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Embryo Specifications at 19-d of Incubation in Different Ross Boiler Breeders Age

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***Abstract* – 480 hatching eggs were obtained from commercial, Ross 308 breeder at 33 and 53 weeks of age, 240 eggs for each breed age. Eggs individually weighed before and after incubation and incubated following usual hatchery practices. Individual embryo weight was recorded at 19d of incubation, relative embryo weight was calculated. A sample of embryo and residual yolk sac was used for measurement of chemical composition in the yolk and albumen base on method of AOAC, 1990. A completely randomized design was used. Eggs weight from Old group was heavier (P<0.05) compared to eggs from Young group, 63.02 and 52.72 g, respectively. Result indicated that embryo from O group had the heaviest weights in compare with the Y group, 36.16 g and 31.48 g respectively. There was no significant differences (P>0.05) between Y and O group in embryo dry matter, ash, fat, crude protein and nitrogen free extract content, although embryo fat content were increased numerically. There was significant differences (P<0.05) in embryo length at 19-d of incubation so embryo from O group breeders are found to had more embryo length than embryo in Y group. There was no significant differences (P>0.05) between Y and O group in heart weight. Our result revealed that as breeder age advanced, embryo weight, length increased, and embryo from old group had more residual yolk sac and this may cause more malabsorbtion of yolk sac during incubation.**

***Keywords* – Embryo, Specification, Ross Broiler Breeder.**

I. Introduction

The avian egg is a package of all essential nutrients required for development ofthe embryo into a viable chick. An egg weighing 60 g comprises, on average, 6.5 g of shell (11%), 18.5 g yolk (31%) and 35 g of albumen (58%) by weight. Further, ~56% of the carbohydrate present in albumen is protein-bound [12]. Breeder hens in the beginning of their productive life tend to produce eggs with a reduced size. These fertile but small eggs when the demand for broiler chicks is great, however, it is common for small eggs to be incubated. Variables related to the composition and incubation of eggs from breeder hens of different ages are still poorly studied [17]. Eggs with more variable weights can be found within a young breeder flock and have been attributed to lack of uniformity in the flock [3]. Avian embryos develop and grow from energy and nutrients stored in the egg by the hen. In this sense, breeder male contribution is not important. Characteristics related to composition of these eggs can be different because of egg size, and their utilization to produce broiler chicks can be acceptable. An increase in egg weight represents an increase in weight of the hatched chick [1]. An evaluation of indices related to egg composition analysis and fertility, is required when these eggs are to be used for the production of broiler chicks. The embryo from each of these parts mobilizes specific nutrients. It is generally agreed that each egg is built with a complete capacity to produce aperfect new organism. However, due to several reasons, this is not always the case. Research on this matter is recent and usually directed to metabolic interventions. Among the broiler strains used for meat production, Ross are actually the most widely produced worldwide. It is not clear if the different post-hatch performances between the breeder different ages are also a reflection of their embryo physiological and hatching parameters. The objective of the present study was to evaluate embryo specification at 19-d of incubation from Ross 308 breeder birds in different ages.

II. Materials and Methods

Two flocks of Ross 308 female broiler breeders at 33(young) and 53 (old) wks. of age were housed in two deep litter curtain sided house system and birds were fed according to the Ross 308 Manual recommendations, lighting in the house was 17 h light: 7 h dark operating from 0400 h to 2100 h each day. The birds were fed at 0400 h daily and water was available for ad libitum consumption.

A. Eggs and incubation

A total of 480 eggs, provided by commercialbroiler breeders' flock were used for this study. Half (240) of the eggs were collected from a commercial flock of Ross broiler breeders flock aged 33 weeks (Young,Y) and the other half (240) were from a commercial flock of Ross broiler breeders at 53 weeks (old,O) of age. Eggs from Ross breeder hen flocks submitted to a similar management and diet program was collected from a commercial hatchery. In all flocks, male breeders were introduced at a male to female ratio of 1:8 and the lighting program was 16h in day. Eggs were stored for 2-d at 18 °C and 75% of relative humidity, then numbered and weighed before incubation.

Eggs from each breeder age were randomly divided into 6 replicate egg trays with 40 eggs each and were incubated at 37.6 °C and 56% of relative humidity.The individually weighed and identified eggs were randomly allotted to incubators (Petersime) tray in same level adjusted to 37.5 °C and 65% relative humidity.Within the experiment, air and humidity condition was similar for all incubated eggs.

B. Experimental observations

Prior to incubation, 10 eggs from each age wereselected and weighed. At19 days of incubation, after the transfer phase, the egg fertility was exanimated by candling then from each group 20 egg that had embryo was selected for further analysis. Selected eggs from each group were bro-

ken from air sac carefully in clean plate. Embryos were separated and weighted. Residual yolk weights (percentage) were separated from embryo and measured and relative residual yolk sac weights were calculated by dividing absolute weight to embryo weight. Embryos were re-moved and weighed then the relative embryo weight was determined by dividing the embryo weight to egg weight. Embryo length (EL) was measured based on length from beak to nail in embryo. Heart weight (HW), liver weight (LW) and gizzard weight (GW) of embryo was measured then after homogenization of embryo body from each replicate, a sample of the embryo was used for measurement of chemical composition based on AOAC, 1990 recommended methods. Embryo dry matter (EMDM), embryo ash (EMASH), embryo crude fat (EMEE), embryo crude protein (EMCP) were determined and embryo nitrogen free extract (EMNFE) was calculated based on difference method(1). Samples of residual yolks were taken from each replicate, to measure the chemical composition of residual yolk. Residual yolk sac dry matter (RYDM), ash (RYASH), crude protein (RYCP) were determined and residual yolk sac nitrogen free extract (RYNFE) was calculated based on difference method(1).Embryo and residual yolk sac lipids were extracted by the Folch method [4] as modified by [19]. It has previously been confirmed that this method results in the exhaustive extraction of all lipids from tissue samples [5], resulting in 99% recovery of lipid. 0.5 g (fresh mass) of yolk was thoroughly homogenized in 20 ml of methanol, then 40 ml of chloroform was added and the homogenization repeated. The homogenate was filtered through filter paper and the filtrate was collected. The solid residue was re-homogenized in 60 ml of chloroform/methanol (2:1, v/v) followed by filtration. The combined filtrates were washed with 30 ml of an aqueous solution of 0.88% (w/v) KCl and the mixture was thoroughly shaken and allowed to settle. Theupper aqueous phase, containing non-lipid components such as sugars and salts, was removed. The lower phase, containing the lipid, was dried by rotary evaporation and the lipid was re-dissolved in 10 ml chloroform. Some 5 ml of this chloroform extract was added to a pre-weighed flask, the sample was dried by rotary evaporation, and the mass of total lipid determined gravimetrically.

III. Statistical Analysis

Quantitative data were analyzed by the PROC GLM procedure, of the SAS software (2006)[14]. Each egg was considered one replicate. Egg weight was assigned as covariate factor in related parameters at 19-d of incubation. Treatment effect ($P \leq 0.05$) were separated using the Duncan multiple range test option of SAS 2006 with an α of 0.05[14].

IV. Result and Discussion

A. Embryo characteristics

Egg weight at pre-incubation stage and embryo weights at 19-d of incubation were shown in table 1. Eggs weight (EW) from Old group was heavier ($P<0.05$) compared to eggs from Young group, 63.02 and 52.72 g, respectively (Table 1). Egg weight is important for their effect on time needed for incubation in broiler breeders so incubation time can be shorter in eggs from young breeders and vice versa for older breeders [15]. Egg size has a positive effect on body weight at hatch and subsequent growth rate of hatchlings; however, this correlation decreases with increasing chick age [1, 2]. Some researchers revealed that an increase in egg weight represents an increase in weight of the hatched chick [16]. This result was in consistent with [6] who found this characteristic exhibited a negative trend-cycle. This may be due to the smaller eggs size of young breeders.

In the present study, a significant effect of breeder age on embryo weights was observed (Table 1).This effect indicated that embryo from O group had the heaviest weights in compare with the Y group, 36.16 g and 31.48 g respectively. As described in the literature, eggs from young breeders produced smaller embryos. Our result revealed that as breeder age advanced, embryo weight increased. This finding is in agreement with previous reports [10, 18, 20, 21]. There was no significant difference ($P>0.05$) between Y and O group in relative embryo weight (Table 1). There was significant difference ($P<0.05$) between Y and O group in residual yolk sac weight (RYW) in the other hands breeder age affected RRYW, which was 9.87 and 12.99 g for Y and O groups, respectively ($P<0.05$). Result revealed that breeder age had significant effect on relative residual yolk sac weight (RRYW) at 19-d of incubation in Ross breeders. Peebles et al. (2001) stated that greater relative yolk sac weight coincided with a lower relative dry matter of embryos from old breeders and this might be further refer to slower embryonic growth in conjunction with a slower utilization of yolk. The residual yolk sac contains 50% lipids and accounts for 20 – 30 % of the chick's body weight at hatch [7]. It is considered it to be the primary energy source for the neonatal chicks, with 90% being utilized within the first two days of hatch. The residual yolk sac is utilized via two routes: 1) transfer to the bloodstream via endocytosis of lipid droplets and 2) transfer to the intestines via the yolk stalk [9].Relative residual yolk sac weights was differ significantly ($P<0.05$) between O and Y group, so the O group (19.38) in compared with Y group (17.01). This result may be due to difference between treatments in residual yolk sac weight (Table 1).

The effect of breeder flock age on embryo specification at 19-d of incubation was shown in table 2. There was significant differences ($P<0.05$) in EL at 19-d of incubation so embryo from O group breeders are found to had more EL than embryo in Y group. There was no significant differences ($P>0.05$) between Y and O group in HW. There was significant different ($P<0.05$) in LW of embryo in different breeder age, so liver weight in O group was more than liver weight in Y group so large egg embryos are found to accumulate more fat in liver compared to small egg embryos [13]. This may be due to this fact that at the end of incubation, young breeders'

embryos accumulate residual lipids in the yolk sac, whereas in older breeder's embryos the highest amount of lipids is found in the liver [8]. Gizzard weights were increased numerically in O group compared to Y group but didn't differ significantly.

Table 1: Effects of Maternal Age on Embryo Parameters at 19-d in RossBroiler Breeder 1

Treatment	Egg Weight2 (g)	Embryo Weight (g)	Relative Embryo Weight (g/g)	Residual yolk sac Weight (g)	Relative Residual yolk sac Weight4 (g/g)
Young-31w	60.42b	31.48 b	54.27	9.87 b	17.01 b
Old- 51w	67.41 a	36.16 a	53.97	12.99 a	19.38 a
CV	7.61	2.08	16.95	17.97	18.08
SEM	1.30	0.651	1.121	0.649	0.848
P-Value	0.0006	<0.0001	0.258	0.0033	0.048

1- Means in the column with same superscript did not differ significantly (P> 0.05).

Table 2: Effects of Maternal Age on Embryo Parameters at 19-d in Ross Broiler Breeder 1

Treatment	Embryo Length (cm)	Heart Weight (g)	Liver Weight (g)	Gizzard Weight (g)
Young-31w	18.69b	0.191	0.6040 b	1.217
Old- 51w	19.71a	0.218	0.6584 a	1.396
CV	4.08	16.95	17.97	18.08
SEM	0.013	0.007	0.012	0.136
P-Value	<0.0001	0.108	0.010	0.102

1- Means in the column with same superscript did not differ significantly (P> 0.05).

B. Embryo and residual yolk sac chemical composition

The effect of breeder flock age on embryo composition was shown in table 3. There was no significant differences (P>0.05) between Y and O group in embryo dry matter, ash, fat, crude protein and nitrogen free extract content, although EMFAT content were increased numerically in O group compared to Y group.The avian embryo is solely dependent on the macronutrient content of the egg to meet its metabolic requirements. Varieties of factors contribute to the relative utilization of these macronutrients for energy and tissue synthesis. These include egg size and composition, breeder age, nutrient supplementation, maternal nutrition, turning of eggs during incubation, etc. so in this study there was no significant effectof breeder age on chemical composition of whole embryo at 18d of incubation. Some research showed that during the latter half of incubation, subcutaneous fat depots start appearing and it isestimated that 25 % of yolk lipids are stored in the subcutaneous adipose tissue of theembryo. These provide a source of energy for hatching. Although there was no significant differences in EMFAT but Large egg embryos are found to accumulate more subcutaneous fat compared to small egg embryos [8].

Table 3: Effects of Maternal Age on Embryo Analysis at 19-d in RossBroiler Breeder 1

Treatment	Embryo Dry Matter (%)	Embryo Ash (%)	Embryo Fat (%)	Embryo CP (%)	Embryo NFE (%)
Young-31w	18.08	0.908	3.360	59.96	44.76
Old- 51w	19.96	0.864	3.907	56.56	38.66
CV	17.52	3.81	24.94	35.39	13.32
SEM	1.054	0.035	0.286	6.56	4.69
P-Value	0.224	0.394	0.194	0.718	0.370

1- Means in the column with same superscript did not differ significantly (P> 0.05).

The effect of breeder flock age on residual yolk composition was shown in table 4. There was no significant differences (P>0.05) between Y and O group in residual yolk sac ash and nitrogen free extract content. Residual yolk sac dry matter and fat content were increased numerically in O group compared to Y group and differ significantly between O and Y group. There was significant differences(P<0.05) in RYFAT so large egg embryos are found to had more fat in yolk sac compared to small egg embryos [13]. Residual yolk sac crude protein content were decreased numerically in O group compared to Y group and differ significantly between O and Y group(P<0.05). This may due to this fact that in old breeder, lipoproteins that transfer fat from liver to yolk

had bigger size and with increasing the lipoproteinsize, protein ratio to fat decreased, so this may cause less protein in O group residual yolk sac in embryo. This finding is in agreement with previous reports [10, 18, 20, 21].

Table 4: Effects of Maternal Age on Residual yolk sac Analysis at 19-d in Ross Broiler Breeder 1

Treatment	Residual yolk sac Dry matter (%)	Residual yolk sac Ash (%)	Residual yolk sac Fat (%)	Residual yolk sac CP (%)	Residual yolk sac NFE (%)
Young-31w	47.87 b	0.965	13.77b	65.05 a	26.63
Old- 51w	51.79a	0.969	18.15a	50.25 b	32.61
CV	5.53	0.714	11.66	26.44	14.32
SEM	0.864	0.002	0.689	4.10	3.16
P-Value	0.0052	0.236	0.003	0.020	0.198

1- Means in the column with same superscript did not differ significantly (P> 0.05).

V. Conclusion

Important differences exist among breeder age from the beginning to the end of the egg production cycle.These differences account for the smaller embryo or chick of embryos from young breeders. This result revealed that the increase in liver weight of embryo from old group due to aging may resulted from increase in fat content in their liver. Our result revealed that as breeder age advanced, embryo weight and length increased. Our finding revealed that embryo from old group had more residual yolk sac and this may cause more malabsorbtion of yolk sac during incubation

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