Effects of dietary physical form and dietary inclusion of probiotic and enzyme on growth performance, cellular and humoral immunity, and relative weights of lymphoid organs at early period of broiler chickens fed triticale-based diets

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Abstract

The aim of the present study was to investigate interactions between feed form and dietary inclusion of probiotics (live organisms) and enzymes on growth performance, cellular and humoral immunity, and relative weights of lymphoid organs of broiler chicks fed a triticale-based diet. A total of 640 broiler chicks were allocated to eight treatments when they were one day old, with four replicates (n = 20 chicks in each). The dietary treatments consisted of feed form (pellets or mash) and dietary supplementation with or without a *Bacillus*-based probiotic (0.03% diet) and enzyme (carbohydrase, 0.05% diet) in a 2 × 2 × 2 factorial arrangement. Feed conversion ratio (FCR) was calculated based on feed intake (FI) and weight gain (WG). The WG of the broiler chicks fed the pelleted diets containing the enzyme and probiotic (alone or in combination) was greater than that of the birds fed the mash diets without these additives. Significant interaction effects of the enzyme and probiotic on FCR and FI were observed in the starter period. The heterophil : lymphocyte (H : L) ratio of the chicks fed the pelleted diet containing the enzyme and probiotic was lower than that of the birds fed the other diets. The cellular immunity of the birds fed this diet increased after 24 hours, as shown by the results of a challenge experiment with dinitrochlorobenzene (DNCB) or an injection of phytohemagglutinin (PHA). In conclusion, feed form and supplementation of a triticale-based diet with feed enzymes and probiotics can greatly improve the growth performance and immunity of broiler chicks.

Keywords: Dinitrochlorobenzene, Di-Pro probiotic, phytohemagglutinin, Rovabio enzyme, spleen, viscosity
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Introduction

It is generally accepted that compared with mash, feeding pellets to broilers has a number of advantages, such as increasing feed consumption, decreasing feed wastage and maintenance energy, improving feed hygiene, eliminating antinutritional factors, and improving poultry performance (Shalmany & Shivazad, 2007; Shariatmadari & Mohiti-Asli, 2009; Shabani *et al.*, 2015). Although a number of studies have reported that feeding broilers a pellet-based diet improves growth performance, some research has shown that it has negative effects on gastrointestinal development and physiology (Nir *et al*., 1994; Engberg *et al.*, 2002; Amerah *et al.*, 2007; Abdollahi *et al.*, 2011). According to the literature, these effects may be partly related to grain type. In one study, in birds fed a maize-based diet, the feed form of pellets had no effect on nitrogen and starch digestibility, but significantly improved the ileal digestibility of various nutrients, including fats, calcium (Ca), and phosphorus (P). In contrast, in broilers fed a wheat-based diet, pellet feeding had negative effects on the digestibility of all nutrients (Abdollahi *et al*., 2013). Cowieson *et al*. (2005) suggested that the viscosity properties of soluble fibres changed during processing, with the dietary viscosity of pelleted wheat-based diets being higher than that of mash diets, with no added xylanase.

Triticale can be used as an alternative to corn in a poultry diet, as it contains high amounts of protein and essential amino acids, such as lysine and methionine, in addition to Ca and P (Nourollahi *et al*., 2014). However, a limiting factor in the use of triticale is its antinutritional effect in solution on nonstarch polysaccharides (NSPs) (Beski & Al-Sardary, 2015). As a result, triticale could increase intestinal viscosity and the population of harmful microbes and reduce nutrient availability (Beski & Al-Sardary, 2015). Dietary

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supplementation with probiotics could overcome these adverse effects by increasing the population of beneficial microbes. In addition, probiotics have been shown to have positive effects on digestive enzymes, the immune system, and productivity (Anyaehie & Irole, 2008; Vicente *et al*., 2008). Previous studies have shown that dietary supplementation with exogenous enzymes decomposed arabinoxylans into low-molecular weight substances and lowered the viscosity of the intestinal contents, thereby improving digestion and absorption (Mireles-Arriaga *et al*., 2015; Pirgozliev *et al*., 2015). A combination of exogenous enzymes and probiotics has long been used as an alternative to subtherapeutic doses of antibiotics to improve broiler productivity and immunity (Talebi *et al*., 2008).

Given the limitations of dietary inclusion of triticale, the beneficial effects of enzymes in improving nutritive feed values, and the role of probiotics in improving intestinal conditions, the present study investigated the effects of feed form and dietary inclusion of a probiotic and enzyme on the growth performance, immune system, and relative weight of lymphoid organs in broiler chicks fed a triticale-based diet.

Materials and methods

The study consisted of 640 one-day-old Ross 308 mixed-sex broiler chicks, with an average bodyweight of 44 ± 2 g. The birds were housed in a hall under a 23-hour light, 1-hour dark regime, with unlimited access to feed and water. The temperature at the start of the trial was 33 °C. Over the following 23 days, it was reduced progressively to 24 °C.

The birds were divided into eight treatment groups, with four replicates (n = 20 in each replicate). All the experimental diets were triticale based. These diets and nutritional requirements were adjusted according to the Ross 308 catalogue (Table 1). First, a, basal diet was prepared. Then, 0.03% Di-Pro probiotic (Tak Gen Zist Company, Iran), containing 1.6×10^9 CFU/g *Bacillus subtilis* and *B. licheniformis* (heat stability until 82 °C), and 0.05% Rovabio® enzyme (Adiseo Company, France), containing 200 IU of xylanase and 200 IU of β-glucanase (heat stability until 85 °C), were added. Half of the diets were prepared in mash form, and the other half in pellet form. The pelleting process was performed at a temperature of 78 °C, and the pellets were 2 and 3 mm in diameter in the starter and grower diets, respectively.

The compositions of the prepared diets were as follows: mash form with no probiotic or enzyme; pellet form with no probiotic or enzyme; mash form with 500 g enzyme/per ton; pellet form with 500 enzyme/per ton; mash form with 300 g probiotic/per ton; pellet form with 300 g probiotic/per ton diet; mash form with 300 g probiotic and 500 g enzyme/per ton; and pellet form containing 300 g probiotic and 500 g enzyme/per ton. FCR of starter (0–10 days) and grower (11–24 days) chicks was calculated based on FI and WG.

Blood samples were collected from two birds from each cage at 14 days and 21 days for analysis of Newcastle disease virus and H : L ratio, respectively. Blood samples were kept at room temperature for two hours, and then centrifuged at 3000 rpm for 15 min. To determine skin hypersensitivity reaction, DNCB was applied to a 10 cm^2 area of skin at a dose of 0.25 ml per cm^2. (Skin thickness had been measured prior to the application of DNCB.) After 14 days, the birds were challenged again with 0.25 ml DNC. Skin thickness (three sections in each area) was measured after 24 and 48 hours. In addition, an intradermal injection of 0.01 ml : 10 $mg.mL^{-1}$ acetone (0.01 ml) and olive oil (10 $mg.mL^{-1}$) in a 4 : 1 ratio was administered between the third and fourth digits of the right foot, and skin thickness was measured 24 and 48 hours later. When the chicks were 21 days old, the H : L ratio was determined after staining by Wright's method.

When the birds were seven days old, they received one dose of Newcastle vaccine, type B, in intraocular form, and blood was obtained from all the birds seven days later. The haemagglutination inhibition method was used to detect antibody titers against Newcastle disease virus.

At the end of the trial, two birds per unit (eight birds per treatment), with weight close to the mean, were selected, weighed, and killed. The spleen, bursa of Fabricius, and liver were collected and weighed separately. The relative weights of the organs were expressed as percentages of live BW.

The parameters were investigated using a 2 × 2 × 2 factorial arrangement based on a randomized complete block design. The data were analysed with SAS software. Duncan's multiple range test was used to detect differences (P <0.05) among the groups. All the parameters were analysed according to this formula:

$$Y_{ijklm} = \mu + R_i + A_j + B_k + C_l + (A*B)_{jk} + (A*Cl)\ jl + (B*C)_{k\,l} + (A*B*C)_{jkl} + e_{ijklm,}$$

Where: Y_{ijklm} was the measured characteristic

μ was the overall mean

R_i was the block effect (Hall length)

A_j was the main effect of the probiotic

B_k was the main effect of the enzyme

C_l was the main effect of the feed form
$(A*B)_{jk}$ was the interaction between the probiotic and enzyme
$(A*Cl)\ jl$ was the interaction between the probiotic and feed form
$(B*C)_{k\,l}$ was the interaction between the enzyme and feed form
$(A*B*C)_{jkl}$ was the interaction among the probiotic, enzyme, and feed form
e_{ijklm} was the residual error

Table 1 Ingredients and composition of basal diets for broiler chicks

Ingredients (g/kg)	Starter (0–10 days)	Grower (11–24 days)
Triticale	587	630.8
Soybean meal	299	254.6
Corn gluten	50	50.0
Soybean oil	15	20.0
Limestone	10.3	9.1
Di-calcium phosphate	20.0	17.7
Salt	2.40	2.4
Bicarbonate sodium	2.0	2.0
Vitamin premix[a]	2.5	2.5
Mineral premix[b]	2.5	2.5
DL-Methionine	3.0	2.6
L-Lysine	4.5	4.3
L-Threonine	1.8	1.5
Nutrient composition(g/kg as fed)		
Metabolizable energy (MJ)	12.6	13.2
Crude protein	231.0	220.0
Calcium	10.5	9.0
Available phosphorus	5.0	4.5
Methionine	5.2	4.5
Methionine + cysteine	9.1	8.4
Lysine	14.2	12.4
Threonine	9.3	8.3

[a]Vitamin premix supplied per kilogram diet: vitamin A, 9000 IU; vitamin D3, 2000 IU; vitamin E, 1800 IU; nicotinic acid, 30 mg; vitamin B_{12}, 0.015 mg; vitamin K3, 4 mg; biotin, 0.15 mg; folic acid, 1.0 mg; niacin, 30.0 mg; pantothenic acid, 25.0 mg; pyridoxine, 2.9 mg; riboflavin, 6.6 mg; thiamin, 1.18 mg
[b]Mineral premix supplied the following per kilogram of diet: manganese oxide, 100 mg; ferrous sulfate (FeSO4.7H2O), 50 mg; zinc oxide, 100 mg; copper sulfate, 10 mg; I, 1.0 mg; selenium (Se), 0.2 mg

Results

The data on growth performance are presented in Table 2. Feed form influenced WG in the starter, grower, and total periods (P <0.05). The addition of the probiotic influenced WG in the starter and total periods (P <0.05). In general, the WG of the broiler chicks fed the pelleted diets containing the enzyme and probiotic (alone or in combination) was greater than that of the birds fed the mash diet without these additives (P <0.05). Weight gain increased in the chicks fed the mash diet supplemented with both the enzyme and probiotic (P <0.05). The WG increased in the starter, grower, and total periods in the birds fed the pelleted diet (without additives) compared with birds fed the mash diet (P <0.05). The pelleted diets lowered FCR in the grower period and total period compared with the mash diets (P >0.05). Significant interaction effects of the enzyme and probiotic on FI and FCR were observed in the starter period (P <0.05). The birds fed the pelleted diet containing the probiotic had the lowest FCR, and those fed the mash diet without the added enzyme or probiotic in the starter period had the highest FCR (P <0.05). The FI of the broiler chicks fed the pelleted diet supplemented with the enzyme and probiotic was greater than that of the

birds fed the pelleted diet with enzyme only, showing that dietary supplementation with a probiotic increased the FI of broiler chicks in the starter period.

Table 2 Mean (± SE) relative interactions among the probiotic, enzyme, and feed form on growth performance of broiler chicks fed a triticale-based diet.

PF	E (%)	Pro (%)	WG(g)			FI(g)			FCR		
			Starter	Grower	Total	Starter	Grower	Total	Starter	Grower	Total
Mash	0	0	174.08^{e}	584.18^{c}	758.26^{c}	276.39^{cd}	985.23	1261.62	1.58^{d}	1.68^{c}	1.66^{c}
Mash	0.05	0	190.62^{d}	607.92^{bc}	798.54^{bc}	257.85^{e}	976.78	1234.63	1.35^{c}	1.61^{bc}	1.54^{bc}
Mash	0	0.03	191.29^{d}	617.47^{bc}	808.76^{bc}	258.78^{e}	985.76	1244.54	1.35^{c}	1.59^{bc}	1.53^{bc}
Mash	0.05	0.03	197.31^{cd}	646.88^{b}	844.20^{b}	274.66^{d}	990.77	1265.43	1.39^{c}	1.54^{ab}	1.50^{ab}
Pellet	0	0	210.39^{bc}	722.21^{a}	932.61^{a}	293.56^{b}	1097.27	1390.83	1.40^{c}	1.51^{ab}	1.49^{ab}
Pellet	0.05	0	222.04^{ab}	730.21^{a}	952.25^{a}	281.19^{cd}	1034.51	1315.70	1.26^{ab}	1.41^{ab}	1.38^{a}
Pellet	0	0.03	228.44^{a}	721.19^{a}	949.64^{a}	284.26^{bc}	1020.74	1305.00	1.24^{a}	1.41^{ab}	1.37^{a}
Pellet	0.05	0.03	234.57^{a}	711.92^{a}	946.49^{a}	313.25^{a}	988.71	1301.96	1.33^{bc}	1.39^{a}	1.37^{a}
SEM			3.95	11.41	5.88	3.20	15.40	14.96	0.01	0.027	0.023
***P*-value**			0.000	0.000	0.000	0.000	0.347	0.117	0.000	0.047	0.006
PF			***	***	***	***	NS	NS	***	***	***
E			***	NS	NS	NS	NS	NS	**	NS	NS
Pro			*	NS	*	*	NS	NS	**	NS	NS
PF × E			NS	NS	NS	*	NS	NS	NS	NS	NS
PF × Pro			NS	NS	NS	*	NS	NS	NS	NS	NS
E × Pro			NS	NS	NS	***	NS	NS	***	NS	NS
PF × E × Pro			NS	NS	NS	NS	NS	NS	NS	NS	NS

PF: physical form; E: enzyme; Pro: probiotic
Superscripts (a–e) show significant differences among groups per column
NS: non significant (P >0.05). *(P <0.05), **(P <0.01), ***(P <0.001)
SEM: standard error of means

The effects of interactions among the probiotics, enzymes, and feed form on cellular immunity after 24 and 48 hours in broiler chicks fed a triticale-based diet are presented in Table 3. The feed form and addition of probiotic or enzyme had no significant effects on the reaction to DNCB and PHA 48 hours after stimulation (P >0.05). However, the dietary inclusion of the probiotic and enzyme influenced the reaction to DNCB 24 hours after stimulation (P <0.05). With regard to PHA, significant interaction effects of the enzyme and probiotic were detected 24 hours after stimulation (P <0.05). The birds fed the pelleted and mash diets containing the probiotic/enzyme combination showed a greater reaction to DNCB and PHA than those fed the other diets (P <0.05). There were no significant differences in the reactions of the birds fed the mash and pelleted diets containing no added enzyme or probiotic (P >0.05).

Data on antibody titers against Newcastle disease virus, in addition to the H : L ratio and organ weights, are shown in Table 4. Significant interaction effects of the enzyme, probiotic, and feed form on the H : L ratio were observed (P <0.05). The birds fed the pelleted diets containing both the enzyme and probiotic had the lowest H : L ratio (P >0.05). Significant interaction effects of the enzyme and probiotic on antibody titers to Newcastle disease virus and relative splenic weights were also observed (P <0.05). Antibody titers to the virus were higher in broiler chicks fed the pelleted diets containing the enzyme and probiotic than those fed the mash diet containing the enzyme only. There were no significant differences in antibody titers to Newcastle disease virus between the pelleted diets without additives and the mash diets without additives (P >0.05). The relative splenic weights were higher in the mash diet without the additives than in the other diets (P <0.05). Neither the feed form nor dietary inclusion of the enzyme/probiotic had a significant influence on the relative weights of the bursa or liver (P >0.05).

Table 3 Mean (± SE) relative interactions among the probiotic, enzyme, and feed form on cellular immunity in broiler chicks fed a triticale-based diet

PF	E (%)	Pro (%)	DNCB		PHA	
			24 h	48 h	24 h	48 h
Mash	0	0	0.81[c]	0.22	0.83[b]	0.40
Mash	0.05	0	1.02[b]	0.34	0.83[b]	0.34
Mash	0	0.03	1.05[b]	0.29	0.95[b]	0.31
Mash	0.05	0.03	1.27[a]	0.38	1.26[a]	0.24
Pellet	0	0	0.87[c]	0.26	0.78[b]	0.29
Pellet	0.05	0	0.98[b]	0.29	0.96[b]	0.38
Pellet	0	0.03	1.01[b]	0.32	0.93[b]	0.31
Pellet	0.05	0.03	1.30[a]	0.30	1.38[a]	0.33
SEM			0.030	0.015	0.042	0.020
***P*-value**			0.000	0.221	0.000	0.652
PF			NS	NS	NS	NS
Pro			***	NS	***	NS
E			***	NS	***	NS
PF × E			NS	NS	NS	NS
PF × Pro			NS	NS	NS	NS
E × Pro			NS	NS	*	NS
PF × E × Pro			NS	NS	NS	NS

DNCB: dinitrochlorobenzene; PHA: phytohemagglutinin; PF: physical form; E: enzyme; Pro: probiotic
Superscripts (a–e) show significant differences among groups per column
NS: non significant (P >0.05). *(P <0.05), **(P <0.01), ***(P <0.001)
SEM: standard error of means

Discussion

The WG of the broiler chicks fed the pelleted diets supplemented with the enzyme and probiotic (alone or in combination) was greater than that of the birds fed the mash diets without these additives. Thus, both feed form and dietary supplementation with enzymes and probiotics appear to have significant effects on WG. The importance of feed form in FI and growth performance of poultry during the starter period is well known (Amerah *et al*., 2007). A previous study concluded that broilers fed pelleted diets had higher WG than those fed mash (Attia *et al*., 2014). The same study stated that the higher WG was associated with higher FI in broilers. Thus, increased WG seems to be the result of higher FI.

Thus far, no studies have investigated the effects of feed form and dietary inclusion of enzymes and probiotics in animals fed a triticale-based diet. As reported earlier, triticale has certain major limitations compared with corn, but these can be overcome by feed form and additives. A similar study to the present one concluded that compared with mash diets, a pelleted diet improved the growth performance of broiler chicks fed a sorghum-based diet by increasing FI (Abdollahi *et al*., 2014). In the study, the pelleted diet increased WG in the starter, grower, and total periods, compared with a mash diet. Compared with the mash diet, the pelleted diet lowered the FCR in the grower period and total period. In agreement with the results of the present study, Svihus *et al*. (2004) reported that pelleted diets increased FI and WG, in addition to lowering FCR, compared with mash diets. Another study demonstrated that pelleted diets increased nutrient density and nutrient intake, improved starch gelatinization, and lowered feed waste (Amerah *et al*., 2008). Research also confirmed that finely ground mash diets enhanced unification, whereas ground feed was less palatable than non-ground feed and did not retain its nutritive value (Jahan *et al*., 2006). Nir *et al*. (1994) showed that pelleted feed improved broiler growth rates compared with a mash diet. However, Ahmed & Abbas (2013) stated that low-quality pellets did not improve the growth performance of boilers from 21 to 42 days and that the quality of pelleted diets played a major role in the performance of broiler chicks.

Table 4 Mean (±SE) relative interactions among probiotic, enzyme and feed form on Heterophil:Lymphocyte ratio (21 days), antibody titers against Newcastle disease virus, and relative weights of lymphoid organs of broiler chicks fed a triticale-based diet

PF	E (%)	Pro (%)	H: L ratio	Newcastle titer	Liver (%)	Spleen (%)	Bursa (%)
Mash	0	0	0.127^{a}	5.25^{b}	2.44	0.195^{a}	0.187
Mash	0.05	0	0.092^{c}	4.25^{c}	2.68	0.120^{bc}	0.133
Mash	0	0.03	0.127^{a}	5.25^{b}	2.22	0.100^{cd}	0.122
Mash	0.05	0.03	0.122^{a}	6.00^{ab}	2.41	0.135^{ab}	0.102
Pellet	0	0	0.125^{a}	5.00^{bc}	2.32	0.101^{cd}	0.122
Pellet	0.05	0	0.127^{a}	5.25^{b}	2.47	0.095^{d}	0.109
Pellet	0	0.03	0.108^{b}	5.25^{b}	2.39	0.095^{d}	0.121
Pellet	0.05	0.03	0.057^{d}	6.75^{a}	2.26	0.110^{bc}	0.119
SEM			0.004	0.160	0.05	0.008	0.007
***P*-value**			0.000	0.002	0.432	0.041	0.084
PF			*	**	NS	*	NS
E			*	NS	NS	NS	NS
Pro			**	NS	NS	NS	NS
PF × E			***	NS	NS	NS	NS
PF × Pro			NS	*	NS	NS	NS
E × Pro			NS	***	NS	*	NS
PF × E × Pro			***	NS	NS	NS	NS

PF: physical form; E: enzyme; Pro: probiotic
Superscripts (a–d) show significant differences among groups per column
NS: non significant (P >0.05). *(P <0.05), **(P <0.01), ***(P <0.001)
SEM: standard error of means

In the present study, the addition of the enzyme only to the mash diet in the starter period increased WG in the grower and total periods, showing that dietary enzyme supplementation was superior to a mash diet at lower ages, whereas a pelleted diet with enzyme supplementation was superior at older ages. Latham *et al*. (2016) showed that dietary inclusion of β-mannanase in a reduced-energy pelleted diet lowered growth performance. Meng *et al*. (2005) reported that the addition of dietary enzymes enabled the utilization of NSPs (i.e., unused energy sources). In triticale, NSPs include xylans and arabinoxylans, which are decomposed by xylanase and arabinoxylanase, respectively (Zarghi *et al*., 2012). Thus, NSPs, as unused energy sources in triticale, can be utilized by the addition of dietary enzymes. In common with the findings of previous studies (Zarghi *et al*., 2012; Beski & Al-Sardary, 2015), the results of the present study showed that dietary supplementation with xylanase and arabinoxylanase removed the antinutritional effects of NSPs in triticale. In addition to increasing carbohydrate digestibility, dietary supplementation with enzymes can reduce digesta viscosity, resulting in improved absorption.

In the present study, broiler chicks fed the pelleted diet containing the enzyme and the probiotic consumed more food in the starter period and had lower FCR in the grower and total periods. There are conflicting reports on the ability of probiotic cultures to improve growth performance, with the discord potentially because of the composition of the culture, dose, and duration of use (Gaggìa *et al*., 2010; Rezaei *et al*., 2015). Saleh *et al*. (2012) stated that the probiotic *A. awamori* improved energy and protein digestibility in broilers. In another study, these authors showed that dietary supplementation with *A. awamori* and *S. cerevisiae* improved the growth performance of broilers synergistically by increasing muscle protein metabolism (Saleh *et al*., 2013).

No previous studies have investigated the effects of feed form and dietary supplementation with enzymes and probiotics in broilers. However, a combination of *Lactobacilli* and enzymes increased the efficacy of the digestion of various ingredients in feed (Chen *et al*., 2014). Researchers also reported that when *Lactobacilli* and enzymes were added to feed, they improved FCR, increased the release of essential nutrients, and decreased faecal losses (Wang & Gu, 2010). Based on the findings in the literature and those

of the current study, it seems that a pelleted diet supplemented with enzymes and probiotics produces the best responses, possibly owing to synergistic interactions among the diet, enzyme, and probiotic.

In the current study, the birds fed the pelleted and mash diets containing the probiotic and enzyme showed a more intense reaction to DNCB and PHA than the birds fed the other diets. The birds given the pelleted diet containing the enzyme and probiotic also had the lowest H: L ratio. Antibody titers to Newcastle disease virus were higher in the broiler chicks fed the pelleted diets containing the enzyme and probiotic compared with the birds fed the mash diet containing the enzyme only. The broilers chicks fed the pelleted diets containing both the enzyme and probiotic showed the best responses.

In the present study, feed form (pellets or mash) had no significant impact on the immune system of the chicks, as determined by the H : L ratio. A previous study suggested that a higher H : L ratio in chicks exposed to stress was owing to increased corticosterone levels, whereas a lower H : L ratio was associated with reduced corticosterone levels. Any factor that stimulated the immune system affected the H : L ratio (Vicente *et al*., 2008). Previous research demonstrated that dietary supplementation with the probiotic PrimaLac (Star-Labs, Missouri, USA) lowered the ceruloplasmin concentration and H : L ratio, and increased antibody titers against Gambro vaccine (Nayebpoor *et al*., 2010). Studies showed that probiotics enhanced the immune response against antigens (Teo & Tan, 2007) and that the immune response of birds fed diets containing a mix of probiotics and enzymes was satisfactory compared with that of a control group (Seidavi *et al*., 2017). Sadeghi *et al*. (2015) showed that dietary inclusion of a mixture of probiotics and enzymes had no significant effect on the immune responses of chickens, but that it improved the immune responses of birds challenged with a pathogen. Stringfellow *et al*. (2011) showed that a probiotic containing lactic acid had a positive effect on the immune response of broilers. An in vitro study demonstrated that a probiotic prevented the production of tumour necrosis factor-α and the translocation of nuclear factor β, which decreased the production of proinflammatory cytokines in human intestinal epithelial cells (Dubert-Ferrandon *et al*., 2008). Zou *et al*. (2006) reported that dietary inclusion of enzymes had immunological benefits, such as increasing relative immune organ weights, serum concentrations of IgM, and proliferation of T-lymphocytes. Furthermore, probiotics improved the immune system of broiler chicks by stimulating the lymphatic system and producing changes in the microbial population of the digestive system (Panda *et al*., 2006). Based on the findings of the present study and those in the literature, it seems that a pelleted diet, supplemented with both enzymes and probiotics, provides better welfare conditions, and subsequently reduces stressful conditions for broiler chicks.Page: 782

The gut microbiome is complex, and includes a multitude of bacteria, yeast, and protozoa. The addition of probiotics to the diet plays a major role in maintaining the equilibrium of the intestinal biota and inhibiting colonization by pathogens (Chen *et al*., 2014). Commensal microorganisms in the intestinal tract are thought to be essential for poultry digestion and immunity (Seidavi *et al*., 2017). Good growth and immunity are correlated with a healthy intestine. It seems that feed form (pelleted diet) and dietary supplementation (enzymes and probiotics) can improve the immune system and growth performance of broilers by enhancing the health of the intestinal system.

In the present study, the relative splenic weights of the birds fed the mash diet without the enzyme or probiotic were higher than those of the birds fed the other diets, whereas nutritional modifications had no effect on the relative weights of the liver and bursa. Others reported similar findings (Seidavi *et al*., 2017). The authors of the current study expected that the addition of the enzyme and probiotic would be associated with an increase in the relative weights of organs linked to the immune system. The mechanism underlying the absence of such an association is not known.

Conclusion

In conclusion, in broiler chicks fed a triticale-based diet, a pelleted form supplemented with an enzyme and probiotic improved growth performance and immunity compared with a mash diet without these additives. Improved immunity and growth may be related to synergistic interaction effects between the feed form and dietary supplementation–on the intestine system. Thus, adding enzymes and probiotics to a pelleted diet can alleviate negative effects of NSPs in triticale on growth performance of broilers.

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Authors' Contributions

All the authors were involved in the experimental design. S.M. Hosseini conducted the growth trial and collected the samples from the birds. S.M. Hosseini and M. Chamani performed the statistical analyses. All the authors were involved in writing the manuscript.

Conflict of Interest Declaration

We declare that we have no conflict of interest.

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