Yolk sac nutrient composition and fat uptake in late-term embryos in eggs from young and old broiler breeder hens

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ABSTRACT In the present study, we examined the composition, amount, and uptake of yolk nutrients [fat, protein, water, and carbohydrates (COH)] during incubation of eggs from 30- and 50-wk-old broiler breeder hens. Eggs were sampled at embryonic d 0 (fresh eggs), 13, 15, 17, 19, and 21 (hatch). Egg, embryo, yolk content, and yolk sac membrane were weighed, and the yolk sac (YS; i.e., yolk content + yolk sac membrane) composition was analyzed. From 30 to 50 wk of age, the albumen weight increased by 13.3%, whereas the yolk increased by more than 40%. The proportion of fat in the fresh yolk of the 30-wk-old group was 23.8% compared with 27.4% in the 50-wk-old group, whereas the proportion of protein was 17.9% compared with 15.6%, respectively. During incubation, results indicated that water and protein infiltrated from other egg compartments to the YS. Accordingly, the calculated change in the content of water and protein between fresh yolk and sampled YS does not represent the true uptake of these components from the YS to the embryo, and only fat uptake from the YS can be accurately estimated. By embryonic d 15, fat uptake relative to embryo weight was lower in the 30-wk-old group than in the 50-wk-old group. However, by embryonic d 21, embryos of both groups reached similar relative fat uptake, suggesting that to hatch, embryos must attain a certain amount of fat as a source of energy for the hatching process. The amount of COH in the YS increased similarly during incubation in eggs from hens of both ages, reaching a peak at embryonic d 19, suggesting COH synthesis in the YS. At hatch, the amount of protein, water, and COH in the residual YS, relative to the weight of the yolk-free chick, was similar in eggs from young and old hens. However, chicks from the younger hens had less fat in the YS for their immediate posthatch nutrition compared with those from the older hens.

Key words: yolk composition, yolk sac, fat uptake, breeder age, broiler embryo

INTRODUCTION

The chick embryo derives all of its nutrient requirements during incubation from the albumen and yolk. The albumen represents about 65 to 75% of the egg’s total content and consists of approximately 88% water and 12% protein, both of which are totally consumed by the embryo during incubation (Romanoff, 1960; Shenstone, 1968). The yolk consists of approximately 50% water, 15% protein, 33% fat, and less than 1% carbohydrates (COH); however, this composition depends greatly upon egg weight, genetic strain, and hen age (Shenstone, 1968; O’Sullivan et al., 1991; Vieira and Moran, 1998). During incubation, nutrients pass from the yolk contents (YC) to the embryo through the yolk sac membrane (YSM) and its surrounding vascular system (Noble and Cocchi, 1990). From embryonic d 19, the yolk sac (YS; i.e., YC + YSM) begins to be internalized into the embryo’s body cavity, and at hatch, it constitutes about 15 to 20% of the chick’s BW, providing the hatching with immediate nourishment until exogenous feed is given in the brooder house (Romanoff, 1960; Noble and Oggunyemi, 1989; Noy and Sklan, 2001).

The YS is the main source of energy (via fatty acid oxidation) during embryonic development and the only source of lipids for embryo tissue growth (Speake et al., 1998). The process of yolk fat utilization has been extensively studied. It has been found that most of the yolk’s fat is taken up by means of nonspecific phagocytosis at the apical surface of the endodermal cells of the YSM (Lambson, 1970; Noble and Cocchi, 1990; Speake et al., 1998). Many factors can affect yolk fat utilization, such as incubation conditions (Burnham et al., 2001), genetic strain (Scheideler et al., 1998), breeder hen diet (Latour et al., 1998), and age. Several studies have associated breeder hen age with YS total fat and
Egg, Embryo, and YS Sampling

Embryo throughout incubation and at hatch. Absorption, and the nutritional needs of the developing absorption, the effects of breeder age on YS nutrient breeder hens, to better understand the process of yolk water, and COH, in eggs of 30- and 50-wk-old broiler tion, amount, and uptake of yolk nutrients (fat, protein, amine the changes during incubation in the composi-

Previous research has been conducted on the changes in YS protein (Sugimoto and Yamada 1986; Sugimoto et al., 1999), water (Ar, 1991), and COH (Thomas and Just, 1964; Romanoff, 1967) contents but not with respect to broiler breeder hen age and not with current commercial breeds. Although, as already mentioned, previous studies have demonstrated various aspects of YS nutrients, the focus in most of them was on only one of the major nutrients: research into comparative changes during incubation between YS nutrient levels are lacking in the literature. The current research can elucidate whether broiler breeder eggs have sufficient nutrients for normal embryo development, especially in light of the increased metabolic rate recorded in today’s commercial embryos (Tona et al., 2004; Hamidu et al., 2007). Therefore, the objective of this study was to examine the changes during incubation in the composition, amount, and uptake of yolk nutrients (fat, protein, water, and COH), in eggs of 30- and 50-wk-old broiler breeder hens, to better understand the process of yolk absorption, the effects of breeder age on YS nutrient absorption, and the nutritional needs of the developing embryo throughout incubation and at hatch.

MATERIALS AND METHODS

Egg Supply, Fresh Yolk and Albumen Sampling, and Incubation

Fertile eggs, collected on the same day from hens of young (30 wk) and old (50 wk) broiler breeder hens, were obtained from a commercial breeder farm (Brown, Hod Hasharon, Israel). All eggs (150 from each hen age) were labeled with a serial number and weighed individually before incubation (egg weight at set), and the mean and variance of egg weight from each breeder age were calculated (30 wk: mean = 59.48 g, SD = 3.83 g; 50 wk: mean = 71.55 g, SD = 4.37 g). Twenty eggs from each breeder age, representing the weight distribution of all 150 eggs, were selected for fresh yolk and albumen analysis: fresh yolk and albumen were weighed, homogenized, and stored at −20°C. The remaining eggs were incubated in a hatchery at 37.5°C and 60% RH, with automatic egg turning. After 10 d of incubation, on embryonic d 10, all eggs were candled and unfertilized eggs and eggs with dead embryos were removed.

Egg, Embryo, and YS Sampling

Eight eggs from each hen-age, representing the weight distribution of the eggs at set, were selected in the middle of embryonic d 13, 15, 17, and 19 and subjected to the following procedure: egg, embryo, and YS were weighed. The YC was separated from the YSM and both were weighed, homogenized, and stored at −20°C for further analysis. A day before hatch, each egg was placed in a separate basket for chick identification at hatch. During the hatch day (embryonic d 21), 8 chicks were selected within a 30-min period after hatch. The mean and variance of the egg weight at set of these selected chicks were similar to those of the eggs sampled in all other embryonic days. Embryos and chicks at hatch were killed by cervical dislocation. To obtain the weight of the embryonic d 21 embryos, the YS was removed from the selected chicks’ abdominal cavities; the chicks without YS (chicks yolk-free; CYF) and the YS were weighed. Each YS was separated to YC and YSM and they were handled as mentioned above.

Sample Analyses

Samples (0.1 g each) of homogenized fresh yolk, YC, and YSM were transferred to separate microcentrifuge tubes. Beads were added, along with 1 mL of distilled water, and the mixture was blended using a mini beadbeater. Further analysis of fat, protein, and water content was performed using these prepared homogenates.

Lipid Analysis

Total lipids were extracted using the method of Folch et al. (1957): 10 mL of chloroform-methanol (2:1 vol/vol) was added to 0.5 mL of the fresh yolk, YC, and YSM homogenates. After 30 min, 2 mL of distilled water was added. After overnight incubation at 4°C, 5 mL of the bottom layer was transferred to glass tubes (weighed in advance) and oven-dried at 105°C. The tubes with the remaining fat were weighed and the weight of fat per gram of fresh yolk, YC, and YSM was calculated.

Protein Analysis

Distilled water (100 µL) was added to 50 µL of the homogenate. A 5-µL aliquot of the mixture was transferred to a 96-well ELISA plate and dye reagents were added (Bio-Rad protein assay, Hercules, CA). A standard curve was generated using dilutions of a BSA standard solution. The absorbance at 750 nm was determined, and the weight of protein per gram of fresh yolk, YC, and YSM was calculated.

Water Analysis

Samples of homogenized fresh albumen, fresh yolk, YC, and YSM (0.2 g each) were transferred to separate glass tubes (weighed in advance) and dried in a 105°C oven. The tubes with the remaining DM were weighed and the percentage of water in the fresh albumen, fresh yolk, YC, and YSM was calculated.
COH Analyses

Glucose Analysis. A 300-µL quantity of enzymatic glucose reagent (TR-15103, Thermo Electron) was added to 2 µL of aliquots of the fresh yolk, YC, and YSM homogenates and incubated for 10 min, after which absorbance was read at 492 nm. Glucose content was determined using a standard calibration curve.

Glycogen Analysis. Glycogen contents were determined by a colorimetric method based on the reduction of iodine by the method of Dreiling et al., (1987): 0.1 g of homogenized fresh yolk, YC, and YSM were transferred into separate microcentrifuge tubes. Beads were added, along with 0.5 mL of 8% perchloric acid, and the mixture was blended using a mini bead-beater, followed by a 5-min centrifugation at 15,000 × g at 4°C. The supernatant was transferred, and 0.5 mL of petrol ether was added to it. After the petrol ether fraction was removed from the mixture, 5 µL from the bottom layer was transferred to a 96-well ELISA plate, and 250 µL of color reagent (Dreiling et al., 1987) was added. A standard curve was generated using dilutions of a glycogen standard solution. After incubation for 10 min at room temperature, the absorbance at 450 nm was determined, and the weight of glycogen per gram of fresh yolk, YC, and YSM was calculated.

Nutrient Amounts and Fat Uptake Calculations

All nutrient concentrations were calculated as milligrams per gram of YC or YSM. The total amount of each nutrient was calculated by multiplying the nutrient concentration in the YC or YSM by the weight of the YC or YSM. The amount of COH in the YC or YSM was calculated by adding the glucose and glycogen amounts. The total amount of each nutrient in the YS was calculated by adding the YC and YSM amounts. Yolk components that were not analyzed in this study, such as micronutrients, are referred to as other and were calculated as follows:

Other = (total YS weight) − (YS protein) − (YS fat) − (YS water) − (YS COH).

Fat uptake from the yolk is the difference between yolk fat amount on day of set to YS fat amount on embryonic day X. Because the amount of fat in the yolk on day of set could not be accurately estimated individually for each of the examined eggs during incubation (low correlation between fresh egg weight and fresh yolk fat amount), the uptake of fat from the YS was determined from the group means of fresh yolk fat and the group means of YS fat amount during incubation (Table 1) and was calculated as follows:

Fat uptake = group mean fresh YS fat on d 0 − group mean YS fat on day X.

Statistical Analysis

All data were subjected to 2-way ANOVA with a model that included hen age and day of incubation as main effects and their interaction. Differences among means were tested by contrasts using Student’s t-tests. All statistical analyses were carried out using JMP 8 software (SAS Institute Inc., Cary, NC).

RESULTS

Fresh Egg Composition and Embryo Development

As expected, eggs from the 50-wk-old breeder hens were heavier than those from the 30-wk-old hens on day of set in the incubator (embryonic d 0, fresh eggs) as well as throughout incubation (Table 2). Fresh eggs of the 50-wk-old hens had a larger yolk relative to the total egg content weight (i.e., yolk + albumen) than eggs of the 30-wk-old hens (Figure 1). From 30 to 50 wk of age, the weight of the total fresh egg content increased by 21.5% (from 52.1 to 63.3 g). However, the egg components differed in the magnitude of weight increase: the albumen increased by 13.3%, whereas the yolk increased by more than 40% and the total weight of the water in the egg content increased by 18%, whereas the total weight of the DM in the egg content increased by 34.3% (Figure 1). From embryonic d 17 to 21 (hatch), embryos were significantly heavier in the 50-wk-old group, but relative embryo weights (calculated as % of egg weight at set) did not differ significantly between the 2 hen ages (Table 2). Relative YS weight (% of egg weight at set) and the ratio between YS and embryo weight were significantly higher in the eggs from 50-wk-old breeder hens, but these differences decreased along with embryonic development from embryonic d 13 to 21 (hatch).

YS Weights

Weights of the entire YS, YC, and YSM are presented in Figure 2. Yolk weight was significantly higher in the 50-wk-old group than in the 30-wk-old group at all days during the last week of incubation, from embryonic d 13 to 21 (Figure 2A). On embryonic d 13 and 15, there was a 5.6-g difference between the hen ages, whereas on embryonic d 19 and 21, the difference was only 2.7 g, resulting in a significant hen age × embryo age interaction (P = 0.04). From embryonic d 13 to 21, there was a decline of approximately 9.8 and 6.9 g in the YS mass of the 50- and 30-wk-old hen groups, respectively. From embryonic d 17 to 19, there was a reduction of 1.65 g (P = 0.094) in YS weight of the 50-wk-old group, but no change was observed in the 30-wk-old group (Figure 2A). During this same period, there was almost no change in YC weight in the embryos from 50-wk-old hens versus a 2-g increase in the embryos from 30-wk-old hens (Figure 2B).
Table 1. Means of yolk sac nutrient concentration (mg/g of yolk sac) and amount (fat, protein, and carbohydrates) in the yolk of unincubated eggs (d 0) and in the yolk sac of eggs, on d 13, 15, 17, 19, and 21 (hatch) of incubation, from broiler breeder hens at 30 and 50 wk of age

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<td>Carbohydrates (mg)</td>
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<td>30 wk</td>
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<td>198</td>
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<td>50 wk</td>
<td>94*</td>
<td>89</td>
<td>147</td>
<td>285*</td>
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1Nutrient concentration or amount examined in the fresh yolk.
2Chicks at hatch (no later than 30 min after hatch).
*Significant difference (P < 0.05) between hen ages within day of incubation.

Table 2. Means of egg weight, embryo weight, yolk sac:embryo weight ratio, and percentage of embryo and yolk weight relative to unincubated egg weight (egg weight at set), on d 13, 15, 17, 19, and 21 (hatch) of incubation, from broiler breeder hens at 30 and 50 wk of age

<table>
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<th>Age</th>
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<td>n</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>19</td>
<td>8</td>
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<tr>
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<td>% Yolk sac (of egg weight at set)</td>
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<td>0.54*</td>
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1Sample of fresh eggs that were broken for fresh yolk measurements.
2Chicks at hatch (no later than 30 min after hatch).
3All eggs were weighed before set (start of incubation). For embryonic d 13 to 21, percentage of embryo, percentage of yolk sac, and percentage of egg weight loss were calculated from initial egg weight at set.
*Significant difference (P < 0.05) between hen ages within day of incubation. Main effect of day was significant (P < 0.0001) in all variables except for egg weight at set (P = 0.8292).
Yolk sac membrane weight was higher in the embryos from 50-wk-old hens than in the embryos from 30-wk-old hens on all days during the last week of incubation (Figure 2C). Between embryonic d 13 and 17, YSM weight increased by 2.2 g in both breeder hen ages, whereas from embryonic d 17 to 21 (hatch), YSM decreased by 3.2 and 1.7 g in the 30- and 50-wk-old hens groups, respectively.

**YS Nutrients**

Results of the analysis of nutrient concentrations in the YS of the 2 hen ages are shown in Table 1. During the course of incubation, the concentrations of protein and COH per gram of yolk increased toward hatch, whereas the fat level decreased. The individual levels of these nutrients per gram of YC and YSM were analyzed but are not shown here. The total amounts of the nutrients in the YS, YSM, and YC are presented in Figures 3, 4, and 5.

**COH**

On embryonic d 13, the amount of COH in the YS was approximately 80 mg for both hen ages. Between embryonic d 13 and 19, the amount of COH increased almost 4-fold, reaching 300 mg at embryonic d 19 in both hen ages. From embryonic d 19 to hatch, the amount of COH in YS decreased by 130 and 90 mg in the 30- and 50-wk-old groups, respectively (Figure 3A). A similar pattern of change was observed for the amount of COH in the YC (Figure 3B) and in the YSM (Figure 3C).

![Figure 1](image1.png)

**Figure 1.** Yolk weight (DM and water), albumen weight (DM and water), and shell weight from fresh eggs of broiler breeder hens at 30 and 50 wk of age. Percentage of DM or water refers to their percentage from the total yolk plus albumen. Bars represent fresh egg weight.

Yolk sac membrane weight was higher in the embryos from 50-wk-old hens than in the embryos from 30-wk-old hens on all days during the last week of incubation (Figure 2C). Between embryonic d 13 and 17, YSM weight increased by 2.2 g in both breeder hen ages, whereas from embryonic d 17 to 21 (hatch), YSM decreased by 3.2 and 1.7 g in the 30- and 50-wk-old hens groups, respectively.

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**Water**

In embryonic d 13 through 17, the amount of water in the YS was significantly higher in the 50-wk-old group than in the 30-wk-old group (Figure 4A). From embryonic d 17 to 19, there was a reduction of 1 g in the amount of water in the 50-wk-old group, whereas no change was observed in the 30-wk-old group. During the same period, there was almost no change in the amount of water in the YC of the embryos from 50-wk-old hens, whereas it increased by 0.5 g in the embryos from 30-wk-old hens (Figure 4B).

The amount of water in the YSM increased between embryonic d 13 and 15 and decreased between embryonic d 17 and 21 in both hen ages (Figure 4C). From embryonic d 15 to 17, there was a reduction in the amount of water in the 50-wk-old group, whereas an elevation was observed in the 30-wk-old group.

**Protein**

In the YS, the amount of protein exhibited sporadic changes from one day to the next, with a similar pattern for both hen ages (Figure 4D). On all days of incubation, the amount of protein in the YS of the embryos from 50-wk-old hens was significantly higher than that for the 30-wk-old hens by 0.8 to 1.2 g. The amount of protein in the YC (Figure 4E) was significantly higher in the 50-wk-old group from embryonic d 13 to 17, whereas from embryonic d 17 to 19, there was a significant elevation of 0.7 and 0.4 g in the embryos from 30- and 50-wk-old hens, respectively.

The amount of protein in the YSM was significantly higher in the embryos from 50-wk-old hens versus the 30-wk-old hens only at embryonic d 19 and 21 (Figure 4F). The YSM protein decreased significantly in the 30-wk-old group (by 0.45 g) from embryonic d 17 to 21, whereas no significant reduction was observed in the 50-wk-old group during that period.

**Fat**

Throughout incubation, the amount of fat in the YS of the 50-wk-old group was significantly higher than in that of the 30-wk-old group, but in both groups, there was a similar reduction of about 3 g in YS fat, from embryonic d 13 to 21 (Figure 5A). The reduction in YS fat weight was minimal between embryonic d 13 and 15, somewhat higher from embryonic d 15 to 17, and a similar linear rate of intensive reduction from embryonic d 17 to 21 in the 2 hen age groups.

Significant differences were observed in the amount of fat in the YC between the 2 hen ages at all days during the last week of incubation (Figure 5B): on embryonic d 13 and 15, the amount of fat in the YC was 1.5 g higher in the 50-wk-old group, and between embryonic d 17 and 21, this difference was reduced to about 0.7 g.

The amount of fat in the YSM was significantly higher in the embryos from the 50-wk-old hens versus the 30-wk-old hens but only from embryonic d 17 onward (Figure 5C). Between embryonic d 13 and 17, the amount of fat in the YSM increased in both groups of embryos, reaching 1.9 and 2.4 g in the 30- and 50-wk-old hens, respectively. From embryonic d 17 to 19, there was a significant decrease of approximately 1 g in the amount of YSM fat in both hen ages.

**Ratio of Fat Uptake to Embryo Weight**

The calculated uptake of fat from the YS, the embryo weight during incubation, and the ratio between them are shown in Figure 5D, E, and F, respectively.
At embryonic d 13 and 15, the fat uptake relative to embryo weight (%) was higher in the 50-wk-old group than in the 30-wk-old group, whereas from embryonic d 15 to 21, the difference decreased to almost zero because the rate of relative uptake was higher in embryos from the younger hens (30 wk) than the older hens (50 wk) (Figure 5F).

Nutrients in the Fresh Yolk and in the Residual YS at Hatch

The total amounts of fat, protein, water, and COH in the fresh yolk and in the residual YS of chicks at hatch are presented in Figure 6. During incubation, the amount of fat in the yolk decreased by 76 and 60%, whereas the protein decreased by 33 and 21% in the 30- and 50-wk-old groups, respectively. The amount of COH (less than 0.1 g in the fresh yolk) showed an approximately 60% increase on day of hatch. The calculated amount of the other components in the YS (e.g., micronutrients) is also presented in Figure 6.

The mean ratio between the amount of each nutrient in the residual YS upon hatch and CYF weight is presented in Figure 7. No significant difference was observed between the 2 age groups in the ratio between amount of water, protein or COH in the residual YS, and weight of the CYF; however, a significant difference was found in the residual YS fat:CYF weight ratio—hatching chicks of the 50-wk-old hens had 0.06 g of fat in the residual YS per 1 g of CYF weight, whereas for the 30-wk-old hens, there was only 0.02 g of YS fat per 1 g of CYF.

DISCUSSION

The differences in YS composition and in its uptake during embryonic development were examined in eggs from 30- and 50-wk-old broiler breeder hens. Results indicated that during the last week of embryonic development, the YS is a major gluconeogenic extraembryonic organ that stores COH for provision to the embryo in the days close to hatch. Results demonstrate that due to infiltration of protein and water from the non-yolk egg compartments (albumen or amnion, or both) into the YS, only YS fat uptake can be accurately calculated during embryonic development. Absorption of yolk fat up to embryonic d 15 was lower in embryos from younger hens than in those from old hens, perhaps due to the lower initial yolk fat content in the eggs of the younger hens. This might have created nutritional deficiency and higher nutritional demand, thereby increasing the rate of yolk fat uptake in embryos from the younger hens between embryonic d 15 to 21; by day of hatch, fat uptake relative to embryo weight was
similar in the 2 groups. The current study also presents the yolk nutritional reserves available for the chick at hatch and shows that hatchlings from young breeder hens have less fat content in their residual YS than chicks from older hens.

The eggs selected for the various analyses represented the mean and variance of egg weight at each hen age. Therefore the higher means of fresh yolk weight, embryo weight, YS weight, and the amount of fat, protein, water, and COH in the eggs of the older hens can be attributed to their higher initial egg weight at set. Accordingly, the main focus in this paper was on the patterns of change in the weight of the YS and its components during incubation in each of the 2 hen ages. Fat uptake during incubation and the weight of residual YS fat, protein, COH, and water at hatch were normalized to the embryo’s weight. Nutrient concentrations (mg/g of YS, Table 1) during embryonic development can be influenced by different rates of absorption for each of the nutrients, and by infiltration of nutrients into the YS, consequently their values do not accurately represent true changes in the amount of the nutrients. Therefore, the patterns of change in YS nutrients concentrations will not be discussed.

In agreement with previous studies (Thommes and Just, 1964; Romanoff, 1967), the amount of COH in the YS changed during embryonic development; on embryonic d 13, it did not exceed 100 mg; by embryonic d 19, it had reached a peak of 300 mg for both hen ages; and it decreased by approximately 100 mg at hatch (Figure 3A). These results may be indicative of COH synthesis (as suggested by Willier, 1968) in the YS, which supplies available energy to the chick embryo during the hatching process and after hatch.

In agreement with Romanoff (1967), our study documented an increase in YSM weight between embryonic d 13 and 17 and a significant decrease between embryonic d 17 and 21 (Figure 2C). This apparently reflected the YSM proliferation that takes place between embryonic d 13 and 17 and its degeneration between embryonic d 17 and 21, probably due to apoptotic processes. The YSM degeneration appeared to contribute fat and protein to the YC during the embryonic d 17 to 21 period, and this would explain why there was no change in the amount of fat in the YC (Figure 5B), even though fat was clearly absorbed from the YS (Figure 5A) during this period.

Embryos of both hen ages grew in a linear manner from embryonic d 13 to 21 (Figure 5E) while utilizing a substantial amount of their YS as expressed by the reduction in YS weight during the embryonic d 13 to 21 period (Figure 2A). However, between embryonic d 17 and 19, despite the gain of almost 10 g by the embryos of both hen ages, there was almost no reduction in the YS weight in the 30-wk-old group and a lower reduction (compared with the period between embry-

**Figure 5.** Comparison of (A) fat amounts in yolk sac [yolk content (YC) + yolk sac membrane (YSM)], (B) fat amounts in YC, (C) fat amounts in YSM, (D) fat uptake from the yolk sac, (E) embryo weight, and (F) fat uptake relative to embryo weight (%), between eggs from broiler breeder hens at 30 and 50 wk of age, on different days during embryonic development. Data are expressed as means ± CI. *Significant difference (P < 0.05) between hen ages within days.
onic d 15 to 17) in the 50-wk-old group. These puzzling results, which may appear to suggest that the embryos gained weight without utilizing their YS, can be explained by the different patterns of change in the YS major nutrient components from embryonic d 17 to 19: Figures 4A and D and 5A show that although there was a substantial decrease in the amount of fat in the YS, the amount of protein increased and the amount of water either did not change or was slightly reduced. The sum of the changes in these 3 YS components between embryonic d 17 and 19 was negative in eggs of the older hens but close to zero in eggs of the younger hens. The apparent finding of lower YS utilization between embryonic d 17 and 19 was probably wrong, as indicated by the reduction in the amount of YS fat during this period.

The fluctuations in the amounts of yolk protein and water in the YS can be explained by their infiltration into the YS from other egg compartments, such as the albumen or the amnion (Ar, 1991; Sugimoto et al., 1999). However, although protein changes were found to be similar for the 2 hen ages, water influx to the YS appeared to be higher in the eggs of the 30-wk-old hens than in those of the 50-wk-old hens (Figure 4A). Ar (1991) summarized that water influx to the YS is associated with osmolarity, electrolyte movement, and egg water loss. Although we did not examine osmolarity, it is possible that the higher percentage of DM in fresh egg contents of the older hens (Figure 1) might have caused a change of osmolarity in the egg compartments, which consequently resulted in different rate of water movements between the compartments. In our work (Table 2), and in agreement with Peebles et al. (2001), relative water loss was lower in the eggs of the younger hens. It could be suggested that this lower water loss is related to higher influx of water into the YS in eggs of the 30-wk-old hens compared with eggs of the 50-wk-old hens. However, in similar studies performed by our group, differences in water influx were found in eggs with similar water loss (unpublished data).

It can be concluded that measurements of yolk uptake have to take into consideration the infiltration of protein and water into the YS. Accordingly, the calculated change between fresh yolk weight and sampled YS weight does not represent the true uptake of the YS. Only fat uptake can be accurately estimated from YS measurements.

We calculated fat uptake from the entire YS weight (i.e., YSM + YC) and not just from its content because

Figure 6. Amount of nutrients in the yolk of eggs that were not incubated (fresh yolk), and in the residual yolk sac of chicks at hatch, from broiler breeder hens at 30 and 50 wk of age. Bars represent fresh yolk weight or residual yolk sac weight. COH = carbohydrates.

Figure 7. Ratio between the amount of residual yolk sac nutrients [fat, protein, water, carbohydrates (COH)] and the weight of the yolk-free chick at hatch for the 30- and 50-wk-old hens. Data are expressed as means ± CI. *Significant difference (P < 0.05) between hen ages.
the YC composition is influenced by the development and the breakdown of the YSM. Only a small amount (less than 10%) of the fresh yolk fat had been absorbed by the embryo by embryonic d 13 (Figure 5D), but from embryonic d 15 to 21, fat uptake from the yolk increased considerably, in agreement with Noble and Ogunyemi (1989) and Noble and Cocchi (1990).

On all tested embryonic days, fat uptake from the yolk was higher in eggs of the older hens than the younger ones. The ratio of fat uptake to embryo weight (Figure 5F) was calculated to account for the difference between hen ages in egg weight at set and embryo weight. By embryonic d 15, embryos from the older hens absorbed more fat (relative to their weight) than those from the younger hens. This difference between the 2 hen ages gradually decreased to almost zero on embryonic d 21, indicating that on day of hatch, embryos from the young and old breeder hens had similar relative fat uptake. This suggests that to hatch, the embryo must absorb a certain amount of fat (relative to its weight) as a source of energy for the hatching process, or energy reserves (fat deposits) for later use by the chick, or both. It is possible that the lower fat content in the fresh yolk of the 30-wk-old group (Table 1) led to the lower relative YS fat uptake on embryonic d 13 and 15 and consequently created nutritional deficiency and higher nutritional demands to reach the necessary fat uptake for the hatching process, thereby increasing the embryos’ relative YS fat uptake from embryonic d 15 to 21.

It had been previously suggested by Noble et al. (1986) and Yafei and Noble (1990) that younger parental age is associated with reduced transfer of yolk lipids into the embryo toward the end of incubation. These authors found that on embryonic d 19, yolk fat uptake by embryos from very young breeder hens (25 wk old) was much lower than that of embryos from older hens (41 wk old) and suggested that this might be linked to the higher late mortality of young hens’ embryos. It appears that a lower fat uptake by embryos in eggs of young hens is due to low yolk fat content. Accordingly, elevation of yolk fat in eggs of young hens (e.g., by dietary manipulation of the hens’ feed) may improve the embryos’ hatchability. However, if the lower fat uptake is not associated with the initial egg composition but rather with molecular mechanisms of lipid transport in the YSM that are less efficient in young hens (e.g., an epigenetic effect on the expression of lipoprotein receptor genes), the capacity of fat uptake will not be affected by dietary manipulation of fresh yolk fat.

Fat transport through the YSM is carried out by means of nonspecific endocytosis of lipoproteins (Speake et al., 1998), which is mediated by a member of the low-density lipoprotein receptor gene family called LR8 (a low-density lipoprotein receptor relative with 8 ligand-binding repeats; Hermann et al., 2000). No study has been done regarding the effect of expression of lipoprotein receptors on YS fat utilization during chick embryonic development. Such future studies may elucidate the differences between hen age groups in the ability of their embryos to use fat from the YS, as affected by fresh egg composition, and by the nutritional demands of the embryo.

An accelerated uptake of YS nutrients (COH, protein, water) was observed between embryonic d 19 to 21, yet the rate of YS fat uptake remained the same (compared with embryonic d 15 to 19). Nutrient metabolism in the egg is influenced by oxygen availability. From embryonic d 13 to 16, adequate oxygen is supplied by the corioallantoic membrane and oxygen consumption increases to a plateau stage (embryonic d 16, internal piping; Rahn and Ar, 1974). During mid to late incubation, the oxidation of fatty acids from the YS provides the embryo with its main source of fuel (Speake et al., 1998). Nevertheless, the high demand for energy to support the dramatic physiological changes of the hatching process, and the reduced oxygen availability of the egg, drives the embryo toward anaerobic catabolism of glucose, which is provided from reserves of glycogen in the liver and muscles (Christensen et al., 2001; Uni et al., 2005; De Oliveira et al., 2008). In the current experiment, it seems that an additional source of energy in the form of COH is provided to the embryo from the YS during its accelerated uptake between embryonic d 19 to 21.

It should be duly noted that the accelerated consumption of the YS and its nutrients between embryonic d 19 and 21 is made possible because in addition to the transfer of nutrients from the yolk to the circulation (Lambson, 1970; Noble and Cocchi, 1990), yolk uptake is facilitated by the transport of the YC directly to the embryo’s intestine via the yolk stalk (Romanoff, 1960; Esteban et al., 1991); in the days after hatch, the yolk stalk transports the bulk of the YC to the embryo (Noy et al., 1996).

This study also examined the difference in residual YS composition at hatch between hatchlings from young and old breeder hens to better understand the nutritional needs of the hatching chicks. Noy and Sklan (1999) showed that chicks deprived of feed for 48 h after hatch (average time without feed in commercial hatcheries) used approximately 60% of their residual yolk during that period for body maintenance requirements. Hence, the residual YS is a vital source of feed available to the hatchling until exogenous feed is provided and consumed, and any deficit in its content might affect the chick’s vitality, which can later decrease growth rate even after feed is given in the brooding house. The results in our study (Table 2) as well as others (Peebles et al., 2001; Hamidu et al., 2007) showed that chicks from younger hens hatch with a lower amount of residual YS relative to their weight than chicks from older hens. To determine whether there is a deficit in a specific nutrient in the hatchlings’ residual YS, the ratio between each of the major nutrients of the residual YS and the weight of the CYF at hatch was calculated.
(Figure 7). The ratio between the amount of protein, water, and COH in the residual YS and the weight of the CYF at hatch was similar in the 2 hen ages. However, the weight ratio of fat to CYF was significantly lower in the chicks from younger hens, indicating that these chicks had lower available fat in the residual YS for their immediate posthatch nutrition. Further study is necessary to determine whether this lower fat content leads to a fat deficit in the hatching nutrition; if so, there might be a need to compensate for this in the prestarter diet, or perhaps even in an early feeding diet in the hatchery.

The present study examined YS composition and uptake during chick embryonic development, in eggs of 30- and 50-wk-old broiler breeder hens. We demonstrated that embryos of the 2 hen ages had a different pattern of relative fat uptake, perhaps due to the lower initial yolk fat content in the eggs of the younger hens. It was not possible to estimate the uptake of the other nutrients due to infiltration of protein and water from the other egg compartments into the YS and a possible synthesis of COH in the YS. Nevertheless, it might be possible to estimate the absorptive capacity of the YSM of these nutrients by examining molecular transport systems of protein and COH that are known to transport peptides [e.g., peptide transporter 1 (PepT1)], amino acids [e.g., excitatory amino acid transporter 3 (EAAT3)], and COH [e.g., sodium glucose cotransporter 1 (SGLT1)] through epithelial cells. The synthesis of COH should also be addressed by examining the enzymes responsible for synthesis and breakdown of COH in the YS, as well as examining these enzymes’ gene expression in the endothelial cells of the YSM. Future investigation into YS nutrient uptake and absorptive capacity in embryos from eggs of different size, hen age, and genetic line may facilitate a better tuning of the yolk composition to the nutritional requirements of the developing embryo, perhaps by manipulation of the hens’ diet, thereby reducing embryonic mortality and improving hatchling vitality.

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REFERENCES


