Animal model show physiological characteristics can alter by feeding of different cereal type and exogenous multi-enzyme

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Keywords: Animal model, Physiological characteristic, Cereal type, exo-enzyme.

ABSTRACT. Physiological characteristics of meat-type chicken such as growth traits, serum biochemical metabolites and mineral concentrations, serum enzyme activity, and gut flora were studied by means of 625 day-old chicks which randomly assigned to five treatments with five replicates in a completely randomized design. Four different types of cereal-based diets (wheat, and barley with or without exogenous multi-enzyme supplement) were used as experimental groups and a corn-based diet was also considered to serve as control group. All diets had similar contents of crude protein, energy, and total non-starch polysaccharides (NSP). Results indicated that enzyme supplemented diets improved daily weight gain and feed conversion ratio than barley and wheat diets without enzyme significantly ($P<0.01$). Feeding wheat and barley diets decreased the serum cholesterol, triglyceride and glucose concentrations than control group significantly ($P<0.01$). Also using of these two type of cereals without enzyme supplementation resulted in reduced serum mineral concentration and undesirable effect on gut flora ($P<0.01$). Total gram negative bacteria of the ileum was decreased, however lactic acid and bifida bacteria population were increased by supplementation of wheat and barley diets with exogenous multi-enzyme significantly ($P<0.01$). The serum activities of α -amylase and lipase were increased in chicks fed wheat and barley diets when compared to the control group fed on corn diet, but enzyme supplementation significantly reduced the serum activities of α -amylase and lipase ($P<0.01$).

1. Introduction

Alternative grains can be successfully replaced for corn in poultry diets. Wheat and barley are generally used as major sources of energy in poultry diets. Though the major components of these grains are starch and proteins, they have considerable content of non-starch polysaccharides (NSPs), derived from the cell walls [15,17]. NSPs are generally considered as anti-nutritional factors [7]. The content and structure of NSP polymers vary between different grains, which consequently affect their nutritive value and anti-nutritional impacts [17]. The major part of NSPs of wheat is constituted by arabinoxylan polymers, whereas NSPs in barley comprises polymers of β -glucans [3,7,24]. A review of literature indicates that a complex blend of exogenous multi-enzyme is required to degrade the NSPs present in the diet in order to obtain satisfactory health, performance and nutrient digestibility through the gut [11,17,18,22]. Few researches have shown the magnitude of adverse effect of equal fractions of NSP from different sources. Besides, there is limited information on the efficacy of multi-enzyme supplements under such circumstances. In the present study, equal fractions of NSP from wheat and barley were included in chicken diets with and without exogenous multi-enzyme to compare the negative effects on growth, serum concentration of metabolites and minerals, intestinal bacterial population, and serum enzyme activity of α -amylase and lipase compared to corn-based diet.

2. Materials and Methods

2.1. Animals, management and treatments

A total number of 625 day-old broiler chickens (Ross-308 strain) were randomly assigned to five treatments and five replicates of 25 chicks each. Each replicate was a floor pen of 1.5×2.2 m². Five experimental diets based on corn(C), wheat (W), barley (B), wheat +exo-enzyme (WE) and barley +exo-enzyme (BE) were formulated for birds according to NRC recommendations [16]. The enzyme supplement (COMBO[®] enzyme blend) contained 480 U/g multi-glycanase (including 200 U/g xylanase, 200 U/g β -glucanase, and 80 U/g hemi-cellulase) and 1000 U/g phytase which used at level of 1 g/kg in the experimental diets. Wheat and barley samples were analyzed based on A.O.A.C instructions [1] before the experiment for non-starch polysaccharide constituents, which depicted in Table 1. All cereal-based diets were formulated to have equal fractions of soluble NSP.

Table 1. Non-starch polysaccharide constituents of wheat and barley samples before the experiment [%]

Sample	Cellulose ¹	Hemi-Cellulose ²	Lignin ³ (ADL)	Total NSP	Soluble NSP	Insoluble NSP	DF ⁴
Wheat	1.8	10.4	1.59	13.11	2.45	10.66	14.7
($\pm$ SE)	($\pm$ 0.1)	($\pm$ 0.52)	($\pm$ 0.18)	($\pm$ 0.64)	($\pm$ 0.12)	($\pm$ 0.53)	($\pm$ 0.73)
Barley	4.4	23.62	1.97	16.73	4.12	12.61	18.7
($\pm$ SE)	($\pm$ 0.24)	($\pm$ 1.42)	($\pm$ 0.24)	($\pm$ 0.81)	($\pm$ 0.15)	($\pm$ 0.65)	($\pm$ 0.94)

1: Cellulose=ADF-ADL 2: Hemi-Cellulose=NDF-ADF 3: Lignin (ADL) = ADF-Cellulose 4: Dietary Fiber=Total NSP +ADL.

Feed and water were provided at free access. 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.

2.2. Measurements

Feed intake, weight gain, and feed conversion ratio (FCR) was calculated as daily mean values. On 42 days of ages, 3 birds from each replicate were randomly selected and blood samples were taken from wing vein, blood samples were transferred to vial tubes containing sodium heparin. The tubes were centrifuged at 5000×g for 20 min, and the supernatant was discarded. Serum glucose, cholesterol, triglyceride and mineral concentrations (Ca, P, Mg, and Zn) were measured by use of specific kits (Biosystem Company, Spain). Also the harvested serum was used for serum enzyme assay. The serum activity of α -amylase (EC 3.2.1.1) was determined using a validated kit from Parsazmun Chemical Company (Parsazmun Co., Karaj, Iran) and the serum 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 instructions. The activities of α -amylase and lipase are expressed as International Unit (IU) per one liter of serum [9,10].

At the day of 42, three chicks per pen (15 chickens from each treatment) were randomly selected and killed by decapitation. Intestinal micro-biota was enumerated according to Langhout et al. (1999) with some modification [12]. In brief, 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 on specific media and transferred to an incubator set at 37°C for 24 hours. At the end of incubation period, bacterial colonies were counted. Specific media as described below were used to culture different type of bacteria including Eosin Methylene Blue (EMB) Agar for Gram negative bacteria, Rogosa Agar (RA) for lactic acids, and Eugon Agar (EA) for bifida bacteria, respectively [9,10].

2.3. Statistical procedures

The results were statistically analyzed by GLM procedure of SAS software of SAS Institute Inc [21]. Statistical scheme was based on completely randomized design (CRD). Samples within pens (3 per each unit) were subjected to nested analysis. Duncan's multiple range tests was used to separate the means.

3. Results

3.1. Growth performance

Table 2 depicts the daily mean results of feed intake, weight gain, and feed conversion ratio of chickens throughout the trial. Results indicated that birds fed wheat significantly ($P<0.01$) reduced their feed intake compared to other groups. Supplementing exogenous multi-enzyme 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 exogenous multi-enzyme 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 their counterparts fed on corn or enzyme supplemented diets ($P<0.01$).

Table 2. Effect of different type of cereal grains and enzyme supplementation on growth traits of chickens.

Treatments	Daily feed intake [g/bird]	Daily weight gain [g/bird]	Feed conversion ratio [FCR]
C	101.90 ^b	53.60 ^a	1.90 ^c
W	99.14 ^c	47.98 ^b	2.06 ^a
WE	101.26 ^b	50.45 ^b	2.00 ^b
B	103.17 ^{ab}	50.17 ^b	2.05 ^a
BE	104.57 ^a	52.36 ^{ab}	1.99 ^b
SEM	0.56	0.53	0.02

*^{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.

3.2. Serum biochemical metabolites and mineral concentrations

Table 3 and table 4 depicts the mean changes of serum biochemical metabolites and mineral concentrations of chickens after feeding of wheat and barley with or without enzyme supplement compared to control group.

Table 3. Effect of different type of cereal grains and enzyme supplementation on serum metabolite concentrations of chickens [mg/dl].

Treatments	Cholesterol	Triglyceride	Glucose
C	135 ^a	131 ^a	270 ^a
W	116 ^c	116 ^c	216 ^c
WE	125 ^b	121 ^b	252 ^b
B	114 ^c	115 ^c	201 ^c
BE	122 ^b	119 ^b	241 ^b
SEM	1.52	1.22	6.42

*^{abc} Means with different superscript letters within columns have significant difference ($P<0.01$). ¹U/Lit: Units of enzymes (IU) per one liter of serum.
*C:corn, W:wheat, B:barley, WE:wheat+enzyme, BE:barley+enzyme.

Results indicated that birds fed wheat or barley significantly ($P<0.01$) reduced their serum metabolites and mineral concentration compared to other groups. Supplementing exogenous multi-enzyme to the wheat or barley diet remarkably restored the situation so that the differences were not significant than their counterpart fed on corn. On the other hand, birds fed wheat or barley had numerically lower serum metabolites and mineral concentration than other treatments.

Table 4. Effect of different types of cereal grains and enzyme supplementation on broiler serum mineral concentrations of chickens [mg/dl].

Treatments	Ca	P	Mg	Zn
C	135 ^a	131 ^a	270 ^a	270 ^a
W	116 ^c	116 ^c	216 ^c	216 ^c
WE	125 ^b	121 ^b	252 ^b	252 ^b
B	114 ^c	115 ^c	201 ^c	201 ^c
BE	122 ^b	119 ^b	241 ^b	241 ^b
SEM	1.52	1.22	6.42	6.42

^{*abc}Means with different superscript letters within columns have significant difference (P<0.01). ¹U/Lit: Units of enzymes (IU) per one liter of serum.
^{*}C:corn, W:wheat, B:barley, WE:wheat+enzyme, BE:barley+enzyme.

3.3. Intestinal microbial

According to the results of table 5, total counts of Gram negative bacteria including *E. coli* and clostridia in the intestinal content were higher in birds fed wheat and barley than the control (P<0.01). On the other hand, the number of lactic acid bacteria and bifid bacteria were significantly lower (P<0.01) in birds received wheat and barley diets compared to the control. Inclusion of an exogenous multi-enzyme supplement to the wheat and barley diets modified the situation so that no significant difference was found between the wheat and barley diets supplemented with the enzyme supplemented and the corn diet.

Table 5. Effect of different types of cereal grains and enzyme supplementation on intestinal bacterial population of chickens [log CFU/g digesta]

Treatments	Total gram negative	Lactic acid bacteria	Bifida bacteria
C	5.31 ^b	4.90 ^b	5.40 ^b
W	6.33 ^a	3.87 ^c	4.06 ^c
WE	5.21 ^{bc}	5.19 ^a	5.67 ^a
B	6.24 ^a	4.90 ^b	3.51 ^d
BE	5.26 ^b	5.40 ^a	5.76 ^a
SEM	0.13	0.10	0.11

^{*abc}Means with different superscript letters within columns have significant difference (P<0.01). ¹U/Lit: Units of enzymes (IU) per one liter of serum. ^{*}C:corn, W:wheat, B:barley, WE:wheat+enzyme, BE:barley+enzyme.

3.4. Serum enzyme activity

Based on results depicted in table 6, Serum enzyme activity of chickens fed wheat and barley were significantly (P<0.01) increased compared to the control group fed on corn. Supplementation of exogenous multi-enzyme mixture to the wheat and barley diets significantly reduced the serum enzyme activity of α -amylase and lipase.

Table 6. Effect of different types of cereal grains and enzyme supplementation on serum enzyme activity of chickens

Treatments	Serum enzyme activity [U/Liter] ¹	
	α -amylase	Lipase
C	22.94 ^c	10.24 ^c
W	60.48 ^{ab}	21.83 ^a
WE	49.48 ^b	17.62 ^b
B	66.32 ^a	20.84 ^a
BE	47.71 ^b	18.72 ^{ab}
SEM	2.60	1.31

^{*abc}Means with different superscript letters within columns have significant difference (P<0.01). ¹U/Lit: Units of enzymes (IU) per one liter of serum. ^{*}C:corn, W:wheat, B:barley, WE:wheat+enzyme, BE:barley+enzyme.

4. Discussion

Soluble fraction of non-starch polysaccharides (NSP) have a highlight negative effect on growth and other physiological characteristics of chickens due to impairment of gut efficiency by different cereals cell wall constituents [15,17,24]. However, the comparative effects of the cereal type and structure of NSP on growth and physiological response have not been adequately addressed. Results reported herein indicate that pentosan polymers of wheat's NSP have more deleterious impact on voluntary feed intake of broiler chickens than β -glucans polymers of barley's NSP. Birds fed on wheat diet consumed lower feed and consequently lower body weight gain compared to those fed barley, primarily due to their differences in NSP constituent resulted in increased digesta viscosity in the intestine [9].

Increased viscosity per se creates ideal environment for maximal proliferation of anaerobic and Gram negative bacteria as observed in this study. These observations are in line with other reports [8,12]. According to the results of one research, slow movement of digesta and low oxygen condition due to the presence of NSP provide a stable media, where fermentative microbes such as anaerobic bacteria proliferate easily [12]. Such microbial changes reduce nutrients available for host and produce detrimental by-products [4]. Water soluble NSPs of wheat were reported to have deleterious impact on physicochemical properties and microbial proliferation of digesta in the intestinal tract of chickens [3,4]. Results of this experiment also indicate that NSP polymers of wheat and barley decreased the population of lactic acid bacteria and Bifida bacteria in the intestinal digesta (table 5). These bacteria are associated with beneficial effects to birds and are known as probiotics growth promoters. Impaired live performance of birds fed on wheat and barley can partly be explained by decrease in population of Gram positive bacteria including Lactobacilli and Bifida bacteria. The probiotic-type bacteria modulate innate immune system of the host [5], and they are necessary for the development of gut-associated lymphoid tissue (GLUT) [19].

Supplementation of wheat and barley diets with exogenous multi-enzyme significantly reduced the negative effects of NSP on proliferation of useful bacteria through breakdown of NSP polymers. These results are in accordance with relevant reports. [4,15,24]. Degrading NSP enzymes including xylanases and β -glucanases release the encapsulated nutrients and reduce digesta viscosity which facilitated by the further action of phytase [17,18].

According to results presented in table 3, reduced cholesterol, triglyceride and glucose can resulted from increase of dietary fiber content of wheat and barley in company with NSP and related digesta viscosity which leads to quick changes in the intestinal passage rate and dynamic of digestion and absorption of nutrients due to impaired physico-chemical properties of the intestinal content [20]. Therefore, it is concluded that the lower serum concentrations of biochemical metabolites and minerals induced by wheat and barley diets are related to their dietary fiber and NSP content. Decreased serum concentrations of metabolites and minerals is associated with reduction in intestinal absorptive potential due to impaired nutrient transporters at gut wall, impede digestive enzyme and normal physiological responses [9,10,20].

Serum α -amylase and lipase activities of chickens were significantly increased in birds fed on wheat and barley diets as compared to those fed on corn diet or wheat and barley diets supplemented with enzyme cocktail. This finding reflects the fact that water soluble NSP of wheat and barley impede pancreatic α -amylase and lipase activities, as well as intestinal enzyme secretory organs which further resulted in enzyme leakage into serum [13]. This finding may indicate needs for greater secretion of enzymes due to increased contents of NSP inside the gut and increased substrate levels through the serum that induced high concentration of enzymes [6,23]. Research has shown that intestinal or serum enzyme activity depends on dietary nutrient source and presence of anti-nutrients in the gut [13,15]. Also diet type affects the rate of enzyme secretion. Diets with high fat or carbohydrates increase the secretion rate and serum concentration of amylase and lipase [2,14,25]. Amylase is secreted in saliva, intestinal fluid, and pancreatic juices. As well as lipase is secreted in stomach and pancreatic juices [2]. In normal conditions, pancreas-derived amylase and lipase makes up only a small portion of serum enzymes, but with abnormal conditions such as

change in diet cereal type and high amount of anti-nutritional factors, acute pancreatitis and leakage of enzymes, total serum concentration of mentioned enzymes rise significantly [23].

5. Conclusion

According to the results of the present study NSP solubility of different type of cereals and its contribution to produce high viscose digesta play an important role in chicken growth efficiency and physiological response. In conclusion, various NSP components of wheat and barley have adverse effects on serum concentration of biochemical metabolites, minerals, and population of benefit bacteria. Besides, NSP polymers of wheat and barley increase the serum activities of α -amylase and lipase. These deleterious impacts could restore by supplementing exogenous multi-enzymes to chicken diets.

Acknowledgment

Authors appreciate financial support of the Animal Research Institute, Karaj, Iran and Shahrekord University, Shahrekord Iran.

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