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Effect of *Broussonetia papyrifera* leaf meal on growth performance, antioxidant capacity, and gut health status of growing rabbits

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Abstract: This experiment aimed to study the effect of *Broussonetia papyrifera* leaf meal (BP) on the growth performance, antioxidant capacity, intestinal permeability, and caecal microbiota of growing rabbits. A total of 240 weaned Ira male rabbits were randomly divided into four groups with six replicates of 10 rabbits each. The BP was added at 0% (CON), 3.0% (BP3), 6.0% (BP6), and 9.0% (BP9). All diets were isoenergetic, isonitrogenous, and isofibrous. The feeding trial lasted for 28 days. The results showed that adding BP had no significant effect on the growth performance of rabbits. Compared with the CON group, groups BP6 and BP9 showed the reduced serum diamine oxidase content ($P < 0.01$). The jejunal secretory immunoglobulin A content in BP6 was higher than in the CON group ($P < 0.01$). The addition of BP had no significant effect on the jejunal antioxidant capacity. The BP9 increased the abundance of the caecal Firmicutes; BP3 and BP6 increased Bacteroidota; BP6 and BP9 increased Proteobacteria ($P < 0.05$). It is concluded that the BP can be used as a roughage source to reduce intestinal toxic markers, improve the intestinal immune function and the abundance of caecal microflora in growing rabbits.

Keywords: caecal microflora; intestinal permeability; secretory immunoglobulin A

Broussonetia papyrifera (Moraceae), also known as paper mulberry, is a herbal medicinal woody plant. The BP is widely distributed in most countries, especially in China, with a high adaptability and lush leaves. *B. papyrifera* leaf meal (BP) contains 18% to 24% of crude protein, which is comparable with lucerne meal in nutritional values (Shen and Peng 2017; Peng et al. 2019). Additionally, from the BP branches and twigs 14 compounds can be isolated, including tannins, flavonoids and phenolic acids, and some compounds showed antioxidant and anti-inflammatory capacity (Sun et al. 2012; Malanik et al. 2020). It is well known that plant flavonoids and phenolic acids possess antioxidant functions (Ding et al. 2019; Zhao et al. 2020). As for the tannins, they belong among polyphenols and they are a traditionally antinutritional factor for pigs and chickens, but not for herbivores, including rabbits. On the contrary, proper concentration of tannins in the diet can also act as an antioxidant in the body (Kim et al. 2021). In weaned piglets, *B. papyrifera* leaf extract added at 150 g/t and 300 g/t of diet improved growth performance and antioxidant capacity, reduced the occurrence of diarrhoea, enhanced immune functions and disease resistance, and affected the composition

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of faecal microflora (Chen et al. 2020). Whether the BP can be an alternative for traditional roughages in small herbivores is unclear. Commercial meat-type rabbits grow fast but with a susceptible body constitution, which can cause subclinical health problems in many organs, especially in the small intestine and the caecum (Wang et al. 2021). Furthermore, traditional roughage resources are becoming scarcer and scarcer, so herbal or medicinal plants woody with a high yield as well as an appreciable nutritional value may be a source worthy of being explored.

Hypothetically, the inclusion of BP leaf meal in the diet can improve intestinal health without compromising the growth performance of herbivores. This experiment aimed to study the effect of BP leaf meal as a roughage source for weaned rabbits on the growth performance, antioxidation, intestinal immunity, permeability, and caecal microbiota of meat-type growing rabbits.

MATERIAL AND METHODS

Broussonetia papyrifera leaf meal

B. papyrifera leaf meal was provided by Luoyang Hejia Agriculture and Animal Husbandry Co., Ltd (Luoyang, China; 112°45'N, 34°62'E). The *B. papyrifera* grew to 1.2 meters, and the branches and leaves were harvested 30 cm above the ground, dried at 65 °C, and ground to pass a 20-mesh sieve. The proximate nutrients of the BP are listed in Table 1.

Diets and animals

The experimental protocol was approved by the Committee on the Ethics of the Gansu Agricultural University (Lanzhou, China).

Table 1. Chemical compositions of *Broussonetia papyrifera* leaf meal (%, dry matter basis)

Compositions	Contents	Compositions	Contents
Dry matter	87.59	Ca	1.19
Crude protein	10.61	P	0.21
Crude fiber	10.30	Flavonoids (mg/g)	0.68
Crude fat	3.05	Total tannins (mg/g)	8.70
Crude ash	9.31	Total polyphenols	0.45

A total of 240 weaned Ira male rabbits with similar age and body weight (body weight ± SD, 839 ± 10.62 g) were randomly divided into four groups with six replicates in each group and 10 rabbits in each replicate. The feeding trial lasted for 28 days after a 7-day adjustment. The BP was added at 0% (CON), 3.0% (BP3), 6.0% (BP6), and 9.0% (BP9) to the diet, and the compositions and nutritional levels are shown in Table 2. All diets were considered isoenergetic, isonitrogenous, and isofibrous. Animals were raised in a rabbit house in the same layer in European-style cages. Animal management was in accordance with Technical Specification for Feeding and Management of Meat-type Rabbits (NY/T 4049-2021, Ministry of Agriculture of China). The feed supply increased by 10 g every three days with the age. The room temperature was maintained at 22 °C to 25 °C. Drinking water

Table 2. Ingredients and nutrient levels of diets (air-dry basis)

Items	CON	BP3	BP6	BP9
Ingredients (%)				
BP	0	3.0	6.0	9.0
Corn	24.00	23.43	22.86	22.29
Soybean meal	12.50	13.00	13.50	13.80
DDGS	5.00	4.85	4.70	4.55
Peanut vine	14.00	13.58	13.16	12.74
Alfalfa meal	17.50	15.83	14.16	12.69
Wheat bran	20.00	19.40	18.80	18.20
Soybean oil	3.00	2.91	2.82	2.73
Dicalcium phosphate	2.00	2.00	2.00	2.00
Premix[1]	2.00	2.00	2.00	2.00
Nutrients[2] (%)				
Crude protein	17.00	17.04	17.09	17.14
Digestible energy (MJ/kg)	9.43	9.44	9.44	9.43
Crude fiber	20.00	20.44	20.90	21.34
Lysine	0.76	0.77	0.76	0.78
Methionine + cysteine	0.51	0.54	0.53	0.53
Ca	1.02	1.03	1.00	1.01
P	0.58	0.58	0.59	0.58

BP = *Broussonetia papyrifera* leaf meal; DDGS = distillers dried grains with solubles

[1]The premix provided the following per kg of diets: vitamin A, 12 000 IU; vitamin D, 2 000 IU; vitamin E, 30 IU; Cu, 12 mg; Fe, 64 mg; Mn, 56 mg; Zn, 60 mg; I, 1.2 mg; Se, 0.4 mg; Co, 0.4 mg; NaCl, 6.4 g

[2]Calculated by Xiong et al. (2010)

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was guaranteed twice a day, and the immunization program was carried out as usual. The feed and water were provided *ad libitum*. The rabbit house was cleaned every day, and drinking water pipes and manure trays were regularly cleaned. The feed intake and body weight were weighed on the 1st and 28th days of the trial. The average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated. The diarrhoea and mortality were monitored twice a day.

Sample collection

On the last day of the feeding trial, one rabbit was randomly selected in each replicate and blood (10 ml) was collected from the heart. The serum was collected after centrifugation at 3 500 rpm and stored in a refrigerator (−4 °C) for intestinal permeability testing. Then, the rabbits were euthanized by cervical dislocation and then dissected. The contents of the caecum were collected in a 1.5 ml centrifuge tube and stored at −80 °C for the 16S rRNA sequencing of intestinal microbes. About 2 cm of the jejunum was cut, and the surface fat was removed. The jejunum was rinsed with 4 °C normal saline to remove the contents and fixed in 4% paraformaldehyde solution for later use. Another section of the jejunum was collected, and the jejunum mucosa was scraped with a glass slide and placed in a cryopreservation tube. After frozen in liquid nitrogen, the jejunum was stored at −80 °C. The present study was carried out in strict accordance with the protocol that protects animals used for experimentation and other scientific purposes.

Proximate nutrient analysis

Dry matter, crude protein, gross energy, P, Ca, crude fat, and crude fibre in the BP and diets were determined by the methods of the Association of Official Analytical Chemists (AOAC 2000). Total flavonoids and tannins in the BP were quantified by China National Food Safety GB/T 20574-2006 and GB/T 27985-2011, respectively. Total phenolic acids were detected using a Folin-Ciocalteu assay according to Grzegorczyk-Karolak et al. (2015). All samples were prepared and detected duplicately according to the methods by Wang et al. (2019).

Determination of permeability, immunity and antioxidation

Intestinal permeability was measured by commercial kits for D-lactate (DLA) and diamine oxidase (DAO) in the serum according to their instructions. The secretory immunoglobulin A (SIgA) in the jejunum mucosa was determined using an enzyme-linked immunosorbent assay kit. The total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), catalase (CAT), and glutathione peroxidase (GPX) activities in the jejunum mucosa were determined. The glutathione peptide (GSH), malondialdehyde (MDA), and protein contents in the serum were determined. The units of intestinal toxic markers and antioxidant parameters were expressed as the activity or the content per gram of serum protein to minimize the error that occurred during the determination process. All kits for permeability, immunity, and antioxidation were purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China).

Determination of caecal microorganisms

After the rabbits were slaughtered, caecal contents were squeezed into a 4-ml sterile cryotube under aseptic conditions, frozen in liquid nitrogen, and stored at −80 °C. Samples were then sent to Shanghai Meiji Biomedical Technology Co., Ltd (Shanghai, China) for microbial diversity analysis. The steps of the analysis included genomic DNA extraction, polymerase chain reaction (PCR) amplification, fluorescence quantification, MiSeq library construction, MiSeq sequencing, and biological information analysis procedures, including: (I) the total genomic DNA (Omega Bio-tek, Norcross, GA, USA) was extracted from frozen caecal chyme samples; (II) the NanoDrop 2000 was used to detect the quality and concentration of total DNA, and 1.5% agarose gel electrophoresis was used to detect its integrity; (III) PCR was performed with the universal forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and universal reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR system was Transgen AP221-02 and consisted of TransStart FastPfu DNA polymerase and 20 µl reaction system. PCR was carried out in a 20 µl mixture contain-

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ing 4 µl 5× FastPfu Buffer, 2 µl of 2.5 mM dNTPs, 0.8 µl forward primer (5 µM), 0.8 µl reverse primer (5 µM), 0.4 µl FastPfu polymerase, 0.2 µl BSA, 10 ng template DNA, and a certain amount of ddH_2O to obtain a 20 µl mixture. The following cycle parameters were used: PCR at 95 °C for 3 min initial denaturation; 27 cycles for 30 s at 95 °C, annealing at 55 °C for 30 s, extension at 72 °C for 45 s; and final extension at 72 °C for 10 min (ABI GeneAmp® PCR system 9700; Applied Biosystems, Foster City, CA, USA); and (IV) the purified cDNA underwent end repair, A-tailing, and ligation of sequencing adapters followed by fragment size selection and PCR amplification. After the constructed MiSeq library was checked using the Agilent 2100 Bioanalyzer quality inspection, the MiSeq was used for sequencing and quality inspection. After being qualified, the PE reads obtained were first spliced in accordance with the overlap relationship, and the sequence quality was controlled and filtered at the same time. After the samples were distinguished, the operational taxonomic unit (OTU) cluster analyses and species taxonomy analyses were performed by the Shanghai MAJOR Biological Company (Shanghai, China) according to the method by Zhu et al. (2022).

Data analysis

Test data were analysed using the ANOVA program of the SPSS v22 statistical software (IBM SPSS, Armonk, NY, USA). Duncan's method was used for multiple comparisons if the difference was significant. Linear and quadratic equations of polynomial contrasts were used for the analysis of dose trends of BP at 3.0%, 6.0%, and 9.0%. Data were expressed as mean and SEM, and $P < 0.05$ was used as criterion for judging the significance of the difference.

RESULTS AND DISCUSSION

Growth performance

As shown in Table 3, the BP had no significant effect on the final weight, ADG, ADFI, and FCR of rabbits. Also, the diarrhoea and mortality were not different between groups (data not shown). The diets containing BP at 3.0%, 6.0%, and 9.0% had insignificant ADFI, ADG, and FCR compared with CON, indicating that the BP can be one of the roughage sources for growing rabbits without compromising the rabbit growth. Literature about the effect of BP on the growth performance is unavailable in omnivorous growing farm animals, such as poultry and pigs, due to the high fibre content of *B. papyrifera* leaves and soft twigs. Supplementation of *B. papyrifera* leaf extract at 150 g/t and 300 g/t increased the growth performance of piglets (Chen et al. 2020). Diet with 15% *B. papyrifera* silage increased final body weight, feed intake, and feed efficiency of beef cattle (Tao et al. 2020).

Weaned rabbits grow and develop fast, and their digestive system development is lagging behind, leading to the insufficient secretion of gastric acid and digestive enzymes. This can cause subclinical health problems of the intestine, such as slight diarrhoea (Wang et al. 2019; Wang et al. 2021). The insignificantly low diarrhoea and mortality rate in the BP groups in the present study implies that the BP can alleviate the intestinal subclinical problems. This is supported by the studies that the *B. papyrifera*-derived product inclusion significantly reduced the rate of diarrhoea in weaned piglets (Chen et al. 2020; Deng et al. 2021), which may be attributed to the flavonoids and polyphenols; anyway, more studies are needed.

Table 3. Effect of *Broussonetia papyrifera* leaf meal (BP) on the growth performance of rabbits

Item	BP (%)				SEM	*P*-value	
	0	3.0	6.0	9.0		linear	quadratic
Initial BW (g/rabbit)	838.9	827.8	848.9	838.9	10.62	0.674	0.785
Final BW (g/rabbit)	1 922	1 932	1 908	1 920	9.858	0.168	0.694
ADFI (g/rabbit)	108.8	101.2	98.75	103.8	4.162	0.225	0.107
ADG (g/rabbit)	38.68	39.44	37.83	38.61	2.865	0.489	0.591
FCR	2.813	2.566	2.611	2.688	0.111	0.456	0.002

ADFI = average daily feed intake; ADG = average daily gain; BW = body weight; FCR = feed conversion ratio

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Intestinal permeability

Table 4 shows the effect of dietary BP on the intestinal permeability, SIgA, and antioxidant status. The serum DLA contents of the groups fed BP were not significantly different from those of CON, and the serum DAO contents of BP6 and BP9 were significantly lower than those of CON and the BP3 ($P < 0.05$). There was no difference in DAO contents between BP3 and CON. With the increasing amounts of BP, there were significant linear and quadratic decreases in the DAO contents ($P \leq 0.024$), indicating that dietary BP can decrease the intestinal permeability.

The level of DLA and the activity of DAO in the blood are often used as useful biomarkers to monitor the integrity of the intestinal barrier (Liu et al. 2018a). The DLA is produced by intestinal bacterial fermentation. Thus, the increase in the concentration of serum DLA reflects the amount of damage to the intestinal mucosa. DAO is an intracellular enzyme in intestinal cells that increases when the intestinal integrity and mucosa are damaged (Liu et al. 2018b). In the present experiment, the DLA content in the blood of the BP group was not statistically decreased, whereas the DAO content was statistically decreased, implying that dietary BP can protect the intestinal mucosa. No information about the effect of BP or BP extracts on the intestinal integrity is available. Based on the fact that plants containing flavonoids and polyphenols can protect intestinal health (Ding et al. 2019; Zhao et al. 2020; Wang et al. 2021), it can be deduced that the BP has a similar effect. Furthermore, if the integrity of the intestinal barrier is improved, the absorption of nutrients from the intestine is promoted, but Hao et al. (2020) reported that *B. papyrifera* silage did not affect dry matter digestibility in Holstein dairy cows. Therefore, the effect of BP on the intestinal integrity and nutrient digestibility in rabbits needs further study.

Immunity and antioxidation

The effects of BP on the mucosal immunity and antioxidation are shown in Table 4. The SIgA content in the jejunum of rabbits at BP6 was significantly higher than that in CON and BP3 ($P < 0.05$), but there were no differences between BP6 and BP9. The differences between groups also caused linear and quadratic effects of BP on the SIgA contents ($P \leq 0.016$). The BP had no significant effect on the jejunal antioxidant capacity, including T-AOC, T-SOD, and CAT activities, and MDA and GSH contents.

Immunoglobulin A is the main antibody isotype in the intestinal mucosa and is found most often in the form of SIgA, which is secreted by B lymphocytes in the *lamina propria* of digestive tracts. SIgA protects the intestinal epithelium from pathogenic microorganisms and toxic compounds (Liu et al. 2018a). In the present study, the jejunal SIgA contents of BP6 and BP9 were significantly higher than those of CON, indicating that the BP can improve the mucosal immunity. Few data are available about

Table 4. Effect of *Broussonetia papyrifera* leaf meal (BP) on intestinal permeability, SIgA and antioxidation in growing rabbits

Item	BP (%)				SEM	*P*-value	
	0	3.0	6.0	9.0		linear	quadratic
DLA (mg/l)	1.82	1.88	1.66	1.80	0.110	0.085	0.079
DAO (pg/ml)	800[a]	802[a]	577[b]	565[b]	41.52	0.024	0.032
SIgA (μg/ml)	89.3[b]	94.7[b]	114[a]	99.1[ab]	6.551	0.016	0.014
MDA (nmol/ml)	1.53	1.15	1.08	1.27	0.342	0.205	0.108
T-SOD (IU/mg)	1.62	1.61	1.89	1.81	0.244	0.145	0.521
CAT (IU/mg)	4.51	4.60	4.71	4.44	0.262	0.289	0.347
T-AOC (IU/mg)	0.49	0.58	0.57	0.61	0.243	0.136	0.475
GSH (μmol/l)	4.13	4.88	5.88	6.20	0.812	0.070	0.269

CAT = catalase; DAO = diamine oxidase; DLA = D-lactic acid; GSH = reduced glutathione; MDA = malondialdehyde; SIgA = secretory immunoglobulin A; T-AOC = total antioxidant capacity; T-SOD = total superoxide dismutase

[a,b]The same row with different superscripts means a significant difference, $P < 0.05$

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the effect of BP on the immunoglobulins of animals, but this potential of BP on immunoenhancement can also be deduced from its phytochemicals.

Antioxidant enzymes, such as SOD, CAT, and GPX, are important antioxidants widely distributed among living cells and body fluids and help reduce oxidative stress. The antioxidant capacity of BP was not significant in the present study. This is inconsistent with literature. Malanik et al. (2020) found that one compound with flavonoid ring isolated from branches and twigs of BP showed a significant antioxidant activity in lipopolysaccharide-stimulated macrophages. Similarly, Si et al. (2018) reported that 15% of *B. papyrifera* silage increased the content of serum CAT, SOD, T-AOC, and decreased the content of 8-hydroxy-2'-deoxyguanosine in dairy cows. Also, *B. papyrifera* fruits showed a strong scavenging capacity dependent on its total phenolic content (Sun et al. 2012). Additionally, BP contains tannins which also possess the antioxidant function (Szczurek 2021). In the present study, the exact reason for the non-significant antioxidant effect of BP deserves further study.

Caecal microbial diversity and richness

Table 5 shows the effect of BP on caecal microbial compositions and richness. Shannon and Simpson indices reflect the α-diversity of caecal microorganisms. The nonsignificant differences between groups for Shannon and Simpson indices indicate that dietary BP had no effect on the *α*-diversity of caecal microbiota. ACE and Chao-1 indices reflect the species richness of caecal microbiota. The ACE and Chao-1 indices of the BP6 and BP9 groups were higher than in CON and BP3 ($P < 0.05$), indicating that BP6 and BP9 diets can increase the richness of caecal microbiota. With the increasing BP doses, there were linear increases in the species richness of caecal microbiota for ACE ($P = 0.024$) and Chao-1 ($P = 0.026$).

The OTU is considered as the basic unit used in numerical taxonomy. These units may refer to an individual, species, genus, or class. As shown in Figure 1A, the total number of OTUs contained in CON, BP3, BP6, and BP9 groups was 891, 718, 1 269, and 970, respectively. A total of 89, 26, and 45 unique OTUs were present in BP3, BP6, and BP9 groups, respectively, whereas 29 unique OTUs were observed in CON. Figure 1B shows the relative richness of caecal microbiota at the phylum level. Dietary BP3 decreased the phylum Bacteroides and increased the phylum Firmicutes ($P < 0.05$) compared with CON.

Figure 2 shows the relative richness of caecal microbiota at the genus level in CON, BP3, BP6 and BP9. In CON, the three top genera were Christensenellaceae_R-7_group at 9.22%, norank_f_Muribaculaceae at 8.29%, and NK4A214 at 8.11%. The three top genera in BP3 were Bacteroides at 30.78%, Rikenellaceae_RC9 at 12.12%, and Phascolarctos bacterium at 4.72%. The three top genera in BP6 were Bacteroides at 12.59%, Rikenellaceae_RC9 at 8.46%, and NK4A214 at 4.23%. The top-ranked genera in BP9 were NK4A214 at 18.38%, Christensenellaceae_R-7 at 7.36%, and norank_f_Muribaculaceae at 7.18%.

The intestinal tract of mammals is almost sterile at birth, and the colonization and maturation of bacterial communities are affected by many factors, such as delivery method, milk source, feeding type, and antibiotic treatment. The colonization of the intestine plays an important role in the devel-

Table 5. Effect of *Broussonetia papyrifera* leaf meal (BP) on the α-diversity of the caecal microbiota community of growing rabbits

Item	BP (%)				SEM	*P*-value	
	0	3.0	6.0	9.0		linear	quadratic
Shannon	4.52	4.60	4.67	4.71	0.064	0.082	0.764
Simpson	0.03	0.03	0.03	0.04	0.008	0.807	0.853
ACE	605^{b}	647^{b}	731^{a}	750^{a}	19.63	0.024	0.092
Chao-1	613^{b}	662^{b}	738^{a}	762^{a}	22.92	0.026	0.103

ACE = abundance-based coverage estimator; Chao-1 = estimator of species richness based on a vector or matrix of abundance data; Shannon = Shannon diversity index; Simpson = Simpson index-based measure of evenness

^{a,b}The same row with different superscripts means a significant difference, $P < 0.05$

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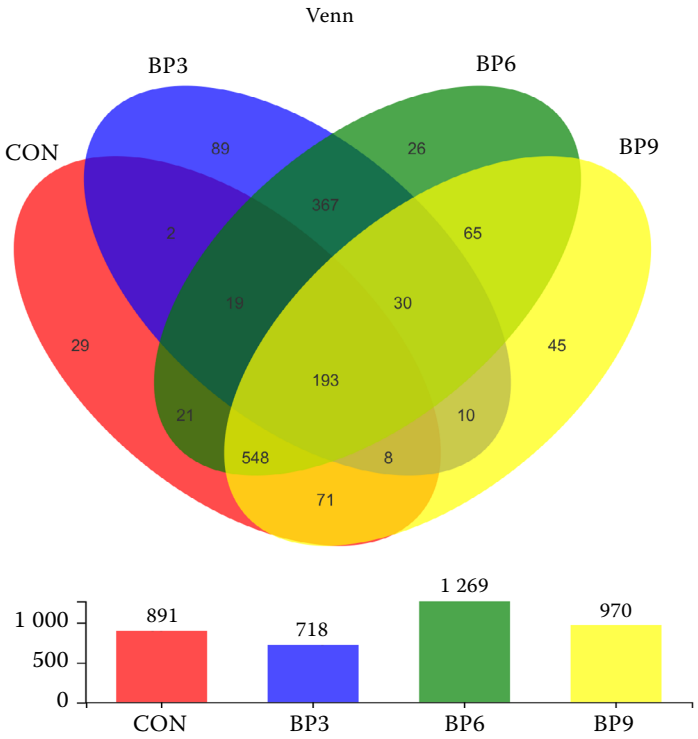
(A)



(B)

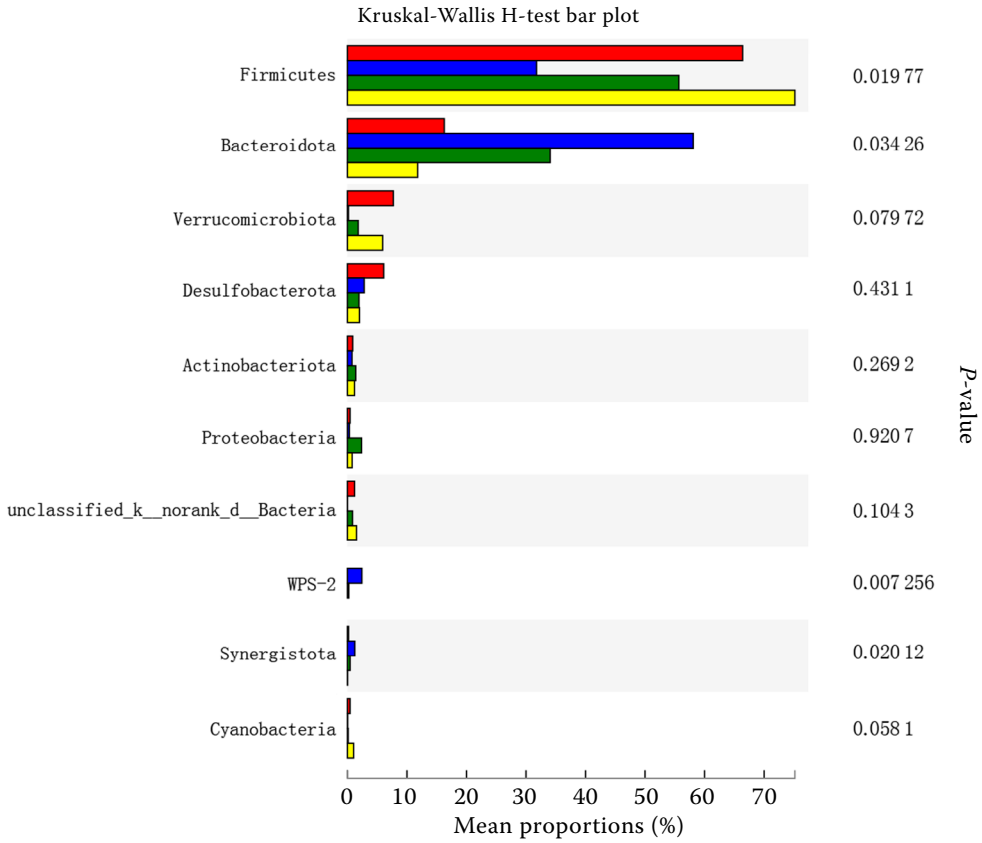


Figure 1. Caecal microbial diversity and richness

(A) Operational taxonomic unit richness and evenness. (B) relative abundance of caecal microbiota at the phylum level; the vertical axis represents the bacterial phylum and the horizontal axis represents the relative abundance. *Broussonetia papyrifera* leaf meal (BP) was added at 0% (CON), 3.0% (BP3), 6.0% (BP6), and 9.0% (BP9) to the diet

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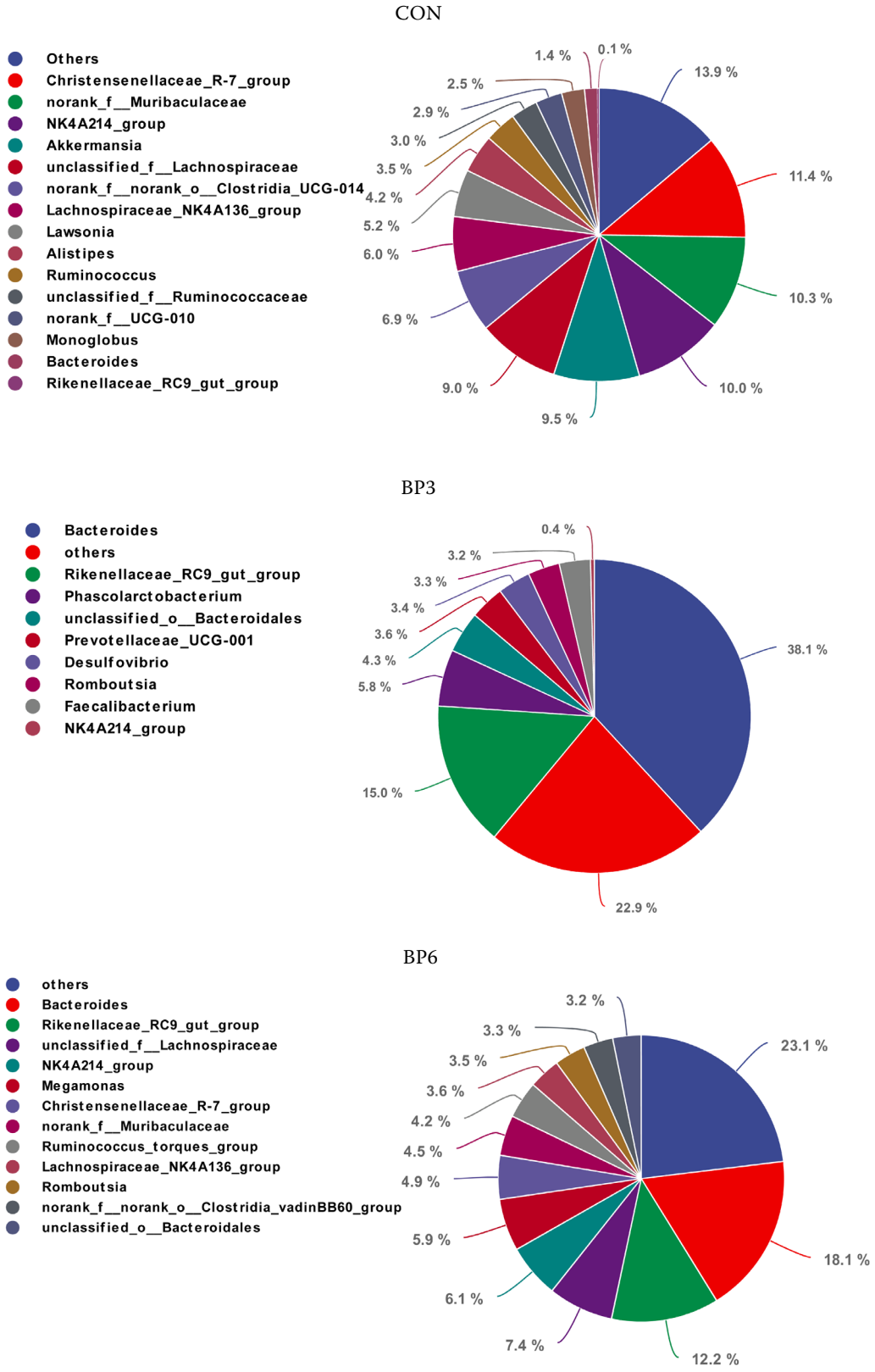

Figure 2. Relative abundance of caecal microbiota at the genus level

Different colours in the pie chart represent different genera. *Broussonetia papyrifera* leaf meal (BP) was added at 0% (CON), 3.0% (BP3), 6.0% (BP6), and 9.0% (BP9) to the diet

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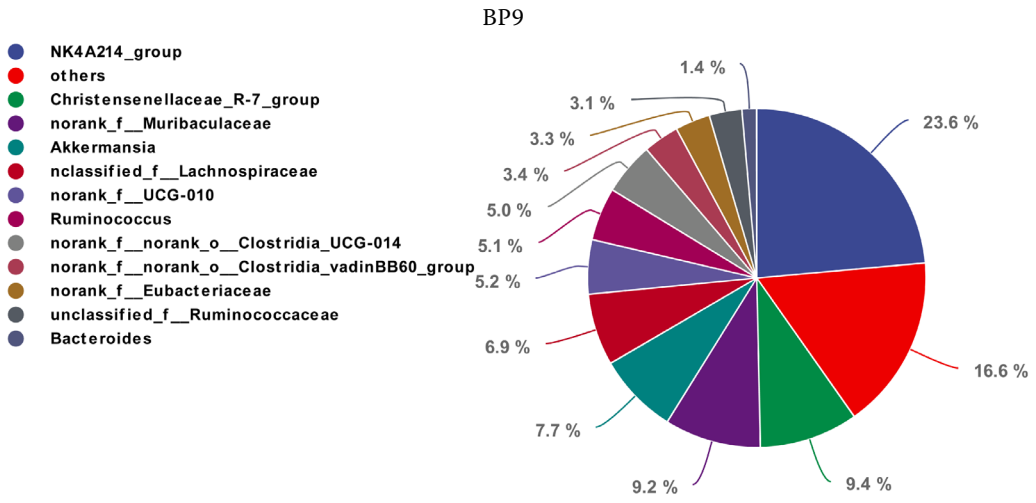

Figure 2 to be continued

opment and health of the intestinal tract of animals. The α-diversity index can often be used as a reference to the diversity and abundance of the intestinal microbial community of livestock and poultry (Deng et al. 2021). In the present study, the caecal microbial diversity of BP groups does not change significantly, but the abundance values of different flora were changed significantly, indicating that the dietary BP can increase the total amounts of flora but not for their classification. Generally, dietary beneficial factors can cause a significant increase in the abundance of gut microbiota but not enough to raise a change of diversity as reported by Tan et al. (2022).

The literature showed that the gut microbiota of rabbits is usually dominated by Firmicutes and Bacteroides (Read et al. 2019). At the phylum level, the advantages of Firmicutes found in the present study are consistent with previous studies on the rabbit caecal flora, and its relative abundance is in line with the previously reported data (Monteils et al. 2008; Bauerl et al. 2014; Zhu et al. 2015). According to Bauerl et al. (2014), the number of Bacteroides in the epizootic rabbit enteropathy (ERE) group increased, which is similar to the results of the present experiment; however, the numbers of Desulfobacterota and Proteobacteria of ERE rabbits increased significantly, and the number of Verrucomicrobia also increased, which are not found in the present study, indicating that the changes in the flora in the BP3 group may not be caused by the epidemic enteropathy. In the present experiment, the drastic changes in the abundance of the caecal microbial flora did not affect the growth performance of rabbits. Therefore, the use of BP is beneficial to improve the intestinal microbes of rabbits.

The most common genus in all groups was NK4A214_group, and its relative abundance gradually increased in the BP groups. The rate of 18.38% in BP9 was 2.27 times that of the CON group (8.11%). Microbial NK4A214_group is generally a producer of short-chain fatty acids, which play a key role in the degradation of various polysaccharides and fibres (Yang et al. 2020). However, the content of NK4A214_group in BP3 and BP6 was both lower, but they both contained a considerable amount of Bacteroides and Rikenellaceae_RC9_gut_group. Both Ruminococcus and Bacteroides are bacteria that digest fibre and polysaccharides, and their overall function was not changed. The CON and the three BP groups had a similar composition of the phyla, Christensenellaceae_R-7_group, NK4A214_group, norank_f_Muribaculaceae, Akkermansia, the total proportion of which exceeded 30%. When young rabbits were fed 9.0% BP, their microbial composition was similar to the CON group.

CONCLUSION

B. papyrifera leaf meal added at 3.0%, 6.0% or 9.0% as a roughage source can protect the intestinal barrier, reflected on an increase in the mucosal immune antigen SIgA, decrease in the gut toxic marker DAO, and improving the abundance

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of the caecal microbiota, but not compromising the growth performance of growing rabbits. It is concluded that *B. papyrifera* as a traditional medicinal woody plant can be used as a roughage source for improving the gut health in herbivores.

Conflict of interest

The authors declare no conflict of interest.

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