

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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Evaluation of Dietary *Aspergillus* Meal on Intestinal Morphometry in Turkey Poults

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Abstract: A trial was conducted to evaluate the influence of dietary *Aspergillus* Meal (AM) prebiotic on intestinal development, morphology in turkey poults. Day-old Nicholas poults (n = 100) were randomly assigned to two groups. Poults in each group were divided and placed in floor pens with 50 poults each. One group served as a control and received a ration containing no added AM prebiotic. Poults in the treatment group were provided a ration supplemented with 0.2% AM prebiotic. Each dietary regimen and water were provided *ad libitum* to 30 days of age. Poults were humanely killed by CO₂ inhalation and specimens of duodenum and distal ileum were collected at 10, 20 and 30 days. Histology sections were cut (5 µm) and stained with haematoxylin and eosin and combinations of either of periodic acid-Schiff with alcian blue, or high iron diamine with alcian blue stains to evaluate 20 intact villi on each section. The morphometric variables analyzed included villi height, villi surface area and crypt depth. In addition, goblet cells, classified as neutral, acidic or sulpho mucin cells, respectively, were quantitative for each treatment group. At all times of evaluation, AM prebiotic significantly increased the number of acid mucin cells in the duodenum, neutral mucin cells in the ileum and sulpho mucin cells in the duodenum and ileum. Villi height and villi surface area of both duodenum and ileum were significantly increased at days 10 and 30 compared to control. The present study suggest that AM prebiotic has an impact on the mucosal architecture and goblet cells proliferation in the duodenum and ileum of neonate poults.

Key words: *Aspergillus* meal, prebiotic, goblet cells, intestinal morphometry, turkeys

INTRODUCTION

The gastrointestinal tract serves as the interface between diet and the metabolic events that sustain life. Intestinal villi, which play a crucial role in digestion and absorption of nutrients, are underdeveloped at hatch (Uni *et al.*, 1995) and maximum absorption capacity is attained by 10 days of age (Noy and Sklan, 1997). Understanding and optimizing the maturation and development of the intestine in poultry will improve feed efficiency, growth and over all health of the bird. Prebiotics are nondigestible food ingredients (e.g. nondigestible oligosaccharides) that are selectively fermented by one or more bacteria known to have positive effects on gut physiology. Some prebiotics have shown to selectively stimulate the growth of endogenous lactic acid bacteria and *Bifidobacteria* in the gut to improve the health of the host (Gibson and Roberfroid, 1995). Prebiotics selectively modify the colonic microflora and can potentially influence the gut metabolism (Gibson and Wang, 1994). The presence of

normal gut microflora may improve the metabolism of the host birds in various ways, including absorptive capacity (Yokota and Coates, 1982), protein metabolism (Salter *et al.*, 1974), energy metabolism, fiber digestion, energy conversion and gut maturation (Furuse and Yokota, 1984; Furuse *et al.*, 1991). Gut microflora also targets goblet cells. Goblet cells reside throughout the length of the small and large intestine and are responsible for the production and maintenance of the protective mucus blanket by synthesizing and secreting high-molecular-weight glycoproteins known as mucins that have tremendous physiological implications in the gastrointestinal tract (Dunsford *et al.*, 1991). Within the mucus gel, other component, including water, electrolytes, sloughed epithelial cells and secreted immunoglobulins, reside. This produces a physical and chemical barrier that protects the epithelium from luminal agents such as enteric bacteria, bacterial and environmental toxins and dietary components that pose a threat to the mucosa (Specian and Oliver, 1991).

Little is known about the maturation and organization of the intestinal cytoarchitecture and goblet cell subpopulations during the dynamic phase of development in young poult, particularly in response to prebiotic supplementation. The objectives of the present investigation were to evaluate the effect of AM prebiotic on morphometric analysis of intestinal mucins and horizontal transmission of SE in neonate poult.

MATERIALS AND METHODS

Diet composition and preparation: Poult were fed a sorghum-soybean starter diet during all the experiments. The diets were formulated without added antibiotics or coccidiostats and contained the levels of nutrients recommended by the National Research Council (1994). The two treatments, similar in energy and protein content, differed only in added prebiotic, *Aspergillus* Meal (AM), a dried extract from the primary fermentation of a non-toxic *Aspergillus niger* Strain (Fermacto™, PetAg Inc. Hampshire, IL 60140 USA). This product is unique in its composition, as it has only 12% protein and 45% fiber. This fiber is not from the normal vegetative source but is rather a mycelium fiber.

Experimental design: A trial was conducted using one-day-old turkey poult (Nicholas) obtained from a commercial hatchery and placed in floor pens located within the Poultry Health Research Experimental Farm of the University of Arkansas, Fayetteville, AR. One hundred poult at 1 day of age were randomly assigned to two groups. Poult in each group were divided and placed in two floor pens with 50 poult each. One group served as a control and received a ration containing no added AM. Poult in the treatment group were provided a ration supplemented with 0.2% AM prebiotic. Each dietary regimen and water were provided *ad libitum* to 30 days of age. Poult were humanely killed by CO₂ inhalation and specimens of duodenum and distal ileum were collected at 10, 20 and 30 days. On each day, five poult were taken randomly from each pen for morphometric evaluations.

Morphometric evaluations: Specimens of duodenum and distal ileum were collected from five poult in each group at 10, 20 and 30 days. Each sample of intestine was cut open longitudinally at the antimesenteric attachment. Samples were fixed on dental wax in 0.1 M-phosphate-buffered formalin solution (40 ml/l), with the villi on the upper side. After the microscopic study, a 3-mm wide zone from the mesenteric site was cut at right angles to the surface of the mucosa and embedded in paraffin wax. Sections were cut (5 µm) and stained with haematoxylin and eosin (HE staining), a combination of the periodic acid-Schiff method (PAS staining) with the basophilic dyes alcian blue (AB staining) and a combination of the basophilic dyes high iron diamine

(HID staining) and the basophilic dyes alcian blue (AB staining), according to routine methods (Kiernan, 2008). From these stained sections, 20 well-oriented villi were selected on each slide. Morphometric indices were determined by computer image analysis (Image-Pro Plus Version 4.0 from Media Cybernetics 8484 Georgia Av. Silver Spring, MD 20910USA). The morphometric variables analyzed included villi height and surface area (from the tip of the villi to the villi-crypt junction) and crypt depth (defined as the depth of the invagination between adjacent villi). In addition, the types and numbers of goblet cells, classified as neutral (red), acidic (blue) or sulpho mucin cells (black), respectively, were determined in 20 intact villi on each slide. Numbers of goblet cells were determined according to the staining with PAS-AB (to discriminate between acid and neutral mucins) or with HID-AB (to separate the acid mucins into sulpho-mucins) (Kiernan, 2008). Sections for villi height, crypt depth and villi surface area were examined under 10x plan objective lens. The number of goblet cells was examined under 40x plan objective lens and were assessed both visually and by image analysis. Prior to image analysis, image brightness was adjusted to standard levels then an autocontrast step was applied to the stored image.

Statistical analysis: Differences on morphometric analysis of villi height, crypt depth, goblet cells and villi area were determined by one way analysis of variance using the General Linear Models procedure. Significant differences were further separated using Duncan's multiple range test using commercial SAS® statistical software (SAS Institute, 2002). Significant differences were set at $p < 0.05$.

RESULTS

The effect of dietary AM prebiotic on histological measurements of the duodenum mucosa of neonate poult is summarized in Table 1. A significant increase in villi height in microns was observed in the AM prebiotic group at 10 and 30 days when compared with the control group. No significant changes were observed in either crypt depth or the total number of neutral mucin cells between AM prebiotic or control group at any time of evaluation. However, a significant increase in the total number of acid mucin cells and sulpho mucin cells in the duodenum mucosa were observed at 10, 20 and 30 days of evaluation on the group that received dietary AM when compared with control. Dietary AM prebiotic also increased villi surface area at 10 and 30 days of evaluation.

The effect of AM prebiotic on histological measurements of the ileum mucosa of neonate poult is summarized in Table 2. Villi height and villi surface area was significantly increased at days 10 and 30 compared with control. Ileum crypt depth was only significant between

Table 1: Effect of dietary *Aspergillus* meal on histological measurements of the duodenum mucosa of poult

Duodenum measurements	DAY 10		DAY 20		DAY 30	
	Control	<i>Aspergillus</i> meal	Control	<i>Aspergillus</i> meal	Control	<i>Aspergillus</i> meal
Villi height (μm)	35.2 \pm 2.9 ^b	56.3 \pm 2.4 ^a	40.7 \pm 3.1 ^a	45.9 \pm 1.9 ^a	42.5 \pm 2.6 ^b	63.3 \pm 2.7 ^a
Crypt depth (μm)	2.7 \pm 0.3 ^a	3.7 \pm 0.4 ^a	5.2 \pm 0.5 ^a	4.8 \pm 0.3 ^a	5.4 \pm 0.7 ^a	8.4 \pm 0.5 ^a
Neutral mucins goblet cells	51.8 \pm 10.8 ^a	28.3 \pm 7.6 ^a	50.4 \pm 9.4 ^a	38.3 \pm 11.4 ^a	71.0 \pm 12.8 ^a	61.8 \pm 14.4 ^a
Acidic mucins goblet cells	55.6 \pm 1.8 ^b	71.2 \pm 4.4 ^a	60.3 \pm 1.8 ^b	80.5 \pm 7.9 ^a	55.4 \pm 10.1 ^b	84.2 \pm 7.8 ^a
Sulfo mucins Goblet cells	41.6 \pm 4.3 ^b	116.0 \pm 16.8 ^a	104.0 \pm 17.1 ^b	155.3 \pm 12.7 ^a	120.1 \pm 14.3 ^b	155.3 \pm 13.0 ^a
Villi surface area (μm^2)	114.1 \pm 10.8 ^b	205.0 \pm 5.6 ^a	98.0 \pm 9.5 ^a	115.6 \pm 12.6 ^a	120.6 \pm 13.7 ^b	251.6 \pm 15.1 ^a

Dietary prebiotic *Aspergillus* Meal (AM), was provided in the diet in a concentration of 0.2%. Five poult (n = 5) were use for histological measurements each day for each group. From each section twenty well oriented villi were selected and pooled. Data expressed as Mean \pm SE. Values in a row within days with no common superscript differ significantly (p<0.05)

Table 2: Effect of dietary *Aspergillus* meal on histological measurements of the ileum mucosa of poult

Ileum measurements	DAY 10		DAY 20		DAY 30	
	Control	<i>Aspergillus</i> meal	Control	<i>Aspergillus</i> meal	Control	<i>Aspergillus</i> meal
Villi height (μm)	9.8 \pm 1.1 ^b	14.6 \pm 3.6 ^a	14.8 \pm 5.4 ^a	16.3 \pm 5.5 ^a	19.8 \pm 8.4 ^b	26.3 \pm 4.2 ^a
Crypt depth (μm)	1.4 \pm 0.1 ^b	3.8 \pm 0.5 ^a	2.1 \pm 0.2 ^a	2.8 \pm 0.4 ^a	5.6 \pm 0.7 ^a	5.4 \pm 0.5 ^a
Neutral mucins Goblet cells	54.2 \pm 10.2 ^b	80.4 \pm 2.6 ^a	60.4 \pm 13.2 ^b	98.3 \pm 15.4 ^a	59.4 \pm 11.8 ^b	103.8 \pm 14.4 ^a
Acidic mucins Goblet cells	20.2 \pm 4.1 ^a	29.4 \pm 7.9 ^a	38.4 \pm 7.9 ^a	27.4 \pm 18.4 ^a	25.9 \pm 13.4 ^a	30.9 \pm 6.3 ^a
Sulfo mucins Goblet cells	42.2 \pm 5.2 ^b	88.4 \pm 9.6 ^a	100.3 \pm 10.1 ^b	155.3 \pm 12.7 ^a	115.8 \pm 13.3 ^b	200.8 \pm 15.4 ^a
Villi surface area (μm^2)	28.0 \pm 0.8 ^{ab}	39.2 \pm 4.6 ^a	22.3 \pm 1.5 ^{ab}	42.0 \pm 2.6 ^a	38.9 \pm 3.7 ^b	51.6 \pm 2.1 ^a

Dietary prebiotic *Aspergillus* Meal (AM), was provided in the diet in a concentration of 0.2%. Five poult (n = 5) were use for histological measurements each day for each group. From each section twenty well oriented villi were selected and pooled. Data expressed as Mean \pm SE. Values in a row within days with no common superscript differ significantly (p<0.05)

synbiotic and control groups at day 10 of evaluation. No significant changes were observed on acid mucin cells, however, a significant increase in the total number of neutral mucin cells and sulpho-mucin of the ileum mucosa were observed at 10, 20 and 30 days of evaluation on the AM prebiotic when compared with control.

DISCUSSION

In the immediate post hatch period birds must undergo the transition from energy supplied by the endogenous nutrients of the yolk to exogenous carbohydrate rich feed. During that critical time dramatic changes occur both in the intestinal size and morphology (Uni *et al.*, 1995; Uni *et al.*, 1996). Goblet cell mucus that covers the epithelial surface of the gastrointestinal tract is an important physical and chemical barrier that interferes with intestinal attachment of luminal antigens. Although intestinal goblet cells are distributed through the entire length of the intestinal tract, their contribution to the total epithelial volume is not constant (Specian and Oliver, 1991). Once propagated from stem cells at the base of the crypt, immature goblet cells rapidly begin to synthesize and secrete mucin granules. Migration to the

villi tip produces not only morphological changes but also changes in the chemical composition of the mucins produced. Although intestinal goblet cells produce multiple mucin species, not all mucin species are secreted equally. Immature goblet cells deep within the crypts in the small intestine produce neutral mucins containing little sialic acid. As they mature and migrate to the villi tip, the mucins become increasingly sialated thus increasing the acidity of the molecule (Filipe, 1979). In the same way, there are differences in mucin composition along the colonic crypt-surface axis (Specian and Oliver, 1991). Goblet cells within the crypt contain predominantly sulpho mucins; the mature goblet cells on the colonic surface contain neutral mucins (Forstner, 1978). Several investigators have reported that the constituents of poultry feed, in particular the consumption of a diet supplemented with enzymes, lead to changes in intestinal viscosity and mucin composition which are associated with alterations in the goblet cell glycoconjugates of the chick intestinal tract (Sharma *et al.*, 1995). Enzyme-supplemented diets or probiotics have been shown to increase the amount of neutral and sulphated mucins in the goblet cells of the small and large intestines and caecum (Dunsford *et al.*, 1991;

Sharma *et al.*, 1995). Similarly, during the present study dietary AM prebiotic produced significant increases in the number of mature acid mucin cells in duodenum and mature neutral mucin cells in ileum, as well as the number of sulpho mucin cells in both duodenum and ileum at all times of evaluation. The metabolism of mucins modulates the growth of intestinal bacteria and may serve to regulate the microecology of the intestinal lumen (Variyam and Hoskins, 1981). Previously, mucins were only seen as water-binding molecules that protected the underlying mucosa against harmful agents. The current picture of these molecules is characterized by the selective interaction with their environment, including epithelial and endothelial cells and microorganisms, thereby regulating a great number of biological processes (Zufarov *et al.*, 1979; Thymann *et al.*, 2007).

In the present study, significant increases in villi height and villi surface area of both duodenum and ileum were observed in the dietary AM prebiotic group when compared with control poult. Elucidation of the mechanisms that alter the biosynthesis, turnover and degradation of intestinal mucins are relevant to the understanding of both the normal gut ecosystem and various intestinal diseases. The present study indicates that dietary supplementation of AM prebiotic has an impact on mucosal architecture and goblet cell proliferation in the duodenum and ileum of neonate poult.

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