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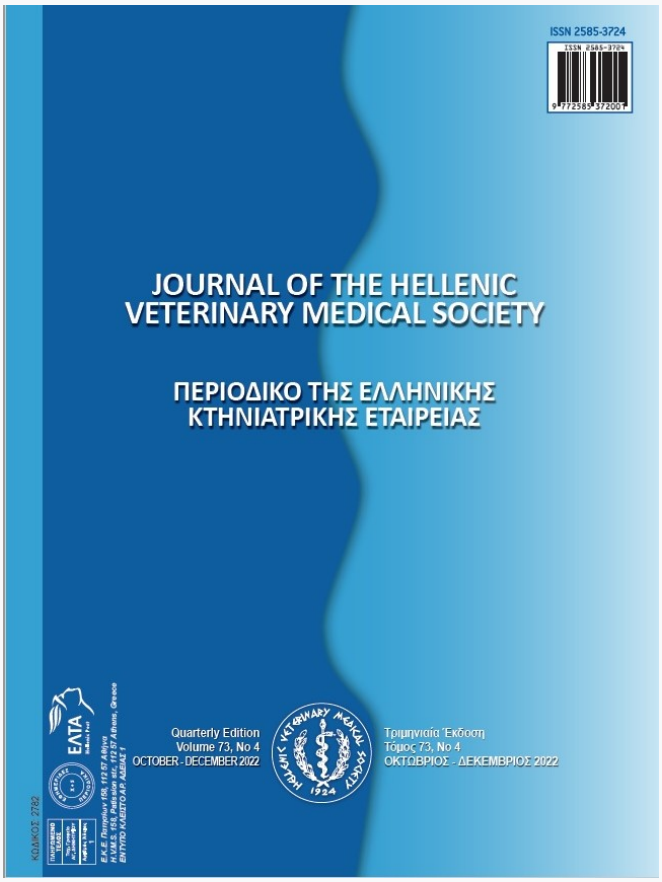

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Research article
Ερευνητικό άρθρο

Effects of Beta Vinasse Supplementation on Performance, Meat Quality and Ilio-Caecal Microflora in Quail Rations

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ABSTRACT: The aim of this study was to evaluate the effects of Beta (β)-vinasse supplementation on the live weight (LW), live weight gain (LWG), feed intake (FI), feed conversion ratio (FCR), carcass yield, meat quality, and ilio-caecal bacteriological flora of quails. A total of 240 5-d-old Japanese (Coturnix coturnix Japonica) quails, including both males and females, were divided into 3 groups 80 quails and treated as follow: a control group (1) with 0 g β-vinasse/kg ration; (2) 15 g/kg β-vinasse and (3) 30 g/kg β-vinasse. The study lasted for 6 weeks. At the end of the experiment, supplementation with β-vinasse did not have a significant effect on FI and FCR. Dietary supplementation with 30 g/kg β-vinasse significantly ($P < 0.05$) increased LW (21 d)and LWG (5 to 21 d). The dietary treatment of quails with different levels of β- vinasse did not affect hot carcass weight, cold carcass weights, hot and cold carcass yields, and breast and thigh pH.The lightness (L*) and yellowness (b*) of breast values were significantly ($P<0.05$) increased by 30 g/kg supplementation. Different levels of β-vinasse significantly increased Lactobacillus spp. in faeces. As a result, it was concluded that β-vinasse (by-product obtained from molasses) can be used in quail diets as an alternative feed source that will meet the nutritional needs of the animal and have positive effects on the digestive system, especially on the intestinal health (an increase in Lactobacillus spp. counts).

Keywords: betaine; β-vinasse; *Lactobacillus* spp.; performance; quails

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INTRODUCTION

In livestock nutrition, by-products can be used to reduce the cost of feed, which is one of the factors affecting profitability.Following this purpose, the desired reduction in feed cost can be achieved by researching by-products and increasing the biological value of existing this (Yang et al., 2021). Beta vinasse, which is the fermentation source of molasses, can be considered a good source of nutrients and minerals (Parnaudeaua et al., 2008).The chemical composition of β-vinas added to the diet in this study was 35.39% crude protein and 31.74% betaine. Due to betaine in its β-vinase structure, β-vinase has gained importance as a valuable feed material in poultry.

Since poultry cannot synthesize methyl groups, methyl group donors such as glycine, a trimethyl derivative of glycine, should be added to poultry diets (Ratriyanto et al., 2017). Moreover betaine functions a major role in the metabolism of protein and energy (Metzler-Zebeli et al., 2009; Ratriyanto et al., 2009). Furthermore, it has the potential to replace some of the dietary methionine by providing a methyl group during the conversion of homocysteine to methionine (Metzler-Zebeli et al., 2009, Cetin et al., 2021). Consequently, it can increase the availability of methionine for protein synthesis, thus a better performance can be obtained (Rao et al., 2011). Betaine supplementation the diet changes the microbial population of the digestive system of poultry (Kettunen et al., 1999). Additionally, betaine supplementation may protect intestinal cells and intestinal microbes from osmotic variation along with the digestive system (Ratriyanto et al., 2009).

The study objective was to evaluate the effects of β-vinasseon performance parameters, carcass characteristics, meat quality, and ilio-caecal microflora in quails.

MATERIALS AND METHODS

Animals, rations and experimental

Totally 240 five-day-old Japanese (*Coturnix coturnix Japonica*) quails, including both males and females, were obtained from the Uludag University Animal Health and Production, Research and Application Centre For quail breeding (Bursa, Turkey). The care and use of animals were following laws and regulations of Turkey and approved by the Ethical Committee of Bursa Uludag University (Decision no: 22.09.2020, 2020-10/09).

The quails were randomly divided into one control group and two treatment groups containing 80 quails each. Each group was randomly distributed into five subgroups, comprised of 16 quails each. The quails in all of the groups were reared under the same growing conditions. All the quails were housed at 32 ˚C for the first three days and decreased by 1 ˚C every three days to 27 ˚C at the end of the second week. The quails were kept under constant artificial lighting for 23 h/d. All quails had *ad libitum* access to water and the form of mash feed along the experimental period. The study lasted for 42 days.

The basal rations were mixed under commercial conditions as one batch, divided into respective parts, and then added to β-vinasse using a horizontal mixer. The quails received a basal ration of 23 % crude protein; 3000 kcal/kg metabolizable energy (ME) that was formulated to meet the National Research Council (NRC, 1994) requirements for nutrients, including vitamins and minerals. The ingredients and chemical compositions of the basal rations are presented in Table 1. The chemical composition of β-vinasse is showed in Table 2. No antibiotics or growth promoters were supplemented to any of the experimental groups. The chemical compositions of basal rations were determined according to the Association of Official Analytical Chemists methods (AOAC, 2006). Group feeding was applied in all experimental groups.The quails were fed either a basal ration (control group) or the basal ration with β-vinasse supplementation at doses 15 (1.5%, group 1) or 30 (3.0%, group 2) g/kg feed. The ME level of basal ratios were estimated using the following equation from Carpenter and Clegg (Leeson and Summers, 2001): ME, kcal/kg = 53+ 38 [(CP, %) + (2.25 × ether extract, %) + (1.1 × starch, %) + (1.05 × sugar, %)].

Performance parameters

At the beginning of the study, the quails were weighed individually after that the chicks were weighed weekly to calculate the individual live weight(LW) and live weight gain(LWG). Mortality was recorded when it happened. Feed intake (FI) was recorded weekly and stated as g per quail per week. The feed conversation ratio (FCR) was calculated as g feed per g live weight gain. At the end of the study, the sex ratio was determined in each group.

Slaughter, carcass characteristics, and meat quality

A total of 75quails, 5 males from each replicate

Table 1. Ingredients (%) and chemical composition of the quail rations

	Control	Group 1	Group 2
Ingredients			
Maize, grain	53.64	53.64	52.76
Soybean meal	28.62	27.12	25.62
Full fat soybean	10.30	10.30	11.08
Corn gluten	1.33	1.33	1.33
Vegetable oil	1.80	1.80	1.85
B-vinasse	-	1.5	3.0
Dicalcium phosphate	1.95	1.95	1.96
Limestone	0.90	0.90	0.90
Salt	0.25	0.25	0.25
Vit-min premix[1]	0.25	0.25	0.25
DL-Methionine	0.34	0.34	0.34
L-Threonine	0.10	0.10	0.10
L-Lysine	0.21	0.21	0.21
Sodium bicarbonate	0.10	0.10	0.10
Choline Chloride	0.11	0.11	0.12
Anticoccidial	0.10	0.10	0.10
Values analysed, %			
Metabolisable energy (kcal/kg)	3030,24	3028,26	3032,30
Crude protein	23,38	23,36	23,32
Ether extract	6.72	6.70	6.75
Ash	10.15	10.35	10.06
Saccharose	4.56	4.55	4.65
Starch	38.08	38.64	38.63
Dry matter	90.82	90.49	90.09
Calcium	1.03	1.12	1.11
Total Phosphorus	0.70	0.72	0.73

[1]Per 2.0 kg premix contains; Vit A 12 500 000 IU, Vit D_3 4 000 000 IU, Vit E 125 000 mg, Vit K_3 3 000 mg, Vit B_1 2 700 mg, Vit B_2 7 000 mg, Vit B_6 4 000 mg, Vit B_{12} 20 mg, Vit C 66 000 mg, Niacine 60 000 mg, Calcium d-pantothenate 15 000 mg, Folic acid 1 500 mg, Biotin 150 mg, Mn 75 000 mg, Fe 15 000 mg, Zn 60 000 mg, Cu 10 000 mg, Co 200 mg, I 1 200 mg, Organic Se 150 mg, Se 150 mg, Crina Poultry Plus 300 000 mg, Fitase 1 000 000 FTU, Xylanase 270 000 U, β-Glucanase 80 000 U, Fungal-1.3-B-Glucanase 70 000 U

pen, were randomly selected and weighed from each pen for carcass characteristics and meat quality evaluation at the end of the study. Quails were randomly slaughtered, stunned by cervical dislocation, and decapitated 6 h after feed withdrawal by cutting between the occipital and atlas bones using scissors. Hot carcass weights were decided with the exclusion of edible internal organs, heads, and feet containing the neck and abdominal fat.Carcasses were kept in plastic bags and stored at 4 °C overnight to determined cold carcass weight and then carcass yields were calculated.

To determine meat pH and meat color 5 quails from each replicate were used. The pH values of the breast and thigh meat samples were determined by homogenizing with distilled water. The homogenates were filtered and the pH of each sample was measured with a previously calibrated portable pH meter (Metler-Toledo, Switzerland) at room temperature. Meat color was get from an average of 3 readings across the surface of the pectoralis minör and pectoralis majör previously exposed to air ambient for 30 min. Immediately before data collection, a Minolta Chromameter CR-300 (Minolta Inc., Tokyo, Japan) was calibrated with a white, and black tile and then the lightness (L^*), redness (a^*), and yellowness (b^*) of breast meat was measured. The mean value from each breast meat was calculated.

Microbiological methods

Sixty ilio-cecal content samples were collected for microbial population analyses. The ileo-caecal region is the area where the ileum ends, obtained by the fixation of the cecum. After the gastrointestinal tract was

removed, the ileo-cecal area was aseptically removed by ligating it with fine twine, and the ileo-cecal area connected through the intestinal tract was transferred to the laboratory by cold chain without separating it. After opening the ilio-caecum for each quail, all contents were placed in sterile stomach bags, and massaged by hand from the outside.

For the isolation and enumeration, 1 g of caecal content homogenized with 9 ml of 0.1% peptone water in a Seward Stomacher 80 Lab System for 2 min. Serial dilutions were made in sterile peptone water and plated in duplicate onto relevant selective media. *Lactobacillus* were grown on de Man Rogosa and Sharpe (MRS, Oxoid CM361) agar and were enumerated after 3 d of incubation at 35°C under 5% CO_2. Total coliform was grown on Violet Red Bile (VRB, Bio life, 4021852) agar using the "pour" plate technique and plates with colonies were used for enumeration after 24–48 h of incubation at 37°C.

Statistical analysis

Statistical analysis was performed using the SPSS software package for Windows (SPSS, 2016). Variance analysis was used to determine the significance of the differences between the statistical calculations of the groups and the mean values of the group, Duncan test was used as a post-hoc test and the level of significance was used in all of the tests $P < 0.05$. Also, in this study, the sex ratio in quails was evaluated by the chi-square test and the level of significance used in all of the tests was $P < 0.05$. Results are expressed as mean ± standard error of the mean.

RESULTS

The current study was carried out to determine the effects of different levels of β-vinasse on the performance, carcass parameters, meat color, and ilio-caecal microflora (*Lactobacillus* spp., Coliform bacteria) of quails. The nutrient composition of β-vinasse is presented in Table 2. The performance values obtained from the groups in this study, which was conducted to define the effects of β- vinasse added to quail rations at different levels on performance parameters are given in Table 3. In this study, there were no differences in the sex ratio, FI and FCR. As shown in

Table 2. Nutrient composition of β-vinass

Nutrients	β-Vinasse (%100 DM)	β- Vinasse (% 63 DM)
Crude protein (%)	35,39	22,30
Crude ash (%)	17,46	11,00
Metabolisable energy (MJ/kg)	6,20	3,91
Crude cellulose (%)	1,26	0,80
Lysine (%)	0,21	0,137
Meth & Cys (%)	0,050	0,032
Methionine (%)	0,050	0,032
Threonine (%)	0,26	0,169
Valine (%)	0,32	0,206
İsoleucine (%)	0,21	0,136
Arginine (%)	0,09	0,061
Tryptophan (%)	0,06	0,039
Calcium (%)	0,04	0,028
Total phosphorus (%)	0,08	0,054
Sodium(%)	2,23	1,41
Potassium (%)	2,87	2,05
Betaine (%)	31,74	20,00
D.Lysine (%)	0,13	0,086
D.Meth & cys (%)	0,31	0,20
D.Methionine (%)	0,33	0,21
D.Threonine (%)	0,16	0,105
D.Valine (%)	0,20	0,130
D.İsoleucine (%)	0,13	0,084
D.Arginine%)	0,06	0,039
D.Tryptophan (%)	0,03	0,024

Table 3. Effects of β-vinasse supplementation on live weight, live weight gain, feed intake and feed conversion ratio in quails

	Control	Group 1	Group 2	*P value*
Sex ratio (M/F)	38/42	37/43	36/44	1.00
Live weight (g) (n=80)				
5 d	10.96±0.17	11.30±0.20	11.56±0.18	0.084
21 d	106.17±1.24[b]	108.67±1.12[b]	112.42±1.06[a]	0.001
42 d	190.86±3.19	192.80±2.66	195.14±3.23	0.612
Live weight gain (g) (n=80)				
5 to 21 d	95.18±1.27[b]	97.36±1.10[b]	100.86±1.07[a]	0.002
22 to 42 d	84.63±3.46	84.13±2.92	82.71±3.35	0.911
5 to 42 d	179.86±3.21	181.49±2.65	183.58±3.24	0.691
Feed intake (g) (n=5)				
5 to 21 d	262.75±7.43	269.12±6.14	263.44±4.31	0.726
22 to 42 d	534.83±20.77	541.65±17.85	508.56±9.59	0.368
5 to 42 d	797.59±22.60	820.77±21.45	772.00±11.17	0.376
Feed conversion ratio (g/g) (n=5)				
5 to 21 d	2.76±0.10	2.77±0.04	2.61±0.04	0.275
22 to 42 d	6.98±0.77	6.43±0.16	6.15±0.15	0.469
5 to 42 d	4.45±0.19	4.47±0.09	4.21±0.06	0.325

[a,b] Mean values with different superscripts in the same row differ significantly ($p<0.05$)
M: Male F: Female
Values are expressed means ±standart deviation Group 1: 15 g/kg β-vinasse, Group 2: 30 g/kg β-vinasse

Table 4. Effects of β-vinasse supplementation on carcass characteristics and meat quality in quails (n=25)

	Control	Group 1	Group 2	*P value*
Carcass Characteristics				
Final live weight (g)	169.29±3.01	174.14±3.07	173.21±3.40	0.520
Hot carcass weight (g)	133.01±2.56	136.66±2.42	134.50±2.87	0.617
Cold carcass weight (g)	129.06±2.50	132.46±2.40	129.13±2.87	0.576
Hot carcass yield (%)	78.63±0.88	78.56±0.76	77.63±0.58	0.582
Cold carcass yield (%)	76.30±0.87	76.13±0.74	74.50±0.59	0.177
Meat Quality				
pH thigh	6.44±0.03	6.52±0.04	6.46±0.03	0.193
pH breast	5.98±0.02	6.01±0.03	6.02±0.02	0.486
L	36.64±0.48[b]	38.61±0.84[b]	47.18±1.41[a]	0.000
A	10.41±0.33[a]	10.40±0.34[a]	7.92±0.46[b]	0.000
B	1.24±0.22[b]	2.53±0.46[b]	6.22±0.60[a]	0.000

[a,b] Mean values with different superscripts in the same row differ significantly ($p<0.05$)
Values are expressed means ±standart deviation
Group 1: 15 g/kg β-vinasse, Group 2: 30 g/kg β-vinasse

Table 3, significant differences ($P<0.05$) LW (21 d) and LWG (5 to 21 d) were observed. Especially, β-vinasse at 30 g/kg caused a significant increase in LW (21 d) and LWG (5 to 21 d). The dietary treatment of quails with different levels of β-vinasse did not affect hot carcass weight, cold carcass weights, hot and cold carcass yields, and breast and thigh pH (Table 4).The lightness (L^*) and yellowness (b^*) of breast values were significantly ($P<0.05$) increased in group 2. The effects of dietary supplementation with different levels of β-vinasse on ilio-caecal bacteriological flora are provided in Table 5. The result of the study showed that the different levels of β-vinasse significantly increased ($P<0.05$) *Lactobacillus* spp. ilio-caecal area. There are no statistical differences in coliform bacteria among the different groups.

DISCUSSION

The nutrient compositions of β-vinasse are presented in Table 2. As shown in Table 2, the β-vinasse used in this research contained 63% dry matter, 22.3% crude protein, 11% crude ash, 20% betaine, and

Table 5. Effects of β-vinasse supplementation on ilio-caecal microflora in quails (n=20)

	Control	Group 1	Group 2	*P value*
Coliform (Log cfu/g)	2.46±0.36	3.25±0.30	2.95±0.37	0.279
Lactobacillus (Log cfu/g)	2.98±0.16[b]	3.69±0.13[a]	3.50±0.15[a]	0.004

[a,b] Mean values with different superscripts in the same row differ significantly ($p<0.05$)
Values are expressed means ±standart deviation
Group 1: 15 g/kg β-vinasse, Group 2: 30 g/kg β-vinasse

2.05% potassium. In another study, ProMass (modified vinasse product) contains 35.47% crude protein, 5.94% crude ash, 10% betaine, and 1.66 % potassium (Yalcın et al., 2010). These levels may vary according to processing conditions and extraction methods.

In the study, it was determined that the addition of 30 g/kg β-vinasse to the diet caused a significant increase in live weight and live weight gain, especially in the initial period.These results are in agreement with those obtained by (Attia et al., 2005; Nofal et al., 2015) who found that the addition of betaine to poultry diet improved significantly body weight.This improvement in performance may be due to the effect of betaine on nutrient digestibility. Also, the total betaine need cannot be met by endogenous metabolism, thus, dietary betaine supplementation may be beneficial to maintain or to improve chicken, health, and productive performance (Eklund et al., 2005). In their study, Cengiz et al. (2019) found that the addition of 30 g/kg bromass (modified vinasse) to the broiler ration caused a significant increase in body weight and body weight gain in broilers at the beginning and development period, while it caused a significant decrease in feed consumption.Lewicki (2001) and Stemme et al. (2005), in their studies, reported that the addition of β-vinasse to the diet caused a decrease in feed cost and a higher level of performance.Studies have also shown that this effect is more significant under stress conditions.Attia et al. (2009) stated that the effect of severe heat stress could partially be overcome by adding 1 kg betaine/ton to chick diets which improved weight gain and feed conversion compared to negative treatment. In contrast to our results, Uzunoglu and Yalcin (2019) showed that betaine dietary supplementation did not affect the growth performance indices of broilers, which could be due to different experimental conditions and the supplemented dose of betaine between the two studies. Different experimental results suggested the need for further experiments to escape the gap in contradictory results due to different levels of betaine used, animal species and environmental conditions, and composition of the experimental diets. Betaine supplement may stimulate protection of intestinal epithelium against osmotic disturbance; improve digestion, absorption, and nutrient utilization in broiler chickens (Mahmoudnia and Madani, 2012).

Our results showed that β-vinasse supplementation did not affect quail carcass characteristics, but caused significant changes in the values of parameters effective in meat quality, such as meat *L**, *a**, and *b** values. Likewise, Al-Sagan et al. (2021), in their study, determined that the addition of betaine at different levels to broiler diets did not cause a significant effect on carcass parameters and meat qualityparameters (*L**, *a** value, and *b** value). In contrast, El-Shinnawy (2015) revealed that broilers that consumed betaine-supplemented diets had increased carcass and carcass part yield percentages. Attia et al. (2009), who indicated that heat stress and dietary betaine supplementation did not affect meat pH and color. Nutautaite et al. (2020) showed that betaine dietary supplementation did not influence breast-muscle colors *L**, *b**, and the water holding capacity (WHC) in broilers meat.

Although there are many studies on the performance of meat and egg-oriented poultry on betaine and its derivatives, studies on the gastrointestinal microbiota population are limited. Betaine also acts as an organic osmolyte by stabilizing intestinal epithelial cells and supporting intestinal microbial growth (Metzler-Zebeli et al., 2009; Weiss et al., 2013).In the digestive tract, there are trillions of microorganisms that cooperate with the host organism with many mechanisms such as nutrient absorption, energy balance, and immunity (Kostadinović and Lević, 2018). Recent studies have focused on this issue, as a healthy and stable microbial community (microbiota) is crucial to animal health and performance. In this study, it was determined that the β-vinasse supplement added to the quail diet caused a significant increase in the beneficial microorganism population. In particular, the *Lactobacillus* species found in the gastrointestinal tract have received tremendous attention due to their

health-promoting properties. Lactic acid bacteria, particularly *Lactobacillus* strains, are frequently used as probiotics which are defined by the FAO/WHO as live microorganisms that when administered in adequate amounts confer a health benefit on the host (Walter, 2008). *Lactobacillus* strains have a high ability to attach to the intestinal epithelium and can establish in the chicken intestine within a day after hatching, so they are considered to be normal bacterial flora of the gastrointestinal tract of chickens (Shokryazdan et al., 2017; Shah et al., 2019). Ahmed et al., (2018) determined that using betaine alone did not have significant effects total count of *E.coli* and *Lactobacillus* bacteria. Although there are data on the small intestine histomorphology of betaine supplementation in the studies (Alahgholi et al., 2014; Klasing et al. 2002) conducted, data on the small intestine microbiota have not been found. In this sense, the data of this study will help reveal the effects on the small intestine microorganism population change.

CONCLUSIONS

As a result, it was concluded that β-vinasse (by-product obtained from molasses) can be used in quail diets as an alternative feed source that will meet the nutritional needs of the animal and have positive effects on the digestive system, especially on the intestinal health**.**It has been demonstrated that the addition of β-vinasse at 15 and 30 g/kg levels to quail diets has a positive effect on lactobacillus type microorganisms that have probiotic effects in terms of intestinal health.

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CONFLICT OF İNTEREST STATEMENT

The authors declare no conflict of interest.

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