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Effects of Pellet and Mesh Diets on the Activity of the Microflora and Morphology of the Small Intestine of Broiler Chicks

[1]M. Rezaian, [2]A. Yaghoobfar and [1]J. Barin
[1]Department of Anatomy, Faculty of Veterinary Medicine, Division of Histology, University of Tehran, Tehran, Iran
[2]Animal Science Research Institute, P.O.Box: 31585-1483, Karaj, Iran

Abstract: In order to evaluate the effects of pellet and mash diets on bacterial count and morphology of the small intestine on broiler chicks, 400 Ross-308 broilers (males) were used. They were divided into 2 treatment groups of 25 randomly selected birds and 8 replications and were housed in 8 pen floors, with controlled temperature and humidity at the rear parts, for 42 days. By termination of the nurturing, when the chicks come of 42 days old, 4 birds per pen were randomly selected and slaughtered. Pellet and mash diets proved no significant effect on the number and activity of small intestinal microflora. Experiments on the morphometry of 3 parts of small intestines showed the increasing in the villus height of ileum, villus width and crypt depth in the jejunum of broilers chicks fed with mashed diet. The results of experiment shown that nutrients and metabolizable energy requirements differences were significantly effects in the villus heights (duodenums and jejunums), crypt depts. (duodenums) and villus widths (ileums and jejunums) of small intestinal of broiler chicks.

Key words: Effect of pellet, mesh diets, activity of microflora and morphoogy, broiler chicks

INTRODUCTION

Dietary ingredients (Proteins, amino acids, energy obtained from fat or starch, raw fiber, minerals and electrolyte balance and original food stuffs) have proved to be the most effective nutrients on intestinal microbial flora and also the most potential substrates for bacterial growth. Effects of food processing on intestinal microflora are clear (Kenny and Kemp, 2003; Gabriel *et al*., 2006). Microbial population of small and large intestine may be 10^9-10^{11} g^{-1} of ileal and cecal contents in broiler chicks. This extraordinary concentration of active organisms not only affects on the hosts nutrients functionability, but also is effective on its rapid growth. Feeding can act as a control factor, the main host dependent factor, on microflora, since microorganisms depend on food stuffs and their nutrients density. Microflora affects the birds' nutrition and can increase energy obtaining of the foods. Meanwhile, results of some researchers indicate higher speed of growth in non bacterial ones nourishing the same diet (Apajalahtib *et al*., 2004; Jafari and Pirsaraie, 2001).

Nir *et al.* (1994b) and Choi *et al.* (1986) reported of an increase in digestive system weight and jejunum and ileum heights of broilers by nourishing mash diet. Annison (1993) showed that the existing differences in physical effects of diet on improving digestion system and difference in fiber particles size of diet can have an important role in determination of diet effects on broilers strength. Using finely grained grains in broilers diets has particularly had harmful effects on their health and activity. Nir *et al.* (1994) in their studies of particle size and absorption activity suggested that a mash diet with large particles is better suited to the chicken's intestinal tract than a mash diet with small particles only and the large particles stimulate peristalsis (Nir *et al*., 1994). Enzberg *et al.* (2002) reported of significant decrease in broilers gizzard weight while increase of gizzard PH of the broilers nourished with pellet diet. The intestinal PH also was less than those nourished by mash diet. The less pancreas weight of broilers nourished with pellet diet and the less activity of pancreatic digestive enzymes (amylase, lipase and chemotropism) indicated of feed-back mechanism which may cause intestine condensation by enzymatic hydrolyzed productions or special digestive enzymes. There was more coli form and entero cocci bacteria in broilers ileum nourished with pellet and there was less clostridium perphyrngens and lacto basil in the cecum and rectum. Microbial fermentation during the processes obtained by accumulation of volatile fatty

Corresponding Author: M. Rezaian, Department of Anatomy, Faculty of Veterinary Medicine, Division of Histology, University of Tehran, Tehran, Iran

acids was significantly more in broilers cloaca nourished with mash diet. Improvement observed by calcium retentions on diet containing coarse maize, however, decrease when pellet diet was used. When the mash diet contained big particles, the phosphorous level of dialyzable plasma increased, however, there was no such reaction when pellet diet was substituted. Food conversation of the fine maize mill was higher, though calcium and phytate phosphorous retention in coarse maize diet were the most. Pelleting, improved food efficiency and growth, while decreased the phytate phosphorous retention (Kilbern and Edwards, 2001). Studying the effects of nutrient and ME requirement and pellet and mash diets on bacterial count and activity and morphology of the three parts of the small intestine of the broilers chicks has been the aim of this experiment.

MATERIALS AND METHODS

Experiment of pellet and mash diets on 400 male broilers chicks (Ross 308) was performed. They were divided into 2 treatment groups of 25 randomly selected birds and 8 replications and were housed in 8 pen floors, for 42 days. The diet was conducted to be adequate in all nutrients and the full nutritional requirements of the Ross 308 in different stages of nourishing. Metabolic energy an crud protein amount of diets were 2900 and 3000 Kcal kg^{-1} or 21 and 23% for 0-21 days of age and 3000 and 3150 Kcal kg^{-1} or 18.50 and 20% for 22-42 days of age receptivity. Other nutrients of the diet were arranged according to the protein and energy level. The broilers diet was prepared for 2 stages of beginning (0- 21 days of age) and termination (21-42 days of age). The experiment was analyzed as Completely Randomized Designs (RCD). Differences among treatment means were using Duncan multiple range methods.

Bacteriological analysis (Intestinal microflora): In order to examine the microflora changes in broilers small intestine, digestive tracts of 42 days of age broilers were resumed immediately after slaughter and the small intestines were separated. One mililitre of their contents was taken by sampler and transferred into a sterile vessel containing preserving solution (50% alcohol) and steered well. 1cc of each prepared samples was transferred into the phosphate buffer pipette and immediately sent to the lab, while kept into the ice box. Different dilutions to be used for further exams. Buffer was prepared as follow: 8.5 g L^{-1} Nacl, 0.68 g L^{-1} Nh_2Po_4, 0.15 g L^{-1} NaOH. Phenol red was used as reagent to regulate PH (7- 7.2). In addition to the total bacterial counts, abundance (incidence rates) of Lactobacilli, coli forms and clostridia in small intestinal contents were determined. Bacterial count was performed in 2 stages. To determine the total bacterial count g^{-1} of sample, 0.01 mL of the original buffered sample was spread over an area of 1 cm^2 of the slide, then dried in the air, fixed over flame and stained with a few drops of crystal violet, washed in tap water after 10 sec (Breed method). Numbers of bacteria were counted in 5 cells of a lattice grid were fixed into the ocular lens of a light microscope. Different cell cultures were used to determine the incidence rates of each respective bacterium, thus ATP, SS and blood agar for Lactobacilli, coli forms and clostridia were used respectively. Different dilutions from -1 to-13 of the original sample were prepared (using P.B.S). Two hundred micro L from each dilution were injected into each special culture and kept 24 h in incubator 37°C. Colony count was used for Lactobacilli and coli forms. Clostridia were put in the fridge for 30 min to enter the spore phase, since colony counting was not possible by culture and spores were counted. Finally, the numbers of bacteria were determined by multiplying the number of colonies by dilution coefficients.

Small intestines morphometry: The whole parts of the small intestine comprising duodenum, jejunum and ileum were removed from the body immediately after death and transverse sections, were successively cut with 2 cm interval and fixed with 10% buffered formalin. Routine histological laboratory methods containing dehydration, clearing, paraffin embedding was used and paraffin blocks were made. Six micrometre thick sections were made by rotary microtome and stained with hematoxylin-eosin and PAS (Luna, 1968) and studied under light microscope. The length and width of the intestinal villi and the depth of the intestinal crypts of lieberkuhn glands were measured with linear scaled graticule. The number of goblet cells mm^{-2} area of the villi and crypts were measured by 25 lattice graticule. Ten slides for each block were randomly selected and 5 fields of microscope in each slide were measured.

RESULTS

Results obtained by statistical analysis of the bacterial count and activity have been shown in the Table 1. Types of diet (pelleted and mashed) showed no significant effect on bacterial activity and numbers ($p>0.05$). Histometrical results of small intestines, including heights and widths of villi, depth of crypts and goblet cell number at 3 different parts of the small intestines (duodenum, jejunum and ileum) are shown in Table 2. No significant difference was observed between

Table 1: Total numbers of bacteria (log 10) g^{-1} of broilers small intestinal contents (in 42 days old)

	Coli forms	Staphylococci	Entero cocci	Total aerobic bacteri	Bifida bacte rium	Lacto bacilli	Bacteroids	Total anaerobic bacteria	Clostridia
Mashed	3.07	3.46	2.66	3.55	3.37	2.96	2.56	3.42	3.01
Pelleted	2.64	3.23	2.21	3.14	2.94	2.54	2.53	3.41	2.99

Table 2: Histometrical results of small intestines

Parts of intestine	Dietary type	Villus height (μm)	Villus width	Crypt depth (μm)	Villus height/ Crypt depth (μm)	Goblet cell number in mucosal epithelium (mm^2)
Duodenum	Pelleted	1108.29	210.86[a]	174.00	6.82[b]	1294.4
	Mashed	1149.43	190.63[b]	176.14	6.97[a]	1253.4
Jejunum	Pelleted	1026.00	146.57[b]	133.71[b]	8.04	2046.7
	Mashed	1057.71	163.71[a]	148.28[a]	7.57	2139.4
Ileum	Pelleted	660.86[b]	162.00	152.57	5.19	2050.1
	Mashed	753.43[a]	164.57	155.14	4.78	2173.8

Table 3: Effects of feeding with 2 different diets (pellet and mash) and nutriment requirements in histological structure of duodenum in 42 days old broiler chicks

	Villus height (μm)	Villus width	Crypt depth (μm)	Villus height/crypt depth(μm)	No. of goblet cell in mucosal epithelium (mm^2)
Requirements					
Minimum level	1170.00[a]	203.48	182.57[a]	6.70[b]	1236.3
Maximum level	1087.71[b]	198.00	167.57[b]	444.10[a]	1311.8
Diet form					
Pelleted	1108.29	210.86	174.00	6.82	1294.4
Mashed	1149.43	190.63	176.14	6.97	1253.4
Mean value of significantly level					
Nutriment requirements	0.0009	0.44	0.039	0.002	0.48
Diet physical forms	0.009	0.005	0.76	0.002	0.69

duodenal and jejunal villus heights by consuming 2 ration of pellet and mash diet ($p>0.05$), however, statistical difference was seen in the ileum ($p<0.05$). Significant difference was observed between duodenal and jejunal villus widths by consuming 2 ration of pellet and mash diet ($p<0.05$), however, statistical difference was not seen in the ileum ($p>0.05$). No significant difference was observed between duodenal and ileal crypt depths by consuming 2 ration of pellet and mash diet ($p>0.05$), however, statistical difference was seen in the jejunum ($p<0.05$). No significant difference was observed between jejunal and ileal villus height/crypt depth ratios by consuming 2 ration of pellet and mash diet ($p>0.05$); how-ever, statistical difference was seen in the duodenum ($p<0.05$). No significant difference was observed between goblet cell number at 3 different parts of the small intestines by consuming 2 rations of pellet and mash diet ($p>0.05$).

In the duodenums, no significant differences were observed between villus width, goblet cell number in mucosal epithelium/(mm^2) by consuming minimum and maximum level of nutrients or metabolizebale energy requirement of diet ($p>0.05$), however, statistical differences were seen in the villus heights and crypt depths ($p<0.05$) (Table 3). No Significant difference was observed between the histological structure of the duodenum by consuming 2 ration of pellet and mash diet ($p<0.05$) (Table 3).

Effects of feeding with two different diets (pellet and mash) and nutriment requirements in histological structure of duodenum in 42 days old broiler chicks was shown in Table 3.

In the jejunums, no significant differences were observed between crypt depths, goblet cell number in mucosal epithelium/(mm^2) by consuming minimum and maximum level of nutrients or metabolizebale energy requirement of diet ($p>0.05$), however, statistical differences were seen in the villus heights and villus widths ($p<0.05$) (Table 4). No Significant difference was observed between the villus heights and Goblet cell number in mucosal epithelium/(mm^2) by consuming 2 ration of pellet and mash diet ($p<0.05$) (Table 4), however, statistical differences were seen in the villus widths and crypt depths ($p<0.05$) (Table 4).

In the ileums, no significant differences were observed between the villus heights, crypt depths, Goblet cell number in mucosal epithelium/(mm^2) by consuming minimum and maximum level of requirement of diet ($p>0.05$), however, statistical differences were seen in the villus widths ($p<0.05$) (Table 5). No Significant difference was observed between villus heights, crypt depths, Goblet cell number in mucosal epithelium/(mm^2) by consuming 2 ration of pellet and mash diet ($p<0.05$) (Table 5), however, statistical differences were seen in the villus widths ($p<0.05$) (Table 5).

Table 4: Effects of feeding with 2 different diets (pellet and mash) and nutriment requirements in histological structure of jejunum in 42 days old broiler chicks

	Villus height (μm)	Villus width	Crypt depth (μm)	Villus height/crypt depth (μm)	Goblet cell number in mucosal epithelium (mm^2)
Requirements					
Minimum level	1002.84^b	141.43^b	139.71^a	7.61^a	1996.2^a
Maximum level	1080.86^a	168.86^a	142.28^a	7.99^a	2190.9^a
Diet form					
Pelleted	1026.00^a	146.57^b	133.71^b	8.04^a	2139.4^a
Mashed	1057.71^a	163.71^a	148.28^a	7.57^a	2046.7^a
Mean value of significantly level					
Nutriment requirements	0.14	0.0001	0.68	0.26	0.07
Diet physical forms	0.39	0. 16	0.02	0.16	0.39

Table 5: Effects of feeding with 2 different diets (pellet and mash) and nutriment requirements in histological structure of ileum in 42 days old broiler chicks

	Villus height (μm)	Villus width	Crypt depth (μm)	Villus height/crypt depth (μm)	Goblet cell number in mucosal epithelium (mm^2)
Requirements					
Minimum level	692.57	180.00^a	139.71	4.82	2002.1
Maximum level	721.71	146.57^b	152.57	5.14	2221.8
Diet form					
Pelleted	660.86^b	162.00^a	155.14^a	4.77^a	2050.1^a
Mashed	753.43^a	164.57^a	152.57^a	5.19^a	2173.8^a
Mean value of significantly level					
Nutriment requirements	0.08	0.75	0.73	0.21	0.05
Diet physical forms	0.0001	0. 0001	0.73	0.10	0.27

DISCUSSION

Food processing, as reported in many scientific resources, can affect on broilers ileum and cecum contents microflora, growth and efficiency in feed utilization (Kenny and Kemp, 2003). Choi *et al.* examined mashed and crumbled diets in 0-4 weeks of age broilers and found more weight gain and more feed consumption on broilers fed with crumbled diet, however, after feeding a mash diet during 0-4 weeks of age, body weight gained in broilers fed with pelleted diet was the same as those fed crumbled diet overall (Choi *et al*., 1986). Engberg *et al.* (2002) reported that the birds nourished by pellet diet, had significant decrease of gizzard weight, increase of gizzard PH and decrease of intestine PH in contrast with those nourished with grinded diet. In addition, birds nourished by pelleted diet, had more coli form and entero cocci bacteria in their ileum and less clostridium perphyrngens and lactobacilli at the rectum and cecum. Accumulation of volatile fatty acid during bacterial fermentation in the coaca of the broilers fed by pellet diet was significantly more than those fed with mashed diet.

Nir *et al.* (1994b) and Choi *et al.* (1986) reported of an increase in broilers digestive tract weight and in height of jejunum and ileum through increasing mashed diet. Annison (1993) showed that the physical affect of food such as size of fiber can improve the digestibility of nutrients and very fine grinded grains have had harmful effects on health and activity of broilers chick. Kilbern and Edwards (2001) suggested that there is a significant interaction between the effects of particle size and food form on the response of metabolisable energy value. Pelleting appears to have a negative effect on phytate phosphorous retention while improving growth and food conversation and improving the metabolisable energy value of a diet (Kilbern and Edwards, 2001). In our finding, increasing the villus height in the ileum, villus width and crypt depth in the jejunum of broilers chicks fed with mashed diet may lead the better growth rate compare with pelleted diet chicks.

Results of some researches show more rapid growth and better efficiency of non bacterial broilers as compared with microbial ones by consuming similar diet (Jafari and Pirsaraie (2001) and Furuse and Okumura (1994). The results of this experiment show that pelleted and mashed diets had no effect on the bacterial activity as total counts of *bacteroidaea*, *Lactobacillus bifidobacterium* and *clostridium* in small intestine of broiler chickens.

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