CORRELATION OF CIRCULATING VITELLOGENIN AT SEXUAL MATURITY WITH SELECTED LAYING TRAITS IN PHILIPPINE MALLARD DUCK (*Anas platyrhynchos domesticus* L.)

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ABSTRACT

Vitellogenin (Vtg) is a large precursor molecule of the two major egg yolk phosphoproteins, namely; lipovitellin and phosvitin. All of the vitellogenic zinc is found in lipovitellin. Several reports had speculated into the probable relationships of Vtg with reproductive phenotype of Philippine Mallard duck (*Anas platyrhynchos domesticus* L.). Hence, it was hypothesized to use the Vtg profile as a nonlethal physiological index of reproductive performance in Philippine Mallard duck. Forty ducks, aged 16 weeks old, were randomly assigned into two treatment groups: 20 ducks fed with 30 ppm zinc-diet (zinc positive) and 20 ducks fed with no added zinc (zinc negative). All ducks were kept individually in cages. The circulating Vtg at sexual maturity (155.11±10.83 days old) was determined from the blood sera. The sera were assayed for Vtg in duplicate using 96-well microplate and the optical density was read at 415 nm. Results show that the circulating Vtg in the blood sera of ducks at sexual maturity was 0.69±0.07 µg Zn/dl. This circulating Vtg was identified to have negatively very weak to weak linear correlation with the majority of the laying traits (11 out of 17) in Philippine mallard duck.

Keywords: laying traits, Philippine mallard duck, sexual maturity, vitellogenin

INTRODUCTION

The Philippine duck industry is contributing significantly to the total value of agricultural production. Earnings of the industry are primarily from egg and meat production. Despite remarkable earnings, the industry still posted decline in duck egg and meat production attributed to the problems in flock performance, management and natural calamities. Moreover, productivity analysis showed various technical and socio-economic constraints affecting technical efficiency in production. The average technical efficiency in duck production was 0.62, which implies that the efficiency in production is 0.38 below the expected technical

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efficiency (Chang and Villano, 2008). This finding indicates that the Philippine duck industry requires technological and scientific interventions to improve farm productivity, specifically the laying performance.

Laying performance is governed by many traits that are influenced by genetics, environment and interaction effects. These major factors can be regulated and modified under the state of current technological advances. However, improving the performance of laying traits entails an understanding of the mechanisms and interactions underlying the expression of these traits. There are various methods utilized to improve laying efficiency from the use of fundamental knowledge in breeding such as selection and mating systems to the application of molecular technology like marker-assisted selection. All of these methods require large population size, large facilities for research, organized systems for handling and evaluation of large data sets and are, therefore, time-consuming. Hence, it is vital that a research procedure that could offer similar results at least cost and at a short period of time be developed. The novel procedure is the association of vitellogenin with the expression of reproductive phenotype (Gorman *et al*., 2009; Han *et al*., 2009).

Vitellogenin (Vtg) is a large precursor molecule of the two major egg yolk phosphoproteins, lipovitellin and phosvitin (Deeley *et al*., 1975). The association of Vtg and its receptors with reproductive phenotype was demonstrated in zebra finch, *Taeniopygia guttata* (a songbird) and greater scaup, order: Anseriformes, *Aythya marila* (Gorman *et al*., 2009; Han *et al*., 2009). These reports created enthusiasm to explore the probable relationships of Vtg with reproductive phenotype of Philippine Mallard duck. Hence, this study investigates the amount of circulating Vtg at sexual maturity and determines its relationship with reproductive phenotype in Philippine Mallard duck.

MATERIALS AND METHODS

Experimental design

Forty 16 weeks old ducks, were kept individually in cages with identification code for recording purposes. Twenty ducks were fed ration supplemented with 30 ppm dietary zinc (zinc positive) (Sahin *et al*., 2002) using feed grade zinc oxide (MW = 81.41 g/mol) from 16 weeks of age until 40 weeks old. The other 20 ducks were fed the control ration (zinc negative). The supplementation of dietary zinc in the diet was considered for purposes of determining the possible influence of zinc on Vtg production or its interference with the Vtg zinc assay (Mitchell and Carlisle, 1991; Salvante and Williams, 2002; Gorman *et al*., 2009).

Collection of serum sample

One milliliter of blood was collected from each duck at sexual maturity. The blood samples were placed in a 1.5 ml microtube and stored in ice chest before transporting to the laboratory. The coagulated blood samples were centrifuged at 1200 rpm for 10 min. About 200 µl serum samples were collected from each blood sample. The samples were placed in properly labeled 1.5 ml microtubes and stored

at -20ºC until vitellogenic zinc assay.

Vitellogenic zinc concentration

Forty serum samples were assayed in duplicate for vitellogenic zinc (Zn; zinc kit, BioAssay System, USA) as surrogate of Vtg following the described protocol using the 96-well microplate. Briefly, 50 µl of serum were transferred into wells where 200 µl working reagent were added. The mixtures were incubated for 30 min at room temperature and the optical density (OD) was read at 415 nm using the microplate reader (Model 680, S/N 123669; Biorad, Hercules, CA, USA.). The OD reading was used in calculating the concentration of vitellogenic zinc in a serum sample. The zinc concentration of the samples was determined from standard values by non-linear regression using the formula $\Delta OD = a \times [Zn^{2+}] / (b + [Zn^{2+}]$. Calculation and graphical formatting were performed in Microsoft Excel. The vitellogenic zinc concentration was expressed in µg/dl using the conversion factor 1µM = 6.5 µg/dl.

Egg production performance

The egg production performance of 40 ducks was determined based on age at sexual maturity, laying duration, egg number, hen-day rate egg production, prime sequence length, sequence length/clutch size, number of sequences, pause length, number of pauses during laying and feed efficiency (Robinson *et al.*, 2001; Chen *et al.*, 2007). The age at sexual maturity was determined by counting the number of days from day-old until lay of first egg. The laying duration, egg number, hen-day egg production rate, prime sequence length, sequence length/clutch size, number of sequences, pause length, number of pauses during laying and feed efficiency were determined from individual daily egg production records from lay of first egg until 40 weeks of age.

The hen-day egg production rate was calculated by dividing the total number of eggs for each hen by the number of days from lay of first egg until 40 weeks of age. Sequence length was measured by counting the number of days by which an egg was laid before a non-laying or pause day. In the event that two eggs were laid on the same day, one egg was recorded as the previous day's egg only if there was no egg laid the previous day and one of the two eggs was laid prior to the first morning collection. Sequence lengths were calculated for the entire laying period. The mean sequence length was the average of all sequences while the prime sequence was the longest uninterrupted laying sequence (Robinson *et al*., 2001).

The egg quality traits measured were egg weight, egg shell appearance (normal shelled eggs, soft shelled eggs and shell-less eggs), incidence of double-yolked eggs (Robinson *et al*., 2001; Chen *et al*., 2007) and egg shape index. Egg weights were recorded daily from lay of first egg until 24 weeks old and once a week thereafter. The egg shape index was calculated as the proportion of egg width to egg length, expressed in percentage.

Data analysis

All data were tested for equality of variances and normality distribution using the Levene's test (Hovtest) and Shapiro–Wilk test (Proc Univariate), respectively. Data with heterogeneous variances or non-normally distributed were cleared for

outliers or were transformed before subjecting to statistical analysis. The vitellogenic zinc concentrations were square root transformed. The hen-day egg production rate, percentage of normal shelled eggs, soft-shelled eggs, shell-less eggs, double-yolked eggs and egg shape index were arcsine transformed. The association of vitellogenic zinc concentration with measures of laying traits was tested using correlation analysis (Proc Corr) in SAS.

RESULTS AND DISCUSSION

Circulating vitellogenin at sexual maturity

The circulating vitellogenin (Vtg, µg Zn/dl) at sexual maturity between ducks fed with or without 30 ppm Zn as zinc oxide in the diet starting at 16 weeks of age was similar. This indicates that the zinc supplemented in the ration did not induce a significant change in the concentration of circulating Vtg. The effect of zinc was tested to determine its possible influence on Vtg production or its interference with the zinc assay (Mitchell and Carlisle, 1991; Salvante and Williams, 2002; Gorman *et al*., 2009).

The mean Vtg concentration in the blood serum of ducks at sexual maturity was 0.69±0.07 µg Zn/dl. Variance component analysis shows that all variations (100%) in Vtg are attributed to individual effects indicating relative differences in hepatic production and demand from the developing ovarian follicle hierarchy (CV = 15.43%). Similarly, this was observed in the greater scaup (*Aythya marila*) with full ovarian follicle hierarchy and non-developed ovaries (Gorman *et al*., 2009). In zebra finches (*Taeniopygia guttata*), the circulating Vtg was detectable only during yolk development and was undetectable at clutch completion (Salvante and Williams, 2002).

Performance of selected laying traits

The age at sexual maturity (in days) was 155.11±10.83 (Table 1). Further observation shows relatively high level of uniformity in the maturity age (CV = 8.88%). However, ducks in this trial had late sexual maturity as compared to the reports of Dagaas (1995) and Deeden (2005). From lay of first egg until 40 weeks of age, ducks had undergone 124.63±13.09 d. A 100% inter-individual variation in laying duration was observed. Ducks laid a total of 109.21±21.08 eggs, higher than that obtained by Deeden (2005).

All of the variations (98.02%) in the hen-day egg production rate were attributed to the inter-individual effect. The highest laying potential was determined at 87.98±14.64%. This observation was higher compared to those obtained from ducks with different plumage patterns subjected to different feeding regimes having 25-75% egg production rate (Romjali, 2001) and those fed different feed brands which had 64.47% and 70.59% production rates (Dagaas, 1995).

The sequence length is the period by which an egg was laid before a non-laying or pause day. It is analogous to clutch size or the number of eggs laid consecutively before a pause day. Ducks were observed to lay consecutively for 17.20±10.25 days. A 100% inter-individual variation was observed in the sequence length. The prime sequence length was 45.91±22.89 d reaching up to 109.30 days.

Table 1. Circulating vitellogenin at sexual maturity and performance of selected laying traits from lay of first egg until 40 weeks of age in Philippine Mallard duck (A*nas platyrhynchos domesticus* L.) (N=40).

Parameter	Mean±SD
Vitellogenin (µg Zn/dl) at sexual maturity	0.69±0.07
Age at first egg, d	155.11±10.83
Laying duration, d	124.63±13.09
Total eggs produced	109.21±21.08
Hen-day egg production rate, %	87.98±14.64
Sequence length, d	17.20±10.25
Number of sequences	9.65±7.42
Prime sequence length, d	45.91±22.89
Pause length, d	2.79±5.40
Feed conversion, kg/doz	3.44±1.09
Average egg weight, g	64.65±4.14
First egg weight, g	49.78±7.78
Last egg weight, g	71.35±4.56
Egg shape index, %	74.61±2.84
Normal shelled eggs, %	97.08±5.95
Soft-shelled eggs, %	2.69±5.93
Shell-less eggs, %	0.23±0.68
Double-yolked eggs, %	0.05±0.19

A 100% inter-individual variation in prime sequence length was observed (CV = 59.27%). The highest sequence length of 418 d was identified in Khaki Campbell ducks (Hutt as cited by Pingel, 1990). Hybrids of ducks were also found to lay 275 eggs from 20 to 72 weeks of age while meat-type duck could produce more than 200 eggs in a 40-week period (Powell as cited by Pingel, 1990). Every pause in laying lasted up to 2.79±5.40 at frequency of 8.76±7.50 times during the laying duration. The variation in pause length is attributed to inter-individual differences (CV = 161.40%). The pause length in ducks was seemingly higher than in chickens (Robinson *et al*., 2001).

Feed conversion ratio (FCR) expressed as the ratio of total feed consumed (kg) to dozen eggs produced was 3.44±1.09. The observed FCR was higher than those obtained by Deeden (2005) and Dagaas (1995). However, the FCR of ducks based on plumage pattern and fed ration of different forms was higher compared to that obtained by Romjali (2001). The average egg weight ($EW_{Ave)}$ was 64.65±4.14 g. The variation (100%) in EW_{Ave} is attributed to inter-individual differences. The observed egg weights conformed to the reports of Dagaas (1995), Romjali (2001) and Deeden (2005). The weight of first egg was 49.78±7.78g. There was relatively high uniformity in the first egg (CV = 15.27%). The weight of last egg (LEW) at 40 weeks of age was 71.35±4.56 g. The EW at 40 week of age was found to be higher than those observed by Deeden (2005).

The egg shape index (ESI) at 40 weeks old was 74.61±2.84%. The variation in ESI is attributed to inter-individual differences (98.36%) and was relatively similar to that obtained by Deeden (2005). Most of the eggs were normally shelled (97.08±5.95%). The variation observed is attributed to inter-individual differences (97.05%). The environmental conditions were found to directly influence the occurrence of abnormal shelled-eggs. Egg production, egg weight, shell weight, shell thickness, and specific gravity were significantly reduced among hens exposed to cyclic daily temperatures (23.9-35ºC) and relative humidity (15-50%) (Mashaly *et al*., 2004). The occurrence of double-yolked eggs was only posted at less than 1% (0.05±0.19%). The variation (100%) in the occurrence of soft-shelled eggs is attributed to inter-individual differences. The occurrence of double-yolked eggs was observed in early maturing hens, suggesting a genotypic influence (Robinson *et al*., 2001).

Relationship of circulating vitellogenin with laying traits

No significant strong association was observed between circulating vitellogenin (Vtg, µg Zn/dl) at sexual maturity and selected laying traits (Table 2). Five laying traits, namely, maturity age, pause length, number of pauses, feed conversion ratio and incidence of shell-less eggs, were identified to have positively very weak linear correlation with Vtg. The other identified laying traits were negatively correlated. This indicates that the majority (11 out of 17) of the laying traits were negatively correlated with the circulating Vtg. This suggests that high uptake rates in large follicles can actually deplete circulating Vtg concentrations (Challenger *et al*., 2001).

On the other hand, report shows that Vtg is strongly correlated with ovary mass and egg weight (Gorman *et al*., 2009). A diet-dependent relationship between Vtg and egg size was identified also in zebra finches (*Taeniopygia guttata*), which shows that low plasma Vtg levels were associated with both very small (<0.90 g) and very large (>1.15 g) egg sizes (Salvante and Williams, 2002).

The above results suggest that circulating Vtg at sexual maturity was identified to have negatively weak linear correlation with the majority of the laying traits (11/17) in Philippine mallard duck.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Department of Agriculture through the Philippine Agriculture and Fisheries Biotechnology Fellowship Program for providing financial assistance and the Department of Science and Technology for the scholarship support to the senior author.

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Table 2. Correlation of circulating vitellogenin at sexual maturity with selected laying traits from lay of first egg until 40 weeks of age in Philippine Mallard duck (A*nas platyrhynchos domesticus* L.) (paired N=40).

Trait	Correlation coefficient
Maturity age	0.06
Laying duration	-0.06
Total eggs produced	-0.11
Hen-day egg production rate	-0.11
Prime sequence length	-0.29
Sequence length	-0.12
Pause length	0.11
Number of pauses	0.13
Feed conversion ratio	0.12
Average egg weight	-0.21
First egg weight	-0.22
Last egg weight	-0.07
Normal shelled eggs	-0.16
Soft-shelled eggs	0.22
Shell-less eggs	0.04
Double-yolked eggs	-0.28
Egg shape index	-0.15

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