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#### **ABSTRACT**

A 2  $\times$  2  $\times$  2 factorial arrangement was conducted to evaluate the effects of cereal type (corn vs. wheat), enzyme supplementation (0 or 0.4 g/kg diet), and sodium butyrate addition (0 or 2 g/kg diet) on growth performance, carcass traits and intestinal morphology of male broilers from 10 to 42 d. A total of 600 male Arbor Acres chickens were assigned to eight dietary treatments in a completely randomized design, providing five replicate pens per treatment. Diets were formulated to meet or exceed NRC nutrient recommendations. When compared with birds fed corn-based diets, chickens fed wheat-based diets had inferior weight gain (1978 vs. 1807 g, p < 0.01) and feed per gain ratio (1.81 vs. 1.93; p < 0.01). Both weight gain (1870 vs. 1915 g; p < 0.01) and feed intake (3511 vs. 3556 g; p < 0.01) were increased by enzyme addition to experimental diets. Feed intake significantly (p < 0.01) increased by sodium butyrate addition to experimental diets; however, no significant differences were observed in weight gain or feed per gain ratio when sodium butyrate added to experimental diets. The birds fed wheatbased diet had higher relative weights of the proventriculus (p < 0.01), gizzard (p < 0.05), liver (p < 0.05), duodenum (p < 0.05), and ceca (p < 0.01) when compared with those fed corn-based diet. Enzyme and sodium butyrate addition had no significant effects (p > 0.05) on the relative weights of the proventriculus, gizzard, pancreas, liver and heart. Cereal type had a significant (p < 0.05) effect on intestinal morphology, including villus height and crypt depth. Jejunal villus height (p < 0.05) and crypt depth (p < 0.01) were improved by sodium butyrate addition. In conclusion, the results of this experiment showed that enzyme and sodium butyrate addition, especially to the wheat based diet, improve growth performance.

### INTRODUCTION

Corn is commonly used as the main source of energy in poultry diets; however, it is sometimes difficult to maintain least-cost diet formulations using corn in the Iranian poultry industry production due to an unstable grain market. When the price difference between wheat and corn is too high, poultry producers frequently substitute corn by wheat. Compared with corn, wheat energy content is lower, but the levels of many other nutrients, including protein and many amino acids, such as lysine, arginine, phenylalanine, tryptophan, are higher in wheat (NRC, 1994). Wheat contains relatively high levels of non-starch polysaccharides (NSP) in the cell wall layers that cannot be digested in the small intestine of monogastric animals due to the lack of endogenous enzymes. The accumulation of NSP (e. g. arabinoxylans) in alimentary tract results in water retention and increases the viscosity of digestive contents. It is well documented that high digesta viscosity has



a negative effect on nutrient digestion and absorption (Choct & Annison, 1992a) and modifies gut microflora (Choct et al., 1996). It has been demonstrated that high dietary soluble NSP is associated with *C. perfringens* proliferation (Annett et al., 2002) and that it may also affect the prevalence of *Salmonella spp.* and other pathogens in the intestines of chickens (Branton et al., 1997).

Considering the reasons mentioned above, enzymes are required to reduce wheat NSP levels. Enzyme (ENZ) supplementation has a direct positive effect on animal performance by improving nutrient digestion and absorption, thereby reducing substrate availability for the growth of microbial pathogens in the ileum (Bedford & Apajalahti, 2001). Generally, these enzymes hypothesized to work in two steps, described as an ileal phase and a cecal phase (Bedford, 2000). During the ileal phase, enzymes remove fermentable substrates. During the cecal phase, degradation products of sugars, such as xylose and xylo-oligomers, are fermented by cecal bacteria, stimulating the production of volatile fatty acids (VFA) and the growth of specific beneficial bacteria. In the process of depolymerization various polysaccharides in the diet, enzymes may produce manno-, galacto-, gluco-, or xylo-oligomers, which are similar to prebiotics and which may facilitate the proliferation of health-promoting bacteria such as Lactobacillus and Bifidobacterium (Monsan & Paul, 1995).

Studies with broilers have indicated a positive response in growth performance from the addition of various organic acids (OA) to diets (Skinner et al., 1991). OA have been considered as a suitable alternative for antibiotics in pig and poultry diets (Dierick et al., 2002). The mechanism by which OA improve performance in broiler chicks has not been clearly determined. It is proposed that OA improve bird performance because of their antimicrobial activity, which enhances protein and energy digestibility by reducing microbial competition with the host for nutrients and endogenous nitrogen losses, and by lowering the incidence of subclinical infections and the secretion of immune mediators (Dibner & Buttin, 2002). Adding OA to animal diets can lower the gastric pH (Ravindran et al., 1993; Garrido et al., 2004). Low gastric pH accelerates the conversion of pepsinogen to pepsin, which enhances the absorption rate of nutrients such as proteins, amino acids and minerals (Youn et al., 2005). Because of the biochemical characteristics of OA, they penetrate the cell membranes of pathogenic bacteria and lower the pH inside the cells. This low

pH kills pathogenic bacteria through the depletion of metabolic energy, the decrease of cell membrane metabolism, the outflow of cell fluids, and the blockage of the use of nutrients (Kirchgessner & Roth, 1982).

Besides its bactericidal activity, butyrate seems to play a role in development of the intestinal epithelium. Butyrate, which is a by-product of microbial fermentation of substances such as resistant starch, is considered to be important for the normal development of epithelial cells (Pryde et al., 2002). Therefore, butyric acid seems to be both bactericidal and a stimulant of intestinal epithelium. Thus, the objective of this study was to determine the effects of the addition of exogenous enzyme (ENZ) supplementation and sodium butyrate (SB) to corn and wheat-based diets on the performance, carcass traits and gastrointestinal morphology of broilers.

#### **MATERIAL AND METHODS**

# **Housing and Management**

A total of 600 male Arbor Acres broiler chickens, vaccinated for infectious bronchitis, were purchased from a commercial hatchery. All birds were fed the same diet until 10 d of age. Forty pens were randomly assigned to eight treatment groups, with 15 birds per pen. Each pen provided 1.5×1.5 m of floor space, with new wood shavings for bedding. Feed and water were provided ad libitum. Water was provided in nipple-type drinkers and feed was provided in trough feeders. At 1 d of age, the temperature was set at 33°C and then the temperature was reduced in 3°C/wk. Lights were on continuously for the first 3 d post-hatching, after which a 23L:1D lighting schedule was maintained for the duration of the experiment.

### **Experimental Design and Diets**

A completely randomized design in a 2  $\times$  2  $\times$  2 factorial arrangement was used to study the effects of cereal type (corn- or wheat-based), ENZ (0 or 0.4 g/kg), and SB addition (0 or 2 g/kg). SB was purchased from Ding Su Co., China. The enzyme [Grindazyme (GP15000)] was supplied by Danisco Animal Nutrition (Marlborough, UK). Grindazyme supplies 18000 U of beta-Endoglucanase activity (EC 3.2.1.4) and 43000 U of xylanase. Diets were free from antibiotics and coccidiostats and were formulated to contain 3,100 kcal of ME/kg and 20.5% CP in the starter phase and 3,200 kcal of ME/kg and 19.5% CP in the grower phase (Table 1). All diets were used in the mash form.



**Table 1** – Composition and calculated analysis of experimental diets

Item	Corn-ba	sed diets	Wheat-based diets			
rtem	Starter	Grower	Starter	Grower		
Ingredient (% of diet)						
Corn	58.4	61.3	24.3	18.4		
Wheat	0	0	40	50		
Soybean meal	30.5	26.35	24.6	18.5		
Vegetable oil	4	4.65	4	5		
Fish meal	4	4.85	4	5.5		
Calcium carbonate	1	1	1	1		
Dicalcium phosphate	1.2	1	1.2	1		
Salt	0.25	0.25	0.25	0.25		
Mineral and vitamin premix <sup>1</sup>	0.50	0.50	0.50	0.50		
DL-Methionine	0.12	0.10	0.12	0.10		
L-Lysine HCI	0.03	0	0.03	0		
Calculated analysis						
CP (%)	20.5	19.5	20.5	19.5		
ME (kcal/kg)	3100	3200	3100	3200		
Lysine (%)	1.14	1	1.11	1		
Methionine (%)	0.46	0.41	0.45	0.41		
Methionine + cystine (%)	0.82	0.75	0.83	0.76		
Calcium (%)	0.95	0.92	0.95	0.96		
Available phosphorus (%)	0.5	0.49	0. 5	0.5		

<sup>1</sup>Mineral and vitamin premix provided the following: Mn, 89 mg; Zn, 88 mg; Fe, 34 mg; Cu, 63 mg; Se, 0.3 mg; I, 1.8 mg;

vitamin A, 6,238 IU; vitamin D3, 2,275 IU; vitamin E, 20 IU; vitamin B12, 0.013 mg; vitamin K, 2.9 mg; niacin, 75 mg;

folic acid, 0.86 mg; biotin, 0.1 mg; riboflavin, 5.5 mg per kilogram of the starter diet; and Mn, 71 mg; Zn, 71 mg; Fe, 27 mg;

Cu, 50 mg; Se, 0.24 mg; I, 1.4 mg; vitamin A, 4,990 IU; vitamin D3, 1,820 IU; vitamin E, 16 IU; vitamin B12, 0.011 mg;

vitamin K, 2.3 mg; niacin, 60 mg; folic acid, 0.69 mg; biotin, 0.08 mg; riboflavin, 4.4 mg per kilogram of the grower diet.

# **Experimental Procedures, Sample Collection, and Chemical Analysis**

The experiment lasted 42 d and consisted of two phases (10 to 21 d, starter; 22 to 42 d, grower-finisher). Feed intake (FI) and weight gain (WG) were measured on a pen basis in weekly intervals, whereas mortality was recorded daily. Fee intake, WG and feed per gain ratio were calculated for each period (d 10 to 21, d 22 to 42, and d 0 to 42). On d 42, two birds per replicate (10 per treatment) were randomly selected and sacrificed by sodium pentobarbital injection. The gizzard, liver, pancreas and total intestinal were excised, and weighed separately for each bird. Then, the small

intestine from each bird was opened, and used for intestinal morphological evaluation. All procedures were approved by the University Institutional Animal Care and Use Committee.

# **Histological Methods**

Histological examinations were carried out according to the method of Iji et al. (2001). Before fixation in Bouin's solution and paraffin embedding, intestinal samples from each section were immersed in formaldehyde. Paraffin sections of 6-mm thickness were made from each sample, stained with hematoxylin and eosin, and examined under light microscopy. Villus height and crypt depth were analyzed from each preparation. The length of the intestinal villi and the depth of the intestinal crypt were measured with linear-scaled graticule.

# **Statistical Analysis**

All data were analyzed by the GLM procedure of SAS 9.1 (SAS Institute Inc., 2003). Dunnett's test was used to compare the means. All statements of significance were based on p < 0.05.

#### RESULTS

The effects of cereal type and ENZ and SB addition on the performance of broilers are presented in Table 2. Chicks fed wheat diets consumed significantly (p. < 0.01) less feed than the corn-fed chicks. Both ENZ supplementation and SB addition significantly increased FI (p < 0.01). There was no significant interaction between cereal type and ENZ supplementation and SB addition. A significant (p < 0.01) interaction between cereal type and ENZ supplementation was observed for WG. Weight gain was reduced (p < 0.01) when the wheat-based diet was fed during the entire experiment compared with the birds were fed cornbased diet. WG was significantly (p < 0.01) influenced by ENZ supplementation. No significant interaction (p > 0.05) was observed for feed/gain ratio on over entire experiment. Feed per gain ratio was affected by cereal type from d 10 to 21 of the experiment (1.59 vs. 1.49; for wheat and corn based diets respectively, p < 0.01), regardless of ENZ and SB additions. ENZ supplementation significantly (1.58 vs. 1.50; p < 0.05) decreased feed per gain ratio from d 10 to 21 of the experiment. Feed per gain ratio was not affected by enzyme supplementation and SB addition during the grower phase and over the entire experimental period. In general, the feed per gain ratio was significantly poorer for chicks fed wheat diet without ENZ.



Table 2 – The effect of cereal type, enzyme and sodium butyrate addition on performance

Item			Feed intake (	g)	\	Veight gain (	g)	Fe	Feed to gain ratio		
item		10-21d	22-42d	10-42d	10-21d	22-42d	10-42d	10-21d	22-42d	10-42d	
Main effects					·		_				
C	W	598ª	2892 <sup>b</sup>	3490 <sup>b</sup>	389.6 <sup>b</sup>	1418 <sup>b</sup>	1807.7b	1.59ª	2.04ª	1.93ª	
Cereal type	С	659.2⁵	2918ª	3578ª	443.7ª	1534ª	1978ª	1.49 <sup>b</sup>	1.90 <sup>b</sup>	1.81 <sup>b</sup>	
SEM		2.36	2.90	3.61	2.68	7.14	7.83	0.012	0.010	0.008	
_	Α0	617.2 <sup>b</sup>	2894 <sup>b</sup>	3511.7 <sup>b</sup>	406.4 <sup>b</sup>	1463.5 <sup>b</sup>	1870.2 <sup>b</sup>	1.58ª	1.98	1.88	
Enz	A1	440a	2916ª	3556.5ª	426.9ª	1488.5ª	1915.5ª	1.50 <sup>b</sup>	1.96	1.86	
SEM		2.36	2.90	3.61	2.68	7.14	7.83	0.012	0.010	0.008	
	В0	616.2 <sup>b</sup>	2896.5 <sup>b</sup>	3513 <sup>b</sup>	420.2	1466.2	1887	1.52 <sup>b</sup>	1.98	1.86	
SB	B1	641ª	2913.5°	3555.2°	413.1	1485.7	1898.7	1.56ª	1.96	1.87	
SEM	D1	2.36	2.90	3.61	2.68	7.14	7.83	0.012	0.010	0.008	
Interaction e	fforts										
Corn	irects	643	2949	3593	440	1521	1962	1.46	1.94	1.83	
Wheat		564	2831	3395	392	1388	1780	1.65	2.04	1.91	
Corn×Enz		650	2908	3558	442.6	1526	1969	1.47	1.90	1.81	
Corn×SB		663	2901	3565	440.7	1529	1970	1.51	1.90	1.81	
Wheat×Enz		608	2898	3506	406.4	1430	1837	1.49	2.03	1.91	
Wheat×SB		599	2895	3494	353.1	1416	1769	1.69	2.05	1.97	
Corn×Enz×SB		681	2914	3596	451.6	1560	2011	1.51	1.86	1.79	
Wheat×Enz×S	В	621	2944	3566	406.9	1438	1845	1.53	2.05	1.93	
SEM		4.72	5.80	7.23	5.26	14.29	15.66	0.025	0.019	0.015	
					Probab	ility					
Cereal type		**	**	**	**	**	**	**	**	**	
Enz		**	*	**	**	*	**	*	ns	ns	
SB		**	**	**	ns	ns	ns	*	ns	ns	
Cereal type×E	nz	ns	ns	ns	**	ns	**	*	ns	ns	
Cereal type×SI	3	ns	ns	ns	ns	ns	ns	ns	ns	ns	
Enz×SB		ns	ns	ns	ns	ns	ns	ns	ns	ns	
Cereal type×E	nz×SB	ns	ns	ns	ns	ns	ns	ns	ns	ns	

W= Wheat-based diet; C= Corn-based diet; A0= Diet without enzyme; A1= Diet containing enzyme; B0= Diet without sodium butyrate; B1= Diet containing sodium butyrate.

Table 3 presents the effects of cereal type, ENZ supplementation and SB addition on the relative organ weights at 42 d of age broilers. Using wheat instead of corn significantly increased the relative weight of the proventriculus (p < 0.01), gizzard (p < 0.05) and liver (p < 0.05) at the end of experiment. In general, ENZ supplementation numerically decreased weights of different segments of the gastrointestinal tract (GIT), but SB addition had no effect (p > 0.05) on the relative weights of the proventriculus, gizzard, pancreas, liver and heart. Type of cereal influenced (p < 0.05) relative weights and lengths of the duodenum and caeca (Table 4); however, cereal type only affected lengths

of the Jejunum and ileum in small intestine. ENZ supplementation reduced duodenum relative length on d 42. Also, the effect of the interaction on cecum weight between cereal type and ENZ supplementation was significant (p < 0.05).

The influences of cereal type, ENZ and SB on intestinal morphology are presented in Table 5. Villus height, crypt depth (p < 0.05) and crypt depth to villus height ratio (p < 0.01) in duodenum were significantly affected by cereal type. This study showed that villi were higher in the duodenum than in the jejunum and ileum in all treatment groups, which its consistent with the major role that the duodenum plays in nutrient

 $<sup>^{</sup>a,b,..}$  Means not sharing a superscript letter in a column are significantly different at p < 0.05.



absorption. Enzyme supplementation did not affect villus height, crypt depth or crypt depth to villus height ratio in the duodenum. Villus height (p = 0.09) and crypt depth to villus height ratio (p = 0.07) in the duodenum were numerically higher in the SB-fed group. Villus height (p < 0.05), crypt depth (p < 0.01) and crypt depth to villus height ratio (p < 0.01) in the jejunum were significantly increased by cereal type and SB addition. There were no significant effects of cereal type, ENZ supplementation and SB addition on the villus height, crypt depth and crypt depth to villus height ratio in the ileum.

#### DISCUSSION

The results of the current study showed that birds fed the cornbased diets had overall superior WG, FI and feed/gain ratio compared with those fed the wheat-based diets. In general, wheat has lower nutritional value than corn because it contains high amounts of watersoluble and viscous NSP (Leeson & Summers, 2005). Arabinoxylans are known to adversely affect nutrient utilization, FI and growth performance of broiler chickens by increasing the viscosity of intestinal (Choct & Annison, 1992a,b), which can be prevented by adding NSP-degrading enzymes (Friesen et al., 1992; Choct et al., 1999; Wang et al., 2005). Previous research showed that the ENZ supplementation was effective in viscosity reduction, which resulted in higher fat, starch, nitrogen, and NSP digestibility in the small intestine of young broiler chickens fed wheat-based diets (Meng et al., 2004). The results of this study are in agreement with these findings, when because enzyme added to the wheat-based diets, an increment in BW and FI was observed over entire experiment. However, the deterioration of feed/gain ratio was significant in birds fed the wheat-based diets during the entire experimental

period, despite the fact that birds at grower phase of production are known to be able to tolerate high intestinal viscosity (Friesen *et al.*, 1992). The results of the current study demonstrated that the cell wall-degrading activities are at least of equal importance as the viscosity-reducing aspects.

The present experiment showed that SB addition did not influence the WG of broilers, only their feed intake. The results of this experiment are consistent with the findings of Leeson *et al.* (2005), who indicated that the dietary addition of fumaric acid did not affect WG of broilers, but did improve FCR. On the other hand, Pinchasov & Jensen (1989) reported that butyric acid, unlike other acids such as propionic acid, did not reduce feed intake. In the current study, the addition

**Table 3** – Relative weight (%) of organs (crop, proventriculus, gizzard, pancreas, liver and heart) of male broilers at 42 days of age

liver and hea	art) c	of male broilers	at 42 days	of age		
Item		Proventriculus	Gizzard	Pancreas	Liver	Heart
Main effects						
Cereal type	W	0.575a	2.46a	0.23	2.18a	0.55
Cerear type	C	0.564b	2.31b	0.22	1.97b	0.51
SEM		0.02	0.08	0.01	0.06	0.02
Enz	A0	0.59	2.45	0.23	2.11	0.53
LIIZ	A1	0.55	2.30	0.22	2.04	0.54
SEM		0.02	0.08	0.01	0.06	0.02
SB	ВО	0.59	2.42	0.22	2.06	0.51
	B1	0.54	2.34	0.22	2.08	0.55
SEM		0.02	0.08	0.01	0.06	0.02
Interaction effe	cts					
Corn		0.59	2.17	0.208	1.93	0.55
Wheat		0.70	2.38	0.222	2.27	0.49
Corn×Enz		0.60	2.38	0.204	1.95	0.47
Corn×SB		0.61	2.3	0.209	2	0.52
Wheat×Enz		0.66	2.44	0.215	2.09	0.53
Wheat×SB		0.68	2.41	0.228	2.21	0.54
Corn×Enz×SB		0.60	2.38	0.205	2.05	0.51
Wheat×Enz×SB		0.68	2.63	0.215	2.12	0.63
SEM		0.107	0.17	0.009	0.18	0.59
				ability		
Cereal type		**	*	*	*	ns
Enz		ns	ns	ns	ns	ns
SB		ns	ns	ns	ns	ns
Cereal type×En		ns	ns	ns	ns	ns
Cereal type×SB		ns	ns	ns	ns	ns
Enz×SB		ns	ns	ns	ns	ns
Cereal type×En:	z×SB	ns	ns	ns	ns	ns

W= Wheat-based diet; C= Corn-based diet; A0= Diet without enzyme; A1= Diet containing enzyme; B0= Diet without sodium butyrate; B1= Diet containing sodium butyrate.

 $<sup>^{</sup>a,b,..}$  Means not sharing a superscript letter in a column are significantly different at p < 0.05.



Table 4 – Relative weight (%) and length (cm kgBW<sup>-1</sup>) of the intestinal tract of male broilers at 42 days of age

		Duode	enum	Jejui	num	lleu	um	Caeca
Item		%	cm kg BW <sup>-1</sup>	%	cm kg BW <sup>-1</sup>	%	cm kg BW_1	%
Main effects	_							
C	W	0.768ª	1.49ª	1.73	3.77ª	1.01	2.45ª	1.55ª
Cereal type	С	0.659⁵	1.28 <sup>b</sup>	1.62	3.41 <sup>b</sup>	0.99	2.27 <sup>b</sup>	1.35 <sup>b</sup>
SEM		0.032	0.036	0.56	0.027	0.050	0.056	0.035
Enz	A0	0.75	1.44ª	1.72	3.63	1.03	2.38	1.49
LIIZ	A1	0.677	1.33 <sup>b</sup>	1.64	3.56	0.968	2.34	1.41
SEM		0.032	0.036	0.056	0.027	0.050	0.056	0.035
CD	ВО	0.725	1.40	1.68	3.63	0.997	2.39	1.45
SB	B1	0.702	1.37	1.68	3.56	1.00	2.33	1.44
SEM		0.032	0.036	0.56	0.027	0.050	0.056	0.035
Interaction effects								
Corn		0.68	1.29	1.63	3.43	0.98	2.27	1.33
Wheat		0.85	1.62	1.79	3.90	1.09	2.53	1.63
Corn×Enz		0.67	1.30⁵	1.61	3.42	0.95	2.34	1.39 <sup>b</sup>
Corn×SB		0.66	1.28	1.07	3.40	1.03	2.23	1.32
Wheat×Enz		0.70	1.39ª	1.66	3.73	0.96	2.41	1.45ª
Wheat×SB		0.81	1.55	1.77	3.77	1.04	2.47	1.66
Corn×Enz×SB		0.62	1.25	1.59	3.38	0.98	2.21	1.34
Wheat×Enz×SB		0.71	1.36	1.68	3.67	0.97	2.40	1.44
SEM		0.09	0.07	0.15	0.05	0.14	011	0.09
				Proba	ability			
Cereal type		*	**	ns	**	ns	*	**
Enz		ns	*	ns	0.07	ns	ns	ns
SB		ns	ns	ns	ns	ns	ns	ns
Cereal type×Enz		ns	ns	ns	ns	ns	ns	*
Cereal type×SB		ns	ns	ns	ns	ns	ns	ns
Enz×SB		ns	ns	ns	ns	ns	ns	ns
Cereal type×Enz×SB		ns	ns	ns	ns	ns	ns	ns

W= Wheat-based diet; C= Corn-based diet; A0= Diet without enzyme; A1= Diet containing enzyme; B0= Diet without sodium butyrate; B1= Diet containing sodium butyrate.

of 0.02% SB to the diet increased FI. In other studies, the use of buffered propionic acid (Izat *et al.*, 1990), commercial organic acid product (Chaveerach *et. al.*, 2004) or 5 and 10 g/kg feed of formic acid (Hernandez *et al.*, 2006) also had no significant effect on the growth performance of broiler chickens.

In general, the use of wheat-based diets significantly altered the morphology of the different GIT segments compared with corn-based diets at the end of experiment. By using wheat in diet, there

was a linear (p < 0.01) increase in relative weights of proventriculus, gizzard and liver on d 42. Presumably the increased weight is accelerated by the presenced of arabinoxylans in wheat-based diets (Annison & Choct, 1991). This NSP can increase the viscosity of the digesta and prevent effective contact between substrates and the digestive enzymes, leading to significant changes of the structure and function of intestine (Dworkin et al., 1976) and organs. The activities of the GIT secretory mechanisms may be increased to adjust to

 $<sup>^{</sup>a,b,..}$  Means not sharing a superscript letter in a column are significantly different at p < 0.05.



these changes. Thus, this may influence the weight of the GIT, proventriculus, gizzard and liver. Brenes *et al.* (1993) indicated that this increased size and weight of intestine and GIT could be an adaptive response to an increased need for ENZ. When supplementing enzymes in the wheat-based diets, a higher proportion of NSP may be digested, which may reduce the secretory function of the responding organs and GIT segments, and therefore, organ size may decrease.

Good intestinal health is essential to achieve the optimal growth rates and feed efficiency. Antimicrobial agents (e.g., antibiotics, SB, etc.) are known to reduce intestinal pathogen levels, which in turn decreases the presence of toxins that causes changes in the intestinal morphology, affecting particularly the villi and the crypts. In general, SB supplementation increased both villus height and crypt depth. It has been demonstrated that short-chain fatty acids stimulate the proliferation

**Table 5** – Effects of cereal type, ENZ supplementation and SB addition on villus height, crypt depth and crypt depth to villus height ratio in different sections of the small intestine of birds.

			Duodenum	1	Jejunum					lleum		
Item		Villi height(µm)	Crypt depth (µm)	Ratio¹(c/v)		Villi height(µm)	Crypt depth (µm)	Ratio¹(c/v)	Villi height(µm)	Crypt depth (µm)	Ratio¹(c/v)	
Main effects					-							
Cereal type	W	1065⁵	389ª	2.81 <sup>b</sup>		942	240	3.93	683	194	3.79	
Cerear type	С	1181ª	315 <sup>b</sup>	3.80ª		978	247	3.97	627	189	3.55	
SEM		33	21	0.18		33	3	0.13	47	27	0.52	
Enz	A0	1117	352	3.30		933	244	3.83	702	192	3.82	
	A1	1129	351	3.31		987	243	4.07	608		3.52	
SEM		33	21	0.18		33	3	0.13	47	27	0.52	
	ВО	1078	371	3.03		920	247	3.73 <sup>b</sup>	644	188	3.62	
SB	B1	1167	333	3.57		1000	240	4.17ª	666		3.71	
SEM	DI	33	21	0.18		33	3	0.13	47	27	0.52	
22111		23	2.	0.10		23	<u> </u>	0.13	17	27	0.32	
Interaction effects												
Corn		1130	320	3.5		980	215	4.6	610	182	3.4	
Wheat		1010	420	2.4		840	258	3.3	593	188	3.2	
Corn×enz		1140	330	3.4		980	217	4.5	609	189	3.2	
Corn×SB		1220	310	3.9		1010	211	4.8	618	178	3.5	
Wheat×Enz		1040	420	2.5		890	247	3.6	595	195	3.1	
Wheat×SB		1100	360	3.1		960	236	4.1	606	183	3.3	
Corn×Enz×SB		1240	300	4.1		1010	206	4.9	609	181	3.4	
Wheat×Enz×SB		1100	350	3.1		970	225	4.3	604	182	3.3	
SEM		98	61	0.54		46	8.2	0.27	27	21	0.51	
							1.20%					
Cereal type		*	*	**		Proba	ability **	**	ns	ns	ns	
Enz		ns	ns	ns		ns	ns	ns	ns	ns	ns	
SB		0.09	ns	0.07		*	**	**	ns	ns	ns	
Cereal type×Enz		ns	ns	ns		ns	ns	ns	ns	ns	ns	
Cereal type×SB		ns	ns	ns		ns	0.09	ns	ns	ns	ns	
Enz×SB		ns	ns	ns		ns	ns	ns	ns	ns	ns	
Cereal type×Enz×SB		ns	ns	ns		ns	ns	ns	ns	ns	ns	

W= Wheat-based diet; C= Corn-based diet; A0= Diet without enzyme; A1= Diet containing enzyme; B0= Diet without sodium butyrate; B1= Diet containing sodium butyrate.

Crypt depth to villus height ratio

 $<sup>^{</sup>a,b,..}$  Means not sharing a superscript letter in a column are significantly different at p < 0.05.



of normal crypt cells, improving healthy tissue turnover and maintenance (Scheppach *et al.*, 1995). This effect was shown by Frankel *et al.* (1994), who found an increase in villus height and crypt depth in the jejunum of rats fed diets supplemented with butyric acid. Also, Garcia *et al.* (2007) reported that formic acid supplementation increased both villus height and crypt depth. Johnson *et al.* (1988) reported that soluble fermentable polysaccharides with high viscosity stimulate crypt cell proliferation rate in the small intestine of rats. It can be concluded that the negative effects of NSP are related to their capability of increasing digesta viscosity, consequently leading to changes in gut morphology and in the efficiency of nutrient utilization by chickens.

# **CONCLUSION**

In summary, enzymes (e. g., Grindazyme) may improve the performance of broilers consuming a wheat-based diet. The beneficial effect of enzyme supplementation was probably due to better nutrient utilization as a consequence of decreased intestinal viscosity and elimination of the nutrient-encapsulating effect of the cell wall polysaccharides. Furthermore, enzyme inclusion may reduce decrease the size of the digestive organs and of the GIT to some extent. The addition of SB may improve intestinal health. These results may aid the development of nutritional strategies to improve the performance of broiler chickens without the use antibiotic growth promoters.

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