Yolk sac fatty acid composition, yolk absorption, embryo development, and chick quality during incubation in eggs from young and old broiler breeders

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ABSTRACT The objective of the present study was to examine the changes in yolk and yolk sac fatty acid composition and also to investigate egg content, yolk absorption, embryo development during incubation, and chick quality at hatch in eggs from 36- and 52-wk-old broiler breeders. The fatty acid profiles of the yolk, the yolk sac of embryos, and the residual yolk sac of chicks were analyzed before incubation, on d 18, and at hatch, respectively. Yolk sac weight, and embryo weight and length were measured on d 18, and chick weight and length were measured at hatch. Egg weight, yolk and albumen weight, yolk percentage, and yolk:albumen ratio increased as breeder age increased, but the albumen percentage decreased. Yolk absorption in absolute value (g) was higher in embryos from the old flock on d 18 and at hatch. Relative yolk absorption was similar between age groups on d 18, whereas it was higher in the young flock at hatch. Breeder age affected the yolk sac weight and was higher in the old flock during incubation. Embryo or chick weight and length, and yolk-free BW were affected by breeder age during incubation. These parameters were higher in the old flock with a difference of 3.7 g, 0.8 cm, and 2.6 g, respectively, on d 18 and 7.4 g, 1.4 cm, and 6.3 g, respectively, at hatch compared with the young flock. The effect of breeder age on fatty acid composition differed significantly by sampling day. Palmitic, stearic, oleic, and linoleic acids were major fatty acids in the fresh yolk, ranging from 13.02 to 29.24%. These were followed by palmitoleic and arachidonic acids ranging from 1.24 to 7.04%, with the remaining fatty acids below 1%. Higher concentrations of myristic, palmitoleic, and oleic acids and lower concentrations of heptadeconoic, stearic, linoleic, and arachidonic acids were found in the residual yolk sac of the young flock than the old flock. The results showed preferentially selective absorption of some fatty acids by the embryo during incubation.

Key words: breeder age, yolk sac fatty acid composition, yolk absorption, embryonic development, chick quality

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INTRODUCTION

Flock age and egg size are major factors in the determination of the albumen and yolk content of eggs (Hamidu et al., 2007; Nangsuay et al., 2011). Researchers have shown that breeder age influences egg weight and consequently egg components and their ratios (Suarez et al., 1997; Tona et al., 2004; Nangsuay et al., 2011; 2013). Yolk and albumen weights and yolk:albumen ratio increase with an increase in breeder age (O'Sullivan et al., 1991). All of the nutrient requirements for embryonic development are stored in the albumen and yolk (Romanoff, 1960; Speake et al., 1998). The yolk consists of approximately 50% water, 15% protein, 33% fat, and less than 1% carbohydrates, but the exact composition varies depending on egg weight, genetic strain, and hen age (Shenstone, 1968; O'Sullivan et al., 1991; Vieira and Moran, 1998a,b).

The developing embryo uses the contents of the egg to develop its body (Meijerhof, 2009). For this process, the yolk is the main source of energy for tissue growth during embryonic development (Noble and Cocchi, 1990; Speake et al., 1998). Embryos use the fats from the yolk sac to initiate body growth (Meijerhof, 2009) and development of the small intestine (Noy and Sklan, 1999), and other organs. From d 19 of incubation, the yolk sac begins to be withdrawn into the abdomen of the embryo, and at hatch, it constitutes approximately 15% of the chick's BW. The residual yolk sac provides nutrients to the newly hatched avian neonate during the first few days of life (Noble and Ogunyemi, 1989; Mikec et al., 2006). The absorption and utilization of nutrients from the yolk sac by the embryo are affected by breeder age (Yadgary et al., 2010). Nangsuay et al.

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(2011) found that yolk absorption in absolute value (g) and percentage on d 18 and at hatch of embryos and chicks of the old flocks were higher than that of the young flocks.

During incubation, optimum embryo development reflects to chick quality at hatch. Also, breeder age affects yolk-free BW, chick weight, and chick length at hatch (Suarez et al., 1997; Hill, 2001; O'Dea et al., 2004; Tona et al., 2004). Recently, chick quality has increasingly gained importance for hatcheries and broiler producers because it has been accepted as an indicator of broiler performance. In practice, chick weight and chick length are largely measured to evaluate chick quality because there is a critical relationship between 1-d-old chick quality and posthatch broiler performance (Tona et al., 2003; Meijerhof, 2009).

The process of yolk fat utilization has been extensively studied (Noble and Cocchi, 1990; Lin et al., 1991; Speake et al., 1998). Yadgary et al. (2010) observed a higher ratio of fat in fresh yolk derived from old flock eggs (50 wk) than that of young flock eggs (30 wk). These results indicated that hens from an older flock deposit more yolk and fat in their eggs compared with eggs from a younger flock. This is associated with changes in yolk size and energy content of the yolk.

Breeder hen age affects the fatty acid content of egg yolks and the utilization of fatty acids by embryos (Nielsen, 1998; Latour et al., 2000; Burnham et al., 2001; Yalçın et al., 2008; Yadgary et al., 2010). Deeming and Ferguson (1991) reported that the ratio of total free fatty acids is 0.9% of yolk lipids. Noble et al. (1988) noted that the oleic acid concentration in the yolk sac of chick embryos decreases with breeder age. Latour et al. (1998) found that palmitic and stearic acid concentrations are higher in the yolks of eggs from breeders of 51 and 64 wk of age than in eggs from breeders of 36 wk of age.

During incubation, numerous changes occur in the yolk fatty acid composition (Noble and Shand, 1985; Noble and Cocchi, 1990). The interconversions of fatty acids in the yolk sac membrane occur during embryogenesis. These changes in yolk fatty acids may result from changes in the activities of various enzymatic processes occurring in yolk sac membranes (Latour et al., 1998). It is known that linoleic acid is converted to arachidonic acid by Δ^6-desaturase activity, and that stearic acid is converted to oleic acid by the Δ^9-desaturase activity of the yolk sac membrane (Noble and Shand, 1985; Noble and Cocchi, 1990; Peebles et al., 1999).

Previous research has been carried out to determine the effects of breeder age on yolk absorption and embryo development (Nangsuay et al., 2011), or changes in yolk and yolk sac lipid and fatty acid compositions by broiler breeder age (Burnham et al., 2001; Yadgary et al., 2010). Little research has investigated the effects of broiler breeder age on changes in fatty acid composition of yolk sac, yolk absorption, embryo development during incubation, and chick quality at hatch. Therefore, the objective of the present study was to examine the changes in yolk and yolk sac fatty acid composition and furthermore to investigate egg content, yolk absorption, embryo development during incubation, and chick quality at hatch in eggs from 36- and 52-wk-of-age broiler breeders.

MATERIALS AND METHODS

A total of 1,500 hatching eggs were obtained from commercial Ross 308 broiler breeder flocks at 36 wk and 52 wk of age on the same day. The breeder flocks received a broiler breeder diet with 2,750 kcal of ME/kg and 14.50% CP. The 2 flocks were kept under the same management conditions according to the breeding company's recommendations (Aviagen, 2010). The eggs ranged from 58.0 to 62 g in the 36-wk-of-age group and from 64.0 to 68.0 g in the 52-wk-of-age group and were weighed with ±0.1 precision one by one. The eggs from each flock age group were randomly placed into incubator trays consisting of 150 eggs (n = 5 trays/each breeder age). Eggs were stored at 16°C and 65% RH for 3 d and were then warmed to room temperature (21°C) for 8 h before setting.

Incubation and Hatching

Eggs were incubated in a commercial hatchery in the same incubator (87,400-egg capacity, multi-stage setters, Fix shelf Chickmaster, Chick Master Limited, Bridgwater, UK) at 37.5°C and a RH of 55 to 60% during the first 18 d of incubation. A total of 10 incubator trays (n = 5 trays/breeder age) with 150 eggs were randomly placed in the incubator. On d 18 of the incubation, incubator trays were weighed to determine egg weight loss and then transferred to the hatcher (87,400-egg capacity, hatchers, Fix shelf Chickmaster). On hatching day, the chicks were pulled out according to standard hatchery procedures.

Measurements

From each breeder age group, 25 egg samples representing the weight distribution of all selected eggs were randomly taken before incubation to determine fresh egg composition. Eggs were weighed and then opened to measure fresh yolk and albumen weight. The yolk samples were stored at −20°C for further fatty acid analysis.

A total of 25 eggs per breeder age were randomly sampled on d 18 of incubation. After sampling, to ensure optimum airflow, the remaining empty places in the trays were settled with nonexperimental hatching eggs that were marked. The eggs were opened and embryos from each treatment group were killed by cervical dislocation. The embryos were carefully separated from the yolk sac. The yolk content was not separated from the yolk sac membrane, and both were recognized as

the yolk sac. Excessive embryonic fluid was dried off with absorbent paper and the embryos were weighed for embryo weight, yolk sac weight, and yolk-free BW to calculate relative embryo and yolk sac weights (Willemsen et al., 2010; Torres et al., 2012). Embryo length was measured from the tip of the beak to the tip of the middle toe by placing the embryo face down on a flat surface and straightening the right leg (Hill, 2001; Nangsuay et al., 2011). The yolk absorption was calculated with the mean initial yolk weight as follows:

$$\text{relative yolk sac weight (\%)} = (\text{yolk sac weight/initial yolk weight}) \times 100;$$

$$\text{yolk absorption (g)} = \text{initial yolk weight} - \text{yolk sac or residual yolk weight (g)};$$

$$\text{relative yolk absorption (\%)} = (\text{yolk absorption/initial yolk weight}) \times 100;$$

$$\text{relative embryo weight (\%)} = (\text{embryo weight/initial egg weight}) \times 100;$$

$$\text{yolk-free BW (g)} = \text{embryo weight (g)} - \text{yolk absorption (g); and}$$

$$\text{relative yolk-free BW (\%)} = (\text{yolk-free BW/initial egg weight}) \times 100.$$

On hatching day, after completing the hatching process, a sample of 5 chicks per hatching basket (total = 25 chicks/breeder age) were killed by cervical dislocation to determine chick weight and length, residual yolk sac weight, and yolk-free BW. Chick length was measured in the same way as the embryo. The yolk sac (on d 18 of incubation) and residual yolk sac (on hatching day) samples were stored at −20°C for further fatty acid analysis.

$$\text{Relative residual yolk sac weight (\%)} = (\text{residual yolk sac weight/initial yolk weight}) \times 100.$$

$$\text{Relative chick weight (\%)} = (\text{chick weight/initial egg weight}) \times 100.$$

$$\text{Yolk-free BW (g)} = \text{chick weight (g)} - \text{residual yolk sac weight (g)}.$$

$$\text{Relative yolk-free BW (\%)} = (\text{yolk-free BW/initial egg weight}) \times 100.$$

Fatty Acid Analyses

A total of 3 g each of the fresh yolk, yolk sac, and residual yolk sac samples (n = 5 samples per each breeder age) was weighed and placed into 50-mL screw-cap tubes. Then, 30 mL of Folch-I solution ($CHCl_3$:CH_3OH, 2:1; Folch et al., 1957) was added. Each tube was vortexed for 1 min and stored overnight at room temperature. After the mixture was filtered with a Whatman 1 filter (FilterLab, Barcelona, Spain) into a 100-mL volumetric flask, the tube was washed twice with 8 mL and 4 mL of Folch-I solution; the filter was also washed with 4 mL of Folch-I solution. Then, 10 mL of saline solution (0.88% NaCl) and 2 mL of Folch-I solution were added into the volumetric flask and vortexed. After the mixture was stored overnight at room temperature for phase separation, the top layer was carefully siphoned off. The bottom layer was moved into a Petri dish and was evaporated under a fume cupboard. Then, it was placed in an oven at 40°C for 2 h. The dried lipids (0.4 mL) were resolubilized in 1 mL of boron trifluoride-methanol (BF_3-methanol; Cherian et al., 2002). The tubes were heated in a water bath at 90 to 100°C for 60 min. When each tube was cooled, 3 mL of hexane and 5 mL of distilled water were added. The sample was mixed and allowed to separate into layers. The hexane (upper) layer (1 mL) was transferred to a vial for analysis. Fatty acid methyl esters (**FAME**) were separated and quantified by gas chromatography. A gas chromatograph (Agilent 6890N Series) was used to analyze the FAME and was equipped with a flame ionization detector (Agilent Technologies Inc., Wilmington, DE). It was operated at a temperature of 120°C for 2 min, followed by heating at 20°C/min to 220°C and holding for 45 min. A HP 5-MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies Inc.) was used for the analyses. The injector and flame ionization detector were maintained at 250 and 300°C, respectively. Identification of sample FAME was performed by comparing the retention times to FAME standards (Mixture ME-100; Greyhound Chromatography and Allied Chemicals, Birkenhead, Merseyside, UK). The fatty acid content of the egg yolk, yolk sac, and residual yolk sac are given as percentages of total fatty acids.

All procedures of this study for the care and use of animals were in accordance with the laws and regulations of Turkey and were approved by the Ethical Committee of the Uludağ University (license number 2012–07/01).

Statistical Analyses

All data were analyzed according to *t*-test (SAS Institute Inc., 1989). Analyses for percentage data were conducted after a square root of arc sine transformation of the data. Data are presented as means ± SE.

Table 1. The effect of breeder age on egg weight and egg content ($\bar{X} \pm$ SEM)

Characteristic[1]	Breeder age (wk) 36	52	*P*-value
Egg weight (g)	60.2 ± 1.22	65.5 ± 0.63	<0.01
Albumen weight (g)	35.3 ± 0.80	37.9 ± 0.35	<0.01
Albumen percentage (%)	58.6 ± 0.77	57.9 ± 0.77	0.001
Yolk weight (g)	19.5 ± 0.45	21.9 ± 0.60	<0.01
Yolk percentage (%)	32.4 ± 0.58	33.4 ± 0.72	<0.01
Yolk:albumen ratio (%)	0.55 ± 0.02	0.58 ± 0.02	<0.01

[1]All variables measured with 25 eggs in each breeder age.

Table 2. The effect of breeder age on the yolk fatty acid composition (%) before incubation[1]

Fatty acid (%)	Breeder age (wk) 36	52	*P*-value
Myristic (14:0)	0.19 ± 0.006	0.11 ± 0.001	<0.01
Palmitic (16:0)	26.80 ± 0.18	24.31 ± 0.02	<0.01
Palmitoleic (16:1n-7)	1.74 ± 0.02	1.24 ± 0.008	<0.01
Heptadeconoic (17:0)	0.18 ± 0.006	0.26 ± 0.002	<0.01
Stearic (18:0)	15.37 ± 0.08	17.43 ± 0.02	<0.01
Oleic (18:1n-9)	29.24 ± 0.67	28.14 ± 0.03	0.013
Linoleic (18:2n-6)	13.60 ± 0.11	13.02 ± 0.03	<0.01
Linolenic (18:3n-3)	0.22 ± 0.005	0.15 ± 0.001	<0.01
Arachidonic (20:4n-6)	5.78 ± 0.08	7.04 ± 0.04	<0.01

[1]n: 5 fresh yolk/breeder age.

In all cases, a difference was considered significant at $P \leq 0.05$.

RESULTS

Egg Content and Yolk Fatty Acid Composition Before Incubation

The mean egg weight was found to be 60.2 and 65.5 g in eggs from breeders of 36 and 52 wk of age, respectively ($P < 0.01$; Table 1). As breeder hens aged, the albumen weight increased, but the albumen percentage decreased. The albumen weight and percentage were determined to be 35.3 and 37.9 g and 58.6 and 57.9% in eggs from the 36- and 52-wk-of-age groups, respectively ($P < 0.01$; $P = 0.001$). Breeder age significantly affected the yolk weight and yolk percentage. The yolk weight and percentage were found to be 19.5 g and 32.4% in eggs from the 36-wk-of-age group and 21.9 g and 33.4% in eggs from the 52-wk-of-age group, respectively ($P < 0.01$). The yolk:albumen ratio was found to be 0.55 and 0.58% in eggs from the 36- and 52-wk-of-age groups ($P < 0.01$).

The effects of breeder age on yolk fatty acid composition before incubation are presented in Table 2. It was found that breeder age significantly affected all of the detected fatty acid concentrations in fresh yolk. In the study, palmitic, stearic, oleic, and linoleic acids were major fatty acids in the yolk and the concentrations of these fatty acids were found to be 26.80, 15.37, 29.24, and 13.60% in the 36-wk-of-age group; and 24.31, 17.43, 28.14, and 13.02% in the 52-wk-of-age group, respectively ($P < 0.05$; $P < 0.01$). The concentration of palmitoleic acid was higher in the 36-wk-of-age (1.74%) group, whereas the arachidonic acid was higher in the 52-wk-of-age (7.04%) group. The remaining fatty acids (myristic, heptadeconoic, and linolenic acids) were found to be below 1%.

Yolk Sac and Residual Yolk Sac Fatty Acid Compositions

On d 18 of incubation, breeder age significantly affected all of the detected fatty acid concentrations of the yolk sac except heptadecanoic and arachidonic acids (Table 3). The concentrations of stearic, oleic, linoleic, and linolenic acids were found to be higher in 36-wk-of-age group (17.20, 32.34, 13.55, and 0.27%, respectively) than the 52-wk-of-age group (16.43, 31.24, 12.83, and 0.14%, respectively; $P < 0.05$, $P < 0.01$). The myristic, palmitic, and palmitoleic acid concentrations were found to be higher in the 52-wk-of-age group (0.24, 26.44, and 2.47%, respectively) compared with the 36-wk-of-age group (0.20, 24.74, and 1.59%, respectively; $P < 0.01$). However, the concentrations of heptadeconoic and arachidonic acids were similar in both breeder age groups.

At hatch, the effect of breeder age on the fatty acid composition of the residual yolk sac was found to be

Table 3. The effect of breeder age on the yolk fatty acid composition (%) on d 18 and at hatch[1]

Fatty acid (%)	On d 18 36 wk	52 wk	*P*-value	At hatch 36 wk	52 wk	*P*-value
Myristic (14:0)	0.20 ± 0.02	0.24 ± 0.005	0.003	0.30 ± 0.001	0.15 ± 0.002	<0.01
Palmitic (16:0)	24.74 ± 0.83	26.44 ± 0.29	0.005	26.08 ± 0.04	26.10 ± 0.23	NS[2]
Palmitoleic (16:1n-7)	1.59 ± 0.06	2.47 ± 0.08	<0.01	1.82 ± 0.008	1.30 ± 0.01	<0.01
Heptadeconoic (17:0)	0.18 ± 0.01	0.19 ± 0.002	NS	0.19 ± 0.006	0.20 ± 0.004	0.023
Stearic (18:0)	17.20 ± 0.03	16.43 ± 0.14	<0.01	16.55 ± 0.07	17.17 ± 0.16	0.001
Oleic (18:1n-9)	32.34 ± 0.78	31.24 ± 0.37	0.039	32.68 ± 0.03	29.93 ± 0.33	<0.01
Linoleic (18:2n-6)	13.55 ± 0.46	12.83 ± 0.10	0.026	13.55 ± 0.04	13.90 ± 0.05	0.001
Linolenic (18:3n-3)	0.27 ± 0.02	0.14 ± 0.02	0.050	—	—	—
Arachidonic (20:4n-6)	5.03 ± 0.07	4.85 ± 0.30	NS	4.57 ± 0.04	5.74 ± 0.08	<0.01

[1]n: 5 yolk sac/breeder age.

[2]NS: $P < 0.05$.

significant except for palmitic and linolenic acids (Table 3). The myristic, palmitoleic, and oleic acid concentrations in the residual yolk sacs of embryos from breeders of 36 wk of age (0.30, 1.82, and 32.68%, respectively) were found to be higher compared with the 52-wk-of-age group (0.15, 1.30, and 29.93%, respectively; $P < 0.01$). On the other hand, heptadecanoic, stearic, linoleic, and arachidonic acid concentrations were found to be higher in the residual yolk sacs of embryos from those of the 52-wk-of-age (0.20, 17.17, 13.90, and 5.74%, respectively) compared with the 36-wk-of-age group (0.19, 16.55, 13.55, and 4.57%, respectively; $P < 0.05$; $P < 0.01$).

Yolk Absorption and Embryo Development

On d 18 of incubation, it was found that breeder age significantly affected yolk sac weight and yolk absorption (Table 4). Yolk sac weight and yolk absorption were found to be higher in embryos from the 52-wk-of-age group, at values of 12.8 and 9.1 g, respectively ($P < 0.01$). However, relative yolk sac weight and relative yolk absorption were found to be similar in embryos from each breeder age group. At hatch, breeder age significantly affected residual yolk sac weight, relative residual yolk sac weight, yolk absorption, and relative yolk absorption ($P < 0.01$; Table 4). These parameters, with the exception of relative yolk absorption, were found to be higher in chicks from the 52-wk-of-age group. The residual yolk sac weight was found to be 3.7 and 4.8 g and the relative residual yolk sac weights to be 19.0 and 21.9% in chicks from the 36- and 52-wk-of-age groups, respectively ($P < 0.01$). The yolk absorption was found to be 15.8 and 17.1 g in chicks from the 36- and 52-wk-of-age groups, respectively. Relative yolk sac absorption was found to be higher with a value of 81.0% in chicks from 36-wk-of-age breeders ($P = 0.010$).

The embryo and chick weight, yolk-free BW, and embryo and chick length on d 18 and at hatch are presented in Table 5 by breeder age. On d 18 of incubation, embryo weight, relative embryo weight, yolk-free BW, relative yolk free BW, and embryo length were found to be higher in embryos from the 52-wk-of-age group ($P < 0.01$). It was found that embryo weight averaged 28.8 and 32.5 g, relative embryo weight 47.8 and 49.6%, yolk-free BW 20.8 and 23.4 g, relative yolk-free BW 34.5 and 35.7%, and embryo length 16.5 and 17.3 cm in chicks from the 36- and 52-wk-of-age groups, respectively. At hatch, chick weight, relative chick weight, yolk-free BW, and relative yolk-free BW were found to be higher in chicks from breeders of 52 wk of age than those of 36 wk of age ($P < 0.01$; Table 5). Chick weight and relative chick weight were found to be 40.4 g and 67.1% and 47.8 g and 73.0% in chicks from the 36- and 52-wk-of-age groups, respectively. Yolk free-BW was found to be 36.7 and 43.0 g in chicks from breeders of 36 and 52 wk of age, respectively. A similar difference was observed for relative yolk-free BW, at 61.0 and 65.6%, respectively. Chick length averaged 19.6 and 21.0 cm in chicks from the 36- and 52-wk-of-age groups, respectively.

DISCUSSION

The differences in fresh egg content, yolk, and yolk sac fatty acid composition, yolk absorption, embryonic development, and chick quality were examined in eggs obtained from 36- and 52-wk-of-age broiler breeders. Our study's results indicated that egg weight, yolk and albumen weight, yolk ratio, and yolk:albumen ratio increased as breeder age increased, but the albumen ratio decreased. This observation is consistent with results from previous studies, which demonstrated that hens progressing through the production period lay larger eggs (Shanawany, 1984; French and Tullett, 1991; Vieira and Moran, 1998a,b; Tona et al., 2004) with larger yolks (O'Sullivan et al., 1991; Suarez et al., 1997) with less albumen. This result is caused by the increase in

Table 4. The effect of breeder age on yolk sac weight and yolk absorption on d 18 and at hatch ($\bar{X} \pm$ SEM)

		Breeder age (wk)		
Incubation day	Parameter[1]	36	52	*P*-value
Before incubation	Initial yolk weight (%)	19.5 ± 0.45	21.9 ± 0.60	<0.01
On d 18	Yolk sac weight (g)	11.5 ± 1.35	12.8 ± 0.51	<0.01
	Relative yolk sac weight[2] (%)	59.0 ± 6.21	58.4 ± 3.24	NS[3]
	Yolk absorption[4] (g)	8.0 ± 1.01	9.1 ± 0.80	<0.01
	Relative yolk absorption[5] (%)	41.0 ± 6.21	41.6 ± 3.15	NS
At hatch	Residual yolk sac weight (g)	3.7 ± 0.96	4.8 ± 0.61	<0.01
	Relative residual yolk sac weight[6] (%)	19.0 ± 5.02	21.9 ± 3.04	0.010
	Yolk absorption (g)	15.8 ± 0.69	17.1 ± 0.79	<0.01
	Relative yolk absorption (%)	81.0 ± 5.03	78.1 ± 3.04	0.010

[1]All variables measured with 25 samples at each breeder age.

[2]Relative yolk sac weight (g) = (yolk sac weight/initial yolk weight) × 100.

[3]NS: $P > 0.05$.

[4]Yolk absorption (g) = initial yolk weight – yolk sac or residual yolk weight.

[5]Relative yolk absorption (%) = (yolk absorption/initial yolk weight) × 100.

[6]Relative residual yolk sac weight (g) = (residual yolk sac weight/initial yolk weight) × 100.

Table 5. The effect of breeder age on embryo and chick weight, yolk-free BW, and embryo and chick length on d 18 and at hatch ($\bar{X} \pm$ SEM)

		Breeder age (wk)		
Incubation day	Parameter[1]	36	52	*P*-value
Before incubation	Initial egg weight (g)	60.2 ± 1.22	65.5 ± 0.63	<0.01
On d 18	Embryo weight (g)	28.8 ± 1.50	32.5 ± 0.68	<0.01
	Relative embryo weight[2] (%)	47.8 ± 3.06	49.6 ± 0.81	0.010
	Yolk-free BW[3] (g)	20.8 ± 0.62	23.4 ± 0.74	<0.01
	Relative yolk-free BW (%)	34.5 ± 1.40	35.7 ± 1.14	0.004
	Embryo length (cm)	16.5 ± 0.26	17.3 ± 0.22	<0.01
At hatch	Chick weight (g)	40.4 ± 1.54	47.8 ± 1.19	<0.01
	Relative chick weight[4] (%)	67.1 ± 3.47	73.0 ± 2.01	<0.01
	Yolk-free BW (g)	36.7 ± 2.18	43.0 ± 1.26	<0.01
	Relative yolk-free BW[5] (%)	61.0 ± 4.65	65.6 ± 2.27	<0.01
	Chick length (cm)	19.6 ± 0.11	21.0 ± 0.32	<0.01

[1]All variables measured with 25 embryos and chicks (n: 25 embryos d 18 and 25 chicks at hatch and each breeder age).

[2]Relative embryo weight (%) = (embryo weight/initial egg weight) × 100.

[3]Yolk-free BW (g) = embryo weight (g) − yolk absorption (g).

[4]Relative chick weight (%) = (chick weight/initial egg weight) × 100.

[5]Relative yolk-free BW (%) = (yolk-free BW/initial egg weight) × 100.

the ratio of yolk:albumen in eggs from older breeders (O'Sullivan et al., 1991; Ahn et al., 1997; Peebles et al., 2000; Nangsuay et al., 2011, 2013).

The yolk has vital importance for embryo development and is the only source of lipids for embryo tissue growth (Speake et al., 1998). Noble and Cocchi (1990) noted that almost 94% of the total energy needs of the embryo during development are provided from the oxidation of fatty acids.

In the study, palmitic, stearic, oleic, and linoleic acids were major fatty acids in the fresh yolk ranging from 13.02 to 29.24%. These were followed by palmitoleic and arachidonic acids ranging from 1.24 to 7.04%, with the remaining fatty acids below 1%. Yalçın et al. (2008) found that palmitic, oleic, and linoleic acids were the major fatty acids in yolk, ranging from 17.95 to 36.88%. Our study demonstrated that breeder age affected the fatty acid contents of yolk before and during incubation. The myristic, palmitic, palmitoleic, oleic, linoleic, and linolenic acid concentrations of fresh yolks were higher in eggs from young breeders than those of old breeders. This observation is consistent with results from previous study which demonstrated that the myristic, palmitic, palmitoleic, and oleic acid concentrations of fresh yolk decrease with increasing hen age (Nielsen, 1998). In the study, the stearic and arachidonic acid concentrations of fresh yolk were higher in the 52-wk-of-age group than 36-wk-of-age group. On the contrary, Burnham et al. (2001) showed higher concentrations of stearic and arachidonic acids in the fresh yolks of eggs from 26-wk breeders than those of 28- and 30-wk breeders.

In our study, embryonic development was determined using embryo weight, embryo length, and yolk-free BW measurements. The present study demonstrated that breeder age affected yolk sac weight. On d 18 and at hatch, the residual yolk sac weights of the embryos and chicks of eggs from the older hens were higher than those of the younger hens. On the other hand, the relative yolk sac weight of the chicks of the older hens was higher only at hatch. Thus, our results are similar to Suarez et al. (1997), Vieira and Moran (1998b), Peebles et al. (2001), Sklan et al. (2003), and Hamidu et al. (2007), who showed that the relative residual yolk sac weight of the chicks from the young hens was lower than that of the old hens.

In our study, on d 18 of incubation, the embryos from the older breeders had a higher yolk absorption than those of young breeders, but the relative yolk absorption was similar. However, at hatch, the chicks of the older breeders had a higher yolk absorption but had lower relative yolk absorption compared with those of younger breeders. Also, at hatch the relative residual yolk sac weight was greater in chicks from 52-wk-of-age breeders. This result would suggest that the rate of yolk absorption by embryos from 52-wk-of-age breeders decreased in comparison with those of 36-wk-of-age breeders. This observation is consistent with results from previous studies, which demonstrated that the absorption and utilization of nutrients from the yolk sac by the embryo are affected by breeder age (Latour et al., 2000; Burnham et al., 2001; Yalçın et al., 2008; Yadgary et al., 2010; Nangsuay et al., 2011).

Withdrawal of the yolk sac into the abdomen of the embryo before hatching provides nutrients to the newly hatched avian neonate during the first few days of life (Meijerhof, 2009). At hatch, chick weight is a combination of the real chick weight and the residual yolk sac weight (Meijerhof, 2009). Noy and Sklan (1999) indicated that chicks deprived of food after hatch used approximately 60% of their residual yolk sac in the first 48 h.

Our study demonstrated that breeder age affected the embryo and chick development parameters on d 18 and at hatch. A higher embryo weight, relative embryo weight, and embryo length were found in the older

flock. This observation is consistent with results from previous studies, which demonstrated that increases in the development and weight of embryos correlate with increasing breeder age (Wilson, 1991). In contrast, Nangsuay et al. (2011) found that the influence of breeder age on embryonic development disappeared in the later stages of incubation. This study indicated that the yolk-free BW on d 18 and at hatch increased with breeder age. This can be explained by a higher yolk absorption and yolk availability more greatly influencing the yolk-free BW from eggs of older hens compared with younger hens on d 18 and at hatch. These findings are consistent with results demonstrating that the larger yolk sac membranes and vascular system of the large yolk compared with the small yolk may affect yolk nutrition utilization, which may cause higher yolk absorption and a heavier yolk-free BW at d 18 and at hatch (Noble and Cocchi, 1990).

The quality of the newly hatched chick is a major factor in determining its livability, growth, and health. At hatch, chick weight, chick length, and yolk-free BW were measured as indicators of 1-d-old chick quality (Deeming, 2000; Wolanski et al., 2003; Willemsen et al., 2008). Our study demonstrated that chick weight and length, and yolk-free BW were higher in the old flock with a difference of 7.4 g, 1.4 cm, and 6.3 g, respectively, at hatch compared with the young flock. This finding is in agreement with the results of other studies that demonstrated a strong relationship between breeder age and chick weight (Suarez et al., 1997; Ulmer-Franco et al., 2010). In addition, it has been stated that chick weight at hatch is an accurate predictor of final broiler BW (Decuypere et al., 2002; Sklan et al., 2003; Meijerhof, 2006). Chick length has a substantially higher positive correlation with broiler performance than 1-d-old chick weight (Meijerhof, 2009). Additionally, at hatch chicks from 52-wk-of-age breeders were found to be longer with higher yolk-free BW than those from 36-wk-of-age breeders. This observation is consistent with results from previous study demonstrating that chick length correlated well with yolk-free BW at hatch (Wolanski et al., 2003).

In this study, the effects of broiler breeder age on yolk sac fatty acid contents were found to be significant on d 18 of incubation and also at hatch. There was an approximate 2.13 and 3.41% increase in total concentration of fatty acids in yolk sac on d 18 compared with fresh eggs in 36- and 52-wk-of-age flocks, respectively. The remarkable changes in fatty acids was observed for oleic and arachidonic acids between initial and d 18. The concentrations of these fatty acids were found to be higher in the yolk sac of the 36-wk-of-age flock compared with the 52-wk-of-age flock. Burnham et al. (2001) found that the oleic acid concentration was higher in the yolk sacs of embryos from breeders at 30 wk of age than from those at 26 wk of age on d 16 and 18 of incubation. Our findings may have resulted from the observed changes in the yolk sac fatty acid

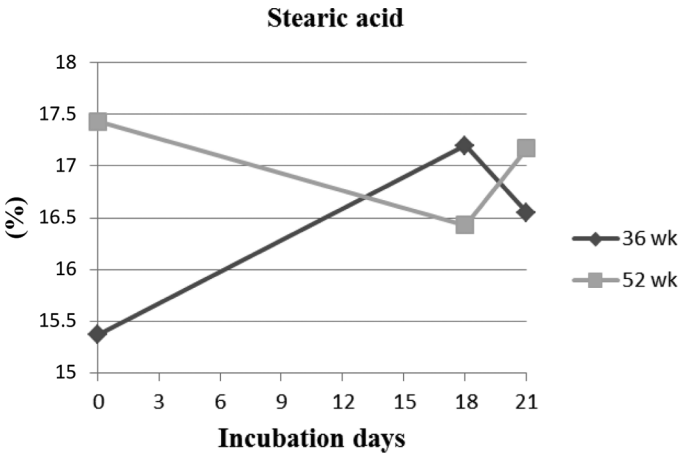

Figure 1. Comparison of changes in stearic acid concentration of yolk during incubation between eggs from flocks at 36 and 52 wk of age.

content compared with the fresh yolk fatty acid content due to broiler breeder age affecting lipid utilization by embryos (Noble and Cocchi, 1990; Latour et al., 1998).

In the present study, there was an approximate 2.8 and 3.04% increase in total concentration of fatty acids in residual yolk sac at hatch compared with fresh eggs in 36- and 52-wk-old flocks, respectively. During incubation, numerous changes occur in the yolk fatty acid composition (Noble and Shand, 1985; Noble and Cocchi, 1990). The palmitic, stearic, oleic, and linoleic acids were found as major fatty acids during incubation. Between d 18 and hatching day, oleic acid concentration decreased by 4.19% in the 52-wk-old flock, whereas it increased by 1.05% in the 36-wk-old flock. This result agrees with Noble et al. (1988), who found a reduction in the oleic acid concentration of the residual yolk sacs of chicks and that this correlated with increasing breeder age. Ding and Lilburn (1996) also reported that oleic acid was a major component of yolk fatty acids during incubation. In the present study, there was a de-

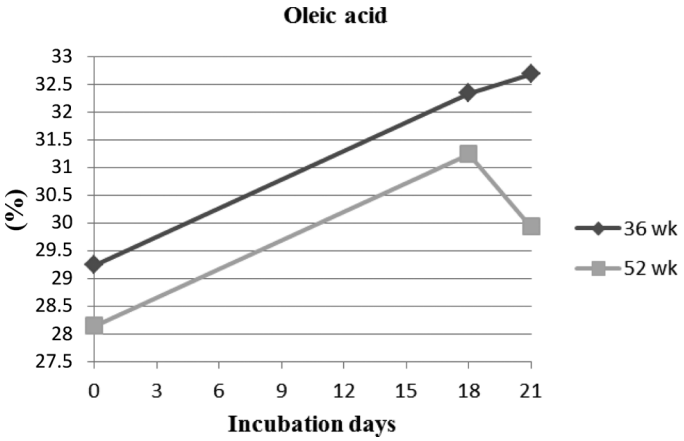

Figure 2. Comparison of changes in oleic acid concentration of yolk during incubation between eggs from flocks at 36 and 52 wk of age.

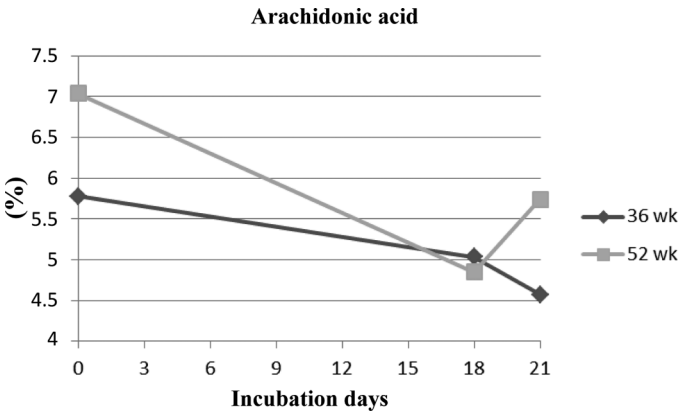

Figure 3. Comparison of changes in arachidonic acid concentration of yolk during incubation between eggs from flocks at 36 and 52 wk of age.

crease in stearic acid concentration, whereas there was an increase in oleic acid concentration in 36-wk-of-age breeders between d 18 and hatching day (Figures 1 and 2). This finding is supported by the results of Noble and Cocchi (1990), who noted that Δ^9-desaturase enzyme activity is functional for converting stearic acid to oleic acid. It is expected that a decrease in stearic acid concentration and an increase in oleic acid concentration occur during incubation.

Arachidonic acid is essential for embryonic development and chick growth and is synthesized from linoleic acid (Speake et al., 1998; Speake and Wood, 2005). In our study, at hatch, the higher arachidonic acid concentration was found in the residual yolk sacs of chicks from 52-wk-of-age breeders (Figure 3). It is uncertain that this finding may result from elevated Δ^6-desaturase activities in the yolk sac membrane or from a lower rate of yolk absorption. In our study, it can be seen that compared with before incubation, there was an approximate 20.94 and 18.47% decline in arachidonic acid concentration of yolk sac from 36- and 52-wk-of-age flocks, respectively. Arachidonic acid was more efficiently transported to the embryo from the yolk sac during incubation. Cherian and Sim (1993) showed that arachidonic acid concentration decreased. The results also showed selective absorption of some fatty acids by the embryo (Cherian and Sim, 1993; Van Elswyk et al., 1994; Yalçın et al., 2008).

In conclusion, in our study, broiler breeder age affected yolk absorption, changes of fatty acid concentrations, the development of embryos during incubation, and chick quality at hatch. We demonstrated that embryos of the younger and older flocks had different patterns of yolk absorption and changes in yolk sac fatty acid concentrations, which may be due to the lower initial yolk weight and differences in yolk nutrients in the younger flock. In our study, higher certain absorption of yolk resulted in a higher chick weight, yolk-free BW, and length in the older flock. Chick weight and length were measured for chick quality assessment. Chick quality is known to be vital for better broiler performance, and it therefore has great importance for the profitability of producers.

It is not clear how broiler breeder age affects the changes in yolk sac fatty acid concentrations during incubation. These changes are still unclear due to differences in the yolk absorption ratio or in the activities of various enzymatic process in yolk sac membranes. Because of this, more detailed studies considering various factors such as breeder age, egg weight, breeder line, and incubation conditions are needed to investigate the changes and utilization of fatty acids in the yolk sac by embryos during incubation.

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