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Effect of Non–Starch Polysaccharide (NSP) of Wheat and Barley Supplemented with Exogenous Enzyme Blend on Growth Performance, Gut Microbial, Pancreatic Enzyme Activities, Expression of Glucose Transporter (SGLT1) and Mucin Producer (MUC2) Genes of Broiler Chickens

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ABSTRACT

The experiment was carried out in a completely randomized design (CRD) with 625 broiler chicks (Ross 308) for 5 repetitions (25 birds per each replicated) on the 5 treatments diet. Treatments included two different types of cereal grains (wheat, and barley) with or without an enzyme supplementation along with a corn-based diet as control group. The experimental diets were formulated to have similar contents of crude protein, metabolizable energy, total non-starch polysaccharides (NSP) and were fed in two periods of starter and grower. Experimental traits were consisted growth performance, ileal flora numeration, villus morphology in 3 parts of the intestine, digesta viscosity and pancreatic enzyme activity, and determining the gene expression level of glucose transporter (SGLT1) and mucin producer (MUC2) in the jejunum. Results indicated that inclusion of wheat and barley to corn-soy based diet with or without exo-enzymes blend on growth performance traits were significant (p<0.01). Feed intake and average daily gain in wheat diet was lower, conversely FCR was higher than other groups (p<0.01). Maximum microbial count were observed in wheat and barley diets and minimum were observed in enzyme supplemented diets respectively (p<0.01). Control group and enzyme supplemented diets had minimum counting of gram negative, coliform and clostridium, but maximum counting of lactobacilli and bifidobacter were observed in enzyme supplemented diets (p<0.01). Viscosity and activities of pancreatic α-amylase and lipase were significantly increased in chicks fed wheat and barley when compared to the control group fed on corn (p<0.01). Feeding wheat and barley diets reduced villus height in different parts of the small intestine when compared to those fed on a corn diet (p<0.01). Gene expression level of glucose transporter (SGLT1) and mucin producer (MUC2) in jejunum was significantly affected by the inclusion of wheat and barley to corn-soy based diet with or without exo-enzymes blend (p<0.01). In conclusion, the inclusion of wheat and barley to corn-soy based diet without enzyme supplementation has an adverse effect on growth, ileal microflora villi morphology, digesta viscosity, pancreatic enzyme activity, and gene expression level of nutrient transporters. However, enzyme supplemented to wheat and barley diets significantly improved those traits, and restored the negative effects.

INTRODUCTION

Wheat and barley as alternative cereals can be successfully replace with corn in poultry diets. These grains could locally grow in many areas of the world and have lower water requirements than corn (Ravindran *et al.* 1999; Lin *et al*. 2010). The major components of wheat and barley are starch and proteins, though they have considerable content of non-starch polysaccharides (NSP), derived from the cell walls (Olukosi *et al*. 2007; Mirzaie *et al*. 2012).

NSP as anti-nutritional factors affected physiological characteristics of broiler chickens (Jamroz *et al*. 2002). The major portion of NSP in wheat is arabinoxylan polymers, whereas NSP in barley are polymers of (1→3) (1→4)-β- glucans (Choct 1997; Yin *et al*. 2000; Jamroz *et al*. 2002). A review of literature indicates that the presence of anti-nutritive compounds in the digestive tract of birds, cause predominant negative changes in the gut such as increasing the product shelf life, physicochemical properties of digesta (viscosity and pH), and impede the action of indigenous enzymes through the gut (Choct & Annison, 1992 a,b; Hetland *et al*., 2004), along with some changes in the intestinal environment and structure of the lining surface with the impact on the quantity and quality of relevant gene expression (Ferraris, 2001; Smirnov *et al*., 2004; Tanabe *et al*., 2005). Associate gene transporting nutrients through the gut enterocytes such as glucose, amino acids, peptides and genes involved in production and secretion of mucin as an important ingredient of mucus coating the intestinal tissue are the most effective factors in the intestinal environment and structural changes (Smirnov *et al*., 2006; Gilbert *et al*., 2008; Horn *et al*., 2009; Gilbert *et al*., 2010).

Consumption level and composition of carbohydrates consumed well over several days increases the concentration of glucose transporters in the cell membrane of the intestine and this increases along with increasing levels of mRNA in these cells, related to the gene SGLT1 (Ferraris, 1997 and 2001). Because high-fiber feeds increase mucin excretion through the gut wall, high consumption of fiber increases the level of gene expression and increases mucin synthesis rate in the intestine (Smirnov *et al*., 2004; Tanabe *et al*., 2005; Smirnov *et al*., 2006; Mott *et al*., 2008). These negative impacts would eliminate use of exogenous enzymes, but the combination of different types of enzymes has a better influence and results in more efficacy (Cowan & Hastrupt, 1995; Ravindran *et al*., 1999; Slominski, 2011). Due to the lack of information on wheat and barley inclusion to applying diets and effects on physiological characteristics of chickens, this study was conducted and for this purpose, equal fractions of NSP from wheat and barley were included in broiler diets with or without multi-carbohydrates to compare the negative effects on growth performance, intestinal bacterial population, villus morphology, digesta viscosity, and pancreatic α-amylase and lipase activities along with impact on expression of glucose transporter and mucin production genes in the intestine.

MATERIALS AND METHODS

Animals, management and treatments

The experiment was carried out in a completely randomized design (CRD) with 625 broiler chicks (Ross 308) for 5 repetitions (25 birds per each replicated) on the 5 treatments diet. Each replicate was a floor pen of 1.5×2.2 m^2. Three experimental diets based on corn, wheat and barley were formulated for the starter (1 to 3 wk of age) and grower (3 to 6 wk of age) stages. Two additional diets were prepared by supplementing exogenous multi-carbohydrates to the wheat and barley based diets (Table 1). The enzyme cocktail contained 180 U/g multi-glycanase and 1000 U/g phytase and were used in the experimental diets at 1 mg/kg. The compositions of the experimental diets during the starter and grower stages are shown in Table 1. All diets were formulated to have the same contents of metabolizable energy, crude protein and dietary electrolyte balance. Wheat and barley samples were analyzed before the experiment for NSP constituents. Total, insoluble and soluble dietary fiber, were determined by using Megazyme assay kits (Megazyme International Ireland Ltd, Wicklow, Ireland) according to Approved Methods of the American Association of Cereal Chemists (AACC, 2000; Table 2).

Wheat and barley-based diets had also equal fractions of non-starch polysaccharides (NSP). Feed and water were provided *adlibitum*. Environmental conditions including temperature and lighting schedule followed the management guideline of the Ross-308 strain. The experimental animals were kept, maintained and treated in accepted standards for the human treatment of animals.

Growth performance and Intestinal microbial Numeration

Feed intake, weight gain, and feed to gain ratio were calculated as gram per bird throughout the period (1 to 42-days). At the day 42, three chicks per pen (15 chickens from each treatment) were randomly selected and slaughtered by cervical dislocation and ileum contents were collected. Gut flora was enumerated according to Langhout, (1999) with some modifications. Briefly, aqueous digesta samples (3 mL) were taken from the distal segment of ileum and immediately transferred to sterile bottles containing 15 mL anaerobic transport medium (TRM; pH=7.0). The samples were weighted and stored at 4°C for further examination. Samples were homogenized and 1 mL of each sample serially diluted 10- fold. An aliquot (0.1 mL) of each diluted sample was then cultivated

Table 1 – The experimental diets and their chemical composition fed to broilers during the starter (1 to 21 days of age) and grower (22 to 42 days of age) stages.

Diets	Starter (1- 21 days)					Grower (22- 42 days)				
Ingredients(%)/Treatment	C	W	B	WE	BE	C	W	B	WE	BE
Yellow corn	56	44.55	45	44.55	45	58	40	42.41	40	42.41
Soy bean meal	36.8	35.1	33.9	35.1	33.9	32	30.5	29.6	30.5	29.6
Soy oil	2	1.35	2	1.35	2	2.9	2.85	3.47	2.85	3.47
Wheat	-	15	-	15	-	-	20	-	20	-
Barley	-	-	15	-	15	-	-	20	-	20
DCP	1.83	1.78	1.78	1.78	1.78	1.81	1.74	1.71	1.74	1.71
Calcium Carbonate	1.12	1.14	1	1.14	1	1.13	1.14	1.13	1.14	1.13
Sodium Chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Potassium Carbonate	0.1	0.13	0.13	0.13	0.13	0.12	0.12	0.11	0.12	0.11
DL-Methionine	0.17	0.15	0.15	0.15	0.15	0.25	0.25	0.05	0.25	0.05
L-Lysine HCL	0.1	-	0.1	-	0.1	0.15	0.1	0.05	0.1	0.05
Vitamin Premix**	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix**	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Inert*	1.08	-	0.14	-	0.14	2.84	2.5	0.67	2.5	0.67
Total	100	100	100	100	100	100	100	100	100	100
Calculated Values										
MEn (MJ/Kg)	12.13	12.13	12.13	12.13	12.13	12.34	12.34	12.34	12.34	12.34
Protein (%)	21	21	21	21	21	19	19	19	19	19
Met + Cys (%)	0.86	0.84	0.82	0.84	0.82	0.85	0.85	0.84	0.85	0.84
Lysine (%)	1.2	1.19	1.18	1.19	1.18	1.2	1.19	1.11	1.19	1.11
Calcium (%)	0.95	0.94	0.92	0.94	0.92	0.95	0.95	0.87	0.95	0.87
AvailablePhosphorus (%)	0.43	0.43	0.45	0.43	0.45	0.45	0.42	0.43	0.42	0.43
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Chloride (%)	0.22	0.23	0.24	0.23	0.24	0.22	0.23	0.23	0.23	0.23
Potassium (%)	0.95	0.95	0.96	0.95	0.96	0.87	0.87	0.88	0.87	0.88
(Na+K)-Cl (meq/kg)	247.85	247.9	247.81	247.9	247.81	231.23	231.56	231.54	231.56	231.54
Total NSP (%)	12.49	12.92	12.95	12.92	12.95	11.73	12.18	12.19	12.18	12.19
Soluble NSP (%)	2.73	2.78	2.77	2.78	2.77	2.62	2.67	2.67	2.67	2.67
Non-Soluble NSP (%)	9.76	10.14	10.18	10.14	10.18	9.11	9.50	9.52	9.51	9.52

*Inert contained sand plus enzyme and enzyme supplementation included 1 kg per 1000 kg of diet for all treatments and contained 1000 active units of Phytase and 480 active units of Multi-glycanase units per gram. **Each Kg of premix which was used in this experiment contained vitamins: 44000 IU A, 7200 IU D3, 440 mg E, 40 mg K3, 70 mg B12, 65 mg B1, 320 mg B2, 290 mg Pantothenic acid, 1220 mg Niacin, 65 mg B6, 22 mg Biotin, 270 mg Choline Chloride, and Minerals: 950 mg Mn, 450 mg Zn, 320 mg Fe, 100 mg Cu, 65 mg Se, 68 mg I and 45 mg Co.

C: corn, W: wheat, B: barley, WE: wheat+enzyme BE: barley+enzyme.

on specific media and transferred to an incubator set at 37°C for 24 hours. At the end of the incubation period, bacterial colonies were counted. Specific media as described below were used to culture different types of bacteria including Nutrient Agar (NA) for total bacterial count, Eosin Methylene Blue (EMB) Agar for Gram-negative bacteria, MacConkey Agar (MCA) for coliforms, Rogosa Agar (RA) for lactic acids, Eugon Agar (EA) for bifidobacteria and Reinforced Clostridial Agar (RCA) for clostridium bacteria, respectively.

Intestinal Villi Morphology

Morphometric characteristics of small intestinal including villus height and width, crypt depth, Villus Height to Villus Width ratio (H/W), and villus height to crypt depth ratio (H/CD) were determined in the duodenum, jejunum, and ileum. Segments of 2-3 cm from the midpoint of the duodenum, the midpoint between the bile duct entry and Meckel's diverticulum (jejunum), and the distal end of the ileum (ileum) were dissected. The segments were flushed with ice-cold phosphate buffered saline (PBS, pH=7.2) and fixed in 10% neutral buffered formalin solution for further histological study. Formalin-fixed tissues were dehydrated, cleared and impregnated in paraffin wax and cut into 6-µm sections with a LEICA RM 2145 microtome. These sections were mounted on 10% Poly-L-Lysine coated slides (Langhout, 1999). The slides were stained with hematoxylin and eosin. Histological indices were determined by use of a computer aided light microscopic image analyzer (Sigma Scan, San Rafael, and CA. USA) according to the method reported by Saki (2011). The mean of 10 measures per section was used for the analysis.

Table 2 – Proximate composition of wheat and barley ingredients (%).

Composition	Wheat	Barley
Dry Matter	95.95	95.67
Crude Protein	14.69	10.92
Ether Extract	1.25	0.96
Crude Ash	1.66	2.84
Neutral Detergent Fiber	13.20	30.20
Acid Detergent Fiber	0.80	6.40
Acid Detergent Lignin	1.00	2.00
Total carbohydrate	82.40	85.28
Cellulose	1.80	4.40
Hemicelluloses	10.40	23.62
Total Dietary Fiber	17.22	30.72
Soluble Dietary Fiber	1.01	4.45
Un-Soluble Dietary Fiber	16.21	25.72
non-starch polysaccharides	18.80	35.17
Non Fiber Carbohydrate	69.20	55.08

Soluble Dietary Fiber (SDF), Unsoluble Dietary Fiber (USDF)

Total carbohydrate (CHO): [100 – (protein + fat + moisture + ash)].

Cellulose content was calculated by difference: ADF – ADL.

Hemicelluloses content was calculated by difference: NDF – ADF.

Non Fiber Carbohydrate is calculated (NFC) by difference [100-(%NDF + %CP + %Fat + Ash)].

Intestinal viscosity and pancreatic enzyme activity

Measuring digesta viscosity was performed by samples and were obtained from selected birds. Ileal digesta was individually collected, homogenized at 4˚C and immediately measured for viscosity (Brookfield viscometer, Model DV-II, MA, USA) according to Langhout *et al*. (1999), with some modifications. For the pancreatic enzyme activity assay, sample sections (3 cm in length) were taken from the middle of the pancreas, rinsed with 0.01 M PBS (pH 7.2), and stored in liquid nitrogen at -80ºC until analysis. The pancreatic samples were homogenized in ice-cold 0.2 mol/L Tris-Hcl buffer, containing 0.05 mol/L NaCl as described by Li *et al* (2004). The homogenates were centrifuged at 3000 rpm for 15 min at 4ºC and the supernatants were saved for ulterior spectrophotometric measurement. The activity of α-amylase (EC 3.2.1.1) was determined using a validated kit from Parsazmun Chemical Company (PARSAZMUN Co., KARAJ, IRAN TS.M.91.4.5). The activity of lipase (EC 3.1.1.3) was measured by a validated kit from ZiestChem Chemical Company (ZIESTCHEM Co., TEHRAN, IRAN) according to the manufacturer's instruction. The activities of amylase and lipase are expressed as unit per milligram of pancreatic crude protein.

Gene Expression of SGLT1 and MUC2

Fragments of the jejunum tissue of the slaughtered chickens were isolated and immediately placed in sterile aluminum foil and were transferred to a freezer with a temperature of -80ºC to subsequent RNA analyzing. Extraction of the RNA from the jejunum samples were done via the standard RNA purification kit (CINNAPURE RNA Purification Kit, Sina gene Co, Catalogue number 50k30341, TEHRAN, IRAN) according to the manufacturer´s directions. Synthesis of cDNA from mRNA samples was done using transcription kit (REVERSE TRANSCRIPTION KIT, QIAGEN Co, Catalogue number 2050311) according to the manufacturer´s protocol. RT-PCR processes were performed by ABI device model 7500, USA using specific primers of β-actin as reference gene and specific primers of poultry as target genes (METABION, CO, GERMANY) and Quanti-Fast produced by Qiagen Co, TM SYBR (Catalogue number 204052). Primers for SGLT1, MUC2 and β-actin were designed by Primer-Blast, publically available in the National Center for Biotechnology Information (NCBI). Details of primers depicted in table 3. To measure the gene expression of the experimental groups, first the Ct index was calculated and then the Ct index for the target and reference genes were compared. Gene expression levels were determined by $2^{-\Delta\Delta C^t}$ criterion (Formula 1), according to the method of Livak & Shcmittgen (2001).

$\Delta\Delta C_t$ = (C_t mean target gene - C_t mean ß-Actin) – (C_t mean control gene - C_t mean ß-Actin) (Formula 1)

Statistical Analysis

All data was statistically analyzed by GLM procedure of SAS software (SAS Institute Inc., 2004). Statistical scheme was based on a completely randomized design (CRD). Samples within pens (3 per each unit) were subjected to analysis. Logarithmic transformation was applied for microbial colony forming unit (CFU). The statistical model used for normal data was $Y_{ij}= \mu+ T_i + e_{ij}$ and for sampling observation within pens was Y_{ijk}

Table 3 – Genes and Primers specifications

Target Gene	Gene code	Gene action	Primers	PCR Product (base pair)
SGLT1	XM_415247	Na+-dependent glucose and galactose transporter	5′-ATACCCAAGGTCATAGTCCCAAAC-3′ 5′-TGGGTCCCTGAACAAATGAAA-3′	169
MUC2	XM_421035	Intestinal mucin2; Gallus Gallus	5′-TCACCCTGCATGGATACTTGCTCA-3′ 5′-TGTCCATCTGCCTGAATCACAGGT-3′	228
β-actin	L-8165	Reference Gene	5′-GTCCACCGCAAATGCTTCTAA-3′ 5′-TGCGCATTTATGGGTTTTGTT-3′	270

= $\mu + T_i + e_{ij} + Se_{ijk}$. In these models, Y_{ij} and Y_{ijk} are observations; μ is the overall mean; T_i is the effect of treatments (different diets); e_{ij} is random error, and Se_{ijk} is the effect of sampling error. Duncan's multiple range tests was used to separate the means.

RESULTS

Growth performance

Table 4 depicts the results of feed intake, body weight, and feed conversion ratio of chickens throughout the trial. Results indicated that birds fed with wheat significantly (p<0.01) reduced their feed intake compared to other groups. Supplementing multiple carbohydrates to the wheat diet remarkably restored feed intake so that the difference was not significant with the control fed on corn. On the other hand, birds fed barley had numerically higher feed intake than other treatments. Supplementing the barley diet with multiple carbohydrates significantly (p<0.01) increased feed intake compared to the wheat and corn diets. Nevertheless, birds fed on barley and wheat diets had significantly lower body weight and higher feed conversion ratio (FCR) than counterparts fed on corn. Enzyme supplementation of barley and wheat diets improved FCR (p<0.01).

Table 4 – Effect of different types of cereal grains and enzyme supplementation on broiler growth performance during 1 to 42 days of age

Dietary treatments	Feed intake (g/b)	Weight gain (g/b)	Feed conversion ratio
C	4280^{b}	2251^{a}	1.90^{c}
W	4164^{c}	2015^{b}	2.06^{a}
WE	4253^{b}	2119^{b}	2.01^{ab}
B	4333^{ab}	2107^{b}	2.05^{a}
BE	4392^{a}	2199^{ab}	1.99^{b}
SEM	23.58	22.30	0.02
p-value	< 0.001	< 0.001	< 0.001

*^{abc} Means with different superscript letters within columns have significant difference (p<0.01). *C: corn, W: wheat, B: barley, WE: wheat+enzyme BE: barley+enzyme.

Intestinal microbial Numeration

Total counts of bacteria and Gram negative including E. coli and clostridia in the intestinal content were higher in birds fed wheat and barley than the control (p<0.01; Table 5). On the other hand, the number of lactic acid bacteria and bifidobacteria were significantly lower (p<0.01) in birds that received wheat and barley diets compared to the control. Inclusion of the multiple carbohydrates to the wheat and barley diets restored the situation so that no significant difference was found between the wheat and barley diets supplemented with the enzyme cocktail and the corn diet.

Intestinal villi morphology

The intestinal villi morphometric indices including villus height, villus width, crypt depth and the related ratio at three parts of the duodenum, jejunum, and ileum were given in Table 6. The height of villus at three parts of the gut was lower, in contrary, villus width, and crypt depth was higher in birds fed wheat and barley than the control or enzyme supplemented diets (p<0.01). On the other hand, the villus height: width and villus height: crypt depth ratios were significantly lowers (p<0.01) in birds that received wheat and barley diets compared to the control or enzyme supplemented diets. The inclusion of the multiple carbohydrates to the wheat and barley diets restored the situation so that no significant differences were found between the wheat and barley diets supplemented with the multi-enzyme and the corn diet.

Intestinal viscosity and pancreatic enzyme activity

The intestinal digesta viscosity and pancreatic α-amylase and lipase activity of chicks fed wheat and barley (p<0.01) increased significantly compared to the control group fed on corn. Inclusion of carbohydrate enzyme mixture to the wheat and barley diets significantly reduced the digesta viscosity and activities of α-amylase and lipase (Table 7).

Table 5 – Effect of different types of cereal grains and enzyme supplementation on ileal bacterial population in broiler chickens (log CFU/g digesta)

Dietary treatments	Total bacterial count	Total gram negative	E. Coli	Lactic acid bacteria	Bifido bacteria	Clostridia
C	6.67^{b}	5.31^{b}	5.07^{bc}	4.91^{b}	5.40^{b}	4.86^{b}
W	7.13^{a}	6.33^{a}	6.32^{a}	3.87^{c}	4.07^{c}	6.29^{a}
WE	6.32^{b}	5.21^{bc}	5.22^{b}	5.20^{a}	5.67^{a}	4.83^{b}
B	7.17^{a}	6.24^{a}	6.28^{a}	4.90^{b}	3.51^{d}	6.65^{a}
BE	6.75^{b}	5.27^{b}	4.56^{d}	5.41^{a}	5.76^{a}	4.50^{bc}
SEM	0.17	0.13	0.12	0.10	0.11	0.17
p-value	< 0.0001	< 0.001	< 0.001	< 0.0001	< 0.0001	< 0.001

* ^{abc} Means with different superscript letters within columns have significant difference (p<0.01).
*C: corn, W: wheat, B: barley, WE: wheat+enzyme BE: barley+enzyme.

Table 6 – Effect of different types of cereal grains and enzyme supplementation on intestinal morphology of chickens

Dietary treatments	VH (µm)	VW (µm)	CD (µm)	H/W ratio	H/CD ratio
C	1530.33^{ab}	115^{c}	108.33^{c}	13.31^{a}	14.39^{a}
W	1360 ^{c}	120^{b}	114.67^{b}	11.33^{b}	11.86^{b}
WE	1668 ^{a}	123.67^{ab}	108^{c}	13.49^{a}	15.44^{a}
B	1440.04^{b}	117^{c}	119 ^{a}	12.31^{ab}	12.1^{b}
BE	1589.82^{ab}	125.33^{a}	109^{c}	12.68^{ab}	14.59^{a}
SEM	63.80	2.18	2.39	0.54	0.52
p-value	<0.001	<0.003	<0.001	<0.0001	<0.0001
	Jejunum				
C	1273.67^{a}	138.67^{b}	109.67^{b}	9.18^{a}	11.61^{ab}
W	1190^{ab}	150^{a}	116^{a}	7.93^{b}	10.26^{ab}
WE	1297^{a}	136^{b}	108^{b}	9.54^{a}	12.01^{a}
B	1025.23^{b}	125^{c}	110.33^{b}	8.25^{ab}	9.29^{b}
BE	1191.33^{ab}	135^{b}	95^{c}	8.83^{ab}	12.54^{a}
SEM	69.62	3.14	4.72	0.61	0.97
p-value	<0.001	<0.001	<0.005	<0.0001	<0.0001
	Ileum				
C	1285.33^{ab}	87^{d}	105^{b}	14.94 ^{a}	12.5^{a}
W	1140.67^{b}	128.33^{b}	115^{a}	8.68^{c}	9.88^{b}
WE	1408^{a}	102.67^{c}	109.67^{ab}	13.33^{ab}	12.61^{a}
B	1172^{b}	142.33^{a}	114.33^{a}	8.23 ^{c}	10.25^{b}
BE	1308^{a}	107^{c}	108^{ab}	12.22 ^{b}	12.12^{a}
SEM	67.67	3.88	3.14	0.71	0.69
p-value	<0.001	<0.001	<0.002	<0.0001	<0.0001

*^{abc}Means with different superscript letters within columns have a significant difference (p<0.01).

*C: corn B: barley, BE: barley + enzyme; VH: Villus Height, VW: Villus Width, CD: Crypt Depth, H/W: Villus Height to Villus Width ratio, H/CD: Villus Height to Crypt Depth ratio.

Table 7 – Effect of different types of cereal grains and enzyme supplementation on digesta viscosity and specific activities of pancreatic amylase and lipase in chickens

Dietary treatments	Digesta Viscosity (cP) [1]	Amylase (U/mg CP) [2]	Lipase (U/mg CP) [2]
C	1.59^{c}	0.71^{c}	0.24^{b}
W	2.17^{a}	1.37^{a}	0.42^{a}
WE	1.60^{b}	0.89^{b}	0.28^{b}
B	1.95^{a}	1.43^{a}	0.42^{a}
BE	1.60^{b}	0.91^{b}	0.26^{b}
SEM	0.04	0.06	0.03
p-value	<0.001	<0.002	<0.001

*^{abc}Means with different superscript letters within columns have significant difference (p<0.01)

[1]cP: centipoises; [2]U/mg CP: Units of enzymes per one milligram of pancreatic crude protein.

*C: corn, W: wheat, B: barley, WE: wheat enzyme, BE: barley+enzyme

Gene Expression of SGLT1 and MUC2

In Table 8, expression of SGLT1 and MUC2 of chicks fed wheat and barley were significantly (p<0.01) higher than other groups. But, expression of SGLT1 and MUC2 of chicks fed enzyme supplemented diets were significantly lower than wheat and barley diets and higher than control group fed on corn (p<0.01).

Table 8 – Effect of different types of cereal grains and enzyme supplementation on expression of SGLT1 and MUC2 genes in jejunum of chickens

Dietary treatments	SGLT1	MUC2
C	1.00^{c}	1.00^{c}
B	1.85^{a}	1.38^{a}
BE	1.23^{b}	1.05^{b}
W	1.78^{a}	1.45^{a}
WE	1.19^{bc}	1.13^{b}
SEM	0.01	0.03
p-value	<0.001	<0.001

*^{abc}Means with different superscript letters within columns have a significant difference (p<0.01).

*C: corn, B: barley, BE: barley + enzyme.

DISCUSSION

The present research has consistently elicited the negative effects of soluble NSP of wheat and barley, as also demonstrated by other researchers (Yin *et al.*, 2000; Olukosi *et al.*, 2007; Mirzaie *et al.*, 2012). However, the comparative effects of different types of NSP on broiler performance and physiological responses have not been adequately cleared. Results reported herein indicate that arabinoxylan polymers

of wheat's NSP have a more deleterious impact on voluntary feed intake of broiler chickens than (1→3) (1→4)-β- glucans of barley's NSP. Birds fed on wheat diet consumed lower feed than those on the barley, corn or enzyme supplemented diets throughout the trial (Table 4). Consequently, birds fed on wheat diet had lower body weight gain compared to those fed barley, corn or enzyme supplemented diets, primarily due to the differences in NSP structure, the size of molecules and the degree of digestion which can affect digesta viscosity and passage rate of gut content (Choct, 1997; 2006). The growth performance data are consistent with the digesta viscosity and pancreatic enzyme activity as depicted in Table 7. The viscosity of intestinal digesta in birds fed on wheat showed the highest value, which was significantly (p<0.01) greater than the control group fed on corn. In fact, NSP of both wheat and barley increased digesta viscosity in the intestine. These observations indicated that every change in the gut environment due to different dietary NSP sources could affect the physicochemical properties of the intestine and consequently the performance and other physiological responses of the birds.

Increased viscosity per se creates an ideal environment for maximal proliferation of anaerobic and Gram-negative bacteria as observed in this experiment. This mode leads to the increased production of volatile fatty acids (VFA) which could result in decreased digesta pH due to the production of a great amount of short chain fatty acid in the lumen. These observations are in line with Jaroni *et al*. (1999), and Langhout *et al*. (1999). Sedentary intestinal digesta and low oxygen condition due to indigestible NSP provide an appropriate environment for fermentative anaerobic bacteria proliferation (Langhout *et al*., 1999). The decrease in nutrient availability and production of detrimental by-products resulted in microbial changes throughout the gut (Choct *et al*., 2006). Water soluble fraction of barley NSP has a deleterious effect on intestinal physicochemical characteristics and microbial proliferation of chickens (Choct, 1997; 2006). Results of this study also indicate that NSP polymers of wheat and barley decreased the count of lactic acid bacteria and Bifidobacteria in the gut digesta. These bacteria are related to beneficial effects on birds and are known as probiotics growth promotants. The impaired live performance of birds fed on wheat and barley can partly be explained by reduced population of Gram-positive bacteria including Lactobacilli and Bifidobacteria. The probiotic-type micro flora modulates innate immune system of the host animal (Christensen *et al*., 2002) and they are necessary for the development of gut-associated lymphoid tissue (GLUT) (Rhee *et al*., 2005).

The negative effects of NSP on the proliferation of bacteria in the intestine were removed significantly after supplementation of wheat and barley diet with exogenous enzymes blend (especially on probiotic-type bacteria). These results are consistent with previous reports. (Yin *et al*., 2000; Choct *et al*., 2006; Mirzaie *et al*., 2012). Digest NSP of wheat and barley by carbohydrases has been successful and promising in broilers (Olukosi *et al*., 2007; Slominski, 2011). Glycanase enzymes including xylanases and β- glucanases release the encapsulated nutrients and decrease digesta viscosity. These events are further facilitated with action of phytases (Ravindran *et al*., 1999; Olukosi *et al*., 2007). Due to the fact that birds don't possess adequate endogenous enzymes to digest NSP components, the addition of exogenous NSp-degrading enzymes seems to be necessary when corn is replaced by wheat and barley.

Results presented in Table 6 cleared that reduced villus height, and vice versa increased villus width and crypt depth can result from an increase in digesta viscosity which leads to quick changes in the intestinal mucosa due to the close proximity of the mucosal surface to the intestinal viscose materials (Saki *et al*., 2011). The crypt can be acted as villus factory and a large crypt depth indicates fast tissue turnover and a high demand for new tissue. Therefore, the addition of a viscose ingredient (NSP) to the diet can result in deeper crypts with a high rate of cell proliferation and tissue renewal (Iji *et al*., 2001). Hence, it is concluded that the shorter villus height induced by wheat and barley diets are related to NSP viscosity. Shorter villus height is associated with a reduction in absorptive potential throughout the intestine, further growth efficiency, and normal physiological conditions (Saki *et al*., 2011).

Pancreas α-amylase and lipase activities of broiler chickens were significantly increased in birds fed on wheat and barley diets as compared to those fed on corn or diets supplemented with enzymes. These results reflect the fact that water-soluble NSP of wheat and barley impede pancreatic α-amylase and lipase activities (Li *et al*., 2004). This mode may indicate needs for greater secretion of pancreatic enzymes (Williams, 1996; Denbow, 2000). Based on relevant research findings intestinal enzyme activity depends on the original of dietary nutrient and quantity or quality of anti-nutrients in the gut (Li *et al*., 2004; Mirzaie *et al*., 2012). Also diet issue affects the magnitude of secretion

in the pancreas. Diets with high fat or carbohydrates content increase the pancreas secretion and serum concentration of amylase and lipase (Brenes *et al*., 1993; Zhao *et al*., 2007; Lin *et al*., 2010). Amylase is secreted in saliva, intestinal fluid, and pancreatic juices. As well as lipase is secreted in stomach and pancreatic juices (Denbow, 2000). In normal conditions, pancreas-derived amylase and lipase constitued only a small portion of serum enzymes, but in abnormal conditions and changing in diet cereal type with high amounts of anti-nutritional factors, acute pancreatitis and leakage of enzymes, total serum concentration of enzymes rise significantly (Williams, 1996).

The results of Table 8 indicated that the decrease in expression of SGLT1 and MUC2 in the jejunum of chickens at the age of 42 days fed on enzyme supplemented diets, may reflect modularly effects of exogenous enzyme blend on gut cell wall, villus structure and restoring the remodeled nutrient transporters which resulted in neutralization of negative effects. In the small intestine, the absorption of nutrients is mediated by transporter proteins expressed in the enterocyte. The nutrients passed through the epithelium of the small intestine and into the blood stream via special transporters. For example, amino acids are transported into the intestinal epithelial cells as di- or tri-peptides by the H+-dependent peptide transporter 1, PepT1 (Chen *et al.*, 2015). Since the nutrient transporters have different gene expression patterns and actions, thus it can be concluded that it is necessary to understand the developmental patterns of intestinal absorptive capacity because of its key function in nutrient intake (Poncet & Taylor, 2013).

According to the literatures, amount and right combination of foods in the intestine is essential for the development of intestinal tissue and the words. Inversely, abundance of anti-nutrients in the gut causes mystification to tissue development or growth and increment of its properties for the sufficient intestinal cell proliferation (Uni *et al.*, 2003a; Tako *et al.*, 2004; Uni & Ferket, 2004). Increasing the level and composition of carbohydrates consumed well over several days increases the density of glucose transmitters in the intestinal cell membranes and this increase along with an increase in mRNA levels of genes in these cells is called SGLT1 (Ferraris, 1997 and 2001). The increase in peptide transmitters and increase in the level of mRNA for the gene MUC2 have also been reported (Gilbert *et al.*, 2008; Mott *et al.*, 2008). Plentitude of nutrient transmitters in the chicken´s intestine well affected by several factors such as genetic selection, the type and amount of feed intake, intestinal tissue growth and development, quality and quantity of dietary protein, as well as dietary fiber level (Chen *et al.*, 2005; Mott *et al.*, 2008; Gilbert *et al.*, 2007, 2008, 2010).

Food deprivation or presence of anti-nutrients such as NSPs especially water-soluble NSP compounds causing delay and disruption of intestinal tissue growth, increased synthesis and secretion of mucin and eventually changing the structure of the intestinal mucus layer (Noy & Sklan, 1996; Uni *et al.*, 2000 Uni *et al.*, 1998, 2003a). The supply of nutrients and absorption of carbohydrates in the early growth period of the chickens has an important role in the normal development of intestinal tissue, mucus layer of the intestinal absorptive cells and other relevant events in the intestine (Uni *et al.*, 1998; Tako *et al.*, 2004; Uni & Ferket, 2004; Moran, 2007).

All of the incidents through the gut are set by regulating the gene expression of nutrient transmitters (glucose, amino acids and peptides) from lumen and through the mucus layer into enterocytes of lining surface at the absorptive area, which created in the intestine and are the main factors limiting further chicken growth (Ferraris, 1997 and 2001; Gilbert *et al.*, 2007, 2010). In this experiment, the barley diet was increased MUC2 gene expression. Because high-fiber food or plentiful NSP content led to the increased excretion of mucin, it is obvious that, the increase in the level of food items containing these substances increases the production of mucin gene expression and mucin synthesis rate (Smirnov *et al.*, 2004, 2006; Montagne *et al.*, 2004; Mott *et al.*, 2008).

Also the lining of the intestinal epithelial mucin plays a vital role to the protection of tissues, including protection of the gut against acidic stomach chyme and hydrolyzing pancreatic enzymes, slippery surface to facilitate the transportation of materials such as roughage and fiber, protection against invasion and establishment of pathogenic microbes as well as the treatment of intestinal absorption and facilitation of the transfer of nutrients to the cells (Smirnov *et al.*, 2004, 2006; Tanabe *et al.*, 2005). MUCIN layers are divided in two parts, including loose and tight layers, which action and composition are different. Tight layers have several membrane conjunction sites to catch and transmit nutrients into enterocyte cells (Montagne *et al.*, 2004; Horn *et al.*, 2009).

Various factors, including the type, quantity and composition of the diet (phytate, fiber and NSP), the number of invasive bacteria, disease, lack of amino acids and environmental factors can change mucin physicochemical properties and its integration. Finally,

the mentioned factors can alter mucin synthesis and mucin disposal rate (Montagne *et al.*, 2003, 2004; Cowieson *et al.*, 2004; Tanabe *et al.*, 2005).

CONCLUSION

Results of the present study suggest that wheat and barley based diet, containing high levels of NSP, due to its contribution to digesta viscosity and impede of endogenous digestive enzymes play an important role to intestinal health and subsequent transmission of hydrolyzed products to the enterocyte cells and nutrient absorption efficiency, as well as villi morphology, bacterial population, and level of nutrient transmitter gene expression in broilers. In conclusion, wheat and barley based diets without exo-enzyme blend supplementation has adverse effects on productive traits, however such changes are remarkably restored by supplementing NSp-degrading enzymes to broiler diets.

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