http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet

Asian Network for Scientific Information

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (4): 659-666, 2006
ISSN 1028-8880

The Effect of Hull-less Barley Dietary on the Activity of Gut Microflora and Morphology Small Intestinal of Layer Hens

[1]A. Yaghobfar, [2]Rezaian, [2]M. Ashrafi-Helan,
[2]J. Barin, [1]H. Fazaeli and [1]Sayed Davod Sharifi
[1]Animal Science Research Institute,
P.O. Box 31585-1483, Tehran, Iran
[2]Faculty of Veterinary Medicine, Tehran University, Tehran, Iran

Abstract: The purpose of the present study was to indicated (evaluate) the effect of hull-less barley dietary on the morphology of the small intestinal wall and ileal microbial activity layer hens fed hull-less barley in diet. The results of experiment shown that the inclusion of hull-less barley in the diet had no effect on the bacterial activity as total counts of *bacteroidaea*, *Lactobacillus bifidobacterium* and *clostridium* in small intestinal of layer hen. In the present study, has shown that the hull-less barley influence on the total number of *aerobes* and *anaerobes* intestinal bacterial. This can be undigested nutrients enter the lower part of the small intestine, where microbial growth will be increased. The hull-less barley had effect on the villus height, villus width and crypt depth in the duodenum, jejunum and ileum of small intestinal of bird compared to control group. Across all groups, the villus height, villus width, crypt depth in the duodenum was greater than those in the jejunum and ileum and this is consistent with the significant role that the duodenum plays in nutrient absorption. The finding of result of present study as the inclusion of hull-less barley in the diet the number of density of goblet cells in the ileum greater than those in the jejunum and duodenum of small intestinal in birds. However, goblet cells are responsible for the secretion of mucin that is used for the mucinous lining of the intestinal epithelium. Based on the results of the present study, it can be stated that NSP as β-glucan content of hull-less barley concentration in diets of birds change microbial activity or population and the morphology of the small intestinal wall. The magnitude of these changes depends on the degree.

Key words: β-glucan, hull-less barley, microbial activity, villus, small intestinal

INTRODUCTION

The carbohydrates can only be utilized after fermentation by the microbial population. It is well established that the NSP fractions present in barley have anti-nutritive properties in poultry diet. It is assumed that the anti-nutritive effects of barley are associated with the water soluble fraction of β-glucans (White *et al*., 1981; 1983; Fincher and Stone, 1986; Classen *et al*., 1985. 1988; Campbell *et al*., 1989). The effect of β-glucan on nutrient digestion, there appears to be secondary effects attributable to higher level of microbial activity (Campbell *et al*., 1986). Literature data indicate that the water-soluble NSP fraction increases microbial activity in the intestinal tract of broiler chicks (Wagner and Thomas, 1978; Smits 1996). Hofshagen and Kaldhusdal (1992) reported that the number of *Clostridia* increased in the small intestine when barley was included in a wheat-and oat-based diet. Sakata (1987) observed an increased gut secretion and a change in the morphology of the gut wall when the bacterial activity was increased in the gastrointestinal tract. The insoluble NSP fraction, on the other hand, may reduce microbial activity by increasing the passage rate (Robertson, 1988). Indirectly through the intestinal microflora, which affect the morphology of the small intestine wall (Southon *et al*., 1987). The slower digesta passage rate would provide a more stable environment for microbial growth and proliferation, thus allowing a major establishment of bacteria to occur in the upper small intestinal. Alternatively, a lower viscosity may be leading to conditions for reduced bacterial populations by altering the digesta components. In additions, high intestinal bacterial populations irritate and thicken the gut lining, damage the microvillus and decrease nutrient absorption (Visek, 1978). The bacteria can contribute to the significantly heavier intestinal tract weight of broilers fed barley (Brenes *et al*., 1993).

Water soluble NSP not only have viscous properties, but are generally also fermentation and affect microbial activity in the intestinal tract. Viveros *et al*., (1994) noted a change in the morphology of the intestinal wall of the jejunum when birds were fed a barley-based compared to

Corresponding Author: A. Yaghobfar, Animal Science Research Institute. P. O. Box: 31585-1483, Tehran, Iran Tel: 982614430010

Table 1: Diet composition of layer ration

	Control	HB 20%	HB 30%	HB 40%	HB 50%	HB 60%
Hull-less barley	0.00	20.00	30.00	40.00	50.00	60.00
Barley	37.00	27.00	22.00	16.00	12.00	0.00
Maize	38.00	28.00	23.00	18.00	13.00	15.00
Other nutrients	25.00	25.00	25.00	25.00	25.00	25.00
Nutrient estimation						
MEn (kcal/g)	2.65	2.63	2.63	2.60	2.60	2.63
CP (%)	15.30	15.02	15.02	15.01	15.01	15.03
CF (%)	3.45	3.87	3.55	2.96	2.96	2.45
Sugar	1.35	1.904	2.36	2.55	2.735	2.745
Starch	16.272	25.013	31.553	34.84	38.124	39.18
β-Glucan	1.05	1.93	2.51	2.88	3.25	3.48
Nutrition determination						
CP (%)	17.10	17.70	16.20	15.60	17.90	19.00
CF (%)	4.00	3.60	3.20	3.80	3.60	3.80
NDF (%)	10.00	808.00	804.00	9.40	9.40	8.60
Sugar	2.50	4.50	3.00	5.03	5.10	5.93
Starch	38.50	33.70	34.80	36.30	41.40	31.29
β-Glucan	1.34	1.89	2.66	2.70	2.64	2.22

a maize-based diet and found shorter and thicker villi and an increase in number of goblet cells in birds fed the barley-based diet. Salih *et al*. (1991) showed that the passage rate was reduced in birds fed hullness barley in the diet compared to birds fed a maize-based diet. When cell proliferation increases microbial activity, the mucin composition of goblet cell may also change. Goblet cells are responsible for the section of mucin (Schneeman, 1982). An increase in cell proliferation will reduce the age and maturity of the goblet cell, which might affect the quality of mucin produced these cells. The maturity of enterocytes may also be reduced when cell proliferation increase, which consequently reduces absorption of fatty acid and nutrients and thus increase the energy requirement for maintenance of the digestive tract. Mathlouthi *et al*. (2002). Reported the small intestine wall showed that villus length, width and surface were decreased in broiler chickens fed the rye-based diet compared with those fed the corn-based diet. Van Leeuwen *et al*. (2004) shown that the pectin affected the mucosal surface by decreasing the area with the zigzag pattern and increasing the area with convoluted, mainly ridge-shaped villi. The *salmonella typhimurium* infection increased the effects of pectin on performance and mucosal morphology.

The main objective of the present study was carried out to investigate the effect of hell-less barley dietary or β-glucans content of hull-less barley on the small intestinal microflrora activity or population and gut morphology in layer hen fed on hull-less barley-based diets.

MATERIALS AND METHODS

Birds, housing and management: The experiment was performed with 220 laying hen Hy-line w36 and birds were housed in 20×20 cm cages (2 birds per each cage). Birds were rearing for 8 month (58 week) then 6 birds per treatments were killed for intestinal bacterial population or gut morphology. Experiment diets was designed to insert hull-less barley in layer ration 0, 20, 30, 40, 50, 50 and 60% or replace 100 percent of the barley (Table 1). Experiment conducted with Randomizes Complete Design by factorial 3×6 with 6 replicated. Treatment mean comparisons were made by Duncan's Multiple Range Tests.

Gut morphology: The whole parts of the small intestine comprising deudenum, jejonom and ileum were removed from the body immediately after death and transverse sections, were successively cut with 2 cm interval and fixed with 10% buffered formalin. Routine histological laboratory methods containing dehydration, Clearying, paraffin embedding was used and paraffin blocks were made. Six micrometer thick sections were made by rotary microtome and stained with heamatoxylin-eosin (1) and PAS (1) and studied under light microscope (Luna, 1968). The length and width of the intestinal villi and the dept of the intestinal crypts of lieberkohn glands were measured with linear scaled graticule. The number of goblet cells mm^{-2} area of the villi and crypts were measured by 25 squared graticule.

Bacteriological counts: After received sample in transport medium (1 g per 9 mL) containing 850 mL distilled water (D.W.)150 mL glycerol (Merck), 5 g yeast extract (Merck) 1 g L^{-1} peptone (BBL), 8.5 g L^{-1} NaCl (Merck) and 0.5 g L^{-1} L-cystine-Cl (Merck) pH = 7.5, Homogenized and divided in 2 Marked cryotubes (2 mL) and stored in liquid nitrogen. After thawing at 37°C, 10-fold dilutions were made in Peptone Physiological Saline (dilutions 10^{-2} to 10^{-6}). Aliquots of 0.1 mL were spread on to the following agar media: Reinforced

Clostridial Agar (RCA, Merck) supplemented with 5 g L^{-1} glucose. And after sterilization, 75 mL sterile horse blood and 75 mL (0.4%) china blue for total *anaerobic bacteria*. RCA agar containing 80 mL kanamycin and 1 mL Vancomycin and after sterilization, 75 mL sterile hors blood for *Bacteroidaceae*. Eugon Agar, (BBL 11230) supplemented with 10 g L^{-1} it maltose (Merck) 400 mL vegetable (tomato) juice and after sterilization, 5 mL sterile propionic acid to bring the pH of 6.0±0.2 for *Bifidobacterium*, incubated *anaerobically* of 37 °C 120 h. Trypton Soy Broth supplemented with 15 g L^{-1} agar (Merck) for total aerobic bacteria. Rogosa agar (Merck) for *lactobacillus*. Perfringens agar base (Merck) with 2 vials perfringens SFP selective supplement (Merck) and 50 mL L^{-1} egg yolk emulsion for *clostridium*. Slanetz and Barlie medium (Merck) for *Enterococcus*. Satmonolla and shigella Agar (Sagor, Merck) for *Enterobacteriaceae*. These culture media were incubated *aerobically* or *an aerobically* at 37°C for 24 to 72 h. After incubation the specific colony on the selective culture media were counted and the numbers of viable colony forming units per g were calculated.

Diet compassion: Diet formulation for layer hens present in Table 1. Therefore nutrient value and non-starch polysaccharide also beta glucan were estimated or determined for ration of layer hens.

RESULTS

Chemical compassion of hull-less barley was 96.6±0.64, 11.3±1.42, 3.2±0.8, 3.2±0.8, 5.33 and 63. 41 (percent), DM, CP, fiber, fat, sugar, starch, respectively. Also anti-nutrients as β-Glucan, SNSP, ISNSP, TNSP, 5.8, 4.6, 12.30 and 16.90 (percent), therefore AME, AMEn, TME and TMEn are 12.42±0.25, 13.39±1.05, 13.72±0.42, 13.64±1.09 (kJ/g). Result indicated that crud fiber content of hull-less barley 49.52% less then barley. Thus, beta glucan content of hull-less 41.38% (5.8%) more than barley (3.4%). Metabolizable energy obtained of hull-less 12.55 kJ and barley was 13.39 kJ it was 10% more then barley.

The inclusion of hull-less barley in the diet had no significantly effect on the total counts of *bacteroidaea*, *Lactobacillus bifidobacterium* and *clostridium* in small intestinal of layer hen. However, the total number of *aerobes* and *anaerobes* counts were significantly increased ($p<0.05$). Addition hull-less barley dietary affected the length of the small intestinal (Table 2). Whereas intestinal weight variety between for birds fed on the hull-less barley diet compared to those present in birds fed on the control diet.

The effect of hull-less barley dietary, individually or in combination, on villus height, villus width, crypt depth, Villus height/Crypt depth ratio and number of goblet cell in different sections of the small intestine of birds fed on hull-less barley are shown in Table 3. Inclusion of hull-less barley in the diet had effect on villus height, villus width, crypt depth and, villus height: crypt depth ratio in the duodenum, jejunum and ileum of small intestinal of layer hen ($p<0.05$). Thus, villus height, villus width, crypt depth and goblet cell number at the duodenum and ileum section of small intestinal mucosa were significantly increased (Fig. 1-4) with increment inclusion of hull-less barley in the diet compared with the control. However goblet cell number tended to be more increased in the ileum of small intestinal of birds, respectively (Fig. 5). Villus height, villus width, crypt depth and goblet cell number at the small intestinal mucosa were significantly increased with increment inclusion of hull-less barley in the diet compared with the control (Table 4). Therefore, villus height: Crypt depth ratios of the small intestinal were reduced with increasing inclusion hull-less barley in diet.

The hull-less barley had effect on the villus height, villus width and crypt depth in the duodenum, jejunum and ileum small intestinal of bird. Across all groups, the villus height, villus width, crypt depth in the duodenum was greater than those in the jejunum and ileum. Thus, numbers of goblet cells in the ileum were greater than those in the jejunum and duodenum of small intestinal in birds.

Table 2: Effects of hull-less barley (HB) on the small intestinal bactroial layer hens

Hull-less dietary barley%	Intestinal weight/g	Intestinal length cm^{-1}	Total aerobes	Total anaerobes	Bacteroidaceae	Lactobacillus	Bifidobacterium	Clostridium
Control	50.64^{ab}	72.00^{d}	6.508^{b}	7.22^{c}	6.33	6.74	6.3	5.83
HB 20%	38.09^{c}	85.67^{c}	6.77^{ab}	7.47^{abc}	6.39	6.85	6.07	5.84
HB 30%	54.73^{ab}	96.67^{b}	7.71^{ab}	7.37^{bc}	5.96	7.45	6.52	2.80
HB 40%	58.90^{a}	117.00^{a}	7.97^{a}	8.16^{a}	5.82	8.11	7.06	6.03
HB 50%	44.37^{bc}	107.33^{ab}	7.36^{ab}	7.98^{ab}	6.09	7.67	6.56	6.82
HB60%	52.40^{ab}	113.67^{a}	6.98^{ab}	7.20^{c}	6.21	7.15	6.15	6.07
p-value	0.012	0.0001	0.174	0.039	0.337	0.293	0.388	0.212

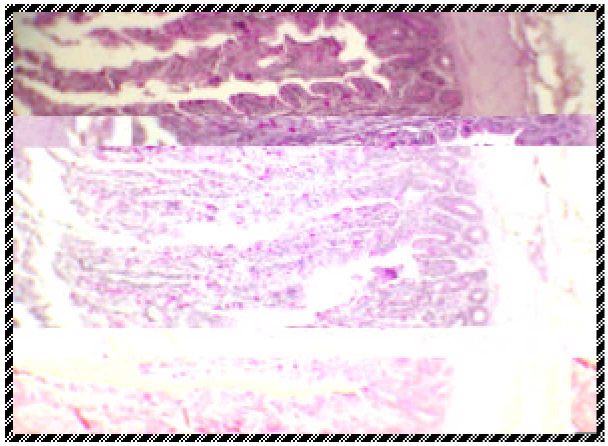

Fig. 1: Microscopic structure of the duodenum of birds fed on the hull-less barley dietary showing increasing the height and width of the villi and depth of the crypts PAS, 19x

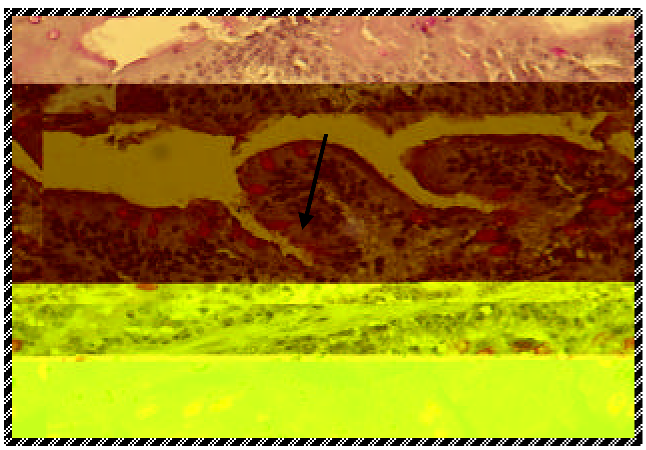

Fig. 2: Microscopic structure of the duodenum villi of birds fed on the hull-less barley dietary showing height amounts of goblet cell (arrow) in the epithelium of a villous. PAS, 88x

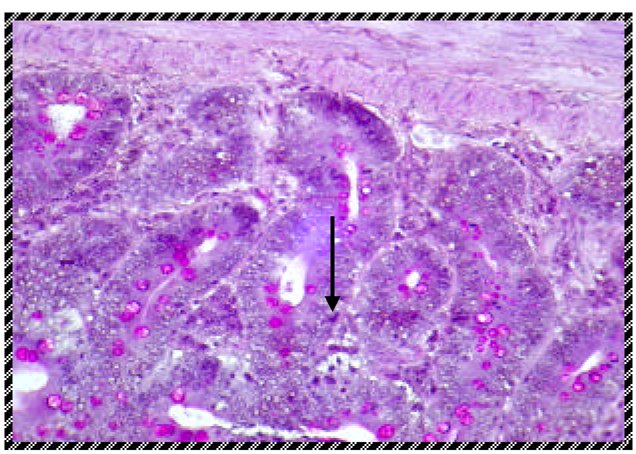

Fig. 3: Microscopic structure of the duodenum crypts of birds fed on the hull-less barley dietary showing height amounts of goblet cells in the epithelium of cryps (arrow), PAS, 88x

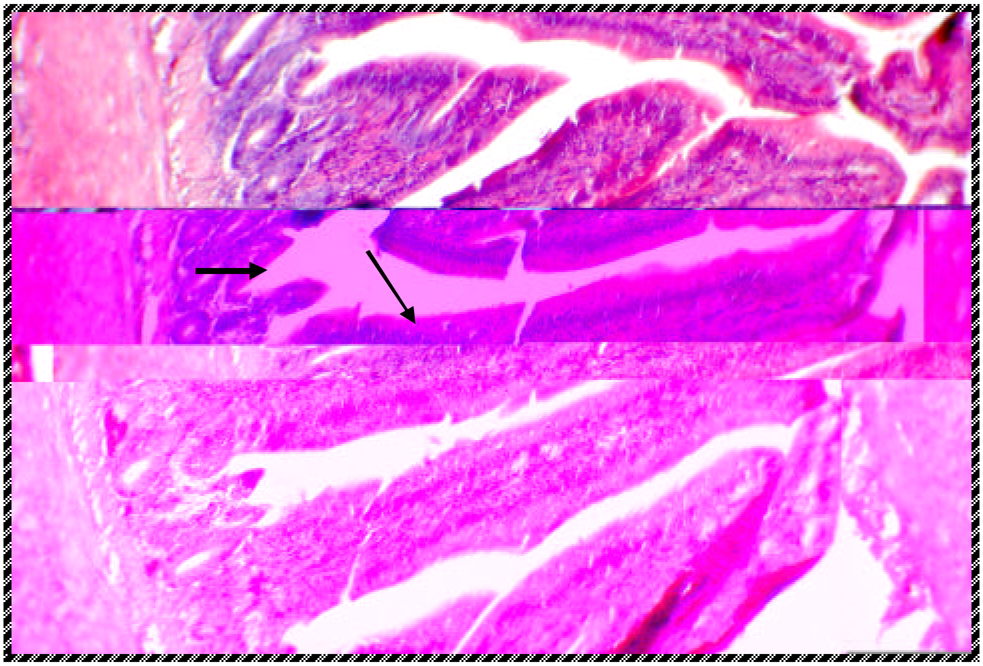

Fig. 4: Microscopic structure of the jejunum of birds (13B) fed on the hull-less barley dietary showing the villi (thin arrow) and crypts (thick arrow), Hand E. 26x

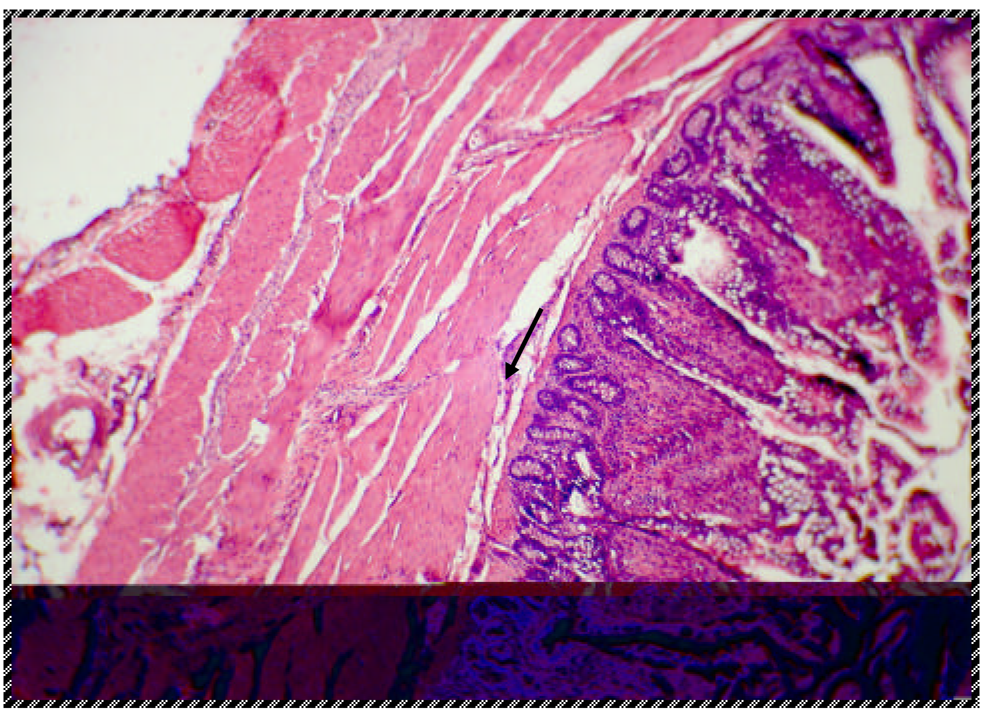

Fig. 5: Microscopic structure of the ileum of birds fed on the hull-less barley dietary showing high amounts of goblet cells in covering epithelia of villi, H and E, 19x

Table 3: Effects of Hull-less Barley (HB) on the morphology of the intestinal mucosa at different sites of the small intestine of layer hens

	Control	HB 20%	HB 30%	HB 40%	HB 50%	HB 60%	SEM
Villus length (μm)							
Duodenum	959.24^{bc}	897.90^{d}	959.14^{bcd}	1075.05^{a}	905.14^{dc}	1004.95^{b}	32.80
Jejunum	881.52^{cb}	915.81^{ab}	915.05^{ab}	947.05^{a}	860.19^{c}	934.10^{ab}	30.61
Ileum	637.71^{c}	717.71^{ab}	758.10^{a}	663.62^{bc}	574.48^{d}	736.00^{a}	36.68
Villus width (μm)							
Duodenum	158.48^{b}	163.05^{b}	137.14^{c}	148.57^{c}	156.95^{b}	185.14^{a}	10.76
Jejunum	80.76^{d}	114.29^{c}	108.95^{c}	131.81^{b}	132.57^{b}	163.05^{a}	8.65
Ileum	109.71^{ab}	99.05^{b}	109.71^{ab}	115.05^{ab}	131.81^{a}	132.00^{a}	12.47
Crypt depth (μm)							
Duodenum	153.90^{b}	134.86^{cd}	148.57^{bc}	176.00^{a}	121.14^{d}	182.86^{a}	9.47
Jejunum	87.62^{c}	119.62^{b}	119.62^{b}	135.62^{a}	124.19^{ab}	124.19^{ab}	8.75
Ileum	96.00^{c}	115.81^{a}	118.09^{a}	112.00^{ab}	102.09^{cb}	100.80^{cb}	7.96
Villus height /Crypt depth (μm)							
Duodenum	7098.9^{b}	6832.2^{b}	6524.3^{bc}	6425.8^{bc}	8275.1^{a}	5543.8^{c}	658.89
Jejunum	1682.9^{a}	1250.1^{b}	1245.5^{b}	1146.0^{b}	1180.4^{b}	1342.1^{b}	135.47
Ileum	1148.60^{a}	1027.99^{ab}	1065.67^{ab}	962.35^{b}	925.22^{b}	1172.99^{a}	97.07
Number of Goblet cell in mucosial epithelial (mm^2)							
Duodenum	1320.6^{ab}	1413.8^{ab}	1489.9^{a}	1413.8^{ab}	1286.8^{ab}	1193.6^{b}	135.98
Jejunum	1684.66^{a}	1591.53^{a}	1625.40^{a}	1583.07^{a}	1693.12^{a}	1532.27^{a}	122.38
Ileum	1811.6^{c}	2522.8^{b}	3885.7^{a}	3572.5^{a}	3513.2^{a}	3758.7^{a}	336.27

$^{a-d}$Mean values within a row with different letter(s) differ significantly ($p<0.05$)

Table 4: Effects of hull-less barley (HB) on the morphology of the total small intestinal of layer hen (μm)

Hull-less Barley dietary	Villus length (μm)	Villus width (μm)	Crypt depth (μm)	Villus l height / Crpt depth (μm)	Goblet cell number in mucosial epithelial (mm^2)
Control	826.16^{b}	116.32^{d}	112.51^{e}	3310.1^{ab}	1608.6^{c}
HB 20%	843.81^{b}	125.46^{cd}	123.43^{bc}	3036.8^{bc}	1825.7^{b}
HB 30%	875.43^{a}	118.60^{d}	128.76^{bc}	2945.2^{bc}	2308.3^{a}
HB 40%	895.24^{a}	131.81^{cb}	141.20^{a}	2844.7^{c}	2195.4^{a}
HB 50%	779.94^{c}	140.44^{b}	115.81^{de}	3460.2^{a}	2184.1^{a}
HB 60%	895.24^{a}	160.51^{a}	136.13^{ab}	2689.5^{c}	2124.9^{a}
Duodenum	965.90^{a}	158.22^{a}	152.89^{a}	6783.3^{a}	1353.08^{c}
Jejunum	908.95^{b}	121.90^{b}	118.48^{b}	1307.8^{b}	1618.34^{b}
Ileum	683.05^{c}	116.44^{b}	107.56^{c}	1052.0^{b}	3164.44^{a}
SEM	33.50	10.75	8.74	392.3^{6}	230.13

$^{a-d}$Mean values within a row with different letter(s) differ significantly ($p<0.05$)

DISCUSSIONS

The results of experiment show that the inclusion of hull-less barley in the diet had no effect on the bacterial activity as total counts of *bacteroidaea*, *Lactobacillus bifidobacterium* and *clostridium* in small intestinal of layer hen. Contrasted to Hofshagen and Kaldhusdal (1992) reported that the number of *Clostridia* increased in the small intestine when barley was included in a wheat-and oat-based diet. Although some authors ascribed the problems with broiler diet partly to changes in the intestinal microflora and in particular to increased numbers of *Clostridium perfringens*. An increased number of *Clostridium perfringens* was associated with growth depression (Stutz and Lawton, 1984). The insoluble NSP fraction, on the other hand, may reduce microbial activity by increasing the passage rate (Robertson, 1988).

In the present study, has shown that the hull-less barley influence on the total number of aerobes and anaerobes intestinal bacterial. This can be the increasing digesta viscosity, more undigested nutrients enter the lower part of the small intestine, where microbial growth will be increased and the competition between the host and the bacteria for nutrients will favor the bacteria. Wagner and Thomas (1978) showed an increase in anaerobic bacteria in the small intestine of chicks when rye or citrus pectin was included in the diet. They suggested that the increase in microbial activity in the ileum might indirectly be responsible for the anti-nutritive effects of rye and citrus pectin. Thus, hull-less barley dietary affected the height of the small intestinal it would be bacteria contribute to the heavier intestinal tract weight or length of birds fed hull-less barley (Brenes *et al*., 1993). In additions, high intestinal bacterial populations irritate and thicken the gut lining, damage the microvillus and decrease nutrient absorption (Visek, 1978). The anti-nutritive effects of barley are associated with the water soluble fraction of β-glucans (White *et al*., 1981; 1983; Fincher and Stone, 1986; Classen *et al*., 1985. 1988; Campbell *et al*., 1989).

The effect of β-glucan on nutrient digestion, there appears to be secondary effects attributable to higher level of microbial activity (Campbell *et al*., 1986). The result of experiment indicated that the counts of aerobes and anaerobes microbial activity resulting from hull-less barley in the diet may influence gut morphology and consequently affect nutrient absorption. Indirectly through the intestinal microflora, which affect the morphology of the small intestine wall (Southon *et al*., 1987). A change in the mucosa of the small intestine of birds was also observed by including barley in the diet (Viveros *et al*., 1994; Silva and Smithard, 1996). Sakata (1987) observed an increased gut secretion and a change in the morphology of the gut wall when the bacterial activity was increased in the gastrointestinal tract.

This study shown, the hull-less barley had effect on the villus height, villus width and crypt depth in the duodenum, jejunum and ileum small intestinal of bird compared to control group. Across all groups, the villus height, villus width, crypt depth in the duodenum was greater than those in the jejunum and ileum and this is consistent with the significant role that the duodenum plays in nutrient absorption. This is in accordance with the finding of Viveros *et al*. (1994) who showed that the villi were shorter and thicker and atrophy of the villi that the number of goblet cells was increased in the barley-fed birds.

The latter conclusion corresponds with the finding of present study as the inclusion of hull-less barley in the diet increased in the number of goblet cells in duodenum, jejunum and ileum small intestinal of birds. Alternatively, data of experiment shown that the density of goblet cells in the ileum greater than those in the jejunum and duodenum of small intestinal in birds. However, goblet cells are responsible for the secretion of mucin that is used for the mucinous lining of the intestinal epithelium (Schneeman, 1982). An increase in cell proliferation will reduce the age and maturity of the goblet cell, which might affect the quality of mucin, produced these cells. The maturity of enterocytes may also be reduced when cell proliferation increase, which consequently reduces absorption of fatty acid and nutrients and thus increase the energy requirement for maintenance of the digestive tract.

Thus, villus height, villus width, crypt depth and goblet cell number at the small intestinal mucosa were significantly increased (higher) with increment inclusion of hull-less barley in the diet compared with the control. In contrast, Mathlouthi *et al*. (2002) reported the small intestine wall showed that villus length, width and surface were decreased in broiler chickens fed the rye-based diet compared with those fed the corn-based diet. Jaroni *et al*. (1999) found that the shortening, thickening and atrophy of the villi in the jejunum of laying hens fed on diets based on wheat middling. This could not be confirmed in our study.

Based on the results of the present study, it can be stated that β-glucan content of hull-less barley concentration in diets of birds change microbial activity and the morphology of the small intestinal wall. The magnitude of these changes depends on the degree of β-glucan content of hull-less barley.

REFERENCES

Brenes, A., M. Smith, W. Guener and R. Marquardt, 1993. Effect of enzyme supplementation on the performance and digestive tract size of broiler chickens fed wheat and barley-based diets. Poul. Sci., 72: 1731-1739.

Classen, H.l., G.l. Campbell, B.G. Rosnagel, R. Bhatty and R.D. Reichert, 1985. Studies on the use of hulles barley in chick diets: Deleterious effects and methods of alleviation. Can. J. Anim. Sci., 65: 725-733.

Campbell, G.l., H.l. Classen and G.M. Balance, 1986. Gamma irradiation treatment of cereal grains for chicks diet. J. Nutr., 116: 560-569.

Classen, H.l., G.l. Campbell and W.B. Grootwassink, 1988. Improved feeding value of saskatchewangrown barley for broiler chickens with dietary enzyme supplementation. Can. J. Anim. Sci., 68: 1253-1259.

Campbell, G.l., B.F. Rossnagel, H.l. Classen and P.A. Thacker, 1989. Genotypic and environmental differences in extract viscosity of barley and their relationship to its nutritive value for broiler chickens. Anim. Feed Sci. Technol., 26: 221-230.

Fincher, G.B. and B.A. Stone, 1986. Cell Walls and their Components in Cereal Grain Technology. In: Advances in Cereal Science and Technology. Vol. 8 (Pomeranz, Y., Ed.), Minnesota, AACC, pp: 207-295.

Hofshagen, M. and M. Kaldhusdal, 1992. Barley inclusion and avoparein supplementation in broiler diet, 1. Effect on small intestinal bacterial flora and performance. Poul. Sci., 71: 959-969.

Jaroni, D., S.E. Scheideler, M.M. Beck and C. Wyatt, 1999. The effect of dietary wheat middling and enzyme supplementation. II: Apparent Nutrient Digestibility, Digestive Tract Size, Gut Viscosity and Gut Morphology in Two Strains of Leghorn Hens. Poul. Sci., 78: 1664-1674.

Luna, Lee G., 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Edn., McGrow-Hill Book Company, New York, USA, pp: 32-39, 158-160.

Mathlouthi, J.P. N Lalles, P. Lepercq, C. Juste and M, Larbier, 2002. Xylanase and β-glucanase supplementation improve conjugated bill acid fraction in intestinal contents and increase villus size of small intestine wall in broiler chickens fed a rye-based diet. J. Anim. Sci., 80: 2773-2779.

Robertson, J.A., 1988. Physicochemical characteristics of food and the digestion of starch and dietary fiber during the gut transit. Proc. Nutr. Soc., 43: 83-88.

Schneeman, B.D., 1982. Pancreatic and Digestive Function. In: Dietary Fiber in Health and Disease (Vahoung, G.V. and D. Kritchevsky, Eds.) Plenum Pres, New York, pp: 73-83.

Stutz, M.W. and G.C. Lawton, 1984. Effect of diet and antimicrobials on growth, feed efficiency, intestinal clostridium perfringens and Ileal weight of broiler chicks. Poul. Sci., 63: 2036-2042.

Sakata, T., 1987. Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: A possible explanation for trophic effects of fermentable fiber, gut microbes and huminal trophic factors. Bri. J. Nutri., 58: 95-103.

Southon, S., J.M., Gee and I.T. Johnson, 1987. The effect of dietary protein source and guar gum on gastrointestinal growth and entroglucagon secretion in the rat. Br. J. Nutr., 58: 65-72.

Salih, M.E., H.l. Classen and G.l. Campbell, 1991. Response of chickens fed on hull-less barley to dietary β-glucanase at different ages. Anim. Feed Sci. Technol., 33: 139-149.

Silva, S.S. and R.R. Smthard, 1996. Exogenous enzymes in broiler diet: crypt cell proliferation, digesta viscosity short chain fatty acids and xylanase in the jejunum, Br. Poul. Sci., 37: S77-S79.

Smits, C.H.M., 1996. Viscosity of dietary fiber in relation to lipid digestibility in broiler chickens. Ph.D Thesis, Agricultural University Wageningen, The Netherlands.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci., 46: 1447-1469.

Viveros, A., A. Brenes, M. Pizarro and M. Castano, 1994. Effect of enzyme supplementation of a diet based on barley and autoclave treatment on apparent digestibility growth performance and gut morphology of broilers. Anim. Feed Sci. Technol., 48: 237-251.

Van Leeuwen, P., J.M.V.M. Mouwen, J.D. Van Derklisand M.W.A. Verstegen, 2004. Morphology of the small intestinal mucosal surface of broilers in relation to age, diet formulation, small intestinal microflora and performance. Br. Poul. Sci., 45: 41-48.

Wagner, D.D. and O.P. Thomas, 1978. Influence of diets containing rye or pectin on the intestinal flora of chicks. Poul. Sci., 57: 971-875.

White, W.B., H.R. BIRD, M.L. Sunde, N. Prentice, W.C. Burger and J.A. Marlett, 1981. The viscosity interaction of barley β-gucan with trichoderma viride cellulose in the chick intestine. Poul. Sci., 62: 853-862.

White, W.B., H.R. Bird, M.L. Sunde and J.A. Marlett, 1983. Viscosity of β-d-glucan as a factor in the enzymatic improvement of barley for chicks. Poul. Sci., 62: 853-862.