Mucin Gene Expression and Mucin Content in the Chicken Intestinal Goblet Cells Are Affected by In Ovo Feeding of Carbohydrates

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ABSTRACT The protective mucus layer covers the entire surface of the gastrointestinal tract. The mucus layer also acts as a medium for molecule transport between the luminal contents and the enterocytes; therefore it has a major role in nutrient absorption. The main mucus layer component, mucin glycoproteins, is produced by mucous-secreting goblet cells. In chicken small intestine, functional development of goblet cells and enterocytes occurs in the late embryonic and immediate posthatch period. Presence of the nutrient is crucial for mucosal development. Feed deprivation immediately after hatch caused delayed mucosa development and perturbed mucin dynamics. Recent studies showed the intraamnionic nutrient supply (in-ovo feeding; IOF) accelerated mucosa functional development. In this study, the effect of IOF on the mucin mRNA expression and mucin content in the goblet cells was studied. The feeding solution containing carbohydrates was administered to the amnionic fluid of the Cobb embryos at d 17.5 of incubation. Samples from the jejunum were taken at d 17 of incubation (before IOF), and then 10 embryos from each group were sampled at 19 d of incubation, at hatch, and at d 3 posthatch. Following IOF, villus surface area increased at day of hatch and 3 d posthatch by 27 and 21%, respectively. In addition, the proportion of goblet cells containing acidic mucin increased 36 h after injection by 50% compared with the controls. The mucin mRNA expression increased gradually from d 17 of incubation to 3 d posthatch. Enhanced expression of the mucin mRNA was found at the day of hatch in chicks that received carbohydrate solution into the amnionic fluid in comparison with the control group. The results showed that providing the carbohydrates as an energy source to the late-term embryo had a trophic effect on the small intestine and enhanced goblet cell development.

Key words: in ovo feeding, mucin, chicken, small intestine

INTRODUCTION

The entire surface of the chicken gastrointestinal tract is covered by a layer of mucus that functions as a diffusive barrier between the intestinal lumen and absorptive cells. The mucins are the main component of the mucus layer, which produces and secretes by goblet cells. The mucus layer is part of the innate host response, protecting against luminal microflora, preventing gastrointestinal pathologies, and participating in the processes of nutrient digestion and absorption (Forstner et al., 1995).

The mucosa functional development in the chicken small intestine was studied recently. In the chicken, 2 d prior to hatch the small intestine has villus structure and perturbed mucin dynamics. Recent studies showed the intraamnionic nutrient supply (in-ovo feeding; IOF) accelerated mucosa functional development. In this study, the effect of IOF on the mucin mRNA expression and mucin content in the goblet cells was studied. The feeding solution containing carbohydrates continues after hatch. During the first week posthatch, the chicken small intestine grows rapidly, villus height and crypt depth increase, and the ability to digest and absorb carbohydrates also increases (Sell et al., 1991; Uni et al., 1995; Uni et al., 1998; Uni et al., 1999; Sklan, 2001).

Development of the small intestinal mucus-secreting cells in chicks occurs in the late embryonic and immediate posthatch period. Mucin-producing cells were observed in the small intestine from 17 d of incubation and at this time contained only acidic mucin. After hatch and until d 7 posthatch, the small intestine contained similar proportions of goblet cells producing acidic and neutral mucins. An increased goblet cell density was observed along the duodenal to ileal axis (Uni et al., 2003a).

The presence of the nutrient in the gastrointestinal tract is essential for enteric development. Feed deprivation for the first 48 h after hatch resulted in delayed development of the mucosal layer and perturbed processes of mucin synthesis and secretion in the small intestine of young chicks (Uni et al., 2003a). In contrast, intraamnionic nutrient administration (in-ovo feeding; IOF) accelerated small intestine development and had an enhanced effect on
the function of enterocytes (Tako et al., 2004; Uni and Ferket, 2004).

The purpose of this study was to determine the effect of IOF on the mucin content, goblet cell development, and levels of mucin mRNA in the chicken small intestine at the pre- and posthatch periods.

MATERIALS AND METHODS

Birds and Experimental Design

Viable Cobb chicken eggs were obtained from a commercial hatchery (Brawn Hatchery, Hod Hasharon, Israel) from the 32-wk-of-age breeding flock. Eggs were incubated according to the standard hatchery practice at the hatchery facility of our institute. Upon hatching the chicks were maintained at the Faculty of Agriculture growing facilities. Each floor pen was bedded with soft pine wood shavings. Chicks were given ad libitum access to a typical chicken starter diet (Matmor Feed Mill D.N., Israel) that met or exceeded NRC recommendations (1994). All procedures were approved by the Animal Care and Welfare Committee of our Institute.

In Ovo Feeding Procedure

The eggs were incubated under optimal conditions. At 17.5 d of incubation (17.5E), 200 eggs containing viable embryos were weighed and divided into 2 groups with equal weight frequency distribution of 100 eggs each with average egg weight of 58 ± 1.1 g. The IOF procedure was performed as described by Tako et al. (2004). Each group was injected with suitable in-ovo feed treatment solutions (1 mL/egg) with a 21-gauge needle inserted into the amniotic fluid, which was identified by candling. The 2 treatment groups included the following: 1) IOF solution: 15 g of maltose/L, 15 g of sucrose/L, 150 g of dextrin/L, and 5 g of NaCl/L, and 2) control solution: 75 g of NaCl/L. After the eggs were injected, the injection holes were sealed with cellophane tape, and eggs were placed in hatching trays such that each treatment was equally represented in each location of the incubator. The hatchability of fertile eggs of IOF and control groups was not significantly different.

Sample Collection

Ten eggs were randomly sampled at d 17 of incubation (17E; before IOF), and then 10 eggs from each group were sampled at 19 d of incubation (19E), at hatch, and at 3 d posthatch. Intestine was removed and gently flushed with 150 mmol of NaCl/L to remove the intestinal contents. Samples of jejunum (~2 cm) were taken at the midpoint between the point of entry of the bile duct and Meckel’s diverticulum.

Total RNA Isolation

Tissue samples from the jejunum for total mRNA extraction were frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated (Chomczynski and Sacchi, 1987) from the intestinal segments using TRI reagent (10 mL/g of tissue) according to the manufacturer’s protocol (MRC Molecular Research Center, Cincinnati, OH).

Mucin mRNA Analysis

The RT-PCR was carried out with primers from chicken similar to Mucin 2 precursor (Intestinal mucin 2; Gallus gallus, XM_421035; forward: 5’-GCTGATTGTCACTCAGCCTT-3’; reverse: 5’-ATCTGCTTGAATCAGAGTGCTCG-3’) and with primers from the Gallus gallus 18S ribosomal RNA gene with (GI 7262899; forward: 5’-CGATGCTTTGAACTGAGTGCT-3’, reverse: 5’-CAGCTTGGACCATTCATCCTC-3’). Determination of the linear phase of the amplification RT-PCR was performed with Access RT-PCR System (Promega Corporation, Madison, WI), and pooled total RNA aliquots were removed at 10, 15, 20, 25, 30, 35, 40, and 45 cycles. Amplification of the chicken intestinal mucin gene was performed for 29 cycles, which consisted of denaturation (95°C, 30 s), annealing (54°C, 1 min), and extension (68°C, 1 min), and 18S was amplified at 25 cycles under the same conditions in a different tube. The 18S (426 bp) and chicken intestinal mucin (441 bp) PCR products were separated by electrophoresis on 1.5%
Figure 2. Effect of in-ovo feeding (IOF) on periodic acid-Schiff (PAS)-positive (A) and Alcian blue- (AB)-positive (B) goblet cell density in the chicken jejunum. Values are means ± SEM, n = 10. **Means without a common letter are significantly different (P < 0.05). 17E = embryonic age, d 17 of incubation; 17.5E = embryonic age, d 17.5 of incubation; 19E = embryonic age, d 19 of incubation.

agarose gel, stained with ethidium bromide, and quantified using Gel-Pro Analyzertm version 3.0 (Media Cybernetics, LP, Silver Spring, MD). The relative amount of mucin mRNA was determined by normalizing density of its PCR products to the density of the 18S PCR product by densitometric scanning, and results are presented as arbitrary units as previously described (Smirnov et al., 2004).

Morphological Examination

Intestinal segments were fixed in 4% (vol:vol) buffered formaldehyde, dehydrated, cleared, and embedded in paraffin. Four serial sections were cut at 3 μm, deparaffinized in xylene, rehydrated, and stained with hematoxylin and eosin. Sections were examined by light microscopy. The data from the examination of serial sections were pooled from a mean for each bird.

Mucin Staining

Determination of neutral mucin was done via staining sections with periodic acid-Schiff reagent (PAS; American Forces Institute of Pathology, 1992; McManus, 1948). Slides after deparaffinization and rehydration were incubated in 5 g/L of periodic acid for 15 min, then washed and incubated with Schiff’s reagent (Sigma Chemicals Co., St. Louis, MO) for 30 min. After washing in warm water, slides were dehydrated and mounted. The number of PAS-positive cells along the villi was determined by light microscopy. Acid mucin was determined by staining sections with Alcian Blue pH 2.5 (American Forces Institute of Pathology, 1992; Lev and Spicer, 1964). Slides after deparaffinization and rehydration were incubated in 0.5 mol/L of acetic acid for 3 min and then in Alcian Blue solution (10 g/L in 0.5 mol/L of acetic acid, pH 2.5). After washing in water, slides were dehydrated and mounted. The number of Alcian Blue-positive cells along the villi was counted by light microscopy.

Morphometric Measurements

Density of goblet cells was calculated as the number of goblet cells per unit of surface area (mm²). All measurements were performed with an Olympus light microscope using EPIX XCAP software (Epix Inc., Buffalo Grove, IL).

Statistical Analysis

Data were analyzed by 2-way ANOVA in which the main effects were intestinal segment and treatment (control or IOF) using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Differences between means were tested using Tukey’s test (Tukey, 1953). Differences were considered significant at P < 0.05 unless otherwise stated.

RESULTS AND DISCUSSION

The novel approach of IOF (Uni and Ferlet, 2003) was used to determine the effect of in ovo nutrient administration on goblet cell development and mucin mRNA expression. Amnionic fluid is swallowed by the chicken embryo during the last period of incubation (Romanoff, 1960); therefore intraamnionic injection of nutrients enables the estimation of the effects of early nutrient ingestion at an early stage of intestinal development.

The chicken embryo has limited carbohydrate resources because of the low glucose concentration in the
amniotic fluid and the yolk sack (Noble and Ogunyemi, 1989; ten Busch et al., 1997; Noy and Sklan, 1998). Similar to the previous study of Tako et al. (2004), providing the carbohydrates as an energy source to the late-term embryo led to a 3.2% increase of BW of in-ovo-fed hatching compared with the controls (47.1 ± 0.45 g vs. 45.6 ± 0.55 g and 61.7 ± 0.8 g vs. 65.1 ± 0.9 g, respectively). In addition, IOF had a trophic effect on the jejunum, caused a villus surface area increase of 27% on day of hatch and 21% on d 3 posthatch relative to controls (Figure 1). One of the hypotheses of this trophic effect of IOF on intestine is that ingested carbohydrates caused high levels of blood insulin. In addition to the anabolic effect of insulin, recent studies have found that it is a potent stimulator of intestinal epithelial proliferation (Chao and Donovan, 1996). Furthermore, Eizaguirre et al. (2000) demonstrated increased jejunal and colonic mucosal thickness and proliferative activity in rats treated with insulin in animals receiving only parenteral nutrition.

Mucin consists of a peptide backbone with attached polysaccharide chains. The glycosylated region comprises 70 to 80% of the polymer (Gendler and Spicer, 1995). According to the types of the polysaccharide chain, mucins are classified into neutral and acidic subtypes and are also distinguished by sulfated or nonsulfated groups (Neutra and Forstner, 1987). The IOF of carbohydrates led to an increased proportion of goblet cells containing acidic mucin compared with controls, whereas the proportion of the goblet cells containing neutral mucin did not change. On d 19 of incubation, 36 h after injection, the density of goblet cells containing acidic mucins (Alcian Blue-positive cells) was 50% greater in the in-ovo-fed group than in the controls (4558 ± 360 cells/mm² and 3027 ± 380 cells/mm², respectively; Figure 2). Glycosylation is regulated by bioavailability of the donor substrates for glycosyltransferases, the transport of glycoproteins, and their degradation. An experiment with radiolabeled glucose-H3 showed that the goblet cells can utilize glucose for conversion to nucleotide-sugars followed by incorporation into the carbohydrate portion of the mucin molecules (Neutra and Leblond, 1966). Therefore, the presence of available glucose in the intestinal lumen of the chick embryo might be a trigger for enhancement in the proportion of acidic mucins in the goblet cells. In addition, the increased insulin levels in response to carbohydrate feeding may trigger modifications in glycoprotein glycosylation (Biol-N’garagba and Louisot, 2003).

In addition to changes in mucin content, enhanced expression of mucin mRNA was found on day of hatch in chicks that received carbohydrate solution into the amniotic fluid. The expression of mucin mRNA increased gradually from d 17 of incubation to 3 d posthatch (Figure 3). The in-ovo-fed chicks exhibited a higher level of mucin mRNA expression on day of hatch compared with the controls (Figure 3). Environmental influences such as diet play an important role in gut differentiation and function (Pacha, 2000). The presence of food in the alimentary canal is important to normal mucosal function and triggers signal-transduction pathways, in particular the protein kinase C (PKC)-signaling pathway, which might be involved in the mechanism of mucin gene upregulation following IOF. In in-vitro studies, a high level of glucose-induced activation of the PKC-signaling pathway was found in various organ cell lines (Knott et al., 1998; Park et al., 2001; Cosentino et al., 2003). In the intestine, glucose transport through SGLT1 activates a PKC-dependent pathway in the jejunal loop in vitro (Kellett, 2001). The PKC has been found to mediate increases in MUC2 and MUC5AC expression in cultured colonocytes (Hong et al., 1999). It is possible that enhanced expression of the mucin intestinal gene in IOF embryos is due to the activation of PKC-dependent pathways by glucose.

The mechanisms by which dietary components influence mucin dynamics in developing chicken intestine are poorly understood. Changes in the mucin type and mucin gene expression in goblet cells of the chicken small intestine may be due to the direct and indirect effects of in-ovo-administered carbohydrates.
REFERENCES


