Pre- and Posthatch Development of Goblet Cells in the Broiler Small Intestine: Effect of Delayed Access to Feed

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ABSTRACT Mucin glycoproteins play a key role in the regular function of the epithelium of the gastrointestinal tract, and in this study, the ontogenesis and development of mucin producing cells was examined in the broiler. Mucin-producing cells were observed in the small intestine from 3 d before hatch, and at this time contained only acidic mucin. After hatch and until Day 7 posthatch, the proximal, middle, and distal segments of the small intestine contained similar proportions of goblet cells producing acidic and neutral mucins. A gradient of goblet cell density was observed increasing along the duodenal to ileal axis. Delayed access to feed for 48 h posthatch resulted in an increase in intestinal intracellular mucins, which might have been due to impaired mucin secretion or enhanced mucin production. Changes in mucin dynamics could affect absorptive and protective functions of the small intestine.

(*Key words*: chick, development, embryo, goblet cells)

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INTRODUCTION

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. Goblet cells arise by mitosis from pluripotential stem cells at the base of the crypt (Cheng and Leblond, 1974) or from poorly differentiated cells in the lower crypt referred to as oligomucous cells (Cheng, 1974). These cells migrate from the crypt toward the villus tip where they are sloughed into the lumen, a process that takes 2 to 3 d (Geyra et al., 2001). Thus goblet cells are short-lived and are constantly undergoing replacement. The mucus layer acts both as a medium for protection of the brush border against damage by chemicals or microorganisms and influences transport between luminal contents and the brush border (Forstner and Forstner, 1994).

Mucins consist of a peptide backbone containing alternating glycosylated and nonglycosylated domains, with the O-linked glycosylated region composing more than 50% of the mass (Allen, 1981). N-acetyglucosamine, N-acetylgalactosamine, fucose, and galactose are the four primary mucin oligosaccharides (Forstner et al., 1995). Mucin exhibits a high level of heterogeneity, which results from diversity in the length, composition, branching, and degree of sulfation and acetylation of the oligosaccharides (Neutra and Forstner, 1987). Acid mucins can be detected by Alcian Blue (AB) pH 2.5 staining and neutral mucins by periodic acid-Schiff (PAS) staining (McManus, 1948; American Forces Institute of Pathology, 1992). Mucin molecules have a net negative charge due to their carbohydrate content. Mucin subtypes and goblet cell distribution vary spatially throughout the gastrointestinal tract and temporally during development in many mammalian species (Sheahan and Jervis, 1976; Hill et al., 1990; Dunsford et al., 1991; Enss et al., 1992; Sharma and Schumacher, 1995; Kandori et al., 1996). There is, however, no information on mucin subtypes and the ontogeny of goblet cell formation and distribution in the small intestine of broiler chickens.

The purpose of the present study was to study the developmental pattern of goblet cells before hatch and during the first week posthatch and to examine the effect of delayed access to feed on mucin production in the chicken small intestine.

MATERIALS AND METHODS

Animals and Experimental Design

Fertile eggs were obtained from a commercial hatchery[2] (Ross × Ross) on Days 18 and 20 of incubation. Male

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Abbreviation Key: AB = Alcian Blue; PAS = Periodic acid-Schiff's reagent.

broiler chicks were taken from the hatchery within 1 h of clearing the shell and were transported within 30 min to a battery brooder where feed and water were available ad libitum. Chicks were divided into experimental groups on the basis of BW, equalizing BW, and variance between groups. The control group had free access to water and to a commercial diet formulated to meet or exceed NRC recommendations (National Research Council, 1994). The fasted group was maintained without access to food and water for 48 h, after which access to food and water was as in the control group. All chicks were maintained in temperature-controlled brooders for 7 d. Each dietary treatment was applied to five replicate groups of 10 chicks. All procedures were approved by the Animal Care and Welfare Committee of our Institute.

Tissue Sampling

Samples were taken from five chicks at 18 and 20 d of incubation, at hatch, and from control and fasted chicks (one per pen) each day during the first week posthatch. Samples (approximately 2 cm) were taken from the midpoint of the duodenum, from the midpoint between the point of entry of the bile duct and Meckel's diverticulum (jejunum), and midway between Meckel's diverticulum and ileocecal junction (ileum). Segments were gently flushed with 0.9% (wt/vol) NaCl to remove the intestinal contents and were fixed in fresh 4% (vol/vol) buffered formaldehyde.

Morphological Examination

Intestinal samples were dehydrated, cleared, and embedded in paraffin. Serial sections were cut at 5 μm and placed on glass slides. For all assays, sections were deparaffinized in xylene and rehydrated in a graded alcohol series. Sections were examined by light microscopy (Uni et al., 1998).

Neutral Mucin Staining

Determination of neutral mucin was by staining 5-μm sections with PAS (McManus, 1948; American Forces Institute of Pathology, 1992). Briefly, following deparaffinization and rehydration, slides were incubated in 0.5% periodic acid for 15 min then washed and incubated with Schiff's reagent[3] for 30 min. After being washed in warm water, slides were dehydrated and mounted. The number of PAS positive (PAS+) along the villi was counted by light microscopy.

Acid Mucin Staining

Determination of acid mucin was by staining 5-μm sections with AB pH 2.5 (Lev and Spicer, 1964; American Forces Institute of Pathology, 1992). Briefly, slides after deparaffinization and rehydration were incubated in 3% acetic acid for 3 min and then in AB solution (1% in 3% acetic acid, pH 2.5). After being washed in water, slides were dehydrated and mounted. The number of AB positive cells (AB+) along the villi was counted by light microscopy.

TABLE 1. BW of chicks with access to feed within 1 h (fed) or 48 h (fasted) posthatch

	Treatment	
Age, d	Fed	Fasted
0	47 ± 1	47 ± 1
1	48 ± 2	44 ± 1*
2	58 ± 2	40 ± 2*
3	83 ± 3	52 ± 2*
5	94 ± 4	63 ± 3*
6	115 ± 5	85 ± 5*

*Differs from fed chicks ($P < 0.05$).

Measurements

Cell length was the distance from brush border membrane to the basolateral membrane. Villus surface area was calculated from villus height and width at half height. The area of the goblet cell was calculated from length and width of goblet cell "cup" in cross-sections of the villi. Density of goblet cells was calculated as the number of goblet cells per unit of surface area (mm^2). All measurements were performed with an Olympus light microscope using Epix XCAP software.[4]

Statistical Analysis

An ANOVA using the general linear models procedures of SAS software (SAS Institute, 1986) was used to examine differences between treatments within ages with significance at $P < 0.05$ unless otherwise stated.

RESULTS

Epithelial cells were examined during late incubation and during 7 d posthatch to determine cell numbers and the types of goblet cells present. In the embryonic duodenum, at 18 d of incubation, 13% of the epithelial cells were goblet cells. This proportion of goblet cells was maintained through the first week posthatch. In the final stages of incubation the goblet cells contained acid mucins, whereas less than 1% were PAS positive (Figure 1A). In the jejunum and ileum a similar pattern was observed (Figure 1 B, C) where at 18 d of incubation all goblet cells (about 19%) contained acidic mucin (AB) but fewer or no PAS positive cells were found. Goblet cells comprised about 23% of the intestinal epithelial cells in the jejunum and 26% in the ileum on the day of hatch and this proportion remained similar during the 7 d posthatch (Figure 1 B, C). Micrographs of the jejunal mucosa at 18 d of incubation and at hatch stained with either AB or PAS are shown in Figure 2.

[3]Sigma Chemical Company, St. Louis, MO.
[4]Epix Inc., Buffalo Grove, IL 60089.

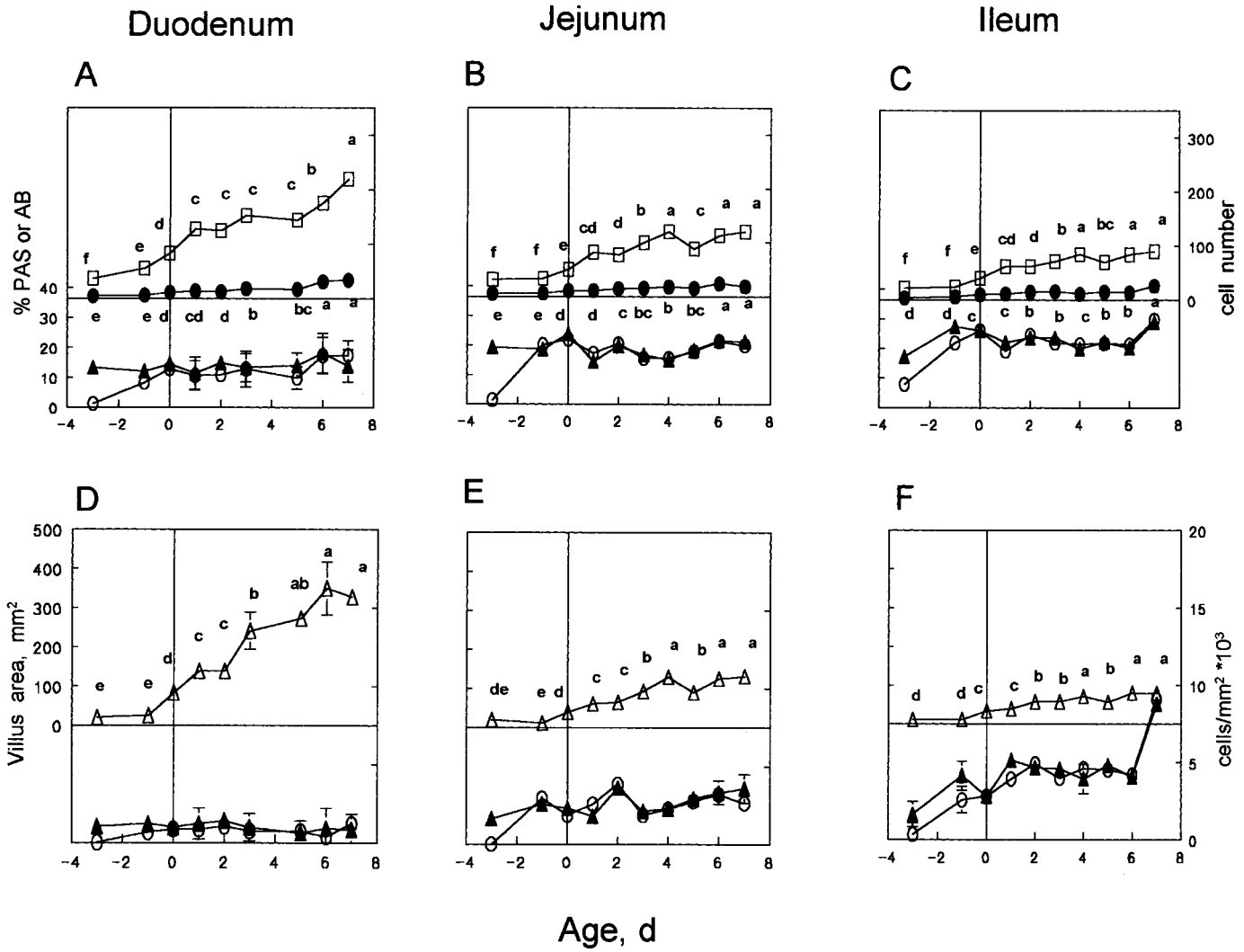

FIGURE 1. Panels A to C: Number of enterocytes (□) and goblet cells (●) (right axis) and the percentage of cells staining positive for Alcian Blue (AB+,▲) or periodic acid-Schiff (PAS+, ○) (left axis) per villus column in various regions of small intestine of broiler embryos and chicks. Panels D to F: Villus surface area (△) (left axis) and density of goblet cell populations AB+ (▲), PAS+ (○), (right axis) in various regions of small intestine of broilers embryos and chicks. Values are means with standard errors represented by vertical bars (when they do not fall within the symbols). Ages not marked with the same letter are significantly different ($P < 0.05$) for numbers of enterocytes and goblet cells. The percentage and cell density of PAS+ cells with age were significantly lower ($P < 0.05$) at 3 d before hatch in the duodenum, jejunum, and ileum; other values did not differ.

The number of the goblet cells per area (Figure 1 D, E, F) increased slightly in the duodenum with development; however, increases in goblet cell density were more rapid in the jejunum and ileum, reaching 50% in the jejunum and 150% in the ileum during late incubation and continuing posthatch. No differences in the density of acidic or neutral goblet cells were observed after hatch.

Chicks with immediate access to feed compared to those with first access to feed 48 h posthatch had increased BW from 1 d posthatch through 6 d (Table 1). Delayed access to feed decreased the villus surface area in the jejunum on Days 3 and 4 and in the ileum on Day 2 compared to fed chicks (Figure 3 A, B, C). After feeding, villus surface area increased and tended to be more like those of fed birds. The density of goblet cells, containing acidic and neutral mucins increased in fasted birds in the duodenum on Day 2 and in the jejunum on Days 2 and 3; PAS-positive cells did not change in the ileum, whereas AB-positive cells increased on Day 1 (Figure 3 D to I). However, not only did the number of goblet cells change, but the volume of the goblet cells also changed in fasted chicks. Typical goblet cells from the jejunums of fasted chicks are shown in Figure 4. The increases in cell area are apparent, and measurements of changes in the area of the "cup" portion of goblet cells in cross-sections of the villus with time are shown in Figure 5. Fed chicks had more mucin-containing longitudinal areas on Day 2 and fewer areas on Days 3 through 6 in the duodenum. Less longitudinal areas were also found in the jejunum from 1 through 4 d and on Day 1 and Days 3 through 5 in the ileum.

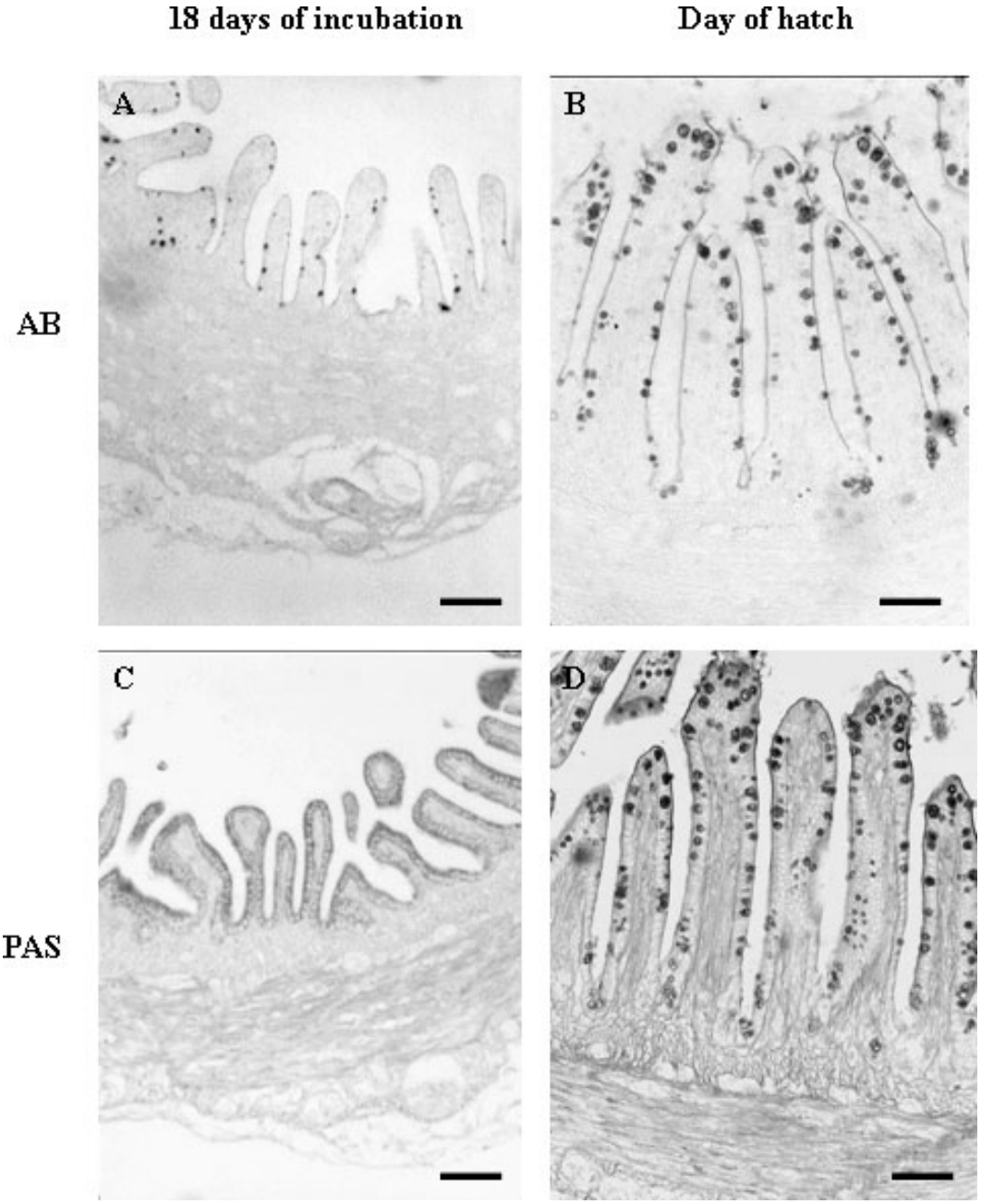

FIGURE 2. Representative light micrographs of jejunum stained with Alcian Blue (A, B) or periodic acid-Schiff (C, D). Goblet cells producing only acid mucin could be observed at 18 d of incubation (A). On the day of hatch (B, D) goblet cells producing acidic and neutral mucin are observed. Crypts are not yet developed, and goblet cells are distributed all along the villi. (Magnification ×200; bar = 50 μm at both ages).

DISCUSSION

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 and, thus, the ontogeny of its development has extensive implications for intestinal function. This study has indicated that goblet cells were observed in the small intestine 3 d prior to hatch, but these cells differed from mature cells in that they contained only acid mucins.

In the late embryonic stages, crypts were not present in the small intestine, and goblet cells were first observed as distributed along the villi. This pattern is similar to that previously demonstrated in poultry with enterocyte proliferation occurring along the villus, at and close to hatch (Uni et al., 2000). Proliferation of enterocytes became localized to the crypts during the posthatch days although in the jejunum approximately 20% of eneterocytes were still proliferating at 7 d posthatch (Uni et al., 1998; Geyra et al., 2001). The location of the stem cells for this proliferation has not yet been clarified. After hatch the proportion of goblet cells was similar, increasing in number with age in a constant proportion to enterocytes throughout the small intestines. However, along the duodenal-ileal axis the density of goblet cells increased distally.

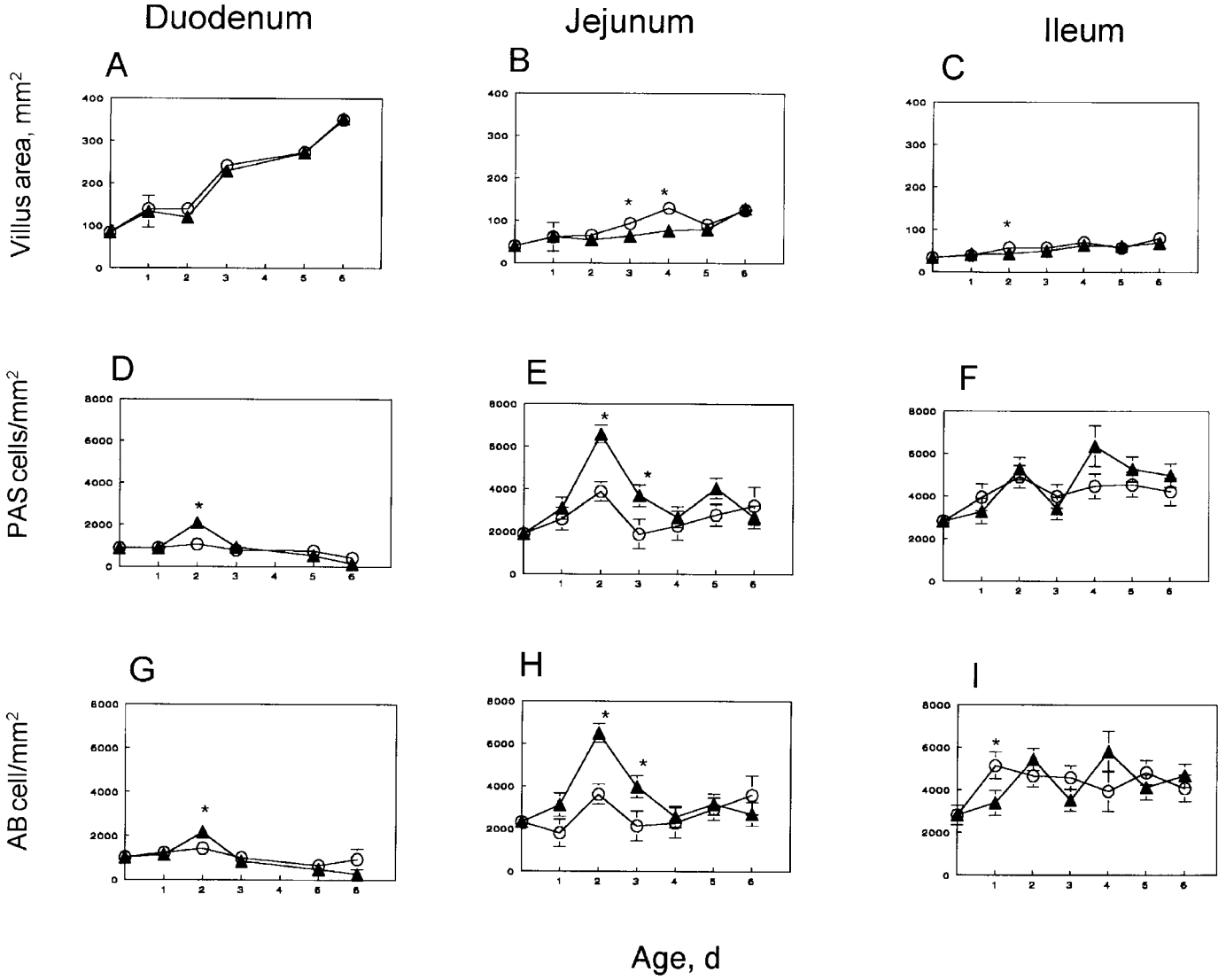

FIGURE 3. Effect of delayed access to feed on villus area (A, B, C) and on density of periodic acid-Schiff (PAS+) (D, E, F) and Alician Blue (AB+) (G, H, I) goblet cells in chicks fed immediately (○) or fasted for 48 h (▲). Values are means and standard errors are represented by vertical bars (when they do not fall within the symbols), asterisks mark significant differences ($P < 0.05$).

In the latter stages of embryonic development, goblet cells contained only acid mucin, and this pattern altered with acid and neutral mucin-containing goblet cells found at hatch. The proportion of goblet cells exhibiting acid and neutral mucin remained constant posthatch. Age-related changes in the chemical composition and properties of mucus glycoproteins in the rat small intestine have been previously reported by Shub et al. (1983) who showed differences in the types of mucus glycoproteins synthesized with growth, and similar developmental patterns have been reported in other mammalian species (Kemper and Specian, 1991; Deplanske and Gaskins, 2001).

The physiological relevance of distinct mucin subtypes is not well understood. It has been suggested that acidic mucins protect against bacterial translocation as sulfated mucins appear to be less degradable by bacterial glycosidases and host proteases (Fontaine et al., 1996; Roberton and Wright, 1997). In this study, acidic mucins were present 3 d before hatch. This finding is similar to observations in mammals (Lev, 1968; Filipe et al., 1989) in which acidic mucin appears to predominate throughout fetal stages, and clear developmental patterns of an increasing ratio of neutral to acidic mucins were found between birth and weaning. The presence of acidic mucins at early developmental stages (Hill et al., 1990; Turck et al., 1993) may be of particular importance as an innate barrier as the acquired immune system is not fully functional in the neonatal intestine (Cebra, 1999).

Delayed access to first feed caused a reduction in the villus surface area, particularly in the jejunum as has been previously reported (Geyra et al., 2001). This finding was accompanied by a decrease in the number of enterocytes and an increase in density of goblet cells producing acid and neutral mucin in the jejunum and ileum villi. In previous reports, rats, pigs, mouse, and chickens (Langhout et al., 1999; Sharma et al., 1997) have shown alternations in the type of mucin produced due to changes in diets or

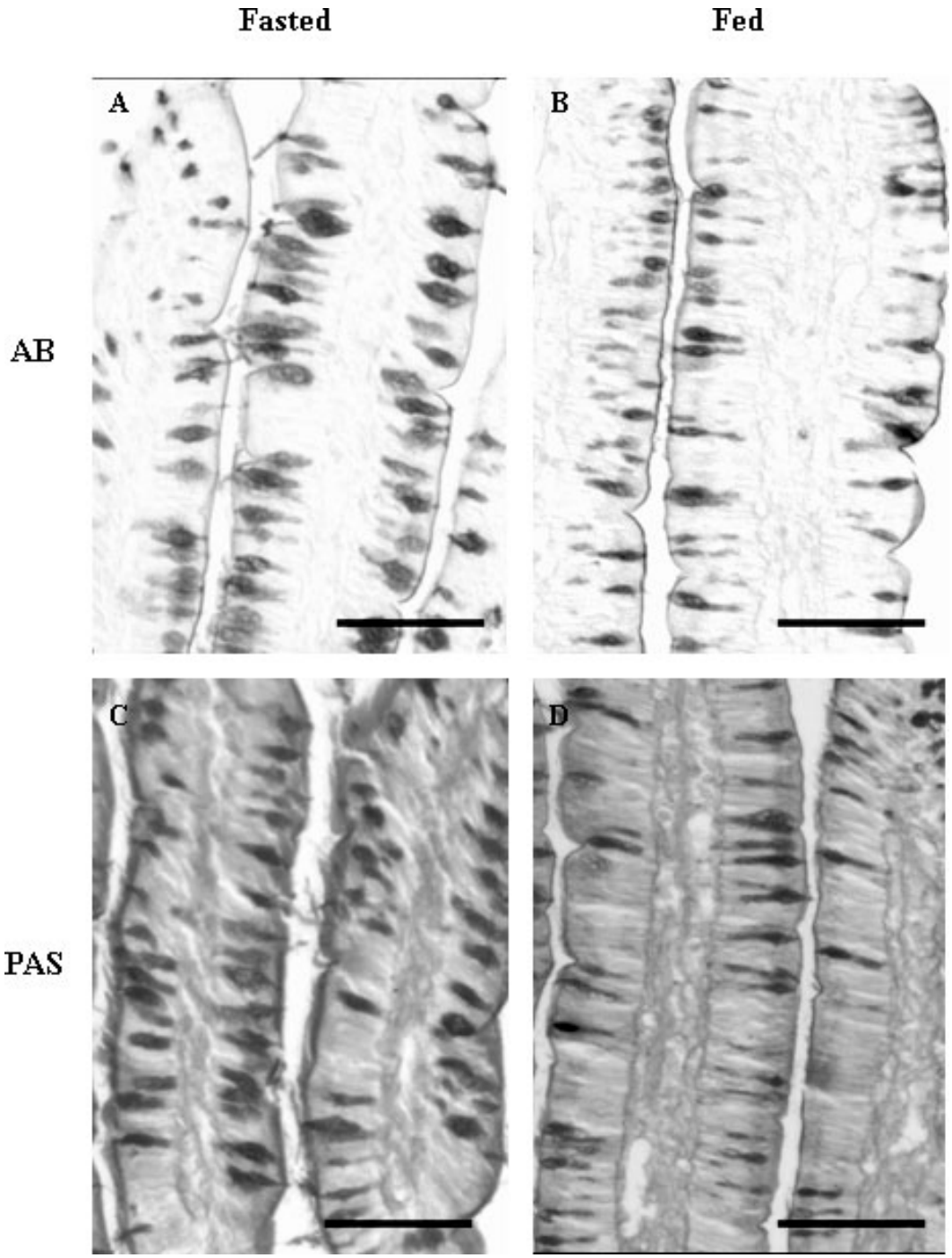

FIGURE 4. Representative light micrographs of jejunum from a 3-d-old chick fed immediately (B, D) or fasted for 48 h (A, C). The jejunum was stained with Alcian blue (AB) (A, B) or periodic acid-Schiff (PAS) (C, D). The stained area of goblet cells in the fasted chicks is greater and contains more acidic and neutral mucins compared to goblet cells in fed chicks ($P < 0.05$). (Magnification ×400; bar = 50× μm).

from malnutrition. Similar results were obtained by Dunsford et al. (1991) who showed, in pigs, that early weaning changed the numbers of goblet cells but did not alter the mucin composition as examined by AB and PAS staining.

Mucin synthesis and secretion is influenced by many factors. Reports of rats, mice, and humans have indicated that mucin production is depressed by agents or conditions that uncouple glycosilation and protein synthesis (De Ritis et al., 1975; O'Doherty and Kuksis, 1975; Sherman et al., 1985). Mucin biosynthesis changes with alterations in the rate of migration of epithelial cells from the proliferating crypt zones (Shea-Donohue et al., 1985) and by perturbations in the rates of differentiation of precursor cells into mature goblet cells (De Ritis et al., 1975; Wattel et al., 1979; Shub et al., 1983). Diet also influences the type of mucoproteins produced, with some components reducing sulfation or the production of individual sugars. In addition, studies have shown that altered mucus-related indexes are observed in germfree animals

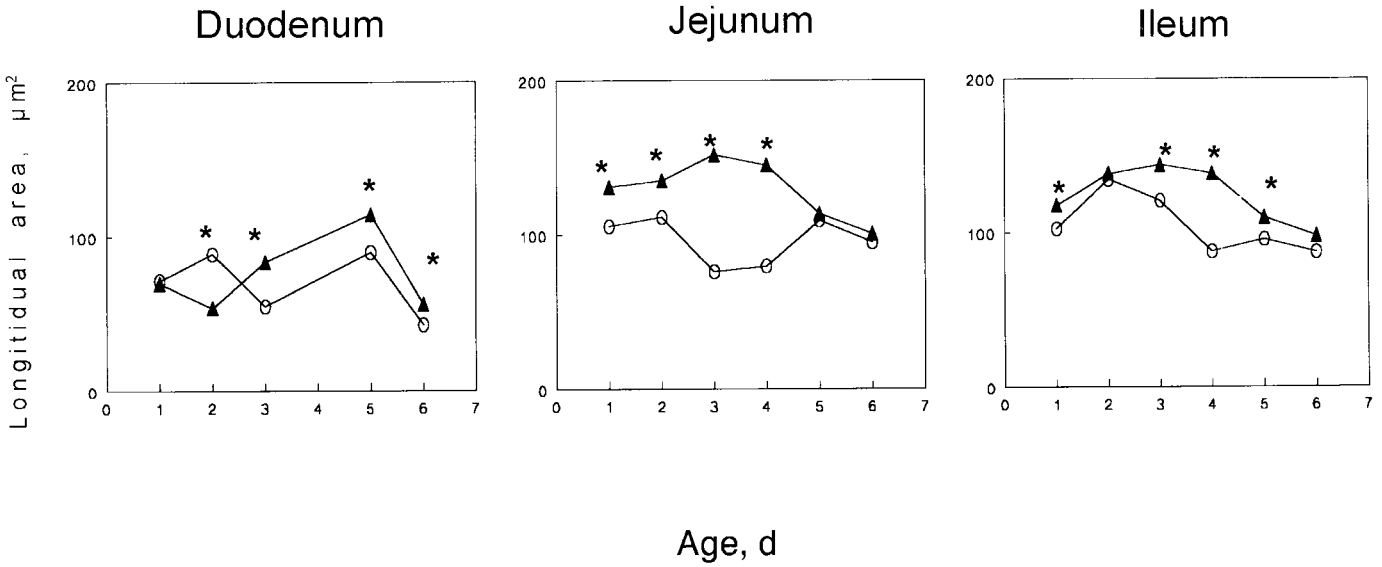

FIGURE 5. Changes in the area of goblet cells determined in longitudinal sections with age in chicks with immediate (○) or delayed access (▲) to feed. Values are means with standard errors represented by vertical bars (when they do not fall within the symbols), asterisks mark significant differences ($P < 0.05$).

(Szentkuti et al., 1990; Enss et al., 1992; Sharma and Schumacher, 1995; Kandori et al., 1996; Gaskins, 1997; Meslin et al., 1999), and evidence of enhanced mucus secretion in response to differing intestinal microbial populations has been presented (Elliot et al., 1970; Chadee and Meerovitch, 1985; Mack et al., 1999). Some or all of these factors may have contributed to the elevated density and larger size of goblet cells in fasted chicks observed in this study.

In addition to the role of the intestinal mucus layer as a defensive barrier, there is evidence suggesting that mucin has an major effect on absorption of cations (Forstner and Forstner, 1975; Powell et al., 1999). Metal ions traverse the mucosally adherent mucus layer with an efficiency of $M^{+}>M^{2+}>M^{3+}$ before transport by membrane proteins into the enterocyte (Powell et al., 1999). Thus Ca^{2+} binds directly to anions of goblet cell mucin before uptake by the enterocytes (Forstner and Forstner, 1975).

The development of the small intestinal mucus production in the broiler occurs in the late embryonic and immediate posthatch period. The mucus layer has protective and transport functions, and its development is influenced by the time of access to feed.

REFERENCES

Allen, A. 1981. Structure and function of gastrointestinal mucus. Pages 617–639 in Physiology of the Gastrointestinal Tract. L. R. Johnson, ed. Raven Press, New York.

American Forces Institute of Pathology. 1992. Laboratory Methods in Histotechnology. E. B. Prophet, B. Mills, J. B. Arrington, and L. H. Sobin ed. American Registry of Pathology, Washington, DC.

Cebra, J. J. 1999. Influences of microbiota on intestinal immune system development. Am. J. Clin. Nutr. 69:1046S–1051S.

Chadee, K., and E. Meerovitch. 1985. *Entamoeba histolytica*: early progressive pathology in the cecum of the gerbil (*Meriones unguiculatus*). Am. J. Trop. Med. Hyg. 34:283–291.

Cheng, H. 1974. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. II. Mucous cells. Am. J. Anat. 141:481–501.

Cheng, H., and C. P. Leblond. 1974. Origin, differentiation and renewal of the four main epithelial cells in the mouse small intestine. IV. Unitarian theory of the origin of the four epithelial cell types. Am. J. Anat. 141:537–561.

De Ritis, G., Z. M. Falchuk, and J. S. Trier. 1975. Differentiation and maturation of cultured fetal rat jejunum. Dev. Biol. 45:304–317.

Deplanske, B., and H. R. Gaskins. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am. J. Clin. Nutr. 73(Suppl.):1131S–1341S.

Dunsford, B. R., W. E. Haensly, and D. A. Knabe. 1991. Effects of diet on acidic and neutral goblet cells population in the small intestine of early weaned pigs. Am. J. Vet. Res. 52:1743–1746.

Elliot, H. L., C. C. Carpenter, R. B. Sack, and J. H. Yardley. 1970. Small bowel morphology in experimental canine cholera. A light microscopic study. Lab. Invest. 22:112–120.

Enss, M. L., H. Grosse-Seistrup, U. Schmidt-Witting, and K. Garner. 1992. Changes in colonic mucins of germfree rats in response to the introduction of a "normal" rat microbial flora. Rat colonic mucin. J. Exp. Anim. Sci. 35:110–119.

Filipe, M. I., A. Sandey, and E. A. Carapeti. 1989. Goblet cell mucin in human foetal colon, its composition and susceptibility to enzyme degradation: A histochemical study. Symp. Soc. Exp. Biol. 43:249–258.

Fontaine, N., J. C. Meslin, C. Lory, and C. Andrieux. 1996. Intestinal mucin distribution in the germ-free rat and in heteroxenic rat harbouring a human bacterial flora: Effect on inulin in the diet. Br. J. Nutr. 75:882–892.

Forstner, J. F., and G. G. Forstner. 1975. Calcium binding to intestinal goblet cell mucin. Biochem. Biophys. Acta 386:283–292.

Forstner, J. F., and G. G. Forstner. 1994. Gastrointestina mucus. Pages 1255–1283 in Physiology of the Gastrointestinal Tract. 3rd ed. P. Leonard and R. Johnson, ed. Raven Press, New York.

Forstner, J. F., M. G. Oliver, and F. A. Sylvester. 1995. Production, structure and biologic relevance of gastrointestinal mucins. Pages 71–88 in Infections of the Gastrointestinal Tract. M. J.

Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant, ed. Raven Press, New York.
Gaskins, H. R. 1997. Immunological aspects of host/microbal interactions at the intestinal epithelium. Pages 537–587 in Gastrointestinal Microbiology. R. I. Mackie, B. A. White, and R. E. Isaacson, ed. Chapman & Hall, New York.
Geyra, A., Z. Uni, and D. Sklan. 2001. The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. Br. J. Nutr. 86:53–61.
Hill, R. R., H. M. Cowley, and A. Andermot. 1990. Influence of colonizing microflora on the mucin histochemistry of the neonatal mouse colon. Histochem. J. 22:102–105.
Kandori, H., K. Hirayama, M. Takeda, and K. Doi. 1996. Histochemical, lectin-histochemical and morphometrical characteristics of intestinal goblet cells of germfree and conventional mice. Exp. Anim. 45:155–160.
Kemper, A. C., and R. D. Specian. 1991. Rat small intestinal mucin: A quantitative analysis. Anat. Rec. 229:219–226.
Langhout, D. J., J. B. Schutte, P. V. Van Leeuwen, J. Wiebenga, and S. Tamminga. 1999. Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Br. Poult. Sci. 40:340–347.
Lev, R. 1968. A histochemical study of glycogen and mucin in developing human foetal epithelia. Histochem. J. 1:152–165.
Lev, R., and S. Spicer. 1964. Specific staining of sulfate groups with alcian blue at low pH. J. Histochem. Cytochem. 12:309.
Mack, D. R., S. Michail, S. Wei, L. McDougall, and M. A. Hollingsworth. 1999. Probiotics inhibit enteropathogenic *E. coli* adherance in vitro by inducing intestinal mucin gene expression. Am. J. Physiol. 276:G941–950.
McManus, J. F. A. 1948. Histological and histochemical uses of periodic acid. Stain Technol. 23:99.
Meslin, J. C., N. Fontaine, and C. Andrieux. 1999. Variation of mucin distribution in the rat intestine, caecum and colon: effect of the bacterial flora. Comp. Biochem. Physiol. A 123:235–239.
National Research Council. 1994. Nutrient Requirements of Poultry. 9th ed. National Academy Science. Washington, DC.
Neutra, M. R., and J. F. Forstner. 1987. Gastrointestinal mucus: synthesis, secretion and function. Pages 975–1009 in Physiology of the Gastrointestinal Tract. 2nd ed. Raven Press, New York.
O'Doherty, P. J. A., and A. Kuksis. 1975. Effect of puromycin *in vitro* on protein and glycerolipid biosynthesis in isolated epithelial cells of rat intestine. Int. J. Biochem. 6:435–441.
Powell, J. J., R. Jugdaohsingh, and R. P. H. Thompson. 1999. The regulation of mineral absorption in the gastrointestinal tract. Proc. Nutr. Soc. 58:147–153.
Roberton, A. M., and D. P. Wright. 1997. Bacterial glycosulfatases and sulfomucin degradation. Can. J. Gastroenterol. 11:361–366.
SAS Institute. 1986. SAS User's Guide. Version 6 Edition. SAS Institute Inc., Cary, NC.
Sharma, R., F. Fernandez, M. Hinton, and U. Schumacher. 1997. The influence of diet on the mucin carbohydrates in the chick intestinal tract. Cell. Mol. Life Sci. 53:935–942.
Sharma, R., and U. Schumacher. 1995. Morphometric analysis of intestinal mucins under different dietary conditions and gut flora in rats. Dig. Dis. Sci. 40:2532–2539.
Shea-Donohue, T., E. D. Dorval, E. Montcalm, H. El-Bayer, A. Durakovich, J. J. Conklin, and A. Dubois. 1985. Alterations in gastric mucus secretion in rhesus monkeys after exposure to ionizing radiation. Gastroenterology 88:685–690.
Sheahan, D. G., and H. R. Jervis. 1976. Comparative histochemistry of gastrointestinal mucosubstances. Am. J. Anat. 146:103–131.
Sherman, P., J. F. Forstner, N. Roomi, I. Kharti, and G. G. Forstner. 1985. Mucin depletion in the intestine of malnourished rats. Am. J. Physiol. 248:G418–G423.
Shub, M. D., K. Y. Pang, D. Swann, and W. A. Walker. 1983. Age related changes in chemical composition and physical properties of mucus glycoprotein from rat small intestine. Biochem. J. 215:405–411.
Szentkuti, L., H. Riedesel, M. L. Enss, K. Gartner, and W. von Engelhardt. 1990. Pre-epithelial mucus layer in the colon of conventional and germ-free rats. Histochem. J. 22:491–497.
Turck, D., A. S. Feste, and C. H. Lifschitz. 1993. Age and diet affect the composition of porcine colonic mucins. Pediatr. Res. 33:564–567.
Uni, Z., A. Geyra, H. Ben-Hur, and D. Sklan. 2000. Small intestinal development in the young chick: crypt formation an enterocyte proliferation and migration. Br. Poult. Sci. 39:544–551.
Uni, Z., R. Platin, and D. Sklan. 1998. Cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the villus. J. Comp. Physiol. 168:241–247.
Wattel, W., G. A. Van Huis, M. F. Kramer, and J. J. Geuze. 1979. Glycoprotein synthesis in the mucous cell of the vasculary perfused rat stomach. Am. J. Anat. 156:313–320.