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A Morphometric Method of Sexing White Layer Eggs

Author(s)

Yilmaz-Dikmen B[1]
Dikmen S[2*]

[1] Department of Animal Science, Faculty of Agriculture, University of Uludag, 16059, Görükle, Bursa, Turkey
[2] Department of Animal Science, Faculty of Veterinary Medicine, University of Uludag, 16059, Görükle, Bursa, Turkey

Mail Adress

Corresponding author e-mail address
Assoc. Prof. Dr. Serdal Dikmen
University of Uludağ, Faculty of Veterinary Medicine, Department of Animal Science, 16059 Görukle, Bursa, Turkey
Phone: + 90 224 2941355
Fax + 90 224 2941202
*e- mail: serdal@uludag.edu.tr

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ABSTRACT

The present study was carried out to determine the sex of the fertilized white layer eggs with using morphological measurements. A total of 300 Super Nick White Layer (54 wks of age) eggs were incubated and sexed in the end of incubation period. Before incubation egg length, width and weight were measured. The egg volume and shape index were estimated with using these measurements for each egg. The effect of egg weight and replicate number was not significant on the sex of the hatching chick ($p > 0.05$). The effects of egg shape index ($p = 0.001$), egg length ($p = 0.0018$), egg width ($p < 0.01$) and volume ($p = 0.004$) of the egg had significant effect on the sex of hatching chick. According to the results of the current study, morphological measurements of the pre-incubated egg might be an indicator of sex of the hatching chick. The shape index and egg volume were more informative for the likelihood of the sex of hatching chick. According to the positive results of the current study, it is possible to incubate more female chicks with using the morphological measurements of egg. This may increase the incubation capacity and decrease the number male chicks that are killed at layer hatcheries.

INTRODUCTION

Many avian species are considered sexually monomorphic. In monomorphic avian species, especially in young birds, sex is difficult to identify based on an analysis of their external morphology. Sex identification in avian species is one of the key points of avian breeding and evolutionary studies (Cerit & Avanus, 2007). Mechanisms for either control or detection of egg sex remain unknown in birds (Krackow, 1999; Komdeur & Pen, 2002), but the nature of the egg size disparity between the sexes may shed some light on the processes involved. Follicles in a bird's ovary, which contain the ovum and yolk deposits, commonly develop approximately 24 h out of phase with one another, and therefore exist in a size hierarchy (Sturkie, 2000). Krackow (1995) speculated that the developmental pace of follicles that ultimately give rise to males and females may differ, so that the faster-growing sex is most likely to end up in the first egg.

According to Mendelian heredity laws, the sex ratio of a given domestic chicken population during hatching is expected to be 1:1 (Li *et al.,* 2008). Female and male broiler hybrid chickens are often reared together and sexing broiler chicks is not essential (Flock & Seeman, 1993; Ellendorf & Klein, 2003). Consequently, day-old male layer chicks are not required and are either used as food for carnivorous birds, reptiles, mammals and fish or are industrially processed for protein production (Kaleta & Redmann, 2008). The premature end of the life of day-old male chicks has raised concerns in welfare-sensitive societies that are demanding an end of this practice. Also, the incubation of eggs that yield only female chicks would be of benefit for the environment (as less energy and other inputs would be needed) and would increase productivity of hatcheries by decreasing costs of incubating eggs and would enhance competitiveness accordingly. Logically, the hatch of only female chicks is a high priority of the poultry industry (Kaleta & Redmann, 2008). The differences in the sale prices and care costs of male and female avian species and the time spent for the reproduction process cause significant financial losses (Cerit & Avanus, 2006). An abundance of scientific and practical efforts have been made in both past and present times to detect the sex in eggs pre-incubation and in embryos as early as possible (Kaleta & Redmann, 2008).

There are different day-old chick sexing methods; these are; cloacal or vent sexing, feather sexing, feather colour sexing, sexing by the number of digital pad scales and ultrasonography methods (Bosk, 1974; Kaleta & Redmann, 2008). In addition to these methods, detection of oestrogen *versus* androgen in incubated eggs, microscopic cytogenetic analysis of chromosomes, ultrasonography *in ovo*, embryonic heart beat and temperature variation during incubation (Kaleta & Redmann, 2008) are also used to determine the sex of the chick in the egg. But these methods need more intense labor and neither suitable nor practical for commercial use. Because of these reasons the use of morphological measurements of egg to determine of sex of the chick would be more practical and useful for commercial use, also minimize issues related to animal welfare in poultry industry.

Egg geometrical calculations are important for the poultry industry and in biological studies, as they can be used in research on population and ecological morphology (Mand, 1988). Egg shape, which can be easily described in terms of the ratio of the maximum width and length, remains constant during the whole period of incubation. The size, length, width, volume and shape of eggs are important life-history variables in birds as hatching mass is highly correlated with egg size for a large number of bird species (Coulson, 1963; Parsons, 1976; Howe, 1977; Hegyi, 1996; Mead *et al.,* 1987).

For every egg-laying hen confined in a battery cage, there is a male chick that was killed at the hatchery. Because egg-laying chicken breeds have been genetically selected exclusively for maximum egg production, they don’t grow fast or large enough to be raised profitably for meat. Therefore, male chicks of egg-laying breeds are of no economic value and they are literally discarded on the day of hatching. An evidence of relationship with morphological characteristics of the egg and sex of the chick might be helpful for poultry industry in several different ways. When more female chicks are incubated, incubation capacity increases and less male layer chicks are killed.

There are very few studies on egg geometrical traits and its relationship with sex in birds (Cordero *et al.*, 2000; Burnham *et al.,* 2003; Magrath *et al.,* 2003; Lislev *et al*., 2005). The objective of this study was to determine the morphological characteristics of egg and its relationship with the sex of the layer eggs.

MATERIALS AND METHOD

This experiment was conducted in accordance with the principles and specific guidelines presented in *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 3rd edition, 2010 (Association Headquarters, Champaign, IL 61822).

Experiment was carried out in a private hatchery located in northwest region of Turkey (26°06’15” N, 103°26’15” W). The eggs used in the study were obtained from Super Nick white layer breeder flock at 54 weeks of age. A total of 300 eggs were incubated and a total of 244 chicks were sexed in the end of incubation period. All eggs were randomly allocated into replicate groups (1, 2 and 3). All eggs were collected at the same day, on the same row of breeder flock. The breeder flock consisted of 431 male and 3746 female Super Nick white layer at the time of egg collection.

The flock, from which the eggs were collected, was reared and kept during lay under standard management conditions in a standard commercial layer breeder house. The layer breeders were fed with layer breeder feed (18% crude protein, 2450 kcal ME). The diet was formulated to meet National Research Council (NRC, 1994) specifications. Water was supplied *ad libitum*.

The eggs were sanitized and stored at 17-18 °C and 85% RH for 4 days. Eggs were numbered and weighed individually on an electronic scale with ±0.01 g precision before setting. The eggs were incubated in an incubator (Jamesway Incubator Company Inc., Ontario, Canada) at 37.2 °C and 55% RH for 18 days. The eggs were turned every hour in an angle of about 45° for 18 day. On the 18th day of incubation, all eggs were candled and fertile eggs placed in a separate chamber in the hatchery cabinet maintained at 36.7 °C and 60% RH until hatching. The vent sexing method was used for sexing day-old chicks by a well-trained staff.

The maximum widths and lengths of each egg were measured with using Mutitoyo digital calliper (Mutitoyo, Japan) (±0.01 mm) and their shape index was calculated using the formula:

SI = (W / L) × 100 (Yannakopoulos & Tserveni-Gousi, 1986).

where SI= shape index, W= width of the egg, and L= length of the egg

And egg volume was calculated using the formula;

$V = (\pi/6) \times L \times W^2$ (Hoyt, 1979).

where V: volume of the egg, π: phi constant (3.1416).

Data Analysis

Multiple logistic regression analysis was used to determine the effects of each factor. The data were analyzed with using Proc Logistic procedure of the Statistical Analysis System (SAS version 9.1.3; SAS Institute, Inc., Cary NC). Since the data contained numerous independent variables, a preliminary analysis was conducted to remove any insignificant independent variables with using multiple logistic regressions in SAS. While selecting the best model for predicting the sex of the hatching chick; replicate (1,2 and 3) was entered as a class variable. Then, the effects of shape index, width, length, weight and volume of the egg was entered as continuous variable. Stepwise procedure was chosen to find the best model in multiple logistic regression analysis. A $p < 0.05$ was used to test for significance of variables entering to the models and variables with $p > 0.2$ was elected from the model. The odds ratio was calculated with using regression coefficients between pairs of variables (odds ratio = anti-natural logarithm of the regression coefficient of a dichotomous independent variable in the exponential function of the logistic regression) and its 95% confidence interval (Proc Logistic Procedure in SAS, 2007). Odds ratios are a multiplicative measure of risk that range from 0 to infinity. An odd ratio of "1" indicates that the factor examined does not alter risk. Odds ratios (OR) > 1 are predisposing and imply a great association. Odds ratios < 1 are less association. Therefore, OR = 2 and 0.5 have the same magnitude but imply a different association (Fleiss, 1981).

According to the logistic regression analysis the probability of being a female chick incidence probability (IP1) was estimated. A second series of multiple regression analysis was performed using forward selection, backward elimination, and stepwise selection procedures with replicate, shape index, width, length, weight and volume of the egg as variables in the model. In these series of analyzes IP1 was entered as dependent variable. All selection procedures gave an $R^2 = 0.25$. In forward and stepwise selection only shape index remained in the model ($p < 0.001$), where as in the backward elimination shape index, volume and length of egg were remained in the model ($R^2 = 0.26$).

RESULTS

Stepwise, multivariate logistic regression analyses (SAS, 2007) were used to evaluate the likelihood of sex of the hatching chick, given replicate and shape index, width, length, weight and volume of egg. All morphological measurements were tested for determining the likelihood of sex of the hatching chick. In order to discard confounding effects a variable 'replicate' was included into the models to check for potential confounding effect associated with the sampling of the eggs, but confounding of replicate was not detected ($p > 0.05$). The best fit model for determining the sex of the hatching chick was found with using stepwise procedure in multiple logistic regression method. According to the stepwise analysis of all factors (shape index, width, length, weight, volume and replicate), the shape index was the only factor that was remained in the model ($p = 0.001$). The effect of shape index on the hatching chick sex was the most prominent factor for determining the sex of the egg in white layers. Hosmer & Lemeshow goodness (GOF) of fit (Hosmer & Lemeshow, 1989) test was found 0.923 for the model selected in stepwise selection. The effect of egg weight and replicate number was not significant on the sex of the hatching chick ($p > 0.05$). The effects of shape index ($p = 0.001$), egg width ($p < 0.01$), egg length ($p = 0.0018$) and volume ($p = 0.004$) of the egg had significant effects on the sex of hatching chick.

According to the stepwise multiple regression analyses only shape index was left in the model for determining the probability of being a female chick. Afterwards, an index was developed to determine the probability of the female chick;

Female chick sex probability (IP1) = - 0.39531 + (0.01214 × shape index) (R^2=0.25)

According to this model, the higher the shape index values results the higher the probability of hatching a female chick.

Descriptive statistics of the eggs that were used in the current study was shown in Table 1. Estimates for the parameters obtained through the maximum likelihood estimation method with 95% Wald's confidence limits for the factors significantly associated ($p < 0.05$) with the sex of the hatching chick (Female) are shown in Table 2. In the preliminary analyses only the shape index ($p = 0.001$) remained in the model. The overall logistic regression model was significant ($p = 0.01$) but the R^2 and the maximum rescaled R^2 values of the model was low ($R^2 = 0.06$ and 0.8, respectively).

Table 1 – Descriptive statistics of the eggs used in the study.

Variable	Sex	Mean	Standard Error	Min	Max
SI, (%)	Male	75.1	2.6	64.4	80.1
	Female	75.5	3.0	63.2	82.7
	Total	75.3	2.8	63.2	82.7
EWI, (mm)	Male	43.4	1.1	40.3	46.4
	Female	43.5	0.9	40.7	46.4
	Total	43.5	1.01	40.3	46.4
EL, (mm)	Male	57.9	1.8	53.5	64.0
	Female	57.8	2.1	52.4	67.7
	Total	57.8	2.0	52.4	67.7
EW, (g)	Male	60.8	4.2	51.3	70.6
	Female	60.9	3.7	49.3	68.6
	Total	60.8	3.9	49.3	70.6
V, (mm3)	Male	57198.9	4025.8	47493.6	65737.7
	Female	57342.0	3442.7	46652.8	65622.4
	Total	57272.8	3729.0	46652.8	65737.7

SI: Shape index, EWI: Egg width, EL: Egg length, EW: Egg weight, V: Egg volume.

Pair-wise comparisons of groups for significant effects were shown in Table 2. The results of shape index values showed that eggs with less shape index values (< 74 vs. > 77) less likely have female chicks. And also eggs with medium versus greater egg shape index values tend to produce less female chicks. According to the egg width results; eggs with greater egg width (< 42 vs. > 44 mm) were 12% more likely to be the female chicks. The same results were also estimated for 42-44 vs. > 44 mm egg width, which was 33% more likely to have the female chick. When egg length was considered, shorter eggs (< 56 mm) were less likely (30%) to produce female chicks when compared to longer eggs (> 60 mm). Egg volume was also another factor that affects the likelihood of the sex of hatching chick. Eggs with smaller volume were more likely to have female chicks but the eggs with greater volume 71% more likely to have the male chicks.

Table 2 – Logistic regression coefficient estimates of various factors associated with female hatching egg in white layers.

Parameters	OR [a]	95% CI (OR)[b]
SI, (%)		
<=75.2 vs. >75.2	1.01	0.44 - 2.27
EWI, (mm)		
<42 vs. >44	1.12	0.22 - 5.74
<=43.4 vs. >43.4	1.06	0.49 - 2.29
EL, (mm)		
<56 vs. > 60	0.30	0.07 - 1.36
<=57.8 vs. >57.8	0.99	0.49 - 2.01
V, (mm^3)		
< 53013 vs. > 59374	0.87	0.19- 4.05
<=57272 vs. >57272	0.71	0.37 - 1.36

[a] OR: odds ratio

CI [b]. Confidence interval.

SI: Shape index, EWI: Egg width, EL: Egg length, V: Egg volume.

The comparison of less versus greater values of shape index, egg width, length and volume were also determined and the odds ratio of egg width, length, and shape index were found to be around 1 (1.06, 0.99 and 1.01, respectively). This means that the changes in these values were not so informative on the sex of the hatching chick. But when the eggs with less volume were compared with the greater volume eggs, the odds ratio was estimated as 0.71. This means that eggs with less volume values were less likely to have female chicks.

In Figure 1, it shows that the estimated probability of hatching a female chick increases with the increase of shape index. The eggs with greater shape index values were more likely to produce female chicks ($p = 0.001$).

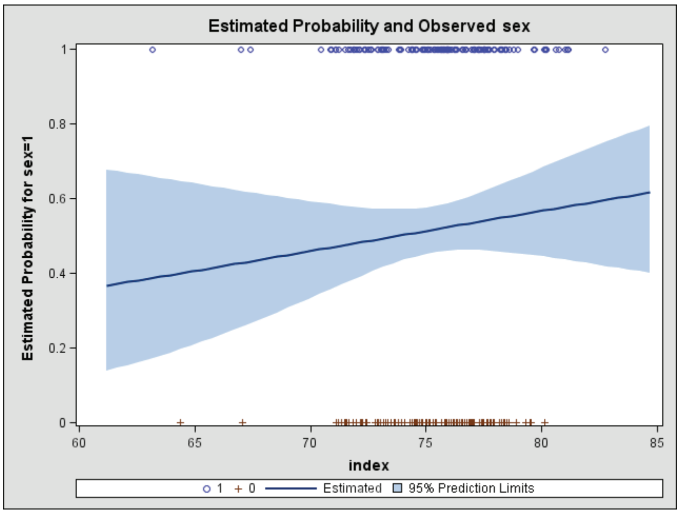

Figure 1: Estimated probability of hatching Female chick (sex = 1) versus shape index of the hatching egg.

Estimated probabilities of having a female chick increased with the increase of the width of the hatching egg from 41 to 47 mm ($p = 0.08$). Rounded shaped and wide eggs were more likely to produce female chicks. While pointed shaped and narrow eggs were more likely to produce male chicks (Figure 1 and 2).

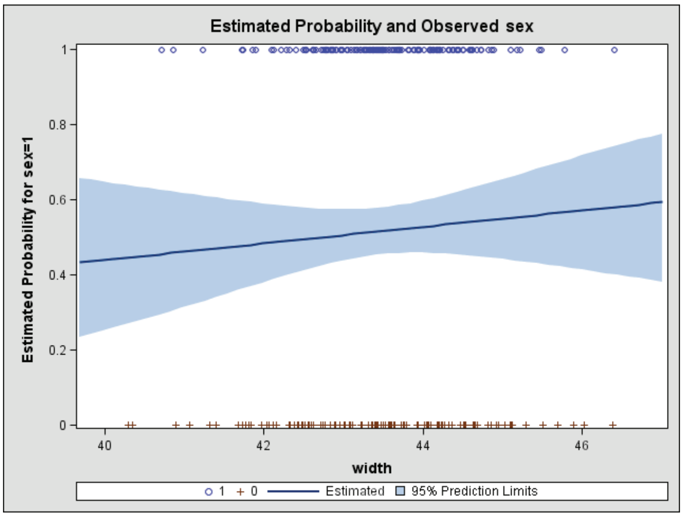

Figure 2: Estimated probability of female chick sex versus width of the hatching egg (0= male; 1= female).

In Figure 3, it shows that the probability of hatching a female chick was decreased with the increase of the length of the hatching egg ($p < 0.05$).

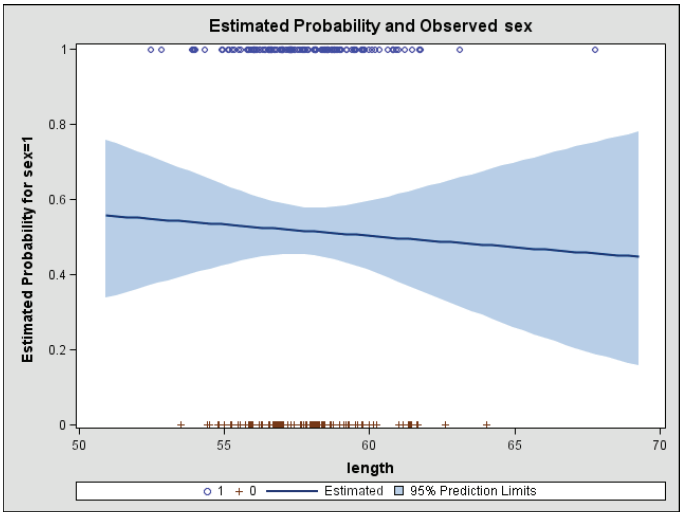

Figure 3: Estimated probability of female chick sex versus length of the hatching egg (0= male; 1= female).

In Figure 4, it shows that the probability of hatching a female chick was not changed with the increase of the weight of the hatching egg ($p > 0.05$). A probability of having a female chick was not affected by egg weight before incubation ($p > 0.05$). And also the effect of replicate was not found to be significant ($p > 0.05$).

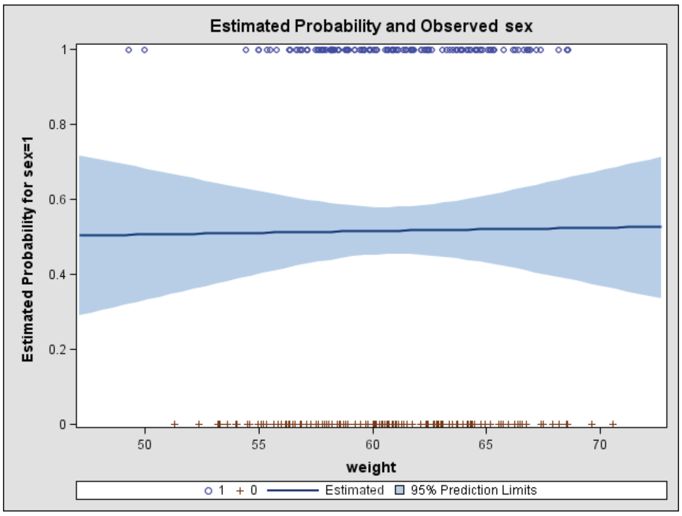

Figure 4: Estimated probability of female chick sex versus egg weight (0= male; 1= female).

In Figure 5, it shows that the probability of hatching a female chick less likely to change with the increase of egg volume (OR = 1.005). Probability of having a female chick was increased with the increase of the egg volume ($p = 0.004$).

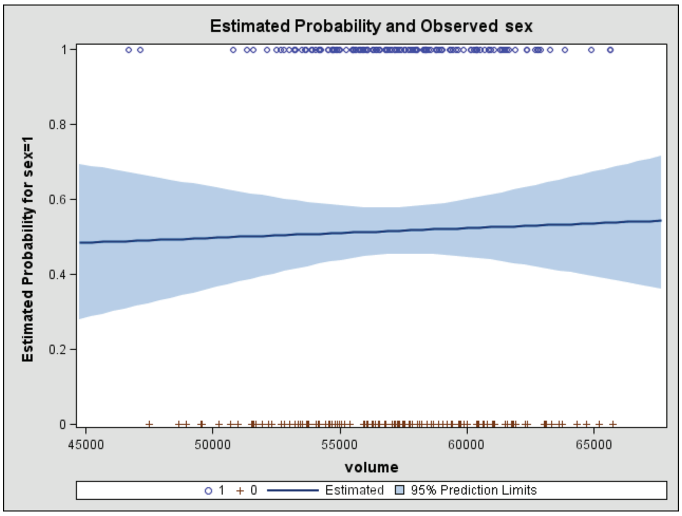

Figure 5: Estimated probability of female chick sex versus egg volume (0= male; 1= female).

DISCUSSION

In the chicken, sex is determined shortly before ovulation (Romanoff, 1960), during the first meiotic division, when segregation of the sex chromosomes (in birds the female is the heterogametic sex) consigns either the W or Z chromosome to the ovum and the remaining sex chromosome to the polar body (Sturkie, 2000). Shortly following ovulation, the ovum is fertilized by sperm present in the infundibulum, before passing down the oviduct over a period of around 24h, when albumin and shell are secreted around it. In species that lay an egg each day, follicles are ovulated at approximately 24 h intervals (Sturkie, 2000; Pike & Petrie, 2003).

The results of the current study indicated that there is a direct relationship with shape index. Eggs with greater egg shape index values are more likely to produce female chicks. The effects of egg length, width and volume were significant but not so informative (OR = 1.062, 0.99 and 1.005, respectively).

Egg shape index (SI)

Considerable intra-clutch variation exists in egg size and shape (Ligon & Ligon, 1978) and in some species egg size apparently correlates with sex: either those eggs that produce males are larger than those that produce females (Ankney, 1982; Ryder, 1983) or vice versa (Fiala, 1981; Weatherhead, 1985). Either of these two variables, size or shape, could provide incubating birds with a simple means of 'choosing' offspring sex by selectively denying incubation to some eggs. Ligon & Ligon (1990) observed single eggs of the green woodhoopoe (*Phoeniculus purpureus*) away from the rest of the clutch to one side of the nest cavity, suggesting selective incubation. It is important to remember that the relative size and shape of eggs produced are, either actively or passively, under maternal control and may provide a mechanism of sex detection for species unable to manipulate prior to laying (Pike & Petrie, 2003).

In our study shape index was found to be related to the probability of the sex of the hatching chick ($p = 0.001$). The eggs with greater shape index value were more likely to produce female chicks ($p = 0.001$). However, this significant effect means that the changes in these values were not so informative on the sex of the hatching chick and it is less likely to change with the increase of shape index (OR=1.005). Pointed shaped eggs were more likely to produce male chicks and the rounded shaped eggs were more likely to produce female chicks ($p < 0.0001$). Similarly, **Mulder & Wollan (1974) reported that rounded (oval) shaped eggs most likely to produce pullets (female) and pointed end (pointy) shaped eggs were most likely to produce cockerels (male).**

Egg width (EWI) and Egg length (EL)

The pair-wise analysis of male and female eggs within the same clutch indicated that the sex of the embryo explained 7% of the variance for relative egg length (Cordero *et al*., 2000).

In our study probability of hatching a female chick was increased with the increase of the width of the hatching eggs with rounded shape and with greater width were more likely to produce female chicks. On the other hand, pointed shape and narrow were more likely to produce male chicks. The probability of hatching a female chick was decreased with the increase of the length of the hatching egg ($p < 0.05$). Similar to our study, Cordero *et al.* (2000) demonstrated that in house sparrow eggs although egg width was not related to egg sex, male eggs were significantly longer than female eggs. However, Jull & Quinn (1924) found that there is no relationship between the length of the egg and the sex of the chick hatched from it. Burnham *et al.* (2003) found that Peregrine Falcon egg length and width were poor predictors of chick sex.

Egg Weight (EW)

Larger eggs might be considered to contain absolutely more nutrients and produce more vigorous and larger chicks than smaller eggs (Meathrel & Ryder, 1987). Under such conditions, Trivers & Willard (1973) predicted that most of the larger eggs would be male. That large eggs tend to produce more male chicks and small eggs tend to produce more female chicks was the observation of Lienhart (1919). However, Jull & Quinn (1924, 1925) and Jull (1931) reported that egg weight bears no relation to sex ratio. The effect of egg size was also found in the white crowned sparrow (*Zonotrichia leucophrys oriantha*) (Mead *et al.,* 1987), American kestrel (*Falco sparverius*) (Anderson *et al.,* 1997) and the house sparrow (*Passer domesticus*) (Cordero *et al.,* 2000). Anderson *et al.* (1997) speculated that laying American kestrel females may thus control not only egg size but also sex of their offspring.

In contrast to these studies, Magrath *et al.* (2003) found that female brown songlarks were hatch from larger eggs than their brothers, independently of laying order. Coerdero *et al.* (2001), reported that in the spotless starling (*Sturnus unicolor*) female eggs were heavier than male eggs and these differences were independent of laying sequence because the interaction between sex and laying order was not significant. In our study probabilities of having a female chick was not affected by egg weight before incubation ($p > 0.05$). Similarly, Rutkowska & Cichon (2005) found that sex of the zebra finches offspring was not related to egg mass. Sagar *et al.* (2005) found that in Buller's albatross (*Thalassarche bulleri*) egg size had no detectable influence on resulting chick sex.

Meathrel & Ryder (1987) in a study of ring-billed gulls (*Larus delawarensis*) also determined that egg size and mass could not be used to predict chick sex, although in some seasons chick sex was related to egg sequence in completed clutches of three eggs. Several authors (Sagar *et al.*, 2005; Burnham *et al.,* 2003) found that Larger Peregrine Falcon chicks hatched from larger eggs but there was no relationship between chick mass at hatching and sex of chicks.

Egg Volume (EV)

Mead *et al.* (1987) found that volume of male eggs in Mountain White-crowned Sparrows (*Zonotrichia leucophrys oriantha*) was slightly larger than that of female eggs in every year of a 5-year study. The volume difference was highly significant ($p < 0.01$) when data for all years were combined. A similar result was also obtained in the current study; probability of having a female chick decreased with the increase of the egg volume ($p = 0.004$, OR = 0.71). However, Sagar *et al.* (2005) found that in Buller's albatross (*Thalassarche bulleri*) egg volume was 228.5 ± 2.2 cm^3 for females and 230.2 ± 1.4 cm^3 for males. The difference in means (1.4 ± 2.5 cm^3) was not statistically significant ($p = 0.49$). Lislev *et al.* (2005) found that there was no relationship between offspring sex ratios and intra-clutch egg volumes and no differences were found in egg size related to embryonic sex.

There are several different factors affecting the sex ratio in chicken (Pike & Petrie, 2003). Further studies could be conducted which takes in to account different age (Blank & Nolan 1983), season (Zijlstra *et al.,* 1992), incubation period (Cook & Monaghan, 2004), incubation conditions (Feng *et al.,* 2006), breed, clutch sequence (Heinsohn *et al.,* 1997) and other factors (Pike & Petrie, 2003).

In conclusion, morphological measurements of the pre-incubated egg might be an indicator of the hatching chick sex. Especially the shape index values were more informative for the likelihood of the hatching chick. These results, according to the animal welfare legislations of EU, might have an important impact on layer hatchery management. Also, a machine that estimates the shape index under commercial conditions might be developed to decrease the hatching male chicks which are also being disposed at the hatching day. Furthermore, more data would be helpful to increase the reliability of the model and exact interval for each significant factor of likelihood of the hatching chick might be estimated more preciously.

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