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RESEARCH ARTICLE

Effects of Dietary *Saccharomyces cerevisiae* and Butyric Acid Glycerides on Performance and Serum Lipid Level of Broiler Chickens

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Summary

This experiment was conducted to evaluate the effects of dietary supplementation of live yeast *Saccharomyces cerevisiae* (SC) and butyric acid glycerides (BAG) on broiler performance and serum lipid composition. One-day-old ROSS 308 female chicks (n=378) were randomly distributed in a 3×3 factorial arrangement with three replicates for each increasing levels of (0, 0.002, and 0.004 g/g) BAG and (0, 0.003, and 0.006 g/g) SC respectively. The experiment lasted 42 d, consisting of starter (1-21 d) and grower (22-42 d) periods. Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were determined by the period for each treatment. On d 42, serum concentrations of triglycerides, cholesterol, and HDL were determined. *Saccharomyces cerevisiae* had no effect on performance in the starter period. However, chicks fed 0, 0.002, and 0.004 g/g BAG had higher BW and better FCR than control diet (P<0.05). In the grower period BW of chicks fed 0.006 g/g SC was higher than other treatments (P<0.05). For BAG both levels improved (P<0.05) BW and FCR. In serum composition, both BAG and SC decreased cholesterol concentrations (P<0.05), but the HDL levels were higher (P<0.05) only in 0.006g/g SC fed chicks. There were no significant effects in triglyceride levels among treatments. There were no SC and BAG interaction effect on response variables. In conclusion, dietary BAG improves growth performance in starter and grower periods but SC was only effective in grower period. Moreover, both BAG and SC had positive effect on serum lipid composition.

Keywords: *Saccharomyces cerevisiae, Butyric acid glycerides, Performance, Lipid composition, Broiler*

Broiler Tavuklarda *Saccharomyces cerevisiae* ve Butrik Asit Gliseridlerinin Performans ve Serum Lipid Değerleri Üzerine Etkileri

Özet

Bu çalışma; diyete canlı maya *Saccharomyces cerevisiae* (SC) ve butrik asit gliseridleri (BAG) ilavesinin broilerlerde performans ve serum lipid değerleri üzerindeki etkisini değerlendirmek amacıyla yapılmıştır. Bir günlük ROSS 308 dişi hayvanlar (n=378) her bir grup 3 kez tekrarlanacak şekilde rastgele dağıtıldıktan sonra faktöriyel olarak 0, 0.002, 0.004 g/g BAG ve 0, 0.003, 0.006 g/g SC verildi. Deneme süreci srater (1-21 gün) ve büyüme/22-42 gün) olmak üzere toplam 42 gün sürdü. Vücut ağırlığı (VA), yem tüketimi (YT) ve yem konversiyon oranı (YKO) her bir deneme için belirlendi. 42. günde trigliserit, kolesterol ve HDL konsantrasyonları tespit edildi. *Saccharomyces cerevisiae* starter döneminde performans üzerine herhangi bir etki göstermedi. Ancak, 0, 0.002 ve 0.004 g/g BAG ile beslenen tavuklar kontrol grubundakilere oranla daha yüksek VA ve daha iyi YKO değerlerine sahipti (P<0.05). Büyüme döneminde 0.006 g/g SC ile beslenen tavukların VA değerleri diğer gruplarınkinden daha yüksek idi (P<0.05). BAG verilen hayvanlarda hem VA hem de YKO değerleri gelişme gösterdi (P<0.05). Hem BAG hem de SC verilen hayvanlarda serum kolesterol konsantrasyonları düşme gösterirken (P<0.05) HDL seviyeleri sadece 0.006g/g seviyesinde SC verilenlerde daha yüksek idi (P<0.05). Gruplar arasında triglisert değerleri yönünden herhangi bir fark tespit edilmedi. Sonuç olarak; diyette BAG verilmesi starter ve büyüme dönemlerinde büyüme performansı üzerinde olumlu etki gösterirken SC verilmesi sadece büyüme devresinde olumlu etki göstermektedir. Hem BAG hem de SC serum lipid değerlerinde pozitif etkiye neden olmaktadır.

Anahtar sözcükler: *Saccharomyces cerevisiae, Butrik asit gliseridleri, Performans, Lipid Değerleri, Broiler*

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INTRODUCTION

Antibiotics benefit animal growth, performance and health. However it has been increasing pressure to reduce or even eliminate antibiotic usage in poultry due to the development of antibiotic resistance in consumers [1]. Thus, there is an increasing interest in finding other antibiotic replacements such as prebiotics, probiotics, aromatic oils and organic acids in poultry production [2]. A probiotic was defined as a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance [3]. Effects of yeast products on production and their mode of action in monogastrics have been reported in poultry [4,5]. *Saccharomyces cerevisiae* yeast has biologically valuable proteins, vitamin B-complex, important trace minerals and other plus factors [6]. Some studies have confirmed the effects of yeast products in increasing concentration of commensal microbes or suppressing pathogenic bacteria [5]. However, these effects were not reported by Van Heugten *et al.*[7].

Reduction in circulating levels of cholesterol and LDL with supplemental yeast was reported by other researchers [8,9] who stated that probiotics could contribute to the regulation of serum cholesterol concentrations by deconjugating bile salts.

Organic acids, including butyric acid are also considered potential alternations to antibiotics growth promoter [10,11]. Butyric acid has been widely reported as the major development promoter of the gut wall tissues and an important growth modulator of symbiotic intestinal microflora [10,11]. Also, butyric acid is known the main energy source for enterocytes. Kwon and Ricke [12] showed butyrate and valerate to have the greatest efficacy. Moreover, Lesson *et al.*[10] and Antongiovanni *et al.*[13] reported positive beneficial effects of butyric acid on performance traits of broilers. It has been also reported that dietary inclusion by BAG had significant reducing effect on serum compositions of total cholesterol and LDL levels [14]. Effects of SC and BAG on the performance and lipid serum composition of broiler chickens are similar. Therefore, the objective of this study was to evaluate the SC by BAG interaction effects on performance and lipid composition in broilers.

MATERIAL and METHODS

Experimental Animals and Management

Three hundred and seventy eight day-old female chicks (ROSS 308) were randomly assigned into 9 treatments, each composed of 42 birds. Birds in each treatment were placed in 3 pens, each containing 14 birds. All birds were raised on floored pen (1.2×1.8 m) and had access to feed and water *ad libitum*.

Experimental Design and Diets

Birds were distributed in a completely randomized design with 3×3 factorial arrangement with pen as the experimental unit. The composition and nutrient analysis of basal diet are shown in *Table 1* and *Table 2*. The basal diet was a typical corn-soybean meal diet as mash form that increasing amount of BAG (0, 0.002 and 0.0044 g/g) and SC (0, 0.0033 and 0.0066 g/g) were added to basal diet.

The chicks were fed the starter diet until d 21 and grower diet from d 22 until 42 based on NRC [15] nutrient requirements of chicken. The used BAG (product of BABY-C4. Silo Company, Italy) contained 25-35% monoglycerides in the 1 or 3 positions, 50-55% diglycerides in the 1 or 3 positions and 15-25% triglycerides. Unlike butyric acid,

Table 1. *The chemical composition of Saccharomyces cerevisiae*
Tablo 1. *Saccharomyces cerevisiae'nin kimyasal kompozisyonu*

Composition	*Saccharomyces cerevisiae*
Dry matter, %	93
ME, kcal/kg	1990
Crude protein, %	44.4
Crude fat, %	1
Crude fiber, %	2.7
Ca, %	0.12
AP, %	1.4

Table 2. *Composition of the basal diet fed in broilers (%)*
Table 2. *Broilerlere verilen bazal diyetin kompozisyonu (%)*

Ingredients	Starter	Grower
Corn	54.5	61
Soybean meal (44% CP)	38	32.5
Soy oil	3.5	3
Dicalcum phosphate	1.8	1.15
Oyster shell	1.3	1.45
Salt (NaCl)	0.2	0.3
DL-Methionine	0.2	0.1
Vitamin Permix[1]	0.25	0.25
Mineral Permix[2]	0.25	0.25
Calculated Composition		
ME, Kcal/kg	2990	3032
CP, %	21.45	19.48
Ca, %	1	0.9
Available P, %	0.48	0.35
Lys, %	1.1	1
Met, %	0.5	0.4

[1] Vitamin premix contained the following per kilogram of diet: vitamin A, 1100 IU; vitamin D_3, 240 IU; vitamin E, 6 IU; vitamin B_{12} 0.004 µg; biotin, 0.15 mg; folic acid, 0.2 mg; nicotinic acid, 50 mg; D-pantothenic acid, 5 mg; pyridoxine hydrocholesteroloride, 1.2 mg; riboflavin, 2.2 mg; thiamine mononitrate, 1.6 mg. [2] Mineral premix contained the following per kilogram of diet: Fe, 80 g; Cu, 8 mg; Mn, 60 mg; Zn, 40 mg; I, 0.4 mg; Se, 0.2 mg

the butyrate glycerides used in this study had only a mild buttery type odor and not the rancid odor often associated with butyric acid. The live yeast SC (containing 1×10^9 CFU/g) was provided from Klar Maya (powdery form, Iran) and chemical composition of SC is presented in *Table 1*. No antibiotics and any coccidiostate were included in the experimental diets.

Growth Performance Traits

Body weights (BW) were recorded for each replicate on days 1, 21, and 42 of age and feed intake (FI) was measured in order to calculate feed conversion ratio (FCR) for each feeding periods. Mortality ratio was recorded daily and FCR was corrected for mortality by adding body weights to the total pen weight at the end of each period.

Blood Collection and Analysis

Blood was collected at 42 day old from wing vein of 9 birds per treatment (3 birds/ replicate) and serum was separated at 5000×g for 10 min. The serum concentrations of total triglycerides, cholesterol and high-density lipoprotein (HDL) were analyzed by an automatic biochemical analyzer (Technicon RA-1000, Spain), following the instructions of the corresponding reagent kit (Pars Azmon Co., Iran).

Statistical Analysis

All data were analyzed by ANOVA using the SAS [16], GLM program. Treatment means portioned by LSMEAN analysis. The mode is:

$$X_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \Sigma_{ij}$$

Where X is the observed response. μ is the overall mean, α_i is the effect of BAG, β_j is the effect of CS, $\alpha\beta_{ij}$ is an interaction between BAG and SC and εij is the error. The level of statistical significance was present at $P\leq0.05$.

RESULTS

Growth Performance

Performance data are detailed in *Table 3*. In starter period, performance of birds were not affected by increasing levels of SC (*P*>0.05). However chicks fed 0.002 or 0.004 g/g BAG had higher BW and better FCR than control diet (*P*<0.05). Also there were no differences (*P*>0.05) between 0.002 and 0.004 g/g BAG fed chicks on broiler performance in starter period. In grower period BW of chicks fed 0.006 g/g SC was than control and 0.003 g/g SC fed chicks higher (*P*<0.05). However, increasing levels of SC had no effect on FI and FCR. For BAG both levels of 0.002 or 0.004 g/g increased BW (*P*<0.05) and decreased FCR (*P*<0.05) compared with control diet. There was no BAG by SC interaction on FI, BWG, and FCR in both starter and grower periods. There was no significant difference in mortality of all treatments.

Serum Lipids

The effects of dietary BAG and SC supplementation on serum lipid composition are shown in *Table 4*. Chicks

Table 3. *Performance of broiler chickens fed diets containing butyric acid glyceride (BAG) and Saccharomyces cerevisiae (SC)*
Table 3. *Butrik asit gliserid (BAG) ve Saccharomyces cerevisiae (SC) içeren diyetlerle beslenen broiler tavukların performans değerleri*

Groups	Starter (1-21 d)			Grower (22-42 d)		
	FI (g/bird)	BWG (g/bird)	FCR	FI (g/bird)	BWG (g/bird)	FCR
BAG, g/g						
0	628.89	477.56^b	1.31^b	2575.78	1120.22^b	2.30^b
0.002	648.33	537.89^a	1.20^a	2628.78	1211.67^a	2.16^a
0.004	663.11	553.44^a	1.20^a	2626.00	1211.00^a	2.17^a
SEM	21.75	20.51	0.02	47.72	28.45	0.03
P-Value	0.563	0.030	0.022	0.639	0.037	0.011
SC, g/g						
0	677.67	555.00	1.23	2581.78	1147.22^b	2.24
0.003	633.89	509.78	1.24	2596.22	1163.22^{ab}	2.24
0.006	628.78	504.11	1.23	2652.56	1232.44^a	2.15
SEM	20.84	22.08	0.03	48.37	29.09	0.03
P-Value	0.260	0.157	0.093	0.500	0.043	0.093
BAG×SC						
P-Value	0.624	0.181	0.449	0.070	0.424	0.865

^{a,b} *Within the same row, means with different superscripts are significantly different (P<0.05)*

Table 4. *Effects of butyric acid glyceride (BAG) and Saccharomyces cerevisiae (SC) on blood serum lipids of broiler chicken at 42 d age (mg/dl)*

Tablo 4. *Butrik asit gliserid (BAG) ve Saccharomyces cerevisiae (SC)'nin 42. günde broiler tavukların serum lipid değerleri (mg/dl) üzerine etkisi*

Groups	Cholesterol	Triglyceride	HDL
BAG g/g			
0	127.44^{b}	87.50	90.42
0.002	120.38^{a}	88.88	87.84
0.004	117.22^{a}	86.33	90.26
SEM	2.70	5.26	1.94
P-Value	0.016	0.943	0.555
SC, g/g			
0	127.22^{b}	94.33	88.52^{b}
0.003	121.55^{ab}	85.38	86.13^{b}
0.006	116.27^{a}	83.00	93.87^{a}
SEM	2.61	4.91	1.83
P-Value	0.011	0.292	0.016
BAG×SC			
P-value	0.202	0.571	0.437

^{a,b} Within the same row, means with different superscripts are significantly different (P<0.05)

fed 0.002 or 0.004 g/g BAG had the lower cholesterol concentrations compared with control diet ($P<0.05$). However there were no differences between 0.002 and 0.004 g/g BAG fed diets on cholesterol concentrations. The cholesterol concentration was lower only 0.006 g/g SC fed chicks ($P<0.05$). There were no differences between 0.003 g/g SC and control diet on cholesterol concentrations. HDL level was higher ($P<0.05$) only 0.006 g/g SC fed chicks. There were no differences between 0.003 g/g SC and control diet on serum HDL levels. Also BAG had no significant effect on serum HDL concentrations in any levels. There were no significant effects in triglyceride concentrations among treatments. There was no interaction effect of SC and BAG on cholesterol, triglyceride and LDL concentrations.

DISCUSSION

The primary role of a diet is not only to provide enough nutrients to fulfill metabolic requirements of the body but also to modulate various functions of the body. Probiotics, prebiotics, and organic acids are either beneficial microorganisms or substrates that facilitate the growth of beneficial microorganisms, which can be suitably harnessed by the food manufacturers and hold considerable promise for health care industry. Results of the present study showed that the inclusion of 0.006 g/g yeast *Saccharomyces cerevisiae* had positive effect on broiler BW gain only in grower period. Although dietary SC had no significant effects on broiler performance in starter period. These results are in agreement with Gao *et al.*[17], who reported positive effects of SC on broiler average daily gain and FCR during grower period. It seems that a period of adaptation is needed before the effects of SC inclusion can be significant because the changes in intestinal morphology and immune responses take time [17]. *Saccharomyces cerevisiae* contains live yeast as well as metabolites such as peptides, oligosaccharides, amino acids, flavor and aroma substances, and possibly some unidentified growth factors which have been proposed to produce beneficial performance responses in animal production by maintenance of beneficial microbial population [3], improving FI and digestion [18] and altering bacterial metabolism [19]. Other studies, however reported that SC had no effect on performance in poultry [4]. Differences in animal response may be related to differences in products formulations; yeast products are interchangeably classified as active dried yeast, live yeast or fermented yeast, making comparisons difficult among studies. Growth performance of birds was positively affected by dietary supplementation of BAG both in starter and grower periods. However, there were no significant differences in BWG, FI and FCR between 0.002 and 0.004 g/g fed chicks in both periods. Also FI in groups fed BAG were not significantly differences from control diet. These results are agreement with those [20-22].

Organic acids like butyric acid maintained a better microbial environment in digestive tract of birds by reducing the number of pathogenic microbes. These enhanced digestion, absorption and efficiency of utilization of feed [2,23]. Bolton and Dewar [24] indicate that free butyric acid is absorbed very quickly in the upper digestive tract, and will likely be of limited effective. By inference, butyrate needs to be stabilized, and hence the testing of butyric acid glycerides used in this study.

The present results showed that broiler chicks fed diet containing BAG or SC had significantly the lower plasma cholesterol concentrations. Similar cholesterol depressing effect due to probiotic and organic acids supplementation in broiler chicken was observed by [14,25]. The findings of our study and previous studies indicated that feeding of probiotics like SC and organic acids such as butyric acid has a cholesterol depressing effect in broiler chicken. Besides, it is reported that some of the microorganisms present in the probiotic preparation could utilize the cholesterol present in the gastro intestinal tract for their own metabolism, thus reduce to absorption the amount cholesterol [26]. Lactobacillus which better survive in low pH environment of intestinal tract specially when organic acids used to decrease pH of intestine has a high bile salt hydrolylic activity, is responsible for deconjugation of the bile salts [27]. Deconjucated bile acids are less soluble at low pH and less absorb in the intestine and are more likely to be excreted in feces [28]. Since the excretion of deconjucated bile acids is enhanced and cholesterol is its precursor, more molecules are spent for recovery of bile acids [8]. As a result of increased synthesis of this acids, it is expected the level of serum cholesterol to be reduced. In addition, probiotic microorganisms inhibit hydroxymthyl-glutaryl-coenzyme A, an enzyme involved in the cholesterol synthesis [29].

In practical term, dietary addition of 2 g BAG per kg diet improved BWG and FCR of broiler chicks in both starter and grower periods. Supplemental SC did not affect performance during the starter period. In the grower period only 6 g SC per kg increased BW. Both agents decreased plasma cholesterol concentration. Their effects were not additive. Each of these agents might be promising alternatives for antibiotic growth promoters. The butyric acid glycerid offers a good alternative to improve poultry production.

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