Poultry Science®

Effect of barley processing and enzyme supplementation on broiler performance, gut morphometry, and the occurrence of ascites syndrome in broiler chickens

Mina Zohrabi ,*,[2] Fariborz Khajali ,*,[1] and Behrouz Dastar[†]

[*]*Department of Animal Science, Shahrekord University, Shahrekord, Iran; and* [†]*Department of Animal and Poultry Nutrition, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*

ABSTRACTS An experiment was conducted to evaluate the nutritive value of processed barley grains and their influence upon growth performance, gut function, and the occurrence of ascites syndrome in broiler chickens. Day-old broilers (n=504) were randomly assigned to six treatments (3 levels of grain processing × 2 levels of enzyme supplementation). Results showed that chicks fed with germinated barley significantly gained more weight compared to unprocessed barley in all feeding stages (P = 0.0001). Chicks received roasted barley showed a slowdown in their growth so that their weight gain was significantly lower than the control and germinated barley groups in the finishing period and the entire experiment (P = 0.0001). Enzyme supplementation of barley-based diets improved weight gain so that a significant improvement was observed in the growing (P = 0.0002) and throughout the trial (P = 0.0098) over the unsupplemented diets. Chickens fed with germinated barley had a higher length of jejunal villi whereas they had a lower crypt depth compared to untreated or roasted barley groups. As a result, the ratio of villus length to crypt depth as well as villus absorptive surface were significantly higher in birds received germinated barley than those fed with unprocessed or roasted barley. Barley processing resulted in a lower viscosity of digesta in the jejunum so that both processing methods showed a significantly lower viscosity compared to unprocessed barley. Birds received unprocessed barley showed a significantly lower concentration of total cholesterol (P = 0.0370) and LDL-cholesterol (P < 0.01) than those received processed barley. Plasma uric acid (P = 0.0350) and total protein (P = 0.0050) concentrations were significantly greater in birds fed with germinated barley as opposed to other experimental treatments. The mortality from ascites was lower in broilers fed with germinated barley compared to untreated barley, particularly in the absence of NSP-degrading enzyme supplement. In conclusion, germinated barley could significantly improve the performance, gut morphometry, and ascites indices of broiler chickens when compared to untreated barley.

Keywords: Ascites, Barley processing, Chicken, Enzyme, Gut

2024 Poultry Science 103:104410
https://doi.org/10.1016/j.psj.2024.104410

INTRODUCTION

Since the ancient time, barley has been cultivated in most part of the world due to its high adaptability to different climates, resistance to drought, and the ability to grow in short growing seasons (Perera et al., 2022). There is a high variation in chemical composition of barley depending on cultivar and environmental factors during harvest, storage and processing. From nutritional viewpoint, the use of barley in broiler diets is limited due to the presence of non-starch polysaccharides (NSPs) including β-glucans and pentosans (arabinoxylans) (Fox et al., 2003). A vast part of barley β-glucans is water-soluble, leading to the formation of gel in aqueous solutions, which causes increased intestinal viscosity in broiler chickens (Papageorgiou et al., 2005). Consequently, feeding barley to broiler chickens results in poor performance (Jacob and Pescatore, 2014). There are several methods to passivate the anti-nutritional effects of NSPs including gamma irradiation (McNab and Smithard, 1992), dehulling (Wang et al., 2017), water extraction and fermentation (Annison and Choct, 1991; Khajali and Rafiei, 2024). However, the application of exogenous carbohydrase enzymes has been the most

Received July 17, 2024.
Accepted October 6, 2024.

[1]Co-corresponding author: Khajali@agr.sku.ac.ir (FK)
[2]Corresponding authors at: Department of Animal Science, Shahrekord University, Shahrekord, Iran. minazohrabi8@gmail.com (MZ)

Table 1. Chemical composition and the CIELAB color space of raw, roasted, and germinated barley (as-fed basis).

Feedstuff	Dry matter (%)	Crude protein (%)	Crude fibre (%)	NSP (mg/g)	Lightness (L*)	Redness (a*)	Yellowness (b*)
Raw barley	90.0	11.7	6.4	160.3	53.66	8.55	25.78
Roasted barley	91.6	12.3	6.1	138.5	44.33	11.80	24.49
Germinated barley	89.0	13.5	2.8	140.1	52.28	9.09	26.36

Duplicate samples were uses to obtain these values.

effective and practical solution to degrade NSPs (Khajali and Rafiei, 2024).

Application of NSP-degrading enzymes has shown to improve broiler performance by modulating gut microbiome (Daneshmand et al., 2023). Under the action of NSP-degrading enzymes, parts of NSPs are completely hydrolyzed to simple sugars, and some parts are partially degraded. Partially-degraded β-glucans (PDβG) of barley can be served as substrates for beneficial bacteria, leading to the proliferation of Lactobacilli and Bifid bacteria (Mitsou et al., 2010). In fact, these compounds exert prebiotic-like activities. In addition, PDβG have shown to play a role as biologically active immunomodulators. Guo et al. (2003) indicated that barley β-glucans enhance proliferation and phagocytizing function of avian macrophages. Moreover, dietary supplementation of broiler chickens with barley β-glucans could enhance humoral (Guo et al, 2003) and cell-mediated immunity (Chae et al., 2006).

Substitution of barley for corn has shown the tendency to increase the relative size of gastrointestinal tract (GIT) due to the high fiber content of barley (Afifian et al., 2023). Although GI tract constitutes a small percentage of the body weight, it accounts for a large portion of oxygen consumption in the body (Vollmar and Menger, 2009). Consequently, such dietary cereal substitution may influence the incidence of ascites syndrome in broiler chickens raised at high-altitude regions. Ascites syndrome is a common metabolic disease, which is associated with reduced oxygen availability (Khajali, 2022). Nevertheless, Afifian et al. (2023) fully replaced corn by barley in broiler diets and they did not find any significant changes in the incidence of ascites. They explained that reduced growth rate and suppressed lipogenesis in barley-based group may counteract the situation.

The present experiment aimed at studying barley processing (germination and roasting) with or without the use of exogenous enzymes on growth performance, gut function, and the occurrence of ascites syndrome in broiler chickens.

MATERIALS AND METHODS

The experiment was performed in the Poultry Research Centre of Shahrekord University, Shahrekord, Iran. All experimental protocols were performed in accord with the Animal Care and Use Committee of Shahrekord University.

Chemical analysis

Before starting the experiment, the chemical composition of feed ingredients and experimental diets including dry matter, crude protein, and crude fibre were determined by Association of Official Analytical Chemists (AOAC) methods 934.01, 992.93, and 962.09 respectively (AOAC, 2000). The content of non-starch polysaccharides and the CIELAB color space of raw and processed barley were also analyzed and presented in Table 1.

Experimental diets, birds, and husbandry

Experimental diets were formulated to be isonitrogenous and isoenergetic and met broiler's requirements recommended by the Ross 308 nutrition guideline (2019). Dietary treatments consisted of an untreated barley (control), germinated barley, and roasted barley. The germination process took 110 h in a temperature set between 18 to 20 °C. The roasting process was performed by gradually increasing temperature to 35, 75, and 110° C. Following barley processing, dietary treatments were prepared with or without enzyme supplementation. The enzyme cocktail consisted of xylanase (2200 U/g), β-glucanase (200 U/g), cellulase (100 U/g), pectinase (100 U/g), and phytase (1000 U/g). The enzyme preparation was supplemented at 0.1 % of diet. Diets were provided in a mash form. Table 2 presents the composition of the experimental diets in different feeding phases.

Day-old sexed broiler chicks (n = 504) were obtained from a local hatchery and randomly assigned to one of 36 floor pens (14 birds/pen; 7 males and 7 females). Six such pens were randomly allotted to each treatment. Feed and water were freely provided to chicks. The lighting program consisted of a continuous light with the intensity of 40 Lux for the first 2 days' post-hatch, and reduced to 21L:3D from day 3 to 6, and set at 18L:6D from day 7 onward with the intensity of 20 Lux. The house temperature was set according to the Ross management guideline (2019).

Measurements

Records of body weight and feed intake of all treatment groups were collected at the beginning and the end of feeding phases. Feed conversion ratio was also calculated taking into account the weight of mortalities.

Blood samples were collected in heparinised tubes from brachial vein of two birds per pen (12 birds/treatment) at the end of trial (42d of age). An aliquot of

Table 2. Composition of the experimental diets containing raw, roasted or germinated barley.

Item (% unless noted)	Starter (1-10 d)			Grower (11-24 d)			Finisher (25-42 d)		
	Raw	Roasted	Germinated	Raw	Roasted	Germinated	Raw	Roasted	Germinated
Raw barley	53.7	-	-	56.7			60.5	-	
Roasted barley	-	53.7	-	-	56.6	-	-	60.5	-
Germinated barley	-	-	54.5	-	-	59.3	-	-	63.1
Soybean meal (44 % CP)	34.0	34.0	32.0	30.5	30.5	28	25.8	25.8	23.2
Soy oil	7.0	7.0	7.5	8.3	8.3	8.1	9.3	9.3	9.0
Di-calcium phosphate	1.6	1.6	1.6	1.2	1.3	1.3	1.2	1.2	1.2
Calcium carbonate	1.5	1.5	1.5	1.4	1.4	1.35	1.3	1.3	1.3
L Lysine HCl	0.25	0.25	0.35	0.20	0.20	0.20	0.20	0.20	0.15
DL Methionine	0.40	0.40	0.45	0.30	0.30	0.30	0.30	0.30	0.60
L Threonine	0.30	0.30	0.30	0.20	0.20	0.20	0.25	0.25	0.30
L Arginine	0.40	0.40	0.40	0.4	0.4	0.4	0.3	0.3	0.3
Vitamin supplement[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral supplement[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium chloride	0.35	0.35	0.35	0.30	0.30	0.35	0.35	0.35	0.35
Nutrient composition									
ME (Kcal/Kg)	2900	2900	2900	3000	3000	3000	3100	3100	3100
Crude protein	22.1	22.1	22.1	20.7	20.7	20.7	19	19	19
Met+Cys	1.04	1.04	1.04	0.95	0.95	0.95	1.14	1.14	1.14
Lys	1.37	1.37	1.37	1.25	1.25	1.25	0.89	0.89	0.89
Thr	0.97	0.97	0.97	0.84	0.84	0.84	0.74	0.74	0.74
Arg	1.45	1.45	1.45	1.30	1.30	1.30	1.15	1.15	1.15

[1]Provided the following per kg of diet: vitamin A (trans retinyl acetate), 3600IU; vitamin D3 (cholecalciferol), 800 IU; vitamin E (dl-α-tocopherol acetate), 7.2 mg; vitamin K_3, 1.6 mg; thiamine, 0.72 mg; riboflavin, 3.3 mg; niacin, 0.4 mg: pyridoxin, 1.2 mg; cobalamin, 0.6 mg; folic acid, 0.5 mg; choline chloride, 200 mg.[2]Provided the following per kg of diet: Mn (from MnSO4-H2O), 40mg; Zn (from ZnO), 40mg; Fe (from FeSO4-7H2O), 20 mg; Cu (from $CuSO4\text{-}5H_2O$), 4 mg; I [from Ca (IO3)2-H2O], 0.64 mg; Se (from sodium selenite),0.08 mg.

blood was also collected into heparinised microcapillary tubes and centrifuged in a microcentrifuge (BMC 24 Microhaematocrit centrifuge, Pars Azma Co., Isfahan, Iran) for 5 min to obtain haematocrit. A second aliquot of blood was also collected on glass slides to prepare the blood smear for the determination of differential leukocyte counts. Blood samples were centrifuged at 2500g for 10 minutes to separate plasma. Plasma samples were used for the determination of nitric oxide (NO), malondialdehyde (MDA), total protein (TP), uric acid (UA), total cholesterol, LDL-cholesterol, HDL-cholesterol, and alanine transferase (ALT). All chemical reagents were obtained from Sigma-Aldrich Co. (Sigma-Aldrich Co. St Louis, MO, USA).

All floor pens were checked daily for mortality and necropsied for the recognition of the cause of death. The ratio of right ventricle to total ventricles (RV/TV) were determined and all values above 0.27 were diagnosed as ascites (Khajali, et al., 2011).

At the end of trial (Day 42), 12 birds from each treatment were slaughtered for carcass characteristics and gut morphometric criteria. Jejunal segments were cut (about 1 × 1 cm^2), flushed with PBS (pH 7), fixed in the Clarke' solution (75 % ethanol: 25 % acetic acid) for 45 min, and stored in ethyl alcohol (50 %). These segments were then exposed to a periodic acid-Schiff staining. Muscle layers were separated from mucosa and rows of villi were cut and transferred onto glass slides to examine with a microscope using an optical lens 1,000 × . Morphometric parameters such as villus length (VL), villus width (VW), and crypt depth (CD) were measured and the villus absorptive surface area was calculated using the formula= $(2\pi) \times (VW/2) \times (VL)$. Jejunal digesta were also collected in 50 mL tubes, centrifuged at 500 × g for 15 minutes. The viscosity was then measured using a viscometer (Brookfield DV-II +pro, Middleboro, MA USA).

Statistical analysis

Data were analyzed in a completely randomized design with 3 × 2 factorial layout. The SAS software (Version 9.4) was used to analyze data. When there was sampling within pens, data were subjected to a nested design. Means were separated with the Duncan's multiple range test.

RESULTS

Enzyme supplementation of barley-based diets improved weight gain so that a significant improvement was observed in growing (P=0.0044) and throughout the trial (P=0.0346) over unsupplemented diets. The interaction between barley processing and enzyme supplementation was significant in growing period and entire trial (Table 3). This significant interaction could be seen as a greater improvement in weight gain by the enzyme supplement in untreated barley than in processed barley.

In the starting period (1-10 d), body weight gain of chicks fed with unprocessed barley (the control) was significantly (P = 0.0001) lower than chicks in processed barley groups. There was a divergent response of weight gain to barley processing in the growing (11-24 d) and finishing (25-42 d) periods. Chicks fed with germinated barley significantly (P = 0.0001) gained more weight compared to unprocessed or roasted barley. Chicks received roasted barley showed a slowdown in their growth so that their weight gain was significantly

Table 3. The effect of barley processing and enzyme supplementation on body weight gain of broiler chickens.

Main effect		Body weight gain (g/bird)			
Barley processing	Enzyme	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Total (1-42 d)
Control		84[c]	462[b]	1275[b]	1818[b]
Roasted		100[b]	453[b]	1111[c]	1664[c]
Germinated		109[a]	493[a]	1411[a]	2013[a]
SEM		1.92	8.38	18.21	21.86
Enzyme					
	-	97	448[b]	1246	1797[b]
	+	98	490[a]	1285	1866[a]
SEM		1.55	6.84	18.21	17.85
	Interactions				
Control	-	81	417[b]	1238.6	1737[b]
Control	+	88	507[a]	1311.3	1900[ab]
Roasted	-	97	439[b]	1098.5	1662[c]
Roasted	+	103	467[ab]	1123.4	1665[c]
Germinated	-	108	489[a]	1376.5	1988[a]
Germinated	+	110	497[a]	1444.8	2037[a]
P-value					
Barley processing		0.0001	0.0056	0.0001	0.0001
Enzyme		0.6819	0.0002	0.0754	0.0098
Interactions		0.0900	0.0044	0.1188	0.0346

[abc]Means with different superscripts within each section have significant difference.

(P = 0.0001) lower than the control and germinated barley groups in finishing period and entire trial (1-42 d) (Table 3).

Table 4 shows feed intake of broilers received unprocessed and processed barley with or without enzyme supplementation. Enzyme supplementation did not significantly change feed intake of broilers during feeding trials. The interaction between barley processing and enzyme supplementation was insignificant during all feeding phases (Table 4). Feed intake results followed a similar trend as body weight gain. In starting period, chicks on the control diet consumed significantly less feed compared to those on treatment groups. Chicks fed with roasted barley significantly consumed less feed than those fed with germinated barley in growing and finishing periods as well as entire trial. Chicks received roasted barley had even lower feed intake than their counterparts on the control group during finishing period and throughout the experiment.

The interaction effect between barley processing and enzyme supplementation for FCR was significant in starting and growing periods (Table 5). This significant interaction could be seen as a greater improvement in FCR by the enzyme supplement in untreated barley than in processed barley groups. In starting and growing phases, birds fed with roasted barley showed a significant increase in FCR compared to other groups. In finishing phase ($P = 0.0133$) well as the entire study ($P = 0.0050$), broilers received germinated barley had the best FCR, which was significantly lower than other experimental groups (Table 5). Enzyme supplementation of barley-based diets significantly improved FCR in all feeding phases of the experiment ($P < 0.01$).

Table 4. The effect of barley processing and enzyme supplementation on feed intake of broiler chickens.

Main effect		Feed intake (g/bird)			
Barley processing	Enzyme	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Total (1-42 d)
Control		136[b]	783[b]	2377[b]	3306[b]
Roasted		166[a]	798[b]	2099[c]	3062[c]
Germinated		173[a]	830[a]	2530[a]	3550[a]
SEM		3.14	14.82	32.98	40.65
Enzyme					
	-	156	794	2320	3295
	+	161	814	2351	3317
SEM		2.76	12.10	32.98	33.19
	Interactions				
Control	-	133	748	2358	3328
Control	+	138	819	2396	3284
Roasted	-	162	800	2055	3015
Roasted	+	170	796	2143	3109
Germinated	-	172	824	2514	3542
Germinated	+	174	837	2546	3558
P-value					
Barley Processing		0.0001	0.0903	0.0001	0.0001
Enzyme		0.1676	0.2359	0.4224	0.6454
Interactions		0.8223	0.1231	0.4421	0.4966

[abc]Means with different superscripts within each section have significant difference.

Table 5. The effect of barley processing and enzyme supplementation on feed conversion ratio of broiler chickens.

Main effect		Feed conversion ratio			
Barley processing	Enzyme	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)	Total (1-42 d)
Control		1.61[b]	1.70[b]	1.86[a]	1.81[a]
Roasted		1.66[a]	1.76[a]	1.88[a]	1.84[a]
Germinated		1.58[b]	1.68[b]	1.79[b]	1.76[b]
SEM		0.017	0.013	0.021	0.015
Enzyme					
	-	1.65[a]	1.77[a]	1.89[a]	1.84[a]
	+	1.58[b]	1.66[b]	1.81[b]	1.76[b]
SEM		0.014	0.011	0.017	0.012
	Interactions				
Control	-	1.71[a]	1.79[a]	1.93	1.88
Control	+	1.52[c]	1.61[b]	1.80	1.75
Roasted	-	1.67[ab]	1.81[a]	1.91	1.87
Roasted	+	1.65[ab]	1.71[ab]	1.87	1.81
Germinated	-	1.60[b]	1.71[ab]	1.82	1.79
Germinated	+	1.56[bc]	1.65[b]	1.76	1.74
P-value					
Barley Processing		0.012	0.0008	0.0133	0.0050
Enzyme		0.001	0.0001	0.0037	0.0001
Interactions		0.001	0.0098	0.304	0.1013

[abc]Means with different superscripts within each section have significant difference.

The effects of barley processing and enzyme addition on the morphometry of jejunum as well as jejunal viscosity are presented in Table 6. The interaction effect between barley processing and enzyme addition was significant on crypt depth ($P = 0.0162$) and the viscosity ($P = 0.0001$) (Table 6). Chickens fed with germinated barley had a higher length of jejunal villi whereas they had a lower crypt depth compared to other groups. As a result, the ratio of villus length to crypt depth ($P = 0.0001$) as well as villus absorptive surface ($P = 0.0118$) were significantly higher in birds received germinated barley than those fed with unprocessed or roasted barley. Barley processing resulted in a lower viscosity of digesta in the jejunum so that both processing methods had a significantly ($P = 0.0001$) lower viscosity compared to unprocessed barley. Moreover, there was a significant ($P = 0.0001$) difference between barley processing methods with respect to jejunal viscosity so that germinated barley showed a lower viscosity that roasted group. Enzyme addition had a significant effect on crypt depth ($P = 0.0041$), the villus length to crypt depth ($P = 0.0008$), and viscosity ($P = 0.0001$). The viscosity of jejunal digesta was significantly reduced by enzyme supplementation to barley-based diets.

Barley processing did not cause any significant difference in circulatory levels of heterophiles (H), lymphocytes (L), H/L ratio, ALT, MDA, and NO. However, birds received unprocessed barley showed a significantly lower total cholesterol ($P = 0.0370$). and LDL-cholesterol ($P < 0.01$) than those received processed barley.

Table 6. The effect of barley processing and enzyme supplementation on intestinal morphometry and viscosity of jejunum in broiler chickens (42 days of age).

Main effect		Variables					
Barley processing	Enzyme	Villus length (VL;μm)	Villus width (VW;μm)	Crypt depth (CD;μm)	VL/CD	Villus absorptive area (mm^2)	Viscosity (cP)
Control		605.82[b]	351.28	116.55[a]	5.20[b]	0.66[b]	5.72[a]
Roasted		590.80[b]	369.71	119.61[a]	4.95[b]	0.68[b]	4.59[b]
Germinated		691.22[a]	372.70	110.29[b]	6.40[a]	0.812[a]	3.49[c]
SEM		15.66	15.25	2.434	0.16	0.036	0.11
Enzyme							
	-	615.3	377.9	119.7[a]	5.17[b]	0.729	5.17[a]
	+	643.2	351.2	111.3[b]	5.86[a]	0.713	4.02[b]
SEM		12.79	12.45	1.988	0.13	0.029	0.09
	Interactions						
Control	-	591.0	365.6	119.2[a]	4.96	0.676	6.72[a]
Control	+	620.6	336.9	113.8[ab]	5.45	0.657	4.71[b]
Roasted	-	565.6	380.1	119.6[a]	4.75	0.670	5.18[ab]
Roasted	+	615.9	359.3	119.6[a]	5.16	0.703	4.01[bc]
Germinated	-	689.3	388.1	120.3[a]	5.82	0.840	3.63[c]
Germinated	+	693.1	357.2	100.2[b]	6.98	0.781	3.35[c]
P-value							
Barley Processing		0.0001	0.5640	0.0283	0.0001	0.0118	0.0001
Enzyme		0.1283	0.1339	0.0041	0.0008	0.7010	0.0001
Interactions		0.5794	0.9703	0.0162	0.2275	0.6468	0.0001

[abc]Means with different superscripts within each section have significant difference.

Plasma uric acid ($P = 0.0350$) and total protein concentrations ($P = 0.0050$) were significantly increased in birds fed with germinated barley as opposed to other experimental treatments. Enzyme supplementation did not make any significant changes to blood and plasma variables. The interaction effect of barley processing and enzymes was significant for H, L, and H/L (Table 7).

Table 8 indicates the effects of barley processing and enzyme supplementation on carcass characteristics. The highest breast yield was observed in germinated barley treatment ($P = 0.0186$). Relative weight of heart ($P = 0.0115$) and the right to the total ventricles weight ratio ($P = 0.0001$) were the lowest in germinated barley treatment. Chicks fed with germinated barley deposited more fat in the abdominal than other treatments ($P = 0.0001$).

The effect of enzyme supplementation was only significant for breast yield so that it improved this trait compared to unsupplemented barley diets. The interaction of barley processing and enzyme supplementation on carcass traits were not significant.

The rate of mortality from ascites was significantly lower in broilers received germinated barley than those fed with untreated barley. When enzyme was supplemented to these diets, no significant difference was observed (Fig. 1).

DISCUSSION

Performance criteria measured in the present study were generally inferior to the Ross performance objectives. The reason lies behind the poor performance was high altitude (2100 m) of the location where the study was carried out. Research has shown that commercial broiler strains hardly gain performance objectives when raised at high altitudes (Khajali, 2022).

The higher weight gain of birds fed with germinated barley compared to those on untreated or roasted barley was associated with a significant increase in feed intake. Germination causes several physiological changes in barley grain including the synthesis and release of the gibberellins from the scutellum. The gibberellins act on DNA in the aleurone layer and facilitate the transcription of several genes encoding hydrolyzing enzymes including amylase and phytase (Habschied et al., 2020; Sung et al., 2005). Amylase breaks starch into sugars, which are used by embryo (Jamar et al., 2011). The breakage of starch granules and the degradation of NSPs by germination of barley result in improved feed intake and subsequent growth of broiler chickens (MacGregor et al., 1994). The main part of barley NSPs consist of mixed $\beta(1\rightarrow 4)$ and $\beta(1\rightarrow 3)$ glucans, which are water-soluble (Sinha et al., 2011). These polysaccharides form highly viscous aqueous solutions in the gut, which enhance the viscosity of digesta and impact nutrient assimilation. These changes in the gut environment are linked to reduced weight gain. Germination of barley breaks water-soluble NSPs and improved growth performance in birds fed germinated barley was

Table 7. The effect of barley processing and enzyme supplementation on blood and plasma variables of broiler.

Treatments		Variables									
Barley processing	Enzyme	ALT (mg/dl)	LDL-c (mg/dl)	HDL-c (mg/dl)	H (%)	L (%)	H/L	Chol (mg/dl)	NO (μMol)	MDA (μMol)	TP)g/dl(	UA (mg/dl)
Control		29.8	27.6[b]	95.8[c]	22.9	75.3	0.31	154.6[b]	7.77	4.33	3.40[b]	4.42[b]
Roasted		30.6	32.3[a]	105.2[a]	20.9	75.1	0.28	178.8[a]	7.21	4.07	3.31[b]	4.25[b]
Germinated		33.8	33.2[a]	95.8[b]	20.4	76.2	0.26	168. 9[b]	7.29	4.59	3.63[a]	4.76[a]
SEM		1.69	1.66	2.33	1.03	1.15	0.09	6.39	0.58	0.31	0.87	0.107
Enzyme												
	-	50.56	50.56	107.07	22.16	74.60	0.30	172.4	7.63	4.20	3.46	4.55
	+	32.37	32.37	106.29	20.65	76.48	0.27	162.8	7.22	4.40	3.44	4.40
SEM		1.383	1.361	1.950	0.84	0.94	0.015	5.22	0.48	0.25	0.07	0.87
Interactions												
Control	-	28.5	29.7	95.67	25.0	79.11	0.35	155.7	7.77	4.57	3.45	4.53
Control	+	31.3	25.2	95.88	17.0	71.80	0.25	153.7	7.76	4.10	3.36	4.32
Roasted	-	27.5	31.8	102.53	25.0	73.80	0.34	184.7	6.72	4.12	3.18	4.05
Roasted	+	33.7	32.9	107.90	21.0	76.40	0.28	172.9	7.58	4.02	3.45	4.45
Germinated	-	35.7	34.0	119.62	20.4	76.80	0.27	178.9	7.06	4.38	3.68	4.64
Germinated	+	32.0	32.4	117.43	20.3	75.60	0.27	158.8	7.54	4.80	3.59	4.88
P-value												
Barley Processing		0.220	0.051	0.0001	0.183	0.769	0.240	0.037	0.773	0.501	0.035	0.005
Enzyme		0.376	0.405	0.675	0.207	0.169	0.183	0.180	0.538	0.475	0.766	0.239
Interactions		0.120	0.504	0.508	0.062	0.055	0.072	0.483	0.906	0.784	0.249	0.129

[abc]Means with different superscripts within each section have significant difference.ALT: alanine transferase; H: heterophils; L: lymphocytes; Chol: cholesterol; NO: nitric oxide; MDA: malondialdehyde; Pro: total protein; UA: uric acid.

Table 8. The effect of barley processing and enzyme supplementation on carcass characteristics of broiler chickens (42 days of age).

	Treatments				Carcass traits				
Barley processing	**Enzyme**	**Carcass yield (%)**	**Breast yield (%)**	**Heart (%)**	**RV/TV**	**Bursa (%)**	**Spleen (%)**	**Liver (%)**	Abdominal fat (%)
Control		64.3	22.6[a]	0.567[a]	0.24[a]	0.17[a]	0.14	2.07	1.58[a]
Roasted		63.0	21.5[b]	0.528[a]	0.18[b]	0.16[a]	0.13	2.19	1.04[b]
Germinated		65.2	23.1[a]	0.494[b]	0.17[b]	0.13[b]	0.12	2.12	1.02[b]
SEM		0.434	0.158	0.015	0.008	0.009	0.007	0.058	0.047
Enzyme									
	-	63.5	21.9[b]	0.544	0.207	0.16	0.13	2.12	1.20
	+	64.7	22.8[a]	0.515	0.191	0.15	0.13	2.13	1.22
SEM		0.289	0.106	0.018	0.008	0.009	0.007	0.055	0.045
	Interactions								
Control	-	64.2	22.4	0.590	0.227	0.163	0.140	2.16	1.47[ab]
Control	+	64.2	22.8	0.544	0.260	0.176	0.139	1.97	1.66[a]
Roasted	-	63.7	20.8	0.537	0.175	0.150	0.144	2.18	1.01[b]
Roasted	+	62.3	22.1	0.519	0.185	0.172	0.118	2.21	1.06[b]
Germinated	-	66.4	22.5	0.506	0.168	0.124	0.116	2.05	0.98[b]
Germinated	+	64.1	23.7	0.438	0.177	0.138	0.132	2.19	1.07[b]
P-value									
Barley processing		0.0631	0.0186	0.0115	0.0001	0.020	0.322	0.29	0.0001
Enzyme		0.1205	0.0403	0.1330	0.0969	0.158	0.661	0.92	0.6299
Interactions		0.3841	0.7110	0.8148	0.5614	0.939	0.127	0.13	0.0373

[ab]Means with different superscripts within each section have significant difference.RV/TV: right to total ventricular weight ratio.

expected. Dastar et al. (2014) demonstrated that germination of barley reduced crude fiber content of the grain from 5 to 2 %, which was associated with increased feed intake and improved weight gain. Similar results were reported by Svihus et al. (1997). In a similar study, Moses et al. (2024) germinated white sorghum to remove the anti-nutritional factors (i.e NSP and tannins). These researchers found a comparable performance between broiler chickens that fed with germinated white sorghum and the control group fed on a corn diet.

As mentioned earlier, several studies have reported that phytate can be effectively removed from cereal grains through germination (Sung et al., 2005; Purohit et al., 2023). As phytate exerts anti-nutritional activity by lowering the availability of many nutrients, its removal by germination process explains improved growth performance that was observed in the present study.

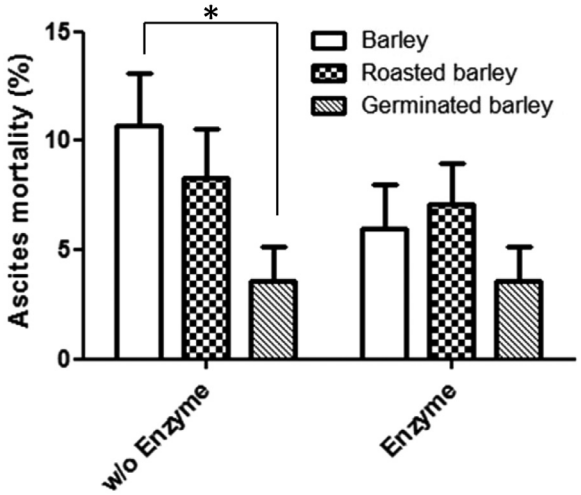

Fig. 1. Effects of barley processing and enzyme supplementation on the occurrence of ascites mortality ($P < 0.05$).

The explanation why roasted barley did not improve weight gain or even reduced growth performance far below the untreated barley group has yet to be determined. A look into the contents of NSPs in raw and roasted barley shows that NSPs were remarkably reduced compared to raw barley (138.5 vs 160 mg/g). This extent of reduction was comparable to germinated barley (140 mg/g). Although NSPs have remarkably reduced by roasting process of barley, chicks did not show willingness to consume it. The lack of disposition of birds in consuming roasted barley may be linked to the dramatic change in its color. In the CIELAB color space, L*, a*, and b* values were determined to be 53.66, 8.55, 25.78 for untreated barley, 44.33, 11.80, 24.49 for roasted barley, and 52.28, 9.09, 26.36 for germinated barley, respectively. In roasted barley, L*, the lightness, was considerably reduced but a*, the redness, was remarkably increased. It is likely that the dark color of diet containing roasted barley may have impacted feed intake of birds, which resulted in poor performance. In line with this finding, Falah et al. (2016) fed broiler chickens with untreated and fermented soybean meal. They reported that broilers on fermented soybean group consumed significantly less feed relative to the control (136 *vs.* 150 g/d) and they blamed this observation on the darkness of fermented product. There are a few reports in the literature that studied the influence of diet's color on poultry performance. Leslie et al (1973) reported that broiler chickens preferred a non-colored feed over a colored feed, when they were given a choice. Gulizia and Downs (2021) evaluated the effects of a starter feed color on broiler performance and they found that weight gain of chicks was decreased when the redness of the feed was increased (467 *vs* 474 g/chick in the control). However, more research needs to be done to address such hypothesis.

Improved FCR of broilers fed germinated barley over other treatments is linked to a better nutrient

utilization. As previously discussed, the germination of barley breaks key anti-nutritional factors in the grain (i. e NSPs and phytic acid). The degradation of anti-nutritional factors is the reason for a significant improvement in FCR as a result of enzyme addition in different feeding phases of this experiment. Enzyme supplementation improved FCR without any significant change in feed consumption. It has been reported that NSPs enhance mucin secretion and endogenous amino acid loss, and the use of NSP-degrading enzymes could improve FCR by offsetting the situation without compromising feed intake (Morel et al., 2003). This finding is in agreement with previous reports (Zhu et a., 2014).

Chickens fed with germinated barley had the highest length of villi and the lowest crypt depth in jejunum segment of the gut. This finding was in parallel with the lowest digesta viscosity in germinated barley group. Enzyme supplementation caused a significant reduction in jejunal viscosity. In accord with these findings, Dänicke et al. (2009) indicated that an increased length of jejunal villus as a result of NSPase supplementation was paralleled by a decrease in jejunal viscosity. Svihus et al. (1997) reported that chickens fed on enzyme−treated or germinated barley had intestinal contents with a lower viscosity than chickens fed on unprocessed barley. In the chicken, jejunum is the place where most digestive functions take place. Feed assimilation in jejunum is a rapid process taking place within 40 to 60 min (Slawinska et al., 2019). Therefore, a longer villus length is translated to a higher absorptive surface area and a higher rate for nutrient absorption. These findings suggest the higher performance of birds fed with germinated barley compared to those fed with unprocessed barley. Findings of the present study are also in line with those reported by Afsharmanesh et al. (2012). These authors reported that the crypt depth in jejunal segment was significantly lower in birds fed with sprouted barley compared an unprocessed barley. Villus length, crypt depth and the villus length/crypt depth ratio are used as biomarkers for evaluating the intestinal health status in the chicken (Ducatelle et al., 2018). A higher content of NSPs in untreated barley has caused the loss of villus epithelial cells, which resulted in a shorter villus length. Increased proliferation of these cells resulting in an increased crypt depth is a physiological response to compensate such situation (Tehrani et al., 2024; Afifian et al., 2024).

Feeding unprocessed barley was related to reduced lipogenesis as reflected in a significant decline in the levels of total and LDL-cholesterol and a lower accumulation of fat in the abdomen when compared to germinated barley. These findings are related to higher contents of dietary fiber and NSPs in unprocessed barley as opposed to germinated barley. Dastar et al. (2014) demonstrated that germination of barley reduced crude fiber content of the grain from 5 to 2 %. Dietary fiber suppresses lipogenesis through an increase in biliary clearance or a decreased feed intake (Brockman et al., 2014).

The proportion of heart weight relative to live body weight and the right ventricular weight ratio (RV/TV) showed a significant decrease in broilers that consumed germinated barley as opposed to their counterparts fed with untreated barley. The occurrence of ascites mortality was significantly higher in birds on the control (untreated barley) than those consumed germinated barley. RV/TV is an indicator of pulmonary hypertension and it is highly correlated with the mortality from ascites in broiler chickens (Khajali, et al., 2011), and this is what we observed in the present study. There are several reasons as to how germinated barley could prevent ascites mortality. First, birds in the germinated barley group had a higher concentration of plasma uric acid. This observation explains the removal of anti-nutritional effects of barley (NSPs and phytic acid), which resulted in improved utilization of nutrients including amino acids. Consequently, more uric acid has been produced as a result of greater amounts of amino acids that are metabolized. Uric acid is a potent antioxidant and a decline in its circulatory level has been shown to be linked to ascites syndrome (Behrooj et al., 2012). Therefore, a higher level of uric acid in germinated barley group could be linked to a lower rate in ascites mortality. Second, a lower depth of jejunal crypts in the germinated barley group compared to the unprocessed barley group could imply a decrease in the turnover of enterocytes, which is associated with a decrease in oxygen consumption by the gut and it explains a lower rate of ascites mortality.

CONCLUSION

Barley processing may have a diverse effect on broiler growth performance. While germinated barley could significantly improve the performance, roasted barley severely affected performance criteria of broiler chickens. Barley processing could influence the right ventricular hypertrophy and the occurrence of ascites in broilers particularly when no exogenous enzymes included in the diet.

ACKNOWLEDGEMENTS

The authors would like to appreciate Behmalt Zagros Ltd. For the provision of unprocessed and processed barley for this research. The authors would appreciate Dr. Bogdan A. Slominski from the University of Manitoba, Winnipeg, Canada, for his assistance in analyzing non-starch polysaccharide contents of barley samples.

CONFLICT OF INTERESTS

The authors declare to have no conflict of interests.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2024.104410.

REFERENCES

Afifian, A., H. Hassanpour, M. A. Karimi Torshizi, M. R. Akbari, and F. Khajali. 2024. Growth performance and gut function of broiler chickens received barley-based diets with or without an enzyme mixture. J. Poult. Sci. Avian Dis. 2:66–72. doi.org/10.61838/kman.jpsad.2.3.6.

Annison, G., and M. Choct. 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. World Poult. Sci. J. 47:232–242.

Behrooj, N., F. Khajali, and H. Hassanpour. 2012. Feeding reduced-protein diets to broilers subjected to hypobaric hypoxia is associated with the development of pulmonary hypertension syndrome. Brit. Poult. Sci. 53:658–664, doi:10.1080/00071668.2012.727082.

Brockman, D. A., X. Chen, and D. D. Gallaher. 2014. High-viscosity dietary fibers reduce adiposity and decrease hepatic steatosis in rats fed a high-fat diet. J. Nutr. 144:1415–1422. doi.org/10.3945/jn.114.191577.

Chae, B. J., J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, and T. W. Hahn. 2006. Effects of supplementation of β-glucan on the growth performance and immunity in broilers. Res. Vet. Sci. 80:291–298. doi.org/10.1016/j.rvsc.2005.07.008.

Daneshmand, A., A. Kumar, S. K. Kheravii, G. A. M. Pasquali, and S. B. Wu. 2023. Xylanase and beta-glucanase improve performance parameters and footpad dermatitis and modulate intestinal microbiota in broilers under an Eimeria challenge. Poult. Sci. 102:103055. doi.org/10.1016/j.psj.2023.103055.

Dänicke, S., E. Moors, A. Beineke, and M. Gauly. 2009. Ascaridia galli infection of pullets and intestinal viscosity: consequences for nutrient retention and gut morphology. Brit. Poult. Sci. 50:512–520, doi:10.1080/00071660903124530.

Dastar, B., A. S. Moghaddam, M. S. Shargh, and S. Hassani. 2014. Effect of different levels of germinated barley on live performance and carcass traits in broiler chickens. Poult. Sci. J. 2:61–69, doi:10.22069/PSJ.2014.1653.

Ducatelle, R., E. Goossens, F. De Meyer, V. Eeckhaut, G. Antonissen, F. Haesebrouck, and F. Van Immerseel. 2018. Biomarkers for monitoring intestinal health in poultry: present status and future perspectives. Vet. Res. 49:1–9, doi:10.1186/s13567-018-0538-6.

Falah, M., B. Dastar, F. Ganji, and A. Ashayerizadeh. 2016. Effects of fermented soybean meal and dietary protein level on performance and gasterointestinal microbial population in broiler chickens. Res. J. Livestock Sci. 28:53–66. (in Persian with English abstract) doi.org/10.22092/ASJ.2016.106085.

Gulizia, J. P., and K. M. Downs. 2021. The effects of feed color on broiler performance between day 1 and 21. Animals 11:1511. doi.org/10.3390/ani11061511.

Guo, Y., R. A. Ali, and M. A. Qureshi. 2003. The influence of β-glucan on immune responses in broiler chicks. Immunopharmacol. Immunotoxicol. 25:461–472, doi:10.1081/IPH-120024513.

Habschied, K., A. Lalić, D. Horvat, K. Mastanjević, J. Lukinac, M. Jukić, and V. Krstanović. 2020. β-glucan degradation during malting of different purpose barley varieties. Fermentation 6:21.

Jacob, J. P., and A. J. Pescatore. 2014. Barley β-glucan in poultry diets. Ann. Transl. Med. 2:20, doi:10.3978/j.issn.2305-5839.2014.01.02.

Jamar, C., P. du Jardin, and M. L. Fauconnier. 2011. Cell wall polysaccharides hydrolysis of malting barley (Hordeum vulgare L.): a review. Biotechnol. Agronomy Soc. Environ. 15:301–313.

Khajali, F. 2022. Managing broiler production challenges at high altitude. Vet. Med. Sci. 8:1519–1527, doi:10.1002/vms3.784.

Khajali, F., M. Tahmasebi, H. Hassanpour, M. R. Akbari, D. Qujeq, and R. F. Wideman. 2011. Effects of supplementation of canola meal-based diets with arginine on performance, plasma nitric oxide, and carcass characteristics of broiler chickens grown at high altitude. Poult. Sci. 90:2287–2294. doi.org/10.3382/ps.2011-01618.

Khajali, F., and F. Rafiei. 2024. A review of plant anti-nutritional factors in animal health and production: the classification, biological properties, and the passivation strategy. J. Agric. Food Res. 18:101290.

MacGregor, A. W., L. G. Dushnicky, S. W. Schroeder, and G. M. Balance. 1994. Changes in barley endosperms during early stages of germination. J. Inst. Brew. 100:85–90.

McNab, J. M., and R. R. Smithard. 1992. Barley β-glucan: An antinutritional factor in poultry feeding. Nutr. Res. Rev. 5:45–60, doi:10.1079/NRR19920006.

Mitsou, E. K., N. Panopoulou, K. Turunen, V. Spiliotis, and A. Kyriacou. 2010. Prebiotic potential of barley derived β-glucan at low intake levels: a randomised, double-blinded, placebo-controlled clinical study. Food Res. Int. 43:1086–1092.

Morel, P. C. H., R. M. Padilla, and G. Ravindran. 2003. Effect of non-starch polysaccharides on mucin secretion and endogenous amino acid losses in pigs. Asian-Australas. J. Anim. Sci. 16:1332–1338.

Moses, C., F. Manyeula, M. V. Radikara, M. H. D. Mareko, and O. R. Madibela. 2024. Malted sorghum as a maize substitute in broiler diets: effect on feed utilisation, growth performance and haemo-biochemical parameters. Ital. J. Anim. Sci. 23:416–425. doi.org/10.1080/1828051X.2024.2326307.

Papa Georgiou, M., N. Lakhdara, A. Lazaridou, C. G. Biliaderis, and M. S. Izydorczyk. 2005. Water extractable (1→ 3, 1→ 4)-β-D-glucans from barley and oats: An intervarietal study on their structural features and rheological behaviour. J. Cereal Sci. 42:213–224. doi.org/10.1016/j.jcs.2005.03.002.

Perera, W. N. U., M. R. Abdollahi, F. Zaefarian, T. J. Wester, and V. Ravindran. 2022. Barley, an undervalued cereal for poultry diets: limitations and opportunities. Animals 12:2525. doi.org/10.3390/ani12192525.

Purohit, P., H. Rawat, N. Verma, S. Mishra, A. Nautiyal, S. Bhatt, and A. K. Gupta. 2023. Analytical approach to assess anti-nutritional factors of grains and oilseeds: a comprehensive review. J. Agric. Food Res. 100877. doi: 10.1016/j.jafr.2023.100877

Sinha, A. K., V. Kumar, H. P. Makkar, G. G. De Boeck, and K. Becker. 2011. Non-starch polysaccharides and their role in fish nutrition. Food Chem. 127:1409–1426, doi:10.1016/j.foodchem.2011.02.042.

Slawinska, A., A. Dunislawska, A. Plowiec, M. Radomska, J. Lachmanska, M. Siwek, and G. Maiorano. 2019. Modulation of microbial communities and mucosal gene expression in chicken intestines after galactooligosaccharides delivery in ovo. PLoS One 14:1–23, doi:10.1371/journal.pone.0212318.

Sung, H. G., H. T. Shin, J. K. Ha, H. L. Lai, K. J. Cheng, and J. H. Lee. 2005. Effect of germination temperature on characteristics of phytase production from barley. Bioresour. Technol. 96:1297–1303. doi.org/10.1016/j.biortech.2004.10.010.

Svihus, B., R. K. Newman, and C. W. Newman. 1997. Effect of soaking, germination, and enzyme treatment of whole barley on nutritional value and digestive tract parameters of broiler chickens. Br. Poult. Sci. 38:390–396, doi:10.1080/00071669708418008.

Tehrani, A. A., F. Khajali, M. A. Karimi Torshizi, and M. R. Akbari. 2024. Does the type of cereal grain in broiler diets affect the susceptibility to ascites syndrome? Poul. Sci. J. 12:35–41. doi.org/10.22069/PSJ.2023.21428.1944.

Vollmar, B., and M. D. Menger. 2009. The hepatic microcirculation: mechanistic contributions and therapeutic targets in liver injury and repair. Physiol. Rev. 89:1269–1339, doi:10.1152/physrev.00027.2008.

Wang, H. L., M. Shi, X. Xu, L. Pan, L. Liu, and X. S. Piao. 2017. Partial dehulling increases the energy content and nutrient digestibility of barley in growing pigs. Asian-Australas. J. Anim. Sci. 30:562–568. doi.org/10.5713/ajas.16.0429.

Zhu, H. L., L. L. Hu, Y. Q. Hou, J. Zhang, and B. Y. Ding. 2014. The effects of enzyme supplementation on performance and digestive parameters of broilers fed corn-soybean diets. Poult. Sci. 93:1704–1712. doi.org/10.3382/ps.2013-03626.