Cadmium in Feeds and Tissues of Female Mallard Ducks in Selected Farms of Victoria and Candaba

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Abstract

This study was undertaken to determine the cadmium (Cd) in feeds and tissues and to document the physiologic condition at peri-pubertal (4-8 months), mid (10-14 months) and late laying (≥18 months) stages of ducks from selected farms in Victoria (V) and Candaba (C), Philippines. The commercial and conventional feeds (i.e. snails), liver, ovary muscle and fat samples of ducks were assayed for Cd using AAS-FLAME. The body condition score (BCS), hepatosomatic, ovary-somatic and oviductosomatic indices were measured. Plasma vitellogenin was established and assayed using ELISA kit, using bird vitellogenin antibody. Results showed that Cd was detected in the liver (0.68 ppm) and ovary (0.07ppm), but negligible in muscle and fats tissues. The mean Cd in commercial feeds was 0.29 ppm (51.7%) and 0.4 ppm (100%) in Victoria and Candaba, respectively. Morphological abnormalities in the liver (V=49%, C=35.9%) and ovary (V=13.6%, C=15.6%) of female ducks were comparable in both locations. This study confirmed the presence of Cd in feeds and tissues of ducks in both sites. Interestingly, in Candaba where Cd in feeds and organs was higher than in Victoria, the plasma vitellogenin was lower (P<0.05), which can be related to possible negative effect of Cd on egg production.

Keywords: *cadmium, feeds, liver, ovary, mallard duck, vitellogenin*

Introduction

Duck production is one of the most profitable poultry industries in the Philippines mainly because of the increasing demand for duck eggs (fresh, *balut*, *penoy*, salted eggs, and century eggs). Although it ranks only second to chicken in terms of egg and meat production, its crucial importance in the Philippine poultry industry lies in its provision of employment and income-generating opportunities for

Filipinos, particularly those in the rural areas (PCARRD, 2008). However, despite the promising opportunities for the industry, there has been a consistent decline in the duck industry performance for years (BAS 2010). The 2010 duck industry performance report by the Bureau of Agricultural Statistics shows that the country's total duck population was down by 1.96% in comparison with the total duck population in 2009. Consequently, the total volume of production dropped by 8.21% and the duck egg production went down by 7.42% from the previous year's level.

The reported decline in the Philippine duck production performance can be attributed to several problems and constraints confronting the duck industry, including disease outbreak, lack of industry organization, scarce and unpredictable quality of locally available feed materials, lack of information on the nutrient requirement of ducks, high cost of commercial feeds, lack of policies directly supporting duck production, low quality breeding stocks, and limited space of herding (Lejano, 2009; BAS, 2010; Lambio, 2010; and FFTC, 2009). Recently, one of the major concerns in the Philippine duck industry is the negative effects of the endocrine-disrupting chemicals (EDCs) in the physiological processes of ducks. These chemicals may be present in the sources of feeds for ducks including rice fields brought about by the more intensive rice cultivation practice and use of pesticides.

The U.S. Environmental Protection Agency defines EDCs as "exogenous agents that interfere with the synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process (Diamanti-Kandarakis *et al.*, 2009). These substances comprise a growing number of compounds which include exogenous natural substances, human-made chemical compounds and heavy metals (Soyano *et al.*, 2010 and Ghijsen and Hoogenboezem, 2000). Cadmium (Cd) is considered one of these compounds which can cause alterations in the endocrine status of different species.[34]

This compound has been conclusively found to have adverse effects on a number of organs in animals and humans (Takiguchi and Yoshihara, 2006). In ducks, a number of studies that have been conducted on the effects of Cd, along with organochlorine pesticides (OCPs) in duck feeds on the reproductive performance of the mallard ducks, showed that the compounds negatively affect the

[34] Cadmium is a lustrous, silvery-white, ductile and a very malleable metal. It is a common environmental and industrial contaminant dispersed throughout the environment today mainly as pollution from a variety of sources. This metal has no known beneficial biological function; yet prolonged exposure to it has been linked to toxic effects in both humans and animals (Henson and Chedrese, 2004). Recently, it has been reported that Cd exhibits estrogen and androgen-like activities in vivo and in vitro and binds to estrogen and androgen receptors; hence, it is regarded as a potential endocrine disruptor. These observations suggest a direct action of Cd-enhancing cellular responses to estrogen and androgen.

growth and development of mallard ducks, particularly those of their reproductive organs (Vega, 2009).

Since the Philippine domestic mallard ducks may be considered exposed to these substances, the effects on their reproductive and sexual development may, in turn, have affected the entire performance of the industry. Since previous studies found the negative effects of EDCs on ducks and have hypothetically regarded Cd as one of the culprits in the consistent decline of the Philippine industry's performance, this research focused on assessing the real status of a number of duck farms in selected sites to determine if the feeds used are indeed contaminated and the ducks, really affected with Cd. In order to validate this claim, this study examined the both the Cd levels in duck feeds as well as the general physical and physiologic condition of sexually mature female ducks in the Victoria and Candaba areas.

Materials And Methods

Sampling Procedures

A total of 123 ducks at peripubertal (4-8 months, n=43), mid laying (10-14 months, n=40) and late laying (18 months and above, n=40) stages were sampled in Victoria and Candaba, Philippines. The ducks were sacrificed for the extraction and isolation of organ samples which include the liver, ovary, and oviduct. Fat and muscle samples were also taken during the sacrifice of ducks. Each sample was wrapped in a labeled aluminum foil and kept inside the refrigerator at -40°C to prevent natural chemical degradation of the sample components until actual analysis for Cd residue levels. Sampling of commercial and conventional feeds used by farmers in both sites was also done. Cadmium content analysis of the samples using atomic absorption spectrophotometry was carried out at the Natural Sciences Research Institute (NSRI) in the University of the Philippines Diliman, Quezon City, Philippines (ISO-17025:2005).

Live Weight and Body Condition Score

The live weights and body condition scores of ducks were recorded before they were sacrificed. Whereas the live weight of the ducks were obtained using a weighing scale, the body condition score or level of fatness on the body of ducks was evaluated by visual appraisal and palpation. The ducks were held by the legs in one hand and the palm of the other hand was used for palpating and grading the protuberance of the keel, development of the breast muscles and the convexity or concavity of the breast muscle contour (NR International, 2006). The scoring system for layer hens developed by Gregory and Robins (1998) in Massey University was adopted in assessing the body condition of ducks in this study.

Photo-documentation and Organ Measurements

Photos of abnormalities observed in the organ samples were taken with the use of a digital single lens reflex (DSLR) camera to obtain high quality image. Observations and cases of abnormalities were recorded at the same time for documentation purposes. The percentage of abnormality among the organs was then calculated.

Organosomatic Indices

Each organ sample was weighed in order to determine the organosomatic indices of the female domestic mallard ducks in both locations. The formula below by Gundersen (2002) was followed in computing for ratios of the organ to body weight.

$$\text{HSI (\%)} = \frac{\text{fresh organ weight (g)}}{\text{live weight (g)}} \text{ X } 100$$

Hepatosomatic Index (HSI)

The HSI, which is an indicator of hepatic development according to age and physiological or physiochemical status of the liver, was obtained using the ratio between the fresh liver weight and the live weight, multiplied by 100 and expressed in percent (%) of the body weight of ducks.

Ovary-Somatic Index (OSI)

The OSI was used to estimate the condition of the reproductive system of the ducks. The ovary to live body weight ratio was computed in a way similar to the manner by which the HSI was obtained and was also expressed in percent of the body weight.

Oviductosomatic Index (ODSI)

The ratio of the oviduct weight to the live body weight of ducks was computed in the same manner that the other organosomatic indices were computed.

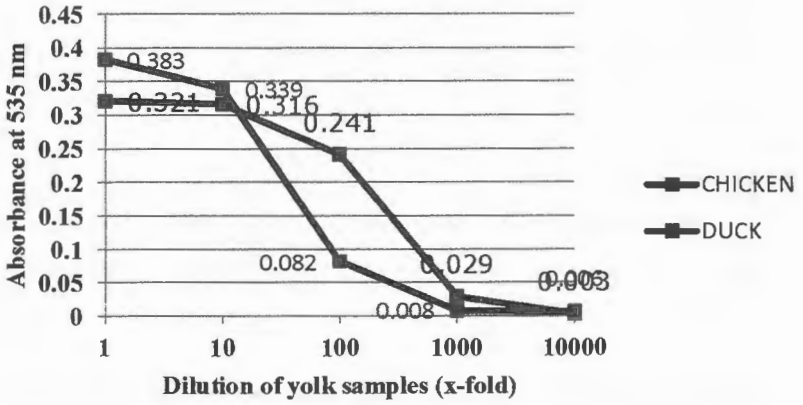
Blood Collection

Preprandial blood collection was carried out during the sacrifice of the mallard ducks. Five (5) ml of blood was drawn from the jugular vein of the duck samples and placed in heparinized tubes. The blood samples were placed in ice and transported immediately to the Animal Physiology Laboratory, ADSC, UP Los Baños for centrifugation at 3000 rpm for 20 minutes to separate plasma from cellular fraction. The aliquot of the samples was stored in the refrigerator at -40°C until the scheduled assay for plasma vitellogenin.

Vitellogenin Analysis

The frozen plasma samples were thawed out and pooled before they were transferred into the 96 – well assay plate which was supplied with the ELISA kit. The determination of plasma vitellogenin was carried out using the Enzyme-Linked Immunosorbent Assay kit purchased from the Biosense Laboratories AS (Norway). The standard used was vitellogenin from duck egg yolk (vitellogenin yolk equivalent) since duck vitellogenin has not yet been sequenced and reported. The bird vitellogenin antibody parallelism/cross-reactivity was accomplished successfully with chicken and duck egg yolk as standards. The cross-reactivity of the mouse anti-bird vitellogenin monoclonal antibody ND-3C3 to duck yolk vitellogenin is presented in Figure 1.

Figure 1: Parallelism of mouse anti-bird vitellogenin monoclonal antibody, ND-3C3 (Biosense Laboratories – Bergen, Norway) to duck yolk vitellogenin



Statistical Analysis

The values were analyzed using PROC GLM of SAS (Cary USA) and factors in the lay-out were considered which include two locations and the stages of maturity of the ducks. Some of the parameters did not fit the normal distribution as depicted in the software output and required data transformation. The statistical design used was 2 X 3 factorial design in CRD (2 locations and 3 stages). The values obtained in the measurement procedures were presented in Mean±SEM, and comparison of means between and within different factors was done using DMRT.

Results And Discussion

Cadmium Content Analysis of the Organ Samples

The results varied greatly for the organ and feed samples were detected with different levels of Cd. Table 1 shows the Cd concentrations in mg/kg detected in the pooled samples of liver, ovary, muscle

and fat of the mallard ducks at different stages of maturity in Victoria and Candaba. Different patterns were observed in the levels of Cd in the organs of ducks but the highest concentrations were detected in the liver and ovary samples of ducks at the late laying stage in both locations. The liver of ducks showed the highest Cd levels detected among the different biological samples subjected to analysis.

ORGAN/STAGE		LOCATION		MEAN
		Laguna	Pampanga	
Liver	Peripubertal	0.17^{bz}	0.75^{ay}	0.46^{b}
	Mid Laying	0.55^{ay}	0.35^{bz}	0.45^{b}
	Late Laying	1.1^{bx}	1.17^{ax}	1.14^{a}
	Mean	0.61	0.75	0.68^{x}
Ovary	Peripubertal	0.05^{by}	0.09^{ay}	0.07
	Mid Laying	0.04^{y}	< EMDL*	< EMDL
	Late Laying	0.11^{bx}	0.15^{ax}	0.13
	Mean	0.07	0.08	0.07^{y}
Muscle	Peripubertal	< EMDL	< EMDL	< EMDL
	Mid Laying	< EMDL	0.07	< EMDL
	Late Laying	< EMDL	0.09	0.04
	Mean	< EMDL	0.053	< EMDL
Fats	Peripubertal	< EMDL	< EMDL	< EMDL
	Mid Laying	< EMDL	< EMDL	< EMDL
	Late Laying	< EMDL	< EMDL	< EMDL
	Mean	< EMDL	< EMDL	< EMDL

*EMDL – estimated method detection limit

Table 1: Mean Cd content (mg/kg) in pooled samples of liver, ovary, muscle and fat at different stages of female domestic mallard ducks in Laguna and Pampanga with estimated method detection limit (EMDL) of 0.04 mg/kg

In contrast, muscle and fat are less likely to accumulate Cd, based on the results. In some studies, Cd has been shown to accumulate preferentially in the inner organs (Sckalicka *et al.*, 2002). Furthermore, the results confirmed the World Health Organization's report that Cd accumulates largely in the liver and not in the muscle tissue. Also, the Cd levels in the liver and ovary of ducks at the late laying stage in both locations were relatively higher than the quantities of the heavy metal detected in the organs of ducks at the peripubertal and mid laying stages. The results reinforced the report by Irwin *et al.* (1997) as cited by Sckalicka *et al.* (2002) that Cd clearly accumulates with increasing age of the animal. Higher Cd concentrations were detected in most of the biological samples in Candaba as compared to the samples taken from Victoria. Moreover, Cd in the muscle samples were observed only in ducks at mid and late laying stages in Candaba.

According to the Codex Alimentorum of the Slovak Republic No. 98/1996 and with the literature presented in some European countries, the maximum permissible hygiene limits for Cd are 0.1 mg/kg in meat and 0.5 mg/kg in liver (Sckalicka *et al.*, 2002). The results in this study showed that the Cd residue levels detected in some muscle samples of ducks were below the maximum permissible hygiene limits for Cd in poultry. Three (3) of the pooled liver samples of ducks, however, exceeded the maximum permissible hygiene limits for Cd in the internal organs for poultry (0.5 mg/kg).

Cadmium Content Analysis of the Feed Samples

The mean Cd contents (mg/kg) in the commercial and conventional duck feeds in Victoria and Candaba are given in Table 2. Surprisingly, the commercial feeds had much higher cadmium residue levels than the concentrations detected in the conventional feeds (snails). All the commercial feed samples taken from Candaba were found to contain Cd while some of the samples in Victoria were observed with Cd below the EMDL adopted by the laboratory. In addition, the highest Cd level detected in the commercial feeds from Candaba (0.84 mg/kg) was relatively higher than that of the highest Cd concentration detected in the sample from Victoria (0.46 mg/kg).

Cadmium enters the environment from natural processes such as erosion and abrasion of rocks and soils, forest fires and volcanic eruptions, and is also released in large amounts by anthropogenic activities like agriculture, industry, mining activities, smelting of metalliferous ores with high Cd content and industrial application of Cd in pigments, plastic stabilizers and nickel cadmium batteries (The International Cadmium Association, 2005; Bharavi *et al.*, 2010 and Zhai *et al.*, 2008). Bharavi *et al.* (2010) further emphasized that increased concentrations of Cd in agricultural soils are known to come from application of phosphate fertilizer, sewage sludge and waste water. Cadmium is easily transferred from soil to plants that may affect target species, if there is intake of feed ingredients from a contaminated plant source. Cd can be absorbed by plants from the soil and accumulate in it. Poultry such as ducks are highly susceptible to Cd toxicity because Cd intoxication may occur through feed ingredients of plant origin and also from dicalcium phosphate, fish meal, and shell grid (Bharavi *et al.*, 2010). The retention of the trace elements can even be compounded by the composition of the feed.

COMMERCIAL FEEDS	**LOCATION**		**MEAN**
	Laguna	Pampanga	
A	< EMDL	0.16	0.08
B	0.44	0.29	0.37
C	0.46	0.16	0.31
D	-	0.12	0.12
E	-	0.84	0.84
F	-	0.83	0.83
G	0.14	-	0.14
H	< EMDL	-	< EMDL
I	0.12	-	0.12

J	< EMDL	-	< EMDL
Conventional Feeds			
"Suso"[a]	0.036	0.03	0.033
"Kuhol"[b]	-	0.07	0.07

Table 2: Mean Cd content (mg/kg) in commercial and conventional duck feed samples in Laguna and Pampanga with EMDL of 0.02 mg/kg

Absorption and accumulation of Cd in tissues seems to be determined by a wide range of factors: nutritional and vitamin status, age and sex (Ger Vos *et al.*, 1986 and Torra *et al.*, 1995 as cited by Sckalicka *et al.*, 2002). Although some of the primary feedstuffs used for livestock and poultry feeds are sourced exclusively in the country, such as rice bran and copra meal, the Philippines relies totally on imported soybean oil meal mainly from the USA, India, China, Australia, and Brazil; only limited amounts of fish meal and meat and bone meal are produced locally (FAO, 1997). Thus, it is difficult to track where the Cd contamination actually took place and what specific feedstuffs contained considerable amounts of Cd due to limitations in the analyses conducted.

Body Condition Score

The body condition score (BCS) of ducks at different stages in both locations did not differ (Table 3). Technically, the body condition scores of ducks fell under the same category in the scoring system developed by Gregory and Robins (1998). The scores were slightly higher than 0 and slightly lower than 1. This result would imply that the BCS of duck, regardless of the differences in the stage of maturity and the source from where they sampled, had more or less the same body condition score.

BODY CONDITION SCORE / LOCATION	STAGES OF MATURITY			MEAN±SEM
	Peripubertal	Mid Laying	Late Laying	
Laguna	0.16	0.45	0.40	0.34±0.06
Pampanga	0.25	0.35	0.32	0.31±0.06
Mean±SEM	0.20±0.07	0.40±0.07	0.36±0.08	0.32±0.04

Table 3: Mean±SEM of body condition score of female domestic mallard ducks at different stages of maturity in Laguna and Pampanga

Live Weight

The mean live weight of ducks at different stages in Victoria and Candaba is shown in Table 4. There were age-related differences in the live weight of ducks in both sites; these may be attributed to their growth and development. An interaction between location and stage of maturity of ducks was observed in ducks at the late laying stage -- ducks in Victoria had a higher live weight than ducks in Candaba. Factors which might have contributed to the difference include the inherent capability of

ducks to convert the feed energy to live weight gain, environmental factors, management system, feed composition, level of egg production, genetic factors, etc. (Ministry of Agriculture, Ontario, 2011).

LIVE WEIGHT / LOCATION	STAGES OF MATURITY			MEAN±SEM
	Peripubertal	Mid Laying	Late Laying	
Laguna	931.58^{c}	1195.0^{ab}	1252.50^{ax}	1126.36±21.07
Pampanga	1018.75^{c}	1162.50^{a}	1134.21^{aby}	1105.15±20.49
Mean±SEM	975.16±24.84^{b}	1178.75±25.58^{a}	1193.36±25.92^{a}	1115.76±17.37

In row, different letters (^{a,b,c}) are significantly different at 5% level. In column, different letters (^{x,y}) are significantly different at 5% level.

Table 4: Mean±SEM of live weight (grams) of female domestic mallard ducks at different stages of maturity in different locations

Hepatosomatic Index

The mean hepatosomatic index (HSI) of ducks at different stages of maturity in Victoria and Candaba is presented in Table 5. There were no differences observed between the mean HSI of ducks in Victoria as well as that in Candaba.

HEPATOSOMATIC INDEX / LOCATION	STAGES OF MATURITY			MEAN±SEM
	Peripubertal	Mid Laying	Late Laying	
Laguna	2.23	2.81	2.58	2.54±0.03
Pampanga	1.95	3.25	2.53	2.58±0.03
Mean±SEM	2.09±0.04^{c}	3.03±0.04^{a}	2.56±0.04^{b}	2.56±0.03

Table 5: Mean±SEM of hepatosomatic index (%) of female domestic mallard ducks at different st ages of maturity in different locations

Ovary-Somatic Index

As a tool for measuring sexual maturity in female domestic mallard ducks, the OSI was observed to be lowest in ducks at the peripubertal stage. However, the trend of the mean OSI of ducks, as shown in Table 6, differed in two locations. An interaction effect between the location and stage of growth was found in ducks at the late laying stage with ducks in Victoria having higher OSI than their counterpart in Candaba. This can be due to the difference in the live weights of ducks in this stage. [35]

[35] A study on Japanese quails revealed a positive correlation between weight and size of the follicles. The ovaries of the heavier birds had relatively larger ovaries and oviducts and their ovaries contained more large yellow-yolk containing follicles. Ovaries are more active in heavy birds compared to light birds most likely due to the availability of increased amount of gonadotropins and yolk constituents (Arora and Samples 2011).

OVARY-SOMATIC INDEX / LOCATION	STAGES OF MATURITY			MEAN±SEM
	Peripubertal	Mid Laying	Late Laying	
Laguna	0.02^{b}	2.12^{a}	2.56^{ax}	1.57±0.20
Pampanga	0.82^{bc}	2.45^{a}	1.12^{by}	1.46±0.20
Mean±SEM	$0.42±0.24^{b}$	$2.29±0.24^{a}$	$1.84±0.25^{a}$	1.52±0.16

^{a,b,c} mean values within a row with different letter superscripts differ significantly ($p<0.05$)
^{x,y} mean values in the same column with different letter superscripts are significantly different ($p<0.05$)

Table 6: Mean±SEM of ovary-somatic index (%) of female domestic mallard ducks at different stages of maturity in different locations

Oviductosomatic Index

The mean oviductosomatic index (ODSI) of ducks at different stages varied in both locations (Table 7). The highest ODSI was observed in ducks at the late laying stage, followed by ducks at the mid laying stage and lastly, ducks at the peripubertal stage in Victoria. In Candaba, the lowest ODSI was still lowest in ducks at the peripubertal stage and the highest was observed in ducks at the mid laying stage. Influence of the location was found in ducks at the peripubertal and late laying stages. Late laying ducks in Victoria had a higher ODSI than ducks at the same stage in Candaba. However, the ODSI of ducks at the peripubertal stage in Victoria was lower than their counterpart in Candaba. This can be attributed to the differences in the ovary-somatic indices of the ducks at these stages. For oviduct growth depends upon the growth of the ovaries (Jorgensen and Vijayakumar, 1970). As the follicle grows larger, it secretes large amounts of estrogen which, in turn, prepares the ovary and stimulates oviductal growth, differentiation and function (Johnson *et al.*, 1986 as cited by Gibbons *et al.*, 1995).

OVIDUCTOSOMATIC INDEX / LOCATION	*STAGES OF MATURITY*			*MEAN±SEM*
	Peripubertal	*Mid Laying*	*Late Laying*	
Laguna	0.01^{cy}	1.78^{b}	3.52^{ax}	1.77±0.08
Pampanga	0.57^{cx}	2.17^{a}	1.03^{by}	1.26±0.08
Mean±SEM	$0.29±0.09^{b}$	$1.98±0.10^{a}$	$2.28±0.10^{a}$	1.52±0.07

^{a,b,c} mean values within a row with different letter superscripts differ significantly ($p<0.05$)
^{x,y} mean values in the same column with different letter superscripts are significantly different ($p<0.05$)

Table 7: Mean±SEM of oviductosomatic Index (%) of female domestic mallard ducks at different stages of maturity in different locations

Organ Morphological Abnormalities

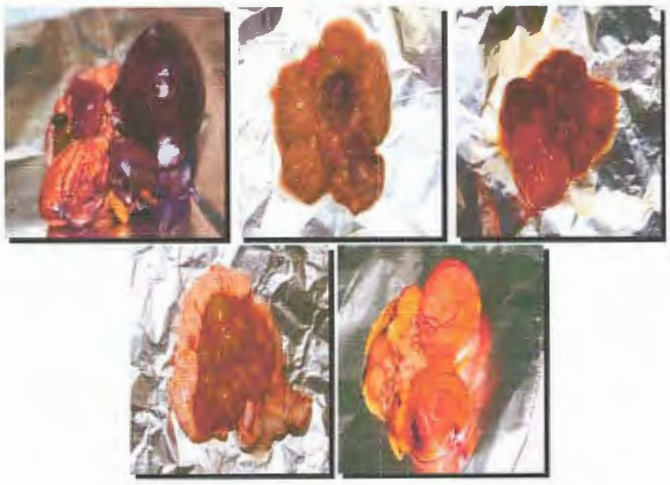
The abnormalities and disturbances observed in the selected organs of the female domestic mallard ducks were comparable in both locations (Table 8). The degree and type of irregularities ranged from mild lesions to severe cases of enlargement, inflammation, hematoma, discoloration etc. (Figure 2). There were no abnormalities noted in ducks at peripubertal stage in Victoria for the obvious reason that only very few samples sacrificed in this stage had developed ovaries. The morphological disturbances in the ovary include necrotic follicles, dispersed yellow follicular pigments, flaccid and collapsed follicles, congested follicular vessels and dark red intrafollicular spaces, displaced empty oocytes, follicular hemorrhage, deformed and vascularized mature follicles (Figure 3). No oviductal abnormalities were observed in ducks in this study.

STAGES OF MATURITY	PERCENT (%) ABNORMALITY					
	Laguna			Pampanga		
	Liver	Ovary	Oviduct	Liver	Ovary	Oviduct
Peripubertal	36.8	0	0	20.8	20.8	0
Mid Laying	55	20	0	35	10	0
Late Laying	55	20	0	55	15	0
Total (%)	49	13.6	0	35.9	15.6	0

Table 8: Frequencies and percentages of morphological abnormalities in the liver, ovary and oviduct of female domestic mallard ducks in Victoria, Laguna and Candaba, Pampanga

Figure 2: Macroscopic abnormalities in the liver of female domestic mallard ducks. Hematoma (A); Hepatic lesions (B); enlarged and discolored liver (C); fatty liver (D); white nodules on the surface (E).

Figure 3: Macroscopic abnormalities in the ovary of female domestic mallard ducks. Necrotic follicles (A); deformed follicles and diffusely pale follicular pigments (B); follicular hemorrhage, flaccid and collapsed follicles – congested follicular vessels and dark red intrafollicular spaces (C); displaced follicles on the oviductal surface (D); vascularized mature follicles and dispersion of the yellow pigment portion of the follicles (E).



Plasma Vitellogenin

Vega *et al.* (2011) published that a low level of Cd (0.10 mg/kg) downsizes and enlarges the liver and oviduct, respectively; resulting in 35 days earlier age of first egg lay without significant increase in egg production. Vitellogenin (Vtg) as a yolk precursor is a good measure of reproductive status in oviparous species. It is synthesized in the liver in response to estradiol stimulation and thus becomes elevated as estrogen concentrations increase during reproduction (IEH 1999). Vtg is tightly coupled with follicle development because it is low during non reproductive stages and then increases quickly and dramatically at the onset of egg production (Salvante, 2002 and Williams, 2003 as cited by Bond *et al.*, 2008).

The concentration of the yolk precursor Vtg in the plasma of ducks is presented in Table 9. The pattern differed in two locations since the lowest plasma Vtg was recorded in ducks at the peripubertal stage and late laying stage in Victoria and Candaba, respectively. However, both were similar in that the plasma Vtg levels were found to be highest in ducks at the late laying stage. The values obtained in this parameter were close to those of the plasma Vtg levels of harlequin ducks studied by Bond *et al.* (2008). Interestingly, the influence of the location on the plasma Vtg levels of ducks was observed and that Vtg levels of ducks in Victoria was higher than ducks in Candaba. The lower plasma Vtg level of ducks at different stages of maturity in Candaba was probably due to the physiological effects of Cd which was detected in higher amounts in the tissues and feeds of ducks in the said location. The observations clearly negate the results of previous studies on the estrogenic effects of Cd in oviparous and mammalian species. However, several researches also suggest that the endocrine-disrupting

tendency of Cd lies mainly in its anti-estrogenic activity; thereby, altering the reproductive status of different species.

PLASMA VTG LEVELS / LOCATION	STAGES OF MATURITY			MEAN±SEM
	Peripubertal	Mid Laying	Late Laying	
Laguna	1.44	3.84	3.72	3.00±0.40^{x}
Pampanga	1.62	1.80	1.41	1.61±0.35^{y}
Mean±SEM	1.53±0.46	2.82±0.48	2.56±0.44	2.30±0.35

^{a,b} mean values within a row with different superscripts differ significantly ($p<0.05$)
^{x,y} mean values in the same column with different superscripts are significantly different ($p<0.05$)

Table 9: Mean±SEM of plasma vitellogenin levels (µg/ml) of female domestic mallard ducks at different stages of maturity in Pampanga and Laguna

Exposure of insect *Oncopeltus fasciatus* females to Cd delayed ovarian maturation and inhibited egg production (Cervera *et al.*, 2005). A study by Pereira *et al.* (1993) showed that Cd reduced the levels of Vtg in winter flounder (*Pleuronectes americanus*). Similarly, Cd together with malathion and 3-methylcholantrene also reduced plasma Vtg in rice eel (*Monopterus albus*) (Schlenk and Benson, 2001). In Cd-exposed white leghorn hens, a reduction in plasma estrogen and Vtg levels was observed while the egg production of laying hens protected with selenium and zinc through supplementation was protected (Nolan and Brown 2000). Rahman *et al.* (2007) explored the possible endocrine-dirupting effect of Cd by testing its role on very-low density apolipoprotein II mRNA (apoVLDL II) expression by diethylstilbestrol in Japanese quail and found that 0.1 mg/kg body of Cd injection contributed an additive effect in DES-stimulated apoVLDL II mRNA expression, whereas Cd injection above this level suppressed the effect.

In the rainbow trout, Cd can affect Vtg gene expression by a direct binding to estrogen receptor followed by the inhibition of estrogen receptor transactivation function. Moreover, Cd also reduces the estrogen receptor mRNA levels in the liver, which is likely to lower the amount of receptors, the main regulatory factor for Vtg gene expression (Vetillard and Bailhache, 2005). A similar study by Olsson *et al.* (1995) indicates that the heavy metal inhibits the transcription of Vtg in rainbow trout. In estrogen-injected flounders (*Platichthys flesus*), a direct effect of Cd on the liver was also shown by the reduction in the RNA to DNA ratio after injection of 2 mg per kg body weight gain suggesting again that cadmium interferes with protein synthesis at the transcriptional level (Povlsen *et al.*,1990 as cited by Kime, 1998).

Conclusion

The results of this study confirmed the presence of Cd in feeds and tissues as well as the presence of abnormalities in the organs and discrepancies in the plasma Vtg levels of ducks in Victoria and Candaba indicative of the negative effects of Cd as an endocrine disruptor on the physiology of the

female domestic mallard ducks. The endocrine-disrupting chemicals, particularly Cd in this study, might have contributed to the decline in the duck industry's performance in the previous years.

However, other endocrine-disrupting chemicals such as organochlorine pesticides (OCP) may have also altered the physiology of the ducks and therefore not ruled out to have caused the discrepancies. It is therefore recommended to examine and compare the OCP levels of ducks in both locations.

Acknowledgments

The authors would like to extend their sincerest gratitude to the Department of Agriculture – Bureau of Agricultural Research and Department of Agriculture – Biotech Program Implementation Unit (DA-Biotech PIU) for the financial support extended to the project entitled "Applied Animal Biotechnology for the Improvement of Philippine Mallard Ducks" and to the support staff of Animal and Dairy Sciences Cluster, UPLB.

Contribution of Individual Authors

Dr. Vega led the project titled "Applied Animal Biotechnology for the improvement of Philippine Mallard Duck." Mr. Octura conducted the experiment, did the analysis, and partially wrote this research article. Dr. Manalo helped in the conceptualization and design of the research and completed the manuscript of the paper for publication in the *NRCP Research Journal*.

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