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Developing predictive models for sex determination based on weight, length and width of eggs in White leghorn laying hens#

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Abstract

In layer industry, females are the preferable gender. This intensification gave rise to killing of billions of day-old male chicks in commercial hatcheries on day-one itself. This practice results in the waste of hatching cost and raises serious concern about animal welfare. The most reliable solution is determination of the sex of embryos prior to hatch, preferably before incubation. In the current study, an attempt was made to identify sex of chick embryo before incubation by using egg morphometry like weight, length and width. About 48 White leghorn laying hens of 30-32 and 50-52 weeks old (24 each) were reared in individual laying cages for a period of 120 days under the division of poultry housing management, ICAR-Central Avian Research Institute, Izatnagar. Birds were inseminated with pooled semen (1:8) at 5 days interval to collect hatching eggs and weight, length and width of eggs were recorded on daily basis. These eggs were incubated and day- old chicks hatched from these eggs were vent sexed and the data from 2800 chicks were collected and statistical analysis was carried out. Egg weight, length and width were found to have a significant effect ($p<0.05$) on determining the sex of chicks. Regression analysis was done using various morphometric parameters of egg for prediction of sex of chick from the above findings. These regression equations were made by using different combinations of morphometric values. Using paired sample test, better predictions were found out which can be further used for predicting the sex of eggs before incubation. .

Key words*: Sex determination, egg weight, egg morphometry*

The poultry sector stands out as the fastest growing and most versatile among all livestock sectors. In recent decades, it has become more specialised and efficient, focusing on maximising profitability. In layer industry, male chicks are culled at day one itself since their rearing is not economically viable. Each year, over 7.0 billion newly hatched male chicks are culled globally in commercial hatcheries (Alin *et al*., 2019). More than 420 million young male chicks are culled in the European Union, while North America accounts for over 370 million annually (Galli *et al*., 2016). Around 45 million day-old male chicks in Netherlands are killed by carbon dioxide asphyxiation and maceration every year. This presents a significant welfare and economic challenge in the global poultry industry (Buhl, 2013). Determining the gender of chick embryos early in incubation and removing surplus male eggs can not only save incubation costs but also reduce the

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waste of the hatching process since the sex ratio in poultry is 50:50 at almost all times (Wei *et al*., 2016).

In-ovo gender identification of domestic chickens and the associated possibility to remove the eggs likely to be male chicks, before they hatch is one method of resolving this situation (Kaleta and Redmann, 2008). But most of the currently available methods are invasive which may affect the hatchability or post hatch development of the chick. So there is a need for cost effective, quick and efficient non-invasive technique. The use of egg geometrical calculations in population and ecological morphology research is crucial for biological investigations. An evidence linking the morphological characteristics of eggs to the sex of the chicks could be beneficial in this regard. The study aimed to identify a simple and cost-effective solution to address the ethical issue of culling day-old male chicks in layer industry, by employing a non-invasive technique that explore effect of various morphometric parameters like weight, length and width of fertile eggs of White leghorn laying hens in determining sex of chick.

Materials and methods

The experiment was carried out in experimental layer farm under the Department of Poultry Housing and Management, ICAR-Central Avian Research Institute, Izatnagar, Bareilly, Uttar Pradesh. Forty eight White Leghorn laying birds were randomly selected and reared. Half of the birds were 30-32 weeks of age (Young group) and remaining were 50-52 weeks of age (Old group). Birds were reared as parents in individual laying cages for recording the egg production for a period of 120 days. The females were inseminated with pooled semen (1 male: 8 females) at 5 days interval to collect hatching eggs. The eggs were collected three times a day; morning (9am), afternoon (1pm) and evening (4pm) and labelled with their parent's wing band number and date. Morphological parameters of each egg were recorded daily, including length (L) and width (W) measured in centimetre with a digital vernier callipers and egg weight (EW) measured in grams using an electronic weighing balance. Eggs were kept for incubation and candling was done on 5th day of incubation in order to segregate infertile eggs. After hatching, sex determination of 2800 day-old chicks were done by conventional vent sexing method.

The percentage of male and female embryo produced by each parent bird during the 120 days was calculated. Based on this, the parent birds were grouped into young group of birds producing high percentage of male chicks (YHM), young group of birds producing high percentage of female chicks (YHF), old group of birds producing high percentage of male chicks (OHM) and old group of birds producing high percentage of female chicks (OHF).

Statistical analysis of data and formulation of predictive equations using morphometric parameters of egg were done using multiple logistic regression analysis tool of SPSS package 20.0. Regression analysis was employed to evaluate the impact of each individual factor and was done based on data of two groups; eggs which produced high no. of male chicks and eggs which produced high no. of female chicks. A preliminary analysis was carried out to eliminate any insignificant independent variables by using multiple logistic regression. A step-wise approach was selected to identify the optimal model in the multiple logistic regression analysis. Morphological parameters of egg including weight, length and width were considered while constructing regression equations. These equations which involve different combinations of morphometric values were used for predicting sex of chicks by putting those values in equations and compared it with actual sex of the bird. Better predictive models were found out by paired sample test.

Results and discussion

Weight of eggs

The average egg weight of various groups and its effect on sex of chick is presented in Table 1. The egg weight was found to be significant ($p<0.05$) in affecting the sex of chick in YHM, YHF and OHM groups. Idahor *et al.* (2015) reported that egg weight can be taken as a significant indicator in determining the sex of keets. From the current study, probability of having a male chick was high with increase in weight of egg which is in line with the observations of Trivers and Willard (1973), Ankney (1982) and Ryder (1983). They hypothesised that the majority of the larger eggs would be identified as male. Mueller *et al*. (2002) noted a positive correlation between the proportion of male eggs and egg mass. Similar observations were reported by Abanikannda and Leigh (2015) in broiler chicken lines like Ross and Marshall. Budi (2021) also reported that proportion of male eggs was positively associated with egg weight in Pekin ducks.

Table 1: Mean (± SE) Average egg weight (g) of male and female chicks in various groups

Sex of chicks	Egg weight			
	YHM	YHF	OHM	OHF
Male	53.21^{a} ± 0.20	51.37^{a} ± 0.46	55.95^{a} ± 0.34	53.95 ± 0.32
Female	51.20^{b} ± 0.31	50.07^{b} ± 0.39	53.25^{b}± 0.56	53.33 ± 0.44
p-value	0.001	0.030	0.001	0.076

Table 2: Mean (± SE) egg length (cm) of male and female chicks in various groups

Sex of chicks	Length of eggs			
	YHM	YHF	OHM	OHF
Male	5.61[a] ± 0.02	5.50 ± 0.02	5.69 ± 0.02	5.75[a] ± 0.02
Female	5.44[b] ± 0.02	5.53 ± 0.02	5.72 ± 0.02	5.56[b] ± 0.02
p-value	0.001	0.203	0.198	0.001

Table 3: Mean (± SE) Egg width (cm) of male and female chicks in various groups

Sex of chicks	Width of eggs			
	YHM	YHF	OHM	OHF
Male	4.25 ± 0.01	4.17[b] ± 0.02	4.41 ± 0.01	4.29[b] ± 0.02
Female	4.26 ± 0.01	4.25[a] ± 0.01	4.42 ± 0.01	4.39[a] ± 0.01
p-value	0.548	0.001	0.759	0.001

Table 4: Prediction of sex of the fertile eggs using regression equations

Predictions	High female group			High male group		
	Regression equation	R^2	p value	Regression equation	R^2	p value
P1	(0.297×L) + (0.018×W)	0.908	0.001	(0.162×L) + (0.122×W)	0.891	0.001
P2	(0.168×L) + (0.046×W) + (0.009×EW)	0.909	0.001	(0.048×L) + (0.086×W) + (0.015×EW)	0.892	0.001

Table 5: Variation between observed and predicted sex

YHF			YHM		
Pairs	p value	t value	Pairs	p value	t value
P1- Observed	0.310	0.137	P1- Observed	0.950	0.062
P2- Observed	0.181	0.748	P2- Observed	0.726	0.351
OHF			OHM		
P1- Observed	0.022	2.304	P1- Observed	0.477	0.712
P2- Observed	0.125	0.937	P2- Observed	0.041	2.048

Length of eggs

The average length of eggs in different groups and its influence on the sex of the chick is shown in Table 2. The length of the egg was found to significant ($p<0.05$) in influencing the sex of chicks in the YHM and OHF groups. The present research study indicates that the likelihood of hatching a male chick increased with longer eggs which is line with the following observations. Cordero *et al*. (2000), found a significant difference in length between the eggs of male and female house sparrows, with the male eggs being noticeably longer than female. According to Yilmaz-Dikmen and Dikmen (2013), probability of becoming a male embryo increases with increase in length of eggs. Similarly, Milojević *et al*., (2019) reported that longer eggs were more inclined to yield male chicks and Budi (2021) documented that shorter eggs have more chance to become female embryos in Pekin ducks.

Width of eggs

Table 3 presents the average width of eggs across various groups and their impact on chick sex determination which demonstrate that width of the egg was found to have a significant ($p<0.05$) effect on chick sex in both the YHF and OHF groups. The probability of having a female chick was found to be high with increase in width. This finding is in agreement with the study of Yilmaz-Dikmen and Dikmen (2013) who found that the likelihood of hatching a female chick was increased with wider eggs. Aslam *et al.* (2013) also observed the same trend in chicken eggs. According to Budi (2021), eggs with comparatively higher width predominantly like to become pullets in case of Pekin ducks.

Predicting sex of chick embryo by using weight, length and width of eggs

Regression analysis was done based on the morphometric data of eggs which produced high no. of male chicks and high no. of female chicks by young and old age parent groups. Equations were made for predicting the sex of chicks in both younger and older groups of birds as described in Table 4. All of the equations were significant ($p<0.01$) and having high R^2 value. By putting corresponding values in these equations, we were able to get values from 1 to 2. It was assumed that values which were nearer to 1 will be males and those nearer to 2 will be females. After prediction using each equation, values were compared with the actual sex of chicks.

In order to identify the better prediction, we have conducted paired sample test in which each prediction value was paired with the observed sex of chicks. From Table 5, prediction 1 was found to be more accurate for young birds since the predicted and observed values are not varying significantly ($p>0.05$) in both high female and male group. In case of older group, prediction 2 was comparatively accurate for high male group and prediction 1 was accurate for high female group.

Conclusion

From the results of the present study, it could be concluded that the morphometric parameters of egg *viz*., weight, length and width of eggs have significant influence in determining the sex of chicks. Based on the current study, the likelihood of producing a male chick was high with the increase in weight and length of egg, while the probability of having a female chick was high when the width of egg was high. The regression equations which were made by using different combinations of morphometric values (weight, length and width of egg) can be used for predicting the sex of chicks from young as well as old birds. The equations, $(0.297\times L) + (0.018\times W)$ and $(0.048\times L) + (0.086\times W) + (0.015\times EW)$ were giving more accurate predictions in high male group and high female group, respectively.

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Conflict of interest

The authors report no conflict of interest.

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