A feed restriction milieu for Pekin meat ducks that may improve gait characteristics but also affects gut leakiness

A. Bentley,* L. Porter,* L. Van Blois,* B. Van Wyk,* C. N. Vuong,† G. Tellez-Isaias‡,† D. Shafer,‡
Z. Tucker,‡ S. M. Fraley,*§ B. M. Hargis,† and G. S. Fraley*‡

*Department of Biology, Hope College, Holland, MI 49423, USA; †Poultry Health Laboratory & Department of Poultry Science, University of Arkansas, Fayetteville, AK 72701, USA; ‡Maple Leaf Farms, Inc. Leesburg, Indiana 46538, USA; and §South Crossing Veterinary Center, Caledonia, MI 49316, USA

ABSTRACT In a previous study, we demonstrated that a 15% feed restriction (FR) during the first 2 wk after hatch could improve gait in Pekin meat ducks, but did result in reduced breast mass. We hypothesized that feed restriction after day 5 following muscle satellite cell development would allow the full growth of the breast meat. To accomplish this goal, 300 1-day-old ducklings (Maple Leaf Farms Inc.) were randomly allocated to 1 of the 3 groups (n = 4 pens, 25 ducks per pen): (1) Control group fed to ad libitum; (2) 85% daily feed intake from days 1 to 14 (FR 85% 1–14); 85% daily feed intake from days 5 to 14 (FR 85% 5–14). All ducks were vaccinated with inactivated Salmonella enteritidis on day 14 and boosted on day 26. The FR 85% 5–14 did show similar production standards to controls, and improved gait characteristics (P < 0.05). To determine if the partial feed restriction would have an impact on intestinal epithelial tight junction integrity, we treated ducks on days 7, 14, 21, 28, and 35 with 8.32 mg/kg FITC-d in water per os and blood samples were obtained via the tibial vein 1 h later. Serum samples were analyzed for presence and quantification of FITC-d. Feed restriction elicited a significant increase in FITC-d permeability at all points of evaluation. Anti-S. enteritidis specific IgY responses were assessed by ELISA from serum collected at 14 D, 28 D, and 35 D. Although all ducks showed an increase humoral immune response to the S. enteritidis, both feed restricted groups showed reduced IgY production compared to ad lib controls. Our data suggest that although the FR 5–14 feed restriction paradigm may reduce gait abnormalities without affecting production rates, some challenges exist due to increased gut leakiness or decreased acquired immune activity. Future studies will look at altering the feed restriction milieu to ameliorate these challenges.

Key words: lameness, welfare, applied nutrition, immunology

INTRODUCTION The Pekin duck (Anas plantyrhynchos domesticus) is one of the most common duck strains in the food industry because of their rapid growth, high egg production, and their calm demeanor (Cherry and Morris, 2008). With the demand for duck meat increasing throughout the world, Pekin ducks are required to reach market weight (3.2 kg) in under 5 wk (Fraley et al., 2013b; Campbell et al., 2015; Schenke et al., 2016; Best et al., 2017). A focus of Pekin duck farmers over the last several years has been to reduce fat content, to increase percent breast yield, and to improve feed conversion ratio (FCR) to achieve market weight in the shortest possible time. Modern Pekin ducks are now the fastest growing among all poultry species. However, this rapid growth has led to increased gait abnormalities (Campbell et al., 2014; Robison et al., 2015; Byrd et al., 2016).

An increase in gait abnormalities that could lead to onset of lameness is a growing concern for all poultry species, and the duck is no exception. Many studies of broilers have determined that feed restriction, specifically a restriction that occurs earlier in the course of the chick’s lifespan, not only reduces the cost of feed but may improve feed efficiency, whereas, decreasing fat content (Zubair and Leeson, 1994; Benyi et al., 2009). Although there has not been extensive research conducted on the effects of food restriction in ducks, a previous study determined that a caloric restriction followed by ad lib access to feed until day 49 could be utilized to improve growth performance as well as body composition in male Pekin ducks (Wu et al., 2012). However, an excessive period of feed restriction could be detrimental, leading to unwanted behaviors such as feather picking/pecking and a reduction in productivity.
(Fraley et al., 2013a; Blois et al., 2019). However, a feed restriction paradigm that benefits the skeletal health of ducks, without having other negative issues that may be possible.

An initial feed restriction study was conducted by our lab that compared 35% and 15% feed restrictions to the ad lib control meat ducks (Blois et al., 2019). That study aimed to reduce the incidence of gait abnormalities in Pekin ducks, whereas, not affecting production targets. Of the 2 feeding milieus, only the 15% feed restricted group was able to successfully reach market weight similar to the controls and appeared to have more uniform gait characteristics. However, both feed restricted groups showed significantly reduced mass of the pectoralis major muscle; a condition not acceptable in the USA duck market. It is known that the satellite cell and myofiber development of the duck breast occurs until day 5 of age (Gille et al., 1998; Zhang et al., 2014), thus, that feed restriction protocol may have adversely impacted pectoral development.

The goal of this current study was to further deduce if the 15% feed restriction could allow ducks to achieve market weight with target breast muscle weight if applied during days 5–14 after hatch. Furthermore, we wished to assess if the feed restriction would have an impact on gut development or on the humoral immune response. Our data suggest that a feed restriction paradigm could be instituted in ducks to help reduce the onset of leg issues, whereas, not negatively impacting growth performance, although some challenges may exist.

METHODS

Animals and Housing

Day-old ducklings (n = 110) were obtained from Maple Leaf Farms Inc. (Leesburg, IN, USA) and placed in an aviary with controlled environment at Hope College (Holland, MI) within 5 h of hatching (Day 0). The aviary conditions adhered to industry standards for 18:6 Light: Dark cycle, temperature, humidity, and initially given ad lib access to commercial feed and pin-metered water (nipple lines). We followed industry guidelines given ad lib control meat ducks (Campbell et al., 2014; Blois et al., 2019). The ducks were placed at 1 end of a paper-covered alley and were allowed to walk at a slow pace to the other end. The alley was made from 2 pieces of 2-meter-long and 0.5-meter-high polystyrene sheets glued on the sides of a third, bottom sheet. The design of the alley was such that the ducks could walk along the bottom sheet but not be able to see over the sides. Ducks were placed at 1 end of the alley and allowed to walk to the opposite, open end of the alley. If a duck ran down the alley the paper was replaced, and the footprint collection was repeated. Each duck had its own sheet of paper for every footprint collection, on which the date and the duck’s identification number were recorded.

The quantitative analyses of gait were performed by at least 2 separate individuals blind to the experimental design and unaware of the results of the qualitative analyses. A total of 3 footprints from the right foot were each connected with a line drawn through the metatarsal pad imprint; 3 corresponding footprints on the left from the left foot were connected with a line drawn through the metatarsal pad imprint. The distance between these 2 lines was measured at 3 positions along the 3 strides and averaged to determine the average width of the duck’s gait. A line was then drawn for each footprint from the metatarsal pad to the second joint of the middle digit. This formed an angle that was then measured for all of the right and left footprints and was averaged to obtain right and left foot angles. This process was repeated for all footprints collected throughout the entire experiment.

Serum Determination of FITC-d

Gut Leakage

Intestinal leakage of FITC-d (MW 3–5 kDa; Sigma-Aldrich Co., St. Louis, MO, USA) and the measurement of its serum concentration was determined, although FITC-d is a marker of paracellular transport and mucosal barrier dysfunction (Yan et al., 2009; Kuttappan et al., 2015; Vicuña et al., 2015a,b). On days 7, 14, 21, 28, and 35, respectively, 1 h before humanely euthanizing the ducks by CO2 inhalation, 15 ducks from each group were given an oral gavage dose of 8.32 mg/kg FITC-d (Baxter et al., 2017), and 5 ducks per group were used as no FITC-d control. FITC-d concentration from diluted sera was measured at an excitation wavelength of 485 nm and an emission wavelength of 528 nm (Synergy HT, Multi-mode microplate reader, BioTek Instruments Inc., VT, USA). Elevated serum FITC-d levels was suggestive of decreased intestinal epithelial tight junction integrity and thus an increased gut leakiness (Baxter et al., 2017).
Effects of Feed Restriction on Humoral Immune Response

When determining whether the feed restriction negatively affected the acquired immune response of the ducks, we utilized approximately 10⁸ inactivated Salmonella enteritidis to vaccinate all of the ducks. The bacteria were injected intramuscularly on day 14, and was boosted via another injection on day 26. Serum was obtained on day 14, 28, and 35 and analyzed for anti-S. enteritidis-specific IgY levels using a commercial ELISA kit (IDEXX, Westbrook, ME).

At the end of the experiment, all ducks were euthanized with CO₂ in accordance with AVMA guidelines (Leary et al., 2013). All ducks were necropsied and total body and organ weights recorded.

Intestinal Morphometric Analysis

Intestinal sections were standardized: for duodenum, a 0.5 cm section was collected from the middle of the descending duodenum; and for ileum, a 0.5 cm section was collected from the mid-ileum at the Meckel’s diverticulum. Duodenal and ileal sections were fixed in 10% neutral buffered formalin and embedded in paraffin, sectioned (5-mm thick), set on a glass slide, and stained with hematoxylin and eosin (H&E), then examined by light microscopy. Photomicrographs of random selected fields of each intestinal sample were acquired using a microscope equipped with a Leica DFC450C camera and Leica V 3.8.0. software (Leica Application Suit) and used for morphometric analysis. ImageJ 1.47v software (Rasband, 1997–2012) was used to take the measurements in the morphometric analysis of the different intestinal sections. For villus height of duodenum and ileum, an average of 10 villi per bird was measured, with a total of 8 ducks per group. Villus length was measured from the luminous apical tip of the basal epithelial surface of the cryptvillus to the top of the lamina propria. Crypt depth was measured from the basal aspect of the crypt at the mucosal–submucosal interface to the luminal aspect of transition from the crypt to villus epitheliumbase of the invagination between crypt and villus (Aptekmann et al., 2001). Data from villus

Figure 1. Production Variables. (A) Feed restricted groups showed an increase in rebound feeding following the feed restriction compared to controls, but showed no further differences in feed intake compared to controls for the remainder of the study. (B) Although the feed restricted groups showed reduced body weight compared to controls during the feed restriction period, no differences in body were observed among the 3 groups by day 28. (C) No differences were observed in feed conversion ratio (FCR) among the 3 groups. * = P < 0.05, ** = P < 0.01, red line = 5–14 D feed restriction, green line = 1–14 D feed restriction, arrow indicates day ad lib feeding resumed.
height and crypt depth were used to obtain the VH:CD ratio. Moreover, villus width was measured at the base area of each villi, and the villus surface area was calculated using the formula $(2\pi)(VW/2)(VL)$, where $VW =$ villus width, and $VL =$ villus length (Sakamoto et al., 2000).

**Statistical Analyses**

All statistical tests were done using SAS software (JMP v9.0.3). All data were averaged within a pen and the pen was considered the statistical unit ($N = 6$). All data were analyzed using an ANOVA (fasting as the independent variable) or repeated measures ANOVA (fasting $\times$ age, with age as the repeating variable) as appropriate followed by a Fisher's PLSD post hoc test. A $P < 0.05$ was considered significant.

**RESULTS**

**Production Data**

At the end of the feed restriction period, both feed restriction groups showed rebound feeding greater than ($P < 0.01$) the control ducks’ feed intake. However, by day 17 all 3 groups showed similar levels of feed intake, which continued throughout the rest of the study. Interestingly, all 3 groups inexplicably had a drop-in feed intake on days 19 and 28, which is similar to what is observed in commercial barns (Kevin Murdoch, Director Live Production, Maple Leaf Farms Inc.). All ducklings had a similar body weight on the day of hatch through day 7. On day 14, both feed restriction groups had a lower body weight ($P < 0.01$) compared to the controls. On day 21, the FR 85% 1–14 group showed lower body weight ($P < 0.05$) compared to controls, whereas, the FR 85% 5–14 group showed similar body weight to controls. By day 28, all 3 groups had similar body weights and this trend continued until the end of the experiment where all 3 groups achieved market weight (~3.2 kgs) by day 35. No differences were observed in the FCR among the 3 groups (Figure 1). Throughout the study only 2 birds were culled due to lameness, both in the control group. No other mortalities occurred.

We have replicated findings from our earlier feed restriction study in that the FR 85% 1–14 groups showed similar body weight, but considerably reduced ($P < 0.05$) breast mass compared to controls. The FR 85% 5–14 group showed similar breast mass to controls. No differences were observed in organ weights for heart, spleen, liver, duodenum, jejunum, ileum, or hindlimbs among the 3 treatment groups (Figure 2).

**Gait Analyses**

Similar to our previous study, we observed that feed restriction did affect gait. The FR 85% 1–15 group had a narrower gait width compared to the other 2 groups. By day 35, the FR 85% 5–14 group showed a greater ($P < 0.01$) width compared to the other 2 groups. Surprisingly, the FR 85% 5–14 group showed a lesser extent, and less variability, of metatarsal adduction at days 10 ($P < 0.001$) and 35 ($P < 0.001$) for both right and left legs compared to the other 2 groups (Figure 3).
Gut Analyses

Except for day 21, both feed restriction groups showed an increase \((P < 0.05)\) in serum FITC-d levels compared to controls throughout the experiment (Figure 4). Histological analyses of duodenum and ileum showed that feed restriction groups showed a decreased \((P < 0.05)\) villus height, width and area, decreased muscular thickness, and villous height to crypt depth ratio \((VH:CD)\) as well as an increased crypt depth compared to controls at day 14. However, no differences were observed among the 3 groups in any histological parameter measured in duodenal and ileal tissues at day 35 (Tables 1 and 2).

Humoral Immune Response

Prior to vaccinations on day 14, no differences were observed in the anti-S. enteriditis-specific serum IgY mean S/N ratio. However, on days 28 and 35 both feed restriction groups showed a reduced \((P < 0.05)\) IgY mean S/N ratio compared to controls in response to the vaccine and booster (Figure 5).

DISCUSSION

A previous study from our lab indicated that a 15% feed restriction during the first 2 wk of life could allow meat ducks to obtain target weight, but adversely affected development of the breast meat (Blois et al., 2019). Our current study aimed to determine whether a 15% feed restriction that was implemented after pectoralis satellite cell development could allow Pekin ducks to obtain target body and breast weights. Additionally, we wished to analyze whether this feed restriction influenced duck gut development, the humoral immune response, and gait score. We found that although there were some alterations in gut absorption, intestinal mucosa, and humoral immune response, a 15% feed restriction from days 5 to 14 of development could meet all production targets and alter gait in a way suggestive of a positive impact on leg development.

In our current study, the FR 85% 5–14 feeding protocol allowed for pectoralis development and altered gait patterns compared to both the control and FR 85%
Table 1. Morphometric analysis of duodenum and ileal tissue in ducks at day 14 of age.\textsuperscript{c}

| Tissue   | Control                  | FR 85\% 1–14 D          | FR 85\% 5–14 D          |
|----------|--------------------------|-------------------------|-------------------------|
| Duodenum | Villus height (\(\mu m\)) | 457.24 ± 4.66\textsuperscript{a} | 337.20 ± 3.07\textsuperscript{b} | 437.20 ± 4.07\textsuperscript{b} |
|          | Villus width (\(\mu m\)) | 44.43 ± 0.22\textsuperscript{a} | 40.07 ± 0.44\textsuperscript{b} | 38.07 ± 0.50\textsuperscript{b} |
|          | Crypt depth (\(\mu m\))  | 55.23 ± 0.41\textsuperscript{b} | 64.07 ± 1.14\textsuperscript{a} | 74.77 ± 0.14\textsuperscript{a} |
|          | Area (mm\(^2\))\textsuperscript{d} | 63.95 ± 0.85\textsuperscript{a} | 42.38 ± 0.52\textsuperscript{b} | 40.38 ± 0.57\textsuperscript{b} |
|          | Muscular thickness (\(\mu m\)) | 60.42 ± 0.40\textsuperscript{b} | 46.79 ± 0.82\textsuperscript{b} | 42.79 ± 0.81\textsuperscript{b} |
|          | VH:CD\textsuperscript{e}  | 8.32 ± 0.11\textsuperscript{a} | 5.34 ± 0.06\textsuperscript{b} | 6.34 ± 0.14\textsuperscript{b} |
| Ileum    | Villus height (\(\mu m\)) | 166.90 ± 3.81\textsuperscript{a} | 140.88 ± 3.06\textsuperscript{b} | 141.88 ± 3.06\textsuperscript{b} |
|          | Villus width (\(\mu m\)) | 39.62 ± 0.62\textsuperscript{a} | 33.91 ± 0.82\textsuperscript{b} | 32.91 ± 0.72\textsuperscript{b} |
|          | Crypt depth (\(\mu m\))  | 38.59 ± 1.00\textsuperscript{b} | 46.88 ± 1.64\textsuperscript{a} | 45.88 ± 0.64\textsuperscript{a} |
|          | Area (mm\(^2\))\textsuperscript{d} | 21.15 ± 0.73\textsuperscript{a} | 15.32 ± 0.59\textsuperscript{b} | 14.32 ± 0.69\textsuperscript{b} |
|          | Muscular thickness (\(\mu m\)) | 43.16 ± 0.64\textsuperscript{b} | 34.86 ± 0.44\textsuperscript{a} | 35.86 ± 0.74\textsuperscript{a} |
|          | VH:CD\textsuperscript{e}  | 4.55 ± 0.13\textsuperscript{b} | 3.02 ± 0.03\textsuperscript{b} | 3.15 ± 0.05\textsuperscript{b} |

\textsuperscript{a,b}Means with no common superscript letter within a row differ significantly at \(P < 0.05\).
\textsuperscript{c}Data are expressed as mean ± SE. \(n = 8\)/group.
\textsuperscript{d}2\(\pi\) × (villus width/2) × villus height (Sakamoto et al., 2000).
\textsuperscript{e}Villus height to crypt depth ratio.

Table 2. Morphometric analysis of duodenum and ileal tissue in ducks at d 35 of age.\textsuperscript{c}

| Tissue   | Control                  | FR 85\% 1–14 D          | FR 85\% 5–14 D          |
|----------|--------------------------|-------------------------|-------------------------|
| Duodenum | Villus height (\(\mu m\)) | 477.24 ± 4.66           | 477.20 ± 3.07           | 467.20 ± 4.07           |
|          | Villus width (\(\mu m\)) | 44.43 ± 0.22            | 44.07 ± 0.44            | 48.07 ± 0.50            |
|          | Crypt depth (\(\mu m\))  | 65.23 ± 0.44            | 64.07 ± 1.14            | 68.77 ± 0.14            |
|          | Area (mm\(^2\))\textsuperscript{d} | 63.95 ± 0.85            | 62.38 ± 0.52            | 66.38 ± 0.57            |
|          | Muscular thickness (\(\mu m\)) | 60.42 ± 0.40            | 66.79 ± 0.82            | 62.79 ± 0.81            |
|          | VH:CD\textsuperscript{e}  | 8.32 ± 0.11             | 8.34 ± 0.06             | 8.84 ± 0.14             |
| Ileum    | Villus height (\(\mu m\)) | 166.90 ± 3.81           | 170.88 ± 3.06           | 161.88 ± 3.06           |
|          | Villus width (\(\mu m\)) | 39.62 ± 0.62            | 38.91 ± 0.82            | 37.91 ± 0.72            |
|          | Crypt depth (\(\mu m\))  | 48.59 ± 1.00            | 46.88 ± 1.64            | 45.88 ± 0.64            |
|          | Area (mm\(^2\))\textsuperscript{d} | 11.15 ± 0.73            | 15.32 ± 0.59            | 14.32 ± 0.69            |
|          | Muscular thickness (\(\mu m\)) | 33.16 ± 0.64            | 34.86 ± 0.44            | 35.86 ± 0.74            |
|          | VH:CD\textsuperscript{e}  | 3.55 ± 0.13             | 3.02 ± 0.03             | 3.15 ± 0.05             |

\(P > 0.05\).
\textsuperscript{c}Data are expressed as mean ± SE. \(n = 8\)/group.
\textsuperscript{d}2\(\pi\) × (villus width/2) × villus height (Sakamoto et al., 2000).
\textsuperscript{e}Villus height to crypt depth ratio.

**Figure 5. Humoral Immune Response.** Serum IgY responses to an inactivated Salmonella enteritidis challenge were reduced in feed restricted groups compared to controls. * = \(P < 0.05\).
in a variety of human disorders, such as osteomyelitis, Rickets, and Blount’s disease (Thienpont et al., 2017). In our current study, the FR 85% 5–14 ducks had a reduced variability in MA in both the right and left legs with fewer extreme angles of MA observed. The more uniform gait characteristics may relate to better hindlimb development and result in reduced gait abnormalities.

Feed restriction has been found to have positive effects on the immune system. Jang et al. (2009) determined that an 85% feed restriction has a beneficial effect on the expression of the cytokine, IL-4, a key regulator in humoral and innate immunity. Early feed restriction paradigms have been shown to have positive effects on immune function in several poultry species (Fassbinder-Orth and Karasov, 2006; Jang et al., 2009; Orso et al., 2019). In this current study, we found that feed restriction elicited an apparent reduction in humoral immune response as evidenced by reduced Salomonella-specific IgY production; however, we showed that feed restriction had no effect on immune organ weight compared to controls. Other studies also found that antibody production and immune organ weight were consistent among both feed restricted and ad lib fed broilers further suggesting that feed restriction did not impact immune function (Liew et al., 2003; Fassbinder-Orth and Karasov, 2006). It is possible that feed restriction in ducks elicited a different temporal release of IgY compared to controls, and therefore our once weekly serum collection following antigen challenge may have missed peak production. Alternatively, altered immune function could be related to altered gut absorption in feed restricted ducks.

We demonstrated that feed restriction between days 5 and 14 after hatch could reach production targets in ducks, although some alterations in gut function may be indicated. Tolkamp et al. (2005) showed that a mild feed restriction on broilers breeders during both rearing and lay was sufficient to allow for desirable growth curves and weight uniformity within a flock of broiler breeders without affecting lay. Novel et al. (2009) also determined that feed restriction improved FCR, increased caloric efficiency, and decreased broiler mortality similar to our current study. The structure of the intestinal mucosa can reveal some information on gut health. Stressors that are present in the digestive system can lead relatively quickly to changes in the intestinal mucosa due to the close proximity of the mucosal surface and the intestinal content. Changes in intestinal morphology, such as shorter villi and deeper crypts, have been associated with the presence of toxins (Yason et al., 1987). A shortening of the villus and a large crypt may lead to poor nutrient absorption and lower performance (Xia et al., 2014). Additionally, others have shown that similar changes in crypt height and villus length have no impact on animal health in piglets (Hampson, 1986; Nabuurs et al., 1993), migrating Black Caps (Karasov et al., 2004), chickens (Smith et al., 1990), or poult (Geyra et al., 2001; Noy et al., 2001). Furthermore, in our current study feed restriction elicited no adverse effects on FCR or weight gain, nor did it elicit any aversive behaviors such as feather picking/pecking (data not shown). All of these observations suggest that a stressor was not a root cause of the altered gut morphology. Previous studies in the duck have shown that excessive feed restriction elicits stress as noted by increased corticosterone levels, and reduced production values (Fraley et al., 2013a). Furthermore, recent study in our lab showed that similar levels of feed restriction did not elicit a corticosterone response (Blois et al., 2019). All of these observations suggest that a mild feed restriction is not a stressor in ducks. If the altered gut morphology at day 14 did allow for increased pathogen absorption that could explain the blunted IgY response to our specific antigen. However, the positive or negative impact of a putative increase in immunological challenge remains to be seen.

The purpose of this study was to determine if a feed restriction milieu could be developed that would allow grow out ducks to achieve market parameters. We further set out to determine if a slight feed restriction would allow better development of the skeletal system to prevent gait abnormalities, without having adverse effects on duck health and welfare. A previous study demonstrated that this level of daily feed restriction does not elicit acute or long-term stress in growing ducks (Blois et al., 2019). Although some effects on gut absorption and humoral immune function were noted that require further investigation, we observed that a feed restriction from 5 to 14 D following hatch allowed for target body weight, breast mass, organ weights, and overall FCR. The FR 5–14 group also showed more uniform gait characteristics, in particular, with metatarsal adduction, which made be associated with limb abnormalities. In conclusion a feed restriction paradigm can be developed to improve grow out duck welfare without adversely impacting the gait.

ACKNOWLEDGMENTS

The authors wish to thank Maple Leaf Farms Inc. for their continued support of our research. This project was supported in part by Agriculture and Food Research Initiative Competitive Grant no. 2018–67016–27616 from the USDA National Institute of Food and Agriculture (to GSF).

REFERENCES

Aptekmann, K. P., S. M. Baraldi Arton, M. A. Stefanini, and M. A. Orsi. 2001. Morphometric analysis of the intestine of domestic quails (Coturnix coturnix japonica) treated with different levels of dietary calcium. Anat. Histol. Embryol. 30:277–280.

Baxter, M. F. A., R. Merino-Guzman, J. D. Latorre, B. D. Mahaf- fey, Y. Yang, K. D. Teague, L. E. Graham, A. D. Wolfenden, X. Hernandez-Velasco, L. R. Bielke, B. M. Hargis, and G. Tellez. 2017. Optimizing fluorescein isothiocyanate dextran measurement as a biomarker in a 24-h feed restriction model to induce gut permeability in broiler chickens. Front. Vet. Sci. 4:56.
Yason, C. V., B. A. Summers, and K. A. Schat. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. Am. J. Vet. Res. 48:927–938.
Zhang, R.-P., H.-H. Liu, Q.-Q. Li, Y. Wang, J.-Y. Liu, J.-W. Hu, X.-P. Yan, H. Gou, L. Li, and J.-W. Wang. 2014. Gene expression patterns, and protein metabolic and histological analyses for muscle development in Peking duck. Poult. Sci. 93:3104–3111.
Zubair, A. K., and S. Leeson. 1994. Effect of varying period of early nutrient restriction on growth compensation and carcass characteristics of male broilers. Poult. Sci. 73:129–136.