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Original article

Effect of diet supplemented with microbe-derived antioxidants on plasma oxidative stress, biochemical parameters and fecal microbiota in Beagle dogs

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Abstract

The present study aimed to explore the effect of dietary microbe-derived antioxidants (MA) supplementation on plasma biochemical parameters, oxidative stress and fecal microbiota in Beagles dogs. Results showed that different dose of MA supplementation did not affect body weight (BW) of dogs after 30 days feeding. 1.5 g/kg MA (HMA) treatment decreased the content of plasma total antioxidant capacity (T-AOC) and activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), increased contents of malondialdehyde (MDA), total cholesterol (T-CHO), total protein (TP) and creatine kinase MB (CKMB) compared with control (CON) group. 1 g/kg MA (MMA) decreased the content of triglyceride (TG), and activity of plasma AST compared with CON group at day 30 (D30). 0.5 g/kg MA (LMA) decreased the contents of malondialdehyde (MDA) at day 14 (D14) and T-AOC at day 7 (D7), and T-CHO at D14 and D7 compared with CON group. Furthermore, in HMA, activities of alanine aminotransferase (ALT) and AST, contents of T-AOC, T-CHO, urea nitrogen (BUN) and direct bilirubin (DBil) decreased at D30 compared with D14 and D7. Consumption of HMA increased the fecal bacterial evenness and modulated microbial profiles at D14 compared with D7. Taken together, appropriate supplementation of MA is a promising candidate to improve nutrients metabolism, hepatic function and fecal microbiota in beagle dogs.

Keywords: dogs, fecal microbiota, microbe-derived antioxidants, oxidative stress

Introduction

Dogs are pets and companion animals that share living environment and food with humans in modern society. However, the prevalence survey of pet obesity reported that about 60% of dogs were overweight or obesity (Ronja et al. 2021). The increasing populations and prevalence of metabolic diseases highlight health, quality of life and well-being of dogs during last decades (Chandler et al. 2017, Xu et al. 2022). Inclusion of functional ingredients such as prebiotics, probiotics, and active compounds in pet foods has become a widespread practice to provide nutritional and health benefits (Di Cerbo et al. 2017, Tanprasertsuk et al. 2022). However, their efficacy, potential action, optimal dosages and application are not completely understood in dogs.

Gut microbiota play an important role in nutrients metabolism, immune systems maintenance and host physiologic regulation through metabolites such as short-chain fatty acids (SCFAs), biogenic amines, neurotransmitters, etc (Flint et al. 2012). The disturbances of gastrointestinal microbiota may lead to many diseases such as oral diseases, diarrhea, gastrointestinal disorders and metabolic syndrome in dogs (Kil and Swanson 2011). Furthermore, the microbial composition of canine is closer to that of humans compared with pigs and mice, which can be used to predict the outcome in human (Coelho et al. 2018), indicating that canine is an important medical model to human diseases. Of note, fecal samples from dogs provide better representation of bacterial taxa unlike human, because of their shorter gastrointestinal tract and fewer mucosa-associated microbiota (Pilla and Suchodolski 2020). Indeed, many of active ingredients in pet foods were designed to beneficially modulate gut microbiota and metabolites, and support gastrointestinal health (Finet et al. 2022, Koziol et al. 2023). Microbe-derived antioxidants (MA) are the blend of Sea buckthorn and Rosa roxburghii fermented by probiotics, containing hundreds of bioactive ingredients such as phenolic acids and flavonoid, which have good 1, 1-diphenyl-2- picrylhydrazyl (DPPH), O_2^-, OH^- and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$) radical scavenging capacity (Luo et al. 2022), anti-inflammatory ability (Shen et al. 2022, Shen et al. 2024), and regulate equilibrium of lipid metabolism (Gao et al. 2023, Yu et al. 2024). However, the application of MA in Beagle dogs, and its effect on physiological parameters and fecal microbiota also remains unknown. Thus, the present study aimed to determine the effect of different concentrations of MA on plasma oxidative stress, biochemical parameters and fecal microbiota in beagle dogs.

Materials and Methods

Animal care

This present experiment was approved by the guidance of Animal Care and Use Committee of Shanghai Vocational College of Agriculture and Forestry (NL. No2023KY001).

Animal and diets

The experiment was conducted in the animal training room from training base of Shanghai Vocational College of Agriculture and Forestry (Songjiang District, Shanghai). MA (liquid) are produced by multistage complex fermentation of the blend of Rosa roxburghii and Sea buckthorn by Bacillus Subtilis, Lactobacillus, Clostridium Butyricum, and Saccharomyces cerevisiae (Shanghai Jiang Han Biotechnology). The main ingredients of MA including flavonoids, polyphenol, phenolic acids, amino acids and derivatives, and minerals were determined in our previous study (Luo et al. 2022). A total of 24 healthy Beagle dogs (10 to 13 kg, 5 years old) were selected and assigned into 4 groups (n=6): the CON group, 0.5 g/kg MA (LMA), 1 g/kg MA (MMA) and 1.5 g/kg MA (HMA). Two dogs were raised in one rearing cage (2 m × 2 m × 3 m). The LMA, MMA and HMA groups were fed with basal diet supplemented with different dose of MA by mixing with chicken oil for spraying after granulation and bulking, respectively. The experimental diet was formulated and provided by Shanghai Shilin Biotechnology according to the nutritional requirements of national standard (GB/T31216-2014). The mean temperature was kept at 25 ± 3°C during the experimental period. The kennel was cleaned once a day, and feed intake was recorded daily. The dogs were fed twice daily (08:00 am and 16:00 pm each day) and had free access to drinking water. The experiment was lasted for 30 days. At the experimental period of Day 7 (D7), D14 and D30, dogs under fasting state were weighed and about 5 mL of blood from hind leg vein were collected into the heparin sodium pro-coagulant tubes. The plasma was collected after centrifuging at 3,000 *g* for 15 min at 4°C. The fresh feces were collected and immediately stored in -80°C.

Automatic biochemical analyzer

The plasma contents of urea nitrogen (BUN), uric acid (UA), total protein (TP), albumin (ALB), creatine kinase (CK), creatine kinase MB (CKMB), lactate dehydrogenase (LDH), total bilirubin (TBil), and direct bilirubin (DBil) were detected by automatic biochemical analyzer (IDEXX Catalyst One Analyzer, USA).

Table 1. Body weight and feed intake in experimental dogs.

Item	Treatments				*p*-value		
	CON	LMA	MMA	HMA	L[1]	Q	C
N (male: female)	6 (2:4)	6 (4:2)	6 (6:0)	6 (2:4)			
BW D7 (kg)	12.008 ± 1.094	11.308 ± 0.656	13.317 ± 1.577^{ab}	10.058 ± 0.91^{2}c	0.449	0.263	0.124
BW D14 (kg)	12.733 ± 0.998	12.205 ± 0.823	13.833 ± 1.4284^{b}	11.075 ± 0.834^{b}	0.484	0.301	0.179
BW D30 (kg)	13.800 ± 0.964	12.780 ± 0.843	14.317 ± 1.479^{a}	11.925 ± 0.845^{a}	0.401	0.527	0.189
Daily feed intake (g)	550.374± 3.455	543.023± 3.189	543.733 ± 3.552	551.748 ± 2.546	0.685	0.008	0.949

BW, body weight. Data were presented as mean ± SEM (n = 6). Different lowercase indicates significance by paired T test along with time. Different capital indicates significance between dietary treatments by ANOVA followed by Duncan.
[1] linear (L), quadratic (Q), and cubic (C) effect of MA.

Oxidative stress parameters, aminotransferases and lipid profiles determination

The plasma oxidative stress parameters, malondialdehyde (MDA) and T-AOC, hepatic function indices aspartate aminotransferase (AST) and alanine aminotransferase (ALT), lipid indices triglyceride (TG) and total cholesterol (T-CHO) were determined. Changes in absorbance at 530, 405, 510, 510, 510 and 510 nm were recorded, according to the manufacturers' instructions (Nanjing Jiancheng Bioengineering Institute, China), as previously described (Luo et al. 2019).

16S RNA sequence

Fecal genome DNA was extracted and monitored on 1% agarose gels for purity. All PCR reactions were conducted and amplified using the specific primer (16S V3-V4/18S V9 rRNA genes). The products of PCR electrophoresed in 2% agarose gel. Bright main strip between 400-450bp were chosen and purified with Gel Extraction Kit (AXYGEN, USA). Sequencing libraries were generated for Illumina (NEB Next®Ultra™ DNA Library Prep Kit, NEB, USA), assessed and sequenced on an Illumina Miseq/ /HiSeq2500 platform. 250bp/300bp paired-end reads were generated, merged using FLASH and assigned to each sample according to the unique barcodes.

Sequences analysis were performed by UPARSE software package and higher than 97% similarity of sequences were assigned to the same OTUs. The representative sequences for each OTU were picked and annotated taxonomic information using the RDP classifier. The relative abundance of bacterial diversity from phylum to species can be visualized using Krona chart. Cluster analysis was preceded by principal coordinate analysis (PCoA) using the QIIME software package. LDA Effect Size (LEfSe) was used to identify the most differentially abundant microbiota taxa.

Statistical analysis

The data in the same dietary treatment were analyzed with one-way analysis of variance (ANOVA) followed by paired sample T test, while the data among different dietary treatments were analyzed by independent sample T-test or ANOVA followed by Duncan's post hoc test depending on the treatments using the statistical software SPSS 20.0 (SPSS Inc., Chicago, US). Data were presented as mean ± SEM. $p<0.05$ was considered statistically significant.

Results

Food intake and body weight (BW)

The BW and daily feed intake of dogs were not significant among dietary treatments in Table 1 ($p>0.05$). But the BW increased significantly ($p<0.05$) at D30 compared to that at D14 and D7 under MMA and HMA.

Oxidative stress, lipid profiles and hepatic function

The content of plasma MDA was lower in LMA at D14, but higher in HMA at D7 compared with CON group (Table 2). The plasma content of T-AOC was significantly decreased ($p<0.05$) at D30 compared with D7 and D14 in HMA. The plasma content of T-AOC was decreased ($p<0.05$) at D7 in LMA and HMA compared with CON group, but there was no difference ($p>0.05$) between dietary treatment at D14 and D30. The plasma TG content was significantly decreased ($p<0.05$) in LMA and MMA at D30 compared to that determined at D7. Also, the plasma TG content was significantly decreased in MMA at D30 compared with CON group ($p<0.05$). The plasma T-CHO content was significantly decreased in HMA at D30 compared with D7 and D14. Also, the plasma T-CHO content was significantly decreased ($p<0.05$) in LMA at D14 compared with CON group. The levels of plasma ALT and AST in HMA were significantly decreased ($p<0.05$) at D30 compared with D7 and D14. The activity of plasma

Table 2. The oxidative stress parameters, lipid profiles and hepatic function determined in dogs.

Item	Treatments				*p*-value		
	CON	LMA	MMA	HMA	L[1]	Q	C
MDA (nmol/mL)							
D7	9.611±0.702^{B}	7.172±0.727^{B}	7.888±0.589^{B}	19.789±1.570^{A}	0.000	0.000	0.006
D14	11.762±1.209^{AB}	8.444±0.779^{C}	9.127±0.585^{BC}	12.524±1.228^{A}	0.685	0.022	0.534
D30	8.646±0.507^{AB}	6.092±0.346^{B}	9.389±1.067^{AB}	15.837±2.325^{A}	0.010	0.071	0.990
T-AOC (mM)							
D7	0.508±0.026^{aA}	0.396±0.032^{abB}	0.448±0.022^{AB}	0.412±0.033^{aB}	0.082	0.206	0.065
D14	0.404±0.037^{ab}	0.420± 0.011^{a}	0.381±0.047	0.370±0.050^{ab}	0.434	0.738	0.637
D30	0.324±0.034^{b}	0.302± 0.036^{b}	0.399±0.023	0.302±0.036^{b}	0.838	0.259	0.044
TG (mmol/L)							
D7	0.714±0.080	1.305± 0.175^{a}	0.958±0.178^{a}	1.643±0.297^{a}	0.011	0.803	0.738
D14	0.990±0.105	0.692± 0.109^{b}	1.186±0.274^{a}	1.032±0.200^{b}	0.614	0.835	0.075
D30	0.718±0.115^{A}	0.547±0.051^{bAB}	0.399±0.045^{bB}	0.725±0.084^{abA}	0.779	0.003	0.178
T-CHO (mmol/L)							
D7	6.055±0.414^{bB}	6.009± 0.296^{B}	6.440±0.375^{AB}	7.423±0.433^{abA}	0.015	0.194	0.965
D14	7.119±0.643^{aA}	5.430± 0.229^{B}	6.442±0.552^{AB}	7.704±0.484^{aA}	0.231	0.008	0.287
D30	5.940±0.371^{ab}	5.223± 0.292	6.082±0.566	6.417±0.305^{b}	0.203	0.192	0.242
AST (U/L)							
D7	15.174±1.028	18.398±2.026	14.501±1.734	19.646±1.661^{a}	0.290	0.682	0.035
D14	15.629±1.885	20.710±1.843	17.907±2.845	16.479±1.473^{ab}	0.976	0.095	0.278
D30	14.669±0.944^{A}	17.568±0.240^{AB}	13.924±1.014^{B}	11.894±1.497^{bB}	0.014	0.019	0.098
ALT (U/L)							
D7	23.965±2.350	23.095±2.380	30.852±6.528	30.392±7.204^{a}	0.137	0.756	0.527
D14	22.799±2.926	20.461±2.854	31.986±6.726	24.013±3.819^{ab}	0.378	0.463	0.061
D30	21.855±2.575	22.971±3.027	27.800±6.229	14.593±4.683^{b}	0.502	0.110	0.250

CON, control; LMA, 0.5 g/kg MA; MMA, 1 g/kg MA; HMA, 1.5g/kg MA. Data were presented as mean ± SEM (n = 6). Different lowercases indicate significance ($p<0.05$) along with time by paired T test. Different capitals indicate significance ($p<0.05$) between dietary treatments by ANOVA followed by Duncan.

[1] linear (L), quadratic (Q), and cubic (C) effect of MA.

AST were significantly decreased ($p<0.05$) in MMA and HMA at D30 compared with CON group.

Plasma biochemical indexes

The plasma biochemical indexes between HMA and CON groups were further determined (Table 3). The contents of UA, ALB, CK and TBil were not affected by dietary treatments and treatment time ($p>0.05$) (data not shown). The content of plasma BUN were significantly decreased ($p<0.05$) at D30 compared with D7 and D14, but there was no significant difference between CON and HMA groups ($p<0.05$). The plasma content of TP was significantly increased in HMA at D14 compared with D7, and increased in HMA compared with CON group at D14 ($p<0.05$). The content of CKMB was higher in HMA than that in CON group at D7 ($p<0.05$), while the activity of LDH was lower in HMA than that in CON group at D7 ($p<0.05$). The content of DBil was lower in HMA at D30 compared with D7 and D14 ($p>0.05$).

Fecal microbioal richness and diversity

Six fecal samples from HMA at D7, D14 and D30 were sequencing to investigate the microbial structural change. A Venn diagram showed that 188 OTUs are shared among these three groups (Fig. 1A). The data revealed that good's coverage was >99.5% for the three groups. Alpha diversity indices such as Shannon and Simpson were not significantly different between D7, 14 and 30 ($p>0.05$) (data not shown). Microbial richness indices Chao1 index was significantly higher ($p<0.05$) at D14 (186.368 ± 6.823) than that at D7 (157.31 ± 13.338) and D30 (165.754 ± 8.865). Beta diversity analysis were listed in different clusters according to PCoA among D7, 14 and 30 based on the unweighted UniFrac metrics (Fig. 1B-1D).

Fecal microbial composition and function

At the phylum level, the dominant microbiota in feces of dogs are *Firmicutes*, *Bacteroidetes*, *Fusobacteria*,

Table 3. Plasma biochemical indexes determined in dogs.

	Reference range	CON	HMA	*p*-value
BUN (mmol/L)	2.1-10.7 mmol/L			
D7		17.645 ± 1.582^{a}	14.571 ± 2.420^{a}	0.260
D14		15.675 ± 1.497^{a}	18.043 ± 0.858^{a}	0.183
D30		7.373 ± 0.673^{b}	7.324 ± 0.522^{b}	0.816
TP (g/L)	52-82 g/L			
D7		63.825 ± 2.432	62.795 ± 2.507^{b}	0.774
D14		62.043 ± 1.141^{B}	69.165 ± 1.902^{aA}	0.009
D30		62.442 ± 2.596	75.272 ± 6.343^{ab}	0.091
CKMB (U/L)	26–310 U/L			
D7		73.345 ± 4.600^{B}	103.92± 9.945^{A}	0.003
D14		81.976 ± 7.738	96.589 ± 10.222	0.178
D30		71.604 ± 5.362	85.582 ± 8.187	0.058
LDH (U/L)	50-495 U/L			
D7		117.25 ± 18.299^{B}	107.5 ± 6.800^{A}	0.614
D14		111.333 ± 14.579	117.150 ± 24.251	0.725
D30		108.283 ± 12.318	110.100 ± 12.646	0.904
DBil (μmol/L)	1.7-5.1 μmol/L			
D7		23.303 ± 5.159	27.531 ± 0.507^{a}	0.082
D14		13.100 ± 2.830	18.057 ± 1.542^{a}	0.111
D30		23.435 ± 5.909	12.352± 5.6620^{b}	0.437

Abbreviations: BUN, Urea nitrogen; UA, uric acid; TP, total protein; CK, creatine kinase; CKMB, creatine kinase MB; LDH, lactate dehydrogenase; DBil, direct bilirubin; Data were presented as mean ± SEM (n=6). Different lowercases indicate significance ($p<0.05$) along with time by paired T test. Different capitals indicate significance ($p<0.05$) between dietary treatments by independent sample T-test.

Actinobacteria, *Proteobacteria*, *Epsilonbacteria*, *Deferribacteres*, *Verrucomicrobia*, *Cyanobacteria*, *Acidobacteria* and others (Fig. 1E). At the genus level, the dominant microbiota in fecal of dogs are *Lactobacillus*, *Veillonella*, *Peptoclostridium*, *Prevotella.9*, *Fusobacterium*, *Blautia*, *Bifidobacterium*, *uncultured*, *Romboutsia*, *Streptococcus* (Fig. 1F). LEfSe analysis revealed that 2 order (*Verrucomicrobiales* and *Selenomonadales*), 2 family (*Akkermansiaceae* and *Veillonellaceae*), 3 genus (*Akkermansia*, *Solobacterium* and *Veillonella*), 2 class (*Verrucomicrobiae* and *Negativicutes*), 1 phylum (*Verrucomicrobia*) were enriched at D14, while 2 order (*Bacillales* and *Enterobacteriales*) and 1 family (*Enterobacteriaceae*) were enriched at D7 (Fig. 1G). No taxa were significantly enriched between D7 and D30. Also, LEfSe analysis revealed that 2 family (*Xanthomonadaceae*, *Veillonellaceae*), 3 genus (*Vulcaniibacterium*, *Cupriavidus* and *Veillonella*), 2 order (*Xanthomonadales* and *Selenomonadales*) and 1 class (*Negativicutes*) were enriched at D14, while 2 genus (*Turicibacter* and *Staphylococcus*), 2 family (*Bacillaceae* and *Staphylococcaceae*) and 1 order (*Bacillales*) were enriched at D30 (Fig. 1H).

Discussion

Evaluating plasma oxidative stress and biochemical parameters is a valuable tool for assessing dog health. MDA reflected the degree of lipid peroxidation mediated by O_2^- (Del Rio et al. 2005). Aminotransferases were indicators of liver cell injury and acute hepatocellular diseases (Hyder et al. 2013). Bilirubin (including TBil, IBil, and DBil) were also conventional liver function indices. An elevated level of DBil may also suggest damage to the hepatic cells, biliary obstruction and hepatitis since DBil was found to be more important than TBil and IBil for metabolic syndrome (Jo et al. 2011). In this study, MDA content was lower in LMA at D14 compared with CON, but its content was higher in HMA at D7 compared with CON. AST, ALT and DBil contents were decreased in HMA at D30 compared with D7. These results suggest that although high-dose MA promoted lipid peroxidation at D7, it could effectively improve hepatic function and health in dogs at D30. Of note, many other indices such as albumin, globulin, ALP and GGT, are also very important indicators of hepatic function. Further study are need to explore the effect of MA on these indices in a large sample size.

In addition, TG content was decreased in LMA and

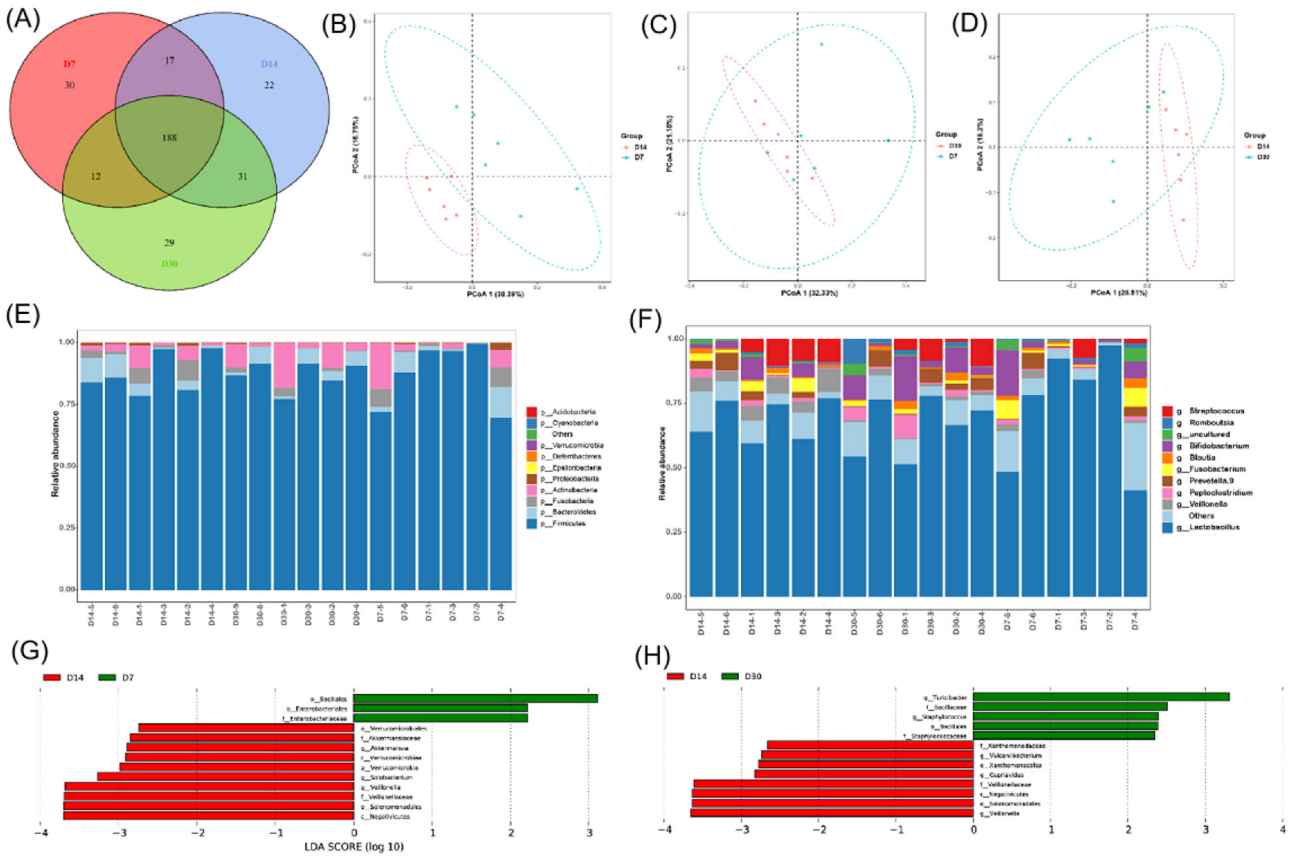

Fig. 1. Effects of 1.5 g/kg MA (HMA) treatment at D7, D14 and D30 on fecal microbial communities and composition in dogs. (A) 188 OTUs Venn diagram; (B-D) Principal coordinates (PCoA) analysis of the microbial communities among D7, 14 and 30 in feces of dogs based on the unweighted UniFrac metrics; (E-F) The intestinal microbiota composition at the phylum and genus levels; (G-H) LEfSe analysis of the differentially abundant microbiota taxa between D14 vs D7, D14 vs D30.

MMA at D30, and in HMA at D14 compared with D7, respectively, suggesting MA was effective to attenuate lipid deposition in dogs. This was consistent with our previous studies, which showed that MA decreased HFD-induced lipid disorders and oxidative stress, and decreased body weight in mice (Gao et al. 2023). Of note, TG content was higher in the HMA than that in LMA and MMA at D30. BW was increased in MMA and HMA at D30 compared with D14 and D7. Previously, MA increased the average daily gain and average daily feed intake, and serum and hepatic total cholesterol in weaned-induced piglets (Yu et al. 2024). The above results indicated that MA probably regulated metabolic balance under different experimental conditions and models, which may be related to synergistic effects of multiple substances including flavonoids, polyphenol, phenolic acids, amino acids and derivatives, and minerals in MA. However, the specifically synergistic mechanism of these different compounds needs further study.

Plasma TP was responsible for maintaining osmotic pressure and pH equilibrium, transporting various metabolites and exerting nutritive function. In this study, TP was increased in HMA at D14 compared with CON group, and at D30 compared with D7, indicating that high dose of MA is able to provide nutritive function since more than 400 compounds including flavonoids, polyphenol, phenolic acids, amino acids and derivatives, have been identified in MA according to our previous study (Luo et al. 2022). BUN was synthesized from hepatic ornithine cycle and removed by renal glomerular filtration, representing renal structural integrity (Jia et al. 2019). CKMB was found almost exclusively in the myocardium. The increased CKMB in blood was highly specific for myocardial cell injury (Cabaniss 1990). LDH mediated the conversion of pyruvate to lactate and its high level was typically associated with a poor prognosis in many solid tumors (Claps et al. 2022). In this study, CKMB increased, LDH decreased and BUN was not affected in HMA compared with CON group at D7, suggesting high dose of MA had little toxic effects on organ function in dogs except for myocardial cell, which need further investigation.

Gastrointestinal microbiota involved in nutrients metabolism and the maintenance of immunological functions, was specific and complex. In this study, Chao1 was significantly increased at D14 than D7, demonstrating an increased microbial richness and

α-diversity after HMA treatment. Indeed, increased alpha diversity was positively correlated with gut health, whereas decreased diversity was linked with disease (Pickard et al. 2017). Previous study reported that dogs with acute diarrhea lowered microbial diversity and beneficial bacteria, increased pathogenic bacteria in feces (Guard et al. 2015). Furthermore, 3 genus (*Akkermansia*, *Solobacterium* and *Veillonella*) were enriched at D14 compared with D7, while *Enterobacteriaceae* family and *Enterobacteriales* order were enriched at D7 compared with D14 in HMA. 3 genus (*Vulcaniibacterium*, *Cupriavidus* and *Veillonella*) were enriched at D14 compared with D30, while 2 genus (*Turicibacter* and *Staphylococcus*) were enriched at D30 compared with D14. *Veillonella*, the gram negative and obligate anaerobes, could co-aggregate with lactic acid bacteria since lactate served as the main carbon and energy source for *Veillonella*. *Akkermansia* genus were potential mucus degrading strain and promising probiotic. Decreased abundance of *Akkermansia muciniphila* was related to multiple diseases such as obesity, diabetes and inflammation (Cani et al. 2022). The genus *Solobacterium* was classified in the family *Erysipelotrichaceae* within the phylum *Firmicutes*. Diets containing Yucca schidigera extract was reported to improve intestinal health and increase *Solobacterium* in the ileum of young pigeons (Sun et al. 2023). The increased *Enterobacteriaceae* family and *Enterobacteriales* order were potentially associated with a higher risk of neuropsychiatric disorders (Zhuang et al. 2020). *Turicibacter* was reported to regulate host bile acids and lipid metabolism (Lynch et al. 2023). All these results suggest that dietary HMA supplementation at D30 and D14 improves gut health and metabolism of dogs through promoting potential beneficial bacteria and inhibiting pathogenic bacteria compared with D7. Of note, the baseline of microbial composition before the introduction of antioxidant feed was not considered, which is the limitation in our study.

Conclusion

Dietary high dose of MA supplementation improved nutrients metabolism, hepatic function and fecal microbiota in beagle dogs at D30 and D14, but had negative effect on oxidative stress parameters at D7, indicating that the appropriate supplementation of MA is a promising candidate for pet food.

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