Dicle Üniversitesi Veteriner Fakültesi Dergisi
https://dergipark.org.tr/tr/pub/duvetfd
Araştırma Makalesi/Research Article
ISSN:1307-9972 Dicle Üniv Vet Fak Derg 2020;13(2):108-113 e-ISSN:1308-0679
DOI: 10.47027/duvetfd.801606

The Effects of Bromass on the Duodenal Histomorphology in the Broilers

Sabire GÜLER[1,a,✉], Şerife Şule CENGİZ[2,b], Kerem ATAM[2,c]

[1]Department of Histology and Embryology, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, TURKEY
[2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, Turkey

[a]ORCID: 0000-0002-7367-6859; [b]ORCID: 0000-0003-0708-3833; [c]ORCID: 0000-0002-6891-4613

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
29.09.2020	26.10.2020	31.12.2020

Abstract

Molasses fermentation vinasse is used in broiler diets directly. The effects of newly developed modified vinasse product ''Bromass'' on body weight gain, feed intake, and broiler performance index, but there is no study about the Bromass effects on intestinal histomorphology. The objective of present study to examine the different doses' effects of Bromass on duodenum histomorphology; total mucosa height, villus height, crypt depth, and villus height to crypt depth ratio. For his purpose, 40 broiler chicks (Ross-308) were used and exposed to different doses of Bromass (5, 30, and 60 g/kg) for 42 days. After trials, duodenum sections were fixed with %10 formalin and after rutine histological procedure they were stained with triple (Crossman) stain and villus height, cript depth and total mucosa measured in duodenum. We found that villus height, crypt depth and total mucosa height increase after Bromass supplementation on diets. Also it was determined that villus height to crypt depth ratio, one of the most important parameters showing digestibility was increased. As the dose increased, it was determined that all parameter values increased. As a result of this study, it was found that Bromass supplementation on broiler diets increase absorption capacity and.has positive effects on intestinal morphology. In addition, this study will be lead of future studies is about different effects of Bromass on the digestive system.

Key Words: Bromass, broiler, duodenum

Bromass'ın Broylerlerde Duodenal Histomorfoloji Üzerine Etkileri

Öz

Melasın fermantasyonu sonucunda oluşan şilempe, broyler diyetlerinde direk olarak kullanılır. Yeni geliştirilen modifiye şilempe ürünü "Bromass" ın vücut ağırlık artışı, yem alımı ve broyler performans indeksi üzerindeki etkileri bilinirken, Bromass'ın duodenum histomorfolojisi üzerindeki etkileri hakkında bir çalışma yoktur. Bu çalışmada farklı doz Bromass uygulamalarının, duodenum histomorfolojisi; total mukoza, villus yükseklikleri, kript derinliği ve villus yüksekliğinin kript derinliğine oranına etkilerinin araştırılması amaçlanmıştır. Histomorfolojik inceleme için, 40 tane broyler tavuk (Ross-308), 4 gruba ayrılmış, bazal diyet (kontrol) ve Bromass'ın 3 farklı dozuyla (5, 30 ve 60 g/kg) 42 gün boyunca beslenmiştir. Uygulamanın sonucunda, duodenum örnekleri %10'luk formalin içerisinde tespit edilmiş, rutin histolojik prosedür sonrasında Crossman'nın üçlü boyasıyla boyanarak, duodenum mukozasında villus yükseklikleri, kript derinlikleri ve total mukoza ölçümleri yapılmıştır. Broyler tavukların Bromass ile beslenmesi sonrası, duodenumda villus ve total mukoza yüksekliklerinin arttığı gözlendi. Ayrıca sindirilebilirliğin en önemli göstergelerinden olan villus yüksekliğinin kript derinliğine oranında da artış saptandı. Bromass uygulama dozunun artışıyla bu parametrelerde artış belirgin olarak gösterildi. Sonuç olarak, Broyler diyetlerine Bromass katkı maddesinin ilavesi sonucunda duodumum emilim kapasitesinde artış olduğu saptanmıştır ve Bromass'ın ince bağırsak morfolojisi üzerine olumlu etkilerinin olabileceği düşünülmektedir. Bu çalışma temel alınarak, sonraki çalışmalarda, Bromass'ın sindirim sistemindeki farklı etkileri incelenmelidir.

Anahtar Kelimeler: Bromass, broyler, duodenum

INTRODUCTION

Food additives and by-products are widely used in animal nutrition (1,2). The use of these substances has become increasingly important as it reduces costs and increases growth performance (3,4). Vinasse is a recovered by product and fermentation substance of molasses (5). Studies have clearly elucidated the effects of vinasse on growth and body weight increase and sexual maturity and reproduction maturation in ruminant, pigs and broilers rations (5-7).

Conventionally vinasse is modified with physicochemical techniques and produced as a new product termed ''Bromass'' (Integro, Food Production and Marketing Incorporated Company., Kocaeli, Turkey) (3). Bromass contains 11.10% purified betaine and betaine has been used to increase nutrient digestibility and growth performance (3). Also, it has been shown betaine protects the intestinal health by protecting the intestinal cells and thus has positive effects on immunity (8).

Dietary feed components play key important roles in intestinal mucosa and histomorphology (9). Especially villus height and villus height to crypt depth ratio are an important parameter of the digestibility and absorptive potential of the duodenum in broilers (10,11). Previous studies have been reported that the villus height and villus height to crypt depth ratio have been increased after feeding with fermented products. (12,13). As a result of feeding with fermented sources, while the mucosal immunity increases, as well as the immune competence positively enhanced by the increase in the number of beneficial bacteria in the intestine (14).

The aim of this study are to determine the effects of different doses of Bromass on intestinal histomorphology; total mucosa height, villus height, crypt depth, and villus height to crypt depth ratio.

MATERIALS AND METHODS

Animals and Feeding

All experiments in this work were carried out after the consent of Bursa Uludag University Animal Researches Local Ethics Committee (HADYEK decision no: 2016 -16/03). One-day-old, 40 male broilers (Ross 308) were acquired from the broiler breeding unit of Bursa Uludag University Animal Health and Production, Research and Application Centre of broiler breeding (Bursa, Turkey). The chickens have housed 10 chicks per pen. Continuous light was provided with fluorescent lamps 23 hours per day and provided with water and feed ad libitum. Each 2.0x1.2 m floor pen was furnished with wood shaving litter. 9-day old broilers were vaccinated against Infectious bronchitis and Newcastle disease (Nobilis MA5+Clone30) and Gumboro disease at 23 day-old. The basal diets chemical compositions and ingredients are presented in Table 1. Bromass chemical composition is presented in Table 2 (94.0% dry matter, 9.12% metabolisable energy, 36.50% crude protein, 10.20%crude cellulose). The treatment diets did not consist of any growth promoters or antibiotics. The Association of Official Analytical Chemists methods is used for analyzing the experimental diets (15). In the present study, broilers, until 21 d of age, fed with a starter diet. The chickens fed with a grower diet between 21-35 d of ages, and a finisher diet until 42 d of age. The chickens were randomly divided into four groups and control group (fed with basal diet) and supplementation of Bromass at doses of 5 (0.5%), 30 (3.0%), or 60 (6.0%) g/kg feed. The studies finished after 42 days.

Table 1. Ingredients (g/kg) and chemical composition of the broiler rations (3).

	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
Bromass3 (%)	-	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 Premix1 (%)	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

(CONTINUE) Table 1 . Ingredients (g/kg) and chemical composition of the broiler rations (3).

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
Bromass3 (%)	-	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 Premix1 (%)	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 Premix2 (%)	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
Bromass3 (%)	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

[a]R.124 STR.VM: 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, Biotin150 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 100 000 FTU, Xylanase 270 000 U, Beta-Glucanase 80 000 U, Fungal-1.3-B-Glucanase 70 000 U.

[b]R.124 GRO. VM: Per 2.0 kg premix contains; Vit A 12 500 000 IU, Vit D_3 3 000 000 IU, Vit E 60 000 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, 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 000mg, Fitase 1 000 000 FTU, Xylanase 270 000 U, Beta-Glucanase 80 000 U, Fungal-1.3-B-Glucanase 70 000 U.

[c]Bromass: Contains 45% β-Vinas 55% Sunflower meal (%36 HP).

Table 2. Nutrient composition of Bromass (3).

Nutrients	Bromass
Dry matter (%)	94.0
Crude Protein (%)	36.50
Crude ash (%)	10.50
Metabolisable Energy (MJ/ kg)	9.12
Crude cellulose (%)	10.20
Lysine (%)	0.90
Meth&Cys (%)	1.00
Methionine (%)	0.50
Threonine (%)	1.00
Valine (%)	1.30
Isoleucine (%)	1.00
Arginine (%)	2.00
Tryptophan (%)	0.30
Calcium (%)	0.30
Total Phosphorus (%)	0.70
Sodium (%)	0.90
Potassium (%)	1.80
Betaine (%)	11.10
D.Lysine (%)	0.41
D.Meth&Cys (%)	0.48
D.Methionine (%)	0.29
D.Threonine (%)	0.43
D.Valine (%)	0.58
D.Isoleucine (%)	0.62
D.Arginine (%)	1.05
D.Tryptophan (%)	0.25

Histomorphometric Analysis of the Duodenum

The broilers were slaughtered at the end of the experimental and duodenum samples were taken out approximately 3-4 cm below the pylorus. The intestinal contents were removed and 10% formalin was used for fixation of the duodenum. After washing under running tap water, specimens were dehydrated through increasing concentrations of alcohol, cleared with xylene and embedded in paraffin. Sections were cut at 5 µm, mounted on slides and dried overnight. After dewaxing and rehydration, and triple (Crossman's) stain was applied for histomorphometric examination to the sections for duodenal mucosa morphology (16).

The villus height, the crypt depth and the total mucosa were measured. Nikon 80i microscope was used for taken micrographs. Randomly 5 villi per section were measured from the villus tip to the villus-crypt junction level for the villus height (Figure1,1). 5 corresponding crypts per section were measured from the villus-crypt junction to the lower limit of the crypt for crypt depth (Figure1, 2) (17). From the top of the villus to the lower limit of the crypt was measured for total mucosa thickness (Figure 1, 1+2). For each experimental group, averages of 5 randomly measured values were determined. Figure 1 illustrates the measurements that were made for each parameter.

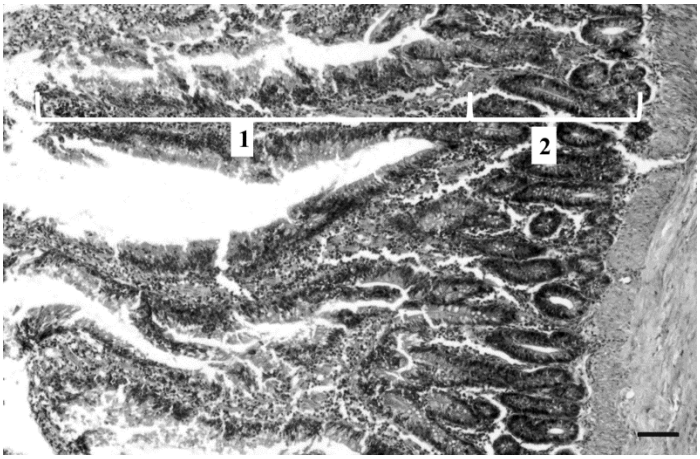

Figure 1. Morphological measurements in the duodenum, villus height (1); crypt depth (2); total mucosa (1+2), 10X, Bar: 50 µm.

RESULTS

Effects of dietary inclusion of different doses of Bromass on intestinal histomorphology; total mucosa height, villus height, crypt depth and villus height to crypt depth ratio of broiler chicks are presented in Figure 2, 3 and Table 3. In all parameters, there was a significant difference between the control and Bromass added groups (Figure 3) ($p \leq 0.05$). Villus heights increased as the dose of Bromass increased. There was no significant difference in villus height between 0.5% and 3% Bromass added groups ($p > 0.05$). In particular, the villus height of 6% Bromass group was found to be 2 times longer than the control group (Figure 3A, Table 3). Crypt depth increased in 0.5% Bromass group but decreased with increasing Bromass doses. While there was a significant difference between the dose groups ($p \leq 0.05$), the difference between the Bromass administration groups (3% and 6% Bromass added groups) and the control group was not significant ($p > 0.05$) (Figure 3B, Table 3). When the ratio between villus length and crypt depth was evaluated, it was found that there was a significant difference between all groups ($p \leq 0.05$) (Figure 3C, Table 3). Total mucosal length measurements were found to be in parallel with villus heights. Decreasing crypt depth at high doses did not affect this parameter negatively. As the dose increased, total mucosal height increased and the difference between the control and dose groups was significant ($p \leq 0.05$) (Figure 3D, Table 3).

Table 3. The effects of Bromass supplementation on villus height, crypt depth and total mucosa

	Villus Height (µm)	Crypt Depth (µm)	Total Mucosa (µm)
Control	885,05 ± 27,78^{c}	206,73 ± 11,12^{b,c}	1091,78 ± 34,37^{c}
0.5% Bromass	1444,50 ± 27,95^{b}	267,31 ± 9,67^{a}	1711,82 ± 27,34^{b}
3% Bromass	1497,39 ± 13,70^{b}	212,69 ± 7,25^{b}	1710,11 ± 14,13^{b}
6% Bromass	1888,39 ± 16,07^{a}	180,98 ± 6,28^{c}	2069,37 ± 17,55^{a}

^{a,b,c} : Different letters show statistical differences between the groups, P≤0.05.

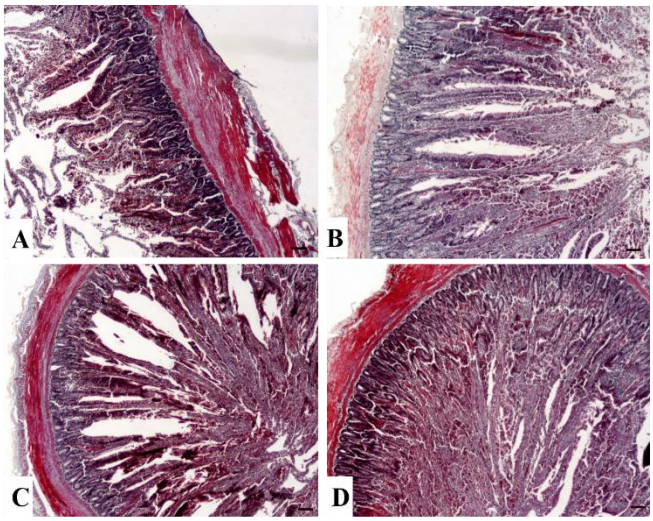

Figure 2: Villus intestinalis, Duodenum, (A) Control group, 4X, Bar 50 µm; (B) 0.05% Bromass supplementation group, 4X, Bar 100 µm; (C) 3% Bromass supplementation group, 10X, Bar 100 µm; (D) 6% Bromass supplementation group, 10X, Bar 100 µm.

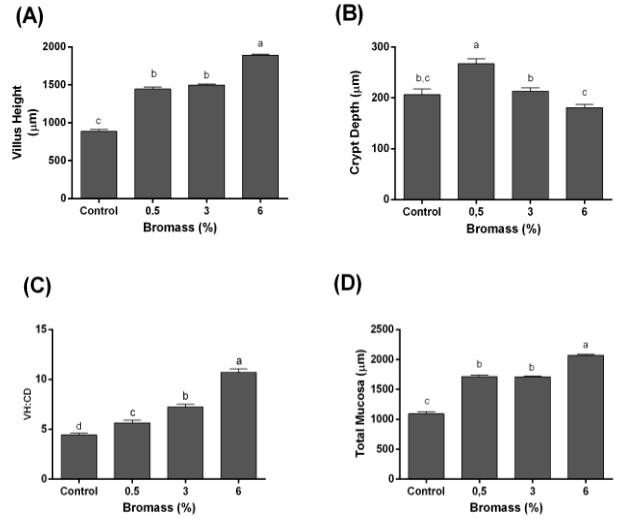

Figure 3. The effects of Bromass supplementation on villus height, crypt depth, villus height to crypt depth ratio and total mucosa.

a,b,c : Different letters show statistical differences between the groups, P≤0.05.

DISCUSSION AND CONCLUSION

Vinasse has been used extensively in animal nutrition as an additive due to its properties such as probiotic effects and being a carrier for premixes in recent years (4,5,18). After vinasse supplementation in diets, positive effects on body weight gain, growth, sexual maturation, and reproduction were observed (4,5). The new modified vinasse product ''Bromass'' added to broiler diets has also been shown to have positive effects on growth and growth (3). This is the first study on the effects of different doses of Bromass on intestinal histomorphology; total mucosa height, villus height, crypt depth and villus height to crypt depth ratio in broilers.

Cengiz et al. fed the broilers with the same doses of Bromass and observed that there was an increase in body weight and body weight gain and these increase was significant especially in the group which added 3% Bromass without any adverse effect (3). In the same study, it was determined that feed consumption decreased with increasing dose of Bromass (3). To support these results, in our study, villus height increased with increasing doses of Bromass. It has been shown that in the intestines that have a place of digestion and absorption, the absorption surface expands with increasing villus height and this is related to body weight gain and mucosal immunity (19-21). In our study, it was observed that villus length doubled in 6% Bromass group. In our study, the villus height-crypt depth ratio, another parameter showing the absorption capacity of the intestines, was found to increase with increasing Bromass doses. In many studies, it has been reported that in broilers fed with fermented feed the villi height increased as well as the villus height-crypt depth ratio (11,12). Zhang et al reported an increase in crypt depth after feeding with fermented foods (12). In our study, it was found that Bromass added in 0.5% dose caused a significant increase in crypt depth. With the increase of villus height, it needs to be renewed in the mucosa and causes high mitotic activity in crypts (22,23). Thus, a significant greater crypt depth is measured. Although it is not statistically significant, there was an increase in the 3% Bromass dose and a decrease in the Bromass dose of 6%. Feng et al fed the broilers with fermented soybean and observed increased villus height in duodenum and jejunum but decreased crypt depth (24).

In many studies, betaine has been shown to increase body weight gain, as well as increase digestibility, absorption of nutrients and water holding capacity in intestinal cells, thus positively affect gut health (14,25,26). Uzunoglu et al added at the level of 0.15% betaine in broilers diet and villus length increased as well as a decrease in crypt depth was observed (14). Similar to this study, Santos et al. determined that villus length increased and width decreased after

betaine supplementation (27). It can be concluded that the purified betaine contained in the Bromass used in our study had an effect on intestinal histomorphology, increased villus length, decreased crypt depth and consequently increased villus height crypt depth ratio. Our results support previous studies on the addition of betaine to the effects of intestinal morphology.

In conclusion, it was observed that villus height, crypt depth, total mucosa height, and villus height-crypt depth ratio increased with 0.5%, 3% and %6 dose of Bromass supplementation in broilers diets. Due to the increase in the absorption area of the duodenum, Bromass has been shown to have positive effects on body weight gain and growth as a result of the use in broiler diets.

REFERENCES

1. Foster WH, Stevenson MH. (1983). The interaction of food additives and protein content in broiler diets. Br Poult Sci. 24(4): 455-462.
2. Sugiharto S, Ranjitkar S. (2019). Recent advances in fermented feeds towards improved broiler chicken performance, gastrointestinal tract microecology and immune responses: A review Anim Nutr. 5(1): 1-10.
3. Cengiz SS, Yesilbag D, Cetın I, Gunes NA, Eren M, Topaloğlu G. (2019). Use of Bromass in broiler rations as a different protein source. Revue Méd Vét. 170(4-6): 95-103.
4. Oliveira MC, Silva DM, Carvalho CAFR, et al. (2013). Effect of including liquid vinasse in the diet of rabbits on growth performance. R Bras Zootec. 42(4): 259-263.
5. Bilal T, Ozpinar H, Gurel A, Ozcan A, Abas I, Kutay HC. (2001). Effects of beet vinasse {desugarized molasses) on performance, blood parameters, morphology, and histology of various organs in broilers. Arch Geflugelkd. 65: 224-230.
6. Abolfazl Zali, Mahdi Eftekhari, Farhang Fatehi, Ganjkhanlou M. (2017). Effect of vinasse (condensed molasses solubles) on performance and meat chemical composition of Holstein male calves. Ital J Anim Sci. 16(3): 515-520.
7. Stemme K, Gerdes B, Harms A, Kamphues J. (2005). Beet-vinasse (condensed molasses solubles) as an ingredient indiets for cattle and pigs – nutritive value and limitations. J Anim Physiol Anim Nutr. 89(3-6): 179-183.
8. Uzunoğlu K, Yalçın S. (2019). Effects of dietary supplementation of betaine and sepiolite supplementation on performance and intestinal health in broilers. Ankara Univ Vet Fak Derg. 66(3): 221-230.
9. Meimandipour A, Hair-Bejo M, Shuhaimi M et al. (2010). Gastrointestinal tract morphological alteration by unpleasant physical treatment and modulating role of Lactobacillus in broilers. Poult Sci. 51: 52-59.
10. Chiang G, Lu WQ, Piao XS et al. (2010). Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. Asian Australas J Anim Sci. 23: 263e71.
11. Mathivanan R, Selvaraj P, Nanjappan K. (2006). Feeding of fermented soybean meal on broiler performance. Int J Poult Sci. 5(9): 868-872.
12. Sun H, Tang JW, Fang CL et al. (2013). Molecular analysis of intestinal bacterial microbiota of broiler chickens fed diets containing fermented cottonseed meal. Poult Sci. 92(2): 392-401.
13. Zhang X, Zhao L, Cao F, Ahmad H, Wang G, Wang T. (2013). Effects of feeding fermented Ginkgo biloba leaves on small intestinalmorphology, absorption, and immunomodulation of early lipopolysaccharide-challenged chicks. Poult Sci. 92(1): 119-130.
14. Gao J, Zhang HJ, Wu SG et al. (2009). Effect of Saccharomyces cerevisiae fermentation product on immune functions of broilers challenged with Eimeriatenella. Poult Sci. 88(10): 2141-2151.
15. A.O.A.C.: Association Of Official Analytıcal Chemists, Official methods of analysis. (2000) 17thed., Maryland, USA.
16. Crossman GA. (1977). Modification of Mallory's Connective Tissue stain with a discussion of the principles involved. Anat Rec. 9: 33-38.
17. Pelicano ERL, Souza PA, Souza HBA et al. (2005). Intestinal mucosa development in broiler chickens fed natural growth promoters. Rev Bras Cienc Avic. 7: 221-229.
18. Weigand E, Kirchgessner M. (1980). Protein and energy value of vinasse for pigs. Anim Feed Sci Technol. 5(3): 221-231.
19. Adibmoradi M, Navidshad B, Seifdavati J, Royan M. (2006). Effect of dietary garlic meal on histological structure of small intestinal in broiler chickens. J Poult Sci. 43: 378-383.
20. Awad WA, Bohm J, Razzazi-Fazeli E, Ghareeb K, Zentek J. (2006). Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on perform-ance and histological alterations of small intestinal villi of broiler chickens. Poult Sci. 85: 974-979.
21. Onderci M, Sahin N, Sahin K et al. (2006). Efficacy of supplementation of-amylase-producing bacterial culture on the performance, nutrient use and gut morphology of broiler chickens fed a corn-based diet. Poult Sci. 85: 505-510.
22. Murakami AE, Sakamoto MI, Natali MRM, Souza LMG, Franco JRG. (2007). Supplementation of glutamine and vitamin E on the morphometry of the intestinal mucosa in broilers chickens. Poult Sci. 86: 488-495.
23. Soltan MA. (2009). Influence of dietary glutamine supplementation on growth performance, small intestinal morphology, immune response and some blood parameters of broiler chickens. Int J Poult Sci. 8: 60-68.
24. Feng J, Liu X, Xu ZR, Wang YZ, Liu JX. (2007). Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. Poult Sci. 86(6): 1149-1154.
25. Ahmed MMN, Zienhom I, Abdel-Wareth AAA. (2018). Application of betaine as feed additives in poultry nutrition-a review. J Exp Anim Sci. 2(3): 266-272.
26. Metzler-Zebeli BU, Eklund M, Mosenthin R. (2009). Impact of osmoregulatory and methyl donor functions of betaine on intestinal health and performance in poultry. World's Poult Sci J. 653: 419-442.
27. Santos TT, Dassi SC, Franco CRC, Costa CRV, Lee SA, Silva AVF. (2019). Influence of fibre and betaine on development of the gastrointestinal tract of broilers between hatch and 14 d of age. Anim Nutr. 5: 115-126.

✉ **Corresponding Author:**
Sabire GÜLER
Department of Histology and Embryology, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, TURKEY
E-mail: sabirepr@uludag.edu.tr