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Comparative Study of the Histomorphological Structure of the Small Intestine of *Lonchura striata* and *Copsychus saularis*

Estudio Comparativo de la Estructura Histomorfológica del Intestino Delgado de *Lonchura striata* y *Copsychus saularis*

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SUMMARY: Six *Lonchura striata* and six *Copsychus saularis* birds were selected in this study, morphological index of the small intestine was measured by quantitative biology and image analysis. The changes of goblet cells and Na^+/K^+ATPase were detected by AB-PAS staining and ELISA to inform the different mechanisms of the digestion and absorption of nutrients between the *Lonchura striata* and *Copsychus saularis*. The villus height, crypt depth and muscle thickness of each segment of small intestine of *Lonchura striata* were smaller than those of *Copsychus saularis*, and the difference of ileum muscle thickness was significant. In addition, the ileum villus height/crypt depth (VH/CD) value of *Lonchura striata* was significantly less than that of *Copsychus saularis*. The number of goblet cells in duodenum and jejunum of *Lonchura striata* and *Copsychus saularis* had no significant difference, but the number of goblet cells in ileum of *Copsychus saularis* was significantly larger than that of *Lonchura striata*. The vitality of Na^+/K^+-ATPase in different intestinal segments of the *Lonchura striata* and the *Copsychus saularis* was different. The vitality of Na^+/K^+-ATPase in the *Lonchura striata* was significantly higher than that of the *Copsychus saularis*. It can be concluded that the digestion and absorption capacity of *Copsychus saularis* and *Lonchura striata* are significantly different, and the reason may be due to their different diets and intestinal floras.

KEY WORDS: ***Lonchura striata*; *Copsychus saularis*; Small intestine; Histological structure; Goblet cells; Na^+/K^+-ATPase.**

INTRODUCTION

The feeding behavior of animals is to choose a certain type of food as the main food in order to meet the needs of survival and reproduction. In general, carnivores and plant-eaters have relatively fixed diets, and the lack of such diets can even lead to extinction. Nevertheless, some species, such as the giant panda, have better ecological adaptability and can change their original feeding habits through long-term natural selection. Some studies found that the carnivore giant panda changed its eating habits, and some intestinal structural characteristics of the plant-eating animals appeared, and the intestinal flora also changed greatly (Zhou *et al*., 2019). The intestinal tract is the main location for animals to digest and absorb food. However, birds have different digestive systems, in order to adapt to flying, the intestinal tract of some birds shrunk, and food was quickly digested and absorbed to help them lose weight. Compared with mammals, birds' digestive system accounts for a smaller proportion of their body weight, so the adaptation of their digestive tract plays a more important role in the regulation of birds' physiological and energy metabolism needs, which is the key to their effective energy and nutrition absorption (Enoki & Morimoto, 2000; McWilliams & Karasov, 2001). The morphological structure of birds' digestive system is closely related to their feeding habits (Koutsos *et al*., 2001; Barton & Houston, 2010; Siddique, 2017).

Lonchura striata is a species of the genus Lonchura of family *Ploceidae*, which is about 10 cm long. It mainly feeds on grains, grass seeds, leaves, buds and other plant foods, especially rice, and occasionally some insects. It is

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easy to domesticate and can be used as an ornamental bird, with great economic potential and research value (Woods *et al*., 2010; Suzuki *et al*., 2012). *Copsychus saularis* is a member of the genus copychus of family Muscicapidae, with a body length of about 20 cm. It mainly feeds on insects and eats a small number of grass seeds and wild fruits. In recent years, due to hunting, the use of pesticides, deforestation and changes in the nature of land use, the habitat of *Copsychus saularis* has been lost, resulting in a sharp decrease in the number of *Copsychus saularis* (Wang *et al*., 2002). In addition, *Copsychus saularis* are often kept as pets by pet bird lovers.

Lonchura striata and *Copsychus saularis* both belong to Passeriformes, and their habitats are relatively similar, but there are some differences in feeding habits. And there were no significant differences in jejunum length, ileum length, whole digestive tract length and weight between adult *Lonchura striata* and *Copsychus saularis* (Huang *et al*., 2020). In this study, our goal is to describe morphological structure of small intestine of *Lonchura striata* and *Copsychus saularis* by paraffin sections, H. E. (Hematoxylin Eosin) staining, micrography, quantitative biology and image analysis. By comparing the height of intestinal villi, the depth of crypt, the thickness of muscular layer and the ratio of the height of intestinal villi to the depth of crypt (VH/CD), the different mechanisms of nutrient digestion and absorption of *Lonchura striata* and *Copsychus saularis* were analyzed. It is also expected to provide certain morphological data reference for subsequent related studies.

MATERIAL AND METHOD

Ethical Statement

Animals and sample collection. Six healthy adult *Lonchura striata* and six *Copsychus saularis* of the same age were obtained in Nanchong city tiange flower and bird market, Sichuan province. After anesthesia and jugular vein, about 3 cm of the duodenum, jejunum and ileum were removed, then the samples were washed with saline and fixed in 4 % neutral paraformaldehyde fixative.

Intestinal morphology observation and measurement. Paraffin embedded tissue sections were deparaffinized using running water followed by graded dehydration using 75 %, 85 %, 95 %, and 100 % alcohol, after which the sections were deparaffinized using toluene and finally, the samples were wax soaked, embedded, and cut into 5 µm thick sections, followed by hematoxylin and eosin (H. E.) staining and resin glue seal.

The tissue sections with clear structure and good staining were observed under optical microscope (BA410, Olympus). Under light microscope with magnification 400 times, the tissue sections were photographed with image-pro software, and 5 visual fields were observed in each section. Then the software was used to take the images of villus height, crypt depth,villus width, and myometrium thickness in each segment of the small intestine and the ratio of villus height and crypt depth (VH/CD) was calculated. Finally, the average measurement of each selected photo was used as the result for statistical analysis (Fig. 1).

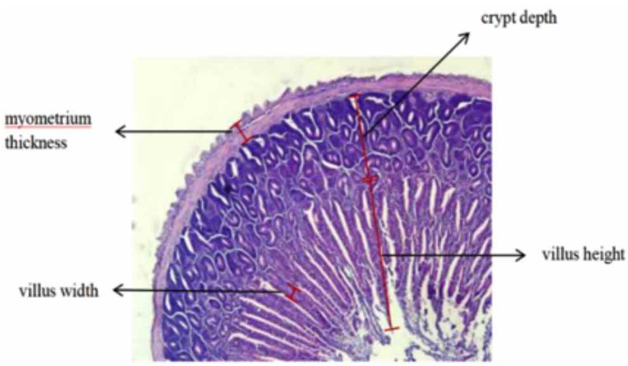

Fig. 1. Manner of measurement of each indicator of intestinal.

Observation of the goblet cells: AB-PAS staining. AB-PAS staining solution (G1285, Solarbio) was used to stain goblet cells, which was consistent with the method used by Saller *et al*. (2020). The goblet cells were stained with Alcian blue-periodic acid Schiff (AB-PAS) staining. Paraffin sections were deparaffinized to water and then sequentially stained with Alcian blue staining solution, 0.5 % periodic acid solution oxidation, Schif's reagent. After hematoxylin re-staining, the nuclei were differentiated with hydrochloric acid ethanol, ammonia turned blue, and xylene was transparent. After that, they were sealed with neutral gum and observed and photographed under a microscope. All measurements were made in µm, at × 200 magnification.

Determination of activity of Na^+/K^+-ATPase. The Na^+/K^+-ATPase controls the transportation of digestive products by controlling the gradient of the Na-concentration. And can be used as an indicator of gut digestion and absorption function (Chen *et al*., 2014). The activity of Na^+/K^+-ATPase was detected based on the information of Na^+/K^+-ATP Kit (bc0056, Solarbio). In short, firstly, the sample enzyme solution was prepared: homogenized in ice bath according to the volume of tissue mass (g) (ML): extracted at the ratio of 1:10. 8 000 g, centrifuged at 4 ºC for 10 min, then placed on ice for clarification. The enzyme activity was measured. The phosphorus content was calculated using the formula of Na^+/K^+-ATPase activity (mmol/h/g) = [C standard tube × V total amount] × (standard tube-a) / (standard tube blank tube) / (w × V total number of samples)/ T ≤ 7.5 × (tube control tube) / (standard tube blank tube) / W (Kisielinski *et al*., 2002; Wang & Peng, 2008).

Statistical analysis. Data were expressed as mean values ± standard deviation (Mean ± SD), and were analyzed using one-way analysis of variance (ANOVA) followed by Post Hoc, Tukey's HSD test. All Statistical analysis was performed using SPSS (SPSS for Windows, version 16.0, SPSS Inc, Chicago, Illinois). Differences were considered statistically significant when the calculated P value was less than or equal to 0.05. P > 0.05 indicated no significant difference.

RESULTS

Intestinal morphology. Figure 2 shows the staining results of the intestinal tissue sections of *Lonchura striata* and *Copsychus saularis*. The structure of villi was distinct and the staining of intestinal tissue was clear. The villus height and crypt depth of the duodenum are longer than the jejunum and ileum. In *Lonchura striata*, the myometrium thickness of the duodenum is the greatest, whereas in *Copsychus saularis*, the myometrium of the ileum has the greatest thickness.

Villus height (VH), crypt depth (CD) and the VH/CD of small intestine between *Lonchura striata* (Ls)and the *Copsychus saularis* (Cs). Table I shows the villus height (VH), crypt depth (CD) and the ratio of them (VH/CD) in duodenum, jejunum and ileum of the *Lonchura striata* and *Copsychus saularis*. The CD in the duodenum of *Lonchura striata* was higher than *Copsychus saularis* (P<0.05), but significantly lower in ileum (P<0.01). There were no significant differences of the VH, VH/CD in duodenum, jejunum and ileum and CD in the jejunum (P>0.05).

Distance of small intestine villus bases (DVB), width (W) and absorption surface area (ASA) between *Lonchura striata* and the *Copsychus saularis*. The width (W) of the villus was significantly wider (P<0.01) and absorption surface area (ASA) was bigger (P<0.05) in the duodenum of the *Lonchura striata* than that of *Copsychus saularis*, and no significant changes were observed in other parameters, which were shown in Table II.

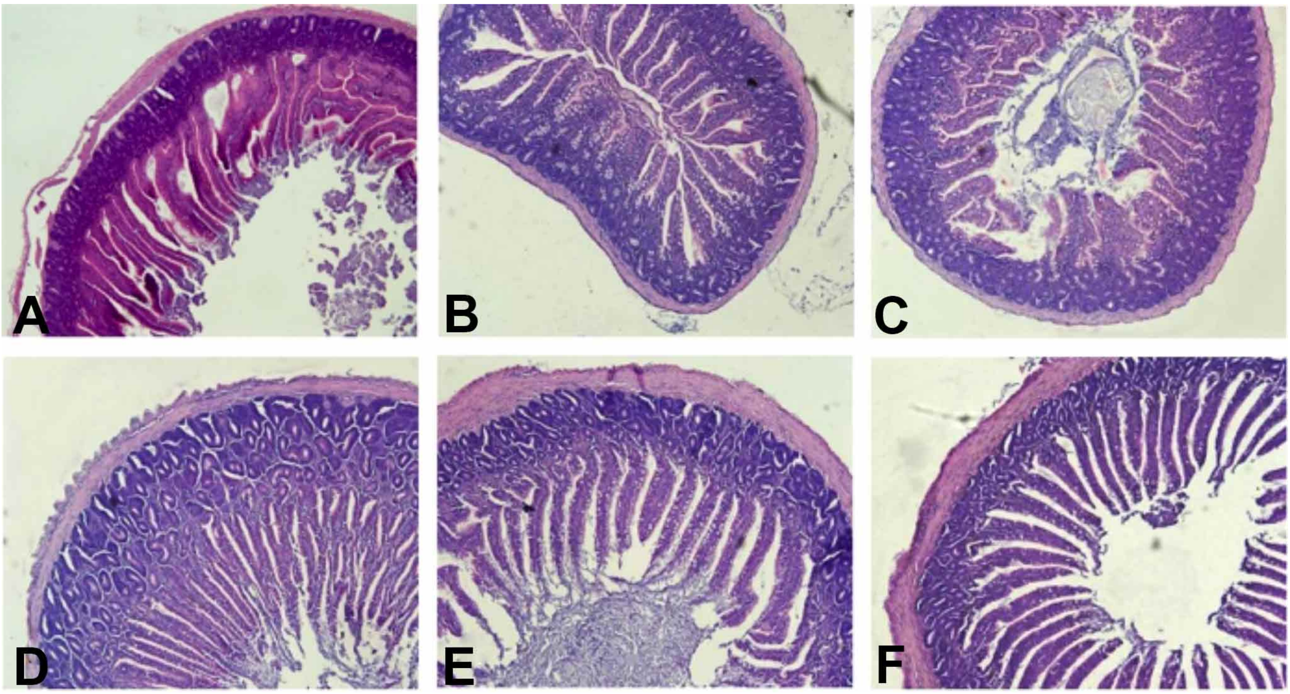

Fig. 2. Duodenum (A, D) jejunum (B, E) and ileum (C, F) of the *Lonchura striata* (A, B, C) and *Copsychus saularis* (D, E, F).

Table I. Comparison of VH, CD and the VH/CD of small intestine between *Lonchura striata* and the *Copsychus saularis* (mm).

	Duodenum			Jejunum			Ileum		
	VH (μm)	CD (μm)	VH/CD	VH (μm)	CD (μm)	VH/CD	VH (μm)	CD (μm)	VH/CD
Lonchura striata	1046.44±36.07	1.99±0.06	547.90±35.93	639.63±11.23	1.73±0.05	401.14±14.71	649.01±11	2.28±0.05	306.77±8.65
Copsychus saularis	1242.41±63.71	1.39±0.12	915.35±43.64	1021.46±17.48	2.20±0.08	477.22±12.48	983.74±8.	2.81±0.14	375.76±11.56
P	0.147	0.018	0.939	0.358	0.089	0.588	0.258	0.01	0.384

Table II. Comparison of the distance of small intestine villus bases (DVB), width (W) and absorption surface area (ASA) between *Lonchura striata* and the *Copsychus saularis*.

	duodenum			jejunum			ileum		
	D	W	ASA	D	W	ASA	D	W	ASA
	(μm)	(μm)	(μm²)	(μm)	(μm)	(μm²)	(μm)	(μm)	(μm²)
Lonchura striata	258.21±29.80	162.55±31.36	161052.30±37279.48	198.54±44.55	125.71±29.96	85123.67±21297.01	224.07±41.02	126.67±27.47	84243.44±25304.95
Copsychus saularis	189.00±31.17	83.42±9.98	104306.06±17036.30	259.83±33.05	155.65±22.41	160189.12±36404.77	231.76±44.72	135.19±21.64	139940.36±34439.59
P	0.807	0.01	0.018	0.363	0.303	0.20	0.592	0.184	0.347

Mucosa thickness (MT), muscularis thickness (MLT) and the small intestinal wall (W) of the small intestinal between *Lonchura striata* and *Copsychus saularis*. As shown in Table III, the muscularis thickness in the ileum of *Lonchura striata* was significantly lower than that of *Copsychus saularis*.

Intestinal secretion function. Goblet cells was the main secretory cells in the gut tract, the number of them determines the strength of secretory function (Fig. 3). The results showed that the number of goblet cells in duodenum and jejunum of *Lonchura striata* and *Copsychus saularis* had no significant difference, but the number of goblet cells in ileum of *Copsychus saularis* was significantly more than that of *Lonchura striata* ($p<0.01$) (Fig. 4).

Changes in the activity of the Na^+/K^+-ATPase. In order to compare the digestive and absorption function between the *Lonchura striata* and *Copsychus saularis*, the Na^+/K^+-ATPase vitality was compared. Na^+/K^+-ATPase provides a driving force for glucose cooperative transport pump and can be used as a monitoring index of gut digestion and absorption function. The experimental results showed that the vitality of Na^+/K^+-ATPase in different intestinal segments of the *Lonchura striata* and the *Copsychus saularis* was different. The vitality of Na^+/K^+-ATPase in the *Lonchura striata* was significantly higher than that of the *Copsychus saularis* (Fig. 5).

Table III. Comparison of the mucosa thickness (MT), muscularis thickness (MLT) and the small intestinal wall (W) of the small intestinal between *Lonchura striata* and *Copsychus saularis*.

	Duodenum			Jejunum			Ileum		
	MT (μm)	MLT (μm)	W (μm)	MT (μm)	MLT (μm)	W (μm)	MT (μm)	MLT (μm)	W (μm)
Lonchura striata	1477.53±57.07	127.64±30.47	1586.83±62.08	1020.31±23.09	108.99±6.43	1142.91±25.94	942.64±27.41	67.11±3.54	997.74±31.18
Copsychus saularis	2013.32±73.89	215.42±20.88	2229.04±76.00	1480.48±33.17	192.16±6.01	1707.49±33.87	1342.48±35.12	207.94±12.34	1665.53±38.81
P	0.536	0.889	0.375	0.113	0.450	0.427	0.465	0.002	0.697

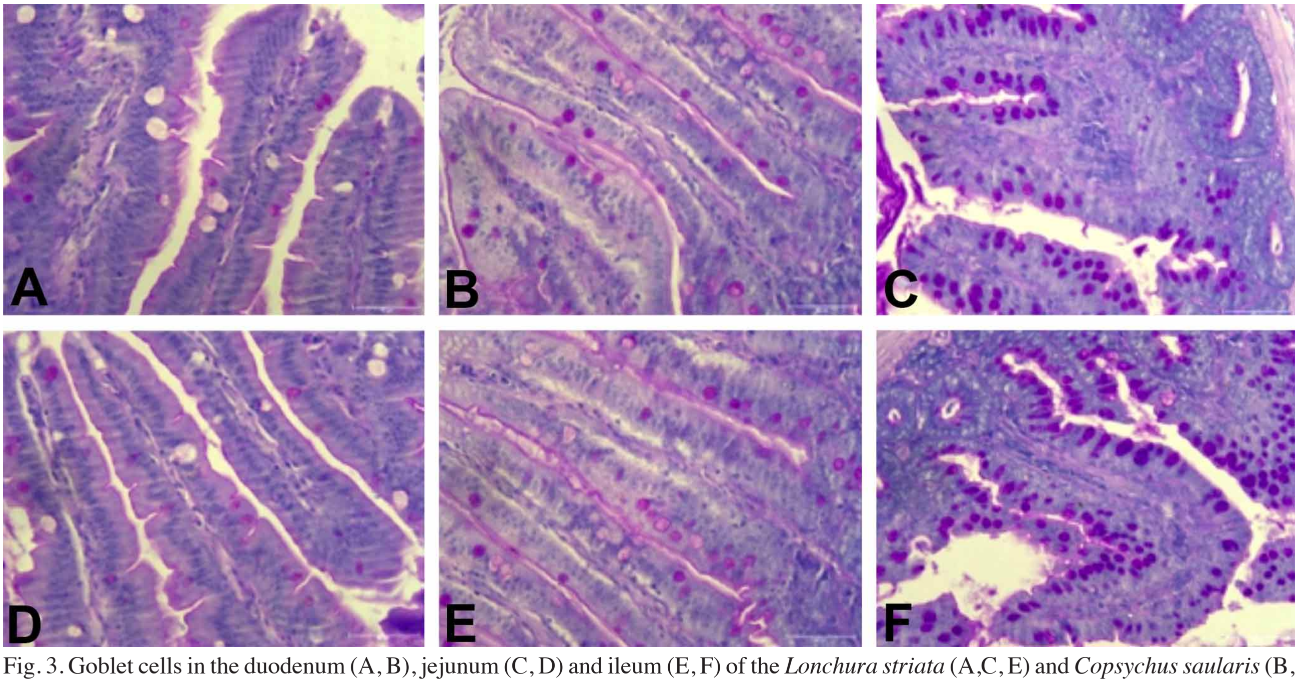

Fig. 3. Goblet cells in the duodenum (A, B), jejunum (C, D) and ileum (E, F) of the *Lonchura striata* (A,C, E) and *Copsychus saularis* (B, D, F).

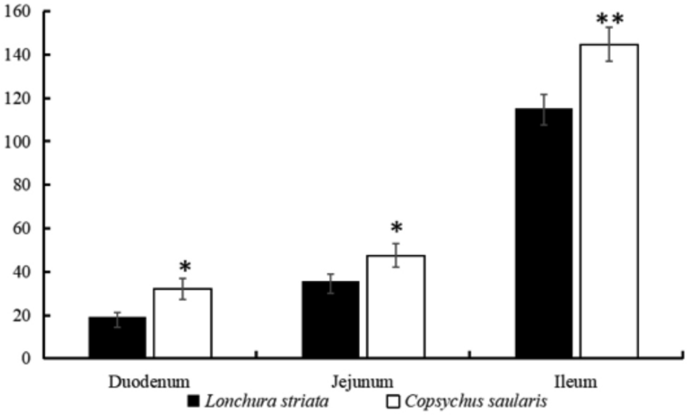

Fig. 4. Comparison of goblet cells in different gut segments between *Lonchura striata* and *Copsychus saularis*
Notes:* indicates that the data are significantly different ($P < 0.05$),
** indicates that the data were significantly different ($P < 0.01$)

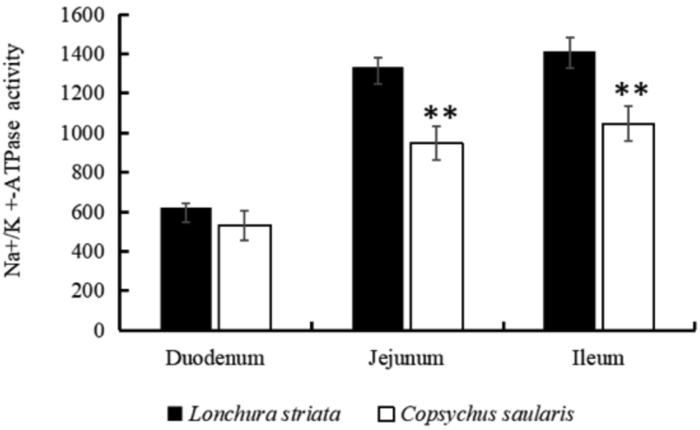

Fig. 5. The vitality of Na^+/K^+-ATPase in different intestinal segments of the *Lonchura striata* and *Copsychus saularis*.

DISCUSSION

The small intestine is the basic structure for nutrients to be fully digested and absorbed. In particular, the length and width of villi, villi surface area, crypt depth and muscular thickness of small intestine are important indicators to measure the digestive and absorption function of small intestine. As a result, it can be seen that *Lonchura striata* mainly feeds on grains, grass seeds, leaves, buds and other plant foods, while *Copsychus saularis* mainly feeds on insects. Although *Lonchura striata* and *Copsychus saularis* belong to Passeriformes and have similar habitats, there is an obvious difference in their feeding habits. Therefore, it is speculated that the differences in the basic intestinal structure and digestive absorption function of *Lonchura striata* and *Copsychus saularis* may be due to their poor feeding habits.

In the duodenum, the villi width, surface area and crypt depth of the small intestine of *Lonchura striata* were larger than those of *Copsychus saularis*, and the differences were significant. Studies have shown that the increase in the height and width of the villi of the small intestine directly reflects an increase of the absorption area of the small intestine, and thus an increase of the mucosal surface area of the small intestine, which help with the entry of digestive enzymes and other substances into the intestine, and lead to better digestion and absorption of the intake (Murakami *et al*., 2007). The results show that the depth of the small intestine can reflect the formation rate of villi cells in the small intestine. The larger the depth of the nest, the higher the cell maturity rate, and the stronger the secretory function; otherwise, the cell maturity rate is low and the secretory function is weak. The intestinal crypt can secrete the small intestine liquid containing various digestive enzymes, which is beneficial to the final decomposition of various nutrients into absorbable forms. A large amount of small intestine liquid can also reduce the osmotic pressure of digestive products by diluting them, which could help with the absorption of digestive products by small intestine (Reynolds, 2010). *Lonchura striata* is a kind of plant-feeding bird, and the cellulose and other substances in the food are more difficult to digest. Therefore, the intestine needs to provide a larger villus area and secrete more intestinal fluid to help with the digestion and absorption. In jejunum and ileum, *Copsychus saularis*' muscle thickness and crypt depth were greater than *Lonchura striata*, and the difference in ileum is most obvious ($P<0.05$). The thickness of the muscle layer can reflect the number of muscle fibers in the intestinal wall and villi. Some studies have shown that the thickness of the muscle layer of the small intestine is closely related to the rhythmic movement of the small intestine and the mechanical digestive efficiency of the chyme. The thicker the muscle layer of the small intestine is, the more muscle fibers are in the intestinal wall and villi, and the stronger the rhythmic movement of the intestinal peristalsis and villi, which is conducive to feeding. The thorough contact and reaction between chyme and digestive juice can improve the mechanical digestive efficiency of chyme, and is also conducive to the absorption of nutrients by intestinal wall cells (Anderson, 1986). The muscularis thickness of small intestine reflects the contractility of small intestine (Dijkstra *et al*., 2007; Udoumoh & Ikejiobi, 2017). *Copsychus saularis* eat insects as its staple food, so it has a large appetite and has a fast digestion and absorption rate. In order to excrete feces in a timely manner to reduce weight, it needs strong contractility and secretion of more mucus in the intestine, especially in the ileum.

Goblet cells are mainly distributed in between the epithelial cells of small intestinal villi, and their secreted mucins enter the intestine to become lubricating mucus and are distributed on the epithelial surface. The mucus can block the destruction of pathogenic microorganisms and harmful substances to a certain extent, prevent toxins from contacting epithelial cells, and pose a protective effect on the epithelium (Forder *et al*., 2007). In addition, goblet cells play an important role in Figurehting infection and the immune response against foreign antigens, and the change in their number can reflect the local immune condition of the digestive tract to a certain extent. As the food moves down, more lubrication is needed to avoid damage to the intestinal wall, so there are more goblet cells from the duodenum to the ileum. In this experiment, the number of goblet cells in duodenum and jejunum of *Copsychus saularis* is more than that of *Lonchura striata* ($P<0.05$), and the difference of goblet cell numbers in ileum is most obvious ($P<0.01$), indicating that *Copsychus saularis* has a stronger ability to digest and absorb nutrients.

Studies have shown that both habitat and food have significant impact on the intestinal length and the related digestive enzymes (Day *et al*., 2011). To compare whether the digestive and absorptive functions of the intestines of the two bird species alter the viability of Na^+/K^+-ATPase, we examined intestinal segments. Na^+/K^+-ATPase is an integral membrane protein found in the cells of all higher-class eukaryotes and is responsible for translocating Na^+ and K^+ ions across the cell membrane. This transportation generates the sodium gradient, which is used to drive numerous transport processes, including the translocation of glucose, amino acids, and other nutrients into cells (Wang *et al*., 2018). In the gut, Na^+/K^+-ATPase is a marker enzyme of the basolateral membrane of enterocyte, which is necessary to drive many transepithelial transportation and ion permeability regulation (Vasilets & Schwarz, 2019). In this experiment, the enzyme activity of all intestinal segments of *Lonchura striata* was greater than that of magpie, and the difference was most obvious in jejunum and ileum ($P<0.01$). Because *Lonchura striata* serves as a type of phytophagous bird, their duodenum is the main food digestion site, and jejunum and ileum are the main absorption sites with greater absorptive capacity. This is consistent with the fact that the duodenal villi are larger in width and surface area and more digestive in *Lonchura striata*in according to previous studies.

Relevant studies have shown that different birds show different digestive tract characteristics due to their different feeding habits. Different birds with the same feeding habits often show the same digestive tract characteristics, while birds with different feeding habits often show different digestive tract characteristics (Yang *et al*., 2013; Ravindran *et al*., 2016). *Lonchura striata* and *Copsychus saularis* show intestinal characteristics that are compatible with them because of different food choices, which leads to differences in the digestive and absorption functions of various intestinal segments in the two species. The findings may provide a reference material to study intestinal differences between phytophagous and mycophagous birds.

It can be concluded that the digestion and absorption capacity of *Copsychus saularis* and *Lonchura striata* are significantly different, and the reason may be related to their different diets and intestinal floras.

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RESUMEN: En este estudio se seleccionaron seis aves *Lonchura striata* y seis *Copsychus saularis*, a las cuales se midió mediante biología cuantitativa y análisis de imágenes el índice morfológico del intestino delgado. Los cambios de las células caliciformes y Na^+/K^+ATPasa se detectaron mediante tinción AB-PAS y ELISA para informar los diferentes mecanismos de digestión y absorción de nutrientes entre *Lonchura striata* y *Copsychus saularis*. La altura de las vellosidades, la profundidad de las criptas y el grosor del músculo de cada segmento del intestino delgado de *Lonchura striata* fueron menores que los de *Copsychus saularis*, y se observó una diferencia significativa en el grosor de la músculatura del íleon. Además, el valor de la altura de la vellosidad del íleon/profundidad de la cripta (VH/CD) de *Lonchura striata* fue significativamente menor que el de *Copsychus saularis*. En el número de células caliciformes del duodeno y del yeyuno de *Lonchura striata* y *Copsychus saularis* no hubo una diferencia significativa, pero el número de células caliciformes en el íleon de *Copsychus saularis* fue significativamente mayor que el de *Lonchura striata*. Hubo diferencias en la vitalidad de Na^+/K^+-ATPasa en diferentes segmentos intestinales de *Lonchura striata* y *Copsychus saularis* . La vitalidad de Na^+/K^+-ATPasa en *Lonchura striata* fue significativamente mayor que la de *Copsychus saularis*. Se puede concluir que la capacidad de digestión y absorción de *Copsychus saularis* y *Lonchura striata* son significativamente diferentes, posiblemente debido a sus distintas dietas y floras intestinales.

PALABRAS CLAVE: ***Lonchura striata*; *Copsychus saularis*; Intestino delgado; Estructura histológica; Células caliciformes; Na^+/K^+-ATPasa**

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