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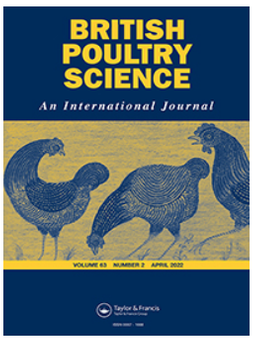

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Dietary amylose and amylopectin ratio changes starch digestion and intestinal microbiota diversity in goslings

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ABSTRACT

1. Research has confirmed that amylopectin (AP) is more easily digested than amylose (AM) because AP polymers have more intramolecular hydrogen bonds and less surface area. Studying the relationship

between the amylose:amylopectin (AM:AP) ratio and intestine digestion in goslings can provide useful information for effective utilisation of starch.

2. A total of 288 healthy male Jiangnan White Goslings, aged three days old, were randomly allotted to four groups, which included six pen replicates per treatment with 12 goslings per replicate. Four diets were formulated with maize, long-grained rice and glutinous rice as starch sources, with AM:AP ratios of 0.12, 0.23, 0.34, and 0.45. *In vitro* starch digestion of the four diets was measured, as well as the effect of AM:AP ratio on growth performance, serum amino-acid concentration and intestinal microbiota diversity of goslings.

3. In terms of *in vitro* starch digestion, the increase in dietary AM:AP ratio resulted in a decrease followed by an increase in both rapidly and slowly digestible starch. The glucose release rate at an AM:AP ratio of 0.34 showed a steady upward trend.

4. The *in vivo* study showed that increasing the AM:AP ratio resulted in a quadratic increase in body weight (BW) and average daily feed intake (ADFI; $P<0.05$). Goslings fed diets with an AM:AP ratio of 0.34 had lower ($P<0.05$) histidine and valine serum concentrations compared with the other three starch sources. Higher AM was beneficial to jejunal microbial and diversity. The species colonisation level of the jejunum microbiota samples at an AM:AP ratio of 0.34 was higher than that in the other groups.

5. The results indicated that diets with an AM:AP ratio of 0.34 improved the growth performance and intestinal microbiota diversity of goslings. This may have been due to the higher level of resistant starch in amylose, which resulted in a slow release of intestinal glucose that acted as a substrate for the microbial species, thus providing conditions that were more conducive to growth.

KEYWORDS: AM:AP ratio; starch digestion; microbiota; goslings

INTRODUCTION

Starch is the most common source of dietary energy in poultry feed and is composed of glucose monomers linked by α-glycosidic bonds. The digestibility of starch mainly depends on its physical structure (including its granule organization), which is especially affected by the ratio of amylose (AM) and amylopectin (AP) (Li *et al.,* 2015; Itani and Svihus 2019). AP is more easily digested than AM because AP polymers have more intramolecular hydrogen bonds, increased branching and lower surface area (Yin *et*

al., 2010). The rate of starch digestion can be classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). AP can be quickly degraded by α-amylase in the gastrointestinal tract of monogastric animals, mainly classified as RDS. The AM cannot be degraded by α-amylase in small intestine or is only degraded very slowly, due to its linear structure, and hence is mainly digested in the end of the ileum and in the large intestine, and is classified as SDS (Englyst *et al.,* 1992). The AM:AP ratio is associated with the digestibility of starch in the gastrointestinal tract (Högberg *et al.,* 2004). However, few studies have investigated the effect of AM:AP ratio on poultry intestinal health. Research has suggested that starch affects the metabolism of protein (Regmi *et al.,* 2011). There is growing interest in starch and carbohydrate nutrition to enhance the efficiency of animal production.

It was inferred that the AM:AP ratio could affect the animal gastrointestinal digestibility of starch in cereals, which, in turn, might affect intestinal health of animals (Choct and Annison, 1992). Notably, the AM:AP ratio and the physical structure are responsible for the digestibility of starch (Regmi *et al.,* 2011). Moreover, Haenen *et al.,* (2013) found that diets containing RS were completely degraded in the caecum and significantly increased the short-chain fatty acid (SCFA) concentration in the cecum and colon. RS plays a key role in protecting gut function and reducing glycaemic concentration in humans and mammals. The AM:AP ratio differs greatly with cereals. Higher AM:AP ratio in the diet might have a detrimental effect on growth performance, partly due to the high AM content which reduces nutrient digestibility (Haenen *et al.,* 2013).

However, the lack of information remains an obstacle to understanding the mechanisms of AM:AP ratio on poultry digestibility of goslings. The present study was conducted to evaluate the effects of AM:AP ratio on starch digestion (*in vitro*), growth performance, serum amino-acid concentration, distribution of sodium–glucose transporter-1 (SGLT-1) in the jejunum mucosa, and intestinal microbiota diversity in goslings.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee (IACUC) of the Yangzhou University Animal Experiments Ethics Committee approved the animal study proposal, permit number SYXK (Su) IACUC 2020-0021. All gosling experimental procedures were performed in accordance with the regulations for the

administration of affairs concerning experimental animals approved by the State Council of the People's Republic of China.

Experiment 1: In vitro starch digestion

The aim of this procedure was to model a mixture of continuous digestion processes in different parts of the digestive tract of the goslings. Test tubes containing four feed samples, glass balls, digestive enzymes, and incubation buffers in a shaking water bath were used (37°C, three tubes per sample). After each of the 12 incubation time-points (0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180 and 240 min), aliquots were removed from the tubes and the amount of glucose was determined using D-Glucose (GOPOD Format) Assay Kit (Megazyme International Ireland, Wicklow, Ireland) (McCleary *et al.,* 1991). The starch digestion coefficient was calculated for each incubation time. The RDS and SDS fractions for goslings were calculated from the starch digestion as measured within 20 min and from 20 min to 2 h, Those could not be digested after more than 2 h were considered RS. Starch digestibility was measured using the in vitro Englyst test to classify the RDS, SDS and RS (Englyst *et al.,* 1992). The AM and AP in the diets was determined according to the colorimetric analysis method. All kits were purchased from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China).

Experiment 2: In vivo study

The study was conducted using 288 healthy, male, Jiangnan White Goslings aged 3 d that were obtained from one commercial hatchery (Changzhou Four Seasons Poultry Industry Co., Ltd., Jintan, China). The Jiangnan White goose is a medium-sized goose species from China with characteristics that include stable performance, a high rate of reproduction, rapid early growth, good meat quality and a strong tolerance and adaptability to coarse feed. Jiangnan White Goslings are suitable for feeding in different regions and seasons, and they can maintain a higher production level under off-season technology.

The goslings were all of similar body weight (BW) and were randomly allocated to one of four diet groups that included six pen replicates per treatment, with 12 goslings per pen. Starch composition of the maize, long-grained rice and glutinous rice are shown in Table 1.

Table 1 here

Four diets were formulated with maize, long-grained rice, and glutinous rice as starch sources, with AM:AP ratios of 0.12, 0.23, 0.34, and 0.45 (Table 2). All the diets were formulated to provide an adequate concentration of all nutrients required by the goslings with similar nutrient and total starch content (NRC, 1994). The geese were fed in separate plastic-floored pens with 2 cm^2 square mesh flooring, which were laid 70 cm above the ground. Faeces under floor were cleaned twice a day with an automatic waste removal belt. Feed was provided in pan troughs and water was available from a nipple drinker. All goslings had free access to feed and water through the trial. The room temperature was approximately 24°C, and no extra heat was provided. The goslings were maintained under natural daylight after 21 d of age. The relative humidity was 65.5%±5.0% and the space allocation was 0.5 m^2/bird.

Table 2 here

Sample collection and determination

In Experiment 2, at 3 and 28 d of age, all birds were weighed measure BW and average daily gain (ADG). Feed intake (FI) by pen was recorded on a daily basis to determine average daily feed intake (ADFI). Feed conversion ratio were calculated at the end of the experiment, and mortality was recorded as it occurred.

Clinical blood parameters

In Experiment 2, at 28 d of age, six goslings from each group were randomly selected, and blood samples were taken. A butterfly needle, with a luer adapter, was inserted into the wing veins of the goslings, and 3 ml of blood was collected into a negative-pressure blood collection vessel. Each blood sample was centrifuged for 10 min at 4500 r/min to obtain serum for subsequent amino-acid (AA) analysis.

The AA content of the serum was determined using a Waters ion-exchange high-performance liquid chromatography (HPLC) system (GC-9A, Shimadzu, Japan) according to AOAC method 994.12. Samples were hydrolysed using 6 M HCL at 110°C for 24 h, and Met and cysteine (Cys) were determined as Met sulphone and Cys acid, respectively, after oxidation with performic acid according to the AOAC method (AOAC, 1990). The following amino acids (AAs) were determined: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe) and lysine (Lys).

Immunohistochemistry

The intestinal segments of the six goslings were flushed and then fixed in 4% paraformaldehyde solution for analysis of distribution of the sodium-glucose co-transporter SGLT-1 in the jejunum mucosa. After 24 h of fixing, paraffin sections were pretreated with 0.03% pronase in 0.05 mol/l Tris-HCl buffer (pH 7.6) for 3 min, rinsed in PBS, treated with 1.5% normal rabbit serum in PBS for 20 min, incubated with mouse anti-BrdU antibody for 14 h at 4 °C, washed in PBS, incubated with biotinylated secondary antibody (Nanjing Baxter biological Co., Ltd, Nanjing, China) for 40 min. Finally, BrdU-labelled cells were identified with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) and counterstained with Mayer's haematoxylin or methyl green (Tiangen Biochemical Technology Co., Ltd., Beijing, China).

16S rRNA gene analysis

In Experiment 2, at d 28 of age, five goslings from each group were selected for 16S rRNA gene analysis. The intestinal tract was removed, and the contents of the jejunum were rapidly squeezed directly into freezing tubes, frozen in liquid nitrogen, and stored at -80°C. A FastDNATM Spin kit for samples originating from the jejunum was used to extract DNA (Sangon Co., Ltd, Shanghai, China). Sequences of the 16S rRNA gene variable 3 (V3–V4) region were amplified according the method described by Wen *et al.,* (2020) and assessed on the Qubit@ 2.0 Fluorometer (Thermo Fisher Technology Co., Ltd, Shanghai, China) and Agilent Bioanalyzer 2100 system (Novogene Co., Ltd, Beijing, China). The library was sequenced on an Illumina NovaSeq platform, and 250 bp paired-end reads were generated (Edgar *et al.,* 2013). Operational taxonomic units (OTUs) were picked with abundant OTU+ (29) with a clustering threshold of 97% sequence similarity. Sequence analysis were performed using Uparse software. OTU abundance information was normalised using a standard sequence number, which corresponded to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity were performed using normalised output data. Total sum scaling was employed to account for variation in sequence depth with the top 10 most abundant taxa assessed for differential abundance for genus-level statistics.

Statistical analysis

The experimental data was preliminarily sorted using Excel 2020, and then analysed with SPSS 20.0 software (Ver. 20.0 for Windows, SPSS, Inc., Chicago, IL, USA). All the data were analysed according to the randomised block design ANOVA, and one-way analysis of variance, linear and quadratic relationships were performed. Significant differences between the treatments were determined at $P<0.05$

using the Tukey test. The results were expressed as mean values and the standard error of the mean. The statistical analysis for 16S rRNA sequencing data was performed in R (version 3.5.2). Total sum scaling normalisation was performed prior to assessing differences in taxa at the genus level within treatment groups, using a two-sided unpaired permutation t-test and corrected for multiple comparisons using the Benjamini-Hochberg procedure (FDR).

RESULTS

In vitro starch digestion of diets with different AM:AP ratios

An increase in dietary AM:AP ratio resulted in a decrease and then an increase in RDS and SDS ($P<0.05$), However, RS tended to increase and then decrease with increasing dietary AM:AP ratios (Table 3). The RDS and SDS in the diet with an AM:AP ratio of 0.34 were significantly lower than those with AM:AP ratios of 0.12, 0.23 and 0.45 ($P<0.05$). Conversely, the content of RS in the diet at an AM:AP ratio of 0.34 was significantly higher than that in the other diets ($P<0.05$).

Table 3 here

Four AM:AP ratios diets showed different glucose release patterns during *in vitro* starch digestion (Figure 1). The diet with an AM:AP ratio of 0.34 had the lowest glucose release rate when incubated up to 15 min. However, between 30 min and 120 min, the glucose release rate showed an upward trend, indicating the release of glucose at a steadier rate.

Fig 1 here

Growth performance

There was no mortality during the experiment. The BW, ADG, and ADFI of goslings at 28 d of age were significantly affected by dietary AM:AP ratios. Increasing dietary AM:AP ratio resulted in a quadratic increase in BW and ADFI in goslings ($P<0.05$; Table 4). The BW of goslings that consumed the feed with AM:AP ratio of 0.34 was significantly higher than that seen in the other three groups ($P<0.05$).

Table 4 here

Amino-acid concentration in plasma

The concentration of most amino acids in the plasma was not affected by the AM:AP ratio ($P<0.05$; Table 5). Goslings fed the diet with AM:AP ratio of 0.34 had lower ($P<0.05$) histidine and valine content in serum compared with the other three diet groups.

Table 5 here

Distribution of SGLT-1 in the jejunum mucosa

There was no significant effect of AM:AP ratio on SGLT-1 expression, but the distribution of SGLT-1 in goslings fed a diet at an AM:AP ratio of 0.34 exhibited a small, but not significant, decrease compared to samples from the other bird groups ($P>0.05$; Figure 2).

Fig 2 here

Microbial profile in the jejunum digesta

A total of 1,991,967 sequence reads were recovered from 20 samples from four treatments with five replicates (Figure 3). The relationships among bacterial communities from different diet treatments were represented by principal component analysis (PCOA).

Fig 3 here

Figure 4 shows the relative abundance of the dominant bacterial communities in the jejunum digesta of 28 d old goslings from each group, at the phylum (a), family (b) and genus (c) levels. At the phylum level, Firmicutes, Proteobacteria, and Bacteroidota in the jejunum digesta were dominant in all treatment groups.

Fig 4 here

The relative abundances of Firmicutes, Proteobacteria, and Bacteroidota in the jejunal microbiota ranged from 65% to 85%, 2% to 12%, and 0.4% to 6%, respectively (Table 6). Figure 5 shows the hierarchically clustered heatmap analysis of the highly represented bacterial taxa found in the jejunum digesta communities of 28 d old goslings. Bacteria in the phylum Proteobacteria exhibited higher abundance in the jejunum samples of goslings fed a diet with an AM:AP ratio of 0.34 than those fed diets with AM:AP ratios of 0.12 and 0.45 ($P<0.05$). Meanwhile, the AM:AP ratio resulted in a quadratic increase in the abundance of Bacteroidota in the jejunum microbiota ($P<0.05$), whereas the abundance of Firmicutes in the jejunum samples of the goslings fed a diet at an AM:AP ratio of 0.34 tended to be lower than that in the other diet groups ($P>0.05$).

Fig 5 and Tables 6 and 7 here

Table 6 shows the number of OTUs and the sample richness and diversity of the jejunum microbiota. Good's coverage index was almost constant (≥0.990) among the four treatments, indicating high coverage. The samples from goslings fed a diet with an AM:AP ratio of 0.34 had a higher number (773.4) of OTUs than those from the other starch groups. The bacterial communities were evaluated using Shannon and Simpson indices (Kim *et al.,* 2017). The Simpson indices of the birds in the jejunum microbiota increased with AM:AP ratio, and the greatest value was observed in the birds fed a diet at an AM:AP ratio of 0.34. The Chao 1 index reflected the richness of the bacterial community, ranging from 656 to 939 across all treatments. However, according to the sequence metric and sample richness, there was no significant difference among all treatments in Chao 1 and abundance-based coverage estimator (ACE) indices.

The abundance of Proteobacteria in the jejunum microbiota increased quadratic with the increase of the AM:AP ratio ($P<0.05$), while the Bacteroidota showed the opposite result (Table 7). The primary bacteria of the birds fed a diet at an AM:AP ratio of 0.12 were Proteobacteria and Cyanobacteria. However, the bacteria in the jejunum of goslings fed a diet with an AM:AP ratio of 0.23 shifted towards Proteobacteria and Fusobacteriota as the main strains. In contrast to other groups, bacteria in the jejunum of the goslings fed a dietary AM:AP ratio of 0.34 had higher numbers of Proteobacteria, Verrucomicrobiota, Firmicutes, and Bacteroidota. The bacteria in the jejunum of the goslings fed a diet with an AM:AP ratio of 0.45 had more Proteobacteria and Firmicutes present. The primary genera of bacteria in the jejunum of the goslings

fed a diet with an AM:AP ratio of 0.23 were *Romboutsia*, *Streptococcus*, *Enterococcus*, and *Turicibacter* spp. Additionally, the number of Romboutsia and Streptococcus decreased in the jejunum of goslings fed a diet with an AM:AP ratio of 0.34. In contrast with the other groups, samples from goslings fed a diet with AM:AP ratios of 0.12 and 0.45 were enriched in *Romboutsia* and *Turicibacter* spp.

DISCUSSION

Many reports have confirmed that AP is more easily digested than AM because the latter polymers have more intramolecular hydrogen bonds, less branching and hence lower surface area (Li *et al.,* 2015). The AM contains more slowly digestible starch, which is mainly digested in the hindgut of the intestine in goslings (Yin *et al.,* 2010; Tayade *et al.,* 2019). In the current study, *in vitro* digestibility of RS in the diets increased and then decreased with the rise in AM:AP ratios. With an increase in AM, the diets had more RS, which was mainly digested in the hindgut of the gastrointestinal tract. Other researchers have shown that the AM:AP ratio affects the formation of RS content, as well as *in vitro* digestibility of starch (Singh *et al.,* 2013; Venkataraman *et al.,* 2016). The results implied that the AM:AP ratio is an important factor facilitating the formation of RS, which might be an important indicator in starch digestibility.

The absorption and the utilisation of glucose in the small intestine is necessary for goslings to achieve their growth potential. After resistant starch is digested and utilised by microorganisms in the hindgut, goslings can slowly and continuously release glucose in the intestine to provide energy for vital activities after being fed a diet at an AM:AP ratio of 0.34. This reduces the amount of AAs oxidised in the intestine for energy supply, enabling AAs to be absorbed for tissue protein synthesis, thus improving the utilisation of nitrogen.

Many studies have shown that different sources of starch affect the growth performance of animals (Del Alamo *et al.,* 2009; Itani *et al.,* 2021). It has been suggested that feeding SDS may improve FCR of birds. Researchers have hypothesised that RDS would not provide enough energy to the intestinal cells in the form of glucose. Consequently, more amino acids could be used as energy for enterocytes rather than for muscle growth. However, due to the longer duration of glucose supply, SDS may spare amino acid oxidation which can improve birds' performance.

The results of the current experiment confirmed this view, whereby BW and ADFI in goslings improved quadratically with rising AM:AP ratio. A higher AM:AP ratio caused SDS to increase gradually, along

with growth performance. Thus, feeding a diet with AM:AP ratio of 0.34 was beneficial to the growth performance of goslings. Sydenham *et al.* (2017) and Itani *et al.* (2021) reported that starch source significantly affected performance and ileal digestion in broiler chickens.

The trend of ADFI was consistent with BW. With an increase in feed intake and energy intake, it was logical that BW increased. So, it is plausible that the effect of starch on the growth performance of birds is related to feed intake.

There is an abundance of studies on plasma concentrations of AAs under various physiological or pathological conditions. However, it is often asserted that plasma AAs levels are difficult or even impossible to interpret, because they undergo various interorgan exchanges (Cynober 2015). Only a limited number of studies have addressed the effects of starch on AA concentration in plasma (Chrystal *et al.,* 2020). In the present study, His and Val content was significantly reduced in the serum of goslings fed a diet at an AM:AP ratio of 0.34. These results suggested that the metabolism of His and Val was significantly influenced by the AM:AP ratio. Further studies with a greater number of replicates are needed to confirm these findings.

The SGLT-1 is gradually expressed on the apical membrane during intestinal cell differentiation and is the major sugar transport system in mature intestinal cells (Cefalo *et al.,* 2019; Zhao *et al.,* 2020). Most glucose is co-absorbed with sodium from the gut *via* SGLT-1 transporters. SGLT-1 mRNA expression occurs in response to high dietary carbohydrate levels, although little information is available on the concentration required to stimulate SGLT-1 upregulation. In the present study, increasing AM:AP ratios had no significant effect on the immunohistochemical expression of SGLT-1 in the jejunum mucosa. However, there was a small unsignificant decrease in the group fed a diet with an AM:AP ratio of 0.34. The SGLT-1 data were similar to those from the study of Yin *et al.* (2019) in broiler chickens. According to Yin *et al.,* (2019), waxy rice in low-protein diets generated the highest jejunal SGLT-1 expression, as it is a source of rapidly digested starch. This phenomenon may reflect that the relative amounts of starch and glucose along the small intestine are dependent on the starch source and its digestion rate.

The intestinal microbiota has multiple functions, including carbohydrate metabolism, fibre degradation, and immune maintenance. However, these functions may be influenced by diet, genotype, feeding practices and disease challenge. Thus, changes in the intestinal environment may affect the dynamics of microbial populations in the gut and, thus, affect function. The source and structure of dietary starch could

alter the microbial profile. According to the hierarchically clustered heatmap analysis, there were significant differences in the microorganisms involved in carbohydrate metabolism. These results revealed that the AM:AP ratio mainly affected glucose metabolism-related pathways, which was consistent with the results for jejunal SGLT-1 expression.

In poultry, Firmicutes, Bacteroidetes and Proteobacteria are dominant at the phylum level (Jami and Mizrahi 2012). Bacteroidetes can use polysaccharides to produce acetic and propionic acid, while an increase in the relative abundance of Proteobacteria leads to an imbalance of the intestinal flora and can cause intestinal inflammation (Shin *et al.,* 2015). In the current study, Firmicutes, Proteobacteria and Bacteroidota in the jejunum digesta were dominant across all treatment groups (Figure 3), which was consistent with the above results. It was found that the abundance of Bacteroidetes and Proteobacteria in the jejunum of the goslings increased linearly with the increase in AM:AP ratio. Tremaroli and Backhed (2012) reported that Bacteroidetes can degrade high-molecular-weight compounds (carbohydrates and proteins) in the intestine, helping the host to acquire more nutrients from the diet. Bacteroidetes have a wide range of mechanisms that enable them to use the complex polysaccharides present in the gut as a source of carbon and energy (McKee *et al.,* 2021), and the higher content of AM feed released by these bacteria can provide nutrition and confer properties that are beneficial to the host. Therefore, it was hypothesised that the presence of large numbers of Bacteroidetes in goslings fed a diet with a higher AM:AP ratio may help in adapting to these diets and improving nutrient digestibility (Wang *et al.,* 2018).

Obviously, at the genus level, the diversity of the intestinal microbiota may be related to the proportion of undigestible components in the diet. Previous experiments found that increased AM content could increase the abundance of Turicibacter and Ruminococcus in the hind intestine (Bretin *et al.,* 2018), and similar results were presented in the current experiment. The number of Romboutsia and Streptococcus decreased in the jejunum of goslings fed a diet with an AM:AP ratio of 0.34, whereas Romboutsia and Turicibacter increased upon exposure to a diet with an AM:AP ratio of 0.45. Turicibacter is considered pathogenic because it increases during enteritis, benefits from the existing inflammatory response in barrier tissues and acts as an opportunistic organism (Cuív *et al.,* 2011; Shin *et al.,* 2015). The decreased growth performance of the goslings fed a diet with an AM:AP ratio of 0.45 may have been related to the increase in Turicibacter in the intestinal flora. Obviously, changes in intestinal microbial diversity may distort homeostasis and affect the immune status of the host.

In conclusion, the AM:AP ratio significantly affected *in vitro* starch digestion, whereas the glucose release rate in goslings fed a diet at an AM:AP ratio of 0.34 showed a steady upward trend. Supplementation with diets with various AM:AP ratios modulated the species colonisation levels in the jejunal microbiota. Diets with an AM:AP ratio of 0.34 improved growth performance and intestinal microbiota diversity in young goslings This may have been due to the high content of resistant starch in AM, resulting in slow release of glucose that increased the microbial species and established conditions more conducive to growth. This may have also been related to the reduction in pathogenic bacteria in the intestine, especially Turicibacter.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Table 1 Starch composition of the maize, long-grained rice and glutinous rice (% dry matter basis).

Treatments	Maize	Long-grained rice	Glutinous rice
Total starch	65.4	74.7	78.3
amylose	16.6	23.4	1.6
amylopectin	48.8	51.3	76.7
amylose/amylopectin	0.34	0.46	0.02

Table 2. Composition and nutrient levels of the basal diets of goslings (dry basis).

Ingredients (g/kg)	AM:AP ratio			
	0.12	0.23	0.34	0.45
Maize	222.1	440.2	631.2	31.0
Long-grained rice	/	/	/	525.5
Glutinous rice	341.7	159.5	/	/
Soybean meal	295.2	292.1	290.0	294.5
Rice husk	26.8	26.3	17.7	35.9
Wheat bran	73.7	41.4	20.6	72.6
Calcium hydrogen phosphate	12.0	12.0	12.0	12.0
Limestone	10.5	10.5	10.5	10.5
Choline chloride	1.0	1.0	1.0	1.0
Sodium chloride	5.0	5.0	5.0	5.0
DL- methionine	2.0	2.0	2.0	2.0
Premix [a]	10.0	10.0	10.0	10.0
Total	1000.0	1000.0	1000.0	1000.0
Metabolisable energy [b] (MJ/kg)	11.46	11.50	11.55	11.30
Analysed nutrient concentration				
Crude protein (g/kg)	184.9	183.0	182.5	184.9
Crude fibre (g/kg)	42.1	42.1	42.1	42.1
Calcium (g/kg)	8.6	8.6	8.5	8.6
Total phosphorus (g/kg)	6.4	6.4	6.6	6.1
Available phosphorus (g/kg)	4.6	4.6	4.8	4.4
Methionine (g/kg)	5.0	4.9	4.8	5.1
Lysine (g/kg)	10.3	9.8	9.4	10.5
Methionine+Cysteine (g/kg)	7.0	7.1	7.2	7.0
Threonine (g/kg)	6.5	6.2	6.0	6.0
Total starch (g/kg)	412.8	412.8	412.8	412.8
AM (g/kg)	42.3	75.6	104.8	128.1
AP (g/kg)	370.5	337.2	308.0	284.7
AM: AP ratio	0.12	0.23	0.34	0.45

AM: Amylose; AP: amylopectin; AM: AP ratio: amylose: amylopectin ratio;

[a] One kilogram of premix contained Vitamin A, 9000,000 IU; Vitamin D, 300,000 IU; Vitamin E, 1,800 IU; Vitamin K, 150 mg; Vitamin B1, 90 mg; Vitamin B2, 800 mg; Vitamin B6, 320 mg; Vitamin B12, 1.2 mg; nicotinic acid, 4.5 g; pantothenic acid, 1100 mg; folic acid, 65 mg; biotin, 5 mg; Fe (as ferrous sulfate), 6 g; Cu (as copper sulfate),1 g; Mn (as manganese sulfate), 9.5 g; Zn (as zinc sulfate), 9 g; I (as potassium iodide), 50 mg; Se (as sodium selenite), 30 mg.

[b] The values were calculated from the ingredient apparent metabolizable energy (AME) values for chickens.

Table 3. In vitro starch digestion of diets with different amylose-amylopectin ratios[a,b,c].

Items[d]	AM:AP ratio				SEM	*P*-value		
	0.12	0.23	0.34	0.45		Between diets	Linear	Quadratic
RDS	8.13[a]	7.34[b]	6.86[c]	8.13[a]	0.168	<0.01	0.307	<0.01
SDS	8.13[a]	6.00[c]	4.21[d]	6.97[b]	0.447	<0.01	0.002	<0.01
RS	0.22[d]	3.70[b]	5.97[a]	1.95[c]	0.647	<0.01	<0.01	<0.01

[a] In the same row, values with different superscript letters indicate a significant difference ($P < 0.05$), while those with the same or no superscript letters indicate no significant difference ($P > 0.05$).

[b] Data were analyzed as a completely randomized block design with three replications in each treatment.

[c]AM:AP ratio: amylose-amylopectin ratio; RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch.

[d] Rapidly digestible starch (RDS) and slowly digestible starch (SDS) fractions for goslings were calculated from the starch digestion as measured within 20 min and from 20 min – 120 min. Those that could not be digested after more than 120 min were considered resistant starch (RS).

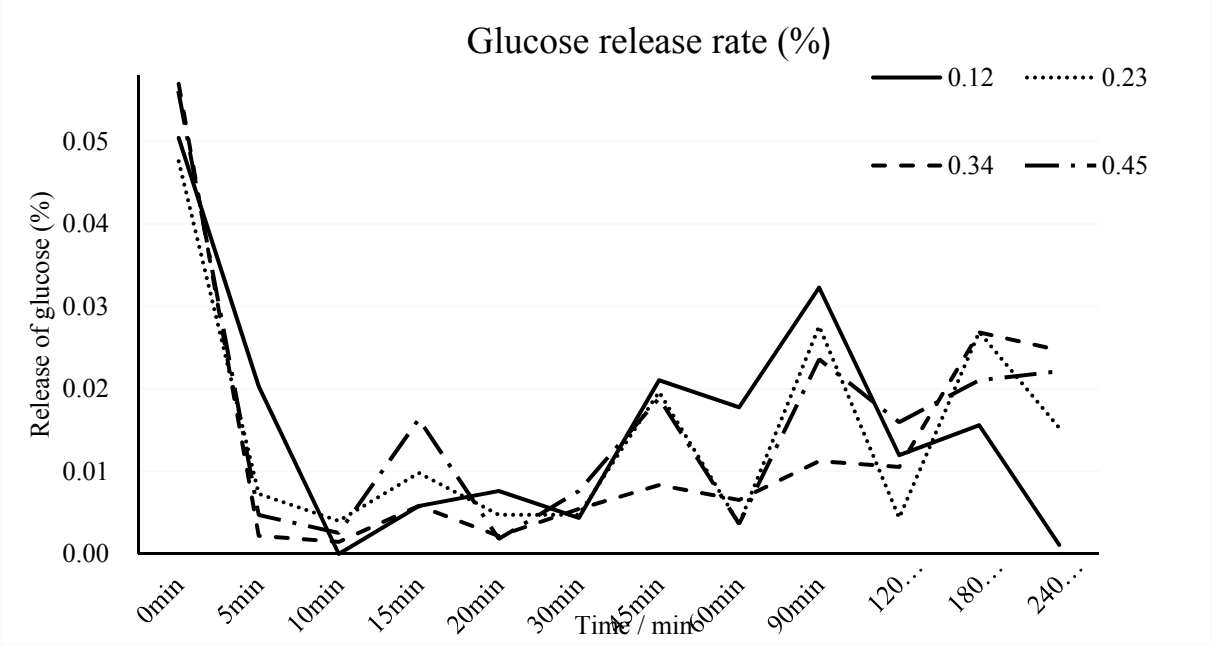


Figure 1. Glucose release patterns from diets with different amylose-amylopectin ratios[a].

[a] Data were analyzed as a completely randomized block design with six replications in each treatment.

Table 4. Effect of different amylose-amylopectin ratios on the growth performance of goslings from 3 to 28 days of age[a,b,c].

Items	AM:AP ratio				SEM	*P*-value		
	0.12	0.23	0.34	0.45		Between diets	Linear	Quadratic
3 d BW (g)	146.4	146.5	146.6	146.7	0.463	0.916	0.485	1.000
28 d BW (g)	1662[b]	1680[b]	1759[a]	1696[b]	26.9	0.011	0.048	0.046
ADG (g)	60.6[b]	61.3[b]	64.4[a]	61.9[b]	1.08	0.015	0.050	0.135
ADFI (g)	125.2[b]	125.5[b]	132.1[a]	127.7[a]	2.15	0.011	0.048	0.046
F/G	2.07	2.05	2.05	2.06	0.030	0.914	0.928	0.491

[a] In the same row, values with different superscript letters indicate a significant difference ($P < 0.05$), while those with the same or no superscript letters indicate no significant difference ($P > 0.05$).

[b] Each value represents the mean of six replicates.

[c] AM:AP ratio: amylose – amylopectin ratio; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; F/G, feed-to-gain ratio; SEM, standard error of the mean.

Table 5. Effect of different amylose-amylopectin ratios on the concentration of amino acids (μg/mL) in the plasma of goslings at 28 days of age[a,b,c]

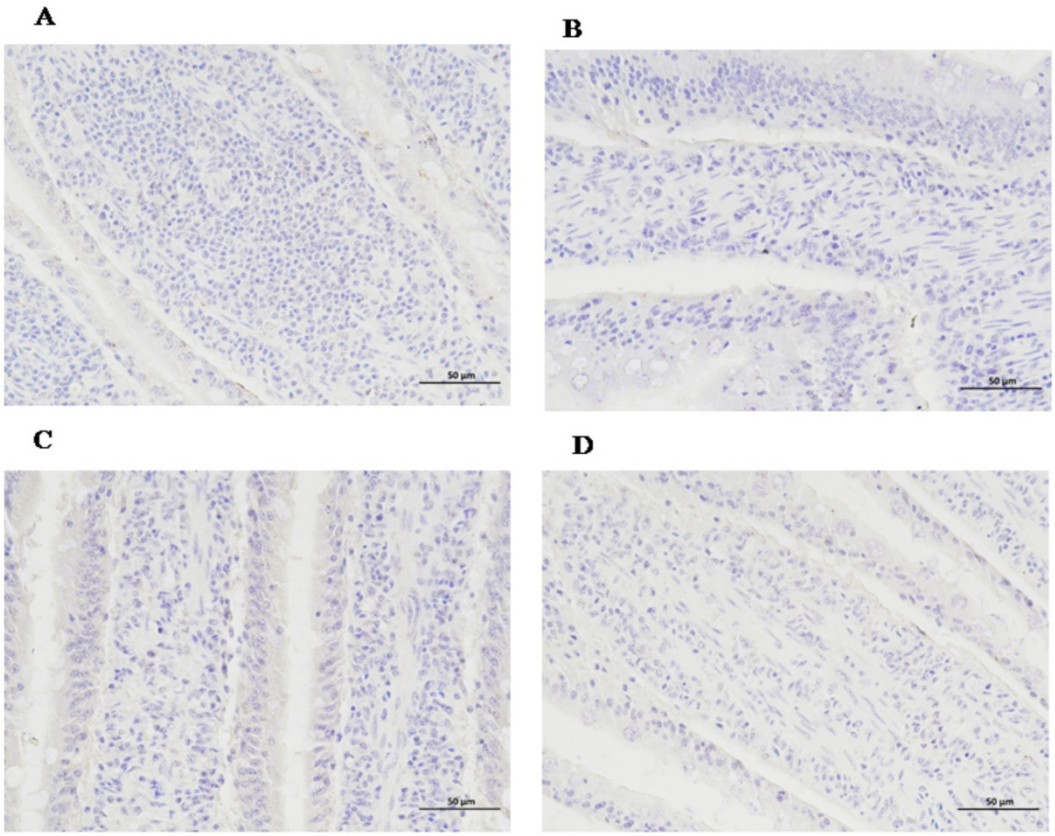
Items	AM:AP ratio				SEM	*P-value*		
	0.12	0.23	0.34	0.45		Between diets	Linear	Quadratic
Aspartic	13.09	15.49	9.75	9.94	1.241	0.312	0.179	0.655
Glutamic	4596	5233	5067	5166	264	0.847	0.541	0.634
Serine	113.8	112.4	98.7	124.6	4.97	0.347	0.672	0.180
Glycine	96.9	80.1	62.1	78.0	4.79	0.074	0.069	0.074
Histidine	50.0[a]	51.3[a]	37.5[b]	40.7[ab]	2.181	0.048	0.025	0.807
Argine	62.05	60.8	52.62	48.34	2.664	0.209	0.043	0.771
Threonine	45.49	48.24	35.9	43.25	2.819	0.475	0.462	0.691
Alanine	45.23	51.12	44.7	50.85	2.816	0.789	0.696	0.983
Proline	32.2	31	26.8	23.7	1.721	0.283	0.061	0.781
Tyrosine	99	118.27	109.56	113.07	5.732	0.707	0.536	0.515
Valine	17.81[a]	15.34[ab]	10.83[b]	12.41[ab]	1.028	0.065	0.021	0.286
Methionine	9.0	12.1	10.3	9.3	0.637	0.326	0.883	0.118
Isoleucine	32.31	32.99	25.62	27.46	1.796	0.406	0.187	0.874
Leucine	29.6	30.7	23.2	24.1	1.718	0.310	0.127	0.981
Phenylalanine	12.4	14.8	11.8	12.6	0.562	0.268	0.662	0.493
Lysine	107.2	121.9	118.4	122.3	6.034	0.818	0.471	0.673

[a] In the same row, values with different superscript letters indicate a significant difference ($P < 0.05$), while those with the same or no superscript letters indicate no significant difference ($P > 0.05$).

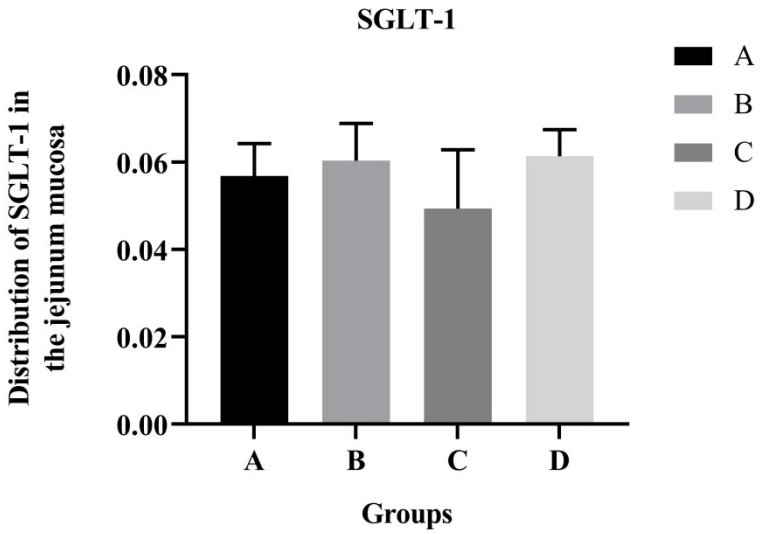
[b] Each value represents the mean of six replicates.

[c] Asp, aspartic acid; Glu, glutamic acid; Ser, serine; Gly, glycine; His, histidine; Arg, arginine; Thr, threonine; Ala, alanine; Pro, proline; Tyr, tyrosine; Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Lys, lysine; SEM, standard error of the mean.

(1)



(2)



FigurFigure 2. Influences of dietary AM:AP ratio on distribution of SGLT-1 in the jejunum mucosa samples of 28-day-old goslings. Each value represents the mean of six replicates. (1) Light microscopy images showed SGLT-1 protein by immumofluorescence staining. The AM:AP ratios of each diet were (A) 0.12, (B) 0.23, (C) 0.34, and (D) 0.45. (2) Protein levels of distribution of SGLT-1 in the jejunum mucosa. Values are mean with their standard errors. Labelled means without a common letter differ, $P < 0.05$ (one-way ANOVA, Tukey test).

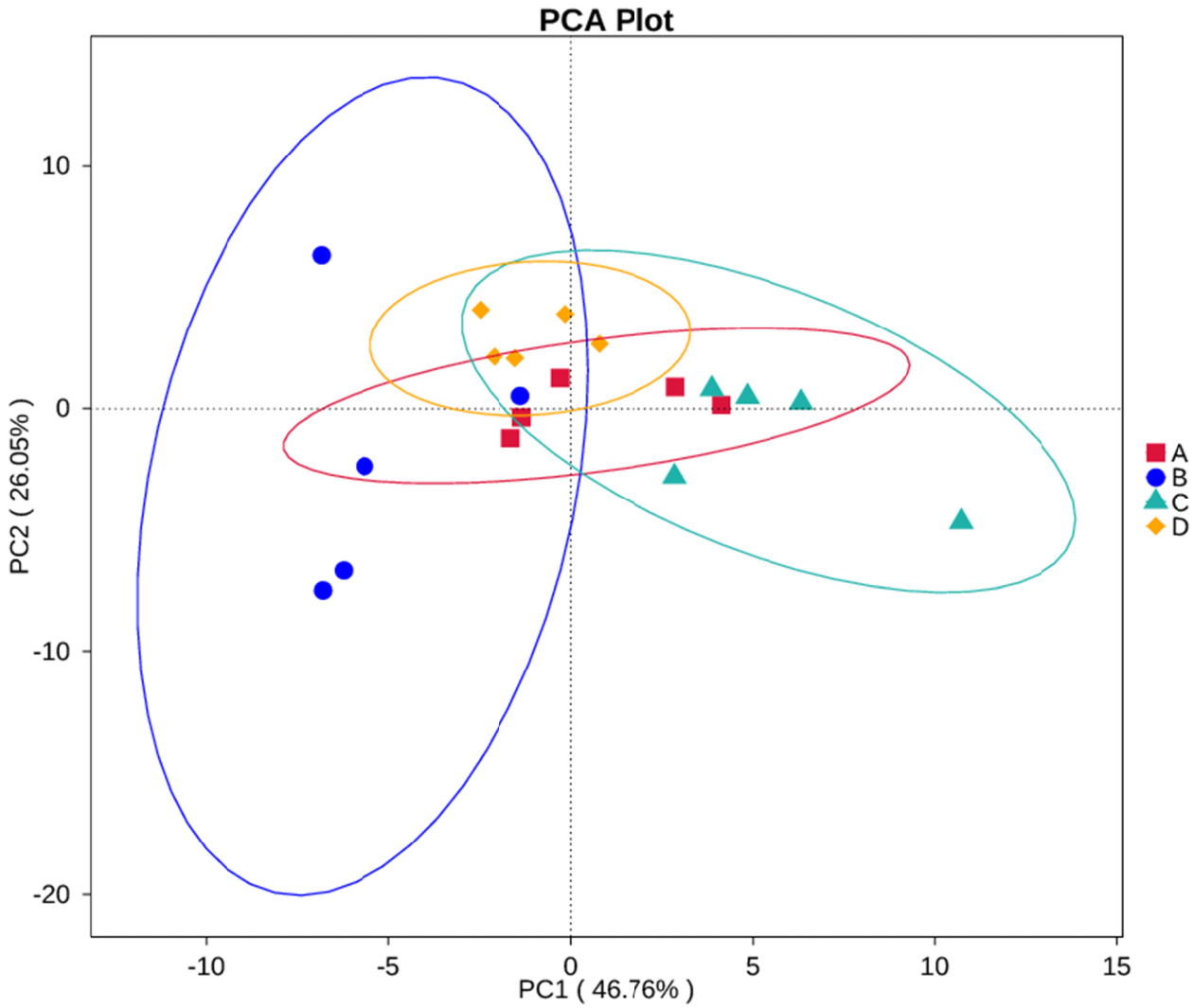


Figure 3. Principal component analysis (PCOA) of bacterial communities in jejunum samples of 28 day old goslings based on the abundance statistics for functional annotation. Circles are drawn around the microbiota of the same treatment. The PCOA score scatterplot depicts the variance in fingerprints derived from different bacterial communities. The AM:AP ratios of each diet were (A) 0.12, (B) 0.23, (C) 0.34, and (D) 0.45.

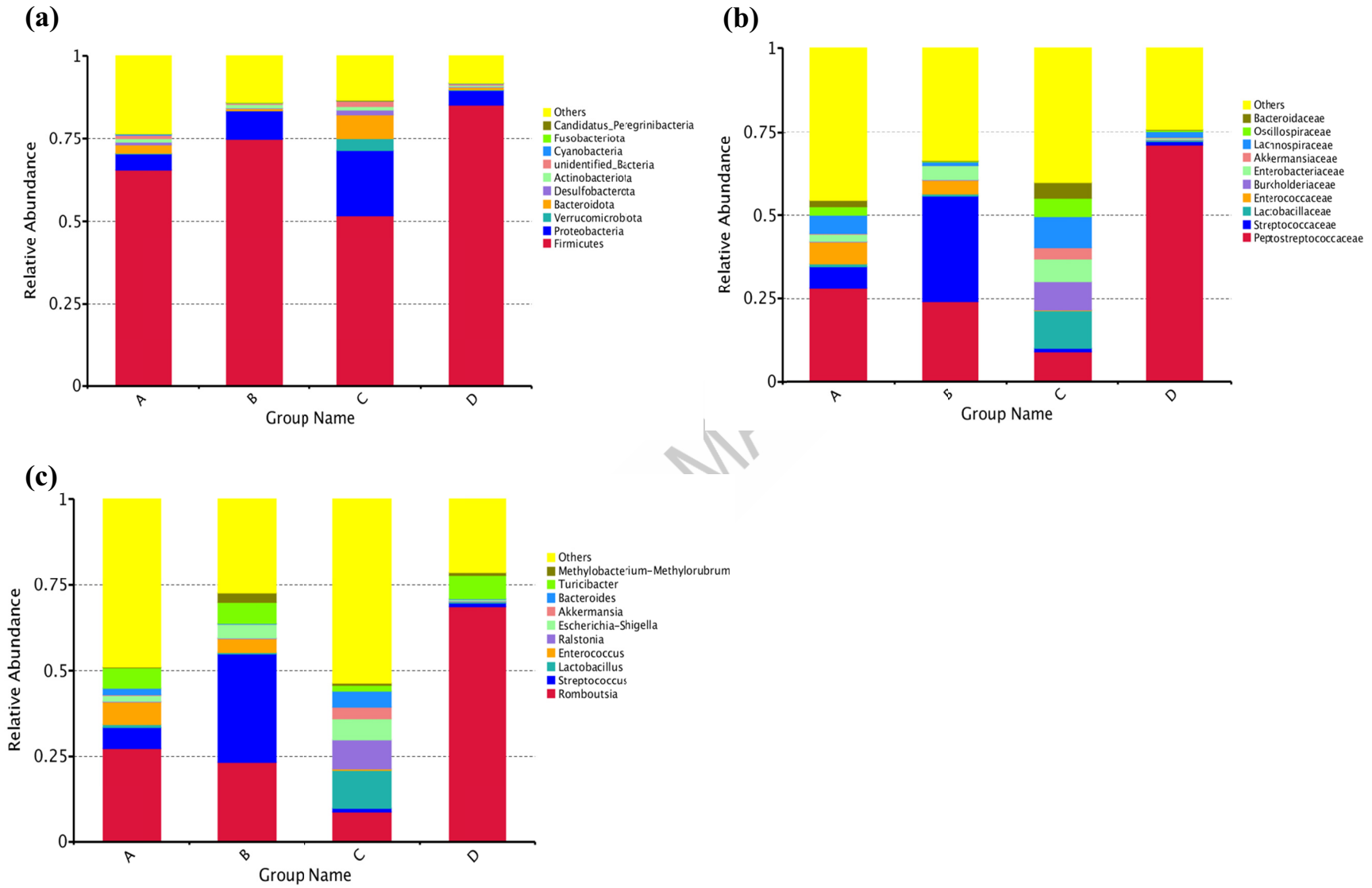

(a)
Relative Abundance
1
0.75
0.5
0.25
0
A
B
C
D
Group Name
Others
Candidatus_Peregrinibacteria
Fusobacteriota
Cyanobacteria
unidentified_Bacteria
Actinobacteriota
Desulfobacterota
Bacteroidota
Verrucomicrobiota
Proteobacteria
Firmicutes
(b)
Relative Abundance
Group Name
Others
Bacteroidaceae
Oscillospiraceae
Lachnospiraceae
Akkermansiaceae
Enterobacteriaceae
Burkholderiaceae
Enterococcaceae
Lactobacillaceae
Streptococcaceae
Peptostreptococcaceae
(c)
Relative Abundance
Group Name
Others
Methylobacterium-Methylorubrum
Turicibacter
Bacteroides
Akkermansia
Escherichia-Shigella
Ralstonia
Enterococcus
Lactobacillus
Streptococcus
Romboutsia

Figure 4. Relative abundance of the dominant bacterial communities in the jejunum digesta of 28 day old goslings at the phylum (a), family (b), and genus (c) levels. Each bar represents the relative abundance of each treatment. Each color represents a particular bacterial phylum. The amylose-amylopectin ratios of each diet were (A) 0.12, (B) 0.23, (C) 0.34, and (D) 0.45.

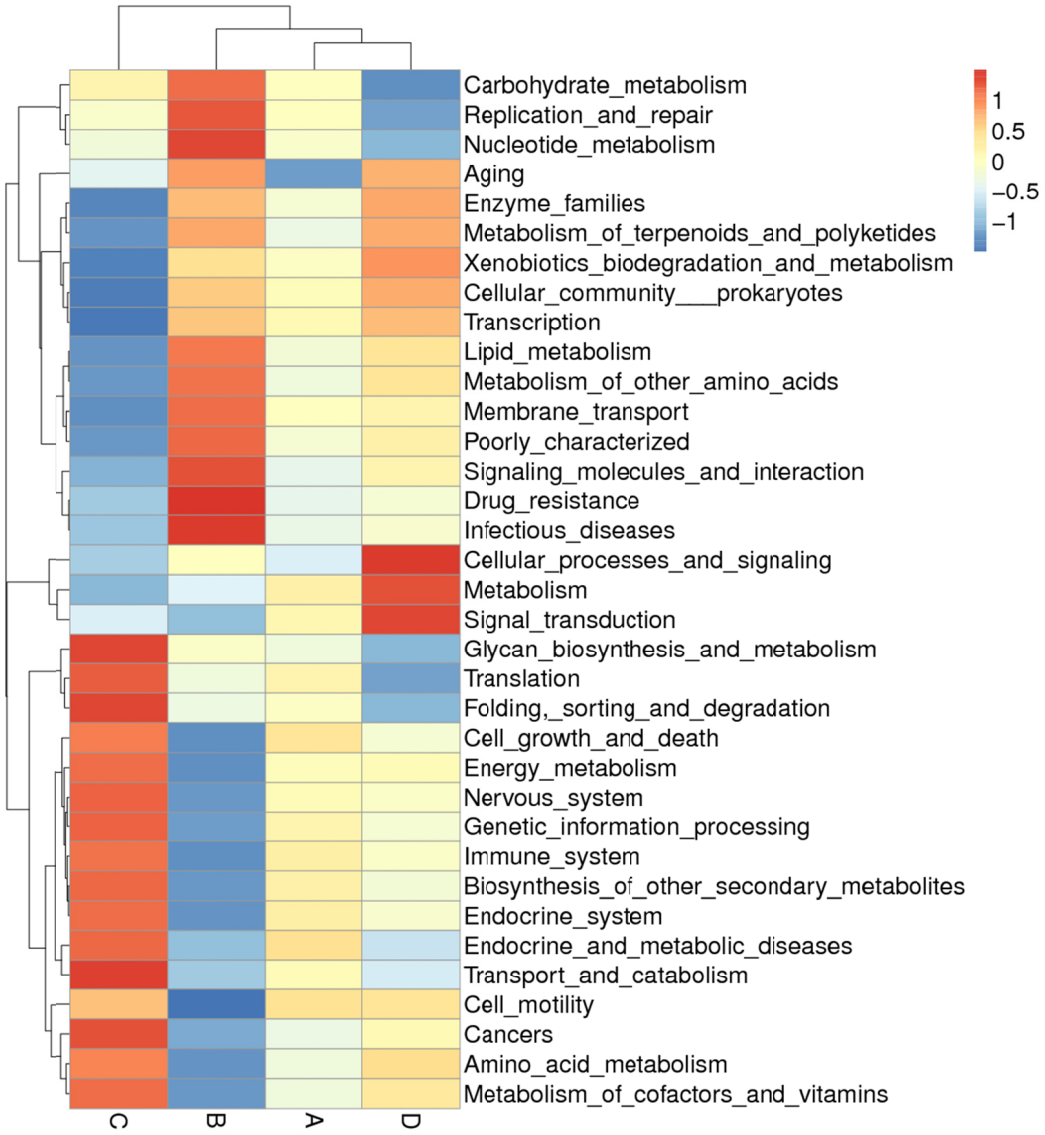


Figure 5. Hierarchically clustered heatmap analysis of the highly represented bacterial taxa found in the jejunum digesta communities of 28 day old goslings (at the phylum level). The relative percentages (%) of bacterial genera are indicated by different color intensities according to the legend at the top of the figure. The names of bacteria are listed on the right side of the heat map, and the tree diagrams at the top and left of the figure show the phylogenetic relationships between treatments and genera. The amylose－amylopectin ratios of each diet were (A) 0.12, (B) 0.23, (C) 0.34, and (D) 0.45.

Table 6. Differences in jejunal microbial species richness and diversity indices per treatment of goslings at 28 days of age (phylum level) [a,b].

Item	AM:AP ratio				SEM	*P*-value		
	0.12	0.23	0.34	0.45		Between diets	Linear	Quadratic
OTUs	585.80	462.20	773.40	543.40	181.086	0.393	0.752	0.683

Shannon	4.25	3.05	5.18	2.56	0.933	0.051	0.336	0.299
Simpson	0.75[a]	0.65[a]	0.88[a]	0.47[b]	0.130	0.044	0.155	0.118
Chao1	701.41	616.88	938.23	656.57	219.589	0.481	0.791	0.535
ACE	718.08	628.55	966.36	666.89	223.103	0.449	0.797	0.515

[a] In the same row, values with different superscript letters indicate a significant difference ($P < 0.05$), while those with the same or no superscript letters indicate no significant difference ($P > 0.05$).

[b] Each value represents the mean of five replicates.

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Table 7. Effects of dietary amylose-amylopectin ratio on species abundance of jejunum microflora of goslings at 28 days of age (phylum level and genus level) [a,b].

Item	AM:AP ratio				SEM	*P*-value		
	0.12	0.23	0.34	0.45		Between groups	Linear	Quadratic
Phylum level								
Firmicutes	65.37	82.85	67.70	85.23	10.250	0.158	0.189	0.997
Proteobacteria	3.27[b]	9.81[a]	11.76[a]	2.61[b]	2.088	0.001	<0.001	0.340
Bacteroidota	3.43[a]	0.55[b]	5.40[a]	0.40[b]	1.333	0.004	0.002	0.001
Actinobacteriota	1.03	0.73	0.92	0.32	0.445	0.429	0.188	0.647
Desulfobacterota	0.35	0.22	1.75	0.32	0.743	0.166	0.097	0.067
Verrucomicrobiota	0.07	0.01	0.10	0.09	0.036	0.109	0.198	0.302
Genus level								
Romboutsia	15.16[b]	13.19[b]	4.65[c]	78.73[a]	4.880	<0.001	<0.001	<0.001
Streptococcus	2.28[b]	25.68[a]	1.02[b]	1.01[b]	1.698	<0.001	<0.001	<0.001
Lactobacillus	0.87	0.56	1.34	0.40	0.460	0.226	0.672	0.349
Enterococcus	0.52	2.00	0.29	0.03	1.155	0.353	0.282	0.223
Ralstonia	0.23	0.21	0.61	0.17	0.255	0.310	0.181	0.137
Escherichia-Shigella	1.66	3.90	1.29	0.31	1.684	0.223	0.222	0.240
Akkermansia	0.18	0.01	0.28	0.09	0.158	0.389	0.231	0.093
Bacteroides	1.93	0.24	1.92	0.24	0.951	0.141	0.114	0.040
Turicibacter	4.07[a]	1.73[b]	1.63[b]	4.97[a]	1.009	0.008	0.430	0.001
Methylobacterium-Methylorubrum	0.18	0.50	0.53	0.92	0.445	0.452	0.903	0.664

[a] In the same row, values with different superscript letters indicate a significant difference ($P < 0.05$), while those with the same or no superscript letters indicate no significant difference ($P > 0.05$).

[b] Each value represents the mean of five replicates.

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