INTRODUCTION

The interaction between feed availability and intestinal growth and development is critical during the first few days posthatch as hatchlings transition from in ovo to enteral nutrition (Uni et al., 1999). In the commercial poultry industry, hatchlings commonly do not have access to feed or water for 24 to 48 h posthatch because of variability in hatching time and transportation distance. This inadvertent delay in feeding may have detrimental effects on early growth, immunity, and intestinal physiology in chicks (Uni et al., 2003; Smirnov et al., 2005). It was reported that chicks that had immediate access to feed at hatch had increased BW gain through 3 wk of age compared with chicks with no access to feed and water for 36 h posthatch (Moran, 1990; Noy and Sklan, 1998). However, chicks with delayed access to feed and water had decreased BW only when the BW was compared on an age basis but not at similar days postfeeding (Geyra et al., 2001).

In poultry, through approximately 4 to 5 d posthatch, the gastrointestinal tract develops at a much faster rate than any other organ (Noy and Sklan, 1997). Delayed access to feed and water may stunt villus and crypt development as well as duodenal length, and it may reduce digestive enzyme secretion and impair mucin secretion (Gonzales et al., 2003). In chicks, delaying access to feed immediately posthatch had increased BW gain through 3 wk of age compared with chicks with no access to feed and water for 36 h posthatch (Moran, 1990; Noy and Sklan, 1998). However, chicks with delayed access to feed and water had decreased BW only when the BW was compared on an age basis but not at similar days postfeeding (Geyra et al., 2001).

In conclusion, delaying access of ducklings with no experimental pathogen infection to feed and water has no long-term effects on early growth parameters, intestinal physiology, and immune responses.

Key words: fasting, posthatch, intestine, CD25+

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The functions and characteristics of avian CD4+CD25+ cells are similar to those of mammalian T regulatory cells (Treg; Shanmugasundaram and Selvaraj, 2011), which are a subset of T cells specializing in immune suppression. In mammals, mucosal tolerance is maintained by preferential enrichment of Treg on the mucosal surface, and Treg are essential for maintaining mucosal tolerance (Izcue and Powrie, 2008). Approximately 15% of CD4+ cells in chicken cecal tonsils are CD25+ (Shanmugasundaram and Selvaraj, 2011), suggesting that these cells are important components in mucosal tolerance. Chicken CD4+CD25+ cells function by producing high levels of IL-10, an anti-inflammatory cytokine.

It has been reported that immediate or delayed access to feed can significantly influence intestinal physiology and growth parameters in chickens (Uni et al., 2003; Smirnov et al., 2006); however, similar studies with other domestic poultry species have not been reported. The objective of this study, therefore, was to study intestinal mucin gene expression, intestinal growth parameters, and selected components of intestinal immune response in ducklings with immediate or delayed access to feed.

**MATERIALS AND METHODS**

Pekin ducklings were obtained on the day of hatch from Maple Leaf Farms (Milford, IN) and transported to the Ohio Agricultural Research and Development Center (OARDC; Wooster). All animal protocols were approved by the OARDC animal care and use committee.

Upon arrival at the OARDC Poultry Research Farm, 80 ducklings were weighed individually and randomly assigned to one of 2 treatments, with 10 replicate pens per treatment. Each pen had 4 ducklings. This was considered d 0, and differences in BW were not significant between treatment groups. In one treatment group (referred to as the fed group), feed and water were provided beginning on d 0, and in the other treatment group (referred to as the withheld group), feed and water were withheld until the morning of d 2. All ducklings were reared in heated Petersime battery brooders (Petersime Incubator Co., Gettysburg, OH) and fed a commercial duck starter diet provided by Maple Leaf Farms.

**Sampling for BW, Duodenum Weight and Length, and Mucin mRNA**

One duckling per pen was selected randomly from 7 pens in both treatment groups on d 2 and 6 posthatch. An extra sampling of withheld ducklings was done at 8 d posthatch so that comparisons could be made with fed ducklings at 6 d postfeeding. The duodenum was removed from each duckling and flushed with ice-cold PBS–saline. The duodenal loop including the pancreas was weighed and the length of the duodenum was recorded. A 3-cm section of the jejunum was removed from each duckling approximately 1 cm proximal to Meckel’s diverticulum and rinsed with ice-cold PBS–saline. After rinsing, the middle 1 cm of each jejunal section was immediately frozen in liquid nitrogen and stored at −80°C until mRNA analysis.

**Sampling for CD25+ Cell Analysis**

Cecal tonsils were collected from the same birds sampled for BW, duodenal weight and length, and mucin mRNA analysis. The cecal tonsils were collected from 3 ducklings in each treatment group on d 2, 6, and 8 posthatch for CD25+ cell analysis. Sampling time points were similar to those of the earlier sampling, except that at 8 d, samples from both fed and withheld ducklings were collected. Single cell suspensions of the cecal tonsils were concentrated for lymphocyte collection by density centrifugation over Histopaque (1.077 g/mL, Sigma Aldrich, St. Louis, MO). Production and phycoerythrin (PE) linking of mouse anti-chicken CD25+ were conducted as described by Shanmugasundaram and Selvaraj (2010). The cross-reactivity of anti-chicken CD25+ with duck CD25+ was verified by both Western blotting and measuring upregulation of CD25+ in duck splenic cells stimulated with concanavalin A (data not shown). Approximately 1 × 10^6 cells were incubated with 10 μg/mL of primary PE-linked mouse anti-chicken CD25+ for 30 min. Unbound primary antibodies were removed by centrifugation at 400 × g for 15 min at 30°C. The percentage of CD25+ cells in different organs was analyzed in a flow cytometer (Guava EasyCyte, Millipore, Billerica, MA) and expressed as percentage of CD4+ cells. The CD25+ population was purified using a magnetic bead sorting technique (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany; Shanmugasundaram and Selvaraj, 2010). Briefly, 1 × 10^7 cecal tonsil lymphocytes were concentrated by density centrifugation at 400 × g for 15 min at 30°C over Histopaque (1.077 g/mL) and incubated with 10 μg/mL of conjugated mouse anti-chicken CD25+. The CD25+ cells were purified using 1:100 anti-PE beads (Miltenyi Biotech) following the manufacturer’s instructions. The purity of CD25+ cells ranged from 65 to 75%, and they were stored at −80°C until mRNA analysis.

**mRNA Analysis**

Intestinal samples collected from the jejunum were analyzed for mucin 2 (MUC2) and mucin 5B (MUC5B) relative mRNA contents, and CD25+ cell samples were analyzed for relative IL-10 mRNA content by real-time reverse transcriptase PCR (Selvaraj and Klasing, 2006). Total RNA was isolated using Trizol reagent (Molecular Research Center, Cincinnati, OH) following the manufacturer’s directions. The mRNA were analyzed for MUC2 (5′-accaagcgaaagactggaa-3′and 5′-cttcagcccacccagcattata-3′), MUC5B (5′-acatgtcgcacgccata-3′).
and 5'-gctcagacaaacagttcc-3'), and IL-10 (5'-catgct- 
gctgctgctgctgtagtg-3'; Shan-
muugasundaram and Selvaraj, 2010) after normalizing 
for β-actin (5'-accggacctacacac-3' and 5'-gacctg-
gctgacacctta-3'). All samples were run in duplicate, 
and PCR products from each gene were visualized by 
gel electrophoresis on 1% agarose stained with ethidium 
brodime to ensure a single product was produced at the 
predicted size. Single-band PCR products for each gene 
were excised and cDNA was purified with a QIAquick 
Gel Extraction Kit (Qiagen, Valencia, CA) and subse-
sequently sequenced at the Molecular and Cellular Imag-
ing Center, OARDC. All PCR products had 95 to 99% 
homology to their respective gene or EST sequence 
standard nucleotide–nucleotide Basic Local Alignment 
Search Tool, National Center for Biotechnology Infor-
mation).

Data Analysis

A 2-way ANOVA (JMP software, Cary, NC) was used to 
examine the main effects of immediate and delayed 
access to feed and water on dependent variables. When 
the main effects were significant (P < 0.05), differences 
between means were analyzed by Student’s t-test least 
squares means comparison.

RESULTS

BW

No significant treatment differences in BW were 
found at 0 d (P > 0.05). The ducklings in the withheld 
treatment lost approximately 25% of their d 0 BW at 
2 d posthatch and weighed significantly less than the 
fed ducklings (Figure 1). Ducklings with delayed 
access to feed and water had significantly reduced BW 
at 6 d posthatch when compared with the fed duck-
lings. When calculated as a percentage of the d 2 BW, 
withheld ducklings gained 228% through 6 d posthatch 
compared with 176% in the fed birds. At 8 d posthatch, 
withheld ducklings had significantly greater absolute weight of 
the duodenum plus pancreas compared with fed ducklings (Table 
1). Withheld ducklings also had a reduced length and 
density (g/cm) of the duodenum. The same treatment 
effects were observed at 6 d posthatch with the excep-
tion of the relative weight of pancreas plus duodenum, 
which was higher in withheld ducklings. At 8 d post-
hatch, which corresponded to 6 d postfeeding, withheld 
ducklings had significantly greater absolute weight of 
the duodenum plus pancreas and an increase in both the 
length and density of the duodenum compared with 
fed ducklings.

Mucin mRNA Content

No significant treatment effects on jejunal MUC2 
mRNA were found (P > 0.05; data not shown). At 2 
d posthatch, withheld ducklings had reduced MUC5B 
mRNA compared with fed ducklings (P < 0.09; Fig-
ure 2). The treatment effects were reversed at 6 d 
posthatch; withheld ducklings had increased MUC5B 
mRNA content (P = 0.07). The differences (withheld 
> fed) were even greater when comparisons were made 
6 d postfeeding (P = 0.07).

CD25+ Cell Properties

Isolation and purity of CD25+ cells isolated from the 
cecal tonsils are shown in Figure 3. The percentage of 
CD25+ cells isolated from withheld ducklings at 2 
and 6 d posthatch and 6 d postfeeding (8 d posthatch) 
was significantly greater than in fed ducklings (Figure 
4). Treatment differences at the latter 2 times progres-
sively declined with onset of feed and water access in 
withheld ducklings. Treatment effects on IL-10 mRNA 
content of CD25+ cells were similar to those observed 
for the percentage CD25+ cell data. The level of IL-10 
mRNA was significantly higher in withheld ducklings 
at 2 and 6 d posthatch, and the treatment differences 
progressively decreased with onset of feed and water access in the withheld treatment (Figure 5). No treat-
ment ($P > 0.05$) differences were found in IL-10 mRNA content at 8 d posthatch (6 d postfeeding in withheld ducklings).

**DISCUSSION**

The intestine in domestic poultry species is immature at hatch and undergoes a rapid period of maturation during the first week posthatch (Uni et al., 1998; Applegate and Lilburn, 1999; King et al., 2000; Applegate et al., 2005). The different segments of the small intestine (duodenum, jejunum, ileum) do not all mature at similar rates and the weight of each segment increases more rapidly than the length (Uni et al., 1999). Noy and Sklan (1999) was among the first studies on early intestinal development to use defined periods of delayed access to feed and water to simulate what often occurs in commercial practice. Geyra et al. (2001) reported that withholding feed for 48 h immediately after hatch significantly reduced enterocyte proliferation within both the crypt and villus. Upon feeding, though, the percentage of proliferating cells rebounded and was actually greater in both the crypt and villus until 6 d posthatch, when it returned to the same levels observed in fed chicks. However, these authors and others who have used various lengths of feed withholding in posthatch studies have limited comparisons to similar ages (days posthatch). In commercial practice, d 1 is when the chick, poult, or duckling arrives at the rearing facility. In the data of Geyra et al. (2001), chicks with delayed feed access for 96 and 144 h posthatch (48 and 96 h postfeeding) had similar BW as fed chicks at 48 and 96 h posthatch. When compared at similar days postfeeding, turkey poults with 48-h delayed feeding had prolonged (up to 5 d postfeeding) reduction in enterocyte proliferation and proportionately greater apoptotic enterocytes (Potturi et al., 2005). The previous data suggest that the intestine is adaptable to its immediate nutritional environment and physiological status at hatch and the days immediately following hatch.

Duckling BW data in the current study are similar in scope to the data of Geyra et al. (2001). The withheld treatment significantly reduced BW at 2 and 6 d posthatch, but when treatments were compared at the same days postfeeding (6 and 8 d), BW was similar in both

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2 d posthatch</th>
<th>6 d posthatch</th>
<th>6 d postfeeding</th>
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<tbody>
<tr>
<td>Total weight (g)</td>
<td>1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
<tr>
<td>Weight (% of BW)</td>
<td>1.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>12.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47</td>
</tr>
<tr>
<td>Weight (g/cm of length)</td>
<td>0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means with no common superscripts within a row differ significantly ($P < 0.05$).

1Ducklings (1 d old) were provided either immediate access (fed) or 48-h delayed access (feed withheld) to feed and water, and samples were collected at indicated times ($n = 7$). Data from 2 d posthatch, 6 d posthatch, and 6 d postfeeding were statistically analyzed separately.
treatments. The duodenum plus pancreas data showed a somewhat different pattern. Whereas the withheld treatment reduced the duodenum plus pancreas weight and duodenal length and density (g/cm) at 2 and 6 d posthatch, all measures with the exception of density increased in withheld ducklings at 6 d postfeeding. This would suggest that the actual process of feed intake and digestion has the greatest influence on early intestinal development and that the early period of feed withholding does not have a prolonged negative effect.

Mucin 2 is the predominant mucin found in the small intestine and both MUC2 and MUC5B are gel-forming, secretory mucins. Mucin 5B is more commonly found in the salivary gland, respiratory tract, and cervix (Van Klinken et al., 1995). The decrease in MUC5B expression at 2 d posthatch in withheld birds could be a reflection of it being a relatively minor mucin and therefore more sensitive to the nutritional environment of the bird. Both fed and withheld ducklings had an age-related increase in MUC5B expression, and the expression at 6 d postfeeding was almost twice as high in the withheld ducklings compared with the fed ducklings. Ducklings in both treatments had similar mucin expression at 6 d posthatch, so it would appear that the transient effects observed at 2 d quickly disappeared once food and water were available. Certainly no long-term adverse effects on intestinal development were observed once the ducklings had access to feed and water.

CD25+ is a T cell activation marker in chickens. Conventional T cells transiently upregulate CD25+ in the periphery during inflammation in chickens (Hála et al., 1986; Kim et al., 2000; Teng et al., 2006). CD25+ is also a Treg-specific marker. A hallmark characteristic of chicken Treg is their ability to suppress an immune response through IL-10 (Shanmugasundaram and Selvaram, 2011). No significant changes occurred in CD25 cell properties in the fed group at 0, 2, and 8 d posthatch; therefore, the data were analyzed to compare changes within a day. Although both non-Treg-upregulating CD25+ and increased Treg migration to cecal tonsils can contribute to the overall CD25+ population, the IL-10 profile suggests that increased Treg migration contributed to the observed increase in CD25+ population in the cecal tonsils. From a nutritional standpoint, energy, amino acids, and several substrates are required to support an immune response (Klasing, 1998). In a nutritionally challenged environment, the host could limit the acute phase response (an energy intensive process) by producing an antiinflammatory type of response (Monk and Woodward, 2009). Feeding the withheld birds decreased IL-10 to a basal level, suggesting that long-term changes in immune responses occurred as a result of delayed access to feed and water.

In conclusion, delayed access to feed and water had no long-term effects on duckling BW, selected aspects of intestinal development, or immune responses, at least through 6 d postfeeding. A future logical step would be to study how the birds respond to a pathogen challenge following delayed access to feed and water.

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**REFERENCES**


Applegate, T. J., and M. S. Lilburn. 1999. Effect of turkey (Meleagris gallopavo) breeder hen age and egg size on poult development.