 1.06	28.9^{b} ± 1.30

Means with different letters at the same column differ significantly at ($p \leq 0.05$).

Table 5: Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on feed conversion ratio (g) of Japanese quail.

Feed conv. (g) / Bird groups	1st week	2nd week	3rd week	4th week	5th week	6th week
G1(Control)	3.08^{a} ± 0.93	2.55^{a} ± 1.24	3.92^{a}± 0.20	4.65^{a} ± 0.26	6.92^{a}± 0.33	13.64^{a} ± 0.30
G2 (0.5g Hemicell®) / kg diet	2.50^{a} ± 0.58	2.51^{a} ± 0.84	3.54^{ab} ± 0.01	3.94^{a} ± 0.50	6.78^{a} ±0.03	11.41^{b} ± 0.23
G3 (1.0 g Hemicell®)/ kg diet	2. 30^{a} ± 0.89	2.56^{a} ± 0.05	3.54^{ab} ± 0.41	3.88^{a} ± 0.09	6.74^{a} ±0.35	10.13^{b} ± 0.13
G4 (1.5 g Hemicell®) / kg diet	2.41^{a} ± 0.86	2. 50^{a} ± 0.18	2.58^{b} ± 0.18	3.70^{a} ± 0.09	5.61^{a} ±0.06	8.89^{c} ± 0.30

Means with different letters at the same column differ significantly at ($p \leq 0.05$).

Table 6: Effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on intestinal morphometry (villi) of Japanese quail.

	Height (µm)	Base width (µm)	Crypt depth (µm)
G1 (Control)	917.14 ± 30.49^{a}	134.15 ± 9.85^{a}	120.59 ± 6.21^{a}
G2 (0.5 g Hemicell®)/kg diet	990.43 ± 30.33^{ab}	112.15 ± 5.33^{ab}	100.58 ± 2.49^{b}
G3 (1.00 g Hemicell®)/kg diet	1130.16 ± 48.52^{bc}	108.30 ± 5.83^{b}	111.81 ± 5.71^{ab}
G4 (1.5 g Hemicell®)/kg diet	1236.16 ± 73.97^{c}	163.90 ± 5.50^{c}	115.60 ± 6.40^{ab}

Table 7: Effect of Endo-β-D-mannanase (Hemicell®) on the relative expression of IL-1 gene in quail spleen.

Group	Fold change mean ± SEM	
	21 Days	42 Days
G1 (Control)	1.00± 0.07 ^{d}	1.00± 0.8 ^{d}
G2 (0.5 g Hemicell®)/kg diet	2.38±0.13 ^{c}	3.76±0.22 ^{c}
G3 (1.0 g Hemicell®)/kg diet	4.66±0.21 ^{b}	5.35±0.28 ^{b}
G4 (1.5 g Hemicell®)/kg diet	5.46±0.25 ^{a}	7.57±0.35 ^{a}

Table 8: Effect of Endo-β-D-mannanase (Hemicell®) on the relative expression of IL-2 gene in quail spleen.

Group	Fold change mean ± SEM	
	21 Days	42 Days
G1 (Control)	1.00± 0.07 [d]	1.00± 0.8 [d]
G2 (0.5 g Hemicell®)/kg diet	3.16±0.25 [c]	5.74±0.22 [c]
G3 (1.0 g Hemicell®)/kg diet	6.82±0.21 [b]	8.88±0.34 [b]
G4 (1.5 g Hemicell®)/kg diet	8.22±0.31 [a]	11.24±0.49 [a]

Means within the same column of each time point carrying different superscript letters are significantly different (P≤ 0.05).

4. DISCUSSION

The obtained results revealed that feeding Japanese quails on diet supplemented with different levels of prebiotic (Endo-*β*-D-mannanase (Hemicell®)) had a significant ($p<0.05$) increase in live body weight throughout the entire experimental period in a dose dependent manner, in comparison with control group. These results are in agreement with the results obtained by Tolba *et al.*, (2007) who found that, pullets provided with prebiotics had a higher live body weights compared to those fed the control diet.

The improvement in body weight for Japanese quails fed dietary enzyme (Hemicell) supplemented diet may be attributed to improvement in nutrient extraction in the small intestine by the host through accelerated digestion and reduced microbial activity (Apajalahti *et al*., 1995; Bedford and Apajalahti, 2001), overcome some of the reasons for poor starch digestion (Classen, 1996), significant improvement in chicks apparent metabolizable energy (Marquardt *et al.,* 1996 and Kocher *et al*., 2003), and elimination of the nutrient encapsulating effect of the cell wall polysaccharides (Jia *et al*., 2009). The negative effects of NSP and phytates in poultry can be alleviated by adding dietary enzymes degrading those compounds (Choct, 2006) and enzymatic supplementation can reduce environmental problems (Hana *et al.,* 2010). These results could be in agreement with Greenwood *et al.* (2002) and Pourreza1 *et al*. (2007) who found that enzyme supplementation improved body weight.

Hemicell is a fermentation product of *Bacillus lentus*; its active ingredient is *β*-mannanase, which hydrolyzes *β*-mannan. Because soybean meal contains *β*-mannans such as *β*-galactomannan and *β*-glucomannan, addition of *β*-mannanase may improve utilization of soybean meal and this is reflected on the live body weight of the Japanese quail in the obtained results and these results were in agreement of Lee et al. (2003) who reported marked improvement in growth parameters including live body weight of broilers after addition of *β*-mannanase to the diet. We suggest that the increase of the live body weight in the Japanese quails fed diet supplemented with *β*-mannanase (Hemicell®) is presumably due to the ability of beta-Mannanase to degrade beta-mannan, which is an antinutritional factor existing in many legumes, including soybean and this was also reported by previous studies as

Jackson *et al.* (1999), Lee *et al.* (2003) and Zou *et al.*, (2006).

Regarding the weight gain, the obtained results revealed a significant increase in weight gain from the 1st week and attain the maximum values during the 3rd and 4th week then decline during the 5th week then increase again especially during the last week of finishing period (the 6th week) in quails fed on diets supplemented with Hemicell® in comparison with control group. These results were in agreement with the results obtained by Petty *et al.* (2002); Lee *et al.* (2003) and Wu *et al.*(2005) who reported that addition of *β*-mannanase (Hemicell®) increased average daily weight gain compared with that of birds fed the control (no *β*-mannanase supplementation) diets.

The improvement in body weight gain in Japanese quails fed on diets supplemented with Hemicell may be attributed to that enzyme (Hemicell) supplementation reduced intestinal viscosity, improved nutrient availability and digestibility. Enzyme supplementation improved growth (Mikulshi *et a.*,1998 and La´zaro *et al*., 2003), increase in the diffusion rates of nutrients and endogenous enzymes enabling the bird to digest and absorb more nutrients and breakdown of water-soluble Non-starch polysaccharides (NSPs) into oligo and monosaccharides (Pawlik *et al.*, 1990). Response to enzymes could be due to hydrolysis of NPS and consequent elimination of the negative effects of those polysaccharides on birds. This may cause greater digestion and absorption of nutrients within the small intestine (Onu *et al*., 2011).

The results of live body weight and body weight gain were in agreement with Zhengkang (1997), La´zaro, *et al*. (2003) and Hana *et al*. (2010) who found that enzyme supplementation to the diet result in improvement of the body weight gain. These results were supported by Hajati *et al.* (2009) who stated that enzyme supplementation significantly improved growth and growth parameters. Also, Mikulshi *et al.* (1998) and Jia *et al*. (2009) reported that dietary enzymes result in faster growth rate of broilers.

Our results revealed that the effect of Hemicell supplementation to the diets of Japanese quails varies according to the age of supplemented birds and the level of Hemicell supplemented to the diets. We suggest that the feed intake is decreased either non-significantly in young age or significantly in adult birds in concomitant with significant increase in body weight and weight gain might be attributed to that Hemicell contributes to the basal metabolic rate of the birds by reducing the bird heat production and improve the net energy of the diet resulting in the significant increase in weight gain and live body weight.

From the obtained results, there was non-significant decrease in feed intake in groups supplemented with higher levels of Hemicell (1.5 g/kg diet) except at the 4th week of age in adult birds compared with non-supplemented birds, while other groups supplemented with lower levels showed non-significant increase in feed intake compared with control birds. The obtained results in this study were in agreement with Zou *et al.*, (2006) and Hana *et al*. (2010) who found that enzyme supplementation had no significant effect on feed intake of birds. However, the results of feed intake except at the 4th week were disagreed with the results reported by Alam *et al*. (2003), La´zaro *et al*. (2003) and Pourreza1 *et al*. (2007). This proves that effect of supplementation of enzymes as prebiotics depends on several factors such as condition of animals, animal species, environment conditions, composition of food

and level and type of prebiotic included in the mixtures.

The increased body weight and body weight gain of the Japanese quails fed enzyme supplemented diets was reflected in reduced feed conversion ratio as observed in this study. There was no difference in feed intake throughout the entire experimental period when the treatments with lower levels (0.5 g/kg diet) of supplemented enzymes but the significant difference appeared after 4 weeks age in higher levels of the enzyme (1.5 g/kg diet) supplemented diets compared with non-supplemented group. The results were inconsistent with the findings of McNaughton *et al*. (1998); Jackson *et al*. (2004) and Zou *et al.* (2006) who reported that Hemicell improved weight gain and feed conversion ratio in chicken broilers.

The results of the present study showed that the Endo-*β*-D-mannanase (Hemicell®) supplementation did not improve performance during the first 3 weeks of age, and the results of this study corroborate those of Zou *et al.* (2006), Li *et al.* (2010), Mehri *et al.* (2010) who did not observe improved broiler growth until the animals reached 3 weeks of age. This may have been caused by the physiologically immature state of the animals preventing them from benefiting from the enzymatic supplement or the animals being close to their genetic potential. However, *β*-D-mannanase supplementation increased the body weight and body weight gain and reduced feed intake and feed conversion ratio values during the 4- to 6-week old age and throughout the entire experiment. Additional studies reinforce the function of *β*-D-mannanase in improving broiler performance (Lee *et al.,* 2003; Daskiran *et al.,* 2004; Li *et al.,* 2010; Cho and Kim, 2013). This improvement is caused by of a series of benefits derived from *β*-D-mannanase supplementation, such as hydrolysis of a mannans class considered one of the main anti-nutritional factors of domestic avian diets. The reduction in the size of these molecules by the enzyme action allows improves the digestibility of nutrients such as dry matter (Cho and Kim, 2013), crude protein, and crude fiber (Li *et al.,* 2010) and produces higher metabolizable energy values (Daskiran *et al.,* 2004; Kong *et al.,* 2011). The Hemicell supplementation increased the AMEn values compared with diets that did not receive the supplement.

Regarding the overall growth parameters after Hemicell supplementation to Japanese quails, we can support the suggestions of the earlier studies who stated that the reduction in foregut digesta viscosity due to enzyme (Hemicell) supplementation was achieved primarily by reducing the molecular weight through hydrolysis of xylan backbone by endo-xylanase into smaller compounds and thus reduction in viscous effects of the feed because foregut digesta viscosity is directly proportional to the molecular weight of wheat arabinoxylans (Bedford and Classen, 1993). As a result of endo-xylanase and ß-glucanase supplementation, the long backbones of the arabinoxylans and ß-glucans are cleaved into shorter fragments, thereby reducing their viscosity (Gruppen *et al*., 1993, Latham *et al.,* 2018) and thereby improving the bird gut physiology which is reflected on the growth performance and growth parameters of the birds.

Regarding the effect of dietary Endo-β-D-mannanase (Hemicell®) supplementation on intestinal morphometry:

The viscous nature of β-mannan in diets, that contain a lot of soybean meal, has been observed to cause physiological and morphological changes to the gastrointestinal tract in avian species, which can impede efficient nutrients utilization (Cassidy *et al*.,

1981; Jacobs, 1983). Some studies have demonstrated that enzyme treatment can influence the morphological development of intestinal villi (Brenes *et al*., 1993; Amat *et al.,* 1996 and Luo *et al.,* 2009). In the results of this study, we observed that dietary supplementation of β-mannanase had a positive effect on duodenum mucosal morphology among Japanese quail sampled at 42 days: villus surface area and tip and base widths were all increased. Although there are few publications demonstrating the effect of β-mannanase on gut morphology, the work of Mehri *et al.* (2010) corroborated our findings. Poultry has a comparatively short digestive tract with a small hind gut and a caecum in comparison to the whole digestive tract (Labier & Leclercq, 1994). Microscopic investigation of the intestinal mucosa shows the presence of extremely more longer and coiled villi with increase in the number of goblet cells in Japanese quails supplemented with Hemicell than non-supplemented control birds, which suggest that Hemicell as a prebiotic could increase the surface area for absorption of digested food and thus improve the nutrient digestibility, efficient feed utilization, growth and the performance of the supplemented birds. These results were also supported by the earlier studies that reported improvement of ME, growth, and feed efficiency after *β*-mannanase supplementation in broilers by about 3% (McNaughton *et al.,* 1998; Petty *et al.* 2002; Daskiran *et al.* 2004).

In poultry, only the amylase enzyme produced by the pancreas can hydrolyze starch into smaller units that can be absorbed; therefore, the presence of exogenous enzymes is needed (O'NEILL *et al.,* 2014). The enzyme β-mannanase is responsible for the hydrolysis of β-mannans, thus reducing intestinal viscosity, promoting better nutrient digestibility which is suggested from the results of intestinal villi in the current study where there was a statistical significant increase in both the height and base width of intestinal villi with higher level of Hemicell supplementation.

Earlier researchers have shown positive effects of ß-mannanase supplementation on performance and nutrient digestibility in poultry fed corn-soybean meal-based diets. Such performance and nutrient digestibility improvements are the result of single or combined modes of action due to ß-mannanase addition. It has become increasingly clear that the modes of action of ß-mannanase might be very complex and versatile (Shastak *et al.,* 2015). The understanding of the mechanisms by which ß-mannanase supplementation affects nutrient digestion, metabolism, overall performance and health of birds, is important for the optimization and broadening of the application of this enzyme in avian nutrition.

Improved gut morphological development of the duodenal mucosal villi could likely contribute to the improved apparent nutrient utilization, which reflects in the better growth performance in poultry often observed when their diets are supplemented with exogenous enzymes. The results reported herein demonstrate that dietary supplementation of β-mannanase can improve gut health and might be effective for activating intestinal absorptive function, and that the functional activation promotes the growth of the Japanese quails as indicated by improved morphological development of the enteric mucosa and number of goblet cells, thereby increasing productive use of nutrients toward better growth performance. Further studies are still needed to evaluate the effect of Hemicell or other supplemental enzymes on gut health, especially as the trend of limiting the use of antibiotic growth promoters in poultry feed continues.

Regarding the effect of Endo-β-D-mannanase (Hemicell®) on the relative expression of IL-1 and IL-2 genes in quail spleen:

Our results revealed a significant (P≤0.05) up regulation of *IL-1* and *IL-2* genes expression level in quail spleen following supplementation of Endo-*β*-D-mannanase (Hemicell®) for 2 different time periods (21 Days old and 42 Days old) as compared to control groups. This up regulation was dose dependent, i.e. higher Endo-*β*-D-mannanase (Hemicell®) dose led to higher increased *IL-1* gene expression. This up regulation was also time dependent, i.e. supplementation for 42 Day resulted in higher expression than supplementation for 21 Day. These results are in partial agreement with those reported in local laying hens by Nofal *et al. (*2006).

Li *et al.,* (2010) indicated that β-mannanase supplementation could increase the coefficient of total tract apparent digestibility (CTTAD) of diet components, while the decrease in immunoglobulin levels is suggestive of a down-regulated immune system that might have allowed nutrients to be redirected towards optimum performance. Panel *et al.,* (2013) also indicated that the *β*-mannanase supplementation in low and high energy diets can improve body weight gain, nutrient digestibility, and relative breast meat weight and decrease the LDL-C in serum. Moreover, Panel *et al.,* (2014) assured that β-mannans induce an immune response that prevents nursery pigs from reaching maximum growth potential and endo-1,4-β-d-mannanase hydrolyzes β-mannans, which allows pigs to realize full growth potential. Hemicell can exhibit immunomodulatory effects through lowering the levels of cytokines, tumor necrosis factor in man and IL-1b and IL-6 and tumor necrosis factor in rat models. These cytokines not only have an essential role in energy homeostasis, but also in the modulation of antibody productions.

Interleukins are cytokines (secreted proteins) on which the function of the immune system largely depends on and rare deficiencies of a number of them have been described to cause autoimmune diseases or immune deficiency. Interleukin 1 participates in the regulation of immune responses, inflammatory reactions, and hematopoiesis (Sims *et al.,* 1988). Moreover, IL-1 receptor 1 mRNA or immunoreactivity was found in the brains of chicken and Japanese quail such as lemnothalamic visual projection areas of the pallium, in the dorsomedial nucleus of the hypothalamus, in the nucleus taenia, in cerebeller Purkinje cells and the motor components of the trigeminal and vagus nuclei suggesting a role for IL-1 in sickness behavior in birds (Wang *et al.,* 2003).

IL-2 is a glycoprotein produced mainly by activated T cells and acts mainly to promote T cell growth, but also activates macrophages and affects B cell growth (Gaffen *et al.,* 1998). IL-2 is a lymphokine that induces the proliferation of responsive T cells. In addition, it acts on some B cells, via receptor-specific binding (Cerretti *et al.,* 1986), also as a growth factor and antibody production stimulant (Mott *et al.,* 1992). Functionally, recombinant chicken IL-2 activates γ & T cells. In an experimental Eimeria infection, high levels of both γ & T cells and expression of IL-2 mRNA were found in the gut of chickens (Choi and Lillehoj, 2000). Interestingly, IL-2 can protect T cells against dexamethasone-induced apoptosis as reported by Guizani *et al.* (1996).

Feeding of prebiotics (beta mannans) was found to increase immune status and enhance the macrophage activity along with

T-helper cell activities (Shohani *et al*., 2013) and increased most of the relative immune organ weights and significantly increased the concentration of serum IgM. Moreover, *β*-mannanase (Hemicell) supplementation can attenuate the immune response of broilers caused by the reduced production of immunoglobulins (IgA) (Li *et al*., 2010 and Ferreira *et al.,* 2016) and leukocytes (Mehri *et al.,* 2010) because of the mannans present in the feed stimulate the immune system. Li *et al*. (2010) and Ferreira *et al.* (2016) found that supplementing diets with *β*-mannanase reduced the weights of immunity related organs such as the thymus and bursa of Fabricius. It has been reported that prebiotics as mannan oligosaccharides from yeast cell wall works by providing specific binding sites to enteric pathogens, thus reduces their chances to attach to the intestinal tract and these oligosaccharides being not digested by the endogenous enzymes of the bird pass through the gut with the pathogens attached thus producing a cleaning up effect (Zopf and Roth, 1996).

Moreover, Hemicell supplementation reduced the IgA, IgG, and IgM values in broiler serum. When found in the intestinal lumen, the mannans are potent stimulators of the immune system. The Hemicell enzyme acts in reducing the mannans content in the intestinal tract resulting in a lower stimulation of the immune system. The present study corroborates Li *et al.* (2010), who found reduced IgG and IgM values in 3-week-old broilers fed 1.0 and 2.0 g Hemicell/kg diet. Additionally, Hemicell supplementation can reduce the concentration of heterophils and lymphocytes in 35-day-old broilers (Mehri *et al.,* 2010). This shows that the Hemicell action is effective only when the animals are under physiological stress, in this case, nutritional deficiency of their diets.

There are advantages of using Hemicell in diets because it increased body weight gain and improved food conversion ratio throughout the entire experimental period. In addition, increased AM En and immune system attenuation were observed by (Ferreira *et al*., 2016); thus, Hemicell supplementation is an important tool for formulating diets to improve performance parameters. Animals with activated immune systems can redirect nutrients acquired in the diet to produce immune response-related molecules, which reduce the nutrients required for maintenance and growth and are directly reflected in /performance losses.

The significant effect of Hemicell on immunity of broilers may be explained by the findings of Wu *et al*. (2005) who reported that substrate of Hemicell entering the intestinal tract resulted in a reduction of the *β*-mannan content associated with a reduction of innate immune stimulation. One possible reason why *β*-mannanase might improve immunity is that *β*-mannan is degraded to mannan oligosaccharide (MOS). Huang *et al*. (2003) also reported that *β*-mannanase hydrolyzed *β*-mannan in soybean meal to MOS. Mannan oligosaccharide could influence the immune system, which had been observed by Shashidhara and Devegowda (2003), who reported that MOS significantly increased maternal antibody levels of broilers.

From the earlier results of Huang *et al.* (2003); Shashidhara and Devegowda (2003); Sims *et al.* (2004); Wu *et al.* (2005); Nofal *et al.* (2006); Li *et al.* (2010); Panel *et al.* (2013); Shohani *et al*. (2013); Panel *et al.* (2014) and the results of this study where Hemicell supplementation at various levels in Japanese quail upregulated the gene expression of IL-1 and IL-2 in spleen in a dose and time dependent manner, we can strongly suggests the immunomodulatory role

of Hemicell as an exogenous enzyme in avian species.

In a conclusion, we could conclude that the incorporation of β-mannanase in the diet or as a supplement results in improvements to certain growth performance parameters and immune response.

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