

Use of bromass in broiler rations as a different protein source

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SUMMARY

In this study, we aimed to determine the effects of modified vinasse (Bromass) on body weight, body weight gain, feed intake, feed conversion ratio, carcass yield, litter quality, blood serum parameters and broiler performance index. A total of 600 Ross-308 broiler chicks were used in the experiment, and the chickens were divided into the following 4 main groups: control (0 g/kg Bromass), group I (5 g/kg Bromass), group II (30 g/kg Bromass), and group III (60 g/kg Bromass). Additionally, each of the main groups was divided into 10 subgroups of 15 chickens each. The trial lasted for 42 days. Supplementation with Bromass (5, 30 and 60 g/kg) caused significant ($P < 0.05$) increases in the average body weight, body weight gain, feed intake, feed conversion ratio, carcass weights, carcass yield and breast yields. Additionally, Bromass supplementation caused more dry litter at a significant level ($P < 0.05$) and significant ($P < 0.05$) decrease in serum cholesterol concentration. The addition of Bromass to the broiler diets resulted in a significant decrease in feed prices ($P < 0.05$) and an important increase ($P < 0.05$) in the broiler performance index values. At the end of the study, we concluded that the addition of Bromass as a different protein source, especially at the 30 g/kg level, caused positive effects and Bromass makes it possible to produce a more profitable broiler.

Key Words: Betaine, broiler, bromass, cholesterol, performance, protein source

RÉSUMÉ

Utilisation de bromasse dans les rations de poulet de chair en tant que source de protéines

Cette étude visait à déterminer les effets de la vinasse enrichie en bétaine (Bromass) sur le poids vif, le gain de poids vif, la consommation alimentaire, l'indice de conversion alimentaire, le rendement de la carcasse, la qualité de la litière, les paramètres sériques et l'indice de performance des poulets de chair. Un total de 600 poulets de chair Ross-308 a été reparté en 4 groupes de 150 animaux : groupe témoin (0 g/kg de Bromass), groupe I (5 g/kg de Bromass), groupe II (30 g/kg de Bromass) et du groupe III (60 g/kg de Bromass). Chaque groupe principal était divisé en 10 sous-groupes de 15 poulets. La supplémentation alimentaire en Bromass a entraîné une amélioration de la sècheresse de la litière ($p = 0,002$) et une diminution de la concentration en cholestérol sérique ($P < 0,05$). En outre, la supplémentation alimentaire en Bromass a entraîné une diminution significative des prix des aliments ($P = 0,001$) et une augmentation importante des valeurs de l'indice de performance des poulets de chair. Au terme de l'étude, nous avons conclu que la supplémentation alimentaire en Bromass comme source de protéines a eu des effets positifs sur la santé des poulets de chair et amélioré les performances économiques.

Mots-clés : Bétaine, poulet de chair, Bromass, cholestérol, performance, source de protéines

Introduction

Utilizing by-products as a cheap and different sources for animals an attractive possibility due to enhanced environmental and economic concerns because most food by-products pose problems in areas of environmental protection.

Molasses is used directly as an animal feed or to obtain different fermentation products (yeast, ethyl alcohol, lysine and betaine). Another fermentation product of molasses is vinasse [4]. Briefly, vinasse is a by-product from industrial production of yeast, alcohol, citric acid or other substances by fermentation of molasses. The chemical composition of vinasse is 48% nitrogen compounds, 10% betaine, and 5-18% potassium [4]. Therefore, vinasse can be used as an animal feed ingredient and a source of nutrients and minerals [12]. These levels may vary according to processing conditions and extraction methods. Due to the betaine in the vinasse structure, vinasse has gained importance as a valuable

additive in poultry feed. Betaine is the trimethyl derivative of the amino acid glycine [5]. In animal nutrition, betaine is widely discussed as a 'carcass modifier' due to its lipotropic and growth-promoting effects [5]. Hassan et al. [8] suggested that betaine addition at either 0.072 or 0.144% significantly improved body weight gain (BWG) by 4.4 and 4.8%, and feed conversion ratio (FCR) by 4.2 and 6.1 compared to control group, respectively.

With traditionally produced vinasse, high humidity, potassium and NPN compounds are the major factors that limit its use in poultry. However, with modified vinasse (Bromass), which is produced by Integro (Pak Food Production and Marketing Inc., Kocaeli, Turkey), the high potassium level, which inhibits the use of conventionally produced vinasse, has been reduced to 2% using physicochemical techniques, and the betaine content has been purified. Additionally, the high moisture content is absorbed into sunflower seeds at a rate of 45-55%, and the result is then dried with a special process to obtain the product termed "Bromass". Thus, the

dry matter level is increased to 94% in the Bromass product. Here, we added this product to broiler rations to provide original quality research.

In this study, we aimed to investigate the effects of the addition of Bromass (Modified vinasse) to broiler rations as a different protein source on performance parameters, carcass parameters, blood serum parameters, litter quality and economical evaluation parameters.

Material and Methods

ANIMALS, DIETS AND EXPERIMENTAL DESIGN

A total of 600 Ross 308 male broiler chicks were obtained from the Uludag University Animal Health and Production, Research and Application Centre of broiler breeding (Bursa, Turkey). The study protocol was approved by Ethics Committee of Uludag University (HADYEK decision no: 2016 -16/03). One-day-old chicks were obtained from a local hatchery and divided into 4 groups of 150 birds each. The chicks were individually weighed and distributed into 40 floor pens with 15 chicks per pen. Each 2.0x1.2 m floor pen was furnished with wood shaving litter. Fluorescent lamps provided 23 hours of continuous light per day. The chickens were vaccinated against Infectious bronchitis and Newcastle disease (Nobilis MA5+Clone30) at 9 days of age and against Gumboro disease at 23 days of age. The experiment lasted for 42 days.

The formulations were adjusted according to phase-feeding practices (three basal diets) as the chickens advanced in age and weight and as established by the breeder (Ross 308). The basal diets were mixed under commercial conditions as one batch, divided into respective parts and then supplemented with Bromass by means of a horizontal mixer. The provided diets were prepared isocalorically (3030-3200 kcal/kg of diet) and isonitrogenously (22.38-19.43% crude protein). Diets were formulated to meet or exceed the requirements of the National Research Council [15] for broilers at this age. The feeds and water were provided for *ad libitum* consumption. The ingredients and chemical compositions of the basal diets are presented in Table I. The chemical composition of β -vinasse (modified vinasse) is presented in Table II. No antibiotics or growth promoters were added to any of the treatment diets. The experimental diets were chemically analyzed according to the methods of the Association of Official Analytical Chemists [1]. The metabolizable energy (ME) levels of the diets were estimated using the equation of Carpenter and Clegg [11]: $ME \text{ (kcal/kg)} = 53 + 38 [(CP, \%) + (2.25 \times \text{ether extract, } \%) + (1.1 \times \text{starch, } \%) + (1.05 \times \text{sugar, } \%)]$. In the study, the feeding program consisted of a starter diet until 21 d of age, a grower diet until 35 d of age and a finisher diet until 42 d of age. The birds were fed either a basal diet (control group) or the basal diet with bromass supplementation at doses of 5 (0.5%, group I), 30 (3.0%, group II), or 60 (6.0%, group III) g/kg feed.

PERFORMANCE PARAMETERS

The chicks were weighed individually at the beginning of the experimental period, after which the animals were weighed weekly to calculate the BWG. Mortalities were recorded as they occurred. Feed consumption (FC) was recorded weekly and is expressed as kg per chicks per week. The FCR was calculated as kg feed per kg BWG. At the end of the study, to determine the carcass yield, 50 male animals from each group (a total of 200 animals, 5 from each subgroup) were weighed and slaughtered under commercial conditions. The hot carcass weight was taken as the weight of the carcass after processing. The cold carcass weight was taken as the weight of the carcass after it was kept for 18 h at 4 °C. The hot and cold carcass yields were calculated by dividing the carcass weights by the body weights (BW).

INTERNAL ORGAN WEIGHTS

During slaughter, liver, heart, pancreas, thigh and breast samples were taken, and their weights were determined. The parts yields (thighs and breast) were calculated as the ratio between the average part weight (PW) representative of each experimental unit and carcass weight according to the following formula: $\text{Parts yield (\%)} = \text{PW} / \text{carcass weight} \times 100$

ECONOMIC ANALYSES (EUROPEAN PRODUCTION EFFICIENCY FACTOR (EPEF) AND THE EUROPEAN BROILER INDEX (EBI))

For the economic analysis of performance indicators, including the BWG, average daily gain (ADG), FCR, viability. EPEF and EBI values was determined by the following formulas:

$\text{BWG (grams on period)} = \text{BW (g) at the end period} - \text{BW (g) in first d};$

$\text{ADG (g/chick/d)} = \text{BWG/number of days in the growth period};$

$\text{FCR (kg feed/kg gain)} = \text{cumulative feed intake (kg)/total weight gain (kg)};$

$\text{Viability (\%)} = \text{chicks remaining at the end of the period (\%)};$

$\text{EPEF} = [\text{BW (kg)} \times \text{viability (\%)} / \text{FCR (kg feed/kg gain)} \times \text{age (42d)}] \times 100;$

$\text{EBI} = (\text{ADG (g/chick/d)} \times \text{viability (\%)} / (10 \times \text{FCR (kg feed/kg gain)})).$

DETERMINATION OF SERUM BIOCHEMICAL VALUES

At the end of the sixth week of the experiment, 10 birds from each pen were selected randomly, and serum samples were taken from the neck vein by puncture and drawn into vacutainer tubes. Blood samples were collected in glass serum-collecting tubes. The blood samples were then centrifuged at 3000 rpm for 10 minutes and then sera were carefully harvested and stored at -20°C until analysis.

Starter				
	Control	Group I	Group II	Group III
Ingredients				
Corn (%)	53.64	53.64	52.76	51.70
Soybean meal (%)	28.62	28.12	25.62	22.63
Full fat soybean (%)	10.30	10.30	11.08	11.95
Corn Gluten (%)	1.33	1.33	1.33	1.53
Vegetable oil (%)	1.80	1.80	1.85	1.96
Bromass ³ (%)	-	0.50	3.0	6.00
Dicalcium (%) phosphate	1.95	1.95	1.96	1.99
Limestone (%)	0.9	0.90	0.90	0.90
Salt (%)	0.25	0.25	0.25	0.16
Vit-Min Premix ¹ (%)	0.25	0.25	0.25	0.25
DL-Methionine (%)	0.34	0.34	0.34	0.20
L-Threonin (%)	0.10	0.10	0.10	0.12
L-Lysin HCl (%)	0.21	0.21	0.24	0.28
Sodium bicarbonate (%)	0.10	0.10	0.10	0.10
Cholin chloride 60 (%)	0.11	0.11	0.12	0.13
Anticoccidial (%)	0.10	0.10	0.10	0.10
Analysed concentration, %				
Crude Protein (%)	22.38	22.36	22.32	22.37
Ether extract (%)	6.62	6.60	6.65	6.55
Saccharose (%)	4.66	4.65	4.70	4.78
Starch (%)	38.03	38.59	38.59	38.22
Dry matter (%)	90.88	90.54	90.07	90.23
Ash (%)	10.19	10.42	9.86	9.86
Calcium (%)	1.06	1.15	1.15	1.07
Total Phosphorus (%)	0.73	0.78	0.78	0.71
Metabolisable energy (MJ/kg)	12.68	12.67	12.69	12.67
Grower				
Ingredients				
Corn (%)	54.42	54.39	54.00	53.02
Soybean meal (%)	18.03	17.53	15.03	12.03
Full fat soybean (%)	14.00	14.00	14.10	14.50
Corn Gluten (%)	2.70	2.70	2.98	3.38
Wheat (%)	4.42	4.42	4.42	4.41
Vegetable oil (%)	2.75	2.75	2.75	2.90
Bromass ³ (%)	-	0.50	3.00	6.00
Dicalcium phosphate (%)	1.61	1.61	1.65	1.69
Limestone (%)	0.82	0.82	0.80	0.78
Salt (%)	0.20	0.20	0.14	0.07
Vit-Min Premix ¹ (%)	0.25	0.25	0.25	0.25
DL-Methionine (%)	0.16	0.16	0.15	0.16
L-Threonin (%)	0.12	0.13	0.15	0.16
L-Lysin HCl (%)	0.17	0.18	0.22	0.27
Sodium bicarbonate (%)	0.17	0.17	0.17	0.17
Cholin chloride 60 (%)	0.08	0.09	0.09	0.11
Anticoccidial (%)	0.10	0.10	0.10	0.10
Analysed concentration, %				
Crude Protein (%)	20.42	20.25	20.82	20.92
Ether extract (%)	8.94	8.08	7.56	7.28
Saccharose (%)	5.30	5.88	5.88	5.14
Starch (%)	38.73	40.30	40.80	41.85
Dry matter (%)	90.33	90.35	90.27	90.58
Ash (%)	8.58	9.27	8.58	9.77
Calcium (%)	0.78	0.78	0.78	0.79
Total Phosphorus (%)	0.65	0.65	0.66	0.66
Metabolisable energy (MJ/kg)	13.37	13.39	13.38	13.39

Finisher				
Ingredients				
Corn (%)	62.23	62.21	61.55	61.11
Soybean meal (%)	14.50	14.00	11.50	8.62
Full fat soybean (%)	14.41	14.43	14.40	14.60
Vegetable oil (%)	2.00	2.00	2.10	2.20
Corn Gluten (%)	3.23	3.23	3.82	3.82
Limestone (%)	0.80	0.80	0.80	0.76
DCP 18 (%)	1.56	1.56	1.60	1.65
DL methyonine 99 (%)	0.13	0.13	0.13	0.13
L-Lysin (%)	0.18	0.18	0.23	0.29
Salt (%)	0.20	0.20	0.20	0.12
Sodium bicarbonate (%)	0.20	0.20	0.10	0.10
Vit-Min Premix ² (%)	0.25	0.25	0.25	0.25
Cholin chloride 60 (%)	0.09	0.09	0.10	0.11
L-threonine (%)	0.12	0.12	0.12	0.14
Vit-E (%)	0.10	0.10	0.10	0.10
Bromass ³ (%)	0.00	0.50	3.00	6.00
Analysed concentration, g/kg				
Crude Protein (%)	19.49	19.78	20.28	19.43
Ether extract (%)	7.75	7.45	7.68	7.66
Saccharose (%)	5.60	5.80	4.42	5.42
Starch (%)	41.60	42.00	42.00	42.10
Dry matter (%)	91.63	89.85	90.28	89.96
Ash (%)	8.79	9.93	8.85	7.86
Calcium (%)	0.76	0.75	0.75	0.78
Total Phosphorus (%)	0.62	0.62	0.65	0.66
Metabolisable energy (MJ/kg)	13.34	13.37	13.35	13.36

¹ R.124 STR.VM: Per 2.0 kg premix contains; Vit A 12 500 000 IU, Vit D₃ 4 000 000 IU, Vit E 125 000 mg, Vit K₃ 3 000 mg, Vit B₁ 2 700 mg, Vit B₂ 7 000 mg, Vit B₆ 4 000 mg, Vit B₁₂ 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, Beta-Glucanase 80 000 U, Fungal-1.3-B-Glucanase 70 000 U

² R.124 GRO. VM: Per 2.0 kg premix contains; Vit A 12 500 000 IU, Vit D₃ 3 000 000 IU, Vit E 60 000 mg, Vit K₃ 3 000 mg, Vit B₁ 2 700 mg, Vit B₂ 7 000 mg, Vit B₆ 4 000 mg, Vit B₁₂ 20 mg, Niacine 40 000 mg, Kalsiyum d-pantothenate 15 000 mg, Folic acid 1 500 mg, Biotin 150 mg, Mn 75 000 mg, Fe 45 000 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 160 000 mg, Fitase 1 000 000 FTU, Xylanase 270 000 U, Beta-Glucanase 80 000 U, Fungal-1.3-B-Glucanase 70 000 U

³ Bromass: Contains 45% β-Vinas 55% Sunflower meal (%36 HP)

TABLE I: Ingredients (g/kg) and chemical composition of the broiler rations

Serum Ca, serum P were analysed with a spectrophotometer (Novaspec® II, Mod 4040) using commercial kits (Teco diagnostics, 1268 N.LAKEVIEW AVE. ANAHEIM, CA 92807 USA). Serum concentrations of total proteins (Lot No: 0517 0209 3871), cholesterol (Lot No: 0836 3242 1327) and activities of alanine amino transferase (ALT; LotNo: 6245 0327 4934), aspartate amino transferase (AST; Lot No: 7314 3252 8703) and alkaline phosphatase (ALP; Lot No: 6536 0312 4422) were determined by a Vitros 350 autoanalyser (New York, USA; Product code 680-2153) using their accompanying commercial kits (Vitros Chemistry Products, Ortho-Clinical Diagnostics, Johnson-Johnson Company, New York, USA).

DETERMINATION OF LITTER DRY MATTER

Litter samples were taken from each replicate group, and dry matter analyses were performed. Litter quality was assessed in a series of samples that were obtained from

five different points located at the edges and in the center of each compartment. A designated cylindrical sampler, which was 30 cm long and 8 cm in diameter, was used to obtain vertical core samples of the litter. Each sample was put in a polyethylene bag that was sealed and temporarily kept in a portable refrigerator until it was transferred to the laboratory for analyses. The analyses of the litter samples were performed immediately when the samples arrived at the laboratory of Animal Nutrition and Nutritional Disease Veterinary Faculty of Uludag University of Turkey. Following the AOAC Analytical Methods [1], the moisture content was determined for each individual sample. The moisture contents (% ww) of the samples were determined by drying them at 105°C to a constant weight.

STATISTICAL ANALYSES

The statistical analyses were performed with the SPSS [18] software package (SPSS Inc., Chicago, IL, USA) for Windows.

Nutrients	Vinasse	β -vinasse	Bromass
Dry matter (%)	29.30	63.0	94.0
Crude Protein (%)	3.63	22.30	36.50
Crude ash (%)	7.32	11.00	10.50
Metabolisable Energy (MJ/kg)	-	3.91	9.12
Crude cellulose (%)	-	0.80	10.20
Lysine (%)	0.13	0.137	0.90
Meth&Cys (%)	0.26	0.032	1.00
Methionine (%)	0.034	0.032	0.50
Threonine (%)	0.13	0.169	1.00
Valine (%)	0.20	0.206	1.30
Isoleucine (%)	0.017	0.136	1.00
Arginine (%)	0.052	0.061	2.00
Tryptophan (%)	-	0.039	0.30
Calcium (%)	-	0.028	0.30
Total Phosphorus (%)	-	0.054	0.70
Sodium (%)	-	1.41	0.90
Potassium (%)	8.20	2.05	1.80
Betaine (%)	10.40	20.00	11.10
D.Lysine (%)	-	0.086	0.41
D.Meth&Cys (%)	-	0.20	0.48
D.Methionine (%)	-	0.21	0.29
D.Threonine (%)	-	0.105	0.43
D.Valine (%)	-	0.130	0.58
D.Isoleucine (%)	-	0.084	0.62
D.Arginine (%)	-	0.039	1.05
D.Tryptophan (%)	-	0.024	0.25

TABLE II: Nutrient composition of vinasse, bromass and β -vinasse

Variance analysis was used to determine the significance of the differences between the statistical calculations for the groups and the mean values of the groups, Tukey tests were used as post hoc tests, and the level of significance used in all of the tests was $P < 0.05$. The results are expressed as the means \pm the standard deviation of the mean.

Results

The present study was conducted to investigate the effects of different levels of Bromass on the performance parameters, carcass parameters, blood serum parameters, litter quality and economical evaluation parameters of broiler chickens. The ingredients and chemical compositions of the diets are presented in Table I. The nutrient compositions of Bromass, vinasse and β -Vinasse are presented in Table II. As shown in the table II, the β -vinasse used in this research contained 63% dry matter, 22.3% crude protein, 11% crude ash, 20% betaine and 2.05% potassium. The dry matter level was raised to 94% with the Bromass product. This modification, β -vinasse with high moisture content is absorbed into sunflower seeds at a rate

of 45-55%, and the result is then dried with a special process to obtain the product termed "Bromass".

The results concerning the effects of Bromass on broiler performance are presented in Table III. At the beginning of the study, there were no differences in the BW of the animals in the experimental groups. Briefly, it said that BW are homogeneous. As shown in table III, significant differences ($P < 0.05$) in BW and BWG were observed. Specifically, Bromass at 30 mg/kg caused a significant increase in BW and BWG during the growing and finishing periods. When we performed assessments across the overall duration of the study (1-42 days), the addition of the high level of Bromass resulted in a significant ($P < 0.05$) decrease in FCR. In this study, significant differences ($P < 0.05$) in FCR between the control and experimental groups were identified in the 1-42 d period. The best FCR in the study was observed in the group in which 30 g/kg (group II) of Bromass was added to the ration.

The effects of dietary treatment on carcass weight, carcass yield, visceral organ weights (heart, liver, and pancreas), thigh-breast pH, thigh-breast yield, litter dry matter and mortality

	Control	Group I	Group II	Group III
<i>Average Body Weight (g) (n=150)</i>				
1 day	47.7 ± 3.88	47.3 ± 3.62	47.3 ± 3.66	46.8 ± 3.43
7 day	162.4 ± 21.06 ^b	167.7 ± 20.47 ^{ab}	171.7 ± 17.94 ^a	170.6 ± 18.60 ^a
14 day	432.1 ± 49.60 ^b	439.3 ± 55.07 ^{ab}	449.2 ± 46.75 ^a	443.8 ± 49.98 ^{ab}
21 day	837.6 ± 104.58	848.4 ± 116.06	859.0 ± 101.63	863.6 ± 99.71
28 day	1371.6 ± 201.99	1392.4 ± 201.59	1407.2 ± 195.84	1399.1 ± 172.80
35 day	1971.3 ± 290.61 ^b	2027.4 ± 306.91 ^{ab}	2062.7 ± 215.28 ^a	2028.2 ± 246.49 ^{ab}
42 day	2605.7 ± 263.19 ^b	2644.4 ± 401.86 ^{ab}	2738.7 ± 293.29 ^a	2650.2 ± 305.45 ^{ab}
<i>Body Weight Gain (g)(n= 150)</i>				
1-7 day	114.77 ± 17.81 ^b	120.51 ± 18.55 ^a	124.35 ± 15.11 ^a	123.96 ± 15.90 ^a
7-14 day	269.68 ± 32.99	272.06 ± 45.65	277.89 ± 32.19	273.11 ± 33.39
14-21 day	406.01 ± 64.08	409.02 ± 68.41	410.16 ± 60.52	419.85 ± 55.43
21-28 day	533.98 ± 108.16	545.32 ± 99.42	548.17 ± 104.46	537.30 ± 83.61
28-35 day	604.84 ± 130.22 ^b	635.02 ± 120.28 ^{ab}	658.81 ± 87.74 ^a	629.08 ± 91.50 ^{ab}
35-42 day	652.14 ± 156.53 ^b	616.97 ± 117.71 ^b	716.44 ± 158.34 ^a	643.77 ± 135.55 ^b
1-42 day	2558.02 ± 338.31 ^b	2597.39 ± 399.05 ^{ab}	2692.27 ± 290.89 ^a	2603.83 ± 302.99 ^{ab}
<i>Feed Intake (g) (n=10)</i>				
1-7 day	218.46 ± 60.46 ^a	162.60 ± 17.56 ^b	165.70 ± 14.30 ^b	159.07 ± 21.24 ^b
7-14 day	482.17 ± 57.07 ^a	427.60 ± 42.58 ^{ab}	416.74 ± 38.47 ^b	467.55 ± 41.04 ^{ab}
14-21 day	641.83 ± 94.91	607.66 ± 23.50	604.59 ± 28.78	591.12 ± 38.30
21-28 day	955.79 ± 40.27	972.20 ± 60.05	967.78 ± 64.75	955.80 ± 57.48
28-35 day	1225.90 ± 75.59	1218.64 ± 53.09	1206.85 ± 59.89	1186.20 ± 53.77
35-42 day	1481.13 ± 50.96	1473.25 ± 73.62	1441.62 ± 56.77	1412.29 ± 59.05
1-42 day	5005.28 ± 98.04 ^a	4861.65 ± 204.69 ^{ab}	4803.28 ± 169.65 ^b	4772.02 ± 174.17 ^b
<i>Feed Conversion Ratio (kg/kg) (n=10)</i>				
1-7 day	1.90 ± 0.51 ^a	1.36 ± 0.18 ^b	1.33 ± 0.08 ^b	1.28 ± 0.19 ^b
7-14 day	1.79 ± 0.24 ^a	1.58 ± 0.23 ^{ab}	1.50 ± 0.12 ^b	1.71 ± 0.16 ^{ab}
14-21 day	1.58 ± 0.17	1.49 ± 0.05	1.48 ± 0.10	1.42 ± 0.31
21-28 day	1.80 ± 0.10	1.78 ± 0.09	1.77 ± 0.12	1.79 ± 0.15
28-35 day	2.05 ± 0.29 ^a	1.92 ± 0.04 ^{ab}	1.84 ± 0.13 ^b	1.89 ± 0.10 ^{ab}
35-42 day	2.29 ± 0.24 ^a	2.39 ± 0.12 ^a	2.01 ± 0.19 ^b	2.22 ± 0.31 ^{ab}
1-42 day	1.96 ± 0.11 ^a	1.87 ± 0.04 ^b	1.78 ± 0.04 ^c	1.84 ± 0.07 ^{bc}

^{a, b, c} Different superscripts in each row shows the significant difference between the groups ($P < 0.05$). Data are shown as Mean ± Standard Deviation

Table III: Effects of bromass supplementation on average body weight, body weight gain, feed intake and feed conversion ratio in broiler

are presented in Table IV. The dietary treatment of 1-day-old chicks with different concentrations of Bromass for 6 weeks did not affect ($P > 0.05$) visceral organ weights, breast-thigh pH or mortality. Significant differences ($P < 0.05$) between the control and experimental groups were observed in the parameters of carcass value (carcass weight, carcass yield and carcass shrink). The breast yield increased in parallel with the increase in Bromass in the ration ($P < 0.05$). Additionally, Bromass addition to the broiler rations at the 30 and 60 g/kg levels caused a significant increase ($P < 0.05$) in the dry matter of the litter.

The results concerning the effects of Bromass on some blood parameters are presented in Table V. In this study, the serum ALT, AST, ALP, total protein, calcium and phosphorus levels were not affected by the levels of Bromass in the broiler diets. In contrast, Bromass supplementation significantly ($P < 0.05$) decreased the serum cholesterol concentrations.

The effects of Bromass on the economically relevant parameters of the broilers in the trial groups are presented in Table VI. In this study, differences in the total FC, feed cost, average BW, EPEF and EBI economic parameter were determined to be statistically significant. For the overall experimental period (1 to 42 d), the Bromass supplemented broilers ate less feed ($P < 0.05$), required lower feed costs ($P < 0.05$) and reached greater BW ($P < 0.05$). Production efficiency was assessed using the EBI and EPEF. The best EPEF and EBI values in this study were observed in the experimental groups that received feed with added Bromass.

Discussion

Bromass, which was added to the broiler rations in this study, contains 36.5% crude protein, 10.5% crude ash, 11.1% betaine and 1.8% potassium. In another study, ProMass

	Control	Group I	Group II	Group III
Final body weight (g)	2715.3 ± 469.6 ^b	2944.5 ± 312.5 ^a	2990.3 ± 240.6 ^a	2933.4 ± 216.6 ^a
Hot carcass weight (g)	2013.1 ± 359.4 ^b	2212.6 ± 230.9 ^a	2264.2 ± 184.5 ^a	2221.8 ± 169.1 ^a
Cold carcass weight (g)	1962.8 ± 369.6 ^b	2186.5 ± 230.8 ^a	2232.9 ± 182.7 ^a	2194.0 ± 161.8 ^a
Carcass yield (%)	72.4 ± 6.30 ^b	74.3 ± 1.60 ^{ab}	74.7 ± 1.90 ^a	74.8 ± 2.40 ^a
Carcass Shrink (%)	2.7 ± 2.40 ^a	1.2 ± 0.60 ^b	1.4 ± 0.40 ^b	1.2 ± 0.50 ^b
Heart (%)	0.4 ± 0.06	0.4 ± 0.05	0.4 ± 0.04	0.4 ± 0.05
Liver (%)	1.9 ± 0.14	2.0 ± 0.17	2.0 ± 0.18	2.0 ± 0.19
Pancreas (%)	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.04	0.2 ± 0.05
pH thigh	6.2 ± 0.16	6.3 ± 0.20	6.2 ± 0.25	6.2 ± 0.23
pH breast	5.7 ± 0.10	5.7 ± 0.12	5.8 ± 0.15	5.7 ± 0.10
Thigh yield (%)	18.5 ± 2.01	19.4 ± 1.24	19.4 ± 1.33	19.4 ± 1.21
Breast yield (%)	26.2 ± 2.22 ^b	26.7 ± 2.02 ^b	29.6 ± 1.49 ^a	30.6 ± 1.98 ^a
Litter dry matter (%)	22.6 ± 6.20 ^b	31.6 ± 6.00 ^{ab}	35.8 ± 2.20 ^a	34.2 ± 12.60 ^a
Mortality (%)	2.7 ± 3.44	3.3 ± 4.72	3.3 ± 4.72	2.7 ± 3.44

^{a, b, c}: Different superscripts in each row shows the significant difference between the groups ($P < 0.05$)
Data are shown as Mean ± Standard Deviation

TABLE IV: Effects of Bromass supplementation on carcass characteristics, litter dry matter, mortality rate in broiler

Parameters	Control	Group I	Group	Group III
ALT(U/L)	2.54 ± 1.02	3.09 ± 2.13	4.85 ± 5.60	5.47 ± 7.13
AST (U/L)	205.80 ± 22.42	213.20 ± 29.54	204.00 ± 27.92	204.30 ± 20.71
ALP (U/L)	17.33 ± 11.78	22.36 ± 14.45	22.44 ± 10.84	24.09 ± 11.31
Cholesterol (mg/dL)	199.27 ± 13.26 ^a	197.12 ± 8.02 ^a	190.40 ± 11.52 ^{ab}	186.95 ± 11.51 ^b
Total Protein (g/dL)	4.88 ± 0.47	4.68 ± 0.61	4.76 ± 0.57	4.65 ± 0.53
Calcium (mg/dL)	7.93 ± 0.82	7.64 ± 1.54	6.71 ± 2.21	6.77 ± 1.26
Phosphorus (mg/dL)	4.89 ± 0.96	5.09 ± 0.72	5.26 ± 0.82	5.51 ± 0.79

^{a, b, c}: Different superscripts in each row shows the significant difference between the groups ($P < 0.05$)
ALT: Alanin Aminotransferase AST: Aspartate aminotransferase ALP: Alkaline Phosphatase
Data are shown as Mean ± Standard Deviation

TABLE V: Effects of Bromass supplementation on some blood serum parameters.

Parameters	Control	Group I	Group II	Group III
Total Feed Consumption (g/chick)	5005.28 ± 98.04 ^a	4861.65 ± 204.69 ^{ab}	4803.28 ± 169.65 ^b	4772.02 ± 174.17 ^b
Feed Cost (Euro/chick)	1.51 ± 0.029 ^a	1.46 ± 0.061 ^{ab}	1.44 ± 0.051 ^b	1.41 ± 0.060 ^b
Average Body weight (kg/chick)	2605.70 ± 338.20 ^b	2644.41 ± 398.97 ^{ab}	2738.72 ± 291.37 ^a	2650.16 ± 303.15 ^{ab}
EPEF Value	309.86 ± 38.19 ^b	326.11 ± 16.01 ^{ab}	353.07 ± 17.31 ^a	335.38 ± 30.56 ^{ab}
EBI Value	304.21 ± 37.77 ^b	320.29 ± 15.70 ^{ab}	346.98 ± 17.17 ^a	329.48 ± 30.22 ^{ab}

- Feed prices has been calculated taking into consideration the T.C. Central Bank's exchange rate dated 25.11.2016 (1 Euro=3,64€).
(EPEF) European Production Efficiency Factor = Body weight (kg) x Viability (%) / FCR (kg feed/kg gain)xAge (42 d)
(EBI) European Broiler Index = (Average Daily Gain (g/chick/d) x Viability (%)) / (10 x FCR (kg feed/kg gain))
Data are shown as mean ± standard deviation. When a significant difference between group was observed (ANOVA, $P < 0.05$), Tukey tests were used as post hoc tests. Groups that are significantly different ($P < 0.05$) are marked by different letters.

TABLE VI: Effects of bromass supplementation on economic evaluation

(modified vinasse product) contains 35.47% crude protein, 5.94% crude ash, 10% betaine and 1.66 % potassium [25]. These levels may vary according to processing conditions and extraction methods.

In this study, Bromass at 30 mg/kg caused a significant increase in BW and BWG during the growing and finishing periods. The addition of the high level of Bromass resulted in a significant ($P<0.05$) decrease in FC value in the 1-42 d period. The best FCR in the study was observed in the group in which 30 g/kg (group II) of Bromass was added to the ration. Bilal et al. [4] determined that the effect of the addition of 2.5% vinasse to broiler diets on weight gain was significant ($P<0.05$) from 7 to 14 and 14 to 21 days of age. No difference was observed in the 35-d BW due to vinasse feeding at the 2.5 or 5% levels. Additionally, neither the FC nor the FCR of the broilers was influenced by the treatments. The use of vinasse as a feed additive in poultry has been reported on by Stemme et al. [19], who demonstrated an influence of this additive on animal performance. The positive effects of the addition of vinasse are due to its contents of yeast walls (polysaccharides and beta-glucans), minerals and B-complex vitamins. These compounds, which have been found to increase the efficiency of the utilization of nutrients, can exert effects on the immune systems of the chicken and cause the exclusion of pathogens at the digestive measurement, which therefore, produces better performing birds.

Bromass feeding led to some changes on carcass weight, carcass yield, visceral organ weights (heart, liver, and pancreas), thigh-breast pH, thigh-breast yield, litter dry matter and mortality. Rodriguez et al. [16] determined that the carcass weight and carcass yield of birds on diets that included 30% vinasse torula yeast were lower than those birds that received 10% supplementation, although the 30% group did not differ from the control group or a group that received feed with 20% supplementation. When using vinasse as an additive (5 mL during the starter, 10 mL during the grower, and 15 mL during the finisher phases), vinasse provoked greater carcass weight (1087 and 1242 g/bird), breast weight (281 and 327 g/bird) and thigh + leg weight (391 and 450 g/bird) [9]. Enhanced muscle protein accretion [17] due to improved utilization of dietary methionine that accompanies betaine supplementation [2] might have contributed to higher breast yields with betaine supplementation (Table IV). Similar to the present study, increased carcass yield following betaine supplementation has been reported in broilers [7, 14, 21, 22, 23]. It would be ideal to optimize the quantity of supplemental DL-methionine with betaine, which has a positive influence on carcass meat yield [14, 22]. In the poultry sector, controlling litter moisture is essential for the maintenance of animal health, welfare and production performance. Some feed additives are used with the objective of directly drying litter moisture by maintaining the water balance of the birds. Osmolytes, such as betaine, affect the water balance or osmotic pressure of cells and tissues by regulating the movement of water through the cell. When poultry diets are supplemented with betaine, it is quickly

absorbed by intestinal cells and balances the osmotic pressure of the gut, which contains high concentrations of inorganic salts after a meal [20]. In other words, water loss is reduced, and the integrity of the intestinal cells is maintained. Betaine seems to be effective at maintaining intestinal water balance and drying poultry litter. In the present study, the addition of Bromass to the broiler ration improved the quality of the litter by providing a stable intestinal water balance.

In this study, the serum ALT, AST, ALP, total protein, calcium and phosphorus levels were not affected by the levels of Bromass in the broiler diets. In contrast, Bromass supplementation significantly ($P<0.05$) decreased the serum cholesterol concentrations. The decreased cholesterol concentrations were 197.12, 190.40 and 186.95 in the broilers fed the diets supplemented with 5, 30 and 60 g/kg Bromass, respectively, compared to the control (199.27). These results may have been due to betaine, which plays a major role in lipid metabolism, which in turn is associated with enhanced synthesis of methylated compounds in the liver and muscle including carnitine and creatine [27]. Carnitine functions in the transport of long-chain fatty acids across the inner membrane of the mitochondria where fatty acid oxidation occurs, and thus carnitine has a role in the regulation of fat metabolism [24]. Accordingly, increased hormone-sensitive lipase activity [27] following dietary betaine supplementation results in reduced lipid deposition [5]. These results are in agreement with those obtained by Jahanian and Rahmani [10] who found that betaine enhances lipase activity and decreases the concentrations of plasma triglycerides and cholesterol in broilers and ducklings [3].

The use of Bromass in feed had some effect on the production efficiency assessed using the EBI and EPEF. The best EPEF and EBI values in this study were observed in the experimental groups that received feed with added Bromass. The positive effect of Bromass on these parameters may be due to betaine content. Because, betaine supplementation may stimulate protection of the intestinal epithelium against osmotic disturbances and improve digestion, absorption and nutrient utilization in broiler chickens [13]. Betaine supplementation of diets with adequate methyl group donors improves weight gain and FCR by approximately 3-5% [8]. Ezzat et al. [6] found that economic efficiency was improved by betaine supplementation in the Matrouh poultry strain from 24-36 weeks of age under hot Egyptian summer conditions. Zayed [26] reported that the economic efficiency was increased by feeding turkeys a diet supplemented with 0.75 or 1.5 g betaine/kg in summer conditions.

In conclusion, particularly in recent years, the spread of genetically modified soy varieties has increased the demand for natural and different protein sources. If all of the yield characteristics are taken into consideration, it is possible to safely use Bromass as a different protein source, which is obtained by special methods, at up to 6% in broiler rations as a performance enhancer. In short, the level at which bromass can be used without causing any side effects is determined

as 6% in this study. Moreover, we conclude that the use of Bromass at the 3% level in broiler ratios facilitated the development of chickens by optimizing the use of nutrients in the rations and thus provided an economic benefit.

References

1. - A.O.A.C.: ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, Official methods of analysis. 17th ed., Maryland, USA, 2000.
2. - AUGUSTINE P.C., DANFORTH H.D.: Influence of betaine and salinomycin on intestinal absorption of methionine and glucose and on the ultra-structure of intestinal cells and parasite developmental stages in chicks infected with *Eimeria acervulina*. *Avian Dis.*, 1999, **43**, 89-97.
3. - AWAD A.L., FAHIM H.N., IBRAHIM A.F., BESHARA M.M.: Effect of dietary betaine supplementation on productive and reproductive performance domyati ducks under summer conditions. *Egyptian Poultry Sci. J.*, 2014, **34**, 453-474.
4. - BILAL T., OZPINAR H., GUREL A., OZCAN A., ABAS I., KUTAY H.C.: Effects of beet vinasse {desugared molasses} on performance, blood parameters, morphology, and histology of various organs in broilers. *Arch. Geflügelkd.*, 2001, **65**, 224-230.
5. - EKLUND M., BAUER E., WAMATU J., MOSENTHIN R.: Potential nutritional and physiological functions of betaine in livestock. *Nutr. Res. Rev.*, 2005, **8**, 31-48.
6. - EZZAT W., SHOEIB M.S., MOUSA S.M.M., BEALISH A.M.A., IBRAHIEM Z.A.: Impact of betaine, vitamin C and folic acid supplementation to the diet on productive and reproductive performance of Matrouh poultry strain under Egyptian summer condition. *Egyptian Poultry Sci. J.*, 2011, **31**, 521-537.
7. - FIRMAN K.E., LI J.B., REMUS J.C.: Relationship between cystine and betaine in low methionine diets. *Poult. Sci.*, 1999, **78** (Suppl. 1), 135.
8. - HASSAN R.A., ATTIA Y.A., ELGANZORY E.H.: Growth, carcass quality and serum constituents of slow growing chicks as affected by betaine addition to diets containing 1. Different levels of choline. *Int. J. Poult. Sci.*, 2005, **4**, 840-850.
9. - HIDALGO K., RODRIGUEZ B., VALDIVIE M., EBLES M.: Utilization of distillery vinasse as additive of broiler chickens. *Cuban J. Agr. Sci.*, 2009, **43**, 273.
10. - JAHANIAN R., RAHMANI H.R.: The effect of dietary fat level on the response of broiler chicks to betaine and choline supplement. *J. Biol. Sci.*, 2008, **8**, 362-367.
11. - LEESON S., SUMMERS J.D.: Nutrition of the chicken. University Books, Guelph, Canada, 2001.
12. - LOPEZ-CAMPOS O., BODAS R., PRIETO N., FRUTOS P., ANDRES S., GIRALDEZ F.J.: Vinasse added to the concentrate for fattening lambs: intake, animal performance, and carcass and meat characteristics. *J. Anim. Sci.*, 2011, **89**, 1153-1162.
13. - MAHMOUDNIA N., MADANI Y.: Effect of betaine on performance and carcass composition of broiler chicken in warm weather - a review. *Int. J. Agr. Sci.*, 2012, **2**, 675-683.
14. - MCDEVITT R.M., MACK S., WALLISS I.R.: Can betaine partially replace or enhance the effect of methionine by improving broiler growth and carcass characteristics. *Br. Poul. Sci.*, 2000, **41**, 473-480.
15. - NATIONAL RESEARCH COUNCIL: Nutrient requirements of poultry. 9th revised. National Academy Press, Washington, DC, 1994.
16. - RODRIGUEZ B., VALDIVIE M., LEZCANO P., HERRERA M.: Evaluation of torula yeast (*Candida utilis*) grown on distillery vinasse for broilers. *Cuban J. Agr. Sci.*, 2013, **47**, 183-188.
17. - SAUNDERSON C.L., MCKINLAY J.: Changes in body weight, composition and hepatic enzyme activities in response to dietary methionine, betaine and choline levels in growing chicks. *Br. J. Nutr.*, 1990, **63**, 339-349.
18. - SPSS: Statistical software for Windows version 7.5 Microsoft Windows. SPSS, Chicago, IL, 1997.
19. - STEMME K., GERDES B., HARMS A., KAMPHUES J.: Beetvinasse (condensed molasses solubles) as an ingredient in diets for cattle and pigs – nutritive value and limitations. *J. Anim. Physiol. Anim. Nutr.*, 2005, **89**, 179-183.
20. - TROTT D.: Feed additives for drying poultry litter. *Canadian Poultry*, 15 March, 2013.
21. - VIRTANEN E., ROSIL.: Effects of betaine on methionine requirement of broilers under various environmental conditions. Proceedings of Australian Poultry Science Symposium, 1995, **7**, 88-92.
22. - WALDROUP P.W., MOTL M.A., YAN F., FRITTS C.A.: Effects of betaine and choline on response to methionine supplementation to broiler diets formulated to industry standards. *J. Appl. Poult. Res.*, 2006, **15**, 58-71.
23. - WANG Y.Z.: Effect of betaine on growth performance and carcass traits of meat ducks. *J of Zhejiang Univ (Agriculture and Life Sciences)*, 2000, **26**, 347-352.
24. - WANG Y.Z., XU Z.R., FENG J.: The effect of betaine and DL-methionine on growth performance and carcass characteristics in meat ducks. *Anim. Feed Sci. Technol.*, 2004, **116**, 151-159.
25. - YALCIN S., ELTAN Ö., KARSLI M.A., YALÇIN S.: The nutritive value of modified dried vinasse (ProMass) and its effects on growth performance, carcass characteristics and some blood biochemical parameters in steer. *Revue Med. Vet.*, 2010, **161**, 245-252.
26. - ZAYED S.M.A.: Physiological studies on turkeys. M. Sci. Thesis, Fac. Agri., Mansoura Univ., 2012, Mansoura, Egypt.
27. - ZHAN X.A., LI J.X., XU Z.R., ZHAO R.Q.: Effects of methionine and betaine supplementation on growth performance, carcass composition and metabolism of lipids in male broilers. *Br. Poult. Sci.*, 2006, **47**, 576-580.