Effect of incubation temperature on nutrient transporters and small intestine morphology of broiler chickens

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ABSTRACT This study evaluated the effects of elevated incubation temperature on posthatch nutrient transporter gene expression, integrity of the intestinal epithelium, organ development, and performance in Ross 308 broiler chickens. Ross × Ross 308 fertile eggs (n = 900) were incubated at different eggshell temperatures during development. From embryonic day (ED) 1 to ED12, all eggs were incubated at 37.1°C, whereas from ED13 to ED21, the eggs were divided into 2 groups for incubation at 37.4°C (S) or 39.6°C (H). Performance characteristics were measured at day of hatch (DOH) and d 7, 14, 21, 30, and 42. Small intestine and residual yolk sacs were collected at DOH and d 2, 4, 6, and 10 and weighed individually. Intestinal samples from the duodenum, jejunum, and ileum were evaluated for mucosal morphology and relative nutrient transporter gene expression. No significant differences were found in performance or organ weights. The intestinal morphology results showed a temperature × age interaction in duodenum villus height ($P = 0.02$) and crypt depth ($P = 0.05$) and in ileum villus height-to-crypt depth ratios ($P = 0.02$). There was a main effect of temperature, resulting in deeper crypts ($P = 0.02$) in the jejunum of chicks incubated at H compared with S. In the nutrient gene expression evaluation, peptide transporter (PepT1) showed a temperature × age interaction. On DOH and d 2, 4, and 10, PepT1 expression was similar between chicks incubated at S and H. However, on d 6, chicks incubated at S had significantly higher expression of PepT1 than those incubated at H. This study presents the effects of elevated incubation temperature on small intestine morphology and relative expression of nutrient transporter mRNA in high-yield broiler chicks, which can be important for the availability of nutrients and distribution of energy.

Key words: incubation, broiler, morphology, nutrient transporter

INTRODUCTION

As the commercial poultry industry changed from slower growing to high-yield broilers, the hatchability of fertile eggs began to decline and changes in chick quality were observed (Hulet, 2007). Currently, chickens spend 30 to 40% of their total life span inside the egg (Hulet et al., 2007). Thus, anything that compromises or promotes growth and development during this embryonic period can have a marked effect on overall performance and health posthatch. The increased growth rate of embryos contributes to increased metabolic heat production and output from the eggs, which accumulates within the incubator. The heat production of embryos of modern high-yield chicken strains is 44% higher than that reported for embryos in the 1930s (Hulet et al., 2007). Consequently, even though the incubator is set for a temperature of 37.4°C, if there is inadequate ventilation to dispose of excess heat generated by the embryos, the incubators may reach temperatures of 41.1 to 41.7°C, particularly in the latter half of incubation (Hulet, 2007). As a result, there is commonly decreased hatchability, impaired embryonic development, and poor posthatch performance (French, 2000; Hulet et al., 2007). Several studies have evaluated the effect of elevated incubation temperatures on characteristics such as hatchability, yolk-free BW, chick length, feed intake, and feed conversion (FC; Lourens et al., 2005; Hulet et al., 2007; Leksrisompong et al., 2007). Although the majority of studies indicate reduced performance characteristics caused by elevated incubation temperatures, data reported from other studies also indicate improved growth and performance (Ricklefs, 1987; Christensen et al., 1999) or no performance changes (Leksrisompong et al., 2007). Other measurements from trials evaluating incubation temperature have demonstrated differences in heart and liver weights and carcass and meat yields.
Effect of incubation temperature on nutrient absorption and posthatch performance. (Joseph et al., 2006; Lourens et al., 2007). Interestingly, Lekrisompon et al. (2007) also observed that chicks incubated at elevated temperatures present white down feathers instead of the normal yellow, which the authors suggested could be due to decreased absorption of yolk sac contents. This suggests that the elevated temperatures might have a direct effect on gastrointestinal development to negatively influence absorption, digestive physiology, or nutrient partitioning of the small intestine. The few physiological measurements evaluated in chicks incubated at elevated temperatures have been focused on the cardiovascular system by measuring heart weight, the endocrine system by measuring the levels of triiodothyronine and thyroxine in blood, and the digestive system by measuring maltase and alkaline phosphatase activities in the small intestine (Christensen et al., 2004a,b, 2005). Studies evaluating the effect of elevated temperature during incubation on the development of the gastrointestinal tract, more specifically, small intestinal morphology and nutrient transporter gene expression, are lacking.

Newly hatched broiler chicks have an immature digestive system. The physiological development and maturation (digestive and absorptive function) of the digestive tract occurs through changes in nutrient transporters (Obst and Diamond, 1992) and through remarkable morphological changes, such as increases in villus height and crypt depth (Geyra et al., 2001). The development and functional maturation of enterocytes is accomplished by the presence of feed in the intestinal lumen and the efficient assimilation of yolk nutrients. During the first 10 d of the life of the broiler chicken, the competence of the intestine for nutrient utilization increases to its maximum (Iji et al., 2001). During this time, absorption of the yolk sac contents enhances the development of villus height and enterocyte maturity (Iji et al., 2001), which favor further performance characteristics, given that the growth of the chicken depends on the availability of nutrients. The hypothesis of this research was that elevated incubation temperature (higher than 37.5°C) would impair chick quality and absorption of nutrients from the yolk sac, which in turn would have a detrimental effect on gastrointestinal morphology and physiology and subsequently affect nutrient transporter expression and absorption of nutrients from the diet. This hypothesis was based on reports that nutrient availability and efficient utilization during the first few days of life are favored by yolk nutrient absorption and affect the performance of chickens (Geyra et al., 2001). A deficiency in nutrient availability during this time would depress mucosal development, impede nutrient transporter expression on the brush border membrane of the intestinal epithelium, and thus compromise the availability and distribution of energy and resources for growth and optimal performance of the broiler chicken. The goal of this study was to evaluate the effects of elevated embryonic incubation temperature on development of the small intestine, as measured through morphology, expression of nutrient transporters, and posthatch performance.

MATERIALS AND METHODS

Bird Welfare and Incubation Design

This project was approved and conducted under the guidelines of the Institutional Animal Care and Use Committee at Virginia Tech.

A total of 900 fertile eggs were obtained from a 31-wk-old Ross × Ross 308 broiler breeder flock. Eggs were incubated in single-stage Buckeye Chick Master incubators (Chick Master, Medina, OH) at Penn State University. Treatments were designated as a high temperature (H) of 39.6°C as compared with the conventional standard (S) temperature of 37.4°C. Temperatures were measured on the eggs shells. From embryonic day (ED) 1 to ED12, all the eggs were incubated at 37.1°C. From ED13 to ED21, half of the eggs were incubated at the S temperature of 37.4°C and the other half were incubated at the H temperature of 39.6°C. These temperatures and times of application were selected to simulate the increase in embryonic temperature from the midpoint of incubation and onward that have been reported (Hulet et al., 2007). Relative humidity was monitored and maintained at 55%. During incubation, data on shell temperature, incubator temperature, and incubator humidity were logged on a daily basis. For each treatment, the percentages of hatch of fertile eggs and early, mid, and late incubation dead embryos were recorded. Chicks from both temperature treatments were pulled from the incubators after a total 12-h hatch window.

Broilers and Diets

At hatch, chicks were transported (6 h) to Virginia Tech in a cargo van, with chicks from each incubation treatment equally distributed throughout the van and chick box stacks. After the arrival of chicks at Virginia Tech, 46 chicks/treatment were assigned to floor pens [0.76 ft²/chick (0.07 m²/chick)] with clean pine shavings, nipple drinkers, and hanging feeders in a negative-pressure ventilated house. Each treatment was replicated by 7 pens for the 42-d trial. All birds were provided with feed and water ad libitum throughout the grow-out period. The diets were corn-soybean meal based and formulated to meet or exceed the NRC (1994) requirements. The dietary phases consisted of a starter (d 0 to 14), grower (d 14 to 30), and finisher (d 30 to 42) phase.

Performance Measurements

Chicks were weighed by pen at day of hatch (DOH) and d 7, 14, 21, 30, and 42. Feed intake, BW gain, and mortality-adjusted FC were calculated for each period.
and cumulatively for the grow-out. Mortality was recorded daily.

**Tissue Collection and RNA Extraction**

At DOH (before placement in floor pens at Virginia Tech) and d 2, 4, 6, and 10, four birds per treatment were randomly selected for evaluation of nutrient transporter gene expression in the small intestine. Each bird for sampling was weighed and killed by cervical dislocation. Tissue samples were immediately collected from duodenum (ascendant loop), jejunum (from the pancreatic duct to Meckel’s diverticulum), and ileum (from Meckel’s diverticulum to the ileocecal junction). The intestinal segments were rinsed in ice-cold PBS, minced with razor blades, and thoroughly homogenized. Duplicate samples of 20 to 30 mg of minced tissue were collected in 2-mL microcentrifuge tubes and snap-frozen in liquid N. Samples were stored at −80°C until further analysis. Total RNA was extracted from the intestinal tissue samples using an RNeasy Miniprep Kit (Qiagen, Darlington Laboratory, Valencia, CA) according to the animal tissue extraction protocol provided in the kit. Concentration and purity were determined using the 260/280 and 260/230 ratios obtained from a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The quality of RNA was assessed by visualization of distinct 28 Svedberg sedimentation coefficient and 18S ribosomal RNA bands after gel electrophoresis with ethidium bromide staining.

**Primer Design and Validation**

Specific oligonucleotide primer pairs were designed by Primer Express software (version 3, Applied Biosystems, Foster City, CA) and synthesized according to already published chicken sequences (Gilbert et al., 2007). Three sugar transporters, sodium glucose transporter 1 (SGLT1), glucose transporter 2 (GLUT2), and glucose transporter 5 (GLUT5), the excitatory amino acid transporter 3 (EAAT3), and the peptide transporter (PepT1) were evaluated. Table 1 shows the primer sequences used.

**cDNA Synthesis and Real-Time PCR**

Total RNA was diluted to 0.2 μg/μL in diethylpyrocarbonate-treated water. Reverse transcription into cDNA was performed using a High Capacity cDNA Archive Kit (Applied Biosystems) according to the manufacturer’s instructions included in the kit. The cDNA was diluted 1:30 and stored at −20°C until further analysis.

Relative quantification with real-time PCR was done for 4 samples/treatment with an ABI Prism 7300 Real-Time PCR System (Applied Biosystems). All reactions consisted of 2 μL (10 ng/μL) of reverse-transcribed RNA, 12.5 μL of SYBR Green PCR Master Mix (Applied Biosystems), 1.5 μL (5 μM concentration) of forward and reverse primers, and 8.5 μL of diethylpyrocarbonate-treated water. All samples were run in 96-well plates (Applied Biosystems) in duplicate. Polymerase chain reaction was performed under the following conditions: 50°C for 2 min (1 cycle), 95°C for 10 min (1 cycle), 95°C for 15 s, and 60°C for 1 min (40 cycles). After each run, a dissociation curve was obtained to verify that only 1 product was being amplified. The dissociation curve had the following conditions: 95°C for 15 s, 60°C for 30 min, and 95°C for 15 min. The cycle threshold (Ct) values for each gene were averaged. The

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank ID no.</th>
<th>Description</th>
<th>Sequence$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>NM_204305</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>F: 5’-GCCGTCCTCTCTGCAAAG-3’</td>
</tr>
<tr>
<td>SGLT1</td>
<td>XM_415247</td>
<td>Solute carrier family 5 (Na$^+$-glucose cotransporter) member 1</td>
<td>R: 5’-TGTAACCATGTAGTTCA-3’</td>
</tr>
<tr>
<td>SLC2A5 (GLUT5)</td>
<td>XM_417596</td>
<td>Solute carrier family 2 (facilitated fructose transporter) member 5</td>
<td>F: 5’-GCCATGGCCAGGGCCTTA-3’</td>
</tr>
<tr>
<td>SLC2A2 (GLUT2)</td>
<td>Z22932</td>
<td>Solute carrier family 2 (facilitated glucose and fructose transporter) member 2</td>
<td>R: 5’-GGAGGTGGAGGGAGAAAGC-3’</td>
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<tr>
<td>PepT1</td>
<td>NM_204365</td>
<td>Peptide transporter</td>
<td>F: 5’-CACACTATGGGCGCATGCT-3’</td>
</tr>
<tr>
<td>SLC1A1 (EAAT3)</td>
<td>XM_424930</td>
<td>Solute carrier family 1 member 1 (excitatory amino acid transporter 3 glutamate and aspartate transporter)</td>
<td>R: 5’-AGCATGACTGTAGTGCAAGAAGTATATAT-3’</td>
</tr>
</tbody>
</table>

$^1$Primers designed by Primer Express software (Applied Biosystems, Foster City, CA).

$^2$F = forward; R = reverse.
differences between the average Ct values of the target and reference gene (glyceraldehyde-3-phosphate dehydrogenase) were also calculated ($\Delta Ct$). The $2^{-\Delta\Delta Ct}$ values were calculated using the $\Delta\Delta Ct$ method, with the reference gene as the endogenous control and the average $\Delta Ct$ value for duodenum from the S treatment as the calibrator to express fold changes in gene expression (Livak and Schmittgen, 2001).

**Small Intestine and Yolk Sac Collection**

Twenty birds were randomly selected at DOH and d 2, 4, 6, and 10 to determine BW, yolk sac weight, and small intestine weight. Each bird was weighed and killed by cervical dislocation. The small intestine was collected, digesta were eliminated by gently squeezing the walls of the intestine, the pancreas was excised from the duodenal loop, and the small intestine was weighed with and without the yolk sac. Measurements of the small intestine and yolk sac were calculated as percentage of BW.

**Morphological Measurements**

Ten birds per treatment were randomly selected at DOH and d 2, 4, 6, and 10 for intestinal morphology evaluation. Each bird was weighed and killed by cervical dislocation. Tissue samples (2 cm in length every 4 cm) were collected from duodenum (midsection of the ascendant loop), jejunum (midpoint from the pancreatic duct to Meckel’s diverticulum), and the ileum (midpoint from Meckel’s diverticulum to the ileocecal junction). The intestinal segments were flushed with cold PBS and fixed in 10% neutral buffered formalin. Each tissue was cut into 5 sections (10 mm) and placed into a tissue cassette. The tissues were processed by dehydration through a series of graded alcohols, cleared with xylene, and embedded in paraffin. Paraffin sections (5-μm thickness) were mounted onto slides. The slides were stained using routine procedures for Mayer’s hematoxylin and eosin (Luna, 1968). Measurements of the development of the small intestine were made using SigmaScan Pro 5 software (Olympus America Inc., Melville, NY). Measurement of 3 of the 5 pieces of each intestinal section for each bird included the villus length (villus tip to crypt opening) and the crypt depth (crypt opening to the base of the crypt right before the lamina propria). Villus length-to-crypt depth ratios were also calculated. Four villi and 4 crypts were evaluated for each of the 3 intestinal pieces. The average villus length, crypt depth, and villus length-to-crypt depth ratio per histological slide were analyzed ($n = 12$ measurements/bird, 10 birds/treatment).

**Statistical Analysis**

Performance data were subjected to ANOVA using the MIXED models procedure for a completely randomized design with the SAS program, version 9.1 (SAS Institute Inc., Cary, NC). Small intestine morphology, intestine and yolk weights, and relative gene expression data were subjected to ANOVA using the GLM procedure for a completely randomized design of the SAS program. All values are expressed as least squares means ± SEM. For nutrient transporter expression, because the duodenum from the S treatment was used as the calibrator, the graphs showing the combined values of the 3 intestinal segments do not show a value of 1 for S. Significance values for treatment differences were determined from analysis of $2^{-\Delta\Delta Ct}$ values. Intestine and yolk weights, expressed as percentage of BW data, were arcsine (square root of the percentage) transformed before analysis. The models included the main effects of incubation temperature, age at sampling, segment (when appropriate), and all 2- and 3-way interactions. Segment × age interactions are not discussed in this paper because interest was focused on the effect of temperature × age or temperature × segment. Differences among treatments were further compared using Tukey’s test. Significance was determined at $P \leq 0.05$.

**RESULTS**

No differences were found in small intestine weights, yolk sac weights, or any performance characteristics (BW, BW gain, feed intake, or FC) from DOH through the end of the grow-out period (data not shown). Two-way interactions of incubation temperature and age were found for both villus height ($P = 0.02$; Figure 1) and crypt depth ($P = 0.05$; Figure 2) in the duodenum. At DOH and d 2, chicks from both incubation treatments had similar villus heights. From d 2 to 4, chicks from both incubation temperature treatments had similar increases in villus lengths that persisted through d 10. A greater increase was found in villus height from d 4 to 10 in chicks from the S incubation treatment, and at d 10, chicks that were incubated at the S temperature had the longest villi measured in the duodenum. Similar interactions were observed for crypt depth in the duodenum. The crypts in chicks from eggs incubated at S or H temperatures were similar in depth from DOH to d 2. The crypts in chicks from the H temperature incubation were significantly deeper by d 4 than crypts in the H group at DOH, whereas the crypts were still similar in depth between days in birds from the S temperature treatment. From d 4 to 6, crypts...
remained at similar depths. However, from d 6 to 10, the crypts in H chicks remained relatively unchanged, whereas those of the S-incubated chicks continued to deepen. The crypts were significantly deeper in the S group at d 10 as compared with the depths measured at d 6. A 2-way interaction of incubation temperature and age was seen with villus height-to-crypt depth ratio in the ileum. Ileum villus height-to-crypt depth ratios (Figure 3) in chicks incubated at the S temperature decreased significantly from DOH to d 4, whereas the ratio remained similar from DOH to d 4 in chicks incubated at the H temperature. In contrast, from d 4 to 6, no significant change in the ratio was found in birds incubated at the S temperature, whereas the ratio increased significantly in birds from the H temperature incubation. At d 10, the ratios were again similar between the S- and H-incubated chicks. In the jejunum, only the main effect of temperature was found to affect crypt depths, with deeper ($P = 0.02$) crypts in chicks incubated at the H temperature (0.061 mm) as compared with the S temperature (0.055 mm) from DOH to d 10 (data not shown).

Results of nutrient transporter gene expression for 3 sugar transporters (SGLT1, GLUT2, and GLUT5), 1 amino acid transporter (EAAT3), and a peptide transporter (PepT1) were variable. No significant differences were found for SGLT1 or GLUT5, and only segment × age interactions were observed for EAAT3 ($P = 0.006$) and GLUT2 ($P = 0.02$; data not shown). A temperature × age interaction ($P = 0.0002$) was found for relative PepT1 gene expression (Figure 4). At DOH and d 2, PepT1 expression was similar, and no difference in expression was found between chicks incubated at the S or H temperature. From d 2 to 4, chicks from both the S and H incubation temperatures had a significant increase in PepT1 expression. However, from d 4 to 6, expression in the S-incubated chicks remained at a level similar to that seen at d 4, whereas expression in the H-incubated chicks decreased significantly, which resulted in d 6 being the only day with different expression levels between S- and H-incubated chicks. By d 10, PepT1 expression in S chicks had decreased to a level similar to that of H chicks, which remained relatively unchanged from d 6.
DISCUSSION

This study evaluated the effect of incubating broiler eggs at an elevated temperature (39.6°C) compared with the standard temperature (37.4°C) on posthatch performance, intestinal morphology, and nutrient transporter expression. No significant differences were observed in posthatch broiler performance or small intestine and yolk sac weights. Although some previous studies have reported decreased BW of broilers when embryos were incubated at elevated temperatures (Hulet et al., 2007; Leksrisompong et al., 2007), application of an elevated embryonic incubation temperature with no significant effect on BW performance (Leksrisompong et al., 2007) or improved growth and performance (Rickles, 1987; Christensen et al., 1999) has also been reported. The discrepancy in performance changes related to embryonic incubation temperature may be due to a variety of factors other than temperature that have been shown to influence the response of embryos to incubation temperature, including setter or hatcher RH, eggshell conductance, and broiler breeder age (Hulet, 2007). The timing of the temperature increase and range of temperature increase could also influence the performance response of the hatched broilers (Leksrisompong et al., 2007). The application of a higher incubation temperature in the present study may have resulted in different performance results, similar to those observed in other studies.

In spite of the lack of performance differences, differences were found in the morphology of the small intestine and in nutrient transporter gene expression. Results of villus height and crypt depth measurements, as indices of intestinal posthatch development, suggest that intestinal development was initially similar in chicks from eggs incubated at the S and H temperatures, particularly at DOH and d 2. The increase in villus length was relatively consistent between chicks from the S and H incubation treatments to d 4. From d 4 to 10, chicks from the S group had a greater increase in villus height, and at d 10, villi in the duodenum of chicks from the S incubation temperature were the longest of all those measured. This increase in villus height corresponded to a similar trend observed for crypt depth in the duodenum. A steady deepening of the crypts occurred until d 6, but from d 6 to 10, a significant increase was observed in crypt depth in chicks from the S temperature incubation, whereas crypts of chicks in the H temperature incubation remained at a depth similar to that measured at d 6. The significant increase in villus height was supported by a significant increase in crypt depth to supply enterocytes for villus lengthening. In a study by Uni et al. (2000), hyperplasia in the crypts occurred during the first 4 d of the life of the chicken, and the growth rate of the intestinal epithelium decreased after this time to d 14, at which time the intestine was mostly mature and established. Enterocytes require 2 to 4 d to migrate to the tip of the villi, which coincides with the observations made in this study, in which a consistent increase was observed in villus length to d 10. The increased crypt depth in the duodenum at d 10 may indicate continued mucosal growth that would allow more enterocytes to develop and migrate to increase villus length.

From DOH to d 2, ileum villus height-to-crypt depth ratios in chicks from the S and H incubation temperatures were similar; however, from d 4 to 6 posthatch, a significant increase was observed in the villus height-to-crypt depth ratio in the ileum of those chicks incubated at the H temperature compared with the S-incubated group, and at this time, the residual yolk should have been used to its maximum. According to Nitsan et al. (1991), the residual yolk contributes 50 and 40% of the total energy and protein supplies, respectively, on the first day posthatch, and by d 4, only negligible amounts of nutrients are found in the residual yolk, with 2% of energy and 6% of protein of the total nutrient supplies. Differences in yolk absorption were not observed in this study. Therefore, it could be theorized that even though the chicks from both groups were obtaining basically a similar amount and quality of nutrients, the partitioning of these nutrients was different, or the requirements for protein and energy were different between chicks from the different incubation temperature groups. The energy demands of the gastrointestinal tract and the metabolic activity of the intestine can have a direct influence on the supply of nutrients to the chicken. The energy required for early mucosal growth is acquired through exposure to dietary nutrients and yolk sac con-

Figure 4. Effect of incubation temperature and age on relative peptide transporter (PepT1) mRNA expression in the small intestine of Ross 308 broiler chickens incubated at 2 different temperatures, standard (S, 37.4°C) and high (H, 39.6°C). From embryonic day (ED) 1 to ED12, all the eggs were incubated at 37.1°C. From ED13 to ED21, half of the eggs were incubated at the S temperature of 37.4°C and the other half were incubated at the H temperature of 39.6°C. DOH = day of hatch. Relative gene expression (2−ΔΔCt) ± SEM (where Ct refers to cycle threshold) was calculated using the ΔΔCt method with glyceraldehyde-3-phosphate dehydrogenase as the endogenous control and the average ΔCt value for the duodenum from the S (37.4°C) treatment as the calibrator. Data are represented as least squares means ± SEM (n = 4/treatment). There was a 2-way interaction (P = 0.0002) of incubation temperature [means not sharing a common letter (a, b) are significantly different (P < 0.05)] and age.
tent, and for the acquisition of these, efficient absorption must occur (Moran, 1985; Iji et al., 2001).

From the 5 different nutrient transporters evaluated, only PepT1 had differences in relative mRNA expression related to interactions of incubation temperature and age of the bird. Peptide transporter is a di- and tripeptide Na+-dependent transporter expressed in the brush border membrane of enterocytes. It is more efficient to transport amino acids through PepT1 than through the free amino acid transporters (Daniel, 2004). In this study, PepT1 expression remained at similarly low levels for chicks incubated at S or H temperatures on both DOH and d 2. Expression of PepT1 in chicks from both incubation treatments increased similarly by d 4. However, on d 6, expression in the chicks incubated at the S temperature remained elevated, whereas expression in chicks from the H incubation temperature decreased significantly. By d 10, expression in the S-incubated chicks decreased significantly to a level similar to that of the H-incubated chicks. The increase in both groups at d 4 may be associated with increased absorption of nutrients from the yolk sac at that time, and the continued expression in the S-incubated chicks at d 6 may indicate a prolonged expression of these transporters for continued nutrient utilization from the yolk. Interestingly, after this increase in PepT1 expression at d 4 and 6 in the chicks from the S temperature incubation, an increase was observed in duodenum villus height and crypt depth in this treatment, possibly suggesting increased nutrient utilization for mucosal development. Expression in chicks incubated at the H temperature was decreased at d 6, which may indicate altered energy expenditure for these birds away from expression of this peptide transporter. Chen et al. (2005) reported that the expression of PepT1 depends on diet changes, feed quality, and bird developmental stage. The developmental stage of the chick is most likely affected by the elevated temperature during incubation, and the distribution of nutrients from the residual yolk and external diet may be diverted from fulfilling the necessary energy needs for the transport of peptides into other physiological functions. Utilization of residual yolk and external diet is associated with hypertrophy, hyperplasia, and development of the digestive and absorptive functionality of the small intestine.

Several of the studies reported to date have not revealed significant differences in the performance of slaughter-age broilers resulting from differences in embryonic incubation temperatures. Similarly, we did not observe performance differences. In this research, although no differences were observed in posthatch performance from altering the incubation temperature, differences were observed in intestinal epithelium development related to incubation temperature. Changes in morphology and nutrient transporters may not result in performance differences because of different demands for energy expenditure, metabolism, or other physiological responses. This study, however, does present the effects of elevated incubation temperature on small intestinal development, as measured by morphology and nutrient transporter relative gene expression.

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REFERENCES


