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Effectiveness of a sexing technique on free-range day-old chick

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1 SUMMARY

The comparison of the length of chick Primary wing feathers (PF) and Secondary (SF) wing feathers was used to sex 228 free-range day-old chicks of diverse phenotypes (Normal, Silky and Frizzled feathered), prior to an experimental study. The sex score = 1, When the secondary feathers were longer than the primary feathers. When the two categories of wing feathers were equal in length, the score = 2 and finally, when the secondary feathers are shorter than the primary feathers the sex score = 3. The results showed that 91.30%, 73.3%, 18.1% of the chicks were male, while 8.7%, 26.6%, 81.8% were female in sex score 1, 2 and 3 respectively. The relative risk of determining male chick in sex score 1 versus score 3 was 50.9 ± 31.9. It was 13.7 ± 9.41 in score 2 versus score 3. The sex determination revealed that the chick is male in score 1 and 2 and female in score 3, giving an overall precision rate of 82.1%. Unlike chick phenotype, body weight was significantly affected by the sex ($P < 0.05$), male chick (28.1 ± 0.5) being heavier than female (25.7 ± 0.4). Indeed, the wing primary and secondary feathers comparative lengths were already sexually dimorphic just after hatching, and must be under the control of the synergistic action of both the somatic sex of the feather cells and the gonad released hormones.

2 INTRODUCTION

Sexual dimorphism in animal population is the differentiation and the development of phenotypic characters, controlled by sexual genes that enable to distinguish between male and female individuals. Higher vertebrate sexing, especially the mammals is determined by the presence of vulva in female and testicles in male already visible at birth . Most birds are sexually monomorphic making sex identification based on appearance, difficult (Lih-Chiann *et al.,* 2006). The difference between male and female of some birds become evident at pubescence with the development of sexual secondary characters. Sex in birds is determined genetically by the inheritance of sex chromosomes, male having two Z chromosomes ("homogametic", ZZ), while female having one Z and one W ("heterogametic", ZW) (Clinton, 1998; Mizuno *et al.,* 2002; Smith and Sinclair, 2004; Smith *et al.*, 2007). It is obvious then, that bird's sex is determined by genes carried on Z and W

chromosomes, but the exact sex determination mechanism among birds has remained obscure for many years (Ellegren, 2001; Smith *et al.,* 2007). According to Schmid *et al.* (2005) and Nanda *et al.* (2008), The Z sex chromosome is highly conserved among birds and must be the same in all species suggesting that the W sex chromosome is a degraded version of the Z in most birds and composed of repetitive sequences, with few bona fide genes (O'Neill *et al.,* 2000; Stiglec *et al.,* 2007). Day-old chick sex determination, realized by Gawron and Robert (1980) using the chick plumage color sexual dimorphism constituted the basis of commercial poultry development with the specialization of the sector in layer (female birds) and broiler (male birds) production. Sexing by molecular methods has been achieved based on genetic markers on the avian sex chromosomes, e.g., CHD1 (Griffiths *et al.,* 1996, 1998 ; Fridolfsson and Ellegren, 1999), EE0.6 (Ogawa *et al.,* 1997 ; Itoh *et al.,* 2001) and WPKCI (Hori *et al.,* 2000 ; O'Neill *et al.*, 2000). Day old chick sexing is of paramount importance and will surely contribute to the success of any local avian resources valorization and promotion driven- research programs, where day-old chick gender is a group factor in the study design. The current study come up with the effectiveness evidence of a traditionally used technique of sexing local free-range day-old chick of silky, frizzled and normal feathered phenotypes.

3 MATERIALS AND METHODS

3.1 Day-old chick management: Fertile eggs from silky, frizzled and normal feathered free-range chicken phenotypes were incubated in an egg incubator . Hatched chicks were reared in controlled environment and improved conditions of the feeding system , basic biosecurity measures of vaccination against Newcastle disease, hygiene and periodic deworming. Chicks were identified with numbered rings fixed at the right wing and housed in wire-floored starting pen under 22-h lighting and held at initially 27 °C. Temperature was steadily adjusted to ensure the comfort of the birds. They had free access to feed and drinking water.

3.2 Day-old chick sex diagnosis technique : The sexing was performed on 228 free-range day-old chicks (81 normal feathered chicks, 69 silky feathered chicks and 78 frizzled feathered chicks), from 24 h to 48 h after hatching. The sexing technique consisted of comparing the length of the Primary (PF) and the Secondary wing feathers (SF) and assigning a corresponding score. The feather length was measured using a tape measure with 1 mm of precision. When the secondary feathers (SF) were superior to the Primary feathers (PF) the score was 1, when the PF were equal to the SF, the score was 2 and finally when the SF were inferior to the PF, the score was 3. Chick identification number as well as chick weight and chick phenotypes were recorded. Chicks were sacrificed at 21 day-old and the abdominal region opened for sex identification through either testicles or ovaries observation.

3.3 Statistical analyses: Collected data were stored and managed in MS Excel 2007. The descriptive and inferential statistics were made in SAS (vo. 9.2). The categorical binary response variable in the LOGISTIC regression analysis model is the sex (male and female). The explanatory variables were the sex diagnosis score (1, 2, 3) with a covariate which was the chick phenotypes (1, 2, 3). Phenotype 1 = normal feathered, phenotype 2 = silky feathered and phenotype 3 = frizzle feathered. The LOGISTIC procedure to fit a two-way logit with interaction model for the effect of sex diagnosis and phenotype was used. Male gender was referred to in the regression model with the estimation of the relative risk of being male chick in each diagnosis score, with Wald 95% confidence limits as well as the predicted probabilities of being male chick in each phenotypic category (phenotype effect). The freq procedure with Fisher test was used to

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evaluate the sex diagnosis precision rate. Variance analysis with F test in General Linear Model procedure was applied to day-old chick weight.

4 RESULTS

4.1 Sex diagnosis precision and chick weight: Results in table 1 showed that when the primary wing feathers are shorter than the secondary wing feathers (sex score 1), 91.3% of the chicks were male and 8.7% were female. Also when the primary wing feathers are equal to the secondary wing feathers (sex score 2), 73.3% of the chicks were male and 26.6% were female and finally when the primary wing feathers are longer than the secondary wing feathers (sex score 3), 81.8% of the chicks were female and 18.1% were male. The sex score 1 and 2 gave stronger prognosis for male chicks detection, while the score 3 was favorable for female chick detection, giving an overall sexing precision rate of 82.1%. The mean weight of day-old male chicks (28.1±3.3) was significantly higher than that of female day-old chick (25.7±3.5) as demonstrated on the figure 1 ($P < 0.05$).

Table 1. *Sex diagnosis precision (%)*

Sex score	Male chick (%)	Female chick (%)
Sex score 1	91.30	8.7
Sex score 2	73.3	26.6
Sex score 3	18.1	81.8
Effect	P<0.05	

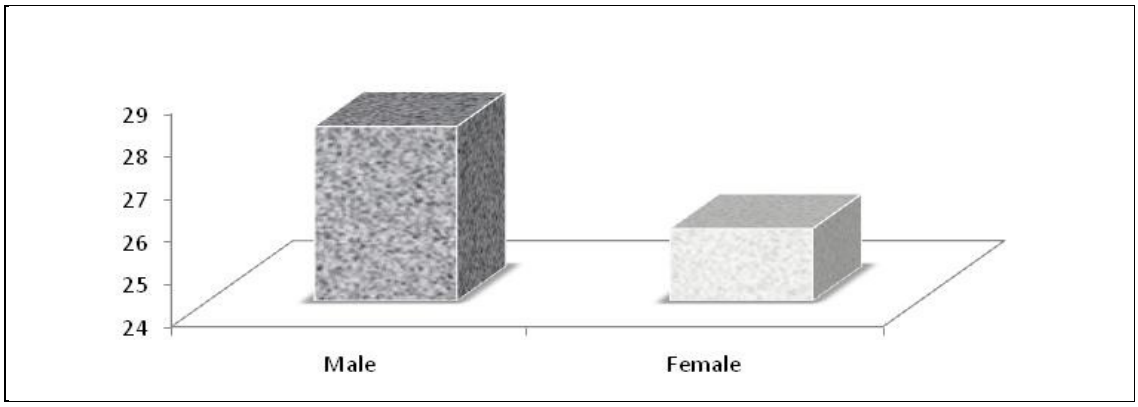

Figure 1. Day-old chick weight

4.2 Relative risk of diagnosing male chick and phenotype effect : Findings in table 2 showed that there was approximately 50.9 fold increase in the risk of having a male day-old chick when the primary wing feathers are inferior to the secondary wing feathers (sex score 1) than the other way round (sex score 3). The relative risk for the chick to be male is 13.7 times higher when the two categories of wing feathers are equal (score 2) than when the primary feather are longer (score 3). The relative risk of determining a male chick is statistically similar in sex score 1 versus sex score 2 ($P > 0.05$). The predicted probabilities of detecting male chicks in each score (1, 2 and 3) were statistically the same regardless of the chick phenotype (silky, frizzled and normal feathered). Therefore chick phenotype effect was not significant on the sexing method used ($P > 0.05$) as demonstrated on figure 2.

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Table 2. Estimates of the relative risk (mean ± standard error) of diagnosing male chick

Effets	Relative risk	95% Wald confidence limits		Pr > Chi-square
Score 1 vs 3	50.9 ± 31.9	14.9	174.1	< 0.0001
Score 2 vs 3	13.7 ± 9.41	3.6	52.5	0.0001
Score 1 vs 2	3.6 ± 2.91	0.7	17.3	0.09

VS: Versus

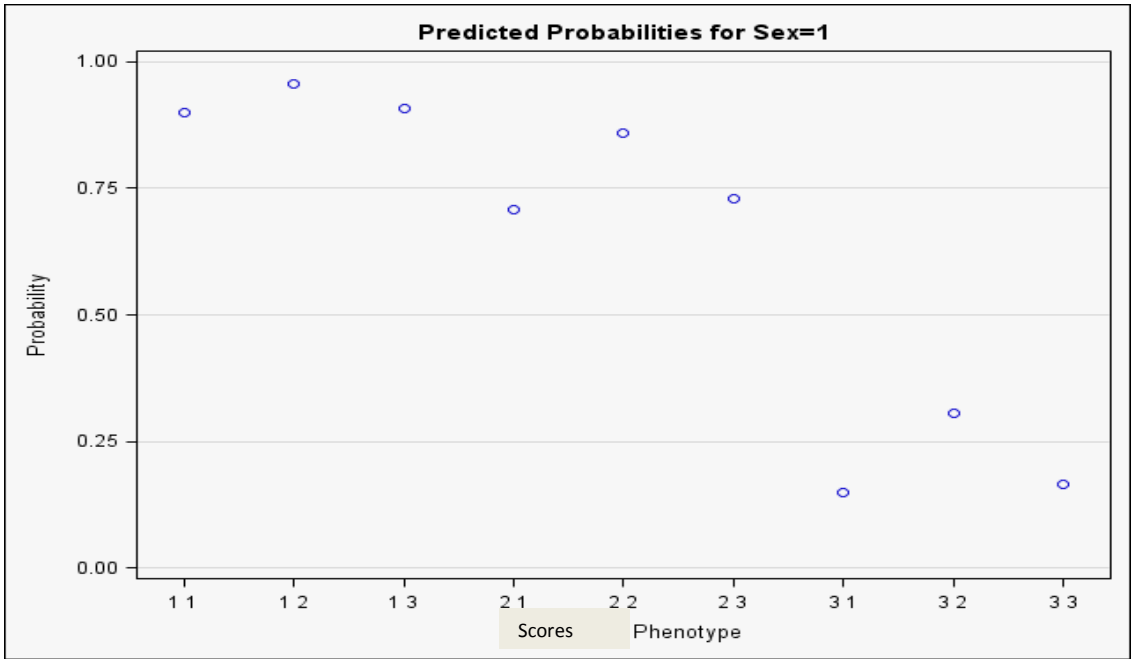

Figure 2: Predicted probabilities with combined effect of the binomial: sex score and male chick phenotype

5 DISCUSSION

These results obviously proved that, when the primary wing feathers are shorter or equal to the secondary wing feathers, the chick is male and when the primary wing feathers are longer than the secondary wing feathers the chick is female. The success or precision rate obtained in the study (82.1%) was slightly inferior to that obtained (97%) by Gawron and Robert (1980), who used the day-old chick plumage color sexual dimorphism to differentiate between male and female. His method has the disadvantage of being layer chicken-breed-specific and therefore, cannot be applied to the entire chicken species. The sex diagnosis was much more precise, detecting male chick when the primary feathers are markedly shorter than the secondary feathers (91.3%). The test was also evident in the favor of female chicks when the primary feathers are longer than the secondary feathers (81.8%). The 26.6% of imprecision rate posted by diagnosis 2 (73.3% of precision rate) could be ascribed to the slight over growth of the wing primary feathers due to late diagnosis. The associated lower relative risk of detecting male sex in sex score 2 versus score 3 (13.7) compared to that of sex score 1 versus score 3 (50.9) was suggestive of the imprecision. This also explains the overall obtained failure rate of 17.7%.

It is well recognized that plumage pattern in bird in general are marked sexual characters that enable differentiation of male from female individuals especially at sexual maturity period. Bird plumage design is therefore controlled by genes located on sexual chromosomes. Sex in birds is determined genetically by the inheritance of the sex chromosomes (Smith *et al.,* 2007). The traditional view that the sexual development in birds and other vertebrates is solely under the control of gonad released hormones during the embryonic life to masculinize or feminize the entire body as originally suggested by some authors, has considerably evolved in the recent years (Smith *et al.,* 2009). studies showed that sexually dimorphic gene expression occurs in embryos before the gonads have differentiated into ovaries or testis (Smith *et al*., 2007; Scholz *et al.,* 2006). It was strongly suggested that all organism cells possess somatic sex that controlled the sexual differentiation of the host cells irrespective of the gonads (Agate *et al.,* 2003). Therefore sex-determining genes may operate not only in the gonads, to produce testes or ovaries, but also throughout cells of the body. The sexual dimorphism of chick wing primary and secondary feather length might be synergistically controlled by both their own somatic sex chromosome gene/s and by the gonad released hormones.

The phenotype has had no effect on day-old chick sex determination in the current study. With the effectiveness of 82.1%, the sexing technique developed herein, based on the comparison of the primary and secondary wing feather length has the advantage of not being chick breed-specific method like other technique earlier highlighted by Gawron and Robert (1980) and Campo (1991). In contradiction to the phenotype, sexual dimorphism of body weight is demonstrable by the significant difference ($P < 0.05$) between male and female, male chick (28.1 ± 0.5) being heavier than female chick (25.7 ± 0.4). The chick body weight results paralleled that obtained by Lombo *et al.* (2011). The consideration of day-old chick body weight in the sex diagnosis technique accessed in this study will significantly improve the effectiveness of the method. Surely, the sexing technique herein experimented proved to be efficient, practically simple with no related operation cost. However the sexing has to be done within 24 and 48 hours after hatching to increase its precision

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