MOLECULAR AND CELLULAR BIOLOGY

Structure and age-dependent development of the turkey liver: a comparative study of a highly selected meat-type and a wild-type turkey line

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ABSTRACT In this study the macroscopic and microscopic structure of the liver of a fast growing, meat-type turkey line (British United turkeys BUT Big 6, n = 25) and a wild-type turkey line (Wild Canadian turkey, n = 48) were compared at the age of 4, 8, 12, 16, and 20 wk. Because the growth plates of long bones were still detectable in the 20-week-old wild-type turkeys, indicating immaturity, a group of 8 wild-type turkeys at the age of 24 wk was included in the original scope of the study. Over the term of the study, the body and liver weights of birds from the meat-type turkey line increased at a faster rate than those of the wild-type turkey line. However, the relative liver weight of the meat-type turkeys declined (from 2.7 to 0.9%) to a greater extent than that of the wild-type turkeys (from 2.8 to 1.9%), suggesting a mismatch in development between muscle weights and liver weights of the meat-type turkeys. Signs of high levels of fat storage in the liver were detected in both lines but were greater in the wild-type turkey line, suggesting a better feed conversion by the extreme-genotype birds i.e., meat-type birds. For the first time, this study presents morphologic data on the structure and arrangement of the lymphatic tissue within the healthy turkey liver, describing two different types of lymphatic aggregations within the liver parenchyma, i.e., aggregations with and without fibrous capsules. Despite differences during development, both adult meat-type and adult wild-type turkeys had similar numbers of lymphatic aggregations.

Key words: liver, morphology, wild turkey line, BUT Big 6, lymphatic aggregation

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INTRODUCTION

Global poultry meat production has increased by 18% over the past five years, and in 2014 was estimated to be more than 100 million tons (USDA International Egg and Poultry Review, 2013). At least 70% of the global poultry livestock population is kept in "intensive" production systems, where the selection pressures in their breeding programs are focused on growth rate augmentation and concomitant decrease in feed conversion ratio (FAO, 2009). In recent times, poultry farming profits have become marginal because of the increasing prices of feed ingredients. Consequently, enhancing farm productivity by improving feed utilization has become a core issue (Dutta, 2010).

In birds, the liver, the largest accessory gland of the digestive tract, is interposed between the gastrointestinal tract caudally and the heart and lung complex cranially. It has key functions in the storage and conversion of many metabolites as well as detoxification and toxin/waste product removal from the circulation. Under modern farming practices in highly productive animals (Grummer, 2008; Gross et al., 2013), and particularly domestic chickens and turkeys, the liver is often adversely affected (Whitehead et al., 1978; Hansen and Walzem, 1993; Hermier, 1997; Crespo and Shivaprasad, 2003; Julian, 2005; D'Andre et al., 2013). While the vertebrate liver has enormous functional reserves (Böhm et al., 2010), when limited hepatic capacity occurs, it has large-scale consequences such as suboptimal growth as well as non-specific clinical symptoms (Duke, 1986; Reavill, 2005; Grunkemeyer, 2010).

Although the microscopic and ultrastructure organization of the mammalian liver has been examined

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Table 1. Age and gender (male/female) allocation within the two turkey type groups.

Age in wk	Wild-type	Meat-type
4	7/1	4/1
8	6/2	2/3
12	5/3	2/3
16	7/1	5/0
20	2/6	5/0
24	2/6	–
Total	29/19	18/7

thoroughly (Naito et al., 2004; Senoo, 2004; Gaudio et al., 2006; Yokomori, 2008; Baratta et al., 2009), little is known about the avian liver, especially under intensive production conditions, which is particularly surprising considering the global economic significance of the poultry industry. Most studies of the avian liver are of the domestic chicken (Purton, 1969; Hodges, 1974; Ghoddusi and Kelly, 2004; Nishimura et al., 2009; Yoshida et al., 2010; D'Andre et al., 2013; Guo et al., 2013), domestic duck (Abdelwahab, 1987; Yoshida et al., 2010), and even the ostrich (Stornelli et al., 2006). There are few studies of the turkey (Malewitz and Calhoun, 1958; Bhatnagar and Singh, 1982). Additionally, growing turkeys differ to other avian species in their lipid metabolism and hepatic triglyceride synthesis because contrary to mammals and the chicken, feeding n-3 polyunsaturated fatty acids does not decrease their hepatic triglyceride synthesis and secretion. In turkeys, n-3 polyunsaturated fatty acids appear to influence HDL metabolism causing a reduction in muscle growth (Kouba et al., 1993; Kouba et al., 1995; Mossab et al., 2002).

Clinical studies report higher susceptibility to infectious diseases such as *Histomonas meleagridis* negatively affecting the livers of highly selected meat production turkey lines compared to wild turkey lines (Abdulrahman and Hafez, 2009). The aim of the present study was to investigate the structure of the liver in relation to age in a highly selected elite size meat-type turkey and to compare this to that of a wild-type turkey line.

MATERIAL AND METHODS

Animals

Forty-eight wild-type turkeys (Wild Canadian Turkeys) were purchased as unsexed 1-day-old-chicks from a wildlife park (Wild- und Freizeitpark Ostrittrum, Germany) (Table 1).

Twenty-five meat-type turkeys from a highly selected line (British United Turkeys BUT Big 6) were purchased as unsexed 14-day-old poults from a commercial grow-out farm (Gut Jäglitz GMBH & Co. Agrar KG, Roddahn, Germany) (Table 1).

This study was approved by the responsible Animal Care Committee (Landesamt für Gesundheit und Soziales, Berlin, Germany).

Husbandry

The study is part of a larger project aiming at comparing the performance of wild-type turkeys and meat-type turkeys under husbandry conditions that are "typical" for each turkey type, thus reflecting common practice conditions. Briefly, the 1-day-old wild-type turkey poults were housed in a pen on a substrate of wood shavings with a light regime of 10 h light per day until wk 8. From wk 1 to 4, the temperature was kept at 30 to 31°C, and from wk 4 to 7 the temperature was decreased to 25°C. However, birds had access to an infrared heater set at 35°C within the illuminated area. From wk 8 onwards, the wild-type turkeys were kept in an outdoor compound having both grassed and concrete areas with wooden perches installed at a height of 80 cm.

The meat-type turkey poults were also housed in a pen on wood shavings with an initial light regime of 15 h light per day. After d 14, the light regime was reduced to 10 h. The pen temperature was 24°C in the first wk and slowly reduced to 20°C by wk 6. The humidity within the pen was 60%. An infrared heater set at 34°C was installed to heat the resting area.

All birds were fed a commercial pellet diet (Ströh Hobbersdorf, Pansdorf, Germany) using a three-phase feeding regimen. This consisted of starter feed (type 015) for wk 1 to 6, then growers feed (type 016) from wk 7 to 12, and finishers feed I (type 017) from wk 13 onwards. Components and chemical analyses of the three feed types are summarized in Table 2. The respective feed type and water were provided ad libitum.

Processing

Sample groups of both wild-type and meat-type birds were euthanized at 4, 8, 12, 16, and 20 wk. Because the growth plates of long bones were still detectable in the 20-wk-old wild-type turkeys (Mainzer, 2011) indicating that they had not reached maturity, an additional group was sacrificed at 24 wk. Meat-type turkeys had matured by wk 20 according to long bone structure. Sample sizes were 8 animals for the wild-type turkeys and 5 animals for the meat-type turkeys per group (Table 1). Note that, as the turkeys were unable to be sexed at the time of purchase that at the time of slaughter, animals were selected at random from their respective flocks. Live body weights were measured to an accuracy of 0.1 kg using a mechanical scale (Sartorius, Göttingen, Germany). The birds were then killed according to Germany's animal welfare standards by stunning and then exsanguination. They were sexed when dissected.

Morphological Examination

Immediately after a bird's death, its liver was dissected free from the carcass. The liver was bisected

Table 2. Content and chemical analysis of the three different feed types employed in the study according to manufacturer information.

	Starter feed T 015	Grower feed T 016	Finisher feed I T 017
Components	35.50% soy coarse meal 5.00% colza cake 2.50% maize 5.00% barley 3.00% feed oils 2.00% molasses 7.00% maize gluten 1.50% Ca-Na-phosphate 1.00% vitamin mix 1.00% calcium carbonate 31.50% wheat 5.00% wheat middlings	30.00% soy coarse meal 4.58% colza cake 10.00% pea 1.80% feed oils 3.00% molasses 1.60% Ca-Na-phosphate 1.00% vitamin mix 1.00% calcium carbonate 42.00% wheat 5.00% wheat middlings	11.40% soy coarse meal 4.58% colza cake 3.00% barley 5.50% maize gluten 8.30% pea 3.00% molasses 1.73% Ca-Na-phosphate 1.00% vitamin mix 1.00% calcium carbonate 60.00% wheat 5.00% wheat middlings
Analysis	11.46 MJ/ME G 26.51% crude protein 5.27% crude fat 3.93% crude fiber 6.73% crude ash 0.73% phosphorus 0.18% sodium 1.22% calcium 0.52% methionine	11.352 MJ/ME G 22.53% crude protein 3.77% crude fat 4.05% crude fiber 6.80% crude ash 0.73% phosphorus 0.19% sodium 1.24% calcium 0.42% methionine	11.403 MJ/ME G 16.50% crude protein 1.96% crude fat 3.30% crude fiber 6.14% crude ash 0.69% phosphorus 0.21% sodium 1.23% calcium 0.39% methionine
	13,500 IU Vit. A 5,000 IU Vit. D3 40 mg Vit. E 15.99 mg copper (II)sulfate	13,500 IU Vit. A 5,000 IU Vit. D3 40 mg Vit. E 15.89 mg copper (II)sulfate	13,500 IU Vit. A 5,000 IU Vit. D3 40 mg Vit. E 15.69mg copper (II)sulfate

into left and right halves by dividing the organ in the interlobar region between the cranial and caudal interlobar notches. Each half was weighed to an accuracy of 0.01 kg on an electronic laboratory balance (Sauter-Cumulus, Freiburg, Germany). Then samples of the different liver lobes were taken and prepared for morphological examination. For light microscopy, 1 × 1 × 0.5 cm tissue samples were excised from the apex of the left liver lobe, washed in 0.9% sodium chloride solution, and fixed in phosphate buffered formalin (4%, pH 7, 24 h, room temperature). They were then dehydrated in a graded series of ethyl alcohol and embedded in paraffin wax. Serial sections were cut at 5 to 6 μm and stained with hematoxylin and eosin (H&E). For the morphometric studies semithin (1 μm) epoxy resin sections were cut out after standard fixation for electron microscopy (2.5% glutaraldehyde), followed by staining according to the Richardson method (Romeis, 2010). Basic microscopic examination such as the overall histological arrangement of the liver's cellular components, including lymphatic aggregations as well as intracellular lipid depositions were undertaken using a Diaplan microscope (Zeiss, Oberkochen, Germany). Relevant images were recorded using a DXM 1200 camera (Nikon, Düsseldorf, Germany).

Quantitative Assessment and Statistical Analysis

From each age group, samples from three animals were examined morphometrically. Here 20 liver cell plates from each individual were evaluated. Morphometry was undertaken using a light microscope (original magnification × 40; Axioskop, Zeiss, Oberkochen, Germany) with an integrated digital camera (3 CCD, Color Video Camera, Sony, Berlin, Germany). Using the image-processing program Lucia 32-G Corona 4.11 (Laboratory Imaging Ltd., Prague, Czech Republic), the number of hepatocytes comprising a liver cell plate (Figure 3B) as well as the area that these hepatocytes occupied were determined. Each area to be measured was circumscribed using a computer mouse and cursor (Figure 3B). The defined area was calculated by the program using its 'Fill-Area' function. Data was statistically analyzed using IBM SPSS Statistics (IBM, Munich, Germany) to determine the arithmetic mean values and standard deviations.

The number and type of lymphatic aggregation per section was counted. At the same time, the surface area of each section of liver (roughly triangular in shape) was determined and number of the lymphatic aggregation per 1 cm^2 was calculated.

Lipid Deposition in Hepatocytes

All hepatocytes in each of the histological samples were examined for the presence or absence of lipid droplets. The degree of lipid storage in each field of view, at magnification of 400×, was determined using the semiquantitative approach of Brunt et al., 1999. The following scale was used:

Score 1: Lipid droplets comprising less than 20% of hepatocyte cytoplasm per field of view.

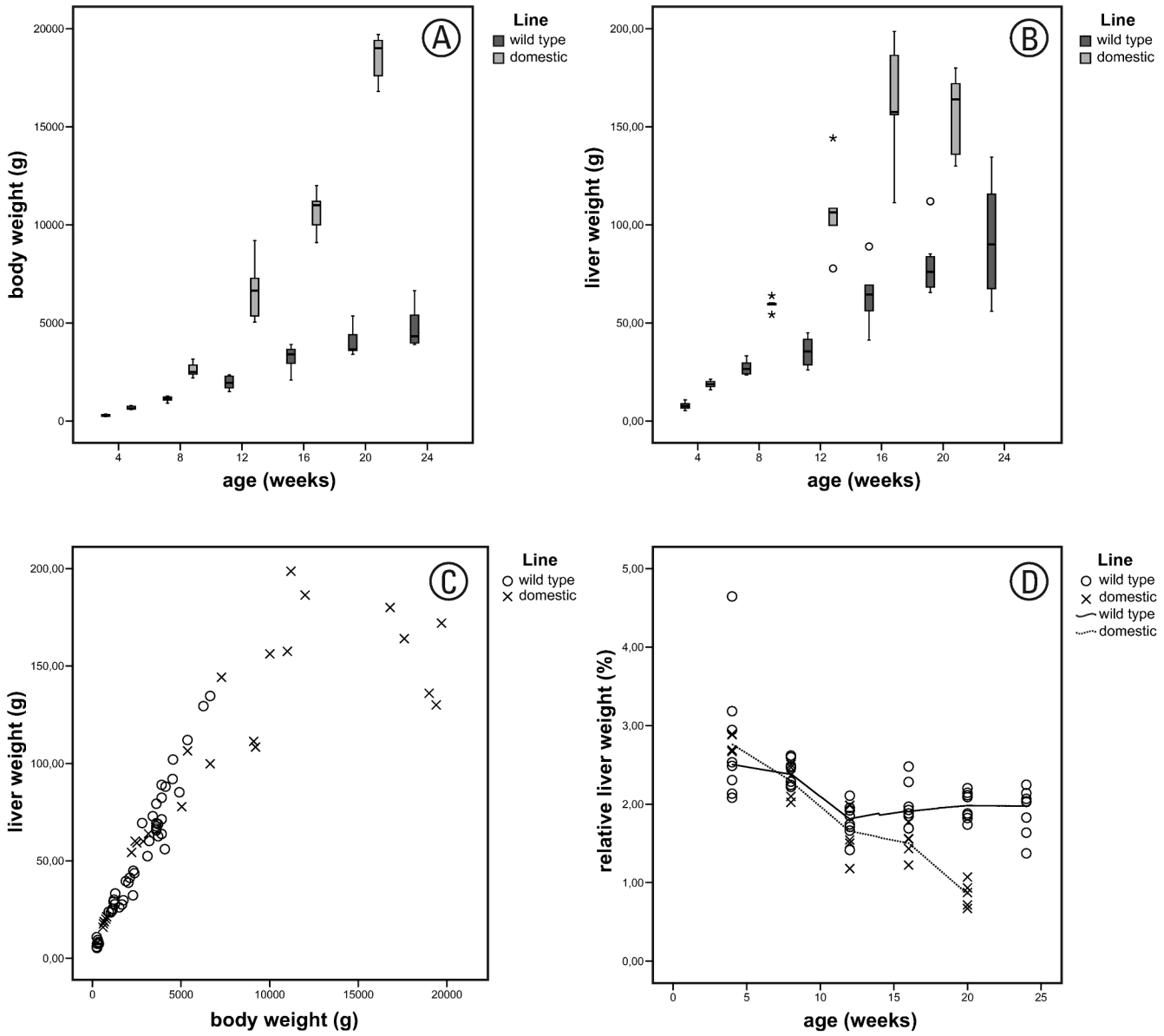

Figure 1. Body weight (A) and liver weight (B) over time of wild-type (n = 48) and meat-type (n = 25) turkey lines. Results are presented as boxplots with medians and data ranges. Single values of liver weight to body weight (C). Relative liver weight (% to body weight) over time (D).

Score 2: Lipid droplets comprising between 20% and 50% of hepatocyte cytoplasm per field of view.

Score 3: Lipid droplets comprising more than 50% of hepatocyte cytoplasm per field of view.

RESULTS

Age-Related Body Weight Changes

The average increase in body weight of the meat-type turkeys was much greater than that of the wild-type turkeys. At wk 4, the meat-type birds were about double the weight of the wild-type birds. Both turkey lines nearly quadrupled their average body weight between wk 4 and 8. From wk 4 to 16, the average weight of the wild-type turkeys increased by about 1 kg per month, while in the meat-type turkeys the average weight gain from wk 8 onwards was about 4 kg per month. At 16 wk, the average weight of the meat-type turkeys was 10.7 kg, i.e., about 7.4 kg higher than the average weight of the wild-type turkeys (Figure 1A). The study showed that the wild-type turkeys of all age groups had a lower body weight than the meat-type turkeys.

Liver Weight

Within each age group, the liver weight of the meat-type turkeys was more than double that of the wild-type turkeys. The mean liver weight of the fully mature wild-type turkeys at wk 24 was basically the same as the meat-type birds at wk 12 (Figure 1B).

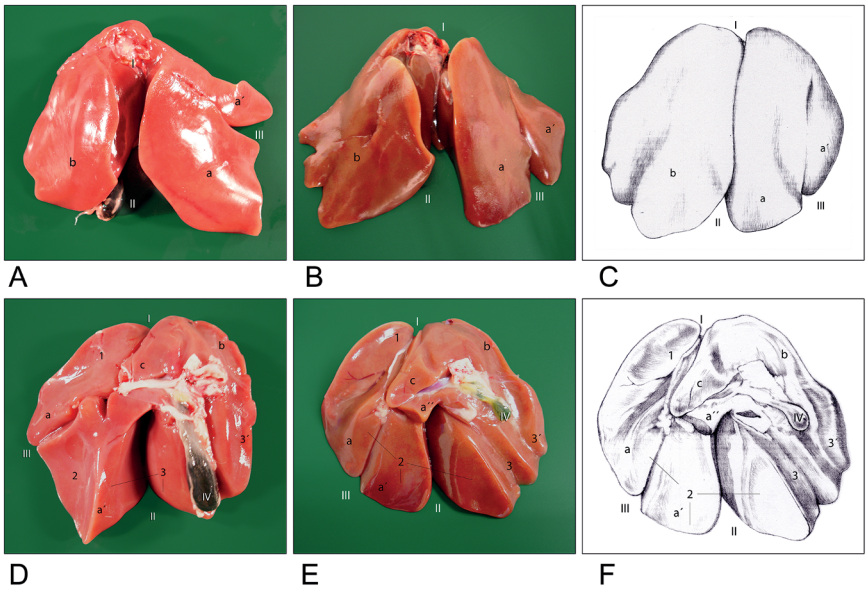

Figure 2. Parietal surface of turkey liver (A: BUT, 12 wk; B: WCT, 12 wk; C: schematic drawing WCT, 12 wk). Visceral surface of turkey liver (D: BUT, 12 wk; E: WCT, 12 wk; F: schematic drawing WCT, 12 wk). Where; I, cranial interlobar notch; II, caudal interlobar notch; III, lobar notch; IV, gallbladder; a/a′, main left lobe (a, caudodorsal part; a′, caudoventral part); a″, the left intermediate process; b, main right lobe; c, interlobar part; 1 proventricular impression; 2, ventricular impression; 3/3′, duodenal impression (3 descending, 3′ ascending).

In wild-type turkeys, the mean liver weight nearly quadrupled between wk 4 and 8. Then between wk 8 to 12 the increase declined to 30%, then from 12 to 16 wk the weight increased by 80% followed by 24% between 16 to 20 wk and 16% between 20 to 24 wk (Figure 1B).

In the meat-type turkeys the mean liver weight tripled between wk 4 and 8 and doubled between wk 8 and 12. Subsequently between 12 and 16 wk the increase was 51% and between 16 and 20 wk liver weight decreased by 3.5% (Figure 1B).

Changes in Liver Weight Relative to Body Weight over Time

Liver weight and body weight are strongly correlated, where r = 0.958, $P = 0.01$ in wild-type turkeys and r = 0.922, $P = 0.01$ in meat-type turkeys (Figure 1C). In both turkey lines, the liver weight relative to body weight dropped with age (Figure 1D). The relative liver weight of meat-type birds declined steadily over the 20 wk of the project from a high of 2.7% at wk 4 to a low of just below 1% at 20 wk (Figure 1D). In contrast the relative liver weight of the wild-type birds declined from an average high of about 3% to about 2% at wk 12 and from there on virtually plateaus at that level for the remaining 12 wk of the study.

Gross Anatomy of the Turkey Liver

In both turkey lines, the liver is prominently located in the cranial part of the mid-coelomic cavity between the caudal aspect of the heart and lungs, and the cranial aspect of the stomach and duodenum.

Grossly, the liver is nearly bisected by a shallow cranial interlobar notch and a deep caudal interlobar notch into right and left lobes, connected by an interlobar part (Figure 2A-F). In wild-type turkeys, the average weight of the right liver lobe (51 g) is a little greater than the left lobe (47 g) at 20 wk of age, whereas in meat-type turkeys, the left liver lobe with a weight of 82 g was heavier than the right one (75 g). A pear-shaped gall bladder was present on the visceral surface of the right lobe that, depending on its degree of fill, could extend beyond the liver's ventral margin. Bile from both major liver lobes drained via a common hepatoenteric duct directly into the adjacent duodenum. The cysticoenteric duct, only present in birds that have a gall bladder, such as galliformes, ran from the gall bladder to the small intestine. Both bile ducts drained into the ascending part of the duodenum close to the pancreatic ducts' orifices.

The liver color changed with age from dark red-brown in turkeys at wk 4 and 8 to become increasingly orange as the birds matured. Associated with the color change, the liver texture altered. After wk 8, the livers became increasingly brittle. The hepatic color change and altered consistency were more pronounced in the wild-type turkeys than in the meat-type turkeys.

Microscopic Examination of the Turkey Livers

General Histology Microscopically, the gland was covered by a serous tunic overlying a thin capsule of connective tissue. The paucity of connective tissue in the parenchyma obscured any visible functional tissue partitioning so that liver lobulation was not detectable.

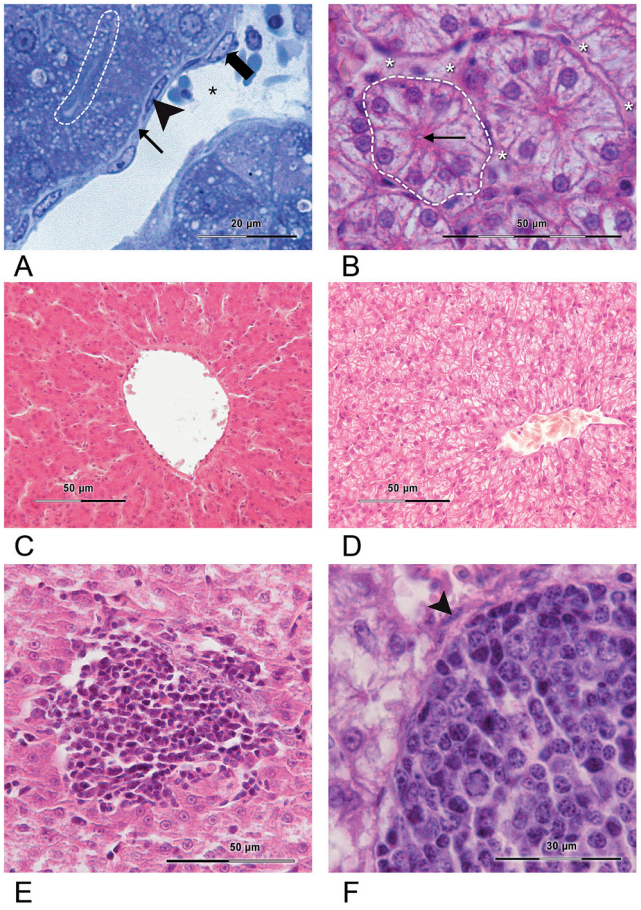

Figure 3. (A) Liver sinusoid of a 20-week-old meat-type turkey (Richardson stain); asterik marks lumen; broken line, bile canaliculus; arrow, perisinusoidal space (space of Disse); wide arrow, Kupffer cell; arrowhead, endothelial cell. (B) Hepatic plate of a 12-week-old wild-type turkey (H&E) where broken line outlines a hepatic plate, asterix is hepatic sinusoid and arrow is a bile canaliculus. (C) Liver lobule of a 12-week-old meat-type turkey and (D) Liver lobule of a wild-type bird at wk 8 showing a centrally located terminal hepatic venule with sinusoids draining into it. Note the nucleated erythrocytes in the venule and the lipid droplets within the hepatocytes (D). (E) Nonencapsulated lymphatic aggregation in liver parenchyma of a 4-week-old wild-type turkey. (F) Capsulated lymphatic aggregation (arrow head) in liver parenchyma of an 8-week-old domestic turkey.

Intralobular structures (the centrally located terminal hepatic venule) and interlobular structures of the portal triad (interlobular vein/artery and bile duct) were distributed within the liver parenchyma without any recognizable pattern. The interlobular vein and artery drained blood via the hepatic sinusoids (Figure 3A) into the terminal hepatic venule, while bile continuously produced in the hepatocytes drained conversely from the centre of the lobule to the bile duct of a portal triad, which was lined by cuboidal epithelium. Stromal connective tissue was only detectable around interlobular vessels and other large caliber vessels. The hepatocytes accounted for 80 to 85% of the liver parenchyma. In cross section the hepatic plates were formed by 6 to 7 pyramidal liver cells that formed tubular structures around a central bile canaliculus confined by the apical sides of the hepatocytes, while contact with the sinusoids occurred at the basal side of the hepatocytes (Figure 3B). A single round basophilic nucleus was located basally in each hepatocyte. Thus, the arrangement of the hepatocytes within the liver cell plates resulted in a tubule or 'glandular-like' appearance in cross section.

The hepatic cell plate area and the number of hepatocytes forming hepatic plates varied between the individual groups as shown in Table 3. In wild-type turkeys size ranged from 917.4 μm^2 to 1,313.4 μm^2. However in the meat-type turkeys the average size of a hepatic plate was 785.02 μm^2 in mature birds and up to 1,334.9 μm^2 in 4-week-old birds.

The liver sinusoids were lined by elongated simple endothelial cells with an acidophilic cytoplasm that was continuous with the cellular lining of the terminal hepatic venule. The endothelial cells each had a basophilic central nucleus that varied in shape from flat to rounded. The sinusoidal lumina contained nucleated erythrocytes as well as fat-storage cells characterized by cytoplasmic lipid droplets. Kupffer cells and mast cells were also present in the liver sinusoids (Figure 3A). The Kupffer cells were about 1.5 times larger than the typical endothelial cells lining the sinusoids. They had a granulated cytoplasm and prominent pseudopodia. Kupffer cells and fat-storing cells also occurred in the lining of the sinusoidal wall.

Scoring of Lipid Droplets in Hepatocytes In all age groups and in both genetic lines, lipid droplets were observed in extracellular sites as well as within the lumen of the vascular sinusoids. Hepatocytes with intracellular lipid vacuoles were found particularly in the hepatic plates surrounding the central veins of each 'liver lobule' (Figure 3C, D). The amount of fat within the cytoplasm ranged from a score of 1.2 in 20-week-old to 2.6 in 8-week-old meat-type turkeys and 1.6 in 16-week-old to 2.3 in 12- and 24-week-old wild-type turkeys (Table 4). Overall, 20 to 50% of the hepatocyte

Table 3. Hepatic cell plate area and the number of hepatocytes forming a hepatic cell plate.

	Area of hepatic cell plates in μm^2 (mean ± SD)		Number of hepatic cells per hepatic cell plate (mean ± SD)	
Age (wk)	Meat-type turkey	Wild-type turkey	Meat-type turkey	Wild-type turkey
4	1,335 ± 447	964 ± 365	6.9 ± 0.9	5.7 ± 0.9
8	1,308 ± 300	917 ± 402	7.1 ± 1.0	6.4 ± 0.9
12	1,035 ± 204	1,313 ± 315	6.7 ± 1.1	7.1 ± 1.0
16	1,137 ± 231	1,182 ± 325	7.0 ± 0.9	7.0 ± 0.9
20	785 ± 158	1,166 ± 274	6.4 ± 0.9	6.9 ± 0.9
24	not assessed	1,014 ± 226	not assessed	6.4 ± 0.8

Table 4. Lipid scoring (fat vacuoles) of meat-type versus wild-type turkeys.

	Score of fat vacuoles (mean ± SD)	
Age (wk)	Meat-type turkey	Wild-type turkey
4	2.2 ± 0.8	2 ± 0.7
8	2.6 ± 0.9	1.9 ± 0.6
12	1.6 ± 0.5	2.3 ± 0.7
16	2.6 ± 0.5	1.6 ± 0.7
20	1.2 ± 0,4	2.1 ± 0.4
24	not assessed	2.3 ± 0.5

Table 5. Number of lymphatic aggregations per 1 cm^2 of histological section of meat-type versus wild-type turkeys.

	Lymphatic aggregations per 1 cm^2 (mean ± SD)	
Age (wk)	Meat-type turkey	Wild-type turkey
4	1.2 ± 0.8	2.8 ± 1.9
8	1.4 ± 1.7	1.4 ± 1.5
12	3.0 ± 2.5	0.6 ± 0.7
16	5.0 ± 2.1	5.8 ± 6.1
20	4.4 ± 1.5	5.8 ± 4.1
24	not assessed	4.0 ± 2.7

cytoplasm in both lines was occupied by fat droplets. Thus, as fat storage increased, the turkey liver histological structure appeared increasingly "foamy" due to the dissolved lipid droplets. No significant difference between wild-type and meat-type turkeys could be determined; however, in some individuals, a larger fat storage was found in the wild-type turkeys (Figure 3c, d).

Lymphatic Aggregations In both turkey genetic lines and in birds of each age group, tissue samples had aggregations of lymphatic cells of varying number, size and irregular distribution throughout the liver parenchyma. While some of these were enclosed by a distinct fibrous capsule, most aggregations did not have a distinct capsule and were either directly embedded in the liver parenchyma or had sparse surrounding connective tissue. Nonencapsulated lymphatic aggregations in liver parenchyma showed an irregular surface and considerable variation in size. This type of lymphatic aggregations mainly consists of lymphocytes (Figure 3E). Encapsulated lymphatic aggregations were surrounded by a thin layer of loose connective tissue with collagen fibers and a few fibroblasts. The lymphocytes were accompanied by a few cells with large nuclei, which are probably macrophages (Figure 3F).

The absolute number of the lymphatic aggregations per 1 cm^2 of histological section are presented in Table 5 as mean values for each age group. The values vary in the different age groups. The number of lymphatic aggregations increased in meat-type turkeys between wk 4 to 16, and decreased slightly subsequently. In contrast, the number of lymphatic aggregations in wild-type turkeys waxes and wanes throughout the period of the study with no apparent pattern. Adult meat-type turkeys and adult wild-type turkeys both had similar numbers of lymphatic aggregations.

DISCUSSION

This study presents data on the gross morphology and fine structure of the livers of Canadian wild turkeys and a meat-producing domestic turkey breed. Apart from the comparison of the two different turkey lines, the present study complements the scarce data on general morphology and fine structure of the turkey liver (Bhatnagar and Singh, 1982). Birds were examined from 4 wk of age until maturity at 24 wk, wild-type, or 20 wk, meat-type, respectively. Because global turkey meat production is a significant source of animal protein (USDA, 2014) and the liver has a central role in body metabolism in both health and disease of high-production animals (Julian, 2005), this study comparing hepatic structure and possible feed-related adaptations between a wild-type and an elite meat-type turkey line is timely.

Body Weight and Liver Weight

The average body weight gain in meat-type turkeys over 20 wk was much greater than in wild-type turkeys principally due to the intensive genetic selection for rapid breast muscle growth in the meat birds (Werner et al., 2008). Over the period of this study, the body weight of wild-type turkeys increased linearly, while that of the meat-type turkeys increased exponentially. After 20 wk, the wild-type turkeys had an average weight of 5 kg, while that of the meat-type turkeys was 18 kg. The meat-type turkeys are extreme examples of the much greater and accelerated growth of a highly selected modern galliform (Werner et al., 2008; Mikulski et al., 2012). Comparative studies of body weight and composition of meat-type turkeys versus wild-type turkeys at different ages revealed that the elite genotypes' muscle volumes and weights are much greater (Andrassy-Baka et al., 2003). It was also found that fat deposition starts earlier in the meat-type turkeys than in the wild-type turkeys; however, the greatest fat deposition was found in the females of both genotypes (Andrassy-Baka et al., 2003).

Over the term of the study, the body and liver weights of birds from the meat-type turkey line increased at a faster rate than those of the wild-type turkey line. However, the relative liver weight of the meat-type turkeys declined (from 2.7 to 0.9%) to a greater extent than that of the wild-type turkeys (from 2.8 to 1.9%), suggesting a mismatch in development between muscle weights and liver weights of the meat-type turkeys. This supports similar findings of mismatched relative growth of other organs including heart and lung in turkeys and other poultry (Shivaprasad et al., 2004; Julian, 2005; Schmidt et al., 2009). Although modern selection has dramatically increased the relative size of the breast muscle, the relative size of liver has decreased. For broiler chickens, it was shown that the liver matured earlier post hatch in modern genetic lines, possibly improving nutrient

utilization as the birds shift from lipid- to carbohydrate-rich feed (Schmidt et al., 2009).

General Morphology of the Liver

The liver morphology of both genetic lines of turkeys examined in this study corroborates the gross anatomy as well as microscopic structure and patterns described for galliform birds (Gille et al., 1999). Apart from small differences in the weights of the right and left liver lobes, the gross anatomy and fine structure of the liver of the meat-type turkeys and wild-type turkeys were similar. This suggests that the higher susceptibility to infectious diseases affecting the liver of elite meat-type turkeys reported by Abdulrahman and Hafez (2009) should not be ascribed to differences in hepatic structure.

In mammals, the classic liver lobule consists of rows of hepatocytes that form radially oriented, branching laminae flanked by blood capillaries (sinusoids) around terminal hepatic venules, and bile canaliculi formed by the cell membranes of two to three adjacent hepatocytes (Fawcett, 1994). However, in turkeys, cross-sections of liver show groups of 6 to 7 pyramidal hepatocytes arranged into interconnected rounded structures associated with tubules surrounding each bile canaliculus. Consequently the branching and anastomosing tubules look like a sponge with the sinusoidal capillaries forming a three dimensional plexus within the spaces. Therefore, as in several avian species reported by Vollmerhaus and Sinowatz (1992) as well as Hodges (1974) and Bhatnagar and Singh (1982), if a bile canaliculus of the turkey is cut transversely the liver "laminae" appear to be 2 cells thick, and not 1 cell thick as is the case in mammals.

This study confirms that turkey liver sinusoids are lined by endothelial cells, Kupffer cells, and fat-storing cells. In this study, few Kupffer cells were found, confirming similar findings in ducklings reported by Abdelwahab (1987). Kupffer and fat-storing cells were also found as mobile cells in the lumen of the sinusoids. In chickens Sugimura et al. (1987) showed that erythrocyte-ingesting Kupffer cells migrated from the liver sinusoids and accumulated in the lymphatic aggregations of the liver tissue. Therefore as reported in mammals and domestic chickens the Kupffer cells are assumed to be part of the mononuclear phagocyte system's immune response (Sugimura et al., 1987; Fawcett, 1994; Hummel, 2000).

Hepatic Fat Storage

The semiquantitative approach to assess hepatic lipid storage (e.g., Brunt et al., 1999) was used due to the simplicity of the method. Although this approach may overestimate the degree of individual steatosis compared to digitized stereological point counting (Franzén et al., 2005), this method is well suited to comparing hepatic fat storage in the two different turkey types of this study.

In birds, lipogenesis takes place primarily in the liver, whereas adipocytes serve as the storage site for triglycerides. Hepatic lipogenesis contributes 80 to 85% of the fatty acids stored in adipose tissue because lipogenic activity is much greater in the liver than in adipose tissue (D'Andre et al., 2013). In the present study, signs of high intracellular fat storage in the liver, mainly around the terminal hepatic venules, were detected in both meat-type turkeys and wild-type turkeys, but were greater in the wild-type birds.

Metabolic fatty liver syndromes occurring in many species including humans, cattle and cats as well as chickens and waterfowl, are triggered by both excesses and deficits of available energy (Hansen and Walzem, 1993; Grummer, 2008; Liu et al., 2010; Molette et al., 2012). Fatty liver in birds occurs when the increase in lipogenesis exceeds the capacity for synthesis and secretion of lipoproteins. Physiologically, this occurs naturally under estrogen dominance, when a dramatic enhancement of lipogenesis occurs in laying females to supply the ovary with lipid components for the growing oocytes. In commercial high-producing enterprises for egg-laying poultry, this condition may result in the fatty liver hemorrhagic syndrome (Hansen and Walzem, 1993; Julian, 2005), one of the most important diseases of laying hens (Scheele, 1997). Hepatic lipidosis in turkeys, also called hepatic steatosis or fatty liver, is different from fatty liver hemorrhagic syndrome in chickens. In turkeys, while highly vacuolated hepatocytes dominate the histopathological picture, hemorrhages and necrosis appear to be involved frequently. The cause of the condition that particularly affects healthy birds has yet to be determined, but genetic components and toxins are possibly involved (Gazdzinski et al., 1994; Aziz, 2008).

Normal physiological liver steatosis is found in wild waterfowl (palmipedes) that fatten up before their migration. Their "seasonal" steatotic liver serves as an energy storage organ for migration. Under these conditions, hepatic lipogenesis is dramatically enhanced and liver steatosis is due to the accumulation of triglycerides within the parenchymal cells (Hermier, 1997). The reason why newly synthesized triglycerides are channeled into intracytoplasmic storage rather than being secreted remains unclear (Hermier, 1997). One hypothesis is that when overeating, hormonal regulation prevents the liver from secreting the excess stored triglycerides into the vascular system. Under normal feeding conditions, temporarily stored triglycerides need further hydrolysis and re-esterification before they can enter the secretory pathway. In permanent overeating, this may be impeded (Hermier, 1997). In the present study, a similar situation may have occurred in the wild-type turkeys under the three-phase feeding regimen of the commercial diets that are specifically formulated for fast-growing, heavy, meat-type turkey lines. The present comparison of the meat-type turkeys

and the wild-type turkey lines suggests that due to long-term selection, the fast-growing, heavy, meat-type turkey line is better adapted to the digestion of the commercial turkey diet. Presumably the commercial diet is too rich for the slow-growing, wild-type turkey line and thus results in their having a more pronounced steatosis. Within our comparative study, despite the post-mortem histological identification of fatty livers, no clinical symptoms of liver dysfunction were observed. This is in agreement with studies on non-alcoholic fatty liver disease in humans, where it is suggested that hepatic triglyceride storage per se is not toxic and may even protect the liver from lipotoxicity by buffering the accumulation of fatty acids (Liu et al., 2010).

Lymphatic Aggregations

The lymphatic aggregations in the liver tissue of birds are part of the peripheral lymphoid tissue that is a major component of the lymphatic system because, with few exceptions (Berens von Rautenfeld and Budras, 1983; Olha and Glick, 1983; Vollmerhaus and Sinowatz, 1992), birds do not have typical lymph nodes. Both Bayyari et al. (1994) and Vickery et al. (2006) describe lymphatic aggregations in the liver parenchyma of domestic turkeys and ducks. However, for the first time, the present study describes two different types of lymphoid aggregations, i.e., capsulated and nonencapsulated within the liver. Both forms appear in the domestic as well as the wild-type turkeys. Referring to Casteleyn et al. (2010) we propose that the encapsulated lymphatic aggregations be called "hepatic lymphatic follicles." The lymphoid tissue that is associated with the intestinal tract, gut-associated lymphoid tissue, is well developed in birds (Tizard, 2002). It is present as aggregations of lymphoid cells, or organized in lymphoid follicles and tonsils. Descriptions of connective tissue capsules have only been made of the latter two (Casteleyn et al., 2010). Contrary to this, Tizard (2002) states that "avian lymph nodes have no capsules."

The two different forms of lymphatic aggregations may have a yet to be determined function in the (hepatic) immune system. It has been suggested that heterophil leukocyte function in wild-type Rio Grande turkeys differs to that of commercial turkey lines: heterophils isolated from wild-type turkeys were found to be functionally more efficient with respect to degranulation and oxidative burst compared to those isolated from commercial heavy-bodied turkeys, suggesting selection pressures for growth have adversely affected immune competence. (Genovese et al., 2006, 2013).

In birds, apart from the liver, lymphoid tissue can be found in other "non-lymphoid" organs such as pancreas, kidney, endocrine glands, gonads, and even in the central nervous tissue (Olah et al., 2013). A major unresolved question is whether these lymphoid tissues represent a burst of lymphomatosis, which is destructive of the non-lymphoid organ, or a normal response to external antigens (Olah et al., 2013). As the hepatic lymphatics in all birds of this study were histopathologically normal, differences in lymphatic aggregation numbers are most likely physiological.

According to Genovese et al. (2006, 2013), data suggest that the ongoing selection of commercial lines of turkeys for larger, heavier bodies and faster growth may be associated with subsequent selection for decreased innate immune functions related to intracellular signaling mechanisms and possibly a subsequent increase in susceptibility to disease. In this study, the involvement of the hepatic immune system cannot be confirmed on a morphological basis, as both adult domestic and adult wild-type turkeys had similar hepatic lymphatic aggregations.

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