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Hepatic assessment of duck layers (*Anas platyrhynchos domestica*) from semi-free range farms in Misamis Occidental and Zamboanga del Sur, Mindanao, Philippines

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Abstract

Farmers in the Philippines are accustomed to raising mallard ducks (*Anas platyrhynchos domestica)* in a semi-free range management system where ducks are not totally confined within cages or houses. This study assessed the hepatic status of layer ducks with age range of 13-24 months from two farms practicing semi-free range management system in Misamis Occidental (Group I, n=10) and Zamboanga del Sur (Group II, n=9). Determination of hepatosomatic index (HSI), identification of external manifestations of liver lesions, and histopathological examination were conducted after a 30-day period of rearing in confinement in Iligan City. The HSI of the sexually mature and laying hens from Group II (2.47±0.832%) was higher than that of Group I, (2.22±0.57%). Approximately 70% of the liver samples from the two farms were observed to have signs of necrosis and fatty liver conditions. Histological examinations of liver showed hepatocytes with minimal to extensive vacuolations, dilated hepatic sinusoids and canaliculi, and lymphocytic infiltrations, which are all signs of hepatic tissue damage caused by still unknown toxicants. Environmental factors brought about by the type of farm management could have adverse effects on the hepatic status of the duck layers.

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Introduction

In the Philippine poultry industry, ducks rank a distant second to chickens in economic importance as source of animal protein and income (Chang *et al.*, 2003). Needless to say the contribution of ducks to the Philippine economy is not to be underestimated although it is relatively small compared with the broiler and layer chickens. Ducks have relatively higher economic value than carabao, dairy cattle, and goat products (Chang and Dagaas, 2004). They also have high influence in the Philippine culture, unlike other Asian countries where duck eggs are processed only into salted and century eggs, more than 80 percent of duck eggs in the Philippines are processed into "embryonated egg" or the so called "balut" (Chang *et al.*, 2003). "Balut" is considered as a delicacy by the Filipinos for its unique taste and nutritional value. A second feature of the Philippine duck sector is that duck meat production is mainly a by-product of "balut" production, derived from excess males and culled layers, although some meat-type ducks are being raised in the country (Chang and Villano, 2008).

In 2005, the Department of Agriculture reported that the country's total volume of duck production decreased by 7.14% and duck egg production by 5.94%, as compared to previous years. This decline in duck egg production continues up to the present (Bureau of Agricultural Statistics, 2012). The Philippine duck sector has received little attention from either the researchers or policymakers in terms of its economic and social values. The reasons for such trend in duck production are attributed to many factors most especially in farm management (Chang *et al.,* 2008). The duck inventory in the Philippines is classified into commercial and backyard farming (Bureau of Agricultural Statistics, 1987; Chang and Villano, 2008). The two most common types of farming management practiced by duck farmers in the Philippines are the commercial feed management and semi-free range management system. In the former, ducks are confined in house or modified shelter and feed is restricted only to commercial branded feeds. In the semi-free ranging management system, which is more common especially for backyard farming, ducks are not totally completely confined in houses or cages. At daytime ducks are released within the perimeters of the farm and are allowed to scavenge on feed items. In some cases, commercial feeds and other available feedstuff are also provided, which expose the ducks to various environmental contaminants such as pesticides, toxic chemicals, or other unknown contaminants in the soil and in the water where the ducks waddle. These environmental toxins in the farm have been reported to have antagonistic effects on the quantity and quality of duck eggs and meat production (Vega *et al.*, 2009). This predicament leads to the need of evaluating the health of ducks and find solutions in order to shun the continuing decline in the total volume of duck production.

The assessment of the duck reproductive health and the physiological conditions including the organosomatic indices of the liver and gonad are useful tools in studying the growth performance of ducks. The liver is the primary organ responsible for detoxification and elimination of toxic substances that enter the system. Aside from metabolism and waste disposal, the liver also has fundamental role in the development of the avian embryo where it is the site for the manufacture of yolk proteins of birds and other oviparous species (Chang *et al.*, 2003). Furthermore, the liver also serves to integrate the egg-white production in the oviduct of avian species (Chambon *et al.,* 1984). The latter function of the liver makes the organ useful in determining the physiologic state of the bird. The function of the liver as clearance organ harbors the danger that the substance that should be degraded or eliminated could lead to tissue damage (Ramadori *et al.*, 2008). Physiological alteration of the organ may indicate the presence of too much harmful substance inside the body. Kalisinska *et al.* (2013) studied mallards and concluded that ducks exhibit a measurable response to environmental mercury (Hg) pollution and meet the requirements as a bioindicator of toxicants. Florijančić (2009) suggested that mallard duck is a good indicator of the presence of Cadmium (Cd) as environmental pollutant since significant concentration of the element was detected in the liver tissues of the samples. Hepatotoxicity studies look into the causes of impaired reproduction, reduced growth, and histopathological lesions (Hoffman, 2002). The morpho-histopathology of the liver can yield clearer evidence of the reproductive health of the ducks, which in turn contribute to the understanding of decline in the output of duck products.

As of the present, there has been barely a number of studies regarding duck reproduction especially in the Philippines. Initial studies have been conducted only in Luzon. In Mindanao, Ortizo *et al.* (2014) studied the plasma biochemistry levels and hematological parameters in mallard ducks from selected semi-free range duck farms and have found significant effects of stress level, genetics, feed types, feed nutrition, and age to the general physiological status of Pateros ducks. This study aims to assess the physiologic status of duck layers using the hepatosomatic index (HSI) and histopathologic parameters in the liver of *A. platyrhynchos domestica* with age range of 13-24 month old from semi-free range duck farms in Misamis Occidental and Zamboanga del Sur.

Materials and methods

Samples

Nineteen randomly selected *Anas platyrhynchos domestica* ducks, aged 13–24 months were obtained from semi-fee range farms located in Bonifacio, Misamis Occidental and Pagadian City, Zamboanga del Sur (Fig. 1).

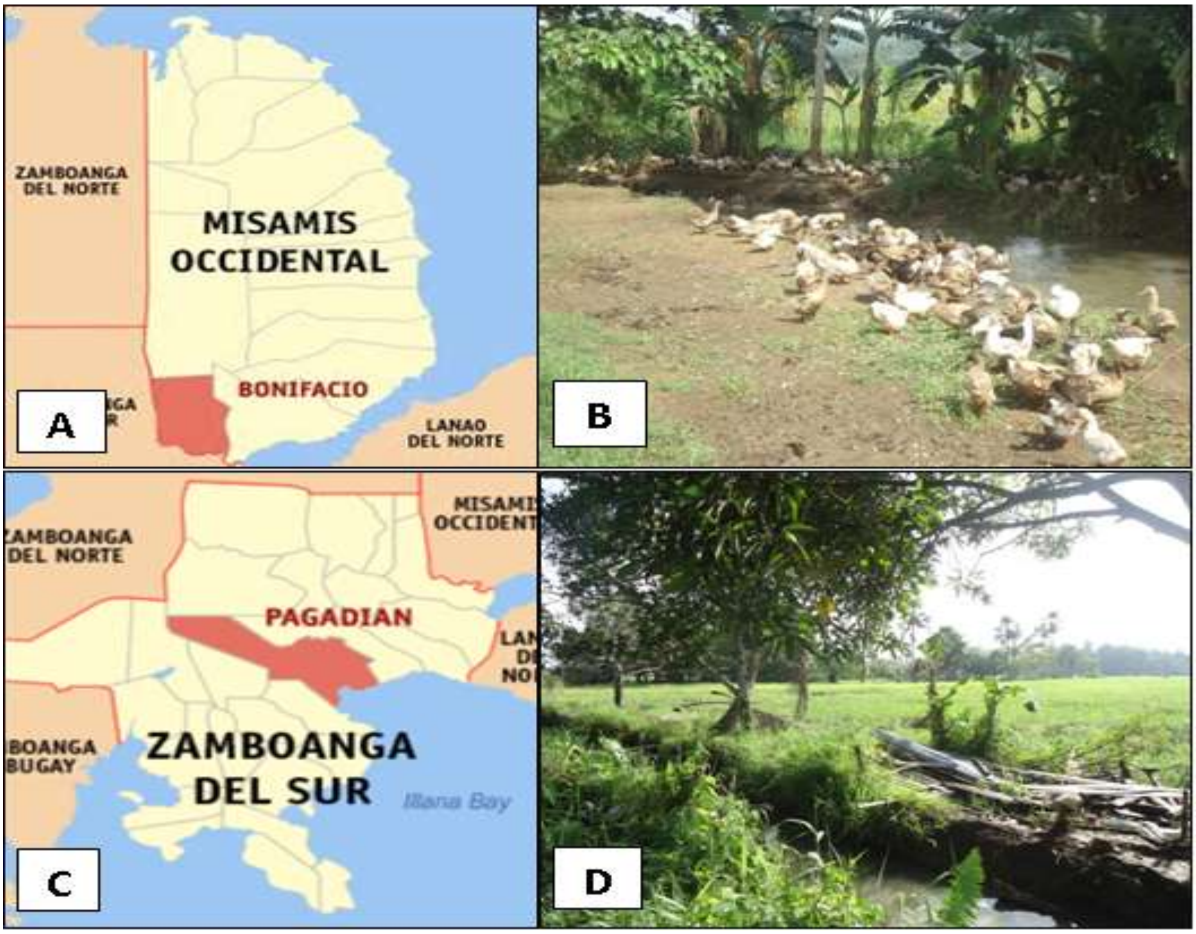

Fig. 1. Location of the first sampling site. Bonifacio, Misamis Occidental (A) (https://en.wikipedia.org, 2016c). *Anas platyrhynchos domestica* ducks freely swimming in the duck farm in Bonifacio (B). Location of the second sampling site in Pagadian City, Zamboanga del Sur (C). Backyard area of the duck farm in Pagadian City (D) (https://en.wikipedia.org, 2016b).

Collection of samples was done on September 17-18, 2012. Proper handling and transportation of the ducks was observed to reduce the stress to the birds. The birds were then divided into two groups upon arrival in Iligan City: Group I with 10 duck layers from Misamis Occidental and Group II with nine duck layers from Zamboanga del Sur.

Data collection and analysis

Dissection of all the ducks was according to the protocol of Vega *et al*. (2009) which suggested cutting through the jugular vein. The live body weight of each duck was obtained and recorded in grams before dissection. The livers obtained upon dissection were immediately weighed in grams.

The Hepatosomatic Index (HSI) was determined. HSI is a reliable indicator of hepatic growth and development according to age and physiological or physiochemical status of the liver. HSI is defined as the ratio between the liver or hepatic weight (HW) and total live body weight (LW) of the organism sampled multiplied by 100 and expressed in percent (i) (Tuene *et al.,* 2002; Vega *et al*., 2011; Zheng *et al*., 2012; and Sadekarpawar and Parikh, 2013). Further analysis of the HSI values was conducted through correlation coefficient analysis.

$$(i) \qquad HSI\ (\%) = \frac{HW}{LW} x\ 100$$

Macro-photographs of the livers were taken after subjecting the samples to the general morphological assessment. Tissues were sectioned from right, mid-section, and left lobe of each three randomly selected liver, which is the broader part of the hepatic tissue. The tissue samples were processed for sectioning by embedding in paraffin and subjected to qualitative histological assessment using a 7□m thick sections stained with the standard Hematoxylin and Eosin stain for animal tissues. Stained liver tissue sections were viewed under 40X and 100X pan-achromatic objectives over a 10X ocular with 1mm micrometer and were digitally photomicrographed for documentation and subjected to ImageJ software (National Institute of Health, 2002) analysis. Microsoft® Excel (v.2010) was used for data management and analysis.

Results and discussion

A. platyrhynchos domestica from Pagadian City, Zamboanga del Sur (Group II) showed relatively higher mean live weight and hepatic weight, 1,455.56±178g and 35.11±8.75g respectively, than those of Bonifacio, Misamis Occidental (Group I) samples with 1387±106g mean live weight and 30.75±7.76g mean hepatic weight. A higher mean HSI of 2.47±0.832% was obtained from the Group II ducks and was higher than the 2.22±0.57% HSI of Group I, as indicated in Fig. 2.

HSI is associated with liver energetic reserves and metabolic activity (Pyle *et al*., 2005; Lenhardt *et al*., 2009). Group II samples also had higher HSI range. However, livers from both groups with HSI greater than 3.0% were observed to be enlarged. An increase in HSI could mean that the liver was growing faster in relation to the body weight (Malik *et al*., 2012).

Table 1. External morphological abnormalities observed in the livers of female Mallard ducks from Misamis Occidental (Group I) and Zamboanga del Sur (Group II).

No.	Groups	
	I	II
1	necrotic, enlarged	fatty liver
2	fatty liver, necrotic, enlarged	Necrosis
3	*relatively normal*	Necrotic
4	necrotic, hemorrhage	Irregular edge, enlarged
5	hematoma, lesion	discoloration, fatty liver, necrotic, lesion, enlarged
6	Tumor	Hematoma
7	necrotic, hemorrhage	*relatively normal*
8	necrotic, hematoma, tumor	necrotic, enlarged
9	fatty liver, necrotic, tumor	hematoma, yellow discoloration
10	necrotic, tumor	-

The difference between the two groups could be attributed to the farm resources, the management, or even the food availability in the locality. Moreover, this variation of HSI values could also be attributed to the different environmental factors. In the present study, the sampled ducks were from semi-free ranged farms where the ducks could have had prior exposure to chemicals that have been contaminating the feed stuff scavenged from the rice fields.

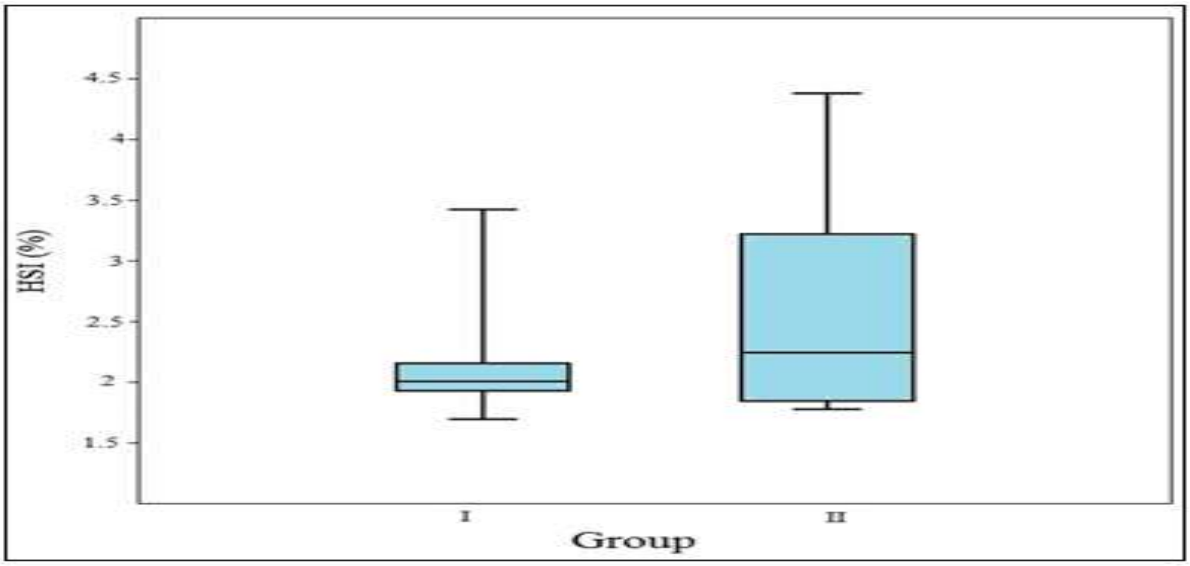

Fig. 2. Box-and-whisker plot showing the variation of hepatosomatic index (HSI) values of *A. platyrhynchos domestica* from Misamis Occidental (Group I) and Zamboanga del Sur (Group II).

The LW and HW of the ducks for the two locations both showed a linear relationship but with opposing trends (Fig. 3). Group I ducks had a negative correlation between the LW and HW indicating that the gain in the live body weight of ducks is inversely proportional to the fresh weight of the liver. However, the LW has weak negative correlation with the HW (R=-0.3219). On the other hand, ducks belonging to Group II showed a directly proportional relationship between the LW and HW but of little to no relationship at all between the two variables (R=0.0971).

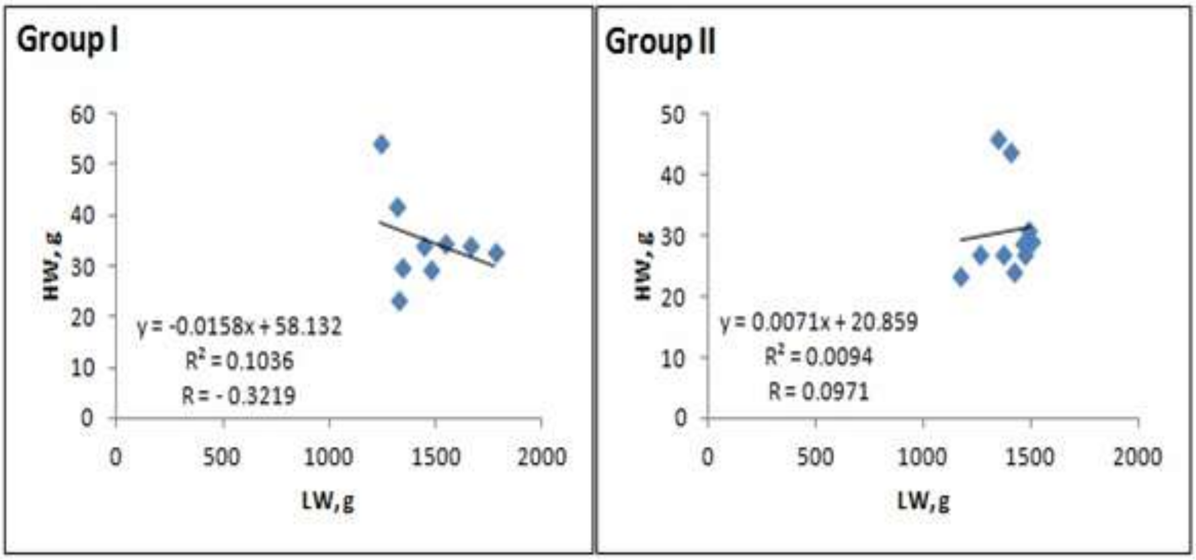

Fig. 3. Correlation coefficient plot of hepatic weight (HW) versus live weight (LW) of ducks from Misamis Occidental (n=10) (Group I) and Zamboanga del Sur (n=9) (Group II).

These observations were related to the varying value of HSI. The increase or decrease in HSI, which is the relationship of LW and HW, can also be due to the degree of damage observed in the hepatic tissues because of environmental factors. Malik *et al.* (2012) stated that changes in HSI values in quails can also be due to the damage of the hepatic tissue, which could be because of the presence of the excessive amounts of glucosinolates that have been in the feed rations of the samples. The presence of glucosinolates in the rations of mammals and quails had shown to induce severe liver damage, liver hemorrhage, and greenish-yellow coloration (Hill, 1979; Malik *et al.,* 2012). Moreover, a study on the Philippine mallard duck (*A. platyrhynchos domestica*) showed that organochlorine pesticide residues, especially γ-BHC (Benzene hexachloride), present in commercial feeds significantly reduce body weight gain and average daily gain of the mallards, while cadmium (Cd) downsizes the liver (Vega *et al.*, 2011).

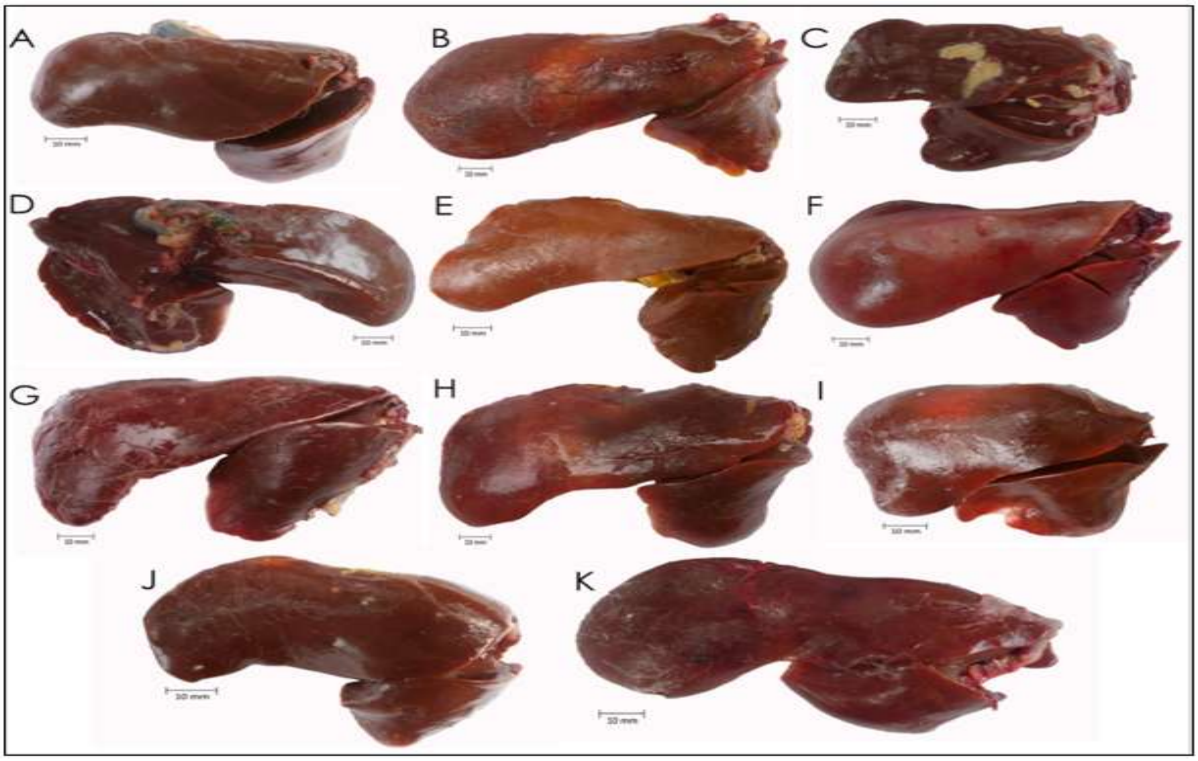

Fig. 4. Macrographs of the fresh liver samples. A relatively normal liver (A) and various conditions of duck liver as observed from the samples: fatty liver (B and C), hematoma (indicated by the arrow) (D and E), lesion and discoloration (E and F), necrotic liver (G), necrotic with hemorrhage (H and I), tumors (indicated by the arrows) (I and J), and black spots on liver presumably necrosis (K).

Table 1 shows the morphological assessment of the livers of ducks obtained from semi-free range farms of Zamboanga del Sur and Misamis Occidental. Abnormalities observed in the liver samples from both sampling sites are presented in Fig. 4. Histopathological alterations were further noted on liver sections as shown in Figs. 5 and 6. There were similar kinds of deviations in the liver observed among the samples from the two locations, and only two livers out of the 19 samples (one each group) were recorded to be relatively normal in morphology. Abnormalities documented in the livers can be considered as evidence of hepatic damage.

Approximately 70% of the liver samples from the Misamis Occidental and Zamboanga del Sur farms were observed to exhibit signs of necrotic tissues or cells. Necrotic liver lesion is characterized as yellowish area, an indication of tissue death or necrosis along the edge of the liver and/or scattered grey spots or as small yellow circumscribed spots within the liver itself that sometimes are surrounded by a light yellow halo effect (Friend, 1999). In this study, signs of necrosis were mostly concentrated along the margins of the liver especially on the left lobe and appeared to be lighter in color. One of the livers from Group II was observed to have dark spots on the right lobe. Histological examination of the sample showed evidence of a necrotic liver with the thickening or proliferation of the connective tissues equated to fibrosis (Klatt, 2013). Consequently, reduced number of hepatocytes per unit area in the lobe was also observed.

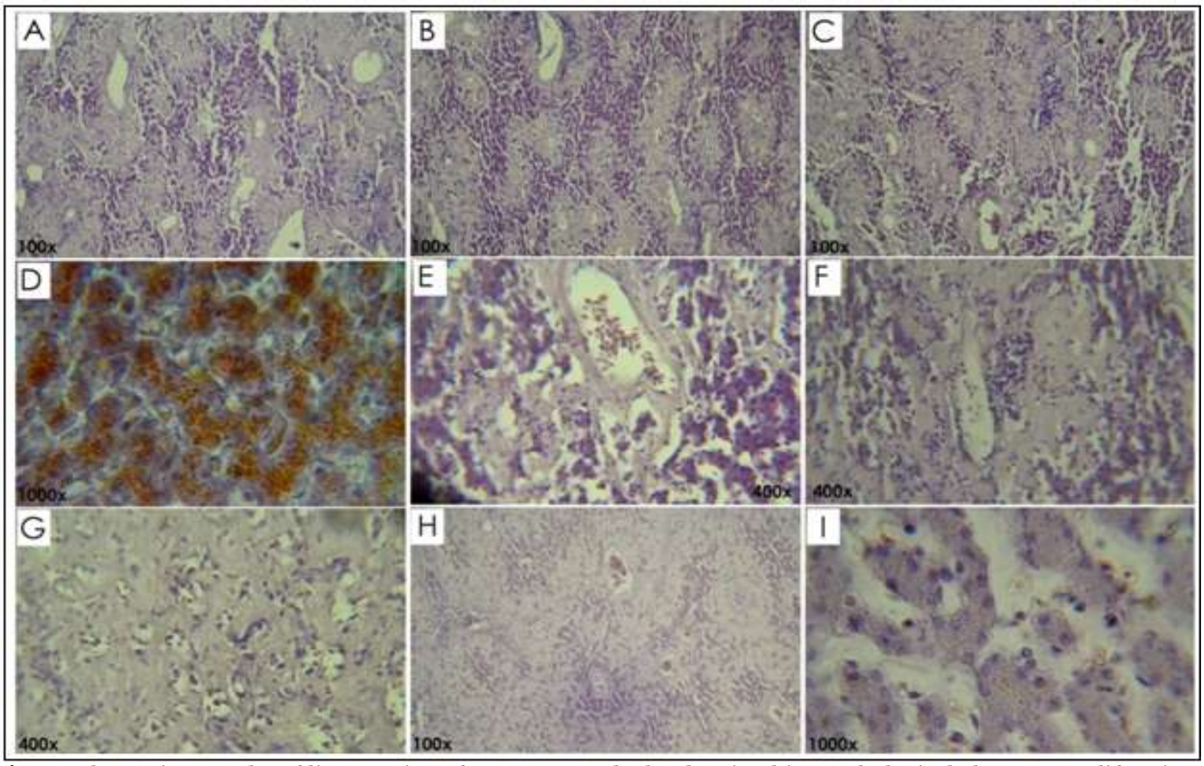

Fig. 5. Photomicrographs of liver sections from Group I ducks showing histopathological changes. Proliferation or thickening of connective tissues (thick black arrows) with reduced number and altered hepatocytes (A, B, and C). Intracellular fat droplets (brownish cytoplasmic discoloration) on the hepatocytes (D). Congestion (arrow) and a dilated canaliculi (E and F). Extensive proliferation of connective tissue and epithelial lifting (G). Hepatocytes formed islets or tumor-like (H). Intracellular heterochromatic pigmentation (brownish coloration) associated with extensive dilation of canaliculi (white arrows) (I).

The hepatocytes of ducks normally appear as strings of cells composed of two to three columns and have the tendency to form islets as they are pushed by the connective tissues or followed by the dilation of canaliculi. Hepatocytes also were observed to have lost their normal shape. Connective tissues proliferate in response to the organ's need for nourishment or blood supply. Fibrosis or connective tissue proliferation is considered a model of wound-healing response to liver injury (Friedman, 2003). Liver injury, especially necrosis, is mostly caused by repeated attempt of the host's immune response; such as in the control of Hepatitis-B virus (HBV) (Yang *et al.*, 2012). It is also known from experimental studies that necrotic liver is present in organisms due to the presence of toxic substances, including cadmium and lead (Bokori and Fekete, 1995; Binkowski *et al.*, 2013). With the same case, necrosis is usually coupled with hemorrhage, which was observed as well in this study. The aforementioned characteristics of a necrotic liver were similarly observed in the duck samples from this study.

Hepatosteatosis was also evident on the samples. Fatty livers or hepatic lipidosis were recorded in ducks from the two sampling areas in Mindanao. The livers of the ducks appeared to be slightly enlarged and were yellowish in color. Histological examination revealed mixed types of hepatosteatosis as often characterized by groups of hepatocytes with centrally placed nuclei and numerous minute lipid droplets in the cytoplasm (Brunt and Tiniakos, 2010). Cellular vacuolation, congestion, and the appearance of intracellular pigments (brownish discoloration of the intracellular region) have been commonly documented through histopathological examination.

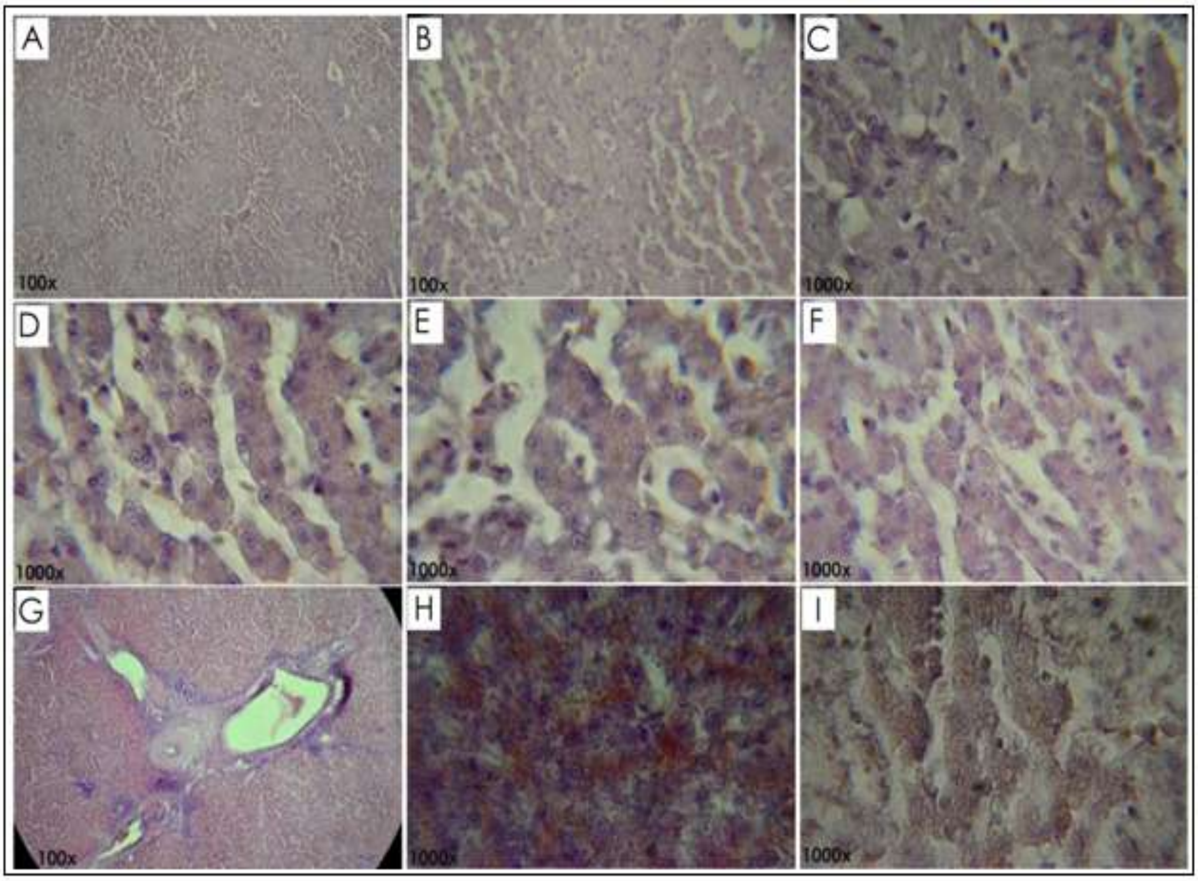

Fig. 6. Photomicrographs of the liver sections of Group II ducks showing histopathological changes. Lesser number of hepatocytes (in islet, black arrows) and thick connective tissue (A, B, and C). Dilated canaliculi (short arrows) and defined vacuolation (arrow heads) (D, E, and F). Hepatic triad with leukocytic infiltration close to the blood vessel (bluish coloration) (G) and intracellular heterochromatic pigmentation (H and I).

It was suggested that liver steatosis is the result of *de novo* lipogenesis by quantifying plasma metabolites and the activities of the hepatic enzymes in the duck (Hermier *et al.,* 2003; Chartrin *et al.,* 2006) Fatty liver is also associated with lipoprotein assembly and secretion and the extra hepatic uptake of plasma lipids (Davail *et al.*, 2003; Andre *et al.*, 2007). Histologic appearance of fatty liver tissues shows slight vacuolation. Lipid accumulates in the hepatocytes as vacuoles; occurs when lipoprotein transport is disrupted and/or fatty acid accumulates (Klatt, 2013). Basically, possible causes of hepatic lipidosis in birds include the high fat content in diet, too frequent feeding, nutritional deficiencies, thyroid disease, toxins such as lead, aflatoxins and phosphorous, and diabetes mellitus. Several researchers reported that aflatoxin-B1 contribute to conditions such as periportal fibrosis, hepatocytic vacuolation, and necrosis (Teleb *et al.*, 2004; Ortatatli *et al.*, 2005; Ellakany *et al.*, 2011; He *et al.,* 2013). Furthermore, fatty liver is said to be caused by increased oxidative stress to the animals with consequent activation of fibrogenesis (Brunt and Tiniakos, 2010), suggesting the relationship between necrosis and fatty liver condition, which was consistent with the present findings in this study.

Another common abnormality noted on the liver was hematoma, which appeared as tiny black dots (blood clots) scattered superficially. Liver is an organ undergoing constant repair in relation to its function and sometimes the repair fails if the damage is extensive and large defect allows for continued bleeding. Hematoma will progress in size if great pressure within the blood vessels continues which tends to leak the blood into the outside (Friend, 1999). Other than necrosis, proliferation of connective tissue or cell proliferation accentuate the histopathological conditions of the liver and may also indicate presence of hematoma or blood clots and the formation of tumors (Calaf *et al.*, 2007).

Tumors in the livers of ducks from this study were consistent with what was described by Calaf *et al.* (2007) emerging as yellowish spots randomly distributed on the two lobes of the organ.

Morphological aberrations in the liver tissues may be caused by several environmental factors. Mallard ducks primarily consume seeds, edible vegetation, parts of any aquatic plants, crop plants, and cultivated grains by dabbling and filtering through sediments (Kalisińska *et al.,* 2004; Florijančić *et al.,* 2009). This behavior of ducks exposes them to the threats of contaminants present in their immediate environment. Ducks can be threatened by numerous xenobiotics and elements that are often easily available from the bottom sediments.

The sediments serve as carriers and deposits of various compounds (Mountouris *et al.*, 2002; Singh *et al.,* 2005, Tylmann *et al.*, 2007; Binkowski *et al.*, 2013;). Moreover, aside from the imminent effects of feeding from the sediments, ducks are also exposed to pure metals such as lead pellets from ammunition and from lead fishing sinkers (Scheuhammer and Norris, 1995; Pain *et al.*, 2005; Binkowski *et al.*, 2013).

In this study, mallard ducks were from semi-free range farms which means that the ducks are exposed to various environmental factors in the rice fields in Misamis Occidental and Zamboanga del Sur. There are no specific environmental chemical factors named so far from this work that could have been the cause of the abnormalities observed in the livers of the randomly selected ducks from the semi-free range farms in the two localities. According to the key informant interviewed, the ducks are intentionally transported almost every three months to areas where there are rice harvests.

This practice uses the ducks as natural control as they feed on snails which are pests in rice fields. With this kind of activity, the ducks are exposed to harmful environmental elements given that pesticides are regularly introduced to these rice fields. Furthermore, not only the contaminants affect the ducks in this particular activity of the farmer. Improper handling in transporting the ducks could also cause stress to the birds. The informant verified the case by the accounts of farmers that recorded a significant decrease on egg production because of such practice.

Conclusion

Liver samples of layer ducks with age range of 13-24 months have abnormalities as evidenced by the low Hepatosomatic Index (HSI), morphological alterations of the liver, and histopathologic conditions of the liver tissues. Of the 19 layer duck samples, only two livers were noted to have relatively normal manifestations. Almost all of the liver samples examined were recorded to have necrotic and fatty conditions, hematoma, hemorrhage, and even tumors. The semi-free range management system of duck farms that majority of the farmers employ in the Philippines apparently has adverse or antagonistic effects on the hepatic status that are expressed in the overall reproductive physiology of the ducks.

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