

# Effect of different types of sugar on gut physiology and microbiota in overfed goose

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**ABSTRACT** To explore the difference of goose fatty liver formation induced by different types of sugar from the intestinal physiology and the gut microflora, an integrated analysis of intestinal physiology and gut microbiota metagenomes was performed using samples collected from the geese including the normal-feeding geese and the overfed geese which were overfed with maize flour or overfeeding dietary supplementation with 10% sugar (glucose, fructose or sucrose, respectively), respectively. The results showed that the *foie gras* weight of the fructose group and the sucrose group was heavier ( $P < 0.05$ ) than other groups. Compared with the control group, the ileum weight was significantly higher ( $P < 0.01$ ), and the cecum weight was significantly lower in the sugar treatment groups ( $P < 0.001$ ). Compared with the control group, the ratio of villi height to crypt depth in the fructose group was the

highest in jejunum ( $P < 0.05$ ); the trypsin activity of the ileum was higher in the fructose group and the sucrose group ( $P < 0.05$ ). At the phylum level, *Firmicutes*, *Proteobacteria* and *Bacteroidetes* were the main intestinal flora of geese; and the abundance of *Firmicutes* in the jejunum was higher in the sugar treatment groups than that of the maize flour group. At the genus level, the abundance of *Lactobacillus* in the jejunum was higher ( $P < 0.05$ ) in the sugar treatment groups than that of the maize flour group. In conclusion, forced-feeding diet supplementation with sugar induced stronger digestion and absorption capacity, increased the abundance of *Firmicutes* and *Bacteroidetes* and the abundance of *Lactobacillus* (especially fructose and sucrose) in the gut. So, the fructose and sucrose had higher induction on hepatic steatosis in goose fatty liver formation.

**Key words:** glucose, fructose, sucrose, intestinal microorganism, fatty liver

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## INTRODUCTION

The intestine is the main place where nutrient digestive absorption takes place and gut flora colonizes, which plays an important role in the growth and metabolism of organisms. When the number of harmful intestinal microbes is increased, their metabolites will lead to changes in the intestinal structure and permeability. Increased permeability also increased the number of microbes, the endotoxin or lipopolysaccharide (**LPS**) (Wu et al., 2020), and harmful metabolites entering the intestine, causing systemic immune response, inflammation, and changes in the function of other organs and tissues (Luci et al., 2019). There is a certain relationship

between intestinal physiology and microorganisms and fatty liver formation induced by sugar (Li et al., 2019). The health small intestine shielded the liver from fructose-induced steatosis (Jang et al., 2020). Glucose metabolism pathway disorder induces liver fatty degeneration and intestinal mucosal barrier dysfunction in metabolism-associated fatty liver disease (**MAFLD**) (Gao et al., 2020; Wang et al., 2020). Wu et al. (2020) reported that gut microbiota modulation can improve glucose tolerance, induce the production of Short Chain Fatty Acids and inhibit the production of endotoxin LPS. Impairment of glucose tolerance aggravates the progression of diet-induced MAFLD (Brandt et al., 2020). Zhao et al. (2020) reported that the dietary fructose induced hepatic lipogenesis via microbiota-derived acetate. Fructose dietary intake affected the composition of the intestinal microbiota and influenced the development of hepatic steatosis (Silva-Veiga et al., 2020). The interaction between fructose and copper in diet regulated the intestinal microbial metabolism in rats, leading to liver injury and liver steatosis

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(Song et al., 2018a, b). Rats fed a diet high in sucrose caused hepatic lipids deposition and gut microbiota change (Okazaki and Katayama, 2020). Ban et al. (2020) reported that rats fed with sucrose and showed a significant increase in insulin resistance and decreased blood glucose regulation level, and a remarkable deterioration in gut microbiota status. However, there was no report whether sugar (glucose, fructose or sucrose) can be used in forced-feeding for *foie gras* production.

The main purpose of force-feeding is to increase fat deposition in liver and produce *foie gras* in ducks and geese. After force-feeding, the waterfowl received high energy, as a result of which the substrates for fatty acid synthesis (glucose) increased substantially in the liver. Meanwhile, the content of producing TG far exceeded the transport capacity of apolipoproteins, and the producing fatty acid far exceeded the degraded fatty acid by  $\beta$ -oxidation, thus leading to the accumulation of lipids in the liver (Wei et al., 2020a). As previously reported, force-feeding induced a significant increase in liver weight (Arroyo et al., 2019). The effect of overfeeding on production performance has been well discussed in waterfowl (Wen et al., 2016). A study of Landes Geese showed that the richness and diversity of the bacterial communities decreased in the ileum and cecum after overfeeding (Tang et al., 2018). Overfeeding caused the oxidative stress in the intestine tract, and the intestine tract and body faced the challenge of oxidative stress in overfeeding process (Wei et al., 2020b). Liu et al. (2016a) reported that *Firmicutes* inhabited the duodena, jejunum and ileum more densely than caeca in the overfed geese, and its abundance was influenced by overfeeding. Gu et al. (2020) reported that maintaining intestinal structural integrity to prevent occurrence of inflammation is a protective mechanism for goose fatty liver during the period of overfeeding.

The carbohydrate feed commonly is used in livestock production, such as corn, wheat and rice, is mainly composed of starch polysaccharide, which is digested in the body and absorbed by the small intestine as glucose and other simple sugars. A large number of animal studies have reported that a high sugar diet can induce the fatty liver (Neuschwander-Tetri, 2013; Vos and Lavigne, 2013). However, at present, whether sugar is suitable for *foie gras* production has been seldom reported. And there have been seldom reports about sugar influence on intestinal physiology and gut flora in the forced-feeding process. Moreover, the differences in liver lipid deposition induced by different types of sugar still lack systematic research. The “gut-liver axis” theory is often used to explain the interaction between the intestine and liver (Miura and Ohnishi, 2014; Miura et al., 2017). In order to understand the differences in the liver lipid deposition induced by different types of sugar from the angle of “gut-liver axis”, the effect of different types of sugar (glucose, fructose and sucrose) on the gut physiology and microbiota in the overfed goose was performed in this study. Not only will understanding this difference mechanism induced by different types of sugar from the angle

of “gut-liver axis” explore the relationship between sugar and the mechanism of goose fatty liver formation, it also opens an approach to improving the *foie gras* production efficiency and *foie gras* quality. Meanwhile, it is not only conducive to animal welfare, but also a reference to the prevention and treatment of fatty liver disease in humans.

## MATERIALS AND METHODS

### Ethics Statement

All procedures in the present study were subject to approval by the Institutional Animal Care and Use Committee (IACUC) of Sichuan Agricultural University (Permit No. DKY-B20141401), and carried out in accordance with the approved guidelines.

### Birds and Experiment Design and Sampling

The forced-feeding experiment was performed in Waterfowl Breeding Farm of Sichuan Agricultural University (Yaan, Sichuan, China). One hundred Tianfu Meat Geese were randomly divided into control group ( $n = 20$ ) and forced-feeding groups ( $n = 80$ ). Forced-feeding groups including maize flour group ( $n = 20$ ), glucose group ( $n = 20$ ), fructose group ( $n = 20$ ) and sucrose group ( $n = 20$ ). Group assignments and forced-feeding diet compositions were shown in Table 1. The daily overfed intake reached 1200–1500 g dry matter (4 meals a day), which lasted 18 d; the geese in the control group were allowed *ad libitum* access to diet. Geese had free access to water at all times. All the experimental geese were reared in cages with a density of 3/m<sup>2</sup>, the temperature was controlled at about 25°C, and light was provided at night (dim light). Geese were weighed individually (before slaughter) after 24 h of fasting. The slaughter weight was measured after slaughter, and the liver was weighed after complete removal. The intestines were carefully separated, then all the intestinal contents were extruded and the weights of the duodenum, jejunum, ileum and cecum were measured. Five geese of each group were killed, then immediately sacrificed for liver, small intestinal tissue and intestinal contents. Two cm segments of the duodenum, jejunum, ileum and liver were removed, and washed with normal saline, then fixed in 4% paraformaldehyde and stored at room temperature. Intestinal contents were collected in sterile Ep tubes and stored at -80°C for subsequent analysis of digestive enzyme activity and intestinal microbial flora.

### Detection of Serum Lactic Acid Content

Lactic acid content determination kit (Nanjing Jiancheng Bioengineering, Nanjing, China) was used to determine the content of lactic acid in serum. The operation procedures were strictly in accordance with the kit instructions.

**Table 1.** Composition and nutrient levels of experimental diets (air-dry basis) %.

Items (%)	Forced-feeding groups				
	C	M	Sugar treatment groups		
			G	F	S
Maize flour	100	94.5	85	85	85
Fish flour	—	2	2	2	2
NaCl	—	1	1	1	1
Soya oil	—	2.5	2	2	2
Glucose	—	—	10	—	—
Fructose	—	—	—	10	—
Sucrose	—	—	—	—	10
Total	100	100	100	100	100
Nutrient levels					
ME/(MJ/kg)	12.87	13.56	13.56	13.56	13.56
Crude protein (CP)	11.3	15.2	15.2	15.2	15.2
Lysine (Lys)	0.84	0.85	0.85	0.85	0.85
Methionine (Met)	0.41	0.41	0.41	0.41	0.41
Calcium (Ca)	0.7	0.7	0.7	0.7	0.7
Total phosphorus (TP)	0.54	0.54	0.54	0.54	0.54

Abbreviations: C, control group; F, fructose group; G, glucose group; M, maize flour group; S, sucrose group (n = 20).

### Analysis of Intestinal Morphology

According to the methods of Hou et al. (2020), the cross-sections from the middle of the duodenum, jejunum, ileum and liver were preserved in 4% formaldehyde-phosphate buffer were prepared using standard paraffin embedding techniques, sectioned (5  $\mu\text{m}$ ) and stained with hematoxylin and eosin (HE), and sealed by neutral resin size thereafter, and then examined by microscope photography system (Olympus, Tokyo, Japan), each slice was observed and 5 visual fields were randomly selected. The selected visual fields were measured via imaging software (Image Pro Plus 6.0, Media Cybernetics, Bethesda, MD, USA). Visual measurements of villus height, crypt depth, intestinal wall thickness and the liver fat droplet area ratio were measured 10 times and taken an average.

### Detection of Intestinal Digestive Enzyme Activity

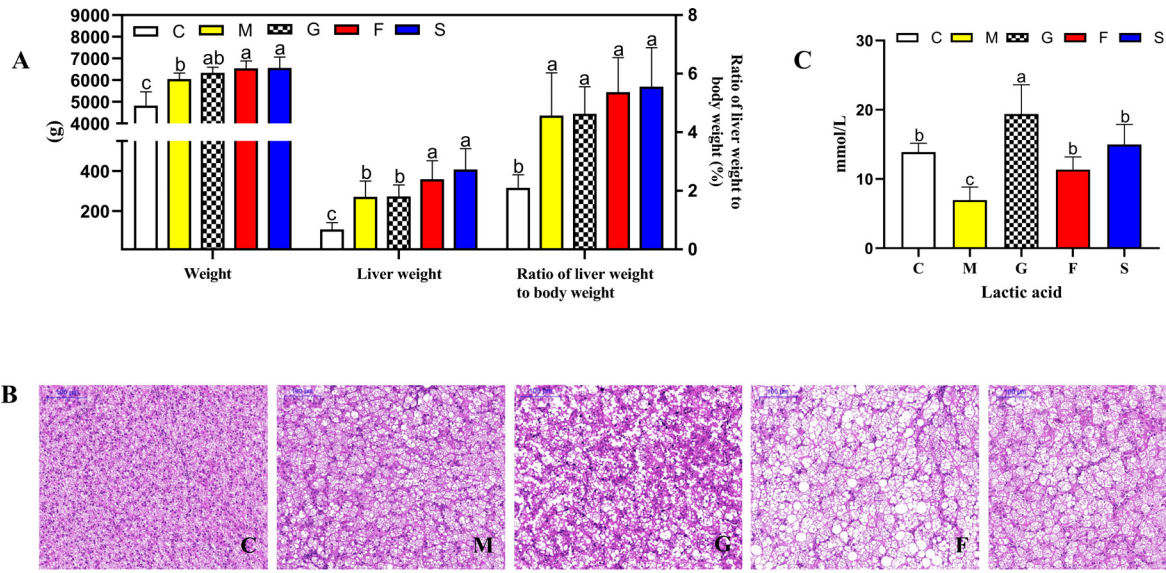
Approximately 0.1 g of frozen intestinal contents were accurately weighed, and placed in sterile Eppendorf tubes containing 9 volumes (w/v) of ice-cold normal saline. The mixture of small intestinal contents and normal saline was homogenized in an ice water bath, at 2500 g for 15 min at 4°C, and the supernatant was obtained and kept at 20°C used for enzyme activity study. Protein concentration of samples was employed to calculate the digestive activities, and assayed using a protein quantification kit (Nanjing Jiancheng Bioengineering, Nanjing, China). The activities of amylase, trypsin and lipase were measured. The kit was purchased from Nanjing Jiancheng biotechnology co., LTD (Nanjing Jiancheng Bioengineering, Nanjing, China). The operation procedures were strictly in accordance with the kit instructions.

### Analysis of Intestinal Flora

The intestinal contents in the duodenum, jejunum, ileum and cecum of 3 geese were selected for microflora analysis respectively from each group. Based on Illumina HiSeq sequencing platform, the Paired-End method was used to construct a small fragment library for microbial diversity sequencing. Sequencing was performed by Beijing Baemai Biotechnology Co. Ltd (Beijing baimike biotechnology co., LTD., Beijing, China). The original sequencing sequence was filtered and the double-end stitching was carried out to obtain the optimized sequence (Tags). UCLUST in QIIME (version 1.8.0) software was used to cluster Tags to obtain OTU at 97% similarity level, and the OTU was taxonomic annotated based on the taxonomy databases of Silva (bacteria) and UNITE (fungi).

### Analyses and Statistics

SPSS software 20.0 (IBM, Armonk, NY) was used to analyze the significance of differences in the relative abundances of intestinal flora between groups. The correlation between liver weight and intestinal weight was analyzed by SPSS software 20.0 (IBM, Armonk, NY) (correlation coefficient Pearson) and the result diagram of correlation analysis was drawn by R Studio. Gut flora diversity analysis was performed via Baimike biocloud platform (Baimike Biological Technology Co., LTD, ID: 1491531197@qq.com; Password: lucangang521123). All experimental data were expressed by mean  $\pm$  SD and showed with graphs created with GraphPad Prism 5.0 software (GraphPad Prism Software Inc., San Diego, CA). We considered  $P < 0.05$  as statistically significant.



**Figure 1.** Influence of different types of sugar on liver physiological. (A) Body weight, liver weight and the ratio of liver weight to body weight ( $n = 20$ ); (B) Liver sections of different treatment groups ( $n = 4$ ); (C) The content of lactic acid in serum ( $n = 6$ ). The different capital letters at the bottom right of the liver section represent different treatment groups ( $10\times$ ). Different letters on the graph indicate significant differences ( $P < 0.05$ ). Abbreviations: C, control group; F, fructose group; G, glucose group; M, maize flour group; S, sucrose group.

## RESULTS

### Effect of Different Types of Sugar on Foie Gras Performance and Intestinal Morphology in Overfed Geese

As shown in Figure 1A and Supplementary S-Table 2, the body weight, the liver weight and the ratio of liver weight to body weight of goose in the 4 forced-feeding groups were significantly higher than those in the control group ( $P < 0.05$ ). Compared with the maize flour group, the liver weight in the fructose group and sucrose group was higher ( $P < 0.05$ ), the hepatic steatosis was more significant in the fructose group and sucrose group (Figure 1B; Supplementary S-Figure 1), and the glucose group showed no significant difference. Compared with the control group, the content of lactic acid in the serum was significantly higher in the glucose group ( $P < 0.05$ ). Compared with the maize flour group, the content of lactic acid in the serum was significantly higher in the glucose group, fructose group and sucrose group ( $P < 0.05$ ) (Figure 1C; Supplementary S-Table 5).

The duodenum weight and jejunum weight in the sucrose group were significantly higher than those in the control group ( $P < 0.05$ ), and the weight of ileum in the 3 sugar treatment groups was significantly higher than those in the control group ( $P < 0.05$ ). The cecal weight of the 4 forced-feeding groups was significantly lower than that of the control group ( $P < 0.001$ ) (Figure 2A; Supplementary S-Table 2). Supplementary (Supplementary S-Figure 2; Supplementary S-Figure 3 and Supplementary S-Figure 4) showed the intestinal tissue slice image of duodenum, jejunum and ileum, respectively. As shown in Figure 2B and Supplementary S-Table 3, compared with the control group, the wall thickness of jejunum decreased significantly, while the villus height and crypt depth of ileum increased significantly in the 3 sugar treatment groups

( $P < 0.05$ ). Compared with the maize flour group or control group, the ratio of villus height to crypt depth of jejunum in the 3 sugar treatment groups showed an increasing trend, and was highest in the fructose group ( $P < 0.05$ ).

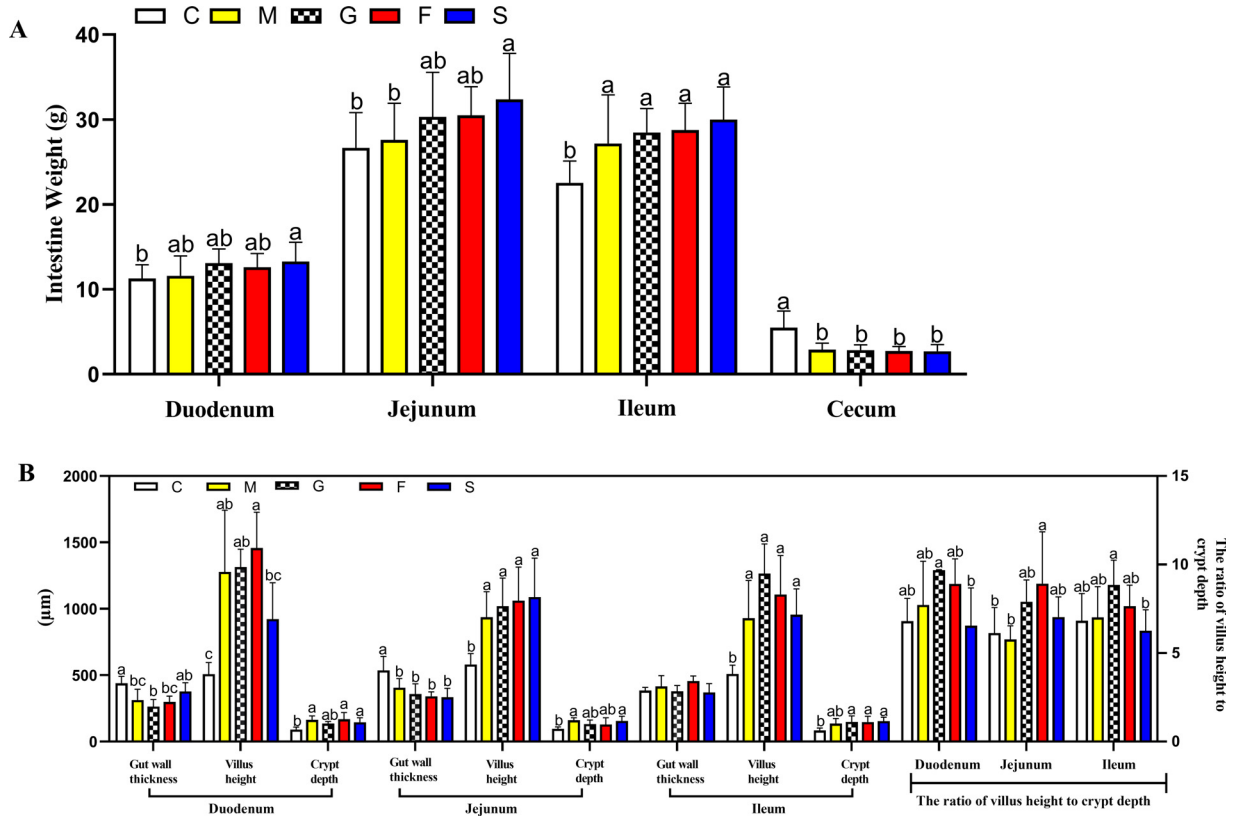
### Correlation Analysis of Liver Weight and Bowel Weight of Intestinal Tract

As shown in Figure 3, the results showed that there was a significant positive correlation between the liver weight and the cecum weight in the control group ( $r = 0.56$ ), and the liver weight was significant negatively correlated with the cecal weight in the maize flour group ( $r = -0.61$ ), glucose group ( $r = -0.74$ ), fructose group ( $r = -0.79$ ) and sucrose group ( $r = -0.78$ ). There was a negative correlation between the liver weight and the ileum weight in the fructose group ( $r = -0.74$ ). There was a positive correlation between the jejunum weight and the ileum weight in the control group ( $r = 0.77$ ), maize flour group ( $r = 0.68$ ), glucose group ( $r = 0.68$ ) and fructose group ( $r = 0.78$ ).

### Effect of Different Types of Sugar on Digestive Enzyme in Overfed Geese

As shown in Figure 4 and Supplementary S-Table 4, compared with the control group, the amylase activity in the duodenum and the ileum was significantly lower in the 3 sugar treatment groups ( $P < 0.05$ ), and the amylase activity in the jejunum was significantly lower in the fructose group and the sucrose group ( $P < 0.05$ ). Compared with the maize flour group, the amylase activity in the cecum was significantly lower in the glucose group and fructose group ( $P < 0.05$ ). Compared with the control group, the trypsin activity in the ileum was significantly

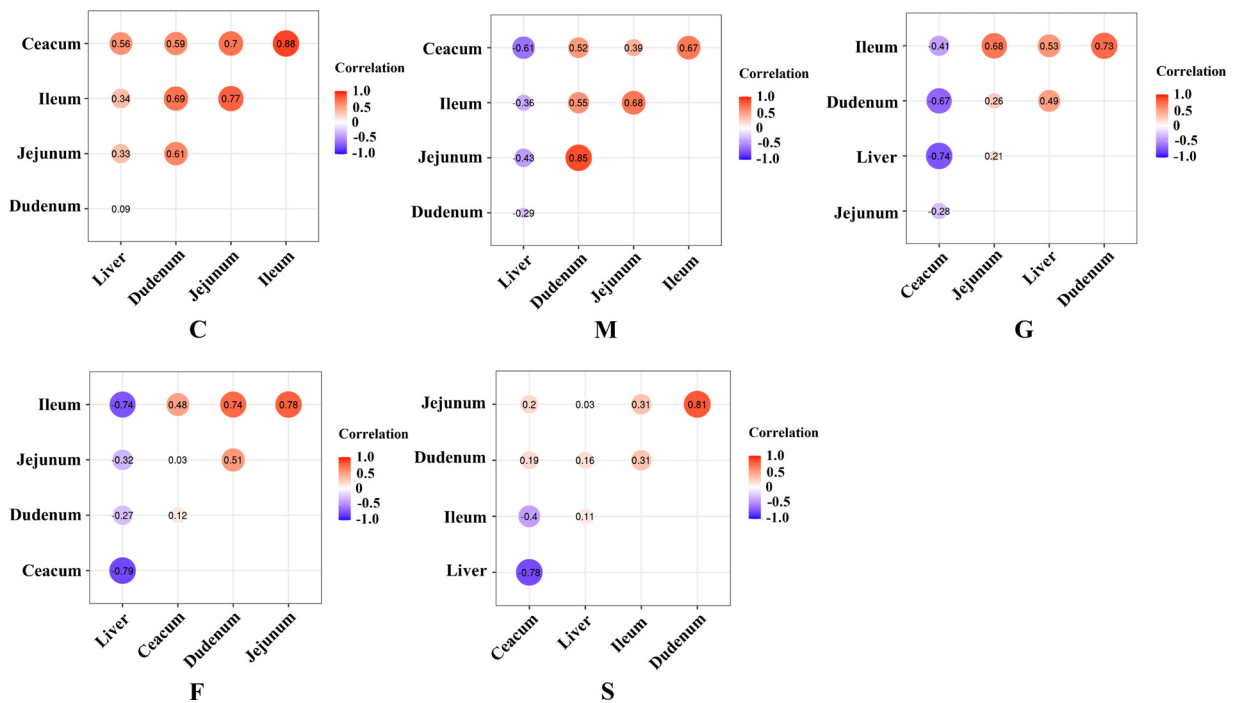




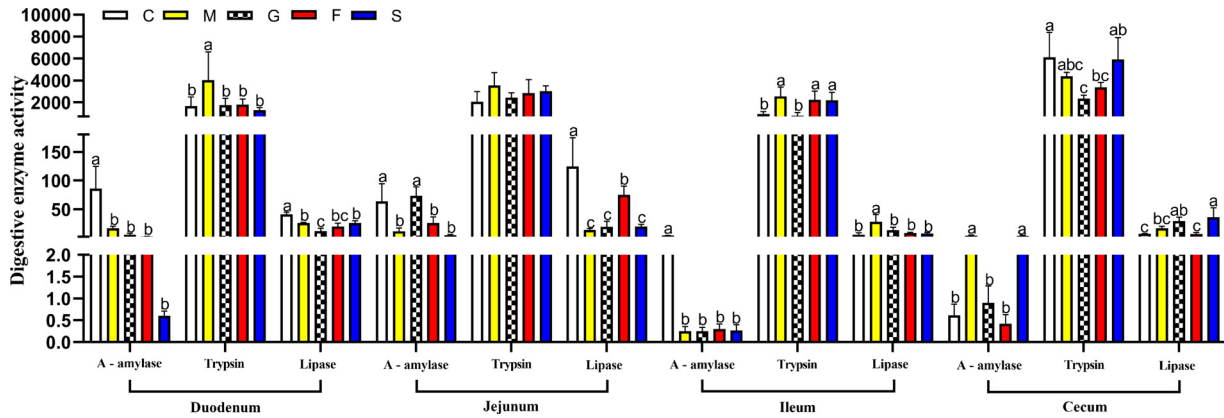
**Figure 2.** Influence of different types of sugar on intestinal physiological. (A) Intestinal weight (n = 20); (B) Intestinal morphology (n = 4). Different letters on the graph indicate significant differences ( $P < 0.05$ ). Abbreviations: C, control group; F, fructose group; G, glucose group; M, maize flour group; S, sucrose group.

higher in the fructose group and sucrose group ( $P < 0.05$ ); the lipase activity in the duodenum and jejunum was significantly lower in the 3 sugar treatment groups ( $P < 0.05$ ); while the lipase activity in the

cecum was significantly higher in the sucrose group ( $P < 0.05$ ). Compared with the maize flour group, the lipase activity was significantly higher in the jejunum of the fructose group ( $P < 0.05$ ) and



**Figure 3.** Correlation between liver weight and intestinal weight in each treatment group (n = 20). Abbreviations: C, control group; F, fructose group; G, glucose group; M, maize flour group; S, sucrose group.



**Figure 4.** Influence of different types of sugar on digestive enzyme activity. The unit of amylase and trypsin is U/mg; The unit of lipase is U/g. Values are means  $\pm$  SD ( $n = 5$ ). Different letters on the graph indicate significant differences ( $P < 0.05$ ). Abbreviations: C, control group; F, fructose group; G, glucose group; M, maize flour group; S, sucrose group.

significantly lower in the ileum of 3 sugar treatment groups ( $P < 0.05$ ).

### Effect of Different Types of Sugar on Gut Flora in Overfed Geese

The dilution curve is used to verify whether the amount of sequencing data is sufficient to reflect the species diversity in the sample (Supplementary S-Figure 5). The VENN diagram was used to calculate the number of OTU common and unique in each group of samples, intuitively showing the similarity and overlap between samples in the number of OTU (Figure 5A). It is known that 0, 1, 4, 0 and 0 OTU were unique to the control group, maize flour group, glucose group, fructose group and sucrose group, respectively, and the 220 OTU were common to the 5 groups. The maize flour group had 283, 312 and 314 OTUs in common with the glucose group, the fructose group and sucrose groups. The control group had 260, 295 and 304 OTUs in common with the glucose group, fructose group and sucrose group, and the OTU classification of the sucrose group was similar to that of the control group.

Chao1 and Ace index measure species abundance, i.e. the number of species. Shannon and Simpson indices are used to measure species diversity. Compared with the control group, the Chao1 index of the glucose group, fructose group and sucrose group showed a downward trend, and the difference of Chao1 index in the fructose group was significant ( $P < 0.05$ ). There was no significant difference in the Ace, Simpson and Shannon index between the control group and the forced-feeding groups and between the forced-feeding groups. Coverage value represents the sequencing depth of the sample. The results showed that the sequencing coverage of samples was high and the experimental data were reliable, because the sequencing coverage of samples in each group was above 0.99 (Supplementary S-Table 1).

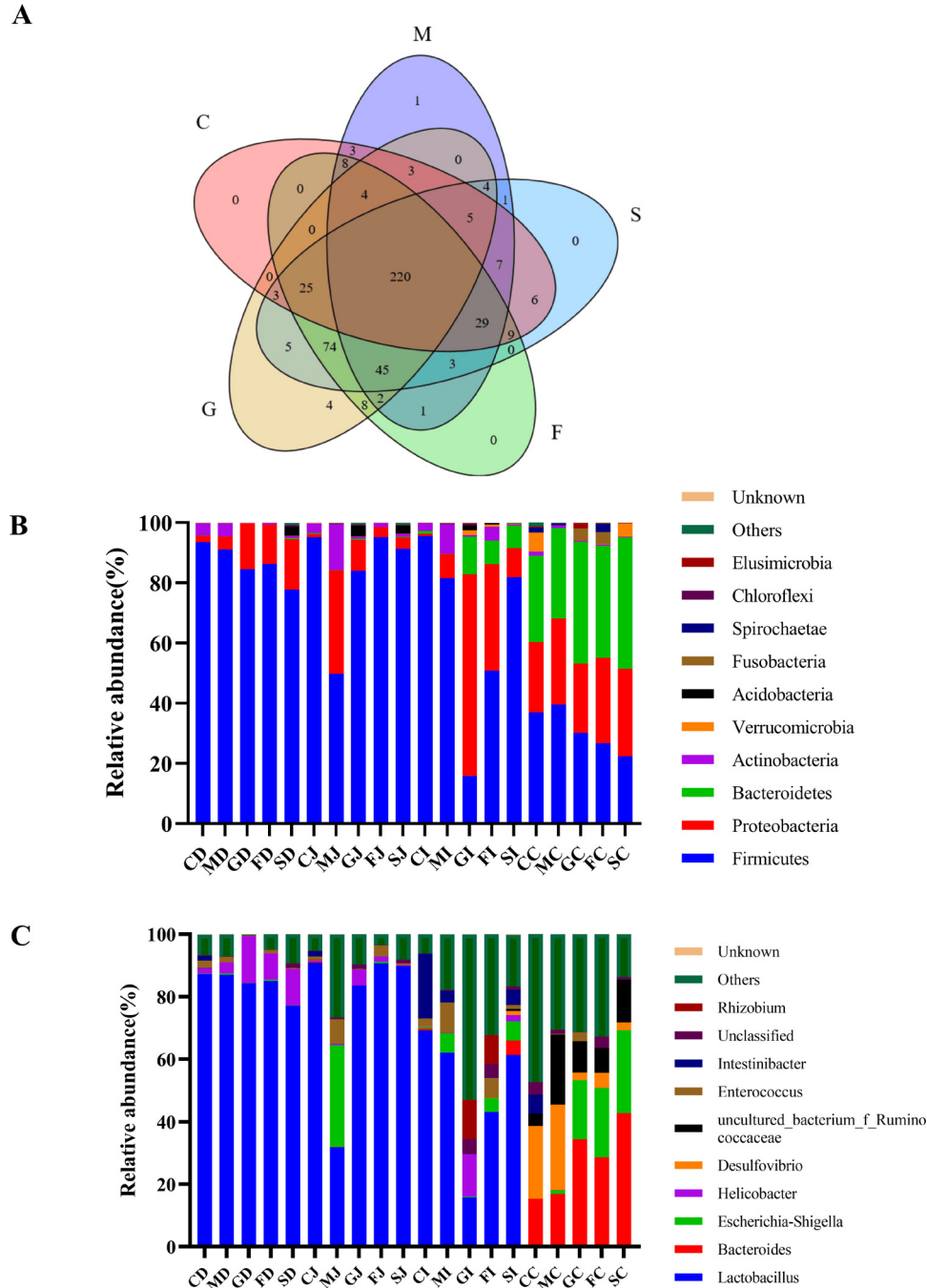
The 16SrDNA sequencing technology was used to analyze the structure of intestinal microflora of Tianfu meat goose. At phylum level, *Firmicutes*, *Proteobacteria* and *Bacteroidetes* were the main intestinal flora of geese, among which the abundance of *Firmicutes* in the

jejunum and *Bacteroidetes* in the cecum of the maize flour group was lower than that of the 3 sugar treatment groups (Figure 5B). In addition, compared to the maize flour group, it was found that the abundance of *Lactobacillus* in the jejunum and *Bacteroides* in the cecum was significantly higher in the 3 sugar treatment groups, visually (Figure 5C).

The changes of bacteria populations of *Lactobacillus* and *Bacteroides* in different intestinal tracts were analyzed, and the result was shown in Figure 6. The results showed that the effect of forced-feeding adding sugar on different intestinal tracts was different. Compared with the maize flour group, the abundance of *Lactobacillus* in the jejunum of the 3 sugar treatment groups increased significantly ( $P < 0.05$ ). Compared with the maize flour group, the abundance of *Bacteroides* in the cecum of the 3 sugar treatment groups showed an increasing trend, and the difference was significant in the sucrose group ( $P < 0.05$ ).

## DISCUSSION

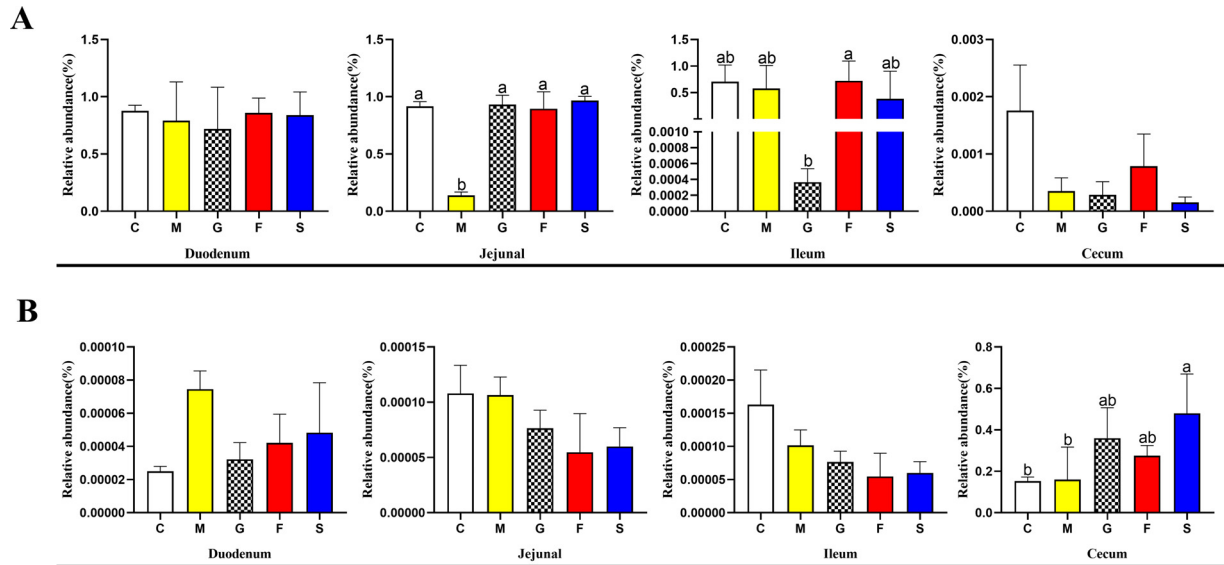
Since the theory of “gut-liver axis” was suggested, the relationship between liver and intestine has attracted a great deal of attention for disease research (Miura and Ohnishi, 2014). There has been increasing evidence that the occurrence of MAFLD in mammals is closely associated with the intestinal environment (Bajaj and Hylemon, 2018). Mitchell and Smith studied 3 broiler strains with different growth rates, and found that the fastest growing strain had the highest absolute intestinal weight and length (Mitchell and Smith, 1991). Overfeeding increased the jejunum length and weight in goose (Liu et al., 2016b). Our results showed that the liver weight of Tianfu Meat Geese after forced-feeding was significantly higher than that of the control group, and there was a significant negative correlation between the liver weight and the cecal weight. It is consistent with another goose overfeeding study reported by Gu et al. (2020), which indicated overfeeding induced atrophy and decline in the function of cecum. The morphological parameters of the intestine, including the



**Figure 5.** Influence of different types of sugar on relative abundance of intestinal bacteria ( $n = 3$ ). (A) Venn diagram of OTU; (B) Column pictures of phyla; (C) Column pictures of genera. The intestinal flora of the top ten relative abundances was studied. Different colors represent different intestinal bacteria; the percentage on the vertical ordinate indicates the relative abundance of intestinal bacteria. Abbreviations: CD, the duodenum of the control group; MD, the duodenum of the maize flour group; GD, the duodenum of the glucose group; FD, the duodenum of the fructose group; SD, the duodenum of the sucrose group; CJ, the jejunum of the control group; MJ, the jejunum of the maize flour group; GJ, the jejunum of the glucose group; FJ, the jejunum of the fructose group; SJ, the jejunum of the sucrose group; CI, the ileum of the control group; MI, the ileum of the maize flour group; GI, the ileum of the glucose group; FI, the ileum of the fructose group; SI, the ileum of the sucrose group; CC, the cecum of the control group; MC, the cecum of the maize flour group; GC, the cecum of the glucose group; FC, the cecum of the fructose group; SC, the cecum of the sucrose group.

villus height, the crypt depth, and the ratio of villus height to crypt depth, are widely used as the standard for evaluating the intestinal health of poultry (Ducatelle et al., 2018; Hosseini-Vashan et al., 2020). It has been reported that a longer villi length and deeper crypts were indicative of the increased nutrient absorption, and the higher ratio of villus height to crypt depth reflects a higher nutrient absorption capacity

(Wang et al., 2018; Qin et al., 2019). The results of this experiment showed that the ratio of villus height to crypt depth in sugar treatment groups was higher than that in the maize flour group, which means that intestinal absorption capacity of the sugar treatment groups was better. This is different from the research reported by Todoric et al. (2020), which showed a large amount of fructose entered the intestine and damaged the



**Figure 6.** Influence of different types of sugar on the abundance ratio of *Lactobacillus* and *Bacteroides* in each intestinal segment. (A) *Lactobacillus*; (B) *Bacteroides*. Values are means  $\pm$  SD ( $n = 3$ ). Different letters on the graph indicate significant differences ( $P < 0.05$ ). Abbreviations: C, control group; F, fructose group; G, glucose group; M, maize flour group; S, sucrose group.

intestinal barrier, resulting in the endotoxemia and the occurrence of nonalcoholic steatohepatitis (NASH). The reason may be that sphingolipid metabolism is involved in the adaptation of intestine to overfeeding, maintaining the intestinal structural integrity (Gu et al., 2020). It is a potential protective mechanism against inflammation in the formation of goose fatty liver. Combined with change of the intestinal weight and morphology index, the gut accelerated its own growth and development to adapt to the high intensity digestion and absorption in the geese of sugar treatment groups.

When the waterfowl is overfed with a large amount of food, the endogenous enzymes of the body is secreted insufficient, and the concentration of digestive enzymes of pancreas and chyme decreases, resulting in the low utilization rate of nutrients (Wen et al., 2013; Wen, et al., 2017). Our results showed that compared with the control group, the activity of amylase and lipase decreased in small intestine significantly after forced-feeding adding sugar. After ducks were overfed, a large amount of carbohydrate-rich diet entered the gut, the increase of amylase activity was not proportional to the increase of filling amount, and some starch was not fully digested and would be excreted out (Wen et al., 2012). White et al. (1983) have reported that the rapid conversion of serum protein and long-term restriction of protein intake caused the decrease of serum albumin content. Corn is the main component of this experiment, and the corn is a high energy and low protein diet, which may lead to the decrease of serum albumin content. In this current study, the trypsin activity of ileum was higher in the geese of the fructose group and sucrose group. Ileum is the main contributor to the enhanced capacity of nutrient digestion and absorption (Gu et al., 2020). Therefore, the reason why the trypsin activity increased may be a compensatory response of digestive glands responding to insufficient dietary protein

(Short and Derrickson, 2020). The dietary protein is essential for liver growth (Li et al., 2020). The results showed that the ileum trypsin activity of geese in the fructose group and sucrose group was higher than that in the glucose group, which was corresponding to the result that the *foie gras* weight was higher in the fructose group and sucrose group.

The intestine is the place not only where nutrients are digested and absorbed, but also where intestinal microbes are colonized. The intestinal microbial ecosystem plays an important role in the nutrition, physiology, and immune defense mechanisms of the animal. Previous studies have implicated the *Eimeria* infection causing intestinal damage including a reduction in the villus height with a consequent decrease in the growth performance and an increased potential for *C. perfringens* to colonize (Wu et al., 2016; Zanu et al., 2020). *Firmicutes* and *Bacteroidetes* can both produce SCFA through fermentation using high fat diets (for *Firmicutes*) or high plant fibers (for *Bacteroidetes*) (De Filippo et al., 2010). Moreover, recent studies showed that the ratio of *Firmicutes/Bacteroidetes* was associated with mammalian obesity, where MAFLD may occur (Turnbaugh et al., 2009; Schwartz et al., 2010). Some studies have found that high-energy (sugar) foods can make animal fat, and the *Firmicute/Bacteroidetes* ratio is higher in obese mice than lean mice (Ley et al., 2005). In this study, the abundance of *Firmicute* of the 3 sugar treatment groups was higher than that of the maize flour group in jejunum, and this abundance of ileum in the fructose group and sucrose group was higher than that in the glucose group. The ileum in poultry, geese is no exception, has a characteristic ileal digestion (Jamroz et al., 2002). In addition, the jejunum and ileum are the major parts of intestinal. It was indicated that the abundance of *Firmicute* in 3 sugar treatment groups was significantly higher than that in the maize flour group; the abundance of *Firmicute* in the fructose group and sucrose group was



higher than that in the glucose group. In addition, in cecum, *Bacteroidetes* abundance of the 3 sugar treatment groups was higher than that of the maize flour group and control group, and its abundance was the highest in the sucrose group. Interestingly, the body weight and the liver weight of the forced-feeding groups were significantly higher than those of the control group; the liver weight of the fructose group and sucrose group was higher than that of the glucose group. It suggested that the abundance of *Firmicute* and *Bacteroidetes* was related to liver weight, which is in line with the research that the abundance of *Firmicute* and *Bacteroidetes* and the liver weight increased in Landes geese after overfeeding reported by Liu et al. (2016b).

This current study indicated that different types of sugar significantly influenced the microbial population structure at the genus level in the cecum. The results showed that the content of *Lactobacillus* in jejunum of the maize flour group was significantly lower than that of the 3 sugar treatment groups, which suggested that the abundance of *Lactobacillus* in jejunum increased significantly after forced-feeding adding sugar. *Lactobacillus* is a normal beneficial bacterium in the gastrointestinal tract of mammals, which can stimulate the body to produce immunoglobulin and enhance immunity (Zhao et al., 2013; Castaneda et al., 2020). The results showed that the content of serum lactic acid in the 3 sugar treatment groups was higher than that in the maize flour group (19.39, 11.37, 14.98 vs 6.96 for the content of lactic acid in the serum,  $P < 0.05$ ) (Figure 1C; Supplementary S-Table 5). Lactic acid is known as the main metabolite of intestinal *Lactobacillus*. Liu et al. (2016b) reported that lactic acid content in the serum and intestine of overfed geese was significantly higher than that in the control group. Therefore, it is speculated that the lactic acid could have a protective effect against the formation of goose fatty liver. In overfed goose, complement system that mediates inflammation was suppressed due to the increasing levels of blood lactic acid produced by the enriched *Lactobacillus*, and *TNF $\alpha$*  was suppressed by the lactic acid via HNF1 $\alpha$ /C5 pathway (Liu et al., 2016b). Thus, “*Lactobacillus*-Lactic acid” also has been regarded as the protective mechanism against inflammation in hepatic steatosis in waterfowl. The geese after forced feeding adding sugar had a greater susceptibility to hepatic steatosis than the overfed geese of the maize flour group. Therefore, it is assumed that protective mechanism plays a great role in the response to forced-feeding for *foie gras* production. The protective mechanism is probably maximized in the fructose group and sucrose group, which may be the reason why the *foie gras* weight of the fructose group and sucrose group was higher than other groups.

## CONCLUSIONS

Glucose, fructose and sucrose can all induce the lipid deposition in overfed goose live, and the inducement by fructose and sucrose is higher. Forced-feeding

with diet adding sugar (especially fructose and sucrose) induced stronger digestion and absorption capacity, and caused *Lactobacillus* enriched in the gut. Gut microbiota (predominantly *Firmicutes*) contributed to shaping the landscape via the gut-liver axis (especially fructose and sucrose). This experiment is only a preliminary exploration of the regulatory mechanism of lipid deposition by different types of sugar in goose liver from gut physiology and gut microbiota. However, the fatty liver formation mechanism induced by sugar in waterfowl remains relatively complex, for which a further study on the mechanism of liver steatosis is deemed necessary. For example, the relationship between lipid deposition, insulin resistance and endoplasmic reticulum stress in the hepatocytic steatosis induced by different sugar types has not been clearly elucidated.

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## DISCLOSURES

The authors declare no conflicts of interest.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.psj.2021.101208](https://doi.org/10.1016/j.psj.2021.101208).

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