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Research Article

Effect of Probiotic and Different Sources of Fat on Performance, Carcass Characteristics, Intestinal Morphology and Ghrelin Gene Expression on Broiler Chickens

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Abstract

The effect of Lactofeed probiotic and different sources of fat on performance, carcass characteristics, intestinal morphology and ghrelin gene expression of broiler chickens was studied in an experiment using a total of 240 one-day-old male chickens from commercial strain (Ross 308) in a completely randomized design via 6 treatments with 4 replicates (10 birds per replicate). The experimental diets included: (1) basal diet (control); (2) diet containing 3% animal fat from tallow (fat); (3) diet containing 3% plant oil from soybean (oil); (4) control + probiotic; (5) probiotic + (fat) and (6) probiotic + (oil). The results showed some improvement in performance in the third group ($P<0.05$). A significant difference in the length, width and depth of crypt was observed between the treatments 3 and 4, and the control group ($P<0.05$). There was a significant difference in ghrelin gene expression of the treatments 2 and 4 in comparison with the control group ($P<0.05$). The results generally showed that there were benefits from the separate use of probiotic and soybean oil in the diet of broiler chicken.

Keywords: *Broiler, Fat, Feed intake, Lactofeed, Performance*

Broiler Tavuklarda Probiyotik ve Değişik Kaynaklı Yağların Performans, Karkas Özellikleri, Barsak Morfolojisi ve Grelin Gen Ekspresyonu Üzerine Etkisi

Öz

Toplam 240 adet bir günlük Ross 308 erkek civciv kullanılarak, tamamen rastgele dizaynda 6 uygulama ve 4 tekrar olmak üzere (her tekrarda 10 hayvan) laktofed probiyotik ve farklı kaynaklı yağ ile beslemenin performans, karkas özellikleri, barsak morfolojisi, bağışıklık sistemi, karaciğer enzimleri, kan parametreleri ve grelin gen ekspresyonu üzerine etkisi incelenmiştir. Deneysel diyetler; (1) bazal diyet (kontrol); (2) %3 donyağı kaynaklı hayvansal yağ içeren diyet; (3) %3 soya fasulyesi kaynaklı bitkisel yağ içeren diyet; (4) kontrol + probiyotik; (5) probiyotik + hayvansal yağ ve (6) probiyotik + bitkisel yağ. Elde edilen sonuçlar üçüncü grupta bazı iyileşmelerin oluştuğunu gösterdi ($P<0.05$). 3. ve 4. gruplarda kontrole göre kript uzunluğu, genişiliği ve derinliğinde anlamlı oranda fark gözlemlendi ($P<0.05$). Kontrol grubu ile karşılaştırıldığında 4. grubun bağışıklık sisteminda anlamlı artış belirlendi ($P<0.05$). 4. grup kontrol grubu ile karşılaştırıldığında AST, ALP ve trigliserid konsantrasyonları anlamlı oranda düşüktü. Kontrol grubuna kıyasla 2. ve 4. grupların grelin gen ekspresyonları anlamlı derecede fark gösterdi ($P<0.05$). Sonuçlar genel olarak broiler tavuklarda probiyotik ve soya fasulyesi yağının ayrı kullanımının daha faydalı olduğunu göstermiştir.

Anahtar sözcükler: *Broiler, Yağ, Yem tüketimi, Laktofed, Performans*

INTRODUCTION

In recent years, the use of additives such as growth promoters has been common in poultry nutrition. In earlier times, using different types of antibiotics in order to protect health, prevent diseases and disorders caused

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by environmental pollution, and growth promoters to increase production, was considered acceptable in livestock and poultry industry. Excessive use of antibiotics in livestock and poultry industry has though caused concerns for consumers, due to an increase of bacterial resistance, in-tissue survival and incidence of dangerous diseases. To find a safe additive to replace antibiotics (to stimulate growth and improve the health of farm animals) is a difficult task. According to different reports, the use of probiotics in poultry nutrition increases performance efficiency [1]. Probiotics, as alternatives to antibiotics and additives, are microbial populations that have a positive effect on improving animal performance and strengthening the immune system by balancing the intestinal flora and preventing gastrointestinal infections [2]. Using fat in the diet could have many benefits. One of these benefits is the longer transit time of food, improving the rate of digestion and absorption of nutrients [3]. It is likely that fat, with the effect on transit time of food, increases digestibility and absorption of other nutrients by enhancing enzyme function and more presentation in the places of absorption [4]. Fat contains high energy, so adding fat to the diet will increase metabolizable energy. However, it is necessary for the absorption of fat into the hepatic portal system that causes micelle formation. Micelles of bile salts, fatty acids, monoglycerides and glycerol bind to fatty acids, monoglycerides and facilitate absorption of fat-soluble vitamins [5].

Thus for absorption of fat, the presence of plenty of bile salts and saturated fatty acids and unsaturated balance are essential. However, the composition of bile acids is changed by the action of microbial flora in the digestive tract. Change in bile acids by intestinal microbial flora (deconjugation and dehydroxylation) damages absorption of fats, and their toxic breakdown products can reduce growth [6]. In addition, today the lactic acid-producing bacteria (*Lactobacillus, Bifidobacterium* and *Streptococci)* are used for the construction of probiotics [7]. These bacteria give enzymic hydrolysis of bile acid and with bile acid dissolution, emulsification of fats and construction of micelles will occur as a result of reduced fat absorption. Therefore, with the use of probiotics in the diet, the small intestine bacterial population increases and this may reduce the digestibility of dietary fat. It seems that the effect of probiotics on the absorption of fat is a function of the amount and type of fat in the diet [8]. On the other hand, it is possible that growth hormone secretion is affected by the additives and dietary ingredients. Ghrelin is one of those hormones. This hormone affects appetite regulation and results in body weight gain [9]. Nowadays, there is less attention to hormones and the factors in dietary ingredients which affect hormone secretion, because of the focus of poultry breeders on performance. Therefore, this study was done to compare the efficacy of probiotic and type fat in the diet on performance, carcass characteristics, intestinal morphology and ghrelin gene expression of broiler chicks.

MATERIAL and METHODS

Chickens, Diets and Management

Research on animals was conducted in Rudsar, Iran (37.1378° N, 50.2836° E) and all the procedures used were approved by the Ethics Committee in Animal Use (Approval date: 10/05/2016; No: 10038). The experiment, in a completely randomized design with 6 treatments and 4 replicates using 240 1-day-old male chicks of strain Ross 308, was conducted in the starter period (1-10 day), grower period (11-28) and finisher period (29-42). Each replication included 10 chicks. The experimental diets were formulated by using Ross-308 *(Table 1, Table 2* and *Table 3)* and animal and poultry feed formulation (WUFFDA) software. During the period, all conditions were similar for chickens, and the feeding was ad libitum for the whole period. The basal diet, based on corn and soybean meal, was balanced. Diets used in the experiment were isocaloric and isonitrogenic. Experimental diets included: (1) basal diet (control); (2) diet containing 3% animal fat from tallow; (3) diet containing 3% plant oil from soybean; (4) basal diet + probiotic; (5) probiotic + diet containing 3% animal fat from tallow and (6) probiotic + diet containing 3% plant oil from soybean. The Lactofeed probiotic preparation was declared to contain *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium* and *Enterococcus faecium* ($1x10^{11}$) c.f.u. per kg by the manufacturer.

Performance

The following growth performance variables were evaluated: production index, feed costs per kg live weight, body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). The birds were weighed on the first day of the experiment, then weighed weekly throughout the remaining experimental period (7 to 42 d of age). Feed was provided weekly and the leftover fed was weighed weekly for calculating the feed conversion ratio. At day 42 of each replicate, a bird was selected and blood samples were collected from the wing veins.

Carcass Characteristics, Intestinal Morphology

At the end of the experimental period, one chicken per replicate (four chickens per treatment) was randomly (close to average weight) selected, and the digestive system was taken out of the carcass after slaughtering. Then, the percentages of different parts including carcass, breast, thigh and abdominal fat were calculated based on live weight. The different parts of small intestine were separated in order to investigate its morphology. Then, one centimeter pieces from the middle parts of duodenum and jejunum were disconnected. The separated pieces were evacuated of the intestinal contents and tissue blocks were prepared from the tissue samples of duodenum and jejunum after stabilization, dewatering, clarification and placement in paraffin [10]. The lams were studied after

Table 1. *Ingredients and calculated analyses of the basal diets in the starter period*

Ingredients (kg)	Starter (0-10 days old)					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Corn grain	63.35	53.76	52.30	63.35	53.76	52.30
Soybean meal	22.57	30.5	35.50	22.57	30.5	35.50
Corn gluten meal	9.3	8	4.20	9.3	8	4.20
Tallow	-	3	-	-	3	-
Soybean oil	-	-	3	-	-	3
Dicalcium phosphate	2.25	2.2	2.15	2.25	2.2	2.15
Calcium carbonate	1.02	1.18	1.50	1.02	1.18	1.50
Sodium bicarbonate	0.29	0.27	0.24	0.29	0.27	0.24
Salt	0.22	0.18	0.20	0.22	0.18	0.20
L-lysine	0.28	0.25	0.20	0.28	0.25	0.20
DL-methionine	0.20	0.14	0.19	0.20	0.14	0.19
Vitamin and mineral permix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Probiotic[2]	-	-	-	0.02	0.02	0.02
Filler	0.02	0.02	0.02	-	-	-
Total	100	100	100	100	100	100
Nutrient						
ME (kcal/kg)	3000	3000	3000	3000	3000	3000
CP (%)	22	22	22	22	22	22
Ca (%)	0.95	1.01	0.95	0.95	1.01	0.95
P (%)	0.47	0.46	0.47	0.47	0.46	0.47
Methionine (%)	0.65	0.47	0.50	0.65	0.47	0.50
Lysine (%)	1.18	1.1	1.13	1.18	1.1	1.13
Methionine + Cysteine (%)	0.98	0.79	0.81	0.98	0.79	0.81
Threonine (%)	0.71	0.72	0.73	0.71	0.72	0.73
Tryptophan (%)	0.21	0.20	0.21	0.21	0.20	0.21
Arginine (%)	1.27	1.18	1.27	1.27	1.18	1.27
Valine (%)	0.91	0.92	0.92	0.91	0.92	0.92
Na (%)	0.16	0.16	0.16	0.16	0.16	0.16
K (%)	0.70	0.72	0.81	0.70	0.72	0.81
Cl (%)	0.21	0.21	0.21	0.21	0.21	0.21

[1] Each kg (DM basis) of vitamin and mineral premix contained: vit A: 11.000 IU; vit D_3: 2.000 IU; vit E: 18 IU; vit K: 4 mg; vit B_{12}: 0.015 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Calcium pantothenic acid: 12.0 mg; Niacin: 30.0 mg; Pyridoxine: 2.9 mg; Folic acid: 1.0 mg; Choline: 260.0 mg; Manganese: 64.5 mg; Zinc: 33.8 mg; Iron: 100.0 mg; Copper: 8.0 mg; Iodine: 1.9 mg; Selenium: 0.25 mg
T1: Basal diet (control); T2: Diet containing 3% animal fat from tallow; T3: Diet containing 3% plant oil from soybean; T4: Basal diet + Probiotic; T5: Probiotic + Diet containing 3% animal fat from tallow; T6: Probiotic + Diet containing 3% plant oil from soybean
[2] 0.02 kg of probiotic was added to starter diet to constitute the probiotic groups
ME: Metabolizable energy; CP: Crude protein

coloring (Alcian blue) by optical microscope and using an Eyepiece Graticule. The length and width of villus and depth of crypt were measured and villus length to crypt depth ratio was determined [10].

Ghrelin Gene Expression

One proventriculus tissue sample was taken from each replicate, washed with 10X phosphate buffered saline solution and transferred to a liquid nitrogen tank. Tissue samples were held at -80°C until the extraction of RNA. The samples were first homogenized for RNA extraction. For this purpose, some of the tissue was smashed and put in a mortar and a uniform powder prepared using liquid nitrogen. The extraction kit, Rneasy Mini Kit (QIAGEN), was used in order to extract RNA from biologic samplesaccording to the protocol. The sequence of the primers used for investigating ghrelin gene included some primers for Real time PCR and some primers related to

Table 2. *Ingredients and calculated analyses of the basal diets in the grower period*

Ingredients (kg)	Grower (11-28 days old)					
	T1	T2	T3	T4	T5	T6
Corn grain	61.37	55.39	59.31	61.37	55.39	59.31
Soybean meal	19.00	28.80	30.80	19.00	28.80	30.80
Corn gluten meal	15	8.80	2.75	15	8.80	2.75
Tallow	-	3	-	-	3	-
Soybean oil	-	-	3	-	-	3
Dicalcium phosphate	2.05	1.90	1.95	2.05	1.90	1.95
Calcium carbonate	0.96	1.00	0.95	0.96	1.00	0.95
Sodium bicarbonate	0.61	0.20	0.23	0.61	0.20	0.23
Salt	0.25	0.22	0.20	0.25	0.22	0.20
L-lysine	0.18	0.12	0.16	0.18	0.12	0.16
DL-methionine	0.15	0.04	0.12	0.15	0.04	0.12
Vitamin and mineral permix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Probiotic[2]	-	-	-	0.01	0.01	0.01
Filler	0.02	0.02	0.02	0.01	0.01	0.01
Total	100	100	100	100	100	100
Nutrient						
ME (kcal/kg)	3050	3050	3050	3050	3050	3050
CP (%)	21.50	21.50	21.50	21.50	21.50	21.50
Ca (%)	0.87	0.86	0.87	0.87	0.86	0.87
P (%)	0.43	0.43	0.43	0.43	0.43	0.43
Methionine (%)	0.69	0.37	0.44	0.69	0.37	0.44
Lysine (%)	0.70	0.96	1.00	0.70	0.96	1.00
Methionine + Cysteine (%)	1.02	0.69	0.72	1.02	0.69	0.72
Threonine (%)	0.69	0.71	0.65	0.69	0.71	0.65
Tryptophan (%)	0.16	0.19	0.19	0.16	0.19	0.19
Arginine (%)	1.00	1.16	1.12	1.00	1.16	1.12
Valine (%)	1.29	0.91	0.81	1.29	0.91	0.81
Na (%)	0.16	0.16	0.16	0.16	0.16	0.16
K (%)	0.54	0.70	0.74	0.54	0.70	0.74
Cl (%)	0.20	0.20	0.20	0.20	0.20	0.20

[1] Each kg (DM basis) of vitamin and mineral premix contained: vit A: 11.000 IU; vit D_3: 2.000 IU; vit E: 18 IU; vit K: 4 mg; vit B_{12}: 0.015 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Calcium pantothenic acid: 12.0 mg; Niacin: 30.0 mg; Pyridoxine: 2.9 mg; Folic acid: 1.0 mg; Choline: 260.0 mg; Manganese: 64.5 mg; Zinc: 33.8 mg; Iron: 100.0 mg; Copper: 8.0 mg; Iodine: 1.9 mg; Selenium: 0.25 mg
T1: Basal diet (control); T2: Diet containing 3% animal fat from tallow; T3: Diet containing 3% plant oil from soybean; T4: Basal diet + Probiotic; T5: Probiotic + Diet containing 3% animal fat from tallow and T6: Probiotic + Diet containing 3% plant oil from soybean
[2] 0.01 kg of probiotic was added to grower diet to constitute the probiotic groups
ME: Metabolizable energy; CP: Crude protein

GAPDH gene as internal control for normalization *(Table 4)*. Gene expression in cDNA samples made of tissue was evaluated using Real time PCR primers and Sybergreen. Apparatus software automatically depicted threshold line at the end of PCR reaction in the Real Time PCR apparatus. The data were analyzed using ABI 7300 sequence detection system and SDS Ver. 1.4 software.

Statistical Analysis

Analysis of the obtained data was conducted by SAS software in a completely randomized design [11]. Differences between means were assessed by Duncan's multiple range test at 5% level.

RESULTS

The *Table 5* and *Table 6* show the effects of trial groups on the performance of broiler chickens. Body weights on treatments 2, 3 and 5 were significantly different from the control group during the entire period (P<0.05). Highest

Table 3. *Ingredients and calculated analyses of the basal diets in the finisher period*

Ingredients (kg)	Finisher (29-42 days old)					
	T1	T2	T3	T4	T5	T6
Corn grain	56.49	61.18	62.42	56.49	61.18	62.42
Soybean meal	30.00	23.23	25.61	30.00	23.23	25.61
Corn gluten meal	9.79	9.0	5.01	9.79	9.0	5.01
Tallow	-	3	-	-	3	-
Soybean oil	-	-	3	-	-	3
Dicalcium phosphate	1.74	1.7	1.69	1.74	1.7	1.69
Calcium carbonate	0.85	0.86	0.98	0.85	0.86	0.98
Sodium bicarbonate	0.20	0.15	0.25	0.20	0.15	0.25
Salt	0.22	0.25	0.19	0.22	0.25	0.19
L-lysine	0.12	0.10	0.22	0.12	0.10	0.22
DL-methionine	0.09	0.03	0.13	0.09	0.03	0.13
Vitamin and mineral permix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Probiotic[2]	-	-	-	0.01	0.01	0.01
Filler	0.02	0.02	0.02	0.01	0.01	0.01
Total	100	100	100	100	100	100
Nutrient						
ME (kcal/kg)	3100	3100	3100	3100	3100	3100
CP (%)	19	19	19	19	19	19
Ca (%)	0.76	0.77	0.81	0.76	0.77	0.81
P (%)	0.38	0.38	0.38	0.38	0.38	0.38
Methionine (%)	0.49	0.32	0.41	0.49	0.32	0.41
Lysine (%)	0.60	0.75	0.95	0.60	0.75	0.95
Methionine + Cysteine (%)	0.91	0.61	0.69	0.91	0.61	0.69
Threonine (%)	0.80	0.64	0.61	0.80	0.64	0.61
Tryptophan (%)	0.15	0.17	0.17	0.15	0.17	0.17
Arginine (%)	0.97	1.02	1.02	0.97	1.02	1.02
Valine (%)	1.08	0.83	0.78	1.08	0.83	0.78
Na (%)	0.15	0.15	0.15	0.15	0.15	0.15
K (%)	0.38	0.61	0.65	0.38	0.61	0.65
Cl (%)	0.20	0.20	0.20	0.20	0.20	0.20

[1] Each kg (DM basis) of vitamin and mineral premix contained: vit A: 11.000 IU; vit D_3: 2.000 IU; vit E: 18 IU; vit K: 4 mg; vit B_{12}: 0.015 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Calcium pantothenic acid: 12.0 mg; Niacin: 30.0 mg; Pyridoxine: 2.9 mg; Folic acid: 1.0 mg; Choline: 260.0 mg; Manganese: 64.5 mg; Zinc: 33.8 mg; Iron: 100.0 mg; Copper: 8.0 mg; Iodine: 1.9 mg; Selenium: 0.25 mg

T1: Basal diet (control); T2: Diet containing 3% animal fat from tallow; T3: Diet containing 3% plant oil from soybean; T4: Basal diet + Probiotic; T5: Probiotic + Diet containing 3% animal fat from tallow and T6: Probiotic + Diet containing 3% plant oil from soybean

[2] 0.01 kg of probiotic was added to grower diet to constitute the probiotic groups

ME: Metabolizable energy; CP: Crude protein

Table 4. *The sequence of primers designed to Real Time PCR*

Real Time PCR Primers	Sequence of the Primers	Product Size
Forward ghrelin	5′-AATTCTCCTTCTCAGCATCCTTGGG-3′	134 pb
Reverseghrelin	5′-CTGTGCCTCGGCGATGTAATCTTG-3′	
GAPDH forward	5′-CTTTGGCATTGTGGAGGGTC-3′	128 pb
GAPDHreverse	5′-ACGCTGGGATGATGTTCTGG-3′	

Table 5. *The effects of Lactofeed probiotic and different sources of fat on body weight (BW) and economical traits*

Treatments	1 to 42 day		
	BW (g)	Production Index	Feed Costs Per kg Live Weight (Rial)
Basal diet (control)	2294.78^{b}	311.63^{cb}	56427.50^{a}
Diet containing 3% animal fat from tallow	2190.60^{c}	288.87^{dc}	58075.00^{a}
Diet containing 3% plant oil from soybean	2431.93^{a}	374.94^{a}	48987.50^{b}
Basal diet + probiotic	2353.25^{ab}	325.83^{b}	55332.50^{a}
Probiotic + diet containing 3% animal fat from tallow	2159.93^{c}	281.00^{d}	57932.50^{a}
Probiotic + diet containing 3% plant oil from soybean	2292.90^{b}	312.40^{bc}	56255.00^{a}
P-value	0.0001	0.0001	0.0001
SEM	21.567	8.126	944.3862

The means within the same column with at least one common letter, do not have significant difference (P>0.05); SEM: standard error of the means

Table 6. *The effects of Lactofeed probiotic and different sources of fat on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR)*

Period	Treatments	BWG (g)	FI (g/hen/starter)	FCR
Starter Period [g/week] 1 to 10 day	Basal diet (control)	158.35^{b}	229.67^{a}	1.46^{a}
	Diet containing 3% animal fat from tallow	196.29^{a}	219.35^{b}	1.11^{b}
	Diet containing 3% plant oil from soybean	184.12^{a}	212.00^{b}	1.16^{b}
	Basal diet + probiotic	198.62^{a}	211.05^{b}	1.06^{b}
	Probiotic + diet containing 3% animal fat from tallow	202.52^{a}	228.22^{a}	1.12^{b}
	Probiotic + diet containing 3% plant oil from soybean	196.53^{a}	210.07^{b}	1.06^{b}
	P-value	0.0022	0.0001	0.0002
	SEM	3.971	2.104	0.033
Grower Period [g/week] 11 to 28 day	Basal diet (control)	1001.05	1257.93^{c}	1.26
	Diet containing 3% animal fat from tallow	977.15	1245.40^{d}	1.28
	Diet containing 3% plant oil from soybean	1042.90	1242.23^{d}	1.20
	Basal diet + probiotic	1013.48	1289.95^{a}	1.27
	Probiotic + diet containing 3% animal fat from tallow	991.57	1292.13^{a}	1.30
	Probiotic + diet containing 3% plant oil from soybean	1030.68	1271.10^{b}	1.23
	P-value	0.8783	0.0001	0.7899
	SEM	15.801	4.235	0.021
Finisher Period [g/week] 29 to 42 day	Basal diet (control)	1087.15^{ab}	2458.50^{a}	2.27^{a}
	Diet containing 3% animal fat from tallow	968.95^{bc}	2410.38^{ab}	2.49^{a}
	Diet containing 3% plant oil from soybean	1157.20^{a}	2229.00^{c}	1.93^{b}
	Basal diet + probiotic	1093.38^{ab}	2465.98^{a}	2.25^{a}
	Probiotic + diet containing 3% animal fat from tallow	918.27^{c}	2344.85^{b}	2.57^{a}
	Probiotic + diet containing 3% plant oil from soybean	1018.00^{abc}	2445.28^{a}	2.42^{a}
	P-value	0.0122	0.0001	0.0037
	SEM	23.143	19.787	0.056

The means within the same column with at least one common letter, do not have significant difference (P>0.05); SEM: standard error of the means

weight was for treatment 3 and the lowest for treatment 5. Production indexes of the treatments 3 and 4 were significantly different from the control group (P<0.05). Feed costs per kg live weight in treatment 3 were significantly lower than for the the control group (P<0.05).

The body weight gains of all treatments in the starter period were significantly different from the control group (P<0.05). While none of the treatments were significantly different from the control in the grower period, but treatment 3 had the highest body weight gain among

Table 7. *The effects of Lactofeed probiotic and different sources of fat on carcass characteristics*

Treatments	Carcass (%)	Breast (%)	Thigh (%)	Abdominal Fat (%)
Basal diet (control)	66.14	31.29^{ab}	26.95^{ab}	1.08
Diet containing 3% animal fat from tallow	64.73	27.31^{b}	24.20^{bc}	1.10
Diet containing 3% plant oil from soybean	65.61	33.49^{a}	29.85^{a}	1.11
Basal diet + probiotic	64.77	29.60^{ab}	25.42^{bc}	1.08
Probiotic + diet containing 3% animal fat from tallow	61.91	29.23^{ab}	21.87^{c}	1.07
Probiotic + diet containing 3% plant oil from soybean	66.60	32.32^{a}	27.44^{ab}	1.06
P-value	0.7106	0.0114	0.0035	0.1032
SEM	0.848	0.699	0.682	0.0029

The means within the same column with at least one common letter, do not have significant difference (P>0.05); SEM: standard error of the means

Table 8. *The effects of Lactofeed probiotic and different sources of fat on intestinal morphology*

Intestine	Treatments	Villus Height (µm)	Villus Width (µm)	Crypt Depth (µm)	Villus Height/Crypt Depth
Duodenum	Basal diet (control)	522.00^{e}	50.80^{d}	96.90^{d}	5.39^{c}
	Diet containing 3% animal fat from tallow	530.80^{de}	55.00^{dd}	108.50^{c}	4.89^{c}
	Diet containing 3% plant oil from soybean	586.30^{c}	72.50^{b}	160.80^{a}	3.53^{d}
	Basal diet + probiotic	856.40^{a}	123.30^{a}	73.50^{e}	11.65^{a}
	Probiotic + diet containing 3% animal fat from tallow	536.70^{d}	64.60^{c}	136.10^{b}	3.94^{d}
	Probiotic + diet containing 3% plant oil from soybean	751.80^{b}	65.40^{c}	94.20^{d}	7.98^{b}
	P-value	0.0001	0.0001	0.0001	0.0001
	SEM	38.910	7.270	8.745	0.849
Jejunum	Basal diet (control)	664.00^{d}	58.20^{c}	72.00^{d}	9.22^{b}
	Diet containing 3% animal fat from tallow	330.00^{f}	51.20^{d}	101.60^{b}	3.24^{f}
	Diet containing 3% plant oil from soybean	812.50^{b}	73.50^{b}	100.20^{b}	8.10^{c}
	Basal diet + probiotic	849.50^{a}	123.30^{a}	73.50^{d}	11.56^{a}
	Probiotic + diet containing 3% animal fat from tallow	442.00^{e}	60.50^{c}	95.40^{b}	4.63^{e}
	Probiotic + diet containing 3% plant oil from soybean	766.60^{c}	73.50^{b}	95.20^{b}	8.05^{c}
	P-value	0.0001	0.0001	0.0001	0.0001
	SEM	58.450	7.164	6.104	0.810

The means within the same column with at least one common letter, do not have significant difference (P>0.05); SEM: standard error of the means

the treatments (P>0.05). Body weight gain in treatment 3 was significantly different from the control in the finisher period whilst treatment 5 had the lowest body weight gain (P<0.01).

There was a significant decrease of the difference in feed intakes between the control group and all the treatments (except treatment 5) in the starter period (P<0.05). Feed intakes for all the treatments were significantly different from the control group in the grower period. The probiotic included treatments had higher feed intake than the control and the treatment merely with fat had lower feed intake than the control. Lowest feed intake was related to treatment 3 (P<0.05). The feed intakes of treatments 3 and 5 were significantly different from the control in the finisher period (P<0.05).

The feed conversion coefficient for all the treatments was significantly different from the control in the starter period (P<0.05). None of the conversion coefficients in the treatments were significantly different from the control group in the grower period (P<0.05). In the finisher period, only treatment 3 had a significantly different conversion coefficient compared to the control group (P<0.05), with more efficient feed conversion

Table 7 shows the effect of experimental groups on carcass characteristics in the finisher period. There were no significant differences from the control group for the percentages of carcass, breast and ventricular fat. The percentage of thigh in treatment 5 had significant decrease compared to the control group (P<0.05).

Table 8 shows the effect of experimental groups on length, width and depth of crypt and villus height to crypt depth ratio of small intestine (duodenum and jejunum)

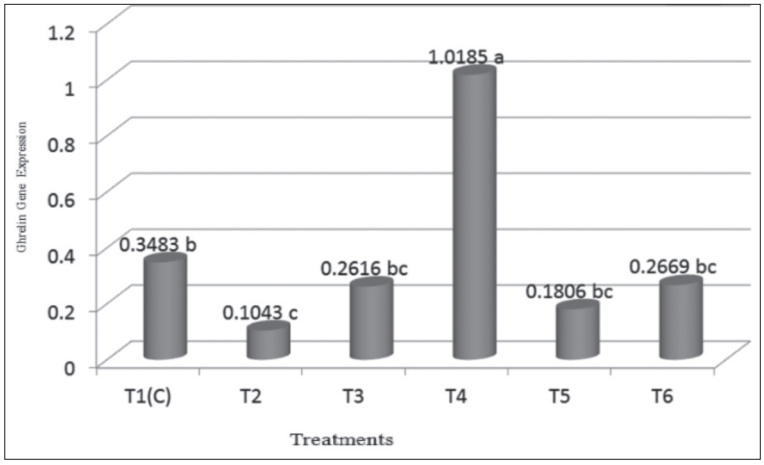

Fig 1. The effects of Lactofeed probiotic and different sources of fat on ghrelin gene expression

in the finisher period. The villi in elemental areas of small intestine had the highest height and the height of villi was lower at the end of intestine. Duodenum villus height and jejunum of all the treatments were significantly different from the control ($P<0.05$). The depths of duodenum villus width and jejunum in treatments 3 and 4 were significantly greater than for the control ($P<0.05$). The length and width of duodenum in treatment 2 was not significantly different from the control. The depth of crypt in duodenum for all the treatments except treatment 6 was significantly different from the control ($P<0.05$). The crypt depth was lowest in treatments of 4 and 6. The depth of crypt in jejunum section for all the treatments except treatment 4 was significantly different from the control group ($P<0.05$).

Villus height to crypt depth ratio of duodenum for all the treatments except treatment 2 was significantly different from the control and the highest value was on treatment 4 ($P<0.05$). Also, villus length to crypt depth ratio in jejunum section for all the treatments differed significantly from the control ($P<0.05$).

Fig. 1 shows the effect treatments on the relative expression of Ghrelin gene at the end of the period. Ghrelin gene expression of treatment 2 was significantly lower than in the control, whilst it was significantly higher in treatment 4 ($P<0.05$).

DISCUSSION

Other studies show that the level of feed intake of broiler chickens in the starter period is lower in fat-included diets [12].

Lower feed intake of fat-included diets, increase in the weight of chickens and improvement in conversion ratio in the starter period, may be because of the decrease in transit speed through digestive system which consequently provides more time for digestive system to absorb nutrients [6].

Leeson and Summers [13] showed that inclusion of fat in the diet causes decrease in feed intake during grower and finisher periods because of decreasing gastric emptying rate. This is consistent with the results of this experiment.

The feed intake with the probiotic-included diet in the grower period (the treatments 4, 5, 6) was increased compared to the control group. Probiotics improve the digestive process via increase of the useful microbial population, enzymatic activity of bacteria and the improvement of intestine microbial balance with consequent effects on food digestion, absorption and intake [14].

Body weight during the entire period, body weight gain and conversion ratio during the finisher period in treatment 3 was improved compared to the control. This is in line with the beneficial effect of fats, specifically soybean oil, on bird body weight gain reported by Shokrollahi et al.[15]. Improvement in weight due to using plant oil-included diets is related to effects on bird feed intake and better use of dietary energy. The better effect of plant oils, such as soybean oil, is due to the high ratio of unsaturated to saturated fatty acids and also better formation of micelle because of creating monoglyceride after its hydrolysis inside intestine results in a better absorption and thus improved performance [15].

Treatment 3 which had the best conversion ratio included only 3% soybean oil. This is related to the effect of fat on feed intake which causes fixed energy absorption of the bird by lower feed intake [16].

Treatment 3 had the best production index and lowest feed cost. These results together with those of den Besten et al.[17] show that soybean oil can improve economc performance, because it is a cheap energy source with beneficial effects on the efficiency of nutrient digestion and absorption resulting from lower rate of transit through the digestive system.

The results of this experiment showed that the effect of dietary fats on performance depends on the type of additive used in the diet; such that there was a lower

performance of birds when both probiotic and fat (tallow or soybean oil) were included in the diets. Intestinal microbial flora *(Lactobacilli, Bifidobacterium* and *Enterococcus)* have been reported to have a role in the decomposition of bile acids. These species are used in the preparation of probiotics and may cause disorder in the bird's fat absorption by creating biologic changes in bile acids and by dehydroxylation and deconjugation. Deconjugation of bile acids by the bacteria of the digestion system was reported by Leeson and Summers [13] to result in less absorption of fats which leadsto decreased absorbed energy and less growth of chickens.

The differences in the weights of carcass, breast and thigh between the treatments and the control were not significant, but the difference in the third treatment was higher. Nobakht et al.[18] stated that the use of soybean oil in the diet increased the weight of the breast and thigh in the poultry, which is consistent with the results of this experiment. The use of soybean oil in the poultry diet, due to the reduced feed transit rate, makes better the digestion and absorption of nutrients, and amino acids are provided in a better position to improve carcass weight.

Also, better absorption of soybean oil than animal fat in the diet results in an increase in carcass weight. The positions of fatty acids in glycerol, as well as the ratio of the fatty acids used in fat formatoin, affect the amount of the metabolizable energy extracted from fat. Non-saturated fatty acids are absorbed more than saturated fatty acids, and thus their metabolizable energy is higher. Since fatty acids used in soybeans were unsaturated, the increase in the absorbed energy led to an increase in carcass weight in the third treatment [19].

Differences in the fat of the abdominal were not significant in any of the treatments compared to the control. The main nutritional factor that can affect the content of abdominal fat is the energy level of the diet and the ratio of diet's energy to protein, and there was no significant difference between the treatments with regard to the energy balance and the energy to protein ratio [20].

Treatments with probiotics and soybean oil alone or in combination had marked effects on the morphology of the intestine. The increase of crypt depth of intestinal wall shows the thickening of intestinal surface. Thickening is due to the body's immune response to the entry of pathogens and toxins. Probiotics prevent thickening of intestinal surface by decreasing intestine's pathogens. Shortening of villi and increase of crypt depth in intestinal surface will decrease absorption from the intestinal wall and decrease of performance [21]. The longer the length of intestinal villus probably results from a lower level of replacing enterocyte cells and renewing intestine tissue. The increase of villus height when probiotics were incluuded is volatile because of their role in increasing fatty acids which are considered as the final product of fermentation by the bacteria used in probiotics *(Lactobacilli* and *Bifidobacteria)*. The aggregation of this material in the intestine decreases intestine's pH and makes the environment inappropriate for Salmonella and Kelly Basil that need pH of about 7. With the decrease of damage to the intestinal wall, the level of renewing intestinal epithelial cells decreases and the length of villi increases [22].

Khatibjoo et al.[23] reported that consuming tallow in the diet instead of soybean oil led to higher values of pH in different parts of the small intestine and increased repelling of bile acids by broiler chickens with increase in intestine pH, higher levels of pathogenic bacteria are expected which result in diarrhea and intestinal tissue destruction. But using unsaturated fatty acids has the opposite effect and causes the decrease of inflammatory responses in the intestine. Therefore, it can be said that using unsaturated fatty acids instead of saturated fatty acid causes the increase in the length and width of villus and also the decrease of intestine's crypt depth with a resulting increase in broiler chicken's performance [24].

As *Fig. 1* shows the lowest level of ghrelin expression in broiler chickens was in the treatment which only had tallow in their diet. Ghazanfari et al.[25] stated in their research that ghrelin plasma concentration decreases when fat is included in the diet indicating that ghrelin secretion is sensitive to diet composition. Salehi et al.[26] found that ghrelin secretion decreased when fat was included in the diet. Cholecystokinin (CCK) is a hormone which is released from intestinal cells during eating fat or protein. This hormone contacts with neural system to announce satiety and at the same time lowers digestion in the digestive system. Since the fat in the diet lowers the rate of feed transit and also digesting saturated fat takes longer than unsaturated fat, the time period of digestion is much slower which causes long-term satiety feeling resulting in less secretion of ghrelin hormone [26].

The level of ghrelin expression in those broiler chickens which only used probiotic in their diet was significantly increased compared with the control. Arosio et al.[27] stated that every factor which increases the capability of digestion and absorption in digestive system and causes faster evacuation of the digestive system, results in the increase of ghrelin secretion. Probiotics keep stomach chymus safe from the damage of pathogenic microorganisms and improve digestion and absorption by removing pathogenic bacteria from the intestine, and consequently increase ghrelin secretion and improve the performance [27]. Probiotics increase ghrelin production via decreasing blood sugar. The bacteria in probiotics use dietary carbohydrates. Therefore, bird's absorption of sugar is reduced which increases the activity of vagus nerve in order to increase the movements of the digestive system. Ghrelin hormone secretion is stimulated by the increase in movements of the digestive system and as the result, feed intake is increased and weight is improved [28].

The results of this work showed that using vegetable fats to supply part of tahe energy in the diet and the separate use of lactofeed probiotic as an additive have beneficial effects in terms of performance of broiler chickens. Separate use of probiotic in the diet of broiler chickens can increase the levels of relative expression of ghrelin gene and this increase improves the weight. This study showed that the energy supplied with 3% animal fat in the diet of broiler chickens and also using the diets of probiotic mixed with (animal and plant) fat did not have adverse effects.

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