Global Veterinaria 13 (2): 221-226, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.02.84115

Comparative Effects of Organic Acids Fed Diet on Morphometry of Visceral Organs and Intestinal Histology of Two Broiler Strains

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Abstract: This study aimed to analyze the effect of organic acids (formic acid and propionic acid) on morphometry of different organs and intestinal microarchitecture of two strains of broilers (Hubbard and Cobb strains) commonly reared in Pakistan. One hundred and twenty day old broiler chicks (N=60 belonging to each strain) were divided into 4 groups (N=02 treatment and N=02 control). Birds of control groups were fed a corn-soya based basal diet while treatment groups received similar diet supplemented with organic acids in the ratio of 1gm/ kg of diet. At d-21 of age, fifteen birds from each group were randomly selected for slaughtering and their visceral organs were collected surgically and weighed. The lengths of small and large intestine were measured. The representative samples from all three segments of small intestine were processed to quantify the histomorphometry. Same procedure was repeated for remaining birds at d-42 of age. The results of this study demonstrated that utilization of organic acids improved the weight of visceral organs as well as the height of intestinal villi in exposed broilers. Moreover, there was no significant variation in terms of tested parameters between the two strains of broilers.

Key words: Organic Acids · Morphometry · Intestinal Histology · Broilers

INTRODUCTION

The poultry industry of Pakistan is experiencing tremendous progress during the last few decades Anonymous [1], thus contributing 1.12% to the national Gross Domestic Product. Feed cost being progressively increasing, accounts for nearly 60-70% of the total expenditure in producing meat and eggs. Organic acids represent safer and cost-effective feed additives capable to reduce the feed cost besides promoting the growth of targeted birds [2].

The nutritional value of diet fed to chicken could be evaluated by growth performance and nutrient digestibility that is regulated by intestinal tract, elaborating the significance of research on intestinal ultrastructure [3]. The morphological and physiological attributes of small intestine are known to be altered by its nutritional profile [4]. The rate of intestinal absorption is directly proportional to the size of villi [5]. Organic acid supplementation markedly enhances the intestinal absorption area by improving the villi growth in height and width at 21 and 42 days [6]. Moreover, these histological alterations appear early (before 21 d.of age) and transiently in duodenum and jejunum but display stability afterward in the ileal portion, hence providing significantly higher magnification rates for ileal villi in exposed birds. Potential benefits of organic acid supplementation encompass improvement in weight gain, carcass characteristics and gut morphology by increasing the histomorphometric features of small intestine [7]. Comparative studies involving different strains of chickens have been useful for recognizing the

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morphological and physiological adaptations [8] thereby providing scientific basis for modern poultry farming. Broilers are exclusively reared for high quality meat production [9]. This project was therefore designed to compare the effects of organic acid supplementation on morphometery of different organs and intestinal histology of Hubbard and Cobb (two strains) of broiler chicken.

MATERIALS AND METHODS

Experimental Birds: A total of 120 d-old broiler chicks were purchased from local commercial hatcheries and divided into two groups (Hubbard and Cobb), each comprising of (n= 60) chicks. Each group was further subdivided into four groups (n=30) according to the following scheme.

Chicks were housed in two environmentally controlled rooms. Temperature of poultry house was maintained on day one at 95°F /35°C which was reduced by 5°F after each week till it reached to 70°F/21°C at the end of 5th week. For the rest of time, the temperature was maintained at 70°F/21°C. Relative humidity was kept at 60% and 24 hours light schedule was followed. Birds were vaccinated according to standard vaccination schedule. Birds of A and C groups received standard starter and finisher rations supplemented with organic acid (formic acid and propionic acid in 7:3 ratio respectively) in concentration of 1g/kg of feed. Whereas, birds of B and D groups were provided with standard starter and finisher corn based basal broiler ration without organic acid supplementation (Table-2).

Sampling and Morphometery: Fifteen birds from each group were randomly selected on day 21 and subsequently slaughtered to remove all visceral organs surgically. Contents from the digestive organs were flushed instantly with normal saline. Different visceral organs including heart, liver, kidneys, gizzard, proventriculus, small and large intestine were carefully weighed after removing the extra attachments. The length of small and large intestines was measured and their representative samples (about 2 cm) were obtained from the midpoint of duodenum and jejunum and distal portion of ileum for histological examination [10].

Tissue Processing: Following fixation, the tissues were prepared for light microscopy using paraffin embedding technique [11]. Microtome (Yamato Kohki Ind. Co. Ltd. PR-50) was used to prepare 4µ thin tissue slices.

Table 1: Grouping plan for experimental broiler chicks

Group	Remarks
A	Organic acid supplemented Hubbard strain group
B	Organic acid deprived Hubbard strain group
C	Organic acid supplemented Cobb strain group
D	Organic acid deprived Cobb strain group

Table 2: Ingredient (%) of basal diet

Ingredients	Starter Feed	Grower Feed
Corn	40.15	57.57
Rice broken	15.0	-
Rice polish	-	4.00
Wheat bran	11.34	-
Soya meal	11.54	9.60
Sunflower meal	12.00	13.00
Canola meal	9.00	5.00
Rape seed meal	5.00	7.60
Guar meal	1.00	-
Molasses	2.00	-
Di-calcium Phosphate	1.73	1.96
Vitamin mineral premix[1]	1.00	1.00
Sodium chloride	0.21	0.21
Soda bicarbonate	0.03	0.065

Table 3: Nutritive value of basal diet

Nutritive parameter	Starter Feed	Grower Feed
DM (%)	87	88
Calculated ME (kcal/kg)	2750	2850
CP (%)	19.6	18.5
Crude Fat (%)	2.16	2.35
Crude Fibre (%)	1.26	1.80
Total Ash (%)	5.77	5.40

Vitamin mineral premix (each Kg containing): Ca, 195g; K, 70g; Na, 18g; Mg, 6g; Zn, 1200 mg; Fe, 2,000 mg; Cu, 400 mg; Mn, 1200 mg; Se, 8 mg; Co, 20 mg; I, 40 mg; vitamin A, 200,000 IU; vitamin D3, 80,000 IU; vitamin E, 1072 IU; vitamin K3, 34 mg; Ascorbic acid, 1300 mg; Thiamine, 35 mg; Riboflavin, 135 mg; Niacin, 1,340 mg; vitamin B6, 100 mg; Folic acid, 34, mg; vitamin B12, 670 µg; and biotin, 3,350 µg.

The slides obtained through tissue processing technique were stained by Haematoxylin & Eosin method [12] for measuring the villus length.

Selection Criteria for Villus: Ten well-oriented villi were selected from each intestinal segment of all samples. Villi were randomly selected based upon the presence of intact lamina properia [13].

Statistical Analysis: The data thus obtained were statistically analyzed using two-way Analysis of Variance Technique [14].

RESULTS

Morphometric Analysis of Different Visceral Organs: Organic acid supplemented birds (groups A and C) exhibited significantly ($P<0.05$) greater weights of heart, gizzard, proventriculus, small intestine and large intestine than non-treated birds (groups B and D) on both day 21 and day 42 of age. Furthermore, the length of small intestine also differed significantly between organic acid supplemented birds and non-treated birds. Dietary inclusion of organic acids substantially increased the length of small intestine in targeted birds. However, significant variation was lacking between the organic acid supplemented birds and non-treated birds in terms of average liver weight and length of large intestine on tested days. The weights of various visceral organs as well as the lengths of intestinal segments remained comparable for both strains of broilers. Nevertheless, the values of all tested parameters progressively increased with the age of broilers.

Effect of Organic Acid Supplementation on Intestinal Histomorphology: The height of villi did not remain uniform with respect to the portion of small intestine. It was maximum in duodenum followed by jejunum and ileum, respectively. Overall, the height of villi increased with increasing age. Duodenum represented the part of small intestine with pronounced positive impact of organic acids on the height of villi. While, ileum showed the least significant results with respect to the action of organic acids on villi length. Contrary to birds of control groups (groups B and D), broilers utilizing the organic acids displayed relatively more height of intestinal villi. Moreover, Hubbard broilers demonstrated comparatively greater height of intestinal villi than Cobb broilers. However, these differences were statistically non-significant.

DISCUSSION

This study demonstrated the beneficial effect of organic acids on the growth of different organs of broiler birds same to the findings of Abdel-Fattah *et al.* [15] after using fed supplemental L-Carnitine in Japanese Quail. Administration of organic acids significantly increased the weight of heart and proventriculus in exposed broilers. However, the average weight of liver did not significantly vary between control and treated birds.

Table 4: Effect of organic acids on average weight (gms) of visceral organs at 21and 42 days of age

	21 days old broiler birds				42 days old broiler birds			
Groups	Heart	Liver	Proventriculus	Gizzard	Heart	Liver	Proventriculus	Gizzard
A	$4.95^{a}\pm0.43$	$27.00^{a}\pm1.99$	$4.71^{a}\pm0.93$	$18.39^{a}\pm2.19$	$9.83^{a}\pm0.35$	$48.15^{a}\pm2.13$	$8.39^{a}\pm0.58$	31.58 ± 4.17
B	$3.72^{b}\pm0.56$	$26.67^{ab}\pm2.87$	$3.18^{b}\pm0.58$	$15.50^{a}\pm1.85$	$7.95^{b}\pm0.81$	$47.13^{ab}\pm3.80$	$6.39^{b}\pm0.43$	25.56 ± 2.75
C	$4.72^{a}\pm0.25$	$26.90^{ac}\pm3.36$	$4.59^{a}\pm0.73$	$16.22^{a}\pm0.89$	$10.12^{a}\pm0.65$	$49.00^{ac}\pm4.30$	$8.29^{a}\pm0.84$	27.32 ± 0.97
D	$3.65^{b}\pm0.47$	$27.09^{bc}\pm2.76$	$3.23^{b}\pm0.49$	$13.13^{a}\pm0.75$	$8.55^{b}\pm0.74$	$47.13^{bc}\pm3.78$	$7.06^{b}\pm0.42$	23.29 ± 5.94

Mean sharing similar superscripts differ non significantly ($P>0.05$) ± SD

Table 5: Effect of organic acids on average length (cm) and weight (gm) of small and large intestines at 21 and 42 days of age

	21 days old broiler birds				42 days old broiler birds			
	Small intestine		Large intestine		Small intestine		Large intestine	
Groups	Length	Weight	Length	Weight	Length	Weight	Length	Weight
A	$88.65^{a}\pm3.34$	$16.32^{a}\pm1.66$	$31.57^{a}\pm4.67$	10.97 ± 1.75	$145^{a}\pm6.17$	$30.28^{a}\pm4.17$	$57.15^{a}\pm2.13$	$23.17^{a}\pm3.46$
B	$77.35^{b}\pm4.67$	$12.54^{b}\pm0.37$	$30.75^{a}\pm2.12$	07.25 ± 0.95	$125.56^{b}\pm2.75$	$22.56^{b}\pm2.75$	$56.13^{a}\pm3.80$	$15.45^{b}\pm1.97$
C	$86.52^{a}\pm5.65$	$15.42^{a}\pm0.85$	$32.07^{a}\pm3.51$	09.13 ± 1.05	$140.32^{a}\pm0.97$	$28.32^{a}\pm0.97$	$56.90^{a}\pm4.30$	$21.23^{a}\pm0.87$
D	$75.83^{b}\pm3.23$	$13.05^{b}\pm1.23$	$30.97^{a}\pm3.78$	05.29 ± 1.94	$123.29^{b}\pm5.94$	$23.29^{b}\pm5.94$	$55.13^{a}\pm3.78$	$16.81^{b}\pm3.68$

Mean sharing similar superscripts differ non significantly ($P>0.05$) ± SD

Table 6: Effect of organic acids on average villi height (μm) of duodenum, jejunum and ileum of 21 and 42 days old broiler in experimental groups

	21 days old broiler birds			42 days old broiler birds		
Groups	Duodenum	Jejunum	Ilium	Duedenum	Jejunum	Ilium
A	875.47 ± 62.76	732.87 ± 13.98	$395.18^{b}\pm32.67$	1360.88 ± 56.32	1124.72 ± 27.35	$749.37^{b}\pm17.79$
B	698.49 ± 39.56	$605.73^{a}\pm23.54$	$385.78b^{c}\pm17.46$	1182.58 ± 29.45	$973.25^{a}\pm33.25$	$739.89^{bc}\pm43.54$
C	751.73 ± 52.67	685.18 ± 19.48	$390.47^{bcd}\pm 21.49$	1290.84 ± 38.53	1074.2 ± 23.58	$743.65^{bcd}\pm23.82$
D	574.79 ± 67.29	$615.19^{a}\pm29.67$	$387.27^{cd}\pm 22.53$	1110.59 ± 30.35	$960.59^{a}\pm17.88$	$736.12^{cd}\pm35.4$

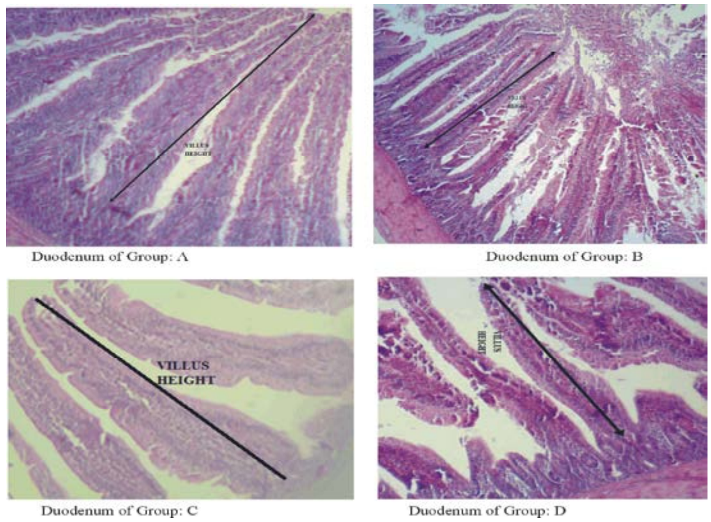

Fig 1: Effect of organic acid on duodenum villus height of broilers in all experimental groups

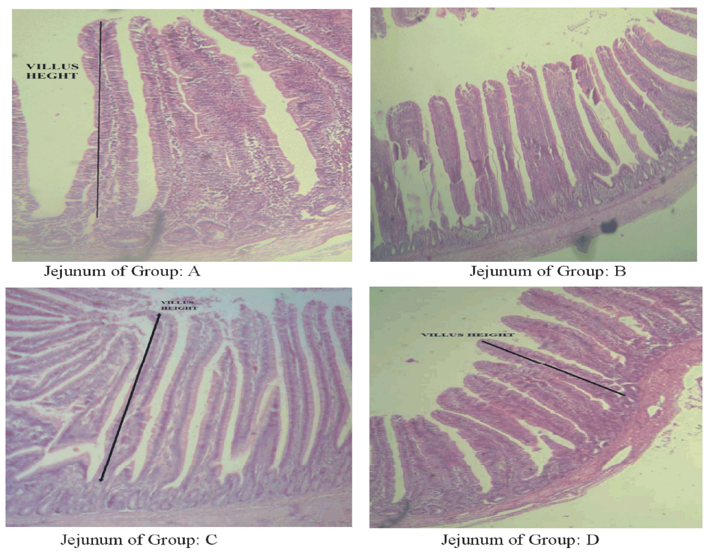

Fig 2: Effect of organic acid on villus height of jejunum of broilers in all experimental groups

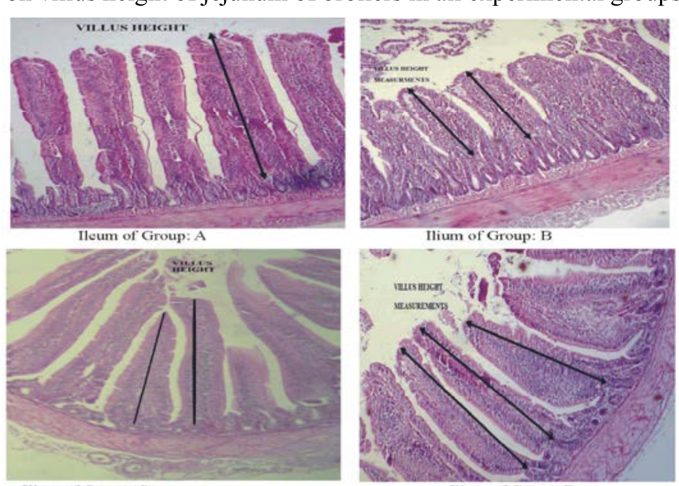

Fig 3: Effect of organic acid on villus height of ilium of broilers in all experimental groups

These results reinforce the findings of Ibardolaza *et al.* [16]. While there is a significant improvement in gizzard weight as a result of organic acid supplementation which is contradictory to the findings of Ibardolaza *et al.* [16]. The length and weight of small intestine increased in broilers fed with organic acids compared to control birds. However, regardless of feed composition, the length and weight of small intestine did not significantly differ between the two strains. Similar results have also been reported by earlier studies (Denli *et al*. [18] and Adil *et al*. [19]. Unlike control birds, broilers provided with supplemented diet possessed a significantly longer length of large intestine, strengthening the findings of Denli *et al.* [18] and Adil *et al*. [19]. But such discrimination was not evident as far as the weight of large intestine was concerned. Adil *et al.* [19] reported a significant increase in the weight of large intestine following the exposure of birds to organic acid supplemented diet. Therefore, this study provided conflicting results regarding the effect of organic acids on the weight of large intestine. Villi present in duodenum and jejunum of small intestine underwent remarkable increase in height as a result of organic acid supplementation. A plethora of studies have previously documented comparable results Paul *et al.* [20] and Mohamed [7]. Addition of organic acids did not affect the height of villi found in ileum part of small intestine. Panda *et al*. [10] and Adil *et al.* [19] also failed to observe any effect on the height of villi found in ileum following the use of organic acid. Inclusion of any level and source of organic acids improved feed digestion and absorption owing to increased small intestine density as an indication of the intestinal villi dimension [21]. Increased height of villi in small intestine has been related to a higher absorptive surface which could facilitate the nutrient absorption to exert a direct impact on growth performance [22]. Our results are in agreement to the previous studies Senkoylu *et al.* [17] indicating the positive effect of organic acids on morphometry of different organs and histomorphology of small intestine of broiler chicks. These histomorphometric changes in small intestine probably increased the surface area for facilitating nutrient absorption and validated the positive effect of organic acids on the growth of birds as described by Loddi *et al.* [22] and Adil *et al.* [23]. Organic acids have been reported to exert a direct stimulatory effect on the proliferation of gastro-intestinal epithelial cell [24] thereby improving the intestinal villi height and overall tissue weight. The beneficial effect of organic acids on growth performance of broiler birds could be also attributed to their potential antimicrobial activity [25] and Saleh *et al.* [26]. Reduction in microbial population improves the digestibility of proteins and imparts a growth promoting potential to organic acids [27].

It could be concluded from the results of this study that organic acids exerted beneficial effect on the growth of different visceral organs of broiler birds besides positively influencing the height of villi present in small intestine. Furthermore, the histomorphology of different organs particularly that of small intestine was appreciably improved which ultimately resulted in efficient absorption and utilization of nutrients to enhance the growth performance of broiler birds.

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