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MORPHOLOGICAL CHARACTERISTICS OF ADIPOSE CELLS IN LIVER TISSUES OF TIANFU BLACK RABBITS

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ABSTRACT
Liver is an important organ of energy metabolism and plays a vital role in regulating the tissue development and body growth. Although it has been widely acknowledged that domestic rabbit is a genetically fat-lacked species in comparison with other farm animals, the related molecular bases remains largely unknown. In order to better understand the fat metabolism profiles in liver, the liver tissue samples of Tianfu black rabbits at 0, 35, 56, 70 and 90 day old was used for morphologic observation with aim to profile the dynamic development processes. Results show that the area of adipose cells in liver tissues of rabbits gradually increased from the age of 56 days, and the diameter of fat cells rapidly increased \( P<0.01 \) after the age of 70 days. The results suggested that 56 days before sales was the optimal stage for fattening and selecting stage, which provided a good basis for further studies on lipid metabolism in liver of rabbits.

Key words: Liver, Adipose cells, Development, HE staining

INTRODUCTION
Liver participates to synthesize types of fatty acids and metabolic enzymes for body needing, and further results in the difference in meat quality (Du et al., 2013). However, the disrupted liver metabolism directly affects the body to absorb nutrients and probably forms liver disease, such as nonalcoholic fatty liver disease, liver enlargement, hepatitis, cirrhosis (Bugianesi et al., 2005; Wieckowska et al., 2006; Yu et al., 2017). In animal growth and development process, the study of liver morphology is helpful to understand the state of body metabolism as indicated by Hamaguchi et al. (2019) and Peixoto et al. (2017). This study mainly focuses on the morphological investigation of liver tissues in different growth stages of rabbits, so as to provide a preliminary study on energy metabolism of rabbits.

MATERIALS AND METHODS
Animals and experimental design
The study was approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University. Tianfu black rabbit was selected from the teaching farm of rabbit in Sichuan Agricultural University. Under the same feeding conditions, the rabbits were fed by commercial feed with nutrition of Crude protein > 16.0, Crude fat > 2.0; Crude fiber: 10.0 ~ 16.0; Ratio of Calcium / Phosphorus: 2/1, Digestive energy: 10.47 > MJ / kg. Fifteen female rabbits with uniform body weight (except 0 day old) were chosen and divided into five groups according to 0, 35, 56, 70 and 90 day old, which is used for collecting liver tissues samples, respectively.
Slice making and HE staining
The liver tissues samples were fixed with 4% paraformaldehyde respectively. After the process of paraffin section production and HE staining, its physiological characteristics were observed and photographed under the microscope (DM1000) for subsequent detection. The specific test steps contain three steps (Embedding, Slice, HE Staining) and refer to this study (Werely W A, 1976).

Statistical Analysis
Image pro plus 6.0 was used to detect the two indexes (Diameter and Area) of adipose cells in HE staining picture (200X). All data analyses were conducted using Graphpad prism 6.0 software and SPASS 22.0.

RESULTS AND DISCUSSION
Body weight and liver are important indexes to measure the growth performance and metabolic profiles of rabbits, which can reflect the physiological changes in rabbits. At each stage of age, the body weight rapidly increased is similar with liver weight changes trends from 35-56 days of age and 70-90 days of age (Figure 1). It showed that the growth metabolism after weaning and fattening later the rabbit is very significant.

Figure1: Body weight and liver weight of rabbits at different age stages
Morphological changes of liver reflects the metabolism state of body, which can be obviously observed in this study. As can be seen from the Figure 2 and Table 1, the lipid droplets (white part) in the liver of rabbits were significantly higher at 0 day age than those in other age stages, and were the least at 56 days age. Meanwhile, the area of adipose cells in the liver was significantly higher at the age of 70 days than that in the other four growth stages, and significantly lower than that in the other four growth stages at the age of 35 days. However, with the increase of age, the diameter gradually becomes higher and reaches the peak at 90 days. The deposition of lipid droplets can reflect the ability of liver fat synthesis and the speed of lipid metabolism, which is directly related to the growth and development of rabbit body. The 56-day-old rabbit belongs to the vigorous metabolic period of youth, during which the body needs more energy materials to meet the energy metabolism needs of various organs. On the other hand, it indicates that 56-day-old rabbit is the key stage of growth and metabolism, and the stability of physiological period provides good conditions for the further development of the body. The lipid deposition tended to be stable between the ages of 70 days and 90 days.
Figure 2: Morphological characteristics of adipose cells in liver at different age stages (HE, 200X, Bar=50 µm).

Table 1: Morphological indicators of adipose cells in liver tissues

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Area (µm² ± SD)</th>
<th>Diameter (µm ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>146.76±11.99</td>
<td>11.38±1.34</td>
</tr>
<tr>
<td>35</td>
<td>58.51±7.72</td>
<td>12.66±1.44</td>
</tr>
<tr>
<td>56</td>
<td>88.07±8.34</td>
<td>10.85±1.24</td>
</tr>
<tr>
<td>70</td>
<td>172.98±6.97**</td>
<td>15.73±5.65</td>
</tr>
<tr>
<td>90</td>
<td>146.37±1.47*</td>
<td>30.25±6.61**</td>
</tr>
</tbody>
</table>

Note: The data use Mean±SD to show difference and the significance data use the symbol ***(P<0.01) and ****(P<0.05).

CONCLUSIONS

In this study, the growth and metabolism of rabbits were studied in liver tissues of different ages. The results showed that lipid deposition in liver tissues of rabbits gradually increased from the age of 56 days, and the diameter of fat cells in liver tissues rapidly increased after the age of 70 days, and lipid accumulation gradually slowed down.

ACKNOWLEDGEMENTS

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Morphological characteristics of adipose cells in liver tissue of Tianfu black rabbits

Yanhong Li*, Songjia Lai**, Mingchuan Gan., Qingling Li., Xuemei Wu., Jie Wang., Xianbo Jia., Shiying Chen. Email: *laisj5794@163.com

Context and objectives: This study used the liver tissue sections of Tianfu black rabbits at five growth stages and analysed the morphological investigation of adipocyte, so as to provide a preliminary study on lipid deposition of rabbits.

Methods: Paraffin section production and HE staining

Experimental design
Fifteen female rabbits at 0, 35, 56, 70 and 90 days of age were chosen and divided into five groups. Liver tissue samples were collected from these rabbits at five growth stages and prepared for observing the histomorphology of liver tissues through the two method of paraffin section production and HE staining.

Result: The liver weight change trends was correlated with body weight from 35 to 56 days and 70 to 90 days.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>0d</th>
<th>35d</th>
<th>56d</th>
<th>70d</th>
<th>90d</th>
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<td>15.73±5.65</td>
<td>30.25±6.61**</td>
</tr>
</tbody>
</table>

The liver tissues at 56 days of age had lower lipid deposition than other stages and its adipocyte diameter and area were significantly increased at 70 and 90 days.

Conclusion: Lipid deposition in liver tissues of rabbits gradually increased from the age of 56 days, and the diameter of adipocyte in liver tissues rapidly increased after the age of 70 days, and lipid accumulation gradually slowed down.