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Dietary supplementation of *Yucca schidigera* and *Gleditsia amorphoides* modulates gut fermentation metabolites, antioxidant status, and inflammatory markers in adult dogs

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Abstract: Saponins are bioactive compounds known for modulating potential inflammatory metabolites produced by the gut microbiome, including ammonia. This study evaluated the effects of the saponin sources, *Yucca schidigera* (7% saponins) and *Gleditsia amorphoides* (21% saponins), on intestinal functionality, antioxidant, and inflammatory markers in adult dogs. Eighteen Beagle dogs (10 males and eight females), with 12.20 ± 1.33 kg body weight were randomly divided into three groups: Control (no saponins), 200 g *Yucca schidigera*/ton of diet (Yucca), and 200 g *Gleditsia amorphoides*/ton of diet (Gleditsia), totaling six replications per treatment. Dogs were fed experimental diets for 20 days. Fresh fecal samples were collected on day 20 to analyze: dry matter, score, pH, ammonia, short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), and biogenic amines. Blood was also collected on day 20 to analyze inflammatory cytokines, antioxidant enzymes, and liver enzymes. Preliminary results showed that the Gleditsia group had higher fecal propionate concentrations and lower histamine concentrations ($P < 0.05$). Both the Yucca and Gleditsia groups presented lower fecal concentrations of ammonia and spermine than the Control group ($P < 0.05$). Additionally, the Yucca and Gleditsia groups showed lower lipid peroxidation and higher catalase activity, whereas only the Gleditsia group showed lower alkaline phosphatase activity than the Control group ($P<0.05$). In conclusion, Yucca and Gleditsia can modulate fecal fermentative metabolites and improve the antioxidant status of dogs. These findings also showed the safety of using Yucca and Gleditsia during the experimental period.
Key-words: Ammonia, Propionate, Saponin extracts.

1. Introduction

Saponins, a class of bioactive compounds found in plants, are recognized for their emulsifying properties and modulatory effects on gut microbiome and its metabolites (Zhang et al., 2023). *Yucca schidigera* extract, the most common saponin source in pet food, has demonstrated efficacy in reducing fecal ammonia concentrations and odor in dogs (Dos Reis et al., 2016). However, *Gleditsia amorphoides* has emerged as a promising alternative to yucca due to its higher saponin concentrations (22% vs. 7–15% in yucca) and additional bioactive components, including galactomannans (with prebiotic functions) and polyphenols (with antioxidant activity) (Lu et al., 2024).

Gleditsia amorphoides (Fabaceae family) is a woody species native to temperate and subtropical regions, traditionally valued for its timber and industrial uses (Cerino et al., 2018). Despite its widespread availability and promising properties, only one in vitro study investigating the effects of gleditsia on the modulation of the human fecal microbiome was found (Wang et al., 2023). This study reported a beneficial shift in the fecal microbiome following gleditsia inoculation, characterized by an increase in bacteria with saccharolytic activity and a decrease in those with proteolytic activity. Additionally, functional analysis revealed elevated levels of metabolites linked to antioxidant and anti-inflammatory activities in feces inoculated with gleditsia (Wang et al., 2023). A study investigating the dietary inclusion of a saponin-rich extract (from *Quillaja saponaria*) also observed a significant reduction in fecal proteolytic bacterial populations in dogs (Zhang et al., 2023), thereby supporting the modulatory potential of saponins on the gut microbiome.

Although there is evidence supporting the potential physiological benefits of saponin sources, data on the effects of *Yucca schidigera* and *Gleditsia amorphoides* on gut function and systemic health parameters in dogs remain scarce. To address this knowledge gap, the present study aimed to evaluate the impact of dietary supplementation with *Yucca schidigera* and *Gleditsia amorphoides* on intestinal fermentation metabolites and systemic inflammatory and antioxidant biomarkers in adult dogs.

2. Materials and Methods

The use of animals for this study was approved by the Ethics Committee on Animal Use of the Agrarian Sciences Sector of the Federal University of Paraná, Curitiba, PR, Brazil, under protocol nº. 013/2024. The study was carried out at the Laboratory of Studies in Canine Nutrition (LENUCAN) in Curitiba, Paraná, Brazil (25º 25' 40" S, 49º 16' 23" W).

2.1. Animals and facilities

Eighteen adult neutered/spayed Beagle dogs (10 males and eight females), 2 years of age, with an average body weight of 12.20 ± 1.33 kg and a body condition score of 5 (scale of 1 to 9) were used. All animals underwent prior clinical evaluation and were considered healthy. The researchers and the veterinarian responsible for the laboratory supervised the animals throughout the experimental period. The dogs were individually housed in brickwork kennels (5 m long x 2 m wide), containing a bed and free access to fresh water. During most of the experiment, dogs had free access to an outdoor area of 1.137 m^2 for 5 h/day for voluntary exercise and socialization.

2.2. Experimental Diets

Dogs were randomly assigned to one of three experimental groups (n = 6/group): Control (no saponins), Yucca (200 g/ton *Yucca schidigera* extract), and Gleditsia (200 g/ton *Gleditsia amorphoides,* Sapcor®, Bioaromas do Brasil, Chapecó, SC). The *Yucca schidigera* extract contained at least 7% saponins, and the *Gleditsia amorphoides* extract contained at least 21% saponins, according to the manufacturers of both products. A commercial extruded dry diet formulated for adult dogs was used for the three treatments. Thus, the experimental treatments differ only in the addition or absence of saponin sources, which were included by coating the diet. The commercial diet used contained no functional additives, such as prebiotics, probiotics, or saponins. Its chemical composition was: 22% crude protein, 9.8% ether extract in acid hydrolysis, 3.1% crude fiber, and 9.9% ash.

The animals were fed the experimental diets for 20 days, twice a day (8:00 a.m. and 4 p.m.). The amount of food was calculated based on their metabolizable energy needs for body weight maintenance, as recommended by the NRC (2006). Dogs were weighed weekly, and the amount of food was adjusted as needed.

2.3. Experimental analyses

2.3.1. Fecal analysis

On day 20 of the experiment, fresh fecal samples were collected from each dog to assess fecal characteristics and metabolites of intestinal fermentation. Fecal samples were collected within 15 minutes of defecation and analyzed for dry matter (fDM), fecal score, pH, ammonia, short-chain fatty acids (SCFA - acetate, propionate, and butyrate), branched-chain fatty acids (BCFA – isobutyrate, isovalerate, and 4-metylvalerate), and other volatile fatty acids (VFA – valerate, heptanoic, and hexanoic), and biogenic amines. The fDM was determined at 105°C according to the AOAC recommendations (1995). Fecal score was assessed by a single researcher to ensure standardization, using a scale of 1 (loose stools, no shape) to 5 (dry, hard feces), as described by Carciofi et al. (2009). Fecal pH was measured using a digital pH meter (Politeste, model 331, São Paulo, Brazil) using 3 g of fresh feces diluted in 30 mL of distilled water. Fecal ammonia was determined according to Brito et al. (2010), for SCFA and BCFA, 10 g of feces was mixed with 30 mL of 16% formic acid, homogenized, and stored at 4°C for 3-5 days. After this period, the solutions were centrifuged at 2500 gx (2K15, Sigma, Osterode am Hans, NI, Germany) for 15 min. At the end of centrifugation, the supernatant was separated and subjected to further centrifugation. Each sample underwent three centrifugations, and at the end of the last one, part of the supernatant was transferred to an appropriately labeled Eppendorf tube for subsequent freezing at –14 °C. Afterward, the samples were thawed and subjected to a second centrifugation at 18000 gx for 15 min (Rotanta 460 Robotic, Hettich, Tuttlingen, BW, Germany). Both centrifugations were conducted under refrigeration (approximately 5 °C). The supernatant was collected and analyzed by gas chromatography (Shimadzu GC-2014, Kyoto, Japan) using a glass column (Agilent HP INNO wax, 30 m × 0.32 mm). Nitrogen was used as the carrier gas (3.18 mL/min), with working temperatures of 200°C in the injection, 240°C in the column, and 250°C in the detector. Biogenic amines were quantified as described by Urrego et al. (2017).

2.3.2. Blood analysis

On day 20 of the experiment, blood samples were collected from each dog to assess inflammatory and antioxidant markers and liver enzymes. Blood samples were collected after an 8-hour fasting period. After physical contention and antisepsis with 70% alcohol on the ventral region of the neck, 7 mL of blood was collected by jugular venipuncture. Blood samples for antioxidant and liver enzyme analysis were collected in heparinized syringes and transferred to citrate tubes. In contrast, those intended for cytokine evaluation were collected in syringes without heparin and stored in anticoagulant-free tubes. Cytokines interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor alpha (TNF-α) were quantified by the ProcartaPlex® multiplex immunoassay, which combines ELISA and flow cytometry via Luminex Xmap®. The antioxidant enzymes analyzed were catalase (CAT), glutathione S-transferase (GST), and reduced glutathione (GSH). Lipid peroxidation (LPO) and total antioxidant capacity (T-AOC) were also analyzed. Enzyme activities were determined according to standard methods: CAT (Aebi, 1984), GST (Habig et al., 1974), and GSH (Sedlak & Lindsay, 1968). The LPO was analyzed by the FOX method (Jiang et al., 1991), which quantifies lipid hydroperoxides based on iron oxidation and binding to xylenol orange dye. The T-AOC was analyzed according to Benzie & Strain (1996). Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were measured on an Olympus AU5400 automated analyzer (Olympus America, Center Valley, PA, USA).

2.4. Statistical analyses

Data were analyzed for normality using the Shapiro-Wilk test. Data with normal distribution were subjected to analysis of variance (ANOVA), totaling six repetitions per experimental group. When the ANOVA F-test indicated a difference ($P<0.05$), means were compared by the Tukey test ($P<0.05$). Nonparametric data (cytokine levels) were analyzed using the Kruskal-Wallis test ($P < 0.05$). P-values between 0.05 and 0.10 were considered a tendency.

3. Results

No adverse food reactions, such as vomiting, diarrhea, or food refusal, were observed throughout the study.

3.1. Fecal characteristics

No differences were observed among treatments in fecal score or pH (P > 0.05; Table 1). However, the Gleditsia group showed a tendency toward higher fDM (P=0.076; Table 1).

Item	Control	Yucca	Gleditsia	SEM	P
Dry matter (%)	31.25	31.47	32.74	0.13	0.076
Score	3.83	3.33	3.83	0.11	0.116
pH	6.97	7.24	6.66	0.13	0.197

Table 1 – Means of fecal characteristics of dogs in the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probability; Fecal score: 1 = liquid feces to 5 = dry feces.

3.2. Intestinal fermentation metabolites

Yucca and Gleditsia groups had lower fecal ammonia concentrations than the Control group (P < 0.05; Table 2). Fecal propionate and total SCFA concentrations were higher, and 4-methylvalerate was lower in the Gleditsia group than in the Yucca group (P<0.05, Table 2). There was a tendency toward higher fecal acetate concentrations in the Gleditsia group (P=0.057, Table 2). No differences were observed in fecal concentrations of other SCFA and BCFA (P>0.05, Table 2).

Item	Control	Yucca	Gleditsia	SEM	P
Ammonia (%)	0.15a	0.11b	0.10b	0.01	0.012
SCFA (µmol/g)					
Acetate	174.60	130.10	194.60	11.60	0.057
Propionate	50.01b	38.51b	67.93a	3.84	0.001
Butyrate	14.55	12.51	14.63	0.78	0.481
Total SCFA	239.16ab	181.12b	277.16a	15.30	0.024
BCFA (µmol/g)					
Isovalerate	5.64	5.44	5.13	0.10	0.110
Isobutyrate	5.34	5.33	4.98	0.07	0.054
4-methylvalerate	0.55ab	0.63a	0.51b	0.02	0.011
Total BCFA	11.53	11.40	10.62	0.21	0.886
Other VFA ((µmol/g)					
Valerate	5.15	4.99	5.05	0.04	0.244
Heptanoic	5.12	5.02	5.40	0.09	0.239
Hexanoic	1.22	1.19	1.79	0.12	0.068
Total VFA	11.49	11.20	12.24	0.19	0.334

Table 2 – Means (based on dry matter) of fecal concentrations of ammonia, short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), and other volatile fatty acids (VFA) in dogs from the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probability.
[a,b]Means followed by different letters differ according to Tukey's test (P<0.05).

Regarding biogenic amines, fecal concentrations of histamine and spermine were lower in the Gleditsia group than in the Control group (P < 0.05; Table 3). There was a tendency towards lower fecal concentrations of cadaverine (P=0.066) and total biogenic amines (P=0.054) in the Gleditsia group, with no differences in other biogenic amines (P>0.05, Table 3).

Item	Control	Yucca	Gleditsia	SEM	P
Serotonin	31.31	27.24	31.73	1.44	0.369
Tyramine	10.07	5.26	3.08	1.39	0.104
Spermidine	77.55	65.90	76.71	3.86	0.413
Cadaverine	373.90	304.30	145.10	42.10	0.066
Spermine	93.00a	50.19ab	39.84b	8.72	0.019
Histamine	38.35a	37.10a	31.25b	1.07	0.006
Putrescine	676.50	604.60	574.10	34.00	0.479
Total	1301.00	1094.60	901.90	69.5	0.054

Table 3 – Means (mg/kg dry matter) of fecal concentrations of biogenic amines in dogs from the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probability.
[a,b]Means followed by different letters differ according to Tukey's test (P<0.05).

3.3. Blood analyses

No significant differences in cytokine levels were observed among treatment groups (P>0.05; Table 4), suggesting that neither *Yucca schidigera* nor *Gleditsia amorphoides* supplementation elicited detectable systemic inflammatory responses.

Item	Control	Yucca	Gleditsia	P
IL-6	18.2 (6,1-69.4)	24.1 (6.1-141.0)	6.1 (6.1-70.7)	0.585
IL-10	6.1 (6.1-76.8)	6.1 (6.1-6.1)	6.1 (6.1-53.04)	0.356
TNF-α	6.1 (6.1-23.50)	6.1 (6.1-65.2)	6.1 (6.1-96.7)	0.885

Table 4 – Medians (minimum-maximum) of cytokines (pg/mL) of dogs in the Control, Yucca and Gleditsia groups.
Note: IL-6: Interleukin-6; IL-10: Interleukin-10; TNF-α: tumor necrosis factor alpha.

There was a reduction in LPO and an increase in CAT in dogs fed diets containing Yucca and Gleditsia, compared with the Control group (P < 0.05; Table 5). There was also a tendency towards higher T-AOC in the Gleditsia group compared to the Control and Yucca groups (P=0.070, Table 5).

Item	Control	Yucca	Gleditsia	SEM	P
LPO	31.96a	26.87b	26.90b	0.922	0.030
GST	8.15	7.28	7.41	0.566	0.827
GSH	66.20	67.31	69.46	0.936	0.380
T-AOC	167.05	168.49	213.63	9.740	0.070
CAT	82.71b	179.29a	157.87a	12.700	0.001

Table 5 – Means of antioxidant system variables of dogs in the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probability; LPO: Lipid peroxidation (mmol/mL); GST: Glutathione S-transferase (mmol/min.mL-1); GSH: Reduced glutathione (µM); T-AOC: Total antioxidant capacity (µM trolox equivalent); CAT: Catalase (mU/mL).
[a,b]Means followed by different letters differ by Tukey's test (P<0.05).

No significant differences were observed in AST and ALT enzymes (P>0.05). However, the Gleditsia group exhibited significantly lower ALP activity than the Control group (P < 0.05; Table 6).

Item	Control	Yucca	Gleditsia	SEM	P
ALP	45.10a	37.70ab	33.30b	2.09	0.049
AST	19.75	23.20	24.57	1.33	0.316
ALT	27.90	30.77	30.06	1.32	0.667

Table 6 – Means of liver enzymes (U/L) of dogs in the Control, Yucca and Gleditsia groups.
Note: SEM: Standard Error of the Mean; P: Probabilities; ALP: Alkaline phosphatase; AST: Aspartate amino transferase; ALT: Alanine amino transferase.
[a,b]Means followed by different letters differ by Tukey's test (P<0.05).

4. Discussion

This study demonstrates that dietary supplementation with *Yucca schidigera,* and particularly with *Gleditsia amorphoides,* results in potential benefits for gut function and systemic health in dogs through possible interconnected mechanisms. One of these potential benefits was the increase in fecal propionate concentrations, along with a reduction in fecal concentrations of ammonia, histamine, and spermine in the Gleditsia group compared to the Control group. This modulation of fecal metabolites is likely due to Gleditsia's composition, which combines triterpenoid saponins with galactomannan prebiotics (Lu et al., 2024), thereby collectively shifting fermentation patterns from proteolytic to saccharolytic pathways. Similar in vitro results were obtained using human fecal samples inoculated with *Gleditsia microphylla* extract (Wang et al., 2023). The authors reported modulation of the fecal microbiome and its fermentative activity in these samples, characterized by a decrease in proteolytic bacteria (phylum Fusobacteriota) and an increase in saccharolytic bacteria (phylum Bacteroidota) (Wang et al., 2023).

Propionate, a saccharolytic metabolite, exhibits potential anti-inflammatory effects in the gut by inhibiting the accessory protein CD14 of toll-like receptor 4 (Hoyles et al., 2018). Conversely, proteolytic metabolites such as ammonia and biogenic amines can be toxic to the gut mucosa and liver, with their effects depending on concentration (Brito et al., 2010; Souza et al., 2025). Although no studies have specifically examined the impact of *Gleditsia* spp. in dogs, research on *Yucca schidigera* has demonstrated a reduction in fecal ammonia concentrations in dogs supplemented with this feed additive (Dos Reis et al., 2016). The saponins found in both Yucca and Gleditsia are thought to lower fecal concentrations of proteolytic metabolites through multiple mechanisms, including inhibition of bacterial urease activity, direct binding to these metabolites, and modulation of the gut microbiome (Dos Reis et al., 2016; Zhang et al., 2023).

The potential inflammatory and pro-oxidant effects of proteolytic metabolites in the gut mucosa may also have systemic health implications (Souza et al., 2025). This could partially explain the observed reduction in ALP activity and LPO, alongside the increase

in CAT activity, in dogs from the Gleditsia and Yucca groups. Other studies have similarly reported associations between gut homeostasis and ALP activity across multiple species (Bilski et al., 2017; Salehian et al., 2019), as well as between antioxidant status and ALP activity in dogs (Souza et al., 2025). Beyond modulating the gut microbiome and its activity, Gleditsia may exert additional antioxidant effects through its polyphenol content. Specifically, quercetin derivatives in Gleditsia may enhance the Nrf2 oxidative stress response pathway, as recently demonstrated in canine hepatocyte cultures (Lu et al., 2024). Supporting this, Wang et al. (2023) observed enrichment of antioxidant and anti-inflammatory pathways in functional analyses of human fecal samples inoculated with *Gleditsia microphylla*.

These multi-systemic benefits, especially those observed in the Gleditsia group, suggest that its supplementation holds promise for supporting overall canine health by modulating the gut microbiota metabolites and antioxidant status. However, to enable broader application, further studies are needed to assess long-term impacts and potential interactions across varying dietary compositions and physiological conditions in dogs.

5. Conclusion

The results of this study demonstrate that dietary supplementation with saponins from *G. morphoides* and *Y. schidigera* supports both intestinal and systemic health in dogs, with *G. morphoides* showing the most pronounced effects. The capacity of Gleditsia extract to increase fecal propionate concentrations and reduce ammonia and biogenic amines, along with the enhancement in antioxidant status, highlights its promising potential as a functional additive in canine nutrition.

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