Kafkas Univ Vet Fak Derg
26 (2): 217-224, 2020
DOI: 10.9775/kvfd.2019.22767

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Effect of β-glucanase on Performance, Carcass Characteristics, Microflora, Plasma Constitutes and Immunity in Local Broiler Hybrid "Golpayegani-Ross"

Mohammadebrahim TASIRNAFAS [1,a] Kazem KARIMI [1,b] Ghobad ASGARI JAFARABADI [1,c]
Fatemeh NOORBAKHSH [2,d] Alireza SEIDAVI [3,e]

[1] Department of Animal Science, Faculty of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin-Pishva, IRAN
[2] Department of Microbiology, Biological Science College, Varamin-Pishva Branch, Islamic Azad University, Varamin-Pishva, IRAN
[3] Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, IRAN
[a] ORCID: 0000-0002-5272-9819; [b] ORCID: 0000-0001-5318-2512; [c] ORCID: 0000-0002-7192-1558; [d] ORCID: 0000-0001-8082-8446
[e] ORCID: 0000-0002-1903-2753

Article ID: KVFD-2019-22767 Received: 30.05.2019 Accepted: 07.11.2019 Published Online: 07.11.2019

How to Cite This Article

Tasirnafas M, Karimi K, Asgari Jafarabadi G, Noorbakhsh N, Seidavi A: Effect of β-glucanase on performance, carcass characteristics, microflora, plasma constitutes and immunity in local broiler hybrid "Golpayegani-Ross". *Kafkas Univ Vet Fak Derg,* 26 (2): 217-224, 2020.
DOI: 10.9775/kvfd.2019.22767

Abstract

The Gram negative bacterium *Fibrobacter succinogenes* lives anaerobically in the bovine rumen and secretes β-glucanase. Barley is a cheap food input for poultry production but it is low in nutrients due to its beta glucan content. Supplementing exogenous β-glucanase (EC 3.2.1.4) to broiler diets can decrease the viscosity of the intestinal content by hydrolyzing the β-glucan and enhancing nutrient digestibility. In the current study, β-glucanase enzymatic activity was assayed after extraction from bovine rumen fluid, and then its effect on broiler performance, carcass characteristics, duodenum microbial flora, hematological and immunological parameters was compared with a commercial enzyme. A total of 120 local broilers (Golpayegani-Ross hybrid) was allocated to 3 treatments with 4 replicates per treatment and 10 birds per replicate/pen. Over a 49-day experimental period, broilers were fed a basal diet (T1), basal diet with 10 IU of extracted β-glucanase and 20% barley (T2) and basal diet with 10 IU of commercial β-glucanase and 20% barley (T3). T2 significantly increased body weight gain and decreased feed intake over the whole experimental period. Treatments had a significant effect on hematological parameters except low density lipoprotein (LDL) concentration ($P<0.01$). Treatments did not affect antibody titration. The highest non-eviscerated carcass weight ($P<0.05$) and eviscerated carcass weight ($P>0.05$) were associated with the T2. The same treatment also caused a significant increase in lactobacilli and *Escherichia coli* in the gastrointestinal tract. Consequently, enzyme addition had a positive effect on broiler performance without any adverse effects on humoral immunity parameters.

Keywords: *Broiler performance, Carcass characteristics, Enzymatic extraction, Fibrobacter succinogenes, Microbial flora*

Lokal Broyler Hibridi "Golpayegani-Ross"da Performans, Karkas Karakteristiği, Mikroflora, Plazma Bileşenleri ve Bağışıklık Üzerine β-glukanazın Etkisi

Öz

Gram negatif bakteri *Fibrobacter succinogenes*, sığır rumeninde anaerobik olarak yaşar ve β-glukanaz salgılar. Arpa, kanatlı hayvan üretimi için ucuz bir besin maddesidir, ancak içerdiği beta glukan nedeniyle besin değeri düşüktür. Broyler diyetlerine eksojen β-glukanazın (EC 3.2.1.4) eklenmesi, β-glukanın hidrolize edilmesi yoluyla intestinal içeriğin viskozitesini azaltarak besinlerin sindirilebilirliğini arttırabilir. Bu çalışmada, sığır rumen sıvısından yapılan ekstraksiyondan elde edilen β-glukanazın enzimatik aktivitesi incelendi ve daha sonra broyler performansı, karkas özellikleri, duodenum mikrobiyal florası, hematolojik ve immünolojik parametreler üzerindeki etkisi ticari bir enzimle karşılaştırıldı. Toplam 120 lokal ırk broyler (Golpayegani-Ross hibrid), uygulama başına 4 tekrar ve her tekrarda 10 hayvan olacak şekilde 3 gruba ayrıldı. Kırkdokuz günlük deney süresince, broylerlere bazal diyet (T1), 10 IU ekstrakte β-glukanaz ve %20 arpa içeren bazal diyet (T2) ve 10 IU ticari β-glukanaz ve %20 arpa içeren bazal diyet (T3) verildi. Tüm deney dönemi boyunca T2 diyeti vücut ağırlığını önemli ölçüde arttırdı ve yem alımını azalttı. Tedaviler, düşük yoğunluklu lipoprotein (LDL) konsantrasyonu ($P<0.01$) dışındaki hematolojik parametreler üzerinde önemli bir etkiye sahipti. Bununla birlikte antikor düzeyi farklı tedavilerden etkilenmedi. İç organları uzaklaştırılmamış ($P<0.05$) ve iç organları uzaklaştırılmış en yüksek karkas ağırlığı ($P>0.05$) T2 diyeti ile beslenen grupta belirlendi. Aynı tedavi gastrointestinal sistemde laktobasil ve *Escherichia coli* düzeyinde de önemli bir artışa neden oldu. Sonuç olarak enzim ilavesi, humoral bağışıklık parametreleri üzerinde herhangi bir olumsuz etkisi olmaksızın, broyler performansını olumlu etkilemiştir.

Anahtar sözcükler: *Broyler performansı, Karkas özellikleri, Enzimatik ekstraksiyon, Fibrobacter succinogenes, Mikrobiyal flora*

İletişim (Correspondence)
+98 919 4449056
karimikazem@gmail.com

INTRODUCTION

The *Fibrobacter succinogenes*, an obligate anaerobic bacterium, is an important degrader of lignocellulosic plant material in the herbivore gastrointestinal tract. Enzymes produced by *F. succinogenes* have the cellulases, endoglucanases and xylanases, which were thought to be involved in cellulose degradation. These membrane vesicles found in *F. succinogenes* cellulose cultures have not a role in cellulose degradation, but they are sign of aging cells [1]. Gong et al.[2] discovered a 180 kDa cellulose binding protein with a role in adhesion to cellulose. Most anaerobic cellulose degrading bacteria rely upon strict binding of the cell to the cellulose fiber. Hence, based on this discovery is proposed the binding proteins termed "fibro-slime" proteins. These proteins are specific to *F. succinogenes* [1].

Corn is the main material used in poultry diets due to its high energy and lack of antinutritive effects but there is competition for consumption of cereals like corn between humans and monogastric animals [3]. There is also interest in replacing corn with other materials, such as barley in diets, due to restricted culture of corn [4].

The use of commercial enzymes in poultry nutrition is of great importance. A proportionate increase in feed ingredients price has been the primary impediment in almost all developing countries. As a result, non-conventional and cheaper feed ingredients should be used which have a higher percentage of non-starch polysaccharides (NSPs) along with starch [5].

To promote growth, protect well-being and maximize the genetic potential of modern broiler and layer hybrids, growth promoting feed additives have been commonly included in poultry diets [6]. The gel-forming characteristic of β-glucan after water absorption is similar to that of other NSPs like pectin and gum arabic [7]. Feeding birds with barley is problematic because of viscosity and adhesivity due to presence of β-glucans during digestion. Supplementing broiler diets with exogenous β-glucanase (EC 3.2.1.4) can decrease the viscosity of the intestinal content by hydrolyzing the β-glucan and enhance nutrient digestibility [7]. B-glucans account for up to 70% of the cell wall in the barley endosperm and β-glucanase can be a valuable tool as a natural way to enhance β-glucan degradation and to improve feed utilization [8].

We know that cereals with high levels of non-starch polysaccharides, like barley, cause electrolytic imbalance of diet. Adding β-glucanase, which can by directly mediate absorption and transmission of minerals through receptors situated on the intestine luminal wall, can prevent acidification of intestine and body fluids, reduce negative effects of body homeostasis imbalance and improve broiler growth and performance through accelerating absorption function of the intestine [9].

Wang et al.[10] investigated the effects of exogenous enzyme supplementation to diets on the growth performance and nutrient digestibility in broiler chickens.

These authors reported that supplementing diets with enzymes can increase digestibility, reduce diet cost, improve growth surface properties and feed efficiency in addition to reducing organic wastes [10]. They also reported that accuracy in selecting enzymes can well improve performance in poultry and supplementing diet with enzyme, apart from economic advantages, can influence environmental conditions and improve diet particles [10]. For investigation of gastrointestinal microbial flora, caecum contents were sampled at the age of 42 days [10].

The aims of current study were to:

a- Extract and purify β-glucanase from the bovine rumen bacterium *Fibrobacter succinogenes*

b- Investigate the effect of supplementation of diets for a local broiler hybrid Golpayegani-Ross with the extracted enzyme on performance, carcass characteristics,

c- Investigate the effect of supplementation of diets for a local broiler hybrid Golpayegani-Ross microbial flora, plasma biochemical parameters and immunity.

MATERIAL and METHODS

Bovine Rumen Fluid

This study started on 22 August 2017 at the microbiology laboratory of the agriculture faculty of Islamic Azad University, Varamin-Pishva Branch, Varamin, Iran. Bovine rumen fluid was collected from a slaughterhouse in Varamin, Iran into sterile tubes and transferred to the microbiology laboratory. Approximately 1 L of rumen fluid was collected, centrifuged for 30 min at 4°C and 1.000 rpm. The transparent supernatant was stored at -20°C.

Preparation of Fibrobacter succinogenes Culture Medium

The composition of the culture medium is shown in *Table 1*.

The solution was heated under vacum to boiling point. Then, the solution was put under CO_2 100% pressure and cooled. Sodium carbonate was then added and the solution was boiled for a further 10 min. The solution was autoclaved with two other separately prepared solutions, 10 mL L-Cysteine. HCl 2.5% and 10 mL $Na_2S.9H_2O$ for 15 min at 121°C. After autoclaving and cooling, the two other solutions (10 mL L-Cysteine. HCl 2.5% and 10 mL $Na_2S.9H_2O$) were added to the main solution. The final pH was set at 6.6±0.1, and the solution was distributed in previously sterilised 50 mL containers. The rumen fluid was injected using a 50 µL sampler. All containers were maintained in 34°C incubator for 2 weeks. The tubes were then taken out and centrifuged at 1.400 rpm for 30 min. Then, 20 µL supernatant was decanted by sampler on microscope lamella and stained for microscopy as explained below:

Table 1. *The composition of theculture medium for bovine rumen bacterium Fibrobacter succinogenes*

Number	Content	Amount
1	KH_2PO_4	0.3 g
2	K_2HPO_4	0.3 g
3	$(NH_4)_2SO_4$	0.3 g
4	NaCl	0.6 g
5	$MgSO_4.7H_2O$	0.12 g
6	$CaCl_2.2H_2O$	0.08 g
7	Tryptone (BD 211705)	1.0 g
8	Yeast extract	0.5 g
9	Resazurin	1.0 mg
10	Cellobiose	4.0 g
11	Vitamin solution[1]	20.0 mL
12	Trace elements[2]	1.0 mL
13	VFA solution[3]	4.65 mL
14	Na_2CO_3	4.0 g
15	Distilled water	960.0 mL

[1] Vitamin solution includes:Lipoic acid, 20.0 mg; Thiamine.HCl, 20.0 mg; Calcium D-(+)-pantothenate, 20.0 mg; Nicotinamide, 20.0 mg; Riboflavin, 20.0 mg; Pyrodoxal hydrochloride 20.0 mg; Pyridoxamine.2HCl, 20.0 mg; p-aminobenzoic acid 1.0 mg; Biotin, 1.0 mg; Cyanocobalamin, 1.0 mg; Distilled water, 100.0 mL; [2] Trace elements include: $ZnSO_4.7H_2O$, 0.1 g; $MnCl_2.4H_2O$, 0.03 g; H_3BO_3 0.2 g; $COCl_2.6H_2O$ 0.2 g; $CuCl_2.2H_2O$ 0.01 g; $NiCl_2.6H_2O$ 0.02 g; $Na_2MoO_4.2H_2O$, 0.03 g; $FeSO_4.7H_2O$ 2.0 g; Distilled water, 1.0 L; [3] Volatile Fatty Acids include: Acetic acid, 17.0 mL; Propionic acid, 6.0 mL; n-Butyric acid, 4.0 mL, n-Valeric acid, 1.0 mL, IsoValeric acid, 1.0 mL, IsoButyric acid, 1.0 mL, DL-alpha-methylbutyric acid, 1.0 mL, Distilled water, 310.0 mL

For preparing smears, 20 µL supernatant was removed from 50 µL containers and put on lamella. Then, crystal violet was poured on lamella for 1-2 min, and washed with distilled water. Then, Lugol's solution was poured for 1 min and washed with distilled water. Ethylic alcohol was poured and washed with distilled water. Safranin was poured for 30 sec to 1 min and washed with distilled water. 100X microscopy was used for identification of bacterium *Fibrobacter succinogenes*.

Enzyme Extraction and Purification

Briefly, 8.7 g potassium phosphate (K_2HPO_4) and 2.5 g citric acid ($C_6H_8O_7$) were mixed with 1 L distilled water to a final pH equal to 6.5.

Substrate Solution

Briefly, 7.5 g β-glucan solution was mixed with 500 mL citrate-phosphate buffer and autoclaved at 121°C for 15 min.

Primary 3,5-Dinitrosalicylic Acid (DNS) Solution

Briefly, 5 g 3,5-dinitrosalicylic acid, 1 g phenol and 5 g sodium hydroxide were dissolved in 500 mL distilled water.

Sodium Phosphate Solution 5%

Briefly, 1 g sodium phosphate (Na_2HPO_4) was dissolved in 20 mL distilled water.

Glucose Solution 5%

Briefly, 1 g glucose was dissolved in 20 mL distilled water.

Secondary 3,5-Dinitrosalicylic Acid (DNS) Solution

Briefly, 0.5 mL sodium phosphate (Na_2HPO_4) and 20 µL glucose solution was completely mixed with 50 mL of primary DNS solution.

Standard Glucose Preparation

The solution was prepared by mixing glucose with distilled water (1 $mg.mL^{-1}$). Briefly, 1080 µL β-glucanase substrate solution was poured into a 1.5-mL microtube and 180 µL centrifuged rumen liquid was added to it. The microtubes were maintained at 37°C in a water bath for 30 min. The microtubes were then removed and the reaction terminated by addition of 1440 µL 3,5-dinitrosalicylic acid. The microtubes were subsequently placed in warm water for staining and after 20 min, the microtubes were removed and immediately cooled. Absorption was measured by a spectrometer at 570 nm. To determine the relationship between absorption and concentration, a standard curve was developed. One $mg.mL^{-1}$ glucose solution was added to the microtubes in volumes 0, 72, 144, 216, 288 and 360 µL. Dinitrosalicylic acid was subsequently added to reach a volume of 2880 µL. The absorption values for samples and standards were read at a wavelength of 570 nm. After reading the absorption of the glucose standard, the concentration was calculated by the formula below:

$y = a + bx$. Enzyme activity is shown in the *Fig. 1*.

According to the calculations, an enzymatic activity of 0.382 IU was observed per g of *Fibrobacter succinogenes*culture medium *(Fig. 1* and *Table 1)*. Consequently, the enzymatic activity of 5.25 g of *Fibrobacter succinogenes*culture equals 2 IU *(Fig. 1)*.

Treatments and Experimental Diets

In this experiment, 120 one-day-old local hybrid broiler chicks (Golpayegani-Ross hybrid) were transferred to the Naranj-Gol Poultry Farm, Rasht, Iran (37.2682° N, 49.5891° E). The experimental protocol was ratified by the Animal Ethic Committee of the Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran, and the experiment was performed with respect to the International Guidelines for research involving animals (Directive 2010/63/EU).

Broilers were randomly allocated to 3 treatments with four replicates/pensper treatment and 10 birds per pen. Chickens were raised under controlled temperature and air condition and continuous 24 h light. Each pen was equipped with a handle pan feeder and a manual drinker. During the whole experiment, water was provided *ad libitum*. The experiment lasted for 49 days and vaccination programs were done regularly.

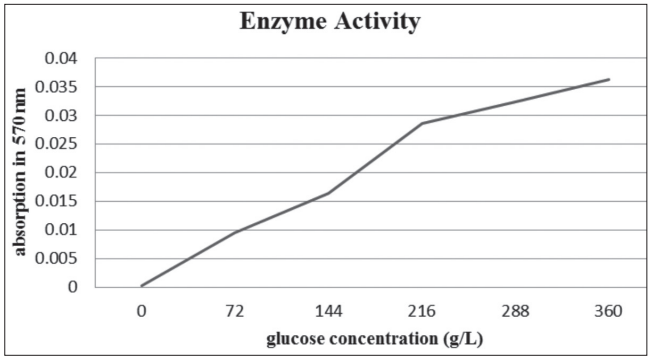

Fig 1. Standard absorption of glucose graph, horizontal axis shows glucose concentration (mg/mL) and vertical axis shows enzyme activity ($mg.min^{-1}.mL^{-1}$)

acid, triglycerides, HDL, HDL cholesterol ratio, LDL, LDL cholesterol ratio, alkaline phosphatase, IgG1, IgG2, IgM1 and IgM2. Tagged birds were injected with 0.2 mL diluted sheep blood (SRBC) and their blood samples were used for hematological tests. At the end of the experiment, 2 birds from each treatment were randomly selected for determination of carcass characteristics including featherless carcass weight, abdomen full carcass weight, eviscerated carcass weight, head weight, breast weight, thigh weight, abdominal fat weight, and neck weight. Microbial flora including anaerobic bacteria, aerobic bacteria, lactobacilli and *Escherichia coli* were counted.

For counting bacteria, colony forming unit method was used. For this purpose, 1 g of caecum contents were added

Table 2. *Ingredient composition of the experimental rations*

Treatments	T1[1]			T2[1]			T3[1]		
	St[2]	Gr[3]	Fn[4]	St[2]	Gr[3]	Fn[4]	St[2]	Gr[3]	Fn[4]
Enzyme (IU)	0	0	0	10 IU Fib.[5]	10 IU Fib.[5]	10 IU Fib.[5]	10 IU com.[6]	10 IU com.[6]	10 IU com.[6]
Barley	20	20	20	20	20	20	20	20	20
Corn	44.675	47.86	51.535	44.675	47.86	51.535	44.675	47.86	51.535
Soybean	30.889	27.045	22.848	30.889	27.045	22.848	30.889	27.045	22.848
Soybean oil	0.238	1.126	1.884	0.238	1.126	1.884	0.238	1.126	1.884
DL-methionine	0.295	0.253	0.236	0.295	0.253	0.236	0.295	0.253	0.236
L-lysine HCl	0.235	0.205	0.211	0.235	0.205	0.211	0.235	0.205	0.211
L-threonine	0.13	0.074	0.068	0.13	0.074	0.068	0.13	0.074	0.068
Di calcium-phosphate	1.783	1.688	1.482	1.783	1.688	1.482	1.783	1.688	1.482
$CaCO_3$	0.804	0.79	0.739	0.804	0.79	0.739	0.804	0.79	0.739
Na-bBicarbonate	0.266	0.265	0.399	0.266	0.265	0.399	0.266	0.265	0.399
NaCl	0.185	0.194	0.098	0.185	0.194	0.098	0.185	0.194	0.098
Vitamin[7] and mineral premix[8]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

[1] basal diet included 20% barley (T1), basal diet included 10 IU extracted β-glucanase and 20% barley (T2) and basal diet included 10 IU, commercial β-glucanase and 20% barley (T3); [2] Starter period (1-14 days); [3] Grower period (15-35 days); [4] Finisher period (36-49 days); [5] β-glucanase enzyme extracted from wild strain F. succinogenes; [6] commercial β-glucanase enzyme; [7] Each kilogram contains: 5.000.000 IU Vit. A, 2.000.000 IU Vit. D_3, 32.000 mg Vit. E, 1.280 mg Vit. K_3, 1.274 mg Vit. B_1, 3.440 mg Vit. B_2, 25.000 mg Vit. B_3, 7.416 mg Vit. B5, 1.944 mg Vit. B_6, 880 mg Vit. B_9, 8 mg Vit. B_{12}, 100 mg Vit. Biotin H_2, Antioxidant 1000 mg; [8] Each kilogram contains: 48.018 mg Mn, 8.092 mg Fe, 44.030 mg Zn, 6.448 mg Cu, 501 mg I, 121 mg Se

The treatments were as follows:

T1 (control): Corn and soybean meal without supplemental enzyme and barley

T2: Corn and soybean meal with 20% barley and 10 IU β-glucanase enzyme extracted from *Fibrobacter succinogenes* (per kg diet)

T3: Corn and soybean meal with 20% barley and10 IU commercial β-glucanase enzyme (Rovabio™) (per kg diet)

Feed ingredients and nutrient percentages are shown in *Table 2* and *Table 3*.

Measured Parameters

Body weight gain and feed consumption were measured during the experiment. The hematological parameters measured were fasting blood sugar (FBS), cholesterol, uric

to 9 mL of phosphate buffer and then serial 10-fold dilutions were prepared. In order to count *Lactobacilli*, sharp agar deman-rogosa was used as the culture medium. To count total anaerobic bacteria, incubation was performed in anaerobic jars for 72 h. For total aerobic bacteria, *Escherichia coli* was extracted from nutrient agar and eosin methylene blue agar aerobically for 24 h [11,12].

On day 20, from each pen 2 birds were tagged and 2 mL defibrinated sheep blood plus 47.5 mL physiologic serum prepared for SRBC were injected intothe breast muscle. One week later, blood was taken from the tagged birds for laboratory tests. Total serum antibody titres to SRBC were determined by hemaglutination assay. For investigating immune response, all birds were vaccinated for Newcastle disease virus (NDV) through drinking water and one week later, blood was taken from 2 birds from each pen and appropriate immunological tests were performed.

Table 3. *Chemical composition of the experimental rations*

Treatment	T1[1]			T2[1]			T3[1]		
	St[2]	Gr[3]	Fn[4]	St[2]	Gr[3]	Fn[4]	St[2]	Gr[3]	Fn[4]
Enzyme (IU)	0	0	0	10 IU Fib.[5]	10 IU Fib.[5]	10 IU Fib.[5]	10 IU com.[6]	10 IU com.[6]	10 IU com.[6]
Dry matter (%)	89.328	89.323	89.299	89.328	89.323	89.299	89.328	89.323	89.299
Crude protein (%)	19.624	18.092	16.527	19.624	18.092	16.527	19.624	18.092	16.527
Energy (ME) (Kcal.kg^{-1})	2800	2900	3000	2800	2900	3000	2800	2900	3000
Calcium (%)	0.840	0.800	0.720	0.840	0.800	0.720	0.840	0.800	0.720
Available phosphorus (%)	0.420	0.400	0.360	0.420	0.400	0.360	0.420	0.400	0.360
Sodium (%)	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160
Lysine (%)	1.1	0.990	0.900	1.1	0.990	0.900	1.1	0.990	0.900
Methionine (%)	0.555	0.498	0.464	0.555	0.498	0.464	0.555	0.498	0.464
Methionine and cysteine (%)	0.825	0.752	0.702	0.825	0.752	0.702	0.825	0.752	0.702

[1] Basal diet included 20% barley (T1), basal diet included 10 IU extracted β-glucanase and 20% barley (T2) and basal diet included 10 IU commercial β-glucanase and 20% barley (T3); [2] Starter period (1-14 days); [3] Grower period (15-35 days); [4] Finisher period (36-49 days)

Data Analysis

The study was conducted using 120 chicks in a completely randomized design with 3 treatments and 4 replicates. The experimental unit was the pen. The results of the study were subjected to one-way analysis of variance (ANOVA) using the GLM procedure of SPSS v 24 for windows [13]. Differences in the experimental treatments were tested using Duncan's Multiple Range Test following ANOVA with significance reported at P≤0.05.

RESULTS

BWG, FI and FCR did not differ between treatments in the starter period, but their effects were significant for BWG in finisher (P>0.05) and whole period (P<0.01), and FCR in whole period (P<0.01). In whole period, the highest BWG (16.2±0.5), the lowest FI (36.3±0.4) and the lowest FCR (2.2±0.1) were all associated with T2 *(Table 4)*.

In the current study, T2 resulted in the lowest FI for whole period but this was not significant (P>0.01). The best FCR in the grower (P<0.01), finisher (P>0.05) and whole periods (P>0.05) was associated with T2 *(Table 4)*.

In the present study, the effects of different treatments were significant for all hematological parameters, except LDL (P<0.01) *(Table 5)*. The highest concentrations of blood cholesterol, HDL and LDL were associated with T2, while the highest concentration of FBS, uric acid, triglycerides, and alkaline phosphatase were associated with T3 (basal diet and 10 IU commercial β-glucanase) (P<0.01).

Treatments did not have a significant effect on antibody titration *(Table 6)*. The highest concentrations of IgG1, IgG2 and IgM1 were associated with T3 (P>0.05) *(Table 6)*.

The highest non-eviscerated carcass weight (P<0.05) and eviscerated carcass weight (P>0.05) belonged to T2 *(Table 7)*. The highest head weight (P<0.05), breast weight (P>0.05), tight weight (P<0.01) and abdominal fat weight (P<0.05) were associated with the T2 *(Table 7)*.

T2 resulted in a significant increase of lactobacilli and *Escherichia coli* in broilers' duodenum (P<0.05) *(Table 8)*.

DISCUSSION

Poultry do not produce enzymes that hydrolyze the NSPs in the cell wall if grains which remain without decomposition and cause a reduction in feed efficiency. Supplementation with preparations of exogenous enzymes in the diet is one approach to overcoming the adverse effects of NSPs. This has a positive effect on feed digestibility and leads to better productivity and performance [5].

Our results contrast with those of Agah et al.[14] who reported that enzyme supplementation had no significant effect on BWG and FI in comparison with an unsupplemented control in the starter, growth and whole periods. The basal diet with 10 IU extracted β-glucanase and 20% barley (T2) resulted in a significant increase in BWG for the whole period (P<0.01). This is in agreement with the finding of Abudabos [15] who reported that enzyme supplementation significantly increased body weight at 42 and 49 d of age. The strain used in that study was Cobb 400 while the strain used in current study was Golpayegani-Ross hybrid. The enzyme used in that study was Bergazyme, a commercial enzyme supplement that contains β-pentosanase, α-amylase, β-glucanases, glucanases, and galactomannases.

Our results are in agreement with Mathlouthi et al.[16] who reported that during the whole experimental period (1-40 d) FCR was decreased (P≤0.05) by β-glucanase supplementation. Our results are however, in inconsistent with those of Kalantar et al.[17] who reported that supplementing wheat and barley diets with multi-enzymes increased total feed intake and decreased feed conversion ratio significantly (P<0.05) compared to non supplementation. Moreover, our results are inconsistent

Table 4. *BWG, FI and FCR in chicks fed the experimental rations; means ± standard deviation*

Items	Experimental Periods	T1[1]	T2[1]	T3[1]	P-value	SEM
Body weight gain (g/bird/day)	Starter (1-14d)	15.6±0.8[a]	16.8±1.1[a]	16.1±0.5[a]	0.19	0.27
	Grower (15-35d)	13.2±1.6[a]	12.7±1.1[a]	13.7±0.7[a]	0.41	0.35
	Finisher (36-49d)	14.0±2.4[a]	20.9±3.8[a]	15.8±3.4[a]	0.03	1.22
	Whole (1-49d)	14.1±0.7[b]	16.2±0.5[a]	15.0±0.6[ab]	0.00	0.30
Feed intake (g/bird/day)	Starter (1-14d)	19.1±1.1[a]	20.8±1.1[a]	20.4±1.4[a]	0.18	0.38
	Grower (15-35d)	37.3±0.7[ab]	36.3±0.3[b]	38.0±0.5[a]	0.00	0.25
	Finisher (36-49d)	53.2±2.0[a]	50.6±0.5[a]	52.2±1.5[a]	0.08	0.50
	Whole (1-49d)	37.0±0.7[a]	36.3±0.4[a]	37.4±0.6[a]	0.07	0.21
Feed conversion ratio (g/g)	Starter (1-14d)	1.2±0.1[a]	1.2±0.1[a]	1.3±0.0[a]	0.67	0.02
	Grower (15-35d)	2.9±0.4[a]	2.9±0.3[a]	2.7±0.1[a]	0.76	0.08
	Finisher (36-49d)	3.9±0.8[a]	2.5±0.5[a]	3.4±0.8[a]	0.05	0.25
	Whole (1-49d)	2.6±0.1[a]	2.2±0.1[b]	2.5±0.1[a]	0.00	0.05

[a-c] Means within each row with no common superscripts differ significantly at P≤0.05; [1] basal diet (T1), basal diet included 10 IU extracted β-glucanase (T2) and basal diet included 10 IU commercial β-glucanase (T3)

Table 5. *Hematological parameters in chicks fed the experimental rations; means ± standard deviation*

Parameters	T1[1]	T2[1]	T3[1]	P-value	SEM
FBS[2] (mg/dL)	1.5±0.1[c]	2.1±0.1[b]	2.4±0.1[a]	<0.001	2.01
Cholesterol (mg/dL)	142.3±1.0[b]	149.5±1.9[a]	125.3±1.0[c]	<0.001	3.08
HDL[3] (mg/dL)	92.00±0.8[b]	97.5±1.3[a]	76.0±0.8[c]	<0.001	2.76
HDL/cholesterol ratio	1.6±0.0[a]	1.4±0.1[b]	1.5±0.1[ab]	<0.001	0.02
LDL[4] (mg/dL)	37.8±1.50[b]	42.5±1.9[a]	34.0±1.6[b]	<0.001	1.13
LDL/cholesterol ratio	0.4±0.1[b]	0.4±0.0[a]	0.4±0.1[a]	0.06	0.01
Triglyceride (mg/dL)	62.8±1.0[b]	64.5±1.3[a]	70.8±2.2[a]	<0.001	4.70
Alkaline phosphatase (U/L)	5055±12.9[b]	5472.5±17.1[a]	5477.5±12.6[a]	<0.001	59.81
Uric acid (mg/dL)	1.5±0.1[c]	2.1±0.1[b]	2.4[a]±0.1	<0.001	0.12

[a-c] Means within each row with no common superscripts differ significantly at P≤0.05; [1] basal diet (T1), basal diet included 10 IU extracted β-glucanase (T2) and basal diet included 10 IU commercial β-glucanase (T3); [2] Fasting blood sugar; [3] High Density Lipoprotein; [4] Low Density Lipoprotein

Table 6. *Immunological parameters (log 10) in chicks fed the experimental rations; means ± standard deviation*

Parameters	T1[1]	T2[1]	T3[1]	P-value	SEM
Immunoglobulin G1	1.8±1.0[a]	2.0±1.4[a]	2.5±1.0[a]	0.65	0.31
Immunoglobulin G2	3.0±1.4[a]	3.0±0.8[a]	4.0±1.4[a]	0.45	0.35
Immunoglobulin M1	3.0±1.4[a]	2.0±1.2[a]	3.3±1.0[a]	0.33	0.35
Immunoglobulin M2	2.5±0.6[a]	3.3±1.0[a]	2.5±0.6[a]	0.29	0.21

[a-c] Means within each row with no common superscripts differ significantly at P≤0.05; [1] basal diet (T1), basal diet included 10 IU extracted β-glucanase (T2) and basal diet included 10 IU commercial β-glucanase (T3)

with those of Zou et al.[18] who reported there were no significant difference in BWG and FI of broilers for enzymes BM (a commercial β-mannanase product), AG (a commercial galactosidase product), and XG (a commercial product containing endo-xylanase and β-glucanase).

In the current study, adding enzymes had a significant positive effect on performance. Enzymes can decrease viscosity and enhance feed digestibility and feed intake.

Since β-glucans are structural carbohydrates and have high molecular weight, they exist in soluble form in small intestine [19]. Therefore, intestinal contents are in viscous soluble form, which prevents free moving of other soluble contents [19]. Under such conditions, water absorption capacity increases in the intestine, which in turn, increases the viscosity of the contents and ultimately restricts nutrient absorption [19].

Studies have shown that adhesive effects of β-glucans can be mitigated through enzymatic hydrolysis by β-glacanases [20]. Enzymes are able to hydrolyze adhesive polymers (soluble non-starch polysaccharides) and convert them into small polymers through complete hydrolysis thereby decreasing viscosity of digesta and releasing nutrients encapsulated by these viscous compounds [20].

Table 7. *Carcass characteristicsin chicks fed the experimental rations; means ± standard deviation*

Items	T1[1]	T2[1]	T3[1]	P-value	SEM
Featherless carcass (g)	559.50±17.6[a]	624.0±58.9[a]	536.5±72.0[a]	0.11	18.11
Non-eviscerated carcass (g)	486.4±6.2[b]	559.0±46.8[b]	471.0±67.0[a]	0.05	16.92
Eviscerated carcass (g)	289.0±97.0[a]	371.8±34.8[a]	298.5±37.2[a]	0.18	19.92
Dressing percentage (%)	42.7±14.2[a]	47.8±4.2[a]	41.4±4.9[a]	0.58	2.50
Head (g)	26.5±1.5[a]	29.2±1.8[ab]	27.1±3.5[a]	0.01	0.72
Breast (g)	80.1±1.2[a]	85.1±8.4[a]	75.2±14.3[a]	0.38	2.78
Tight (g)	117.2±4.8[b]	149.2±18.1[a]	112.0±15.4[b]	<0.00	6.15
Abdominal fat (g)	8.6±3.7[b]	20.9±5.5[a]	12.7±5.9[ab]	0.02	2.04

[a-c] Means within each row with no common superscripts differ significantly at P≤0.05; [1] basal diet (T1), basal diet included 10 IU extracted β-glucanase (T2) and basal diet included 10 IU commercial β-glucanase (T3)

Table 8. *Deodenal bacteria (log CFU/mL) in chicks fed the experimental rations; means ± standard deviation*

Items	T1[1]	T2[1]	T3[1]	P-value	SEM
Aerobic bacteria	6.6±0.3[a]	6.7±0.4[a]	7.1±0.3[a]	0.14	0.10
Lactobacilli	7.3±0.0[a]	7.6±0.3[a]	7.1±0.3[a]	0.03	0.08
Anaerobic bacteria	6.7±0.3[a]	7.0±0.3[a]	7.0±0.3[a]	0.31	0.08
Escherichia coli	6.5±0.2[b]	7.1±0.3[a]	6.5±0.1[b]	0.002	0.10

[a-c] Means within each row with no common superscripts differ significantly at P≤0.05; [1] basal diet (T1), basal diet included 10 IU extracted β-glucanase (T2) and basal diet included 10 IU commercial β-glucanase (T3)

Viscosity increases small intestine length and weight in different ways. First, NSP compositions, by stretching the intestine wall, apply pressure to the wall and its muscular layers, which causes an increase in sarcomere length and the muscular layer myofibrils to combat the applied pressure. Moreover, an increase in the length and width of intestinal wall muscles is an adaptation mechanism by the bird to make viscous materials move in its intestine. The second mechanism by which viscosity increases intestine length is probably bird need to nutrients [5]. The beneficial effects of enzyme addition are probably associated with an increase in nutrient consumption through decreasing intestine viscosity and removing anti-nutritional effects of non-starch polysaccharides [5].

In the case of the triglyceride content our findings comply with those of Abudabos [15] who reported an increase of broilers' triglyceride concentration due to enzyme supplementation.

It has been shown that β-glucan can enhance glucose and insulin hemostasis and decrease blood cholesterol level [21]. For this reason, it can be proposed that in the current study enzyme addition results in an increase in blood cholesterol level through digesting β-glucan content.

According to our results, cholesterol content for treatment 2 was higher than in treatment 3. Serum cholesterol is affected by diet and factors, such as non-starch polysaccharides, can have an effective role in this matter.

Nahas and Lefrancois [22] stated that NSPs in barley are effective in transferring and metabolism of lipids and can reduce blood cholesterol level. Increase of NSP levels in diet causes decrease of lipid digestion and probably destroys cholesterol reabsorption cycle through adhesivity and decreases in blood cholesterol. Moreover, short chain fatty acids resulting from NSP digestion in large intestine can stop cholesterol synthesis through excretion of bile acids and secretion of natural steroids [23].

Cholesterol increase in the group supplemented with enzyme extracted from *Fibrobacter suuccinogenes*, apart from its high purity, increases digestibility, decreases viscosity of digesta in gastrointestinal tract and improves better digestion of it including fatty acids [24]. As a result, cholesterol content is increased in birds consuming enzyme additive extracted from *Fibrobacter suuccinogenes* in comparison with the control group and the third group.

Our results are in agreement with those of Basmacioğlu Malayoğlu et al.[25] who reported that enzyme supplementation had no significant effect on immune response of broilers fed on wheat–soybean meal diets. Our results agree with those of Khaksar et al.[26] who reported antibody production against sheep red blood cell (SRBC) antigen and other haematological analysis were numerically (P>0.05) enhanced in broilers fed wheat-based diet with the addition of Endofeed W enzyme, containing arabinoxylanase and β-glucanse. Seidavi et al.[27] reported significant increase of IgG in broilers fed enzyme and probiotic supplemented diets after the second challenge with SRBC (P<0.01). Changes observed in immune response may be attributed to changes in broilers' intestinal microbial population since microorganisms naturally living in the intestinal tract are crucial for poultry digestion and immunity [27].

Our results are in agreement with those of Agah et al.[14] in that enzyme supplementation had no significant effect on breast weight and tight weight. In the case of dressing percentage, our results are in agreement with Abudabos [15] who reported that this parameter was slightly increased by enzyme supplementation. He suggested that the difference in dressed yield of broilers could be due to the different diets and kind and level of enzyme used [15]. In the case of abdominal fat weight, our results are in agreement with those of Agah et al.[14] and Mathluthi et al.[16] who reported that abdominal fat weight was not affected by the addition of enzyme in broiler feed.

Our results contrast with those of Mathluthi et al.[16] who reported a decrease in the number of *E. coli* in the caeca of broilers due to the addition of xylanase and β-glucanase. Khaksar et al.[26] reported increase in ileal microbial population of Lactobacillus and decrease in ileal microbial population of *E. coli*.

It is concluded that the basal diet with 10 IU extracted β-glucanase and 20% barley (T2) resulted in a significant increase in body weight gain and decreased feed intake over the whole experimental period. This treatment enhanced FCR over the whole experimental period ($P<0.05$).

REFERENCES

1. Burnet MC, Dohnalkova AC, Neumann AP, Lipton MS, Smith RD, Suen G: Evaluating models of cellulose degradation by *Fibrobacter succinogenes* S85. *PLoS ONE,* 10 (12): e0143809, 2015. DOI: 10.1371/journal.pone.0143809

2. Gong JH, Egbosima EE, Forsberg CW: Cellulose binding proteins of *Fibrobacter succinogenes* and the possible role of a 180-KDa cellulose-binding glycoprotein in adhesion to cellulose. *Can J Microbiol*, 42 (5): 453-460, 2011. DOI: 10.1139/m96-062

3. Shahir MH, Moradi S, Afsarian O, Heydarinia A: Effect of adding enzyme and organic acid in corn and wheat based diets on performance and intestinal morphological characteristics of broiler chicken. *Iranian J Anim Sci Res,* 3 (4): 351-362, 2012.

4. Zarghi H, Golian A, Kermanshahi H: The effect of triticale and enzyme cocktail (xylanase and β-glucanase) replacement in grower diet on performance, digestive organ relative weight, gut viscosity and gut morphology of broiler chickens [in Persian]. *Iranian J Anim Sci Res,* 2 (1): 298-312, 2016.

5. Algawany M, Elnesr SS, Farag MR: The role of exogenous enzymes in promoting growth and inproving nutrient digestibility in poultry. *Iranian J Vet Res,* 19 (3): 157-164, 2018. DOI: 10.22099/ijvr.2018.4932

6. Sarangi NR, Babu LK, Kumar A, Pradhan CR, Pati PK, Mishra P: Effect of dietary supplementation of prebiotic, probiotic, and synbiotic on growth performance and carcass characteristics of broiler chickens. *Vet World*, 9 (3): 313-319, 2016. DOI: 10.14202/vetworld.2016.313-319

7. Yu B, Hsu JC, Chiou PWS: Effects of β-glucanase supplementation of barley diets on growth performance of broilers. *Anim Feed Sci Technol,* 70, 353-361, 1998. DOI: 10.1016/S0377-8401(97)00078-3

8. Jian-yi S, Wei-fen L, Zi-rong X, Sai-hong G: Purification and some properties of a β-glucanase from a strain trichoderma reesei GXC. *Zhejiang Univ Sci,* 3 (1): 106-112, 2002. DOI: 10.1007/BF02881853

9. Murakami AE, Franco JRG, Martins EN, Rondon O, Sakamato MI, Pereira MS: Effect of electrolyte balance in low-protein diets on broiler performance and tibial dyschonroplasia incidence. *J Appl Poult Res*, 12, 207-216, 2003. DOI: 10.1093/japr/12.2.207

10. Wang ZR, Qiao SY, Lu WQ, Li DF: Effect of enzyme supplementation on performance, nutrient digestibility gastrointestinal morphology and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. *Poult Sci*, 84, 875-881, 2005. DOI: 10.1093/ps/84.6.875

11. Milanković B, Ćirić J, Krstić M, Starčević M, Baltić B, Šefer D, Đorđević V, Popović M, Marković R: Effect of dietary fatty acid pattern on growth performance, carcass characteristics, fatty acid profile, and serum biochemistry parameters in broiler chickens. *Kafkas Univ Vet Fak Derg,* 25 (4): 507-516, 2019. DOI: 10.9775/kvfd.2018.21205

12. Poorghasemi M, Chamani M, Mirhosseini SZ, Sadeghi AA, Seidavi A: Effect of probiotic and different sources of fat on performance, carcass characteristics, intestinal morphology and ghrelin gene expression on broiler chickens. *Kafkas Univ Vet Fak Derg,* 24 (2): 169-178, 2017. DOI: 10.9775/kvfd.2017.18433

13. SPSS Inc: Statistical Package for Social Sciences Study. SPSS for Windows, Version 24. Chicago SPSS Inc., USA, 2016.

14. Agah MJ, Hashemi SMR, Noorolahi H, Hashemi M, Movasaghi S: Performance, carcass characteristics and some parameters of the gastrointestinal tract of broiler chickens fed with diets based on corn and triticale with or without the addition of enzyme supplement. *Anim Prod Res,* 7 (2): 35-46, 2018. DOI: 10.22124/ar.2018.7300.1201

15. Abudabos A: Enzyme supplementation of corn-soybean meal diets improves performance in broiler chickens. *Int J Poult Sci,* 9 (3): 292-297, 2010. DOI: 10.3923/ijps.2011.157.159

16. Mathlouthi N, Ballet N, Larbier M: Influence of beta-glucanase supplementation on growth performances and digestive organs weights of broiler chickens fed corn, wheat and barley-based diet. *Int J Poult Sci,* 10 (2): 157-159, 2011. DOI: 10.3923/ijps.2011.157.159

17. Kutlu HR, Saber SN, Kutay H, Celik L, Uzun Y, Toy N, Kutlu M, Yucelt O, Burgut A, Thiery P, Yavuz B: Effect of multi-enzyme produced by a single fungus on growth performance and some carcass parameters of broiler chicks fed on maize-soya based diets. *Kafkas Univ Vet Fak Derg,* 25 (2): 221- 230, 2019. DOI: 10.9775/kvfd.2018.20765

18. Zou J, Zheng P, Zhang K, Ding X, Bai S: Effects of exogenous enzymes and dietary energy on performance and digestive physiology of broilers. *J Anim Sci Biotechnol*, 4:14, 2013. DOI: 10.1186/2049-1891-4-14

19. Choct M, Annison G, Trimble RP: Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomized broiler chickens. *J Nutr*, 122, 2457-2465, 1992. DOI: 10.1093/jn/122.12.2457

20. Daymeh S, Afzali N, Bashtini M: Effect of revabio in diets containing wheat bran on growth performance, some blood metabolites and absorbing of mineral elements in broilers chickens. *Res Anim Prod*, 7, 33-43, 2016. DOI: 10.1016/j.carbpol.2005.11.033

21. Ajithkumar A, Andersson R, Siika-aho M, Tenkanen M, Aman P: Isolation of cellotriosyl blocks from barley B-glucan with endo-1,4-b-glucanase from *Trichoderma reesei*. *Carbohydr Polym,* 64, 233-238, 2006. DOI: 10.1016/j.carbpol.2005.11.033

22. Nahas J, Lefrancois MR: Effects of feeding locally grown whole barly with or without enzyme addition and whole wheat on broiler performance and carcass traits. *Poult Sci*, 80, 195-202, 2001. DOI: 10.1093/ps/80.2.195

23. Jenkis DJA, Axelsen M, Kendall CW, Augustin LSA, Vulsa V, Smith U: Dietary fibre, lente carbohydrates and the insulin-resistant diseases. *Br J Nutr*, 83, S157-S163, 2000. DOI: 10.1017/s0007114500001100

24. Smits CHM, Annison G: Nonstarch plant polysaccharides in broiler nutrition toward a physiologically valid approach to their determination. *World Poult Sci J*, 52, 203-221, 1996. DOI: 10.1079/WPS19960016

25. Basmacioğlu Malayoğlu H, Baysal Ş, Misirlioğlu Z, Polat M, Turan N, Yilmaz H: Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat-soybean meal diets. *Br Poult Sci,* 51 (1): 67-80, 2010. DOI: 10.5897/AJB12.1237

26. Khaksar V, Golian A, Kermanshahi H: Immune response and ileal microflora in broilers fed wheat-based diet with or without enzyme endofeed W and supplementation of thyme essential oil or probiotic primaLac. *Afr J Biotechnol,* 11 (81): 14716-14723, 2012.

27. Seidavi A, Dadashbeiki M, Alimohammadi-Saraei MH, van den Hoven R, Payan-Carreira R, Laudadio V, Tuffarelli V: Effects of dietary inclusion level of a mixture of probiotic cultures and enzymes on broiler chickens immunity response. *Environ Sci Pollut Res*, 24 (5): 4637-4644, 2017. DOI: 10.1007/s11356-016-8206-8