PHYSIOLOGY AND REPRODUCTION

Artificial Incubation of Muscovy Duck Eggs: Why Some Eggs Hatch and Others Do Not

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ABSTRACT This study was designed to gain insight into the influence of spraying and cooling, during artificial incubation, on the embryo metabolic rate and hatching ability of Muscovy duck eggs. Three times a week 93 incubated eggs were sprayed and cooled for 0.5 h at room temperature. Daily embryo metabolic rate was measured in 30 eggs with a water vapor conductance ranging from 1.15 to 2.07 mg/day·kPa. Egg weight ranged from 63.73 to 84.52 g; length and breadth ranged from 59.6 to 66.4 mm and 43.2 to 48.2 mm, respectively. According to observed hatching ability, eggs were classified by three categories: eggs that hatched normally; eggs that were assisted during hatching, and nonhatched eggs. Five ducklings were assisted during hatching. Four ducklings died on Day 31, two on Day 32, and two on Day 34. Two functions were derived by discriminant analysis and accounted for 100% of the variation among the three categories of hatching ability. Collectively, these functions were able to classify 93.3% of the eggs in the correct hatching category. Egg length and metabolic rate at Days 21 and 28 of incubation were the most important predictor variables of the two functions. The results obtained in the present study indicate that an incubation temperature of 37.5 C with spraying and cooling seems to be beneficial for larger eggs.

(*Key words*: Muscovy duck, hatchability, artificial incubation, egg length, metabolic rate)

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INTRODUCTION

Optimal incubation conditions may be defined as those leading to maximum hatchability of healthy hatchlings (Ar, 1995). Hatchability is an important economic trait of domestic poultry and represents a major component of reproductive fitness (Hassan and Nordskog, 1971). Hodgetts (1991) has pointed out that hatchability of artificially incubated duck eggs is low (65 to 82%) compared with that of domesticated chickens (81 to 85%). Hogetts reported that the main factors that influence duck hatchability in artificial incubation are variation in sizes, age, and degree of contamination of eggs.

To improve hatchability of eggs from domesticated waterfowl, a common practice has been to spray with water or to cool the eggs periodically. The physiological benefit to the embryo is not well understood (Sarpong and Reinhart, 1985; Ar, 1995). Spraying appears to simulate natural incubation because eggs from Muscovy ducks hatch better with nesting hens (Serbul, 1983; Moraes and Packer, 1988).

There is a substantial difference between natural and artificial incubation in the way heat is applied and regulated. This difference may influence embryonic growth and energy metabolism (Ar, 1995). Embryonic development and metabolic rate also are influenced by egg size and shell properties. During artificial incubation, the embryo temperature is dependent on incubator temperature, embryonic metabolic rate, and thermal conductance of the egg and surrounding air (French, 1997). During natural incubation, embryo temperature is higher in the beginning (closer to the brood patch). In contrast, this situation is reversed with artificial incubation. Likewise, the physiology of the contact-incubated egg is different from eggs incubated by convection (Turner, 1991).

Comparative studies of incubation of waterfowl eggs are scant. Romanoff (1943) studied artificial incubation requirements for Indian Runner Duck eggs and pointed out that the moment of embryonic mortality is affected by such environmental conditions as temperature, relative humidity, and air movement. Previously, we have dem-

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Abbreviation Key: EW = egg weight; GH_2O = water vapor conductance; MR = metabolic rate.

TABLE 1. Mean, SD, and range of egg characteristics for the original sample and the representative subsample

Sample	n	Egg weight (g)	Egg length (mm)	Egg breadth (mm)	Egg shape index (B/L)	GH_2O (mg/day·kPa)
Original sample	93					
Mean		77.04	62.6	46.7	0.75	1.61
SD		5.02	1.9	1.1	0.02	0.22
Range		63.73 – 85.25	58.3 – 66.4	43.2 – 48.7	0.69 – 0.80	1.15 – 2.14
Representative subsample	30					
Mean		76.28	62.6	46.5	0.74	1.58
SD		4.77	1.8	1.0	0.02	0.24
Range		63.73 – 84.52	59.6 – 66.4	43.2 – 48.2	0.71 – 0.77	1.15 – 2.07

onstrated that hatching of Muscovy duck eggs lasts longer during artificial incubation and is an energy-demanding phenomenon often associated with late embryonic death (Harun, 1998). In the present study, we describe embryo development and hatching ability in relation to egg characteristics (weight, length, breadth, and shell water vapor conductance) and metabolic rate. Experimental eggs were categorized as those that 1) hatched normally, 2) contained ducklings that required assistance in hatching, and 3) contained ducklings that failed to hatch. Embryonic metabolic rate and egg characteristics were measured to determine their influence on hatching ability and to predict the three hatching outcomes.

For a good prediction, discriminant analysis was applied because it includes features of analysis of variance as well as multiple regression (Meyer, 1993).

MATERIALS AND METHODS

Incubation and Egg Metabolic Rates

Eggs were incubated in an incubator,[2] with the temperature set at 37.5 C and RH at 58%. Eggs were automatically turned hourly. Three times a week (starting from the beginning of incubation) eggs were sprayed with lukewarm distilled water and cooled at room temperature for 30 min. The water vapor conductance (GH_2O) of Muscovy duck eggs was determined in the incubator at 24, 72, and 120 h after the beginning of incubation, as described by Visser (1991), using the method of Tullett (1981). The original sample size was 93 eggs (*Cairina moschata*; strain R51, Grimand Frères). After determination of GH_2O, a representative subsample of 30 eggs containing live embryos and a GH_2O range of 1.15 to 2.07 mg/day·kPa was used to measure egg metabolic rate. There were no differences between egg characteristics of the original sample and the representative subsample (Table 1). Metabolic rate (MR) was measured on Days 13, 15, 17, and 19 and thereafter daily from 21 to 35 d of incubation. Oxygen consumption and carbon dioxide production were measured in an open flow system, as described by Visser (1991) and Dietz (1995). Each egg was put in a small respiration chamber. The chambers were placed in a water bath at 37.5 C. After 1 h of equilibration, average egg MR was calculated over a period of at least 15 min, by using the formula derived by Romijn and Lokhorst (1961):

$$MR = 4.49 * \dot{V}O_2 + 1.39 * \dot{V}CO_2 \quad [1]$$

where MR is expressed in milliwatts, and gas volumes are expressed in milliliters (standard temperature pressure dry; STPD)/h. At Day 32 of incubation, individual eggs were laid horizontally in mesh wire boxes and further incubated at 37.2 C and 80% RH.

From Day 33 onward, eggs were checked hourly for external pipping and hatching. During hatching, any duckling that had an enlarged pipping hole for more than 48 h without being able to escape from the egg (hatching) was assisted in breaking the eggshell. Thus, eggs were

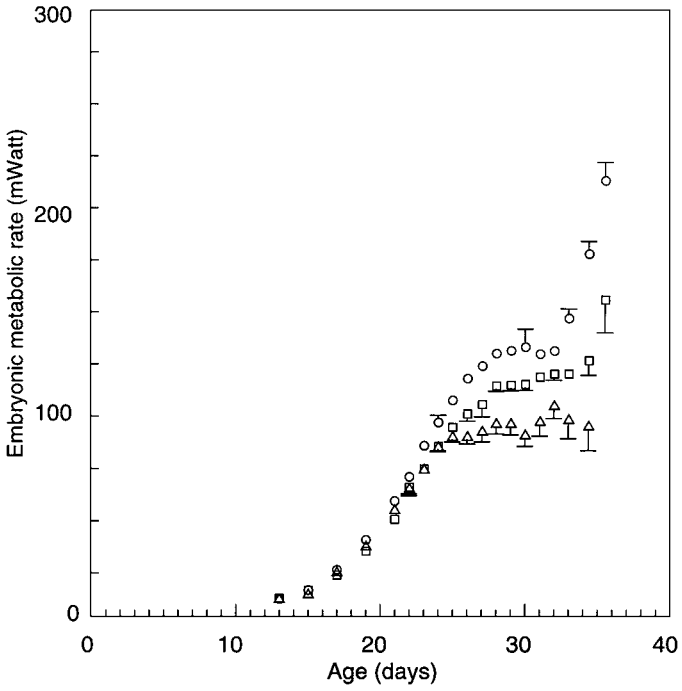

FIGURE 1. The metabolic rate during incubation for the three hatching categories (circles: normally hatched; squares: helped hatchlings; triangles: nonhatched. Vertical bars represent ± SEM).

[2]Pas Reform Combi 41, Pas Reform B.U., 7038 CH, Zeddam, The Netherlands.

TABLE 2. Mean, SD, and range of egg characteristics for the three hatching categories

Hatching categories	n	Egg weight (g)	Egg length (mm)	Egg breadth (mm)	Egg shape index (B/L)	GH_2O (mg/day·kPa)
Normally hatched eggs	17					
Mean		78.49[a]	63.5[a]	46.9[a]	0.74[b]	1.62[a]
SD		3.19	1.4	0.6	0.02	0.26
Range		74.92 – 84.52	62.0 – 66.4	45.8 – 48.2	0.71 –0.77	1.15 – 2.07
Helped eggs	5					
Mean		72.69[b]	60.4[b]	46.1[b]	0.76[b]	1.55[a]
SD		4.05	0.9	0.8	0.01	0.10
Range		67.98 – 78.59	59.6 – 62.0	45.1 – 47.1	0.75 – 0.77	1.42 – 1.65
Nonhatched eggs	8					
Mean		73.83[b]	62.2[c]	45.8[b]	0.73[b]	1.52[a]
SD		5.79	1.7	1.4	0.01	0.26
Range		63.73 – 81.24	59.7 – 64.3	43.2 – 47.7	0.72 – 0.75	1.24 – 1.95

[a–c]Means within a column of hatching categories with no common letters are significantly different ($P < 0.05$).

classified into three hatching categories: 1) eggs from which ducklings hatched normally (normally hatched), 2) eggs that contained ducklings that were assisted during hatching (helped), and 3) eggs that contained ducklings that failed to hatch (nonhatched).

Statistical Analysis

Discriminant analysis (Meyer, 1993) was used to select predictor variables. For other variables, the means and standard deviations were calculated, and differences between means were evaluated using students *t*-test. Significance, if not stated otherwise, is based on a 0.05 level of probability.

RESULTS

The relationship between incubation time and embryo MR for the three categories of hatching ability is shown in Figure 1. Embryonic death was as follows: four embryos died on Day 31, two on Day 32, and two on Day 34. Five embryos were assisted during hatching. Egg characteristics were significantly different among hatching categories, with exception of GH_2O (Table 2).

A stepwise variable selection from egg characteristics EW, breadth, length, GH_2O, and MR (up to Day 31) showed that length and MR at Days 21 and 28 were more important predictor variables. The analysis indicated that the two discriminant functions were statistically significant for hatching categories. The two functions together accounted for 100% (0.692 for function I and 0.308 for function II) of the variation among the three categories (Table 3).

TABLE 3. Discriminant function weights for hatching categories, egg characteristics, and metabolic rate (MR)

	Discriminant functions	
	I	II
Length	0.995	0.886
MR at Day 21	0.943	0.763
MR at Day 28	0.402	–0.975
χ^2	59.381	22.627
P	0.0001	0.0001
r^2	0.692	0.308

The group centroids, plotted in the discriminant functions, are shown in Figure 2. Classification function coefficients for the three categories of hatching ability are presented in Table 4.

Figure 2 shows that the discriminant function I separates the eggs from which ducklings hatched normally from the "helped" eggs and nonhatched eggs. Discriminant function II separates the nonhatched eggs from "helped" eggs. To interpret the nature of each of the two functions, it is necessary to look at the standardized canonical discriminant function coefficients shown in Table 3. The largest coefficient on the first discriminant function was associated with length and MR on Day 21. This discriminant function is essentially a contrast between the ducklings that hatched versus those that required assistance and those that failed to hatch. Discriminant function II is essentially defined by the highest negative coefficient of MR on Day 28 and a positive value for length and MR on Day 21. Assisted ducklings had the lowest score on the second discriminant function, and the ducklings that failed to hatch had the highest score, as is shown in Figure 2. This separation occurred because eggs of assisted ducklings showed lower values for length (Table 2) and higher values for MR on Day 28 compared with nonhatched eggs (Figure 1).

The classification matrix for the actual and predicted hatching categories (group membership) based on two discriminant function scores is shown in Table 5. Hatching categories were predictable (93.3%) on the basis of embryo MR (on Days 21 and 28) and length. There were 28 correct classifications of 30 eggs. Only nonhatched eggs had a percentage (25%) of incorrect classifications. When discriminant analysis was repeated with the egg MR as an average of the whole incubation period, a stepwise variable selection criterion showed that length, MR, and GH_2O were the predictor variables for the three categories of hatching ability, and the overall classification improved from 93.3 to 100% (Table 6).

DISCUSSION

Commercial duck hatcheries commonly select eggs to minimize variation in egg size. Variation may cause prob-

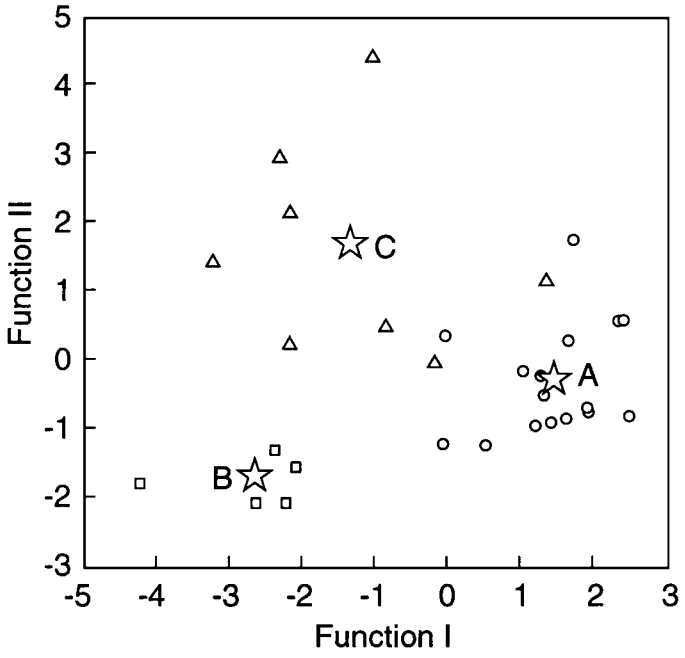

FIGURE 2. Two discriminant functions plotted against each other. Group centroids are marked by a star. A and circles: normal hatched embryos; B and squares: helped hatchlings; C and triangles: nonhatched.

lems with temperature control and ventilation during incubation (Hodgetts, 1991). French (1997) suggested that studies were warranted to determine the effects of incubation temperature and egg size on the metabolic heat production of poultry embryos. The experiment described in this paper was also designed to assess the role played by egg characteristics on hatching ability during artificial incubation of Muscovy duck eggs. Our study demonstrated that a constant incubator temperature, spraying with water, and cooling applied to a batch of eggs of different sizes (in particular in length) induced different patterns of embryo MR during incubation. This phenomenon was observed for the first time in our preliminary experiments of artificial incubation of Muscovy duck eggs, in which 25 ducklings that hatched normally had a higher MR compared with the 12 nonhatched ducklings (Figure 3). The MR measurements could not be analyzed by a two-way analysis of variance design with one repeated factor, because the treatment factors (hatching categories) were assigned after completion of the experiments.

TABLE 4. Classification function coefficients for hatching categories

	Hatching categories		
	Normally hatched	Helped	Nonhatched
Length	64.599	60.850	63.855
MR[1] at Day 21	10.982	10.208	10.803
MR at Day 28	–0.938	–0.955	–1.215
Constant	–2,306.039	–2,031.939	–2,218.583

[1]Metabolic rate.

Discriminant analysis was used to isolate the functions that accounted for differences among hatching categories from embryo MR and egg characteristics. The analysis found a linear combination of length and MR that best separates the hatching categories by maximizing the between-group variance of the linear combination relative to the within-group variance. Discriminant functions were able to predict correctly the hatching categories of 93.3% of the eggs. The model described in this study (egg length and MR at Days 21 and 28) was able to predict correctly the hatching categories of 70.3% of the eggs from our preliminary experiment (37 eggs data; from Figure 3). To our knowledge, discriminant analysis has not been applied previously to predict egg hatching ability of domesticated waterfowl, in particular, and to poultry, in general.

Several factors may explain the low metabolic rate observed in helped and nonhatched eggs. Besides egg size-related characteristics such as length, incubation temperature might have played an important role in this phenomenon. Nichelmann et al. (1994) have shown that Muscovy duck embryos have a thermoneutral temperature zone between 39 and 40.5 C depending on age, whereas the normal incubation temperature (37.5 C) is the temperature of summit metabolism (embryo peak metabolic rate). Hoyt (1987) separated embryonic metabolism into growth and maintenance. He suggested that the change in embryo growth rate due to manipulation of incubation temperature may affect the rate of oxygen consumption per gram of embryo mass. Heat production during development surpasses heat loss by evaporation in the latter part of the incubation process. Consequently, the embryo's temperature rises above incubator temperature (Sotherland et al., 1987), which reverses the difference between internal egg temperature and incubator temperature (Tazawa and Nakazawa, 1985; French, 1997). Larger embryos approach the thermoneutral temperature more closely. Thus, they have a relatively lower metabolic rate and use more energy for growth instead of maintenance. Very large embryos may surpass the thermoneutral temperature and may even have difficulties loosing heat, which may lead to an increase in temperature and heat production, if the incubator temperature is kept constant. French (1997) showed that larger eggs hatch better when the incubation temperature is reduced from 37.5 to 36.5 C during the second half of incubation; however, he did not observe such an improvement in small eggs.

The higher metabolic rate shown by the normally hatched eggs as compared with nonhatched and helped eggs may be related to the fact that those eggs were much heavier, with longer length and higher embryonic growth rate compared with the other two hatching categories (Table 5). Van Kampen et al. (unpublished data) found a relationship between metabolic rate and embryo mass, in Muscovy ducks: from Days 13 to 26 of incubation, the allometric mass exponent was 0.97, but between Days 31 to 33 it was 2.01.

Spraying and cooling of the eggs during incubation may explain why length was one of the predicting vari-

TABLE 5. Classification matrix for actual and predicted hatching categories

Actual hatching categories	Number of cases	Predicted hatching categories: Normally hatched	Helped	Nonhatched
Normally hatched	17	17 (100%)	0	0
Helped	5	0	5	0
Nonhatched	8	2 (25%)	0	6 (75%)
Percentage of cases correctly classified	93.3%			

ables. Length is an important factor for egg heat exchange (cooling and heating) during incubation, according to the mathematical model of Meijerhof and van Beek (1993) for temperature and moisture loss of hatching eggs. At the beginning of incubation, large eggs have cool surfaces. Small eggs have a warm and more uniform surface temperature, but at the end of incubation, blood flow warms the surfaces of all eggs, and the relative increase in surface temperature of large eggs is greater than that of the small ones (Turner, 1987).

The present study offers evidence that leads us to believe that spraying and cooling of the bigger eggs were beneficial in alleviating heat stress. On the other hand, spraying and cooling might have been too severe and, consequently, depressed the metabolic rate and embryo growth for the smaller eggs. Meijerhof and van Beek (1993) have shown that the relationship between egg size and temperature difference between the developing embryo and incubator temperature depends on metabolic heat production and is influenced by air velocity, which means that variations in air velocities directly around the eggs may cause differences in embryonic temperature and therefore in embryonic development.

Data from the present study suggest that the interaction between egg size and incubator environmental conditions accounts for the difference in metabolic rate observed between hatching categories during incubation. There is an optimal egg size for the incubation temperature used. Besides the differences in metabolic rate, the eggs with ducklings that required assistance in hatching were more rounded (high egg shape index; Table 2), which may also have contributed to a difficult hatching process.

The results obtained in the present study indicate that 1) the level of embryonic metabolic rate is very important for the hatching success of an egg, 2) hatching ability of an egg may be predicted by egg characteristics such as length and egg metabolic rate, and 3) an incubation temperature of 37.5 C with spraying and cooling seems to benefit eggs with higher EW, length, and GH_2O.

TABLE 6. Classification function coefficients for hatching categories

	Hatching categories: Normally hatched	Helped	Nonhatched
Length	47.587	45.101	46.371
MR[1]	0.0027	0.0023	0.0021
GH_2O	–24.240	–22.608	–22.871
Constant	–1,499.195	–1,334.043	–1,389.721

[1]MR = Metabolic rate; GH_2O = water vapor conductance.

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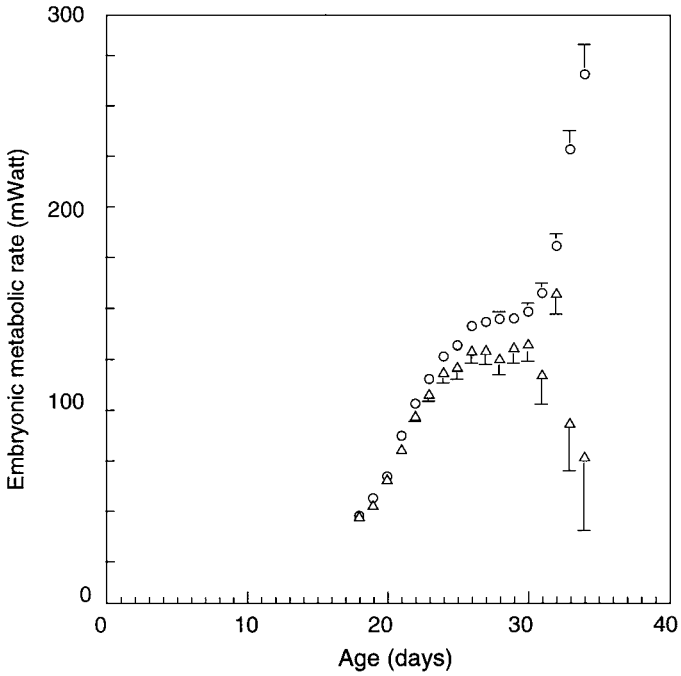

FIGURE 3. The metabolic rate (MR) during incubation from our preliminary experimental data, which showed a higher MR from normally hatched ducklings (circles) and a lower MR from nonhatched ducklings (triangles). (Vertical bars represent ± SEM)

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