ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 6 (12): 944-951, 2007
ISSN 1682-8356
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Effect of Dietary Gelatin Supplementation on the Expression of Selected Enterocyte Genes, Intestinal Development and Early Chick Performance

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Abstract: Day-old Ross 308 chicks were used to evaluate the effect of dietary gelatin on intestinal development and broiler chick performance. Chicks were randomly allocated to two dietary treatments. The first treatment was the control SB diet based on corn-soybean meal and second treatment was the SBG test diet that contained 2% added gelatin with the total sulfur amino acid level remaining the same as in SB. Each treatment consisted of 4 replicate pens with 14 chicks per pen. Total duration of experiment was 0-3 weeks of age. Chick performance (weight gain and feed conversion efficiency) was evaluated on day 7 and 14. To assess intestinal development, abundance of mRNA of selected enterocyte genes (alkaline phosphatase, leucine aminopeptidase and mucin) was quantitatively determined by RT-PCR on day 7. Biochemical indices including DNA concentration, protein:DNA and protein:RNA ratios were also used to assess intestinal development. Results indicated that chicks fed the SBG diet had improved feed conversion efficiency on day 7 ($p<0.05$) and higher body weight gain on day 21 ($p<0.05$) compared to chicks fed the SB diet. Furthermore, eneterocyte genes assessed were upregulated ($p<0.05$) in the duodenum of SBG chicks. Jejunal protein:RNA ratio was also higher for SBG chicks ($p<0.05$), indicating a higher rate of protein synthesis in this tissue. The non-essential amino acids provided by gelatin beyond the most limiting appear to enhance early intestinal development and chick growth.

Key words: Gelatin, mRNA levels, biochemical indices, intestinal development, chick performance

Introduction

Chick growth during the first 7 days of life represents approximately 10% of final market weight (Lilburn, 1998). Preceding hatching, late-term chick embryos recover yolk sac contents in various forms of nutrients (Dibner *et al.*, 1998). As emergence ensues, chicks utilize their reserves (liver glycogen, muscle glycogen and residual yolk sac) to meet the high nutrient demands associated with hatching (Christensen *et al.*, 2001; Uni *et al.*, 2005). After hatching, yolk nutrients diminish and transition to feed must provide the difference. Length of a chick's stay in the incubator after hatching, hatchery procedures and duration of transportation to brooding facilities delay this transition (Donaldson *et al.*, 1994; Noy and Sklan, 1998; Noy and Sklan, 1999). As a result of these delays, chicks preferentially allocate energy for maintenance over growth (Ricklefs, 1987), thereby restricting intestine and muscle development (Uni and Ferket, 2004). Extended delay in providing feed to poultry hatchlings (chicks and poults) has progressive adverse effects that last until marketing (Noy and Sklan, 1999; Dibner *et al.*, 1998; Dibner and Knight, 1999; Bigot *et al.*, 2003).

Functional maturation of the intestine has been found to be a major constraint to early development of precocial birds such as poultry (Konarzewski *et al.*, 1990; Uni and Ferket, 2004) and early feed access has been shown to be especially advantageous (Baranyiova and Holman, 1976; Uni *et al.*, 1998; Geyra *et al.*, 2001). Intestinal epithelium comprises a proliferating compartment in the crypts, an evolving component on the lower half on the villi and a well-differentiated surface at the top (Gordon, 1989; Potten and Loeffler, 1990). Stem cells in the crypt eventually give rise to enterocytes and goblet cells (Chang and Leblond, 1974; Potten and Loeffler, 1990). As enterocytes migrate up the villi, several divisions precede their differentiation into the stage at which they acquire functions that include nutrient digestion and active transport and absorption. The fully differentiated enterocyte is defined by the presence of enzymes that finalize digestion and transport proteins (Dahlqvist and Nordstrom, 1966; Mooseker, 1985; Shields *et al.*, 1986; Ferraris *et al.*, 1992; Uni *et al.*, 2000; Jeurissen *et al.*, 2002). In fact, brush border disaccharidases, alkaline phosphatase and aminopeptidase are some of the few proteins that have been designated as markers of a fully developed intestinal surface (Uni, 1999; Uni *et al.*, 2000; Jeurissen *et al.*, 2002).

Contrary to enterocytes, goblet cells differentiate to become proficient at mucin synthesis (Potten and Loeffler, 1990; Smith, 1993; Uni, 1999). Although goblet cells are the major intestinal cells that secret mucin (Allen *et al.*, 1982; Neutra and Forstner, 1987), enterocytes also express specialized mucin-like glycoproteins that comprise the glycocalyx (Lang *et al.*,2004; Frey *et al.*, 1996). The glycocalyx extends from microvilli to entrap mucin (Fahim *et al.*, 1987; Lang *et al.*,

Table 1: Protein and amino acid composition of soybean meal and gelatin (% "as is")

Nutrient	Soybean meal[1]	Gelatin[1]	Vyse Gelatin[2]
Metabolizable energy (kcal kg^{-1})[3]	2,230.0	2,360.0	2,360.0
Crude protein	45.6	97.4	94.0
Alanine	4.3	10.3	11.0
Arginine	6.2	4.8	8.8
Aspartic acid	11.0	6.3	6.9
Glutamine	17.7	11.5	11.4
Glycine	4.2	12.6	27.5
Histidine	3.2	3.4	0.8
Hydroxyproline	NA[4]	NA	14.5
Leucine	6.5	3.1	3.4
Isoleucine	3.1	1.4	1.8
Lysine	5.2	3.8	5.2
Hydroxylysine	NA	NA	1.0
Methionine	1.4	2.6	0.9
Phenylalanine	10.1	2.0	2.6
Proline	3.8	16.9	18.0
Serine	5.5	3.9	4.1
Threonine	3.5	11.7	2.2
Tyrosine	3.2	0.9	0.9
Valine	3.4	2.3	2.8
Cystine	1.0	0.2	Trace

[1]Nutrient expectations based on Mu *et al.* (2000), [2]Amino acid values provided by Vyse Gelatin Company (Schiller Park, IL). [3]NRC (1994). [4]Not available

2004) and form the unstirred water layer that functions to restrict diffusion and act as a surface protectant (Lang *et al.*, 2004; Frey *et al.*, 1996). Goblet mucin and enterocyte glycocalyx both have exceptionally high proportions of non-essential amino acids (NEAA), particularly glycine, serine and proline.

Gelatin is a high protein ingredient whose crude protein content is higher than that of soybean meal (Table 1) and may therefore enhance intestinal development. In addition, gelatin has similar metabolizable energy to soybean meal and also has particularly high levels of glycine, glutamine, proline, serine and threonine which are the major amino acids found in mucins and the intestinal glycocalyx (Tse and Chadee, 1992; Lang *et al.*, 2004). Thus, this study evaluated the effect of supplementing dietary gelatin into a corn-soybean meal based starter diet on intestinal development and performance of broiler chicks. Intestinal development was evaluated indirectly by measuring the abundance of the mRNA of selected genes that are commonly used as markers of mature enterocytes. The genes include Alkaline Phosphatase (ALP), Leucine Aminopeptidase (LAP) and Mucin. In addition, intestinal biochemical indices such as DNA concentration, RNA: DNA, protein:DNA and protein:RNA ratios were employed as indicators of intestinal development. DNA concentration reflects the rate of mitosis to produce new columnar epithelial cells, RNA:

DNA ratio reflects tissue activity and individual cellular efficiency, protein: DNA reflects cell size and protein: RNA reflects ribosomal capacity for translating amino acids into protein (Cook and Bird, 1973; Waterlow *et al.*, 1978; Uni, 1999; Iji *et al.*, 2001; Jeurissen *et al.*, 2002). These biochemical indices were calculated from total protein, DNA and RNA concentrations determined from the intestine of chicks used in the study.

Materials and Methods

Chicks and diets: One hundred and forty-four male day-old Ross 308 chicks were obtained from Aviagen (Aville, Alabama) and then randomly assigned to two dietary treatments. The control feed (SB) was a typical corn-soybean meal based starter, while the test feed was the same corn-soybean meal basal into which 2% gelatin (spray dried gelatin hydrolysate processed from beef hide collagen; Vyse Gelatin Company, Schiller Park, IL) was included at the expense of corn. Amino acid compositions of soybean meal and gelatin assumed for experimentation are given in Table 1, while estimated dietary compositions are given in Table 2. Diets were pelleted and fed in crumbled form. Each treatment consisted of 4 replicate pens with 14 chicks per pen. Chicks were housed in Petersime batteries at room temperatures of 30±2°C during the first week with a gradual reduction of temperature by 2°C per week until 3 weeks of age. Feed and water were offered ad libitum to all chicks and continuous lighting was provided throughout the experiment (0 to 21 days). All experimental procedures were approved by the Animal Care and Use Committee of Auburn University.

Performance evaluation and tissue sampling: Weight gain, feed intake and Feed Conversion (FC) were measured on days 7 and 21 of experimentation. In addition, on day 7 of experiment, 3 chicks were randomly taken from each pen for sampling of intestinal tissue. Tissue samples (about 4 cm long) were taken at the midpoint of duodenum (from the gizzard to the point of entry of the pancreo-biliary ducts), jejunum (from the pancreo-biliary ducts to the yolk stalk) and ileum (from yolk stalk to the ileo-cecal junction). Each piece of tissue was quickly rinsed in cold saline (0.9% NaCl) to get rid of luminal contents (such as the digesta), snap frozen in liquid nitrogen and kept at -80 C until determination of mRNA levels for selected enterocyte genes [alkaline phosphatase (ALP), leucine aminopeptidase (LAP) and mucin]. A second set of tissue samples were taken from duodenum, jejunum and ileum as previously described, snap frozen in liquid nitrogen and held at -80°oC for subsequent measurement of DNA, RNA and protein concentrations that were in turn used to calculate the biochemical indices (DNA concentration, RNA: DNA, protein: DNA and protein: RNA ratios).

Quantitation of mRNA of selected enterocyte genes: Total RNA was isolated from homogenized intestinal

tissue samples with TRI reagent (1 mL/100 mg tissue), according to the manufacturer's protocol (Molecular Research Center Inc., Cincinnati, OH). RNA from tissue samples obtained from chicks in the same pen were then pooled to make a total of 4 RNA samples per treatment. RNA concentration and integrity were assessed spectrophotometrically (at 260 nm) and electrophoretically (on agarose gel), respectively. RNA (3 µg) was then reverse transcribed into complementary DNA (cDNA) using the BIO-RAD iScript™ cDNA Synthesis Kit (Biorad Laboratories, Hercules, CA). Polymerase chain reaction (PCR) amplification of cDNA of each sample was performed in triplicates using the iQ™ SYBR Green Supermix (Biorad Laboratories, Hercules, CA) along with specific primers (Table 3) designed for each enterocyte gene and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that was used as the housekeeping gene. The PCR reactions were performed as triplicate measurements of each sample in an iCycler Thermal Cycler (Biorad Laboratories, Hercules, CA) under the following conditions: 1 cycle at 95°C for 3 min, followed by 50 cycles at 95°C for 30 sec, appropriate annealing temperature (Table 3) for 30 sec and 72°C for 30 sec, with a final extension at 72°C for 5 min. Data obtained from the PCR reactions provide the threshold Cycle (C_t) value for each gene. The C_t value is the cycle number at which the reporter dye emission intensities rise above background noise (Huang *et al*., 2007). C_t values predict the quantity of target mRNA in the sample (Heid *et al*., 1996). The C_t of each enterocyte gene was normalized against that of GAPDH (housekeeping gene). Fold change in the expression of each gene was calculated by the Pfaffl (2001) method as a change in the expression of a gene in SBG chicks relative to its expression level in SB (control) chicks.

Biochemical indicators of intestinal development: The second set of duodenal, jejunal and ileal tissue samples obtained from chicks in the same pen were pooled to make a total of 4 samples per treatment. Simultaneous extraction of nucleic acids (RNA and DNA) and proteins was performed on homogenates prepared from the second set of duodenal, jejunal and ileal tissues using TRI-Reagent-RNA/DNA/protein isolation reagent (Molecular Research Center Inc., Cincinnati, OH). The isolated RNA and DNA were each quantitated at 260 nm, then the protein pellet respectively obtained was solubilized using the ReadyPrep Sequential Extraction Kit (Biorad Laboratories, Hercules, CA). The ReadyPrep Sequential Extraction Kit contains three reagents for extracting proteins of differing solubilities. Solubilized protein was then quantitated at 595 nm using a modified Bio-Rad protein assay (based on Bradford dye-binding procedure, Bradford, 1976) as described in the ReadyPrep Sequential Extraction Kit. The modified protein assay was certified by Bio-Rad to resist interference from the detergents and chaotropes present in the reagents used for extracting the proteins. From the total tissue DNA, RNA and protein concentrations determined, biochemical indices corresponding to DNA concentration (indicating cell

Table 2: Composition of experimental diets (% "as is")

Ingredient	SB control	SB gelatin
Corn	55.25	53.25
Soybean meal	36.50	36.50
Poultry oil	3.75	3.75
DL-Methionine	0.30	0.30
L-Lysine HCL	0.10	0.10
Limestone	1.30	1.30
Dicalcium Phosphate	1.85	1.85
Vitamin Premix[1]	0.25	0.25
Mineral Premix[2]	0.25	0.25
Salt	0.45	0.45
L-Threonine	0.05	0.05
Gelatin	-	2.00
Calculated analyses		
Crude protein (N x 6.25)	22.00	23.62
Metabolizable energy (kcal g^{-1})	3080.00	3059.00
Total sulfur amino acids (%)	1.00	1.01
Lysine (%)	1.31	1.38
Calcium (%)	0.99	1.00
Available phosphorus (%)	0.48	0.47

[1]Vitamin Premix, supplied per kilogram of diet: vitamin A (retinyl acetate), 7356 IU; vitamin D_3 (cholecalciferol), 2,205 ICU; vitamin E, (8 IU); vitamin B_{12} (cyanocobalamin), 0.2 mg; riboflavin, 5.5 mg; niacin, 36 mg; D-pantothenic acid, 13 mg; choline, 501 mg; vitamin K (menadione sodium bisulfate), 2 mg; folic acid, 0.5 mg; vitamin B6 (pyridoxine), 2.2; vitamin B_1 (thiamin), 1.0 mg; D-biotin, 0.5 mg; and ethoxyquin, 0.13 mg. [2]: Mineral Premix, supplied per kilogram of diet: manganese, 65 mg; zinc, 55 mg; iron, 55 mg; copper, 6 mg; iodine, 1 mg and selenium, 0.3 mg

Table 3: Nucleotide sequences of specific primers

Gene[1]	Gen bank accession No.	Primer sequence	Annealing temperature (°C)
1. ALP	U19108	Forward: 5´-GAACCTCATCCTCTTCCTG-3´	60
		Reverse: 5´-CTACAAATACCAAGGGAGAGTCG-3´	
2. LAP	NM001031336	Forward: 5´-GGATAGCAGCACAGCAGATG-3´	53
		Reverse: 5´-CTACAAATACCAAGGGAGAGTCG-3´	
3. Mucin[2]	XM_421035	Forward: 5´-GCTGATTGTCACTCACGCCTT-3´	60
		Reverse: 5´-ATCTGCCTGCCTGAATCACAGGTGC-3´	
4. GAPDH	L01458	Forward: 5´-GGTGAAAGTCGGAGTCAACGG-3´	56
		Reverse: 5´-TCGATGAAGGGATCATTGATGGC-3´	

[1]ALP = Alkaline phosphatase; LAP = Leucine aminopeptidase; Mucin; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.
[2] Primer sequences were excerpted from Smirnov *et al.* (2006)

population), RNA: DNA (tissue activity or individual cellular efficiency), protein: DNA ratio (indicating cell size) and protein: RNA ratio (indicating ribosomal capacity) were calculated.

Statistical analysis: Performance data (weight gain, feed intake and FC), fold change values (obtained from gene expression data for the different intestinal segments) and the biochemical indices for intestinal growth were analyzed with ANOVA using the General Linear Models procedure (SAS Institute, 2004). Significant differences among means were determined by using the Duncan option of the GLM procedure as a post hoc test. Data are presented as means±SEM or SD. Values were considered statistically different at p<0.05.

Results

Chick growth performance is presented in Table 4. On day 7, no differences in weight gain and feed intake of chicks were apparent between treatments. However, feed conversion was better (p<0.05) for chicks fed the SBG diet than those receiving the SB diet. By day 21, gain of chicks fed the SBG was significantly greater than when fed the SB diet and FC was equivalent.

The effects of gelatin on the mRNA of selected enterocyte genes in different intestinal segments are presented in Fig. 1, 2 and 3. For each enterocyte gene assessed, the fold change in mRNA induced by the SBG treatment was expressed relative to the SB treatment such that SB treatment had a fold change of 1. Therefore, the fold change value for SBG treatment could be >1 or =1. In this study, a gene whose fold change is >1 was upregulated by dietary gelatin supplementation, while a gene whose fold change is <1 was downregulated by dietary gelatin supplementation. Accordingly, in Fig. 1, ALP gene was upregulated (p<0.05) in the duodenum and jejunum of SBG chicks, while the mRNA level of the gene in the ileum was similar for both SB and SBG treatments. In Fig. 2 and 3, LAP and Mucin genes were upregulated (p<0.05) only in the duodenum. Regardless, the expression level of these genes in the jejunum and ileum were similar for both SB and SBG treatments.

Biochemical indices of intestinal development are presented in Table 5. Although these biochemical indices were measured in the duodenum, jejunum and ileum, significant differences between the SB and SBG treatments were observed only in the jejunum. Chicks in the SBG treatment had a higher jejunal protein:RNA ratio (p<0.05), thereby implying greater ribosomal capacity compared to the SB treatment. Furthermore, a higher jejunal protein:DNA ratio that approached significance (p = 0.0575) was observed for chicks given the SBG, while no differences were apparent between both SB and SBG chicks in jejunal DNA concentration and jejunal RNA:DNA.

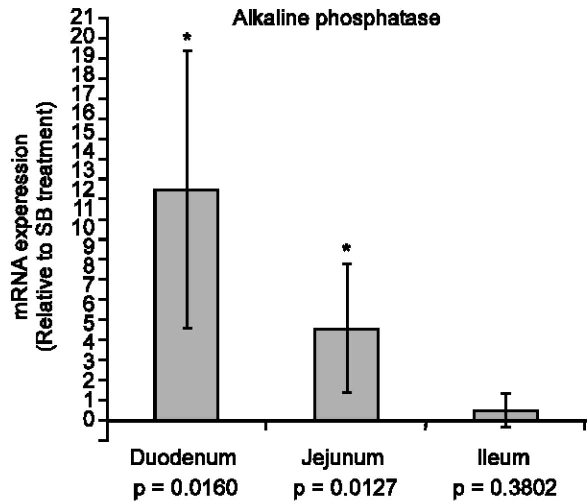

Fig. 1: Differential Expression of Alkaline Phosphatase (ALP) Gene in Intestinal Segments of Chicks Fed the Gelatin-Supplemented Diet. The fold change in ALP mRNA induced by the SBG treatment was expressed relative to the SB treatment such that SB treatment had a fold change of 1. Therefore, ALP fold change values for SBG treatment would be >1 or = 1. Values are means±SD. Number of observations per mean (n) = 4, with each n value representing tissue pooled from 3 chicks from the same pen. * = p<0.05

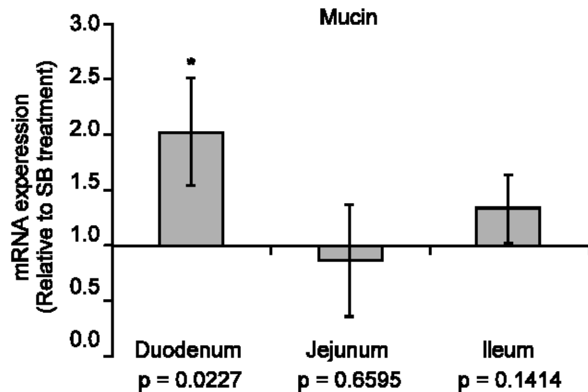

Fig. 2: Differential Expression of Leucine Aminopeptidase (LAP) Gene in Intestinal Segments of Chicks Fed the Gelatin-Supplemented Diet. The fold change in LAP mRNA induced by the SBG treatment was expressed relative to the SB treatment such that SB treatment had a fold change of 1. Therefore, LAP fold change values for SBG treatment would be >1 or =1. Values are means±SD. Number of observations per mean (n) = 4, with each n value representing tissue pooled from 3 chicks from the same pen. * = p<0.05

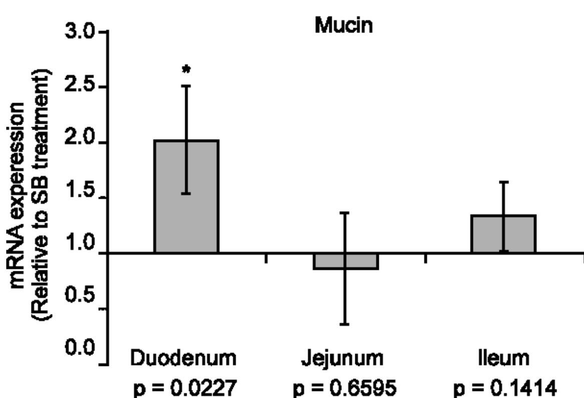

Fig. 3: Differential Expression of Mucin Gene in Intestinal Segments of Chicks Fed the Gelatin-Supplemented Diet. The fold change in Mucin mRNA induced by the SBG treatment was expressed relative to the SB treatment such that SB treatment had a fold change of 1. Therefore, Mucin fold change values for SBG treatment would be >1 or = 1. Values are means±SD. Number of observations per mean (n) = 4, with each n value representing tissue pooled from 3 chicks from the same pen. * = $p<0.05$

Table 4: Effect of gelatin supplementation on chick performance

Treatments[1]	g Weight gain[2]	g Feed intake	FC[3]
Day 7			
SB	81	95	1.266
SBG	86	95	1.197
SEM	2.57	2.59	0.014
p-value	0.2622	0.9634	0.0124
Cummulative (day 0 to 21)			
SB	842	602	1.384
SBG	892	637	1.367
SEM	12.35	9.78	0.024
p-value	0.0293	0.0481	0.6336

[1]SB represents a corn-soybean starter, while SBG contained gelatin. [2]Corresponds to birds not sampled. [3]Feed conversion adjusted for mortality by including the gains of dead birds

Table 5: Effect of Dietary Gelatin Supplementation on Biochemical Indices of Intestinal Development

	DNA Concentration (µg/mg tissue)	RNA:DNA	Protein:DNA	Protein:RNA
Duodenum				
SB[1]	5.17	0.63	8.48	5.49
SBG[1]	5.01	0.57	8.66	6.44
SEM	0.55	0.13	1.12	0.74
P-value	0.8460	0.7419	0.9161	0.4003
Jejunum				
SB	3.21	1.16	8.02	2.51[b]
SBG	3.82	0.93	12.15	4.26[a]
SEM	0.41	0.11	1.25	0.45
P-value	0.3255	0.2002	0.0575	0.0339
Ileum				
SB	3.76	0.88	11.62	4.78
SBG	3.51	1.03	13.56	4.26
SEM	0.46	0.13	1.32	0.44
P-value	0.7112	0.4654	0.3370	0.4043

[a,b]Mean values bearing different superscript letters within a column are significantly different ($p<0.05$). Number of observations per mean (n) = 4, [1]SB represents a corn-soybean starter, while SBG contained gelatin

Discussion

Supplementing 2% gelatin to a common corn-soybean feed was examined in terms of chick intestinal development. Advantage in FC on day 7 and weight gain on day 21 of chicks fed the SBG diet can be attributed to the additional NEAA provided by gelatin, given the constant level of total sulfur amino acids that would limit balance. Sklan and Noy (2003) showed that chicks fed starter diets that contain 23 or 26% crude protein while providing essential amino acids levels recommended by the National Research Council (NRC, 1994) had higher body weights ($p<0.05$) compared to chicks fed starter diets that contain 20% crude protein.

The small intestine is the primary site for nutrient assimilation. The chick's intestine is not fully developed at hatch and early growth is highly dependent on its functional state (Konarzewski *et al.*, 1990; Uni, 1999). Dietary support is central to gut differentiation, development and continued function (Pacha, 2000). Intestinal ALP is a membrane-bound glycoprotein that hydrolyzes a wide variety of monophosphate esters at an alkaline pH optimum (Malo *et al.*, 2004). Because intestinal ALP is commonly used as a marker of enterocyte maturation (Uni *et al.*, 2000; Iji *et al.*, 2001; Jeurissen *et al.*, 2002; Hinnebusch *et al.*, 2004), changes in its mRNA levels can be used as an indirect indicator of enterocyte maturation. Thus, the higher ALP expression in the intestine of chicks fed SBG probably indicates the presence of a higher number of mature enterocytes in these chicks compared to chicks given SB diet.

Other enterocyte genes assessed are LAP and Mucin. LAP is an intestinal brush border exopeptidase that preferentially cleaves amino acids from the N-terminal end of peptides and is specific for peptides with an N-terminal neutral or basic amino acids (Maroux *et al.*, 1973; Sanderink *et al.*, 1988; Gal-Gerber and Uni, 2000). Mucins are high molecular weight glycoproteins that have domains with extensive O-glycan attachment (Thornton and Sheehan, 2004). Mucins are the major components of enterocyte glycocalyx and goblet cell mucus and that act as a diffusion barrier and epithelial surface protectant (Frey *et al.*, 1996; Lang *et al.*, 2004). In this study, dietary gelatin supplementation probably enhanced chick performance in part, by inducing higher expression of LAP mRNA for the synthesis of LAP enzyme that breaks down peptides at the intestinal brush border membrane and also by inducing higher expression of the mucin gene for the synthesis of mucins that form the intestinal glycocalyx and unstirred

water layer. Dietary nutrient composition is known to influence gene expression. For instance, Smirnov *et al.* (2006) observed an increase ($p<0.05$) in the expression of mucin mRNA in the jejunum of chicks injected a mixture of carbohydrate feed during late embryonic stage of development. Thus, the higher abundance of ALP, LAP and Mucin mRNA in SBG chicks is probably due to the high crude protein and NEAA in the gelatin added into the diet of chicks fed SBG.

Intestinal growth is known to occur by hyperplasia and hypertrophy (Morisset, 1993). Nutrient supply from the lumen is also known to foster mucosal metabolism and development (Reisenfeld *et al.*, 1982; Sklan *et al.*, 1996). In this study, only the jejunum showed differences in biochemical indices between the SBG and control SB chicks (Table 5). It has been well established that the rate and pattern of intestinal development varies with intestinal region (Uni *et al.*, 1995; Uni *et al.*, 1999; Jin *et al.*, 1998; Iji *et al.*, 2001; Yamauchi, 2007). Because protein: RNA reflects the ribosomal capacity to translate amino acids into protein (Uni, 1999; Jeurissen *et al.*, 2002), the higher protein:RNA ratio observed in the jejunum of SBG chicks indicates that the higher levels of intestinal ALP, LAP and Mucin mRNA measured in these chicks (Fig. 1, 2 and 3) were probably efficiently translated into proteins at least in the jejunum. This is important because the jejunum functions in nutrient digestion and is also the primary site for nutrient absorption.

Taken together, the results of this study show that dietary crude protein and NEAA provided by gelatin supported intestinal development by enhancing the expression of selected enterocyte genes (ALP, LAP and Mucin) and increasing the rate of protein synthesis in the jejunum. The improved intestinal development could have enhanced nutrient assimilation in chicks fed the SBG diet, consequently resulting in the improved FC on day 7 and higher weight gain by day 21. Dietary gelatin supplementation at 2% level of broiler starter diet appears to be favorable.

Acknowledgments

The Authors wish to thank the Meat Quality Group of the Poultry Department at Auburn University and Shelly Nolin of the Department of Poultry Science at NC State University for technical assistance.

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