Preharvest Feed Withdrawal Affects Liver Lipid and Liver Color in Broiler Chickens

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ABSTRACT An Iowa grain processor attempted to alter the typical 12-h preharvest fasting period by giving broilers cornstarch derivative pellets and water for 6 h followed by 6 h of no feed or water. After slaughter, plant food inspectors determined that livers from the treatment group were lighter in color than normal, and consequently a significant number of chicken carcasses were condemned for human consumption. The study reported herein was conducted to determine the effects of fasting or 3 feeding programs applied before processing on liver color, liver lipids, and liver glycogen of broilers. Dietary treatment groups consisted of 1) full-fed control broilers, 2) fasted broilers, 3) maltodextrin-fed broilers, and 4) chickens given maltodextrin and methionine. Full-fed chickens had lighter liver coloration than chickens without access to feed for 6 or 12 h immediately prior to slaughter (P < 0.05). Lightness values for livers from full-fed control chickens (L* = 54.41) were 38% higher than those for livers from fasted broilers (L* = 39.30). Lighter liver colors in full-fed broilers were associated with higher hepatic lipid concentrations (6.38%) and more total liver lipid (4.96 g/liver) than was found in broilers without feed for 12 h. In contrast, darker livers from fasted broilers had lower levels of lipid (4.42%) and less total lipid (2.68 g/liver) than the full-fed broilers. Feeding maltodextrin pellets resulted in liver colors that were lighter (P < 0.05) than those found in fasted chickens but darker (P < 0.05) than livers from full-fed broilers. If carbohydrate supplements are fed prior to slaughter, producers should notify processing plant officials so that inspectors do not interpret light livers as an abnormal physiological state.

(Key words: chicken, color, feed, liver)

INTRODUCTION

Withholding feed from broiler chickens immediately prior to catching, loading, and transportation to processing plants is a standard management practice. During this time, broilers’ crops are emptied of feed, and the volume of ingesta in the intestines is markedly reduced, but cecal contents may or may not be evacuated (Bilgili, 1988; Hinton et al., 2000a,b). The objectives of feed withdrawal are 1) to reduce fecal excretion and external cross-contamination during transportation, 2) to reduce fecal contamination of poultry carcasses which may occur during automated evisceration procedures in the plant (Bilgili, 1988, 2002; May and Lott, 1990; Papa, 1991; Northcutt et al., 1997), and 3) to reduce feed costs by not providing feed during this period (Farhat et al., 2002a). The environmental conditions (e.g., temperature, season of year, and lighting) during feed withdrawal may influence the length of a feed withdrawal program (May and Lott, 1990).

Feed withdrawal refers to the total length of time the chickens are without feed prior to processing. A typical preharvest fasting period for broiler chickens lasts about 12 h (Papa, 1991; Willis et al., 1996). Chickens are in the broiler house with access to water but without feed for approximately 6 h prior to loading. An additional 6 h are spent on live-haul trucks without feed and water during transit to the processing plant and awaiting slaughter at the processing plant holding sheds. After 4 h of feed withdrawal in the chicken house, broilers begin to consume litter, fecal material, and the associated microorganisms (Northcutt et al., 1997; Corrier et al., 1999a). Feed withdrawal has been shown to increase the incidence of Salmonella isolation from crops and ceca of market-age broiler chickens (Moran and Bilgili, 1990; Ramirez et al., 1997; Corrier et al., 1999a). Similarly, feed withdrawal resulted in increased recovery of Campylobacter recovery from crops and ceca of commercially reared broilers (Willis et al., 1996; Byrd et al., 1998). Contaminated crops result in carcass contamination by Salmonella and other bacteria during processing. Crop contents may contaminate the external carcass after crop removal.
The optimal length for feed withdrawal is the shortest amount of time required for the birds’ digestive tracts to become empty (May and Deaton, 1989). If feed withdrawal time is too short (less than 6 h), the bird’s digestive tract will be full of feed at slaughter, and the intestines will be large and rounded (Northcutt et al., 1997), which greatly increases the likelihood that the intestines will be accidentally cut or torn during evisceration causing contamination (Bilgili and Hess, 1997; Northcutt et al., 1997; Bilgili, 2002). In addition, plants must run slower when processing birds that are full of feed which increases processing costs. If withdrawal time is excessively long (greater than 14 h), carcass contamination by feces increases because the tensile strength of intestinal walls decreases; tearing of intestines occurs more readily during evisceration, and the mucosa sloughs (Bilgili and Hess, 1997; Northcutt et al., 1997). During prolonged absence of feed, bile is retained in gall bladders, which subsequently enlarge and are more easily ruptured during automated evisceration with resultant bile contamination of carcasses (Bilgili and Hess, 1997). Also, longer feed withdrawal periods are economically costly to producers because of weight loss (live shrink) prior to slaughter, which occurs at the rate of 0.25 to 0.35% per hour (Veerkamp, 1986; Buhr et al., 1998).

An Iowa grain processor attempted to reduce the conventional 12-h preharvest fasting period to 6 h by providing broiler chickens a carbohydrate-based supplement during the first 6 h of the traditional feed withdrawal period prior to slaughter. The concept was to maintain live weight prior to slaughter by providing nutrition that contributes no residue in the gastrointestinal tract. Treatment chickens were given access to pellets made from a cornstarch derivative (maltodextrin, which is generally recognized as a safe human food ingredient) and water for 6 h followed by a 6-h period of no feed or water. At processing, however, livers from the treated chickens were markedly lighter in color than those found in broilers following a conventional feed withdrawal program. The unusually light color of the livers resulted in condemnation of significant numbers of chickens by meat inspectors in the processing plant.

The objectives of this study were 1) to objectively measure the surface color, lipid concentration, and glycogen content of livers from broiler chickens on full feed, 2) to determine whether these liver parameters are altered by 12 h of feed withdrawal, 3) to ascertain whether a feed withdrawal supplement of maltodextrin or maltodextrin plus methionine would alter the effect of fasting on broiler liver parameters, and 4) to evaluate the relationship between hepatic concentrations of lipid and glycogen and liver surface color in broiler chickens. Methionine was added to maltodextrin in the ration of one group to determine if dietary availability of an amino acid would enhance mobilization of fat from the livers.

**Experimental Chickens and Diets**

One hundred 1-d-old female broiler chicks were purchased from a commercial hatchery and fed standard broiler rations for 48 d. Diets were based primarily on corn and soybean meal and were formulated to meet or slightly exceed nutrient concentrations recommended by the National Research Council. All birds were wing-banded on d 16. On d 41, 80 broilers were allotted randomly to 16 floor pens with 5 chickens per pen. For 12 h prior to slaughter, starting at 0730 h, diets were fed to 4 pens (experimental units) of chickens. Dietary treatments consisted of 1) full-fed control broilers fed the standard broiler ration and water for the full 12 h, 2) fasted broilers receiving water for the first 6 h and no feed or water thereafter, 3) maltodextrin-fed broilers (M) given this carbohydrate (Maltrin M100)[2] in the form of cornstarch derivative pellets and water for the first 6 h and no nutrition or water thereafter, and 4) maltodextrin + methionine fed chickens (MM) given access to maltodextrin pellets containing 1.05% DL-methionine during the first 6 h and no nutrition or water thereafter. Body weights of the broilers were determined at the start of the experiment (0730 h). Feed consumption of the appropriate experimental groups was measured for the intervals of 0730 to 1330 h and 1330 to 1930 h. All broilers were euthanized by cervical dislocation at the conclusion of the 12-h experimental period.

**Liver Color**

Digital photographs of each liver were taken by a professional photographer immediately after slaughter using the same lighting and black background. CIE color values (Hunt et al., 1991) of the livers, representing lightness (L*), redness (a*), and yellowness (b*), were measured from digital color photographs enlarged to 20x27 cm. The color measurements were conducted using a HunterLab Labscan[3] color meter. The instrument was calibrated using a white tile standard. The D65 light source and 2.54-cm aperture were used for all measurements. Luminance, or lightness, may range from 100 (white) to 0 (black). Positive a* values are a measure of redness, and negative a* values are a measure of greenness. Positive b* values measure yellowness, and negative b* values indicate blueness. Color was measured at 3 random locations on each photograph, and mean color values were calculated for each liver. Color difference (ΔE) was calculated using the following formula: \[ ΔE = \sqrt{(L^*1 - L^*2)^2 + (a^*1 - a^*2)^2 + (b^*1 - b^*2)^2}/10 \]

where L*, a*, and b* represent color parameters measured on livers from chickens with no feed withdrawal, and L^*2, a^*2, and b^*2 represent color parameters measured after one of the feed withdrawal treatments (Francis and Clydesdale, 1975).

Colors of the livers were measured by a second method using a computer and the CMYK model of Adobe Photoshop 6.0 (2000). Each pixel in a digital photograph was
assigned a percentage value by the computer for cyan (blue), magenta (red), and yellow based upon the intensity of the color. Percentage values for a given color are assigned independently of the other 2 colors. For example, a bright red might contain 2% cyan, 93% magenta, and 90% yellow. Total color intensity for a specimen is the sum of the individual colors. In the example given, total color intensity is 189 (2% + 93% + 90%) out of a possible intensity score of 300 (100% + 100% + 100%).

**Glycogen Content**

Glycogen was measured enzymatically using a modified method of Keppler and Decker (1974). Deep-frozen liver sample (approximately 1 g) was homogenized with 5 mL of chilled 0.6 N perchloric acid using a Brinkman Polytron for 30 s at high speed. For glycogen hydrolysis, the homogenate (0.1 mL) was transferred to a 1.5-mL microcentrifuge tube with an indented cap, and 50 µL of KHCO₃ and 1 mL of amyloglucosidase solution (14 units in 0.2 M acetate buffer, pH 4.8) were added. The mixture was then incubated at 40°C for 2 h with occasional shaking. Incubation was stopped by adding 0.5 mL of 0.6 N perchloric acid and centrifuging at 3,000 x g for 10 min. The supernatant (75 µL) was taken into another microcentrifuge tube, and 1.5 mL of ATP/NADP/glucose-6-phosphate dehydrogenase (1 mM ATP/1 mM NADP/140 units G6P-DH in 1 mL of 0.3M triethanolamine buffer, pH 7.5) was added; the absorbance was read at 340 nm against an air blank. The glycogen content (%) was calculated from the amount of hydrolyzed glucose from glycogen per g tissue. ATP/NADP/glucose-6-phosphate dehydrogenase (1 mM ATP/1 mM NADP/140 units G6P-DH in 1 mL of 0.3M triethanolamine buffer, pH 7.5) was added; the absorbance was read at 340 nm against an air blank. The glycogen content (%) was calculated from the amount of hydrolyzed glucose from glycogen per g tissue. A glycogen content (%) was used for a standard curve, and residual glucose in the tissue was subtracted as a blank.

**Lipid Content**

An accurately weighed liver sample (approximately 2 g), butylated hydroxytoluene (50 µL, 7.2% in ethanol), and 30 mL of Folch I solution (chloroform:methanol = 2:1) were added to a 50-mL test tube and homogenized using a Polytron for 20 s at high speed. The homogenate was filtered through a Whatman No. 1 filter paper into a 100-mL graduated cylinder, and the filter paper was rinsed twice with 10 mL Folch I solution. After adding 8 mL (1/4 volume on the basis of filtrate) of 0.88% NaCl solution to each cylinder, the cylinder was capped with a glass stopper, and the content was mixed well. The inside of cylinder was washed twice with 5 mL of Folch II solution (chloroform:methanol:water = 3:47:48). After phase separation, the lipid layer volume was recorded, and the upper layer (methanol and water) of the solution was siphoned completely and carefully off so as not to contaminate the chloroform layer. The organic layer (20 mL) was put in a glass scintillation vial and dried for 5 h at 50°C under a nitrogen flow. Lipid content (%) was calculated from the weight of the dried lipids (g of lipid / g of liver x 100).

**Statistical Analysis**

Experimental chickens were allotted randomly to 1 of 4 dietary treatments groups, and each treatment was independently replicated in each of 4 floor pens. Floor pens were the experimental units and each floor pen contained 5 chickens. Data were analyzed by the GLM procedure using SAS software (SAS Institute, 1995). Student-Newman-Keuls multiple-range test was used to compare mean values of treatments. Mean values and SEM were reported at P < 0.0001. Correlation coefficients between liver lipid and color values were calculated by the CORR procedure of SAS.

**RESULTS**

Full-fed control chickens gained an average of 70 g BW during the 12 h preharvest feed withdrawal period (Table 1). Mean BW of broilers subjected to conventional feed withdrawal for 12 h declined by 110 g. Broilers fed maltodextrin or a combination of maltodextrin and methionine lost an average of 90 g of BW during this period, which was less (P < 0.05) than the weight lost by the fasted group. During the first 6 h of the experiment, consumption of feed by chickens fed a maltodextrin supplement (38 g/chicken) or maltodextrin-methionine (26 g/chicken) was less (P < 0.05) than broilers in the control group eating their normal corn-soybean ration (62 g/chicken).

Complete feed withdrawal for 12 h resulted in less (P < 0.001) lipid per liver (Table 1). Mean BW of broilers subjected to conventional feed withdrawal for 12 h declined by 110 g. Broilers fed maltodextrin or a combination of maltodextrin and methionine lost an average of 90 g of BW during this period, which was less (P < 0.05) than the weight lost by the fasted group. During the first 6 h of the experiment, consumption of feed by chickens fed a maltodextrin supplement (38 g/chicken) or maltodextrin-methionine (26 g/chicken) was less (P < 0.05) than broilers in the control group eating their normal corn-soybean ration (62 g/chicken).
Livers of full-fed control broilers were lighter (L*, P < 0.0001) and had less redness (a* values, P < 0.0001) than was observed in livers of broilers in the groups subjected to a period of feed withdrawal (Table 3). Yellowness (b* values) was numerically greater in livers of the control group broilers than in those from fasted broilers, but the differences were not significant. Liver surface color in broilers fasted for 12 h was different (P < 0.0001) from the color of livers in broilers maintained on full feed (control group) during the fasting period as indicated by color difference (ΔE) results. The color intensity of magenta and yellowness measured by a computer and the CMYK model of Adobe Photoshop 6.0 was greater (P < 0.0001) in livers from the fasted, M, and MM groups than it was in livers of full-fed broilers. Total color intensity of livers in the full-fed group (163.6) was less (P < 0.0001) than the intensity of color in livers from fasted broilers (183.0 to 192.9).

Pearson correlation coefficients for liver fat and liver color measurements are shown in Table 4. The concentration of fat in the livers (% of liver weight) had strong negative correlations (both r = −0.660) with magenta and redness (a*, both r = −0.611). In a similar manner, the quantity of lipid in the livers (g/liver) had strong negative correlations (both P < 0.002) with magenta and redness (a*, both r = −0.705).

**DISCUSSION**

Full-fed chickens had lighter liver color (P < 0.0001) than chickens fasted for 12 h during the feed withdrawal period. Lightness values for control group chickens (L* = 54.41) were 38% higher than for fasted broilers (L* = 39.30). Also, the concept of lighter colored livers in full-fed broilers is supported by computer-generated data indicating that total color intensity of livers from the control group (163.6) was less than the color intensity of livers from broilers subjected to a period of feed withdrawal (182.68 to 192.90). This finding is in agreement with a previous reports wherein broiler liver L* values decreased as feed withdrawal periods increased (Buhr et al., 1998). However, Northcutt et al. (1997) reported no change in liver lightness in broilers after removal from feed for up to 18 h.
Lighter liver colors in full-fed broilers ($L^* = 54.41$) were associated with higher hepatic lipid concentrations (6.38%) and more total liver lipid (4.96 g) than was found in broilers without feed for 12 h. In contrast, darker livers in fasted broilers ($L^* = 39.30$) had lower concentrations of lipid (4.42%) and less total lipid (2.68 g). This finding is consistent with a previous report describing a reduction in liver fat content of chickens given no feed for 12 h or longer (Jensen et al., 1984). Lipid synthesis in the chicken is restricted to the liver with very low lipogenic activity in adipose tissue (Hasegawa et al., 1994). During periods of fasting, de novo triglyceride synthesis from lipid and carbohydrate precursors is reduced by more than 90% in chicken livers (Hasegawa et al., 1994). In addition, the rate of fatty acid oxidation increases more than 4-fold. After feed withdrawal, energy requirements are initially met by use of carbohydrates and later by catabolism of lipid and proteins (Riesenfeld et al., 1981; Hazelwood, 1986). The combination of decreased production and increased removal accounts for the rapid loss of lipid in chicken livers during the preharvest fasting period.

Feeding maltdextrin pellets to broiler chickens during the preharvest feed withdrawal period did result in liver colors that were lighter than those found in fasted chickens but darker than livers from chickens eating a conventional ration. Liver lipid content of group M chickens was numerically intermediate between hepatic lipid concentrations found in full-fed and fasted chickens. Feeding a combination of maltdextrin plus methionine for 6 h yielded livers that were darker colored than those from the M group (41.18 vs. 44.69) but were no different from livers of broilers in the fasted or maltdextrin group with respect to the concentration and quantity of liver lipids.

Live weight losses for the maltdextrin and maltdextrin-methionine groups were less than for the fasted group. This observation is consistent with a previous study in broiler chickens supplemented with maltdextrin (Farhat et al., 2002b). However, even though shrinkage was reduced by feeding these supplements, the chickens did not readily accept either supplement as indicated by reduced feed consumption (maltdextrin, 38 g/chicken; maltdextrin-methionine, 26 g/chicken) as compared with broilers fed a normal ration (62 g/chicken). It is possible that an acclimation period or an improvement in palatability would have increased consumption of the supplements (Farhat et al., 2002b).

![Table 3. Liver color of broiler chickens subjected to conventional feed withdrawal and feed withdrawal supplements](https://academic.oup.com/ps/article-abstract/84/1/137/1593414/137)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Fasted</th>
<th>M</th>
<th>MM</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td>Lipid (%)</td>
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<td>0.959</td>
<td>-0.350</td>
<td>-0.660</td>
<td>-0.482</td>
<td>0.628</td>
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<td>0.0001</td>
<td>0.1834</td>
<td>0.0054</td>
<td>0.0588</td>
<td>0.0092</td>
</tr>
<tr>
<td>Lipid (g/liver)</td>
<td>0.959</td>
<td>1.000</td>
<td>-0.398</td>
<td>-0.705</td>
<td>-0.556</td>
<td>0.766</td>
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<tr>
<td>$P$-value</td>
<td>0.0001</td>
<td>0.0</td>
<td>0.1266</td>
<td>0.0023</td>
<td>0.0254</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

1. $L^*$ value = lightness; $a^*$ value = redness; $b^*$ value = yellowness.
2. Percentage of liver on a weight basis (g of lipid/g of liver × 100).

![Table 4. Pearson correlation coefficients for liver lipid and liver coloration](https://academic.oup.com/ps/article-abstract/84/1/137/1593414/137)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lipid (%)</th>
<th>Lipid (g/liver)</th>
<th>Cyan</th>
<th>Magenta</th>
<th>Yellow</th>
<th>$L^2$</th>
<th>$a^2$</th>
<th>$b^4$</th>
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<td>Lipid (%)</td>
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<td>0.959</td>
<td>-0.350</td>
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<td>-0.482</td>
<td>0.628</td>
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<td>0.0588</td>
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<tr>
<td>Lipid (g/liver)</td>
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<td>1.000</td>
<td>-0.398</td>
<td>-0.705</td>
<td>-0.556</td>
<td>0.766</td>
<td>-0.705</td>
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<td>$P$-value</td>
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dark due to loss of hepatic lipid. Light livers in broiler chickens on full feed represent a normal physiological condition but may not be recognized as such by inspectors following slaughter. Consumption of significant quantities of a carbohydrate feed withdrawal supplement could result in light coloration and retention of hepatic lipid. If carbohydrate feed withdrawal supplements are fed prior to slaughter, it is important that producers notify processing plant officials so that inspectors do not interpret light livers as an abnormal physiological state.

REFERENCES


