

Growth Performance, Mucin2 Gene Expression, Morphology of Small Intestine and Intestinal *Lactobacillus* Population of Broiler Chicks Fed with Triticale-Based Diets: Effects of Dietary Physical Form and Dietary Inclusion of Enzyme and Probiotic ^[1]

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^[1] Financial support for this study was obtained from the Islamic Azad University, Tehran 477893855 (Iran), Grant

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Article Code: KVFD-2017-18629 Received: 20.08.2017 Accepted: 17.01.2018 Published Online: 18.01.2018

How to Cite This Article

Hosseini SM, Chamani M, Mousavi SN, Hosseini SA, Sadeghi AA: Growth performance, mucin2 gene expression, morphology of small intestine and intestinal *Lactobacillus* population of broiler chicks fed with triticale-based diets: Effects of dietary physical form and dietary inclusion of enzyme and probiotic. *Kafkas Univ Vet Fak Derg*, 24 (2): 203-210, 2018. DOI: 10.9775/kvfd.2017.18629

Abstract

This study was conducted to investigate the effects of physical form of diet and dietary inclusion of enzyme and probiotic on growth performance, mucin2 gene expression, morphology of small intestine and intestinal *Lactobacillus* population of broiler chicks fed with triticale-based diets. Six hundred forty 1-d-old broiler chicks were arranged in 8 treatments with 4 replicates (n=20 chicks). This study was done based on a randomized block completely design in a 2x2x2 factorial arrangement with two levels of feed form (pellet and mash), two levels of probiotic (0 and 0.03% diet) and two levels of enzyme (0 and 0.05% diet). Birds fed with pelleted diet had higher weight gain at starter and grower (P<0.001) and also lower feed conversion ratio at starter (P<0.001) and grower periods (P<0.01) than those fed the mash diet. Birds fed with pelleted diets containing enzyme and probiotic consumed the most feed intake at starter period. Mucin2 gene expression was significantly higher in birds fed the pelleted diets containing enzyme or/and probiotic and mash diets containing probiotic than those fed other diets (P<0.01). Intestinal morphology and intestinal *Lactobacillus* population were not influenced by experimental treatments (P>0.05) Thus, physical form of diet plays important role in improving performance and gene expression in birds fed with triticale-based diets.

Keywords: Broiler chicks, Di-pro probiotic, Mucin2 gene, Pellet, Triticale, Weight gain

Tritikale Temelli Diyet ile Beslenen Broiler Cıvcıvlerin Büyüme Performansı, Musin2 Gen Ekspresyonu, İnce Bağırsak Morfolojisi ve Bağırsak *Lactobacillus* Popülasyonu: Diyetin Fiziksel Formu ile Enzim ve Probiyotik İlavesinin Etkileri

Öz

Bu çalışma tritikale temelli diyet ile beslenen broiler cıvcıvlerde diyetin fiziksel formu ile diyete enzim ve probiyotik ilavesinin büyüme performansı, musin2 gen ekspresyonu, ince bağırsak morfolojisi ve bağırsak *Lactobacillus* popülasyonu üzerine etkilerini araştırmak amacıyla gerçekleştirilmiştir. Altı yüz kırk adet 1 günlük broiler cıvcıv 8 uygulama ve 4 tekrar olmak üzere kurgulanmıştır (n=20 cıvcıv). Çalışma rastgele dizayn usulünde olmak üzere 2x2x2 faktöriyel düzende planlanmış ve iki yem formu (pelet ve püre), iki probiyotik dozu (0 ve %0.03) ve iki enzim dozu (0 ve %0.05) denenmiştir. Pelet ile beslenen cıvcıvler püre ile beslenenler ile karşılaştırıldığında başlangıç ve büyüme periyotlarında daha yüksek ağırlık kazanımı gösterirken (P<0.001), başlangıç (P<0.001) ve büyüme (P<0.01) periyotlarında daha düşük yem konversiyon oranına sahipti. Enzim ve probiyotik içeren pelet yem ile beslenen cıvcıvler başlangıç periyodunda en yüksek yem tüketimine sahipti. Musin2 gen ekspresyonu enzim ve/veya probiyotik içeren pelet yem ile beslenen cıvcıvler ile probiyotik içeren püre yem ile beslenenlerde diğer yemlemelere göre anlamlı derecede daha yüksekti (P<0.01). Bağırsak morfolojisi ve bağırsak *Lactobacillus* popülasyonu deneme gruplarında fark göstermedi (P>0.05). Sonuç olarak, tritikale temelli diyet ile beslenen cıvcıvlerde yemin fiziksel formu performans ve gen ekspresyonu üzerine önemli rol oynamaktadır.

Anahtar sözcükler: Broiler cıvcıv, Di-pro probiyotik, Musin2 geni, Pelet, Triticale, Ağırlık kazanımı



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INTRODUCTION

Some cereals grains have been commonly used in poultry nutrition at all over world. Triticale is a hybrid cereal which is produced from crossbreeding of wheat and rye and it can be used instead of corn in poultry diets ^[1]. Triticale and wheat have similar nutrient composition, such as resistance to hard agronomic conditions ^[2,3]. Triticale is known to have high amounts of soluble non-starch polysaccharides (NSPs) ^[1]. It is well known that NSPs initially increase digesta viscosity and then reduce digesta passage, feed intake (FI), nutrient digestion and absorption ^[4], and also increases harmful microbial population ^[5]. Triticale has more NSP content than wheat and rye. Thus, researchers have used some feeding strategies such as enzyme supplementation to improve the nutrient digestion and absorption in triticale-based diets ^[6-8].

Physical form of diet (mash, pellet and crumble) can act as key factor in improving growth performance of broiler chicks. Studies have shown that broiler chicks fed with pelleted diets consumed more feed intake and showed higher weight gain (WG) ^[9,10]. Pelleted diets could improve growth performance via several factors including decreasing feed waste, energy consumption and dustiness of feed and increasing palatability ^[10]. Earlier studies have reported other advantages of pelleted diets in improving growth performance of animals such as lowering ingredient degradation, increasing digestibility, removing pathogenic microbes and thermal modification of starch and protein ^[11,12].

It is shown that diet supplementation with enzyme improved feed conversion ratio (FCR) in diets containing high amounts of NSP ^[6-8]. Dietary inclusion of xylanase to diets containing higher levels of NSP significantly decreased degree of polymerization of NSP and subsequently reduced digesta viscosity and also increased nutritive value of the diet ^[6,13]. Dietary inclusion of enzyme could increase proliferation of beneficial microflora in the final compartments of the gastrointestinal tract by increasing substrate ^[14]. Adding xylanase to diet significantly improved growth performance in broiler chicks fed with triticale-based diet ^[1].

Probiotics are known to have beneficial effects on growth performance of animals ^[15,16]. Dietary inclusion of probiotics, *Lactobacillus acidophilus* and *L. casei*, to deficient-diets in certain nutrients could increase growth performance in broiler chicks ^[17]. Probiotics are also known to have positive effects in balancing intestinal microflora ^[18,19]. In vitro studies have reported different interactions between intestinal mucin2 and intestinal microflora. Studies have also reported adhesion of *Lactobacillus* strains ^[20] and other bacterial strains ^[21] to intestinal mucin2 by competition between pathogenic and beneficial bacteria ^[22].

With regards to adverse effects of NSPs on microflora

population and growth performance in triticale-based diet, positive role of physical form of diet, probiotics and enzymes on microbial population and also relation between benefit bacteria and mucin2, it was hypothesized that dietary inclusion of probiotic and enzyme in pelleted diets can improve mucin gene expression, intestinal *Lactobacillus* population and growth performance in broiler chicks fed the triticale-based diets. Thus, the current research was done to investigate the probable interactions among physical form of diet, dietary inclusion of probiotic and enzyme on growth performance, mucin2 gene expression, intestinal *Lactobacillus* population and morphology of small intestine of broiler chicks fed with triticale-based diets.

MATERIAL and METHODS

Birds and Breeding Conditions

All procedures used in this research were approved by the Animal Ethics Committee of the Islamic Azad University, Science and Research Branch, Tehran-Iran. A number of 640 one-day-old Ross-308 broiler chicks (320 males and 320 females), with average body weight 44 ± 2 g, were purchased from a commercial hatchery. Birds had *ad libitum* access to feed and water. Temperature was kept at 33°C in start of trial and then progressively reduced from 33 to 24°C at 21 days of age. Lighting programs were as follows; 23 h light: 1 h dark during experiment.

Experimental Design and Diets

This experiment was done in a 2×2×2 factorial arrangement based on a randomized completely block design with 8 treatments consisting of 4 replicates and 20 chicks in each pen or replicate. The experiment was lasted for 42 days. The experimental diets were formulated on the basis Ross 308 catalogue. Nutritional requirements were provided based on the standard recommendations ^[23]. Analysis of crude protein was done on basis to Association of Official Analytical Chemists, or AOAC ^[24]. The nutritional requirements were adjusted on basis same catalogue (*Table 1*). Basal diets were firstly prepared and then 0.03% Di-Pro probiotic (Tak Gen Zist Company product-Iran: containing 1.6×10^9 CFU/g *Bacillus subtilis* and *Bacillus licheniformis*) and 0.05% Rovabio® enzyme (Adiseo Co. product-French; containing 200 IU xylanase and 200 IU β-gluconase) were added to them. The half of diets was prepared in mash form and other part in the pelleted form. Non-probiotic and enzyme diets were prepared before probiotic and enzyme containing feeds. The pelleted diets were prepared by BUHLER (DFCP-65909-S, Bühler AG, Uzwil, Switzerland) pellet press. The pelleted diets were prepared at 78°C and had size 2 mm at starter diet and 3 mm at grower and finisher diets and 1.5 mm length. The triticale-based diets were as follows;

1. Diet prepared in mash form without probiotic and

Table 1. Ingredients and composition of the basal diets at starter period (1-10 d), growth period (11-24 d) and finisher period (25-42 d)

Ingredients (%)	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Triticale	58.7	63.08	68.2
Soybean meal	29.9	25.46	20.11
Corn gluten	5.00	5.00	5.00
Soybean oil	1.50	2.00	2.5
Lime stone	1.03	0.91	0.83
Di-calcium phosphate	2.00	1.77	1.60
Salt	0.24	0.24	0.24
Bicarbonate sodium	0.20	0.20	0.20
Vitamin premix ^a	0.25	0.25	0.25
Mineral premix ^b	0.25	0.25	0.25
DL-methionine	0.30	0.26	0.23
L-lysine	0.45	0.43	0.44
L-threonine	0.18	0.15	0.15
Nutrient Composition			
Metabolizable energy (kcal/kg)	3025	3150	3200
Crude protein (%)	23.1	22.00	19.32
Calcium (%)	1.05	0.90	0.85
Available phosphorus (%)	0.50	0.45	0.42
Methionine (%)	0.52	0.45	0.41
Methionine + cysteine (%)	0.91	0.84	0.86
Lysine (%)	1.42	1.24	1.09
Threonine (%)	0.93	0.83	0.75

^a Vitamin premix provided the following per kilogram of diet: Vit. A, 9,000 IU; Vit. D₃, 2,000 IU; Vit. E, 1,800 IU; Nicotinic acid, 30 mg; Vit. B₂, 0.015 mg; Vit. K₃, 4 mg; Biotin, 0.15 mg; Folic acid, 1.0 mg; Niacin, 30.0 mg; Panthotenic 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; FeSO₄. 7H₂O, 50 mg; Zinc oxide, 100 mg; Copper sulfate, 10 mg; I, 1.0 mg; Se, 0.2 mg

enzyme (Treatment 1)

2. Diet prepared in pellet form without probiotic and enzyme (Treatment 2)

3. Diet prepared in mash form containing 500 g enzyme/ per ton diet (Treatment 3)

4. Diet prepared in pellet form containing 500 g enzyme/ per ton diet (Treatment 4)

5. Diet prepared in mash form containing 300 g probiotic/ per ton diet (Treatment 5)

6. Diet prepared in pellet form containing 300 g probiotic/ per ton diet (Treatment 6)

7. Diet prepared in mash form containing 300 g probiotic and 500 g enzyme/per ton diet (Treatment 7) and

8. Diet prepared in pellet form containing 300 g probiotic and 500 g enzyme/per ton diet (Treatment 8)

Performance Parameter

For calculating performance, feed intake (FI) and body

weight (BW) were recorded at 10, 24 and 42 days of age. FI was considered as difference between given feed from residue feed. Mortality was daily registered. Any bird that died was weighed and FCR were calculated by dividing FI by WG of live plus dead birds.

Mucin2 mRNA Gene Expression

At the end of trial, 8 broiler chicks (4 males and 4 females) from each treatment (2 birds per replicate) were randomly selected and killed and intestinal segments were removed. The midpoint between the entry of the bile duct and Meckel's diverticulum was considered as jejunum. The jejunum was washed by normal saline and kept at liquid nitrogen (-196°C) and then transferred to lab and stored at -80°C. RNA was extracted from jejunum samples by Fermentas kit (GeneJET RNA Purification Kit-Russia) on the basis manufacturer company instructions. After removing residual DNA, cDNA was synthesized as primary template. RNA was reverse transcribed to cDNA by a Revert Aid First Strand cDNA Synthesis Kit, as recommended by manufacturer's instructions (Fermentas-Russia). The cDNA samples were served at -75°C until analysis. Real-time PCR was performed by PCR master mix for 5 min at 65°C.

The PCR was done in a reaction volume of 25 µL containing reagents at the following final concentrations: 12.5 µL of Maxima® SYBER Green'ROX qPCR Master Mix (2x), forward primer 0.75 µM, reverse primer 0.75 µM, water free nuclease 10 µL and 1 µL of cDNA sample. Quantity and quality of RNA and cDNA were evaluated using spectrophotometry method by Nano drop apparatus (Thermo Company). After determinate of quality and quantity cDNA, all cDNA were diluted. The primers used in present study (Cynagen Company) were as follows;

Forward for GAPDH: 5'GGTGGTGCTAAGCGTGTAT3'

Reverse for GAPDH: 5' ACCTCTGTCATCTCTCCACA 3'

Forward for Mucin 2: 5'-TCACCCTGCATGGATACTTGCTCA-3'

Reverse for Mucin 2: 5'-TGTCCATCTGCCTGAATGACAGGT-3'

The cycling profiles were as follows: primary denaturation (1 cycle at 95°C for 10 min), denaturation (1 cycle at 95°C for 15 min), annealing (40 cycles at 60°C for 30 min) and final extension (40 cycles at 72°C for 30 min). For per run, a negative control, a calibrator sample, cDNA samples, and endogenous control (GAPDH) were considered. GAPDH samples were evaluated in duplicate and the target genes were analyzed in triplicate. Quality of gene expression was evaluated by using the $\Delta\Delta C_t$ method. The difference between the Ct amount of mucin 2 gene and Ct of GAPDH for each sample was considered as ΔC_t and $\Delta\Delta C_t$ which subsequently calculated as follows;

$\Delta\Delta C_t = \Delta C_t$ of each treatment - ΔC_t control

Intestinal Morphology

At the end of trial, tissue samples from jejunum and ileum

broiler chickens (2 males and 2 females each replicate) were excised and placed in (10%) neutral buffer formalin. Formalin-preserved jejunum and ileum tissues were firstly sectioned and then stained with Alcian blue at pH=2.5. The two intestinal segments from per broiler chick were mounted on a glass slides and villus length, villus surface and crypt depth was evaluated. Morphological criteria were as follows; villus height (from the base of the lamina propria to the villus apex); villus width at its midpoint and crypt depth between adjacent villi. Morphological slides were investigated for villus length and crypt depth by using microscope (NOVEX model-Holland country) and the villus length and crypt depth were measured by using micrometer stage for calibration the objective lens (10X) with ocular micrometer.

Lactobacillus Population

Sixty four intestinal contents were collected from all parts of the intestine and stored in a sterile container and refrigerated at 4°C. Digesta was mixed in a 10 mL pre-reduced salt medium and then diluted as explained by others [25] to examine the count of Lactobacilli (Rogosa, CM 0627, incubated anaerobically 48 h). Gut tissue samples were serially diluted from 10^{-7} to 10^{-3} and then from each dilution, 0.1 mL of the sample was plated into the suitable medium for enumeration of bacteria.

Statistical Analysis

The birds were studied at a 2×2×2 factorial arrangement based on a randomized completely block design. The data were analyzed by SAS software. Duncan's multiple range test was used to detect the differences ($P < 0.05$) among different groups ($P \leq 0.05$). All of the parameters measured were analyzed as follows:

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

Where Y_{ijklm} is the characteristic measured, μ is the overall mean, R_i is block effect (saloon length), A_j is main effect of probiotic, B_k is main effect of enzyme, C_l is main effect of dietary physical form, $(A*B)_{jk}$ is interaction between the probiotic and enzyme, $(A*Cl)_{jl}$ is interaction between the probiotic and dietary physical form, $(B*C)_{kl}$ is interaction between the enzyme and dietary physical form, $(A*B*C)_{jkl}$ is interaction among the probiotic, enzyme and dietary physical form and e_{ijklm} is the residual error. Main effect of factors would not be considered when interaction is significant.

RESULTS

Performance

The data for growth performance are presented in Table 2. The FI, WG and FCR were affected by experimental treatments. Broiler chicks fed in treatments (2, 4, 6 and 8) significantly showed higher WG than those in treatment 1

(mash diet without additives) at starter period ($P < 0.001$) and those in odd treatments (1, 3, 5 and 7) at grower period ($P < 0.001$). Treatment 8 (Pellet diet included with probiotic and enzyme) and treatment 6 (pellet diet included with probiotic) significantly had the highest WG at starter period ($P < 0.01$). A significant interaction between probiotic and enzyme was seen for FI and FCR at starter period ($P < 0.001$). The most feed intake was for broiler chickens receiving the pelleted diets containing enzyme and probiotic (treatment 8) while, those fed the mash diets containing enzyme or probiotic consumed least feed at starter period ($P < 0.001$). Birds receiving mash diet without additives also showed higher FCR than those fed pellet diet containing enzyme and probiotic at starter ($P < 0.01$) and grower periods ($P < 0.01$). As mentioned before, where interaction is significant, main effects would not be discussed.

Mucin 2 Gene Expression

The data for jejunal mucin 2 mRNA expression are presented in Fig. 1. Dietary inclusion of probiotic and enzyme to pelleted diets significantly increased mucin 2 mRNA expression. There was higher gene expression in 4, 5, 6 and 8 treatments compared with treatment 1 ($P < 0.05$). According some papers, difference in the amount of gene expression is more important than significant different. Therefore treatments 2, 3 and 7 did not have significant different with treatment 1, but there was more gene expression.

Intestine Morphology and Lactobacillus Population

As Table 3 and Table 4 shows intestinal morphology parameters and intestinal *Lactobacillus* population were not influenced by dietary form and dietary inclusion of probiotic and enzyme ($P > 0.05$). The images for intestinal morphology are presented in Fig. 2.

DISCUSSION

Previous studies have shown that broiler chicks receiving the pelleted diets consumed more FI and had higher WG compared with broiler chicks fed with mash diet [9,10,26,27]. In contrast to other studies, triticale-based diets were used in this study. In any case, soluble NSP fraction has negative relation with apparent metabolizable energy, because of increased digesta viscosity [28]. Osek et al. [29] reported that broiler chicks fed the whole with wheat and whole triticale diets exhibited the lowest WG. However, the prepared diets in pellet form improved WG in starter period. In sorghum-based diets, Abdollahi et al. [30] stated that the pelleted diets significantly increased performance rather than mash diets. Increase in WG in broiler chicks fed with pelleted diets can be due to increased FI [31]. In this research, increased FI was seen in broiler chicks fed with pelleted diets in starter period.

Dietary supplementing of probiotic and enzyme significantly

Table 2. The interaction among probiotic, enzyme and dietary physical form on growth performance at starter period (0-10 d), grower period (11-24 d) and finisher period (25-42 d) of broiler chicks fed with triticale-based diets

Treatment			WG			FI			FCR		
PF	E (%)	Pro (%)	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
Mash	0	0	174.08 ^d	584.18 ^c	1449.29	276.39 ^c	985.23	2551.52 ^{bc}	1.58 ^d	1.68 ^c	1.76
Pellet	0	0	210.39 ^{abc}	722.21 ^{ab}	1602.84	293.56 ^b	1097.27	2613.38 ^b	1.40 ^c	1.51 ^{bc}	1.63
Mash	0.05	0	190.62 ^{cd}	607.92 ^c	1461.50	257.85 ^d	976.78	2462.53 ^c	1.35 ^{abc}	1.61 ^{abc}	1.69
Pellet	0.05	0	222.04 ^{ab}	730.21 ^a	1511.02	281.19 ^{bc}	1034.51	2399.73 ^c	1.26 ^{ab}	1.41 ^{ab}	1.58
Mash	0	0.03	191.29 ^{cd}	617.47 ^c	1493.13	258.78 ^d	985.76	2406.47 ^c	1.35 ^{ab}	1.59 ^{bc}	1.61
Pellet	0	0.03	228.44 ^a	721.19 ^{ab}	1535.33	284.26 ^{bc}	1020.74	2281.00 ^d	1.24 ^a	1.41 ^{abc}	1.49
Mash	0.05	0.03	197.31 ^{bcd}	646.88 ^c	1554.23	274.66 ^c	990.77	2290.33 ^a	1.39 ^{bc}	1.54 ^{ab}	1.53
Pellet	0.05	0.03	234.57 ^a	711.92 ^{ab}	1570.07	313.25 ^a	988.71	2298.24 ^a	1.33 ^{ab}	1.39 ^a	1.46
SEM			1.96	6.07	24.45	1.13	13.12	33.33	0.01	0.024	0.036
P-Value			0.000	0.000	0.753	0.000	0.347	0.149	0.000	0.047	0.453
PF			***	***	NS	***	NS	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
ExPro			NS	NS	NS	***	NS	NS	***	NS	NS
PF×ExPro			NS	NS	NS	NS	NS	NS	NS	NS	NS

Superscripts (a-e) show significant different at each column. NS: non-significant ($P>0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$; PF: physical form, E: enzyme, Pro: probiotic. SEM: standard error of means

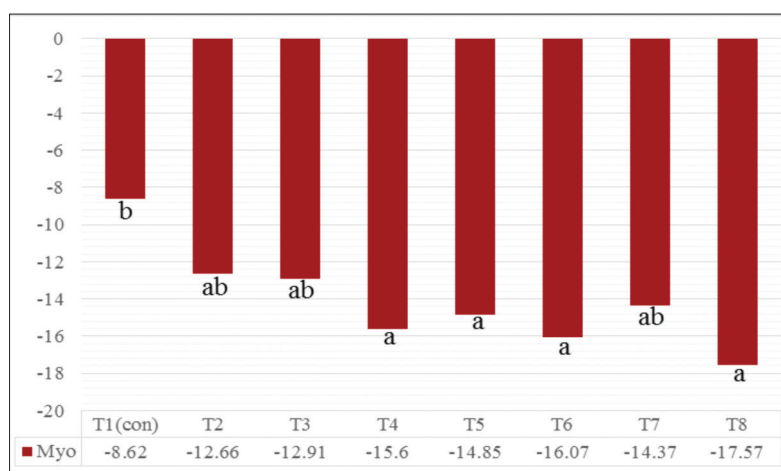


Fig 1. Effect of feed form, probiotic and enzyme on jejunal mucin 2 mRNA expression of broiler chicks fed with triticale-based diets ($P=0.002$, SEM= 0.4.)

increased WG at starter period ($P<0.05$). In the current study Rovabio enzyme is contained 200 IU xylanase and 200 IU β -glucanase. Studies have been shown dietary inclusion of xylanase in diets containing higher levels of NSP significantly reduced the degree of polymerization of NSP which subsequently reduced digesta viscosity and finally increased the nutritive value of the diet [7,13]. It was reported that dietary inclusion of enzyme in diets having high levels of NSP increases the efficiency of feed utilization and WG [7]. It is reported that dietary inclusion of xylanase in wheat, rye, or triticale-based diets reduced

the degree of polymerization of NSP, digesta viscosity and improved the nutritive value of the diet [7] which may increase WG. Regarding probiotic supplementing, it was reported that supplementing of probiotic increased WG which may be due to improved absorption of nutrients and reduced harmful bacteria [32]. However, dietary inclusion of probiotic and enzyme and pelleted form increased WG in lower ages. Broiler chicks in lower ages have more physical limitations in digestive and food consumption. Thus, consuming diets having probiotic, enzyme and in pelleted form increases FI in lower ages and thus increases WG. However, birds fed with pelleted diet showed higher WG and lower FCR than birds fed the mash diet, WG (1554 vs 1489) and FCR (1.54 vs 1.65), in finisher period. However, these differences were not significant ($P>0.05$).

Chickens receiving the pellet diets containing enzyme and probiotic consumed more FI than those fed the mash diet containing enzyme in starter period. This can attributed to synergism interaction effect among pellet, enzyme and probiotic. Studies have shown that NSPs enhance digesta viscosity and decrease FI [4]. It is reported that enzyme supplementing to diet increases the digesta passage rate and subsequently FI [33]. Earlier studies have reported that probiotics act as appetizer supplement [34,35]. Studies have also reported that pelleted diets could increase FI [36].

Table 3. Interaction among feed form, probiotic and enzyme on ileum and jejunum villus length and crypt depth of 42 d old broilers

Treatment			Villus Height, μm		Villus Surface Area, mm^2		Crypt Depth, μm		Villus Height to Crypt Depth	
PF	E (%)	Pro (%)	I	J	I	J	I	J	I	J
Mash	0	0	0.850	1.286	0.830	0.122	0.082	0.085	10.44	15.133
Pellet	0	0	0.795	1.265	0.775	0.127	0.085	0.088	9.44	14.410
Mash	0.05	0	0.836	1.348	0.851	0.121	0.078	0.086	11.05	15.690
Pellet	0.05	0	0.828	1.198	0.813	0.120	0.086	0.080	9.69	14.935
Mash	0	0.03	0.805	1.330	0.826	0.120	0.083	0.084	9.78	15.972
Pellet	0	0.03	0.831	1.302	0.822	0.121	0.084	0.089	10.09	14.993
Mash	0.05	0.03	0.767	1.287	0.820	0.122	0.080	0.086	9.72	15.084
Pellet	0.05	0.03	0.902	1.310	0.887	0.120	0.082	0.088	11.06	14.858
SEM			0.011	0.014	0.014	0.001	0.001	0.001	0.218	0.269
P-Value*			0.168	0.297	0.718	0.769	0.883	0.688	0.416	0.890

* There was no significant different among groups and NS was deleted. I: ileum, J: jejunum, PF: physical form, E: enzyme, Pro: probiotic. SEM: standard error of means

Table 4. Interaction among feed form, probiotic and enzyme on intestinal *Lactobacillus* ($\times 10^6$ CFU) population of 42 d old broilers

PF	E (%)	Pro (%)	Mean
Mash	0	0	3.19
Pellet	0	0	3.80
Mash	0.05	0	2.49
Pellet	0.05	0	3.99
Mash	0	0.03	3.44
Pellet	0	0.03	3.89
Mash	0.05	0.03	3.83
Pellet	0.05	0.03	3.69
SEM			0.42
P-Value*			0.169

* There was no significant different among groups and NS was deleted. PF: physical form, E: enzyme, Pro: probiotic, SEM: standard error of means

Broiler chicks consume FI in pelleted-based diets, because pelleted diets are more density and broiler chicks use lower energy for its consumption.

Broiler chicks fed with pelleted diets containing enzyme and probiotic relatively had lower FCR. Dietary inclusion of *B. subtilis* strain in broilers diet could improve WG at 24 d and reduce FCR at 12-24 days of old in broiler chicks [37]. Improved FCR and the reduced FI in heat-stressed broiler chicks fed with *Lactobacillus* strain have been previously reported [38].

Reduced feed waste, energy consumption and dustiness of feed, the improved palatability [10], the increased digestibility, the removed pathogenic microbes and thermal modification of starch and protein [11,12] can be considered as reasons for the improved FCR in broilers fed the pellet diet. In the present study, the pelleted diet rather than mash diet showed better performance at starter and grower, while it could not improve growth performance in finisher period. Birds receiving mash diet without additive

showed higher FCR than other birds. As mentioned NSPs increase harmful bacteria population and the diets prepared in pelleted form especially with enzyme and probiotic at starter and grower period showed lower FCR compared with mash diet without additive. We believed that the decreased FCR could be achieved by the reduced harmful microflora. It can be concluded that the improved performance in starter period cannot be attributed to *Lactobacillus* population and improved intestinal morphology because neither *Lactobacillus* population and nor improved intestinal morphology were not influenced.

In the current study, mucin2 gene expression was increased in pellet diets containing enzyme (Fig. 1). Previous studies reported adhesion of *Lactobacillus* strains [20] and other bacterial strains [21] to intestinal mucin2 through competition between pathogenic and beneficial bacteria [22]. Investigations have been also shown that diet supplementing with probiotic significantly increased mucin2 gene expression [39]. It also seems that microbial balance becomes more complete with increased age. Nutritional modulation cannot improve growth performance in finisher period. It can be stated that the pelleted diet containing enzyme and probiotic improved growth performance by increasing mucin 2 gene expression. The data are conflicting, since studies have reported that increased mucin2 gene expression can increase *Lactobacillus* strains, but such results were not found. It seems that mucin2 gene expression increases *Lactobacillus* population in lower ages. However, studies have reported that dietary inclusion of probiotic significantly increased *Lactobacillus* population by increasing volatile fatty acids [40]. However, it was expected that enzymes increase NSP digestion and help to increasing *Lactobacillus* population. Sampling at higher ages can be reason for non-changing in *Lactobacillus* population.

Feed form and dietary inclusion of probiotic and enzyme could not change intestinal morphology of small intestine.

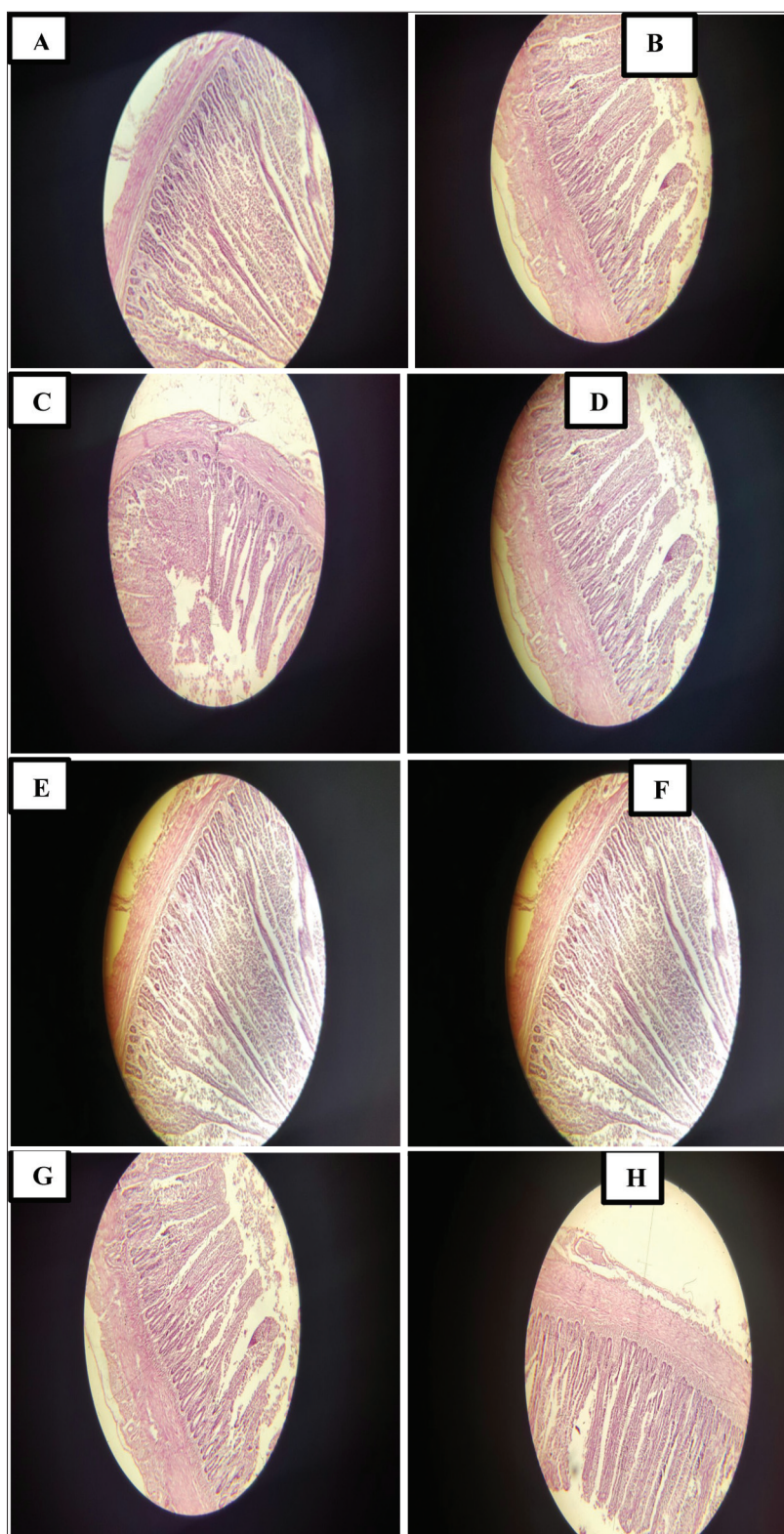


Fig 2. Intestinal morphology of birds, A-H Figures show intestinal morphology in Treatments 1 to 8, respectively

The villus crypt is known as villus factory and deeper crypts are criteria for tissue turnover which allow renewal of the villus and crypt depth is involved in the production of enterokinase that aids to digestion of protein [41]. There are

conflicting reports for effect of nutritional modulation on intestine morphology. Some studies have shown that dietary inclusion of probiotic increases ileum villus height and crypt depth [5,42] while other studies have reported that dietary inclusion of probiotic reduces villus height and crypt depth of the ileum [43,44]. Cereals contain high NSP increase the digesta viscosity and subsequently change bacterial intestinal physiology [45]. It is reported that dietary inclusion of enzymes enhance hydrolysis of NSPs and subsequently increase the height of villi and the proportion height-to-depth of the crypts, and improve the bacterial activity of the intestinal [46].

It can be stated that physical form of diet can efficiently affect growth performance of broiler chicks fed with triticale-based diets. It seems that dietary form improves performance by balancing microbial population at lower ages; however, pelleted diets had higher mucin2 gene expression at 42 d. Measuring nutrient digestibility would be offered in future studies for understanding more mechanisms.

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