

HISTOLOGICAL STUDY OF NATURAL, BETA-AGONIST FREE, AND HYGIENIC PORK LIVERS

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ABSTRACT

A healthy diet for a healthy life has been proposed as one of healthy system. Pork liver is a kind of healthy diet, composed of enriched proteins, vitamins and minerals in which people attend to make a dish. However, the nutrition and characteristics in different domestication of pork livers have not been reported. This study aimed to investigate the histological characteristics of pork liver in terms of hepatocyte modification, inflammatory cell infiltration and collagen fiber accumulation. Pork livers were bought from three different farm enterprises: natural farm, Beta-agonist free farm, and hygienic farm. Fixed pork liver tissues were then histological processed, paraffin embedded, tissue sectioned and Hematoxylin and Eosin stained. Pork liver slides were finally taken photograph and analyzed. The histological characteristics of hepatocyte modification, inflammatory cell infiltration and collagen fiber accumulation showed statistically significant difference between natural, Beta-agonist free, and hygienic pork livers.

Keywords: pork liver, histology, healthy diet

1. INTRODUCTION

To date, the trend of healthy diet for healthy life has been found in all over the world. Pork liver is a kind of healthy diet containing lean protein source, high mineral content, and valuable vitamins [1]. Each 100 grams portion of cooked pork liver provides 25 grams of protein or 50 percent of the daily value based on 2,000 calorie diet [2]. A 100 grams serving of pork liver also provides 17.9 milligrams of iron, or 99 percent of the daily value, in which iron is an essential mineral for maintaining healthy red blood cells in the body [3]. The heme form of iron in pork liver especially helps the iron deficiency persons including adolescents, athletes, and pregnant women.

In addition, pork liver also provides a variety of vitamins and high amounts in zinc, an essential mineral for many enzyme reactions in the body [4]. Each 100 grams pork liver serving provides 360 percent of the daily value for vitamin A, 18.7 micrograms of vitamin B12, or 313 percent of the daily value. Pork liver is also an excellent source of niacin, riboflavin, folate and vitamin C [5].

In Thailand, there are 3 kinds of pork liver dividing by different domestication which are natural pork liver, Beta-agonist free pork liver, and hygienic pork liver. Natural pork liver is from the free-range swine which brought up in the natural range with clean water and food sources and also free from Beta-agonists, antibiotics and chemicals [6]. Beta-agonist free pork liver is from the Beta-agonist free swine which brought up in the certified Beta-agonist free farm [7]. Hygienic pork liver is from the hygienic swine which brought up in the hygienic farm with free from Beta-agonists and diseases [8]. Even, swine farms have been certified by the Department of Livestock Development, however, the different nutritive value and pork liver characteristics in different domestication did not find out.

The hepatocyte modification, inflammatory cell infiltration and collagen fiber accumulation in different kind of pork livers were studied for the histological characteristics leading to the advantage of evaluating the nutritive status and good condition of the choosing and having a healthy diet for a healthy life.

2. MATERIALS AND METHODS

2.1 Ethic statement

The current study was consistent with the Laboratory Animal Use Convention published by the National Institutes of Health. All animal experimental procedures were approved from the animal ethics committee of Center for Animal Research, Naresuan University (NUCAR).

2.2 Sample collection and histological analysis

Twenty-seven pieces of pork livers were bought from three different farm enterprises in Nakhon Pathom,

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Thailand: natural farm, Beta-agonist free farm, and hygienic farm.

Nine pieces of pork liver tissues in each farm were cut into 1x1x0.5 cm size and then fixed in 10% neutral buffered formalin for 72 hours, followed by tissue processing and paraffin-embedded. Three 3 μ m thick sections were cut from each pork liver paraffin block in superficial, middle and deep parts by rotary microtome. All eighty-one tissue slides were Hematoxylin and Eosin stained, then mounted.

Five fields in all slides were taken photo by Olympus BX51 light microscope connected with digital camera, then recorded by ZEN 2012 (blue edition) program.

The histological characteristics of five collected fields in all pork liver tissue slides were microscopically counted independently by two anatomists and one pathologist for quantitative and morphological evaluation to identify and quantify the number in each cell types including normal hepatocyte, changed hepatocyte, Kupffer's cell, and inflammatory cell in 20X field. The thickness of collagen fiber accumulation in all slides were also measured and analyzed in 4X field by the ImageJ program (<https://imagej.nih.gov/ij/links.html>).

2.3 Statistical analysis

IBM SPSS Statistics 22 was used for statistical analysis. The quantities of histological pork liver characteristics and thickness of collagen fiber accumulation in three different kinds were compared by one-way ANOVA method. The data in each experiment were calculated using mean \pm SD. A *p-value* of < 0.05 was considered significant.

3. RESULTS

3.1. Histological characteristics of pork liver

In order to determine the histological characteristics of pork liver, we performed Hematoxylin and Eosin staining to evaluate the hepatocyte and other cells in each kind of pork liver in 4X, 10X, 20X and 40X fields (Figure 1).

Natural pork liver, Beta-agonist free pork liver, and hygienic pork liver had regular structure of hepatic lobule, clearly visible hepatic sinus, as well as normal structure and morphology of hepatic cells, and without lipid droplets deposition when observed in 4X and 10X fields (Figure 1A to 1F). However, numbers of normal hepatocyte, changed hepatocyte, Kupffer's cell and inflammatory cell showed the statistically significant difference in three different kinds of pork liver when observed in 20X and 40X fields (Figure 1G to 1L). The histological characteristic of natural pork liver showed the significantly higher numbers of normal hepatocyte and Kupffer's cell when compared to Beta-agonist free pork liver and hygienic pork liver. On the other hand, the numbers of changed hepatocyte and inflammatory cell

showed the significantly lower in natural pork liver when compared Beta-agonist free pork liver and hygienic pork liver.

3.1.1. Normal hepatocyte

The number of normal hepatocyte was counted from the pork liver in 20X field (Figure 1G to 1I). The criteria of the counted normal hepatocyte included round to polygonal cell, well-defined cell border, abundant cytoplasm, prominent nucleoli, and single-nucleated or bi-nucleated (Figure 1J). The result showed the statistically significant lower percentage of normal hepatocyte in both of Beta-agonist free pork liver and hygienic pork liver (23.63 ± 2.35 and 20.32 ± 1.87) when compared to the natural pork liver (27.99 ± 2.00) at *p-value* < 0.05 and *p-value* < 0.01 , respectively (Figure 1 and 2).

3.1.2. Changed hepatocyte

The number of changed hepatocyte was counted from the pork liver in 20X field (Figure 1G to 1I). The criteria of the counted changed hepatocyte included swelling or shrinkage round to polygonal cell, unwell-defined cell border, insufficient cytoplasm, and invisible nucleoli (Figure 1K and 1L). The result showed the statistically significant higher percentage of changed hepatocyte in both of Beta-agonist free pork liver and hygienic pork liver (43.37 ± 0.99 and 46.82 ± 1.46) when compared to the natural pork liver (34.96 ± 2.14) at *p-value* < 0.01 . In addition, the percentage of changed hepatocyte also showed the significantly higher in hygienic pork liver at *p-value* < 0.05 when compared to Beta-agonist free pork liver (Figure 1 and 2).

3.1.3. Kupffer's cell

The number of Kupffer's cell was counted from the pork liver in 20X field (Figure 1G to 1I). The criteria of the counted Kupffer's cell were the dark brown or black amoeboid-shaped cell which attached to the hepatic sinusoidal endothelial cells (Figure 1J). The result showed the statistically significant lower percentage of Kupffer's cell in both of Beta-agonist free pork liver and hygienic pork liver (1.07 ± 0.85 and 0.73 ± 0.83) when compared to the natural pork liver (5.88 ± 1.46) at *p-value* < 0.01 (Figure 1 and 2).

3.1.4. Inflammatory cell

The number of inflammatory cell was counted from the pork liver in 20X field (Figure 1G to 1I). The criteria of the counted inflammatory cell included neutrophils, lymphocytes and monocytes (Figure 1K and 1L). The characteristics of neutrophils were multi-lobed nucleus with numerous purplish granules in cytoplasm. The characteristics of lymphocytes were large sized white blood cell with a large kidney bean shaped eccentrically placed nucleus, and abundant pink or purple granules in cytoplasm. The result showed the statistically significant higher percentage of inflammatory cell in both of Beta-agonist free pork liver and hygienic pork liver (18.70 ± 1.90 and 22.32 ± 1.07) when compared to the natural pork liver (14.91 ± 1.72) at *p-value* < 0.05 and *p-value* < 0.01 .

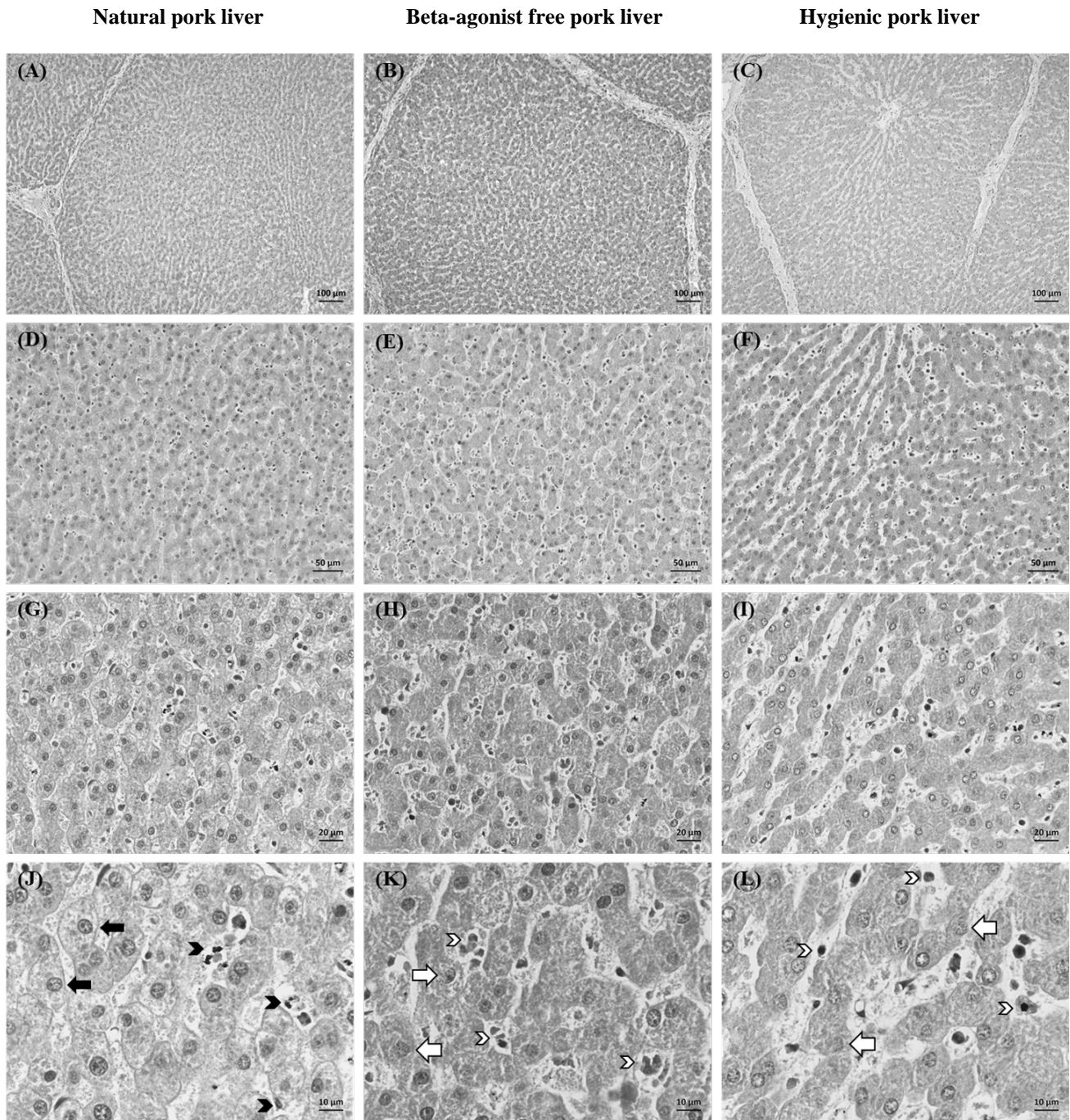


Figure 1. The histological characteristic of natural pork liver, Beta-agonist free pork liver and hygienic pork liver (n=9 each group): (A)-(C) 4X fields, (D)-(F) 10X fields, (G)-(I) 20X fields, (J)-(L) 40X fields. Note: black arrow indicates normal hepatocyte, white arrow indicates changed hepatocyte, black arrow head indicates Kupffer's cell, and white arrow head indicates inflammatory cell.

In addition, the percentage of inflammatory cell also showed the significantly higher in hygienic pork liver at p -value < 0.05 when compared to Beta-agonist free pork liver (Figure 1 and 2).

3.2. Collagen fiber accumulation of pork liver

In order to determine the collagen fiber accumulation of pork liver, we performed Hematoxylin and Eosin staining

to evaluate the thickness of connective tissues lining around the borders of hepatic lobule in each kind of pork liver in 4X field (Figure 3).

Natural pork liver, Beta-agonist free pork liver, and hygienic pork liver showed different characteristic of collagen fiber accumulation. As the average of connective tissue thickness measurement in 4X field, the thickness of collagen fiber accumulation was statistically significant higher in Beta-agonist free pork liver and hygienic pork liver (152.93 ± 5.59 and 162.97 ± 6.31)

when compared to natural pork liver (39.81 ± 6.27) at p -value < 0.01 (Figure 3 and 4).

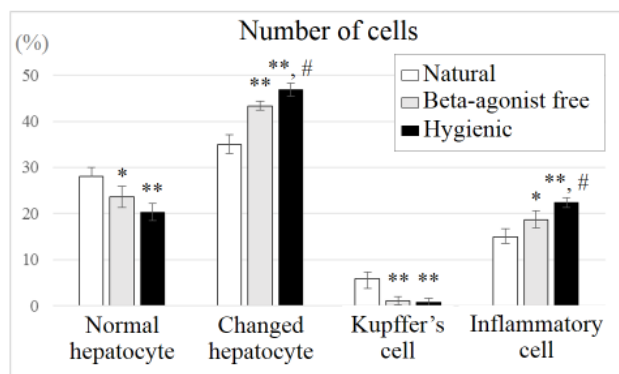


Figure 2. Percentages of normal hepatocyte, changed hepatocyte, Kupffer's cell and inflammatory cell comparison between natural pork liver, Beta-agonist free pork liver, and hygienic pork liver ($n=9$ each group). * and ** indicate p -value < 0.05 and 0.01 compared with natural pork liver. # indicates p -value < 0.05 compared with Beta-agonist free pork liver.

4. DISCUSSIONS

Pork liver ranks among one of the healthiest food which is the favored item and enriched nutritious [9]. Its benefits have played a vital role in babies, children, adolescents, adults and also elderly people in both of male and female [10]. It is a good source of proteins, iron, zinc, niacin, riboflavin, folate, vitamin A, vitamin B-complex, vitamin C and vitamin D [11]. In addition, the essential unsaturated fatty acids were found in the pork liver including linoleic acid, linolenic acid and arachidonic acid which are necessary constituents of mitochondria, cell wall and other active metabolic sites in the cell of body. Docosahexaenoic acid, another necessary fatty acid was also found in pork liver, even it is normally present at low concentration in meat tissues, but this is present in high concentration in fish and fish oils [12]. Although, many studies reported the significant role in fulfillment and maintenance of human health and also preventive role against major and minor nutrients deficiency diseases [13], but whether the different components and characteristics of pork liver in terms of different kinds of domestication have not been found before.

This study aimed to quantify the histological components of different kinds of pork liver in the 3 microns thickness of Hematoxylin and Eosin staining pork liver tissue slides including natural pork liver, Beta-agonist free pork liver and hygienic pork liver.

The natural pork liver histology showed the best characteristics of the normal liver tissue pattern. The regularly hexagonal shape of hepatic lobule, clearly visible hepatic sinus, as well as the normal structure and size of hepatic cords and hepatic cells, and without lipid droplets depositions were observed in 4X and 10X fields under the light microscope.

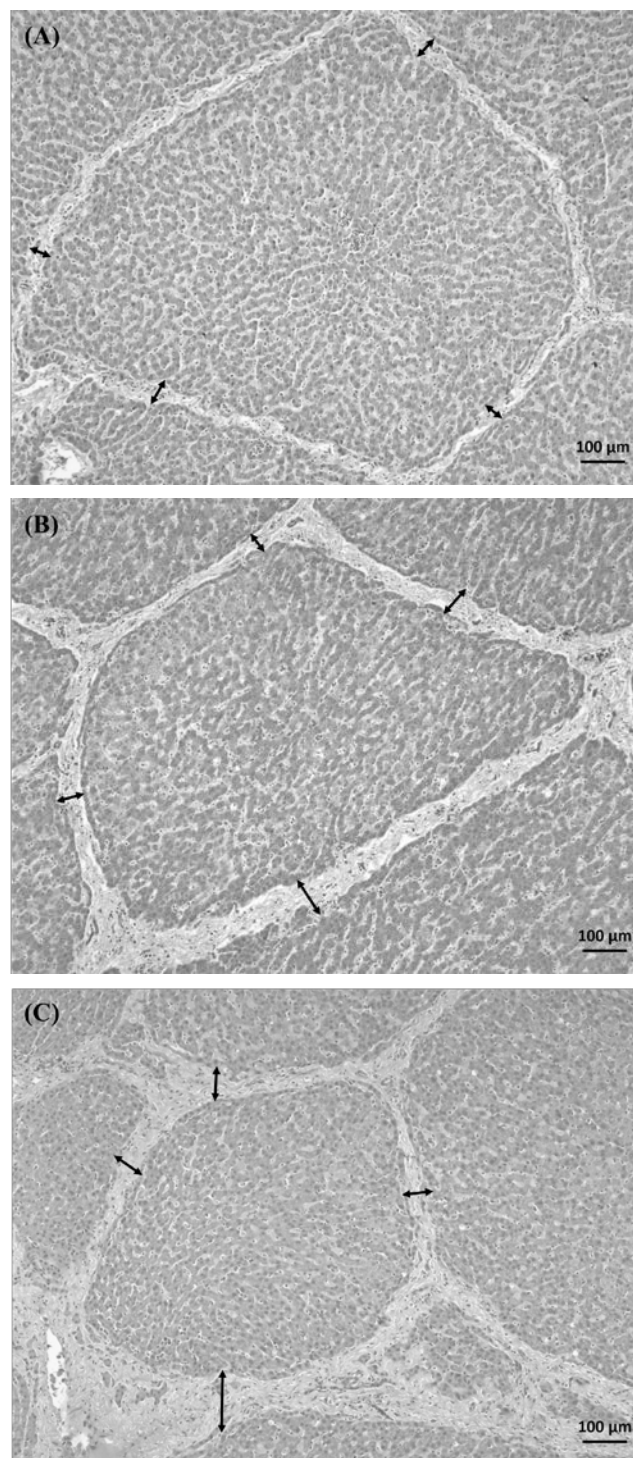


Figure 3. The thickness of collagen fiber accumulation in pork liver tissues, 4X field ($n=9$ each group): (A) natural pork liver, (B) Beta-agonist free pork liver, (C) hygienic pork liver. Note: black arrow indicates the widest thickness of connective tissue lining around each border of hepatic lobule.

In 20X field, the counted normal hepatocyte and Kupffer's cell showed the highest percentage when compared to the Beta-agonist free pork liver and hygienic pork liver. The round to polygonal cell, well-defined cell border, abundant cytoplasm, prominent nucleoli, and

single-nucleated or bi-nucleated characteristics of normal hepatocyte were dominantly observed in 40X field. The few dark brown or black amoeboid-shaped cells which attached to the hepatic sinusoidal endothelial cells called Kupffer's cell were also found in the liver tissue. These higher percentages of normal hepatocyte and few Kupffer's cells defined the normal characteristic of the liver [14-15].

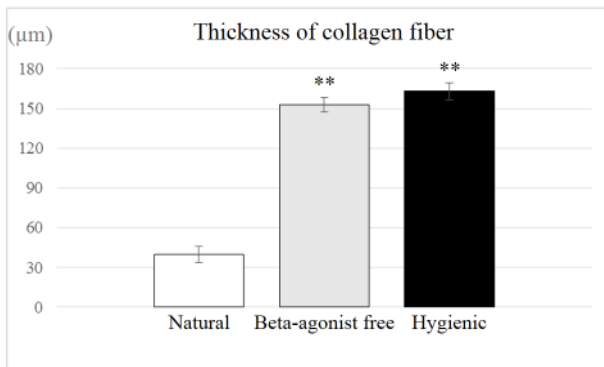


Figure 4. Thickness of collagen fiber accumulation comparison between natural pork liver, Beta-agonist free pork liver, and hygienic pork liver (n=9 each group). ** indicates p -value < 0.01 compared with natural pork liver.

On the other hand, the changed hepatocyte which characterized by the swelling or shrinkage round to polygonal cell, unwell-defined cell border, insufficient cytoplasm, and invisible nucleoli of hepatocyte was also found in less percentage when compared to other two kinds of pork liver. Similarly, the inflammatory cells including neutrophils, lymphocytes and monocytes were also rarely found. These characteristics might be found in normal liver tissue as the cellular adaptation balanced by antioxidant defenses and by other biochemical cytokines to preserve the intracellular environment and normal cell functioning [16]. The small thickness of collagen fiber accumulation in the natural pork liver also defined as the healthy liver tissue [15, 17].

The Beta-agonist free pork liver histology showed the quietly resemble characteristics of the normal liver tissue pattern as found in natural pork liver. The regularly hexagonal shape of hepatic lobule, clearly visible hepatic sinus, as well as the typical structure and size of hepatic cords, and without lipid droplets deposition were observed in low power fields. In high power field, the counted normal hepatocyte and Kupffer's cell showed the less percentage than the natural pork liver in which the number of normal hepatocyte characterized by small-sized round to polygonal cell, abundant cytoplasm, prominent nucleoli, and single-nucleated or bi-nucleated pattern. The less number of few dark brown or black amoeboid-shaped, Kupffer's cells were counted. These lower percentages of normal hepatocyte and barely Kupffer's cells defined the unnatural characteristic of the liver. On the other hand, the higher percentage of swelling or shrinkage hepatocyte with unwell-defined cell border was counted when compared to the natural pork

liver, but smaller percentage presenting when compared to the hygienic pork liver. Similarly, many types of inflammatory cells were also denumerable found as the same trend as the number of changed hepatocyte. These characteristics might be found in unusual liver tissue [15, 17]. Cellular swelling has emerged as an important initiator of metabolic and proliferative changes in cells [18]. The inflammatory cytokines might be then secreted by many kinds of inflammatory cells as the defense mechanism of cellular functioning [19]. In addition, the collagen fiber accumulation in Beta-agonist free pork liver showed the thicker lining when compared to the natural pork liver, but lesser than the hygienic pork liver when observed in the low power field focusing on the hepatic lobule's borders. This characteristic indicates the early sign of fibrosis stage according to the connective tissue accumulation [20].

The hygienic pork liver histology showed the quietly deviate characteristics of the liver tissue pattern when compared to the natural pork liver and Beta-agonist free pork liver. The regularly hexagonal shape of hepatic lobule, enlarged hepatic sinus with inflammatory infiltration, as well as the atypical structure and small size of hepatic cords without lipid droplets deposition. The counted normal hepatocyte and Kupffer's cell showed the less percentage than the natural pork liver. The most finding characteristic of hepatocyte in hygienic pork liver showed the irregularly small oval to polygonal cell with unwell-defined cell border, abundant cytoplasm, prominent nucleoli, and single-nucleated or bi-nucleated pattern which rather defined as changed hepatocyte. Around the hepatic sinusoidal endothelial cells, dark brown or black amoeboid-shaped Kupffer's cell was hardly found. These slight numbers of normal hepatocyte and Kupffer's cells defined the uncommon characteristic of the liver [15, 17]. On the other hand, the higher percentage of shrinkage changed hepatocyte with unwell-defined cell border was counted when compared to both of the natural pork liver and Beta-agonist free pork liver. Similarly, many types of white blood cells including neutrophils, lymphocytes and monocytes were also substantial found according to the inflammatory cell infiltration pattern in the hepatic sinusoids of this hygienic pork liver. These characteristics might be found in injured, infected or inflamed liver tissue [21-22]. Cell volume loss is a fundamental and universal characteristic of programmed cell death, apoptosis. This mode of cell death has been defined by a distinct set of both morphological and biochemical characteristics [23]. The predominant signatures of apoptotic cell include the loss of cell volume or cell shrinkage, nuclear condensation, internucleosomal DNA fragmentation, and apoptotic body formation [23-24]. These phenotypic features distinguish apoptosis from other types of cell death including necrosis [25]. The cause of a shrunken morphology exhibition was from the dramatic reduction of sodium and potassium concentrations that has been shown in apoptotic cells occurring process [26]. The apoptotic programmed cell death is under the influence of hormones, growth factors and cytokines [23, 25]. During

apoptosis the cell membrane is still normal but the cell is broken into apoptotic bodies via the phagocytosis. Then, the damaged cell leaked the cellular content into the adjacent tissues [24]. The accumulation of inflammatory cell infiltration particularly neutrophils release the enzymes, inflammatory cytokines and oxygen radicals enhancing the inflammatory responses [27]. In addition, the collagen fiber accumulation in hygienic pork liver showed the thickest value when compared to the natural pork liver and Beta-agonist free pork liver as measuring in the low power field focusing on the hepatic lobule's borders. This thick collagen fiber accumulation indicates the pathological sign of hepatic injury or hepatic fibrosis according to the connective tissue deposition [28]. However, the fiber accumulation in terms of type, degree and pattern are needed to be specifying as the clinical implication.

5. CONCLUSION

The study found different findings in various domestication or farm enterprises of pork liver in aspect of histological characteristics including the features and the numbers of normal hepatocyte, changed hepatocyte, Kupffer's cell, inflammatory cell, and also the thickness of collagen fiber accumulation. These associated with the health status of pork liver in the different kinds. The qualitative and quantitative evaluation indicated that the natural pork liver has the healthiest characteristics of liver tissue pattern. Further studies regard the special staining in the collagen fiber accumulation to specify the type, degree and pattern of connective tissue deposition leading to the pathological stage clarification of natural, Beta-agonist free, and hygienic pork livers.

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