African Journal of Biotechnology Vol. 8 (20), pp. 5620-5626, 19 October, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effect of dietary levels of a modified meat meal on performance and small intestinal morphology of broiler chickens

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Accepted 3 September, 2009

A locally produced meat meal in Ardabil province in Iran is mixed with 150 g wheat bran, 100 g feather meal and 150 g zeolite per kg. Experimentally, this leads to an improvement in physical texture and preservation characteristics. In this experiment, six hundred 3-week-old Cobb 500 broiler chicks of both sexes were assigned randomly to 24 floor pens with 25 chicks in each pen. Dietary modified meat meal at six inclusions levels of 0 (control diet containing 50 g/kg fish meal), 20, 35, 50, 65 or 80 g/kg, created the treatments of a completely randomized design to survey analysis the performance traits. For histological data, a completely randomized design with a 2 × 6 factorial arrangement was used. The experimental factors were bird sex at 2 levels and dietary modified meat meal at the corresponding levels noted above. The experiment was carried out at 21 to 42 days of age. Dietary modified meat meal did not affect on birds' feed intake and body weight gain, but feed conversion ratio improved in the birds fed diet with 20 g modified meat meal/kg in comparison to the control. Compared to the control, the birds fed 20, 35 and 50 g of modified meat meal/kg diet had a significantly lower villus height, crypt depth and crypt depth to villi height ratio, increased goblet cell number and higher epithelium thickness in duodenum. However, these values significantly altered after feeding of 65 and 80 g of modified meat meal/kg diet and showed an almost similar value to control. The same condition observed in jejunum section, with the exception of the goblet cell number that was not affected by dietary alteration. Ileal histological morphology was not influenced by dietary manipulation. The only significant effect of sex was the higher jejunal epithelial thickness in females compared with males. The results of this study demonstrate that the inclusion of modified meat meal up to 80 g/kg of broiler diets resulted in comparable small intestinal morphometric characteristics to a common commercial fish meal based diet. It seems that the processed meat meal can be used as a suitable alternative to dietary fish meal in broiler chickens diets.

Key words: Broiler chickens, meat meal, performance, intestinal morphology.

INTRODUCTION

Despite the prohibition on the use of mammalian meat in farm animals' nutrition in several countries, it can be allowed in some cases. The protein of meat by-products is of good quality and is particularly useful as a lysine supplement (McDonald et al., 2002). In a research carried out by Leeson and Zubair (1994), meat meal was used successfully as the sole animal protein source in broilers rations (meat meal is eaten readily by poultry and may be included at levels up to 150 g/kg of the diet for laying hens (McDonald et al., 2002).

A locally produced meat meal in Ardabil province in

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Table 1. Composition of the modified meat meal used in the experiment (g/kg).

Ingredient	Concentration
Meat meal	600
wheat bran	150
feather meal	100
Zeolite	150
Chemical Composition	
Moisture	63.5
Crude protein	298.8
Gross energy (kcal/kg)	5115
Metabolisable energy (kcal/kg)	2010
Ash	227
Crude fiber	104.0
Calcium	45.9
Phosphorous	10.5
Sodium	31.5
TVN (mgN/100 g)	102.5
AIA	162.7

TVN: Total volatile nitrogen, AIA: acid insoluble ash.

Iran is a mixture of 60% meat meal, 15% wheat bran, 10% feather meal and 15% zeolite. The thermal and mechanical processes cause a dramatic structural modification that in experiment resulted in a better conservation property and reduced undesirable odors and mycotoxin effects (Seifdavati and Navidshad, 2006). In this modified meat meal, wheat bran increases the fiber content and changes meat meal structure. Feather meal reduces the protein price of product. The effects of zeolite on mycotoxins reduction, may be due to its high molecular sieve adsorption capacity; efficient selectivity for cations and ion-exchange capacity; hydration and dehydration; deodorising properties and acid resistance (Mumpton, 1984; Tsitsishvili et al., 1992). Additionally, natural zeolites can absorb excess ammonium and help to reduce the problem of subsequent ammonia generation (Street, 1994). We observed on adverse effects on broilers performance after 100% replacement of fish meal by the modified meat meal (Seifdavati and Navidshad, 2006). According to Tivey and Smith (1989), any change in the development of enterocytes and in the structure of villi can be determinant on the digestive and absorptive capacity of the small intestine.

Some recent studies have indicated that dietary protein source can affect intestinal morphology. Van Leeuwen et al. (2004) studied the effects of different dietary protein sources on the orientation of the villi, villus shape and the presence of convoluted villi in the small intestine of broiler chickens. They observed that, the diet containing soybean isolate protein supplemented with L-glutamine increased the percentage of the area with zigzag villus orientation. In a study on pigs, Spencer et al. (1997) reported that pigs fed a spray-dried plasma protein diet had longer villi and a greater villous length to crypt depth ratio than pigs fed a no-spray-dried plasma protein diet. We could not find any reported research on the effects of meat meal on the morphology of small intestine in chickens.

The main objective of the present study was to determine the morphological characteristics of the small intestine in 21 to 42 day-old broilers chickens in relation to different dietary levels of modified meat meal.

MATERIALS AND METHODS

Table 1 shows the characteristics of the modified meat meal used in this study. In this experiment six hundred 3-week-old Cobb 500 broiler chicks of both sexes were assigned randomly to 24 floor pens with 25 chicks in each pen.

Chickens had *ad libitum* access to feed during the entire experiment. Birds were managed in accordance with the guidelines of the Cobb 500 manual. Isoenergetic and isonitrogenous diets (Table 2) were formulated according to the Cobb 500 manual. Experiment was carried out at 21 to 42 days. Feed intake and body weight gain were recorded for each pen at the end of the experiment and feed conversion ratio calculated as the ratio of feed intake to weight gain in experimental period.

Dietary modified meat meal at six inclusions levels of 0 (control diet containing 50 g/kg fish meal), 20, 35, 50, 65 or 80 g/kg, created the treatments of a completely randomized design (Table 2). For histological data analysis a completely randomized design with a 2 × 6 factorial arrangement was used. The experiment factors were bird's sex at 2 levels that randomly selected from each cage and dietary modified meat meal at 6 levels.

At the end of experimental period, for morphometric analysis, 3 male and 3 female birds per replicate pen were randomly selected based on apparent characteristics like bird's comb and wattle shape and body size and then killed. Bird's sex was reconfirmed by the post-slather sexual gland examination. The intestinal tract was removed immediately and severed from the gizzard and the pancreas was removed. Three 1 cm tissue samples were cut from the proximal, middle and distal parts of each small intestinal segment (duodenum, jejunum and ileum sections). All samples from each of those birds were taken from the same area of each section of the tract. Samples were stored in 10% buffered neutral formalin for fixation, where they were gently shaken to remove any adhering intestinal contents. Cross sections (5 µm thick) of each intestinal segment were processed in low-melt paraffin and stained with hematoxylin and eosin. Using a light microscope, 15 observations were recorded for each morphometric parameter-villus height, crypt depth, epithelial thickness and goblet cell numbers of duodenum, ileum and jejunum sections were recorded and the records averaged into one value per bird.

Data on performance traits and histological parameters (villus height, crypt depth, crypt depth to villus height ratio, epithelial thickness and goblet cell number (Figure 1)) were analyzed with the general linear model procedure and differences among treatments means were classified by Duncan's multiple range test (SAS, Version 6.12, SAS Institute, Inc.). The Correlation coefficients were calculated using Microsoft Excel software.

Table 2. Composition and calculated nutrient content of experimental diets with different modified meat meal levels.

Nutrient	Control	Dietary modified meat meal (g/kg)				
		20	35	50	65	80
Corn (g/kg)	600	550	550	546	540	530
Soybean Meal (g/kg)	165.4	226.6	217.6	206.8	197.8	189.6
Wheat (g/kg)	150	150	150	150	150	150
Fish meal (g/kg)	50	0	0	0	0	0
Soybean oil (g/kg)	3	16	16	15	16	18
Oyster shell (g/kg)	10.3	9	7	5.4	3.5	1.6
Dicalcium phosphate (g/kg)	10	16	16	16	16	16
Salt (g/kg)	2.2	2.6	2.3	1.9	1.9	190
Lysine (g/kg)	1.7	2.4	2.6	2.7	2.8	3
Methionine (g/kg)	1.8	2.4	2.4	2.4	2.6	2.6
Vitamin and mineral supplement (g/kg)*	5.6	5.5	5.5	5.5	5.5	5.5
Metabolisable energy (kcal/kg)	2990	2990	2990	2990	2990	2990
Crude protein (g/kg)	169.3	169.3	169.3	169.3	169.3	169.3
Ether extract (g/kg)	34.8	44.8	47.3	48.6	51.8	54.4
Calcium (g/kg)	8.5	8.5	8.5	8.5	8.5	8.5
Available phosphorous (g/kg)	4.3	4.2	4.2	4.2	4.2	4.2
Sodium (g/kg)	1.5	1.5	1.5	1.6	1.6	1.6
Met+Cys (g/kg)	7.7	7.8	7.8	7.8	7.8	7.8
Lysine (g/kg)	9.9	9.9	9.9	9.9	9.9	9.9

*Provided per kilogram: vitamin A, 4000000 IU; cholecalciferol 800000 IU; vitamin E, 14000 IU; vitamin K3, 760 mg; vitamin B2, 2800 mg; vitamin B6, 1520 mg; vitamin B12, 7.6 mg; nicotinic acid, 18000 mg; folic acid, 560 mg; pantothenic acid, 4400 mg; choline chloride, 190000 mg; biotin, 45.3 mg; zinc, 16000 mg; manganese, 25600 mg; iron, 12800 mg; copper, 3200 mg; selenium, 64 mg; iodine, 320 mg.

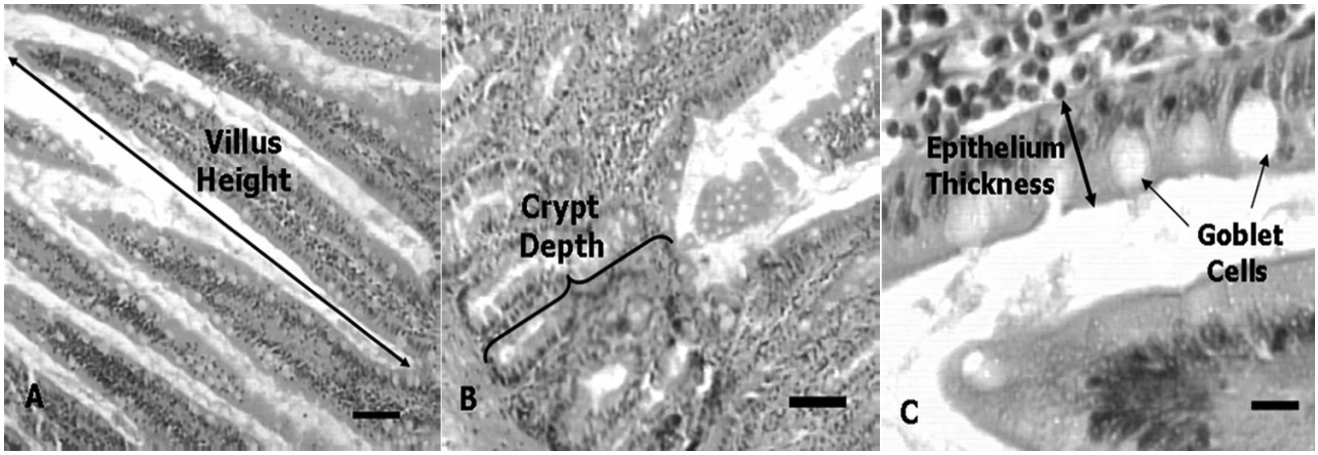

Figure 1. Sample sections of a duodenal tissue of broilers chickens, hematoxilin and eosin stained, showing the measured parameters. A: villus height, Scale bar represent 100 µm. B: crypt depth, Scale bar represent 35 µm. C: goblet cells and epithelium thickness Scale bar represent 25 µm.

RESULTS

The results of the performance and histological studies are summarized in Tables 3 and 4, respectively. Dietary modified meat meal did not affect the bird feed-intake and body weight gain ($P>0.05$). The only statistically

Table 3. Effects of dietary modified meat meal level on performance of broiler chickens.

Dietary Meat Meal g/kg)	Daily weight gain (g per chick)	Final body weight (g)	Daily feed intake (g per chick)	Total feed intake (g)	Feed conversion ratio
control	69	2184	148	3100	2.13[a]
20	74	2270	150	3140	2.02[b]
35	73	2255	152	3190	2.10[ab]
50	72	2262	149	3140	2.10[ab]
65	71	2277	148	3110	2.07[ab]
80	71	2237	146	3070	2.08[ab]
SEM	5.2	98.8	8	128.6	0.11
P value	0.70	0.13	0.32	0.39	0.03

[a - b] Values in the same column in each comparison group, with no common superscript differ significantly ($P < 0.05$).

significant effect was observed in the feed conversion ratio. This parameter improved in the group fed diet with 20 g/kg modified meat meal comparing to the control group ($P < 0.05$).

In the duodenum section, the birds fed 20, 35 and 50 g/kg modified meat meal had a lower villus height, crypt depth and crypt depth to villus height ratio and higher epithelium thickness compared to the control group ($P < 0.05$). However, the birds fed 65 and 80 g/kg of modified meat meal, showed almost similar values to the control ($P<0.05$). For the duodenal goblet cell number, the higher ditary meat meal dosage (65 and 80 g/kg) resulted in a lower goblet cell number in compare to the birds fed diets containing lower meat meal levels (20 and 35 g/kg). Birds fed 20 and 35 g/kg, had more goblet cells than birds fed 65 and 80 g/kg of modified meat meal ($P < 0.05$). The jejunum section showed similar changes ($P < 0.05$), with the exception of the goblet cell number that was not affec-ted by dietary modified meat meal level ($P>0.05$). Ileum histological morphology was not influenced by dietary manipulation ($P >0.05$). The only effect of sex was the higher jejunum epithelium thickness in females in comparison to males ($P< 0.05$). There was no statistically significant interaction between dietary modified meat meal content and birds' sex, on measured parameters.

DISCUSSION

Meat meals contain some beneficial factors like enteric growth factor from the intestinal tract of swine, the Ackerman factor and a growth factor located in the ash (McDonalds et al., 2002). Meat meal is particularly useful as a lysine supplement but is a poor source of methionine and tryptophan (McDonald et al., 2002). Kellems and Church (2001) reported that protein quality of meat meal is lower than fish meal or soybean meal for applications in swine or poultry feeding when used to supplement crude protein in cereal based diets.

In previous research with same age of birds, Seifdavati and Navidshad (2006) used the modified meat meal as the sole animal protein source up to 100 g/kg of a corn-soybean meal based broiler diet successfully (average final body weight of 2218 g and feed conversion ratio of 2.1). In a research conducted by Leitgeb et al. (1998) replacement up to 10% of soybean meal by meat meal in chick diets showed no differences in gain and feed conversion.

According to Table 1, 40% of the modified meat meal used in this study was consisted of non-meat derived components and at the maximum inclusion level, the experimental diets contain about 48 g real meat meal/kg diet, that approximately is comparable to fish meal content (50 g/kg) of the control diet. Based on reports indicating more superior protein quality of fish meals than meat meals (Kellems and Church, 2001; McDonald et al., 2002), the comparable performance recorded for birds fed meat meal and fish meal based diets, in this experiment, can be surprising. Although the meat meal based diets have contained more soybean meal in compare to the control (fish meal based) diet, but a com-parison between the control and the diet containing 80 g/kg meat meal that had closely near soybean meal level can confirm the meat meal effect on bird's performance.

Intestinal morphology changes with nutritional variations, stress, aging and/or disease and consequently affects the physiology of the intestine, specifically nutrient absorption and metabolism (Kristy et al., 2005). Villus height and crypt depth are direct representations of the intestinal functional condition and may be used as indicators of intestinal health (Kristy et al., 2005). Overall gut surface area affects net utilization of dietary nutrients in chicken. This effect is determined by gross morpho-logical features such as length and cross-sectional area of the duodenal, jejunal and ileal segments and by finer morphological features such as villus height and crypt

Table 4. Effects of dietary modified meat meal level and bird sex on small intestinal morphological parameters of broiler chicken.

Duodenum	Villus height (µm)	Crypt depth (µm)	Goblet cell number	Epithelium thickness (µm)	Crypt depth to villus height ratio
Dietary Meat Meal (g/kg)					
control	1756.2^{a}	147.2^{a}	9.6^{ab}	48.2^{b}	0.084^{a}
20	1723.8^{b}	136.6^{b}	10.3^{a}	54.8^{a}	0.079^{b}
35	1727.4^{b}	136.5^{b}	10.6^{a}	52.8^{a}	0.079^{b}
50	1720.1^{b}	135.3^{b}	9.7^{ab}	52.4^{a}	0.079^{b}
65	1759.1^{a}	146.1^{a}	9.4^{b}	47.9^{b}	0.083^{a}
80	1750.1^{a}	145.1^{a}	9.2^{b}	47.3^{b}	0.083^{a}
P_M	0.048	0.046	0.026	0.042	0.023
Sex					
Male	1740.7	141.1	9.9	50.7	0.081
Female	1738.2	141.1	9.6	51.4	0.081
P_S	0.72	0.31	0.34	0.52	0.12
P_I	0.96	0.82	0.4339	0.9450	0.7653
SEM	47.2	7.3	0.22	1.3	0.003
Jejunum					
Dietary Meat Meal (g/kg)					
control	847.9^{a}	117.4^{a}	10.2	39.8^{bc}	0.138^{a}
20	835.6^{b}	111.0^{bc}	10.9	42.8^{a}	0.133^{ab}
35	836.6^{b}	108.9^{c}	9.8	40.9^{b}	0.130^{b}
50	835.9^{b}	110.8^{bc}	10.2	41.4^{ab}	0.132^{ab}
65	845.8^{a}	116.2^{a}	10.4	38.8^{c}	0.137^{a}
80	846.6^{a}	114.2^{ab}	10.1	38.2^{c}	0.135^{ab}
P_M	0.022	0.031	0.083	0.027	0.011
Sex					
Male	841.6	113.9	10.4	39.9^{b}	0.13
Female	841.0	112.2	10.1	40.9^{a}	0.13
P_S	0.091	0.33	0.53	0.021	0.92
P_I	0.93	0.76	0.39	0.035	0.8708
SEM	61.4	4.9	0.55	1.6	0.007
Ileum					
Dietary Meat Meal (g/kg)					
control	794.6	108.9	10.1	36.6	0.137
20	775.7	107.3	10.3	37.9	0.138
35	774.9	105.9	10.0	38.3	0.137
50	774.8	155.9	9.9	38.4	0.199
65	751.1	106.9	9.7	39.5	0.137
80	791.5	107.7	10.1	38.8	0.136
P_M	0.42	0.53	0.44	0.29	0.56
Sex					
Male	785.5	121.7	10.1	37.6	0.150
Female	768.6	109.1	9.9	38.8	0.155
P_S	0.35	0.55	0.88	0.87	0.89
P_I	0.56	0.5	0.63	0.80	0.4
SEM	21.1	5.9	0.23	0.96	0.006

$^{a-c}$ Values in the same column in each comparison group, with no common superscript differ significantly ($P < 0.05$).
P_I = P value of interaction of Dietary Meat Meal (g/kg) and sex; s.e. = Standard error.

depth as indicators of surface area of the epithelium (Jin et al., 1998; Iji, 1999).

The crypt can be regarded as the villus factory and a large crypt indicates fast tissue turnover and a high demand for new tissue (Yason et al., 1987). The villus : crypt ratio is an indicator of the likely digestive capacity of the small intestine. An increase in this ratio corresponds to an increase in digestion and absorption (Montagne et al., 2003). Reduction in small intestinal epithelium thickness can facilitate absorption process. Thinner intestinal epitheliums enhance nutrient absorption and reduce the metabolic demands of the gastrointestinal system (Visek, 1978). The intestinal tract epithelium is covered by a mucus layer composed predominantly of mucin glycoproteins, which are synthesized and secreted by goblet cells distributed along the villi. The mucus layer in the small intestine plays an important role in protection of the small intestinal epithelial cells and in transport between the lumen and the brush border membrane. A fewer goblet cell number in intestinal tract epithelium can be attributed to a less stressful condition that leads to a reduced need for the protective mucus layer (Edens et al., 1997).

There has been little study on the effects of nutrient composition on intestinal development (Kristy et al., 2005). We could not find any research which conducted to determine the effects of feather meal or zeolite on intestinal morphology. On the other hand, Maneewan and Yamauchi (2004) reported the positive effect of fiber free diets on epithelial cells size.

We concluded that following a 21-d feeding period, the diets with the higher levels (65 and 80 g/kg) of modified meat meal, improved small intestinal morphometric characteristics. This effect was in such a manner that increased villus height, crypt depth and crypt depth to villus height ratio and decreased epithelial thickness and goblet cell number. This observation was comparable with the histological effects caused by a common fish meal containing diet as the control.

We could not find any documented report for effects of feather meal or zeolite (components of the modified meat meal) or any negative effect of dietary fiber (provided by wheat bran) on small intestinal morphology. The observed improvement in histological characteristics of the small intestinal mucosa in the birds fed isonitrogenous diets with different levels of modified meat meal can be attributed to the effect of animal protein supplied by meat meal. This caused a comparable performance between birds fed fish meal based diet (control) and experimental modified meat meal contained diets.

In this research, we could not demonstrate any effects of small intestinal morphology on bird's performance, because in despite of the significant differences in some morphological parameters of the small intestine, there were no statistically significant differences for the majority of the performance traits. The feed conversion ratio was the sole performance parameter that affected by dietary meat meal. The correlation coefficients of feed conver-sion ratio and villus height, crypt depth and epithelium thickness were 0.29, 0.31 and - 0.45 for duodenum, 0.38, 0.29 and -0.39 for jejunum and 0.39, 0.23 and - 0.38 for ileum segments, respectively. These data indicate that an increase in villus height and crypt depth increased the feed conversion ratio but the epithelium thickening decreased it.

As the sole logical reason for this condition as noted previously by Xu et al. (2003), it seems that increased villus length means that more energy and nutrients would be required for faster turnover of the gut mucosa. Providing the extra nutrients for a higher mucosal growth maybe resulted in a reduction in energy supply for growth (Rehman et al., 2007).

Conclusion

It can be speculated that dietary meat meal in this experiment enhanced the energy expenditure via raising the turn over of the small intestine tissue (as demonstrated by a deeper crypt), such that this increase was not compensated by the expected improvement in the absorptive process mediated by the taller villi.

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