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Agaricus blazei Murrill stipe promotes growth by improving anti-inflammatory activity and gut function in broilers

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ABSTRACT. *Agaricus blazei* Murrill (AB) is a mushroom known for its medicinal applications. AB stipe (ABS) is a by-product of the mushroom's processing, which is rich in bioactive substances, such as polysaccharides. This experiment was conducted to assess the effects of dietary ABS supplementation on growth performance, short-chain fatty acid production, antioxidant capacity, immune function, meat quality, gut function, and caecal microbiota in broilers. A total of 180 one-day-old male Arbor Acre broilers (46.17 ± 2.47 g) were randomised to 3 treatments, with 6 replicates per treatment and 10 broilers per replicate. The treatments included one basal diet (control group) and two experimental diets supplemented with 1% and 2% ABS, respectively. This trial lasted 42 days and was divided into Phase 1 (day 1–21) and Phase 2 (day 22–42). The results showed that broilers fed ABS exhibited a significant reduction in the feed conversion ratio throughout the study period ($P < 0.05$), and tended to have increased average daily gain in Phase 2 ($P < 0.1$) and the overall period ($P < 0.1$). On day 42, dietary ABS supplementation significantly decreased the concentration of interleukin-1β ($P < 0.05$) and total cholesterol in serum ($P < 0.05$). Moreover, ABS addition significantly increased the villus height-to-crypt depth ratio ($P < 0.05$) in the jejunum and tended to increase villus height ($P < 0.1$) and decrease crypt depth ($P < 0.1$). In addition, the ABS-supplemented groups showed higher microbial diversity and increased proliferation of beneficial bacteria in the caecum. Based on these findings, it can be concluded that ABS supplementation can improve growth performance, anti-inflammatory activity, and intestinal function.

Introduction

The significance of the gut in broiler performance and health has gained increasing recognition. As the subtherapeutic use of antibiotics in poultry rearing has been phased out in various parts of the world, there is a pressing need for alternative methods to prevent bacterial infections and enhance poultry growth (Giannenas et al., 2010b). Natural medicines derived from herbs and fungi have been utilised as feed additives for animals for centuries (Guo et al., 2004). Mushrooms possess immune-stimulating and growth-promoting properties, partially attributed to their high polysaccharide content, which exerts a prebiotic effect (Guo et al., 2003; Giannenas et al., 2010b).

Agaricus blazei Murrill (AB) belongs to the family *Agaricaceae* and is cultivated on an industrial scale in China and Japan (Hu et al., 2021). AB contains many bioactive substances, including proteins, polysaccharides, minerals, and vitamins (Endo et al., 2010). AB is widely applied as

a medicinal and functional food due to its pharmacological properties, such as antimutagenic, antimicrobial, antioxidant, lipid-lowering, and anticarcinogenic effects (Delmanto et al., 2001; Kuroiwa et al., 2005; Lima et al., 2016; Li et al., 2020a).

It has been reported that a bioactive protein derived from AB exhibits pathogen-resistant properties and shows potential benefits in alleviating type 2 diabetes and cancer (Hu et al., 2021). AB protein shows a significant antioxidant effect by scavenging oxygen free radicals and improving antioxidant enzyme activity. Additionally, β-glucan and agaritine purified from AB have been shown to exhibit anti-tumour activity (Endo et al., 2010). AB is also rich in polysaccharides, which have been proven effective in treating cancer, diabetes, hyperlipidaemia, heart disease, arteriosclerosis, and chronic hepatitis (Kaneno et al., 2004; Zhai et al., 2015). An acidic polysaccharide isolated from AB was found to have a hypolipidemic effect, significantly reducing serum total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) cholesterol levels, while increasing serum high-density lipoprotein (HDL) cholesterol concentration (Li et al., 2020b). Furthermore, polysaccharides extracted from AB have been found to possess antioxidant activity in a concentration-dependent manner (Wu et al., 2014).

Agaricus blazei Murrill stipe (ABS) is a by-product of mushroom processing that is typically discarded, resulting in environmental pollution and waste of resources (Liu et al., 2020). We hypothesised that dietary ABS supplementation could improve the growth and health of chickens. Therefore, the objective of this study was to examine the effects of dietary ABS on various parameters, including growth performance, short-chain fatty acid (SCFA) production, antioxidant capacity, immune function, meat quality, gut function, and ceacal microbiota in broilers.

Material and methods

ABS preparation

ABS used in this study was obtained from Guangxi Junbaoyan Food Co., LTD. (Longzhou, China). The raw material was dried at 55 °C in an oven to preserve the bioactivity of ABS. Subsequently, the dried ABS was ground into powder and passed through a sieve (2 mm). The analysed nutritional composition of ABS is presented in Table 1.

Table 1. Chemical composition of *Agaricus blazei* Murrill stipe (ABS), %[1]

Item	ABS
Crude protein	11.70
Neutral detergent fibre	41.50
Acid detergent fibre	22.70
Ether extract	1.58
Ash	5.10
Total polysaccharide	2.97

[1] performed in duplicate

Animals, diets, and experimental design

Animals used in the current study received prior approval from the Institutional Animal Care and Use Committee of the Guangxi Academy of Agricultural Sciences (WSW211001). A total of 180 one-day-old male Arbor Acre broilers, with an initial body weight (IBW) of 46.17 ± 2.47 g, were bought from Shandong Yi Sheng Livestock and Poultry Breeding Company (Yantai, SD, China). All broilers were randomly allocated to 3 treatment groups, with 6 replicates and 10 broilers in each replicate. The experimental treatments included one basal diet (control group) and two experimental diets supplemented with 1% and 2% ABS, respectively. All nutrient levels in the diets met or exceeded the standards recommended by the National Research Council (NRC, 1994). The composition and nutrient concentrations are shown in Table 2. The trial lasted 42 days and was divided into Phase 1 (day 1–21) and Phase 2 (day 22–42). All broilers were inoculated with inactivated Newcastle disease vaccine (Beijing Centre Biological Co., Ltd., Beijing, China) on days 7 and 28, and infectious bursal disease vaccine on days 14 and 21. All broilers were raised in cages in an environmentally controlled room. The lighting conditions were set to provide continuous light at intensities ranging from 10 to 20 lux. All birds had free access to water and feed. During the first 3 days, the ambient temperature was maintained at 33 °C and then gradually decreased by 3 degrees per week to 24 °C.

Growth performance and sample collection

Individual body weight and feed consumption were measured on days 1, 21, and 42 to determine average daily gain (ADG) and average daily feed intake (ADFI). Feed conversion ratio (FCR) was also calculated for each period. On day 42, 1 bird was selected per replicate with a body weight close to the average body weight per pen. Blood samples (n = 6) were collected from the vein on the wing of each broiler into a 10 ml tube. After centrifugation at 3000 *g* for 15 min at 4 °C, serum

Table 2. Composition and nutrient levels in the experimental diets, %, as fed basis

Item	Phase 1 (day 1 to 21)			Phase 2 (day 22 to 42)		
	Control	1% ABS	2% ABS	Control	1% ABS	2% ABS
Ingredients						
maize	57.79	56.44	55.04	65.03	63.68	62.29
soybean meal	30.00	30.00	30.00	24.00	24.00	24.00
soy protein concentrate	2.00	2.00	2.00	2.00	2.00	2.00
ABS	0	1.00	2.00	0	1.00	2.00
fish meal	3.00	3.00	3.00	3.00	3.00	3.00
soybean oil	3.55	3.90	4.27	2.86	3.20	3.56
dicalcium phosphate	1.30	1.30	1.31	0.80	0.80	0.80
limestone	1.42	1.42	1.42	1.47	1.47	1.47
salt	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine	0	0	0.01	0	0	0.01
methionine	0.14	0.14	0.14	0.04	0.04	0.05
threonine	0	0	0	0	0	0.01
tryptophan	0	0	0.01	0	0	0.01
vitamin-mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrient levels[2]						
metabolisable energy, MJ/kg	12.76	12.76	12.76	12.97	12.97	12.97
digestible lysine	1.22	1.22	1.22	1.08	1.08	1.08
digestible methionine	0.50	0.50	0.50	0.38	0.38	0.38
digestible threonine	0.83	0.83	0.83	0.75	0.75	0.75
digestible tryptophan	0.30	0.30	0.30	0.26	0.26	0.26
Analysed nutrient level						
crude protein	22.13	21.98	21.85	19.83	19.75	19.71
calcium	0.98	1.03	0.99	0.93	0.91	0.95
total phosphorus	0.68	0.65	0.67	0.61	0.62	0.60

ABS – *Agaricus blazei* Murrill stipe; [1] premix supplied per kg diet: mg: copper 10, iron 48, zinc 96.6, manganese 101.76, cobalt 0.3, selenium 0.05, iodine 0.96, vitamin E 22, vitamin K_3 2.2, thiamine 1.65, pyridoxine 3.3, riboflavin 6.6, folic acid 0.33, nicotinic acid 22, pantothenic acid 13.2, choline chloride 500; IU: vitamin A 11 000, vitamin D_3 025; µg: cobalamin 17.6, biotin 88; [2] nutrient levels were calculated based on data provided by NRC (1994)

samples were separated and stored at −20 °C until analysis. For sample collection, broilers (n = 6, 1 broiler per replicate) were stunned, euthanized by CO_2, and slaughtered. Segments of the jejunum measuring 3 cm were collected and preserved in 4% paraformaldehyde solution for intestinal morphological analysis. Breast meat samples were also collected for meat quality determination. Caecal contents were frozen in liquid nitrogen for short chain fatty acids SCFA analysis (n = 6) and 16S rRNA sequencing (n = 4).

Chemical analysis

The ABS ingredient and experimental diets were analysed for crude protein, ether extract, ash, neutral detergent fibre, acid detergent fibre, calcium, and total phosphorus contents (Van Soest et al., 1991; AOAC International, 2006). The total polysaccharide content was measured using the phenol-sulphuric acid method developed by Nielsen (2003).

Determination of serum biochemical parameters

The serum biochemical parameters were determined using assay kits purchased from the Jiancheng Institute of Biological Technology, Nanjing, JS, China. The contents of total antioxidant capacity (T-AOC), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) were measured according to the manufacturer's instructions and determined using an automatic biochemical analyser (model 7170, Hitachi Corp, Tokyo, Japan). The concentrations of immunoglobulins (IgA, IgG, IgM), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and interleukin-10 (IL-10) were determined using commercially available ELISA kits. The concentrations LDL, HDL, TG, and total cholesterol (TC) were measured using an automatic biochemical analyser (model 7170, Hitachi Corp, Tokyo, Japan) at the wavelength of 450 nm.

Analysis of intestinal SCFAs

Caecal SCFA concentrations were analysed according to a modified method described by Porter and Murray (2001). Specifically, approximately 1 g of digesta was dissolved in 2.0 ml HCl solution (0.10%) and shaken vigorously. After incubation at 0 °C in a sealed centrifuge tube for 30 min, the tube was subjected to high-speed centrifugation at 12000 rpm for 15 min. The resulting supernatant was collected and mixed with 25% (m/v) phosphoric acid. Next, the mixture was kept on ice for 40 min and filtered through a 0.45 μm nylon filter into a chromatographic sample vial. A 5.0 μl aliquot was injected into a gas chromatograph (6890, Agilent Crop, Santa Clara, CA, USA) with a capillary column (DB-23, 60 m × 0.25 mm, 0.25 μm, Agilent Crop, Santa Clara, CA, USA). The column was maintained at 50 ℃ for 10 min, followed by a temperature increase at a rate of 10 ℃ per min until reaching 230 ℃, resulting in a run time of 9 min. The injector and flame ionization detectors were maintained at 280 ℃. The carrier gas (nitrogen) was set at a flow rate of 1 ml per min.

Intestinal morphology measurements

Morphological measurements of the jejunum were carried out according to the method described by Abdelqader and Al-Fataftah (2016). Formalin-fixed tissue samples collected from the jejunum were washed with physiological saline solution and then embedded in paraffin wax (Shanghai Yiyang, Shanghai, China). Paraffin blocks were cut into 5 μm sections, mounted on glass slides and stained with haematoxylin-eosin. Villus height (VH) and crypt depth (CD) were determined using a light microscope with a morphometric system (Eclipse E100, Nikon, Tokyo, Japan), which allowed to calculate the ratio of villus height to crypt depth (V/C).

Determination of viscera percentage and meat quality

On day 42, the liver, spleen, thymus gland, and bursa of Fabricius were weighed to calculate the percentage of viscera relative to body weight (BW). Fresh breast meat was used to analyse meat quality, including colour, pH, drip loss, and shear force. The pH values of raw breast meat after 45 min and 24 h were determined by a pH meter (IS400, Mingao, Nanjing, JS, China). Meat colour characteristics expressed as lightness (L*), redness (a*), and yellowness (b*) were measured using a Chroma Meter (NR, Mingao, Nanjing, JS, China). All tests were performed in triplicate at three different locations. Drip loss after 24 h was determined according to the method described by Straadt et al. (2007) and was calculated as follows: drip loss, % = [(initial meat weight − final meat weight) / initial meat weight] × 100%. Shear force was determined using a muscle tenderness meter (MAQC-12, Mingao, Nanjing, JS, China), as described by Ciobanu et al. (2004).

16S rRNA sequencing and analysis

For 16S rRNA sequencing, total bacterial DNA from caecal contents was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. The extracted DNA was checked for quality using a 1% agarose gel (Shanghai Yuanye, Shanghai, China) electrophoresis, and DNA concentration was measured using a spectrophotometer (NanoDrop2000, Thermo Fisher Scientific, Waltham, MA, USA). The V3–V4 hypervariable regions of 16S rRNA were amplified using a GeneAmp 9700 thermocycler PCR system (ABI, CA, USA) and the following primers: 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The amplification conditions were as follows: initial denaturation at 95 ℃ for 3 min, 40 cycles of denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, extension at 72 ℃ for 45 s, and final elongation at 72 ℃ for 10 min. Polymerase chain reaction (PCR) products were extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Subsequently, the purified PCR products were pooled in equimolar concentrations and subjected to paired-end sequencing on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA) following the protocols provided by Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China).

Statistical analysis

Data on growth performance, serum biochemical parameters, SCFA contents, morphological indices, viscera percentage, and meat quality values were checked for normality using the UNIVERIATE procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA). Subsequently, they were analysed using the general linear models (GLM) procedure of SAS. For growth performance data, the pen was considered as the experimental unit, while for other data, each individual broiler was treated as the experimental unit. Least squares means (LSMEANS) analysis was performed to separate group means with Turkey's test used for adjustment. The level of significance was set at $P < 0.05$, and a tendency for significance was assumed at $0.05 \leq P < 0.10$.

Results

Growth performance

The data on growth performance are shown in Table 3. ABS supplementation significantly reduced FCR in the overall period ($P < 0.05$) and tended to increase ADG in Phase 2 ($0.05 < P < 0.1$) and over the whole period ($0.05 < P < 0.1$). No differences were found in ADFI between any of the groups during individual stages.

Table 3. Effects of *Agaricus blazei* Murrill stipe (ABS) on broiler growth performance

Item	Treatments			SEM	*P*-value
	Control	1% ABS	2% ABS		
Day 1–21					
initial body weight, g	45.84	46.47	46.21	0.83	0.87
final body weight on day 21, g	578.82	596.73	588.05	6.45	0.20
ADG, g	25.38	26.17	25.80	0.29	0.22
ADFI, g	34.3	34.88	34.84	0.23	0.19
FCR	1.35	1.34	1.35	0.01	0.77
Day 22–42					
final body weight on day 42, g	1829.47	1897.76	1900.93	23.84	0.10
ADG, g	59.57	61.90	62.52	0.84	0.07
ADFI, g	93.31	93.78	94.09	1.14	0.89
FCR	1.57	1.52	1.51	0.02	0.26
Overall period					
ADG, g	42.47	44.84	44.16	0.61	0.051
ADFI, g	63.80	64.33	64.47	0.62	0.74
FCR	1.51^{a}	1.44^{b}	1.46^{ab}	0.02	0.04

ADG – average daily gain, ADFI – average daily feed intake, FCR – feed conversion ratio, SEM – standard error of the mean; ^{ab} different superscripts within a row indicate that the means are significantly different at $P < 0.05$

Determination of serum biochemical parameters

The results of dietary ABS supplementation on serum biochemical parameters are shown in Table 4. There were no differences observed in the content of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), total peroxidase (T-AOC), and malondialdehyde (MDA) in any of the groups. Compared to the control group, ABS supplementation significantly decreased serum concentration of IL-1β ($P < 0.05$), but did not affect the levels of immunoglobulins (IgA, IgG, IgM), tumor necrosis factor-α (TNF-α), IL-6, and IL-10. Broilers fed ABS exhibited lower TC concentrations ($P < 0.05$), while there were no differences in TG, HDL, and LDL levels between individual groups.

Table 4. Effects of *Agaricus blazei* Murrill stipe (ABS) on broiler serum biochemical indices

Item	Treatments			SEM	*P*-value
	Control	1% ABS	2% ABS		
GSH-Px, umol/l	23.11	23.78	24.66	3.09	0.94
T-AOC, U/ml	4.44	4.32	4.65	0.56	0.91
SOD, U/ml	33.96	36.11	35.63	2.75	0.85
MDA, nmol/ml	7.29	6.98	6.62	0.50	0.65
IgA, g/l	58.15	57.44	59.80	4.07	0.92
IgM, g/l	16.97	17.63	18.11	1.05	0.75
IgG, g/l	26.84	26.41	27.80	1.93	0.87
IL-1β, pg/ml	2.59^{a}	2.21^{b}	2.14^{b}	0.10	0.02
IL-6, pg/ml	13.66	13.12	13.17	0.72	0.85
IL-10, ng/ml	134.91	136.47	138.05	5.60	0.92
TNF-α, pg/ml	6.31	6.07	5.84	0.42	0.74
TG, mmol/l	0.66	0.60	0.62	0.05	0.64
TC, mmol/l	2.56^{a}	2.07^{b}	2.03^{b}	0.13	0.02
HDL, mmol/l	1.89	2.07	2.00	0.14	0.66
LDL, mmol/l	0.51	0.50	0.47	0.05	0.81

GSH-Px – glutathione peroxidase, T-AOC – total antioxidant capacity, SOD – superoxide dismutase, MDA – malondialdehyde, IgA – immunoglobulin A, IgG – immunoglobulin G, IgM – immunoglobulin M, IL-1β – interleukin 1β, IL-6 – interleukin 6, IL-10 – interleukin 10, TNF-α – tumour necrosis factor-α, TG – triglyceride, TC – total cholesterol, LDL – low-density lipoprotein cholesterol, HDL – high-density lipoprotein cholesterol, SEM – standard error of the mean; ^{ab} different superscripts within a row indicate that the means are significantly different at $P < 0.05$

Determination of SCFAs in the caecum

As shown in Table 5, ABS supplementation had no effect on the production of SCFAs compared to the control group.

Table 5. Effects of *Agaricus blazei* Murrill stipe (ABS) on short chain fatty acid concentrations in the caecum of broilers, μg/g

Item	Treatments			SEM	*P*-value
	Control	1% ABS	2% ABS		
Acetate	180.77	176.63	181.91	7.08	0.86
Propionate	78.25	76.10	79.20	2.86	0.74
Butyrate	165.85	169.42	171.60	5.02	0.72
Total	424.87	417.15	426.04	7.07	0.64

SEM – standard error of the mean; ^{ab} different superscripts within a row indicate that the means are significantly different ($P < 0.05$)

Intestinal morphology measurements

As shown in Table 6 and Figure 1, diets supplemented with ABS significantly increased the V/C ratio ($P < 0.05$) in the jejunum and tended to increase villus height ($P < 0.1$) and decrease crypt depth ($P < 0.1$) compared to the control group.

Viscera percentage and meat quality determination

The results concerning viscera percentage and meat quality are shown in Table 7 and Table 8.

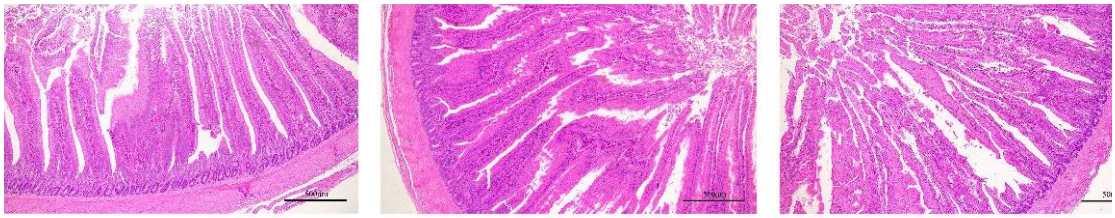

Figure 1. Effect of *Agaricus blazei* Murrill stipe (ABS) addition on the jejunal morphology in Arbor Acres broilers

Table 6. Effects of *Agaricus blazei* Murrill stipe (ABS) on broiler jejunal morphology, μm

Item	Treatments			SEM	*P*-value
	Control	1% ABS	2% ABS		
Villus height	1180.91	1378.52	1245.46	58.92	0.07
Crypt depth	144.83	122.66	112.19	9.12	0.05
Villus height/Crypt depth	8.73[b]	11.51[a]	11.65[a]	0.74	0.01

SEM – standard error of the mean; [ab] different superscripts within a row indicate that the means are significantly different at $P < 0.05$

Table 7. Effects of *Agaricus blazei* Murrill stipe (ABS) on broiler viscera percentage on day 42 (%, body weight)

Item	Treatments			SEM	*P-value*
	Control	1% ABS	2% ABS		
Liver	1.94	1.97	1.98	0.06	0.90
Spleen	0.12	0.14	0.13	0.01	0.36
Thymus gland	0.19	0.20	0.21	0.01	0.34
Bursa of Fabricius	0.21	0.22	0.20	0.01	0.61

SEM – standard error of the mean; [ab] different superscripts within a row indicate that the means are significantly different at $P < 0.05$

Table 8. Effects of *Agaricus blazei* Murrill stipe (ABS) on broiler meat quality

Item	Treatments			SEM	*P*-value
	Control	1% ABS	2% ABS		
pH at 45 min	6.48	6.47	6.44	0.05	0.83
pH at 24 h	5.68	5.66	5.63	0.05	0.69
ΔpH	0.79	0.80	0.81	0.06	0.98
*L** at 45 min	49.12	50.15	51.24	1.35	0.56
*a** at 45 min	5.78	5.27	5.70	1.27	0.96
*b** at 45 min	7.81	7.36	5.69	0.99	0.33
*L** at 24 h	50.58	48.06	49.04	1.64	0.57
*a** at 24 h	5.53	5.38	7.53	1.22	0.41
*b** at 24 h	5.46	4.71	7.32	0.84	0.13
Drip loss, %	2.39	2.54	2.56	0.15	0.71
Shear force, kg	1.28	1.20	1.20	0.26	0.96

ΔpH = pH at 45 min – pH at 24 h; *L** – lightness, *a** – redness, *b** – yellowness, SEM – standard error of the mean; [ab] different superscripts within a row indicate that the means are significantly different at $P < 0.05$

There were no differences in the viscera percentage indices and meat quality between the control and ABS groups.

Caecal microbiota analysis

Figure 2 shows that dietary supplementation with 1% or 2% ABS significantly improved microbiota diversity ($P < 0.01$) in broilers compared to the control diet.

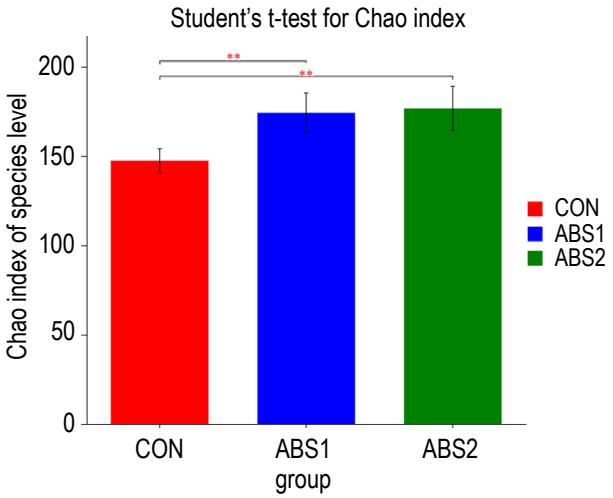

Figure 2. Alpha diversity of the caecal microbiota
CON – control diet, ABS1 – 1% ABS diet, ABS2 – 2% ABS diet

Figure 3 illustrates the results of core operational taxonomic unit (OTU) analysis of the microbiota samples. Among the three groups, there were a total of 13, 26, and 31 core OTUs specific to each group, with a common set of 280 core OTUs shared among all groups. The majority of core OTUs in the control group (280 out of 321) were shared with the ABS-added groups. Additionally, 26 OTUs were only shared with 1% of the ABS group, 31 OTUs were uniquely shared with 2% of the ABS group, and 65 OTUs were common for both ABS groups.

All experimental groups had the same abundance of the 4 most common phyla, but differed in their relative abundance (Figure 4).

At the order level, *Peptostreptococcales-Tissierellales, Erysipelotrichales, Acidaminococcales, Clostridia_UCG-014,* and *Clostridiales* presented significant differences in the abundance between the three groups. Dietary supplementation of

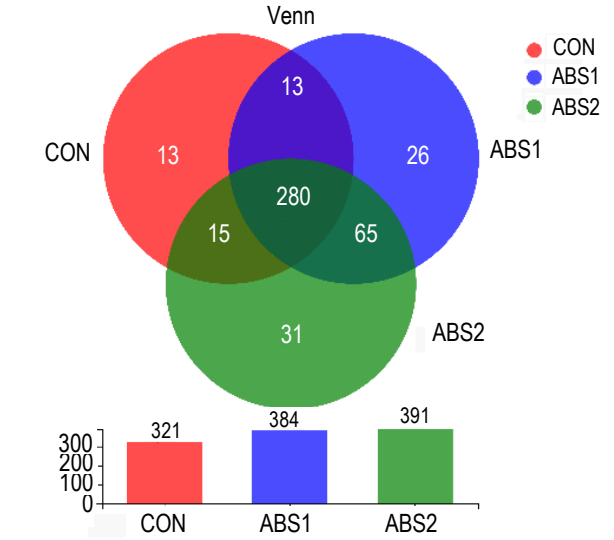

Figure 3. Venn diagram analysis of the caecal microbiota
CON – control diet, ABS1 – 1% ABS diet, ABS2 – 2% ABS diet

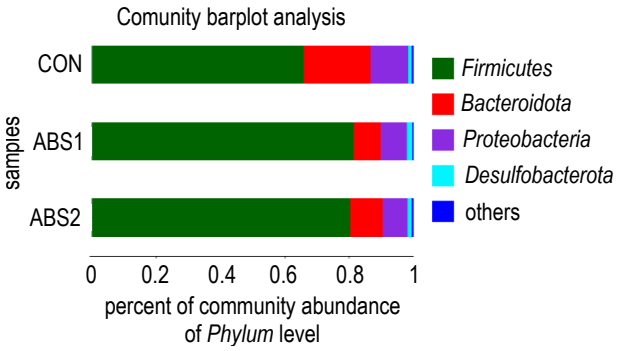

Figure 4. Alterations in the caecal microbiota at the phylum level
CON – control diet, ABS1 – 1% ABS diet, ABS2 – 2% ABS diet

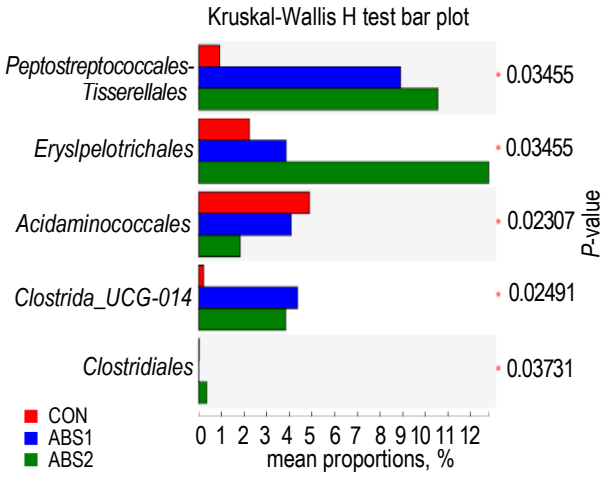

Figure 5. Kruskal-Wallis H test bar plot at the order level of the caecal microbiota
CON – control diet, ABS1 – 1% ABS diet, ABS2 – 2% ABS diet

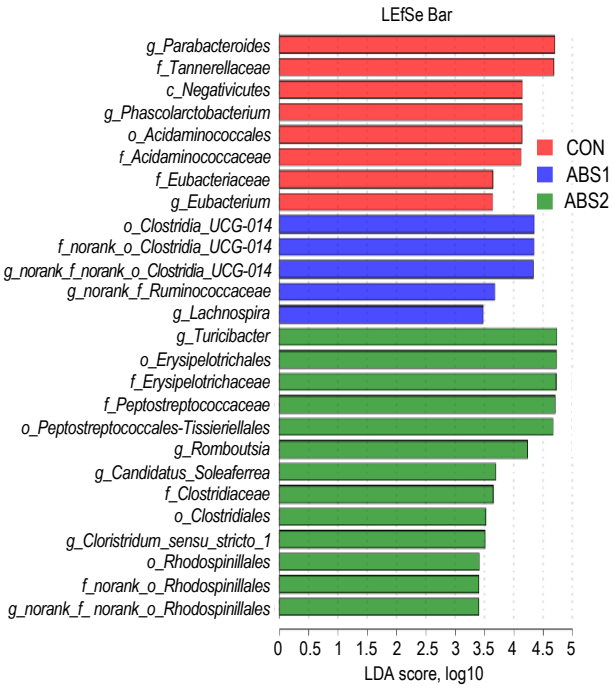

Figure 6. Linear discriminant analysis effect size (LEfSe) of the caecal microbiota
CON – control diet, ABS1 – 1% ABS diet, ABS2 – 2% ABS diet, LDA – linear discriminant analysis

ABS significantly increased the abundance of *Peptostreptococcales-Tissierellales, Erysipelotrichales,* and *Clostridia_UCG-014* compared to the control diet (Figure 5).

As illustrated in Figure 6, the relative abundance of *o__Clostridia_UCG-014*, *f__norank_o__Clostridia_UCG-014*, *g__norank_f__norank_o__Clostridia_UCG-014*, and *g__norank_f__Ruminococcaceae*, *g__Lachnospira* was significantly increased after 1% ABS supplementation in relation to the control group. Broilers fed a diet with 2% ABS showed a significant increase in the relative abundance of *g__Turicibacter, o__Erysipelotrichales, f__Erysipelotrichaceae, f__Peptostreptococcaceae, o__Peptostreptococcales-Tissierellales, g__Romboutsia, g__Candidatus_Soleaferrea, f__Clostridiaceae, o__Clostridiales, g__Clostridium_sensu_stricto_1, o__Rhodospirillales, f__norank_o__Rhodospirillales*, and *g__norank_f__norank_o__Rhodospirillales*, compared to the control group.

Discussion

In this study, the incorporation of ABS in broilers' diets decreased FCR compared to the control diet. The underlying mechanisms could be attributed to bioactive compounds present in ABS. According to Giannenas et al. (2010a), polysaccharides derived from mushrooms, such as those found in ABS, have the potential to enhance the activity of

gut microflora and promote the production of beneficial metabolites like SCFA, thereby improving gastrointestinal health.

The latter authors demonstrated that the inclusion of 1% and 2% *Agaricus bisporus* in broiler diets resulted in improved feed efficiency without affecting feed intake. Additionally, broilers fed 2% *A. bisporus* showed increased body weight and weight gain on day 42, which was partly consistent with our results. Another study reported that weaned piglets fed diets with 0.1% AB extract showed an 11% increase in productivity (Maribo, 2004). Similarly, mice fed with AB also showed improved performance, and AB addition increased villous height and V/C ratio in the small intestine. These studies implied that AB could be vital in enhancing villous development, consequently improving intestinal absorption (Shen et al., 2012).

The present work demonstrated that dietary ABS supplementation did not affect the antioxidant capacity of broiler serum. A previous study (Wang et al., 2013) reported that AB ethanolic extracts exhibited strong reducing power, inhibition of lipid peroxidation (LPO), scavenging capacity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, and ferrous ion chelating capacity. Similarly, Jia et al. (2013) found that five polysaccharides obtained from AB using various methods, involving hot water, single-enzyme extraction, and compound-enzyme extraction exhibited antioxidant activity in a concentration-dependent manner. All extracts showed scavenging activity towards hydroxyl radical, DPPH radical, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical, indicating their potential as natural antioxidants. It is important to recognize that *in vitro* studies provide valuable insights into the antioxidant properties of substances. However, translation of these findings to *in vivo* experiments and their effects on living organisms may not always align.

Inflammation can have both beneficial and detrimental effects on the body. While it can help combat pathogens and initiate the healing process, an excessive or prolonged inflammatory response can lead to cell damage and tissue dysfunction, particularly when it occurs in situations where it is not necessary or desired, such as normal physiological conditions (Lee et al., 2019). Administration of diets with ABS to broilers could result in a decrease in IL-1β level. The anti-inflammatory effect exerted by ABS may be attributed to the presence of polysaccharides in ABS. Polysaccharides are the main bioactive substances in AB and are known to possess antioxidant, anti-inflammatory, anti-hyperlipidaemic, and immune properties (Da Silva et al., 2013). Human studies have also shown that consumption of AB extracts can lead to a decrease in serum levels of TNF-α, IL-1β, IL-2, and IL-17 (Johnson et al., 2009). AB contains high levels of β-glucan, and dietary supplementation with this compound could reduce the levels of proinflammatory cytokines. Previous studies have even suggested that a reduced immune response may enhance nutrient utilisation and promote accelerated tissue growth (Tzianabos, 2000; Ohno et al., 2001).

Concentrations of serum TG, TC, HDL, and LDL are commonly used indicators of lipid metabolism (Dechesne et al., 2016). We observed that dietary ABS supplementation decreased serum TC concentration in broilers. In a study by Li et al. (2020b), AB polysaccharides decreased serum TC levels in hyperlipidaemic rats. The possible mechanism behind this effect could be an increase in the expression of 7α-hydroxylase (CYP7A1) protein after the administration of AB polysaccharides. As previous studies have indicated, CYP7A1 is an enzyme limiting the transformation of cholesterol into bile acids in the liver and plays a central role in maintaining the dynamic balance of cholic acid and cholesterol (Lee et al., 2018; Hasegawa et al., 2019). Additionally, Wei et al. (2019) have reported that AB extracts have a positive regulatory effect on blood lipids.

We observed that ABS supplementation did not affect SCFA contents in broilers. A possible reason could be that ABS contains more insoluble fibre. Angkanaporn et al. (1994) reported that the indigestible dietary fibre components could be subjected to limited fermentation and, therefore, exert insignificant effect on SCFA contents in the caeca of broilers. However, other study indicated that dietary fibre could modulate caecal SCFA concentrations (Lin and Olukosi, 2021). The production of SCFA in the caeca in chickens is influenced by microbial colonisation, nutrient flow, and gut maturation. Broilers, compared to other species, exhibit greater variability and lower efficiency in extracting energy from non-starch polysaccharides, which can be attributed to the absence of fibre-digesting enzymes and their relatively short gastrointestinal tract and faster transit time of digesta (Walugembe et al., 2015). Considering these factors, it is plausible that different dietary conditions, such as the composition and digestibility of fibre, could contribute to the discrepancy observed in SCFA contents between studies.

A certain amount of dietary fibre is necessary for normal physiological function of the intestine in

poultry (González-Alvarado et al., 2007). The effects of dietary fibre can vary based on factors such as particle size, chemical structure, and supplementation level. One of the key indicators of intestinal health is the morphology of the intestinal lining, including parameters such as villus height, crypt depth, and the V/C ratio. These morphological indices are closely associated with the intestinal absorption capacity and can have a significant impact on the growth performance of animals (Pluske et al., 1996; Montagne et al., 2003). Changes in gut morphology have been associated with higher tissue turnover or the presence of toxins, resulting in shorter villi and deeper crypts (Miles et al., 2006). In the current study, ABS supplementation could have improved small intestine morphology. Fard et al. (2014) also observed that the villus height of jejunum in broilers (Ross 308) was significantly increased after dietary addition of 1% and 2% oyster mushroom waste.

Meat quality yield of broilers, including parameters such as water holding capacity, pH value, and meat colour, did not show significant differences between the three treatments in the present work. This finding was consistent with a study conducted by Moon et al. (2016), where the addition of dietary β-glucan at different concentrations did not affect these meat quality characteristics in chicken breast muscle. The intestinal microbiota plays a crucial role in the growth and health of broilers, and its composition can be influenced by dietary factors. The relationship between dietary carbohydrates and bacterial populations in the gut has been extensively studied (Yang et al., 2009). Polysaccharides present in the diet can be degraded by the gut microbiota, leading to the production of metabolites, primarily SCFA. The latter serve as an energy source for intestinal epithelial cells and play a role in regulating microbial diversity, pH value, intestinal peristalsis, and barrier function (Li et al., 2020a).

In the current study, the addition of ABS could decrease the abundance of *Bacteroidota* and increase the number of *Firmicutes* in the gut of broilers. It has been observed that higher abundance of *Firmicutes* is positively correlated with animal obesity, whereas *Bacteroides* are associated with host body maintenance (Simpson and Campbell, 2015). Simpson and Campbell. (2015) have also demonstrated that dietary fibre could promote *Bacteroides* proliferation and reduce the population of *Firmicutes* in the gut of mammals. This result is contradictory to our findings. The discrepancy may be partly due to differences in digestion and absorption between chickens and mammals.

According to the results of the linear discriminant analysis effect size (LEfSe) method, ABS supplementation could promote the proliferation of beneficial bacteria, many of which exhibit anti-inflammatory, antioxidant, and SCFA-producing abilities. The family *Ruminococcaceae* are SCFA producers (Bonvegna and Cilia, 2023); *Lachnospira, Erysipelotrichaceae,* and *Candidatus_Soleaferrea* can be considered as potential probiotics (Bian et al., 2020; Zhang et al., 2022; Zou et al., 2022); *Turicibacter* has been found to display anti-inflammatory activity related to SCFA production and associated with glucolipid metabolism (Wu et al., 2020); the family *Peptostreptococcaceae* has the ability to ferment polysaccharides and produce SCFA (Bernad-Roche et al., 2021), while *Romboutsia* is beneficial bacterium, and its metabolites have been shown to have anti-inflammatory, antioxidant, and immune-protective properties (Zhang et al., 2022). We conclude that bacteria with anti-inflammatory activity may also play a role in reducing serum IL-1β levels in broilers fed ABS diets.

Conclusions

Dietary supplementation of *Agaricus blazei* Murill stipe (ABS) resulted in a decrease in feed convertion ratio, as well as serum interleukin-1β and total cholesterol concentrations. Broilers fed ABS diets also showed higher microbial diversity in the caecum, enhanced proliferation of beneficial bacteria, and improved the villus height to crypt depth ratio in the jejunum. Based on these findings, it can be inferred that ABS has the potential to be applied as a viable feed ingredient for broilers. Its supplementation can improve feed efficiency, reduce inflammation, lower cholesterol levels, enhance microbial diversity, and promote a healthier gut environment.

Data availability statement

The raw sequence data generated in this study have been deposited in the Genome Sequence Archive in National Genomics Data Center (accession number: CRA011364). The data can be found at: https://bigd.big.ac.cn/gsa/browse/CRA011364.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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