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Research Article

Effects of Dietary Methylsulfonylmethane (MSM) Supplementation on Apparent Nutrient Digestibility, Blood Parameters and Faecal Fermentation in Golden Retriever Dogs

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Abstract

Methylsulfonylmethane (MSM) is a naturally occurring organosulfur compound whose effects in dogs are poorly characterised. This study evaluated dietary MSM on nutrient digestibility, blood parameters and faecal fermentation in Golden Retriever dogs. Twenty-four healthy adults (2-8 years; 30.7±3.3 kg) were randomly allocated to three groups of eight: a basal diet without MSM (Control) and the same diet top-dressed with 4 g (MSM4; 127 mg/kg) or 8 g (MSM8; 259 mg/kg) MSM per head daily for 30 days. Digestibility (acid-insoluble ash), blood (days 0 and 30) and faecal short-chain fatty acids (SCFA) and ammonia were assessed. Apparent digestibility of dry matter, organic matter, crude protein and crude fat did not differ among groups (P>0.05). Serum glucose and creatinine differed among groups (P<0.01); triglyceride showed only a linear trend (overall P=0.064; linear P=0.026). The relative monocyte proportion rose and the neutrophil proportion fell with dose. Faecal consistency and pH were unchanged, but faecal acetate was lower in MSM8 than in the control (P=0.021), total SCFA declined linearly (P=0.011) and faecal ammonia-N was higher in MSM8 (P=0.014). In this small 30-day study, MSM at 4 or 8 g/head/day was not associated with detectable differences in digestibility or faecal consistency; some clinical-pathology and faecal metabolite measures differed or showed dose trends, but their biological significance is uncertain and the fermentation findings require confirmation.

Keywords: Methylsulfonylmethane, Dog, Nutrient digestibility, Blood parameters, Faecal fermentation, Short-chain fatty acids

Introduction

Functional feed ingredients are increasingly incorporated into premium pet foods to support gastrointestinal function, antioxidant status and overall wellbeing of companion animals [1,2]. Among these, methylsulfonylmethane (MSM; dimethyl sulfone, $(CH_3)_2SO_2$) has drawn particular attention. MSM is a naturally occurring organosulfur compound that contains approximately 34% sulfur by weight and represents a stable oxidised metabolite of dimethyl sulfoxide within the biological sulfur cycle [3,4]. It is readily absorbed, widely distributed across tissues and is able to cross the blood-brain barrier, and it has been granted "Generally Recognized as Safe" status [3,5]. As an organic sulfur source, MSM is considered more bioavailable and metabolically advantageous than inorganic sulfur salts, which are often poorly utilised by animals [4,6].

A range of biological activities has been ascribed to MSM, including antioxidant and anti-inflammatory effects mediated through enhancement of glutathione-dependent defences and inhibition of nuclear factor-κB signalling [7-9]. In production animals, dietary MSM has improved growth performance, antioxidant capacity and immune indices in broilers and Pekin ducks, and has increased ileal protein digestibility in poultry [10,11]. In companion animals, most available evidence concerns integumentary and microbial endpoints: MSM improved hair quality and modulated the faecal microbiota in Ragdoll kittens and in

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poodles, without adverse effects on growth, feed intake or faecal output [1,12]. In laying hens, and particularly under the oxidative and metabolic stress of feed restriction, dietary MSM has improved performance and egg quality, reduced serum and yolk malondialdehyde, and altered serum biochemical variables such as glucose and alkaline phosphatase [13]. Reviews of MSM in animal nutrition have pointed to its potential to support intestinal health and modulate immune and metabolic responses [4,14].

Despite this interest, the effects of MSM on nutrient digestibility, serum biochemistry and haematology in dogs remain largely unexplored, and dose-response information is scarce. The canine evidence to date rests on a single trial in poodles [1], whereas the digestibility, blood and antioxidant findings summarised above derive from poultry, rodents and humans and cannot be assumed to transfer to dogs. Because dogs are frequently supplemented with MSM for joint and skin health, characterising its influence on digestive efficiency and routine clinical-pathology variables is important both for efficacy and for safety. The two doses were chosen to represent a practical supplementary level (4 g/head/day, about 130 mg/kg) and a two-fold multiple of it (8 g/head/day, about 260 mg/kg), both far below the no-observed-adverse-effect level of 1.5 g/kg/day established in rats [15]. The primary objective was to determine whether these doses altered the apparent total-tract digestibility of dry matter, organic matter, crude protein and crude fat; the hypothesis was that digestibility would be maintained. Secondary objectives were to document serum biochemical and haematological variables and faecal consistency and pH under the same regimen, with the expectation of no clinically relevant change. Faecal short-chain fatty acids and ammonia were measured as exploratory indicators of hindgut fermentation without a directional hypothesis.

Material and Methods

Ethical Statement

The animal experiments were approved by the Selçuk University Faculty of Veterinary Medicine Experimental Animal Production and Research Centre Ethics Committee (SÜVDAMEK; decision no. 2025/165; date 30/10/2025). Every procedure was carried out in accordance with the relevant laws and standards, and the study was conducted in accordance with the 3Rs principle (Replacement, Reduction, Refinement) of animal welfare.

Animals and Housing

The study was carried out at the Dog Unit of the Prof. Dr. Hümeyra Özgen Research and Application Farm, Faculty of Veterinary Medicine, Selçuk University (Konya, Türkiye), where the dogs are bred and maintained for scientific studies. Twenty-four clinically healthy adult Golden Retriever dogs of mixed gender (2-8 years; body weight 30.7±3.3 kg, range 24.5-37.4 kg; body condition score 4-7 on a nine-point scale) were housed individually in pens comprising a closed indoor (190 × 190 cm) and an open outdoor (510 × 230 cm) concrete-floored area, with free access to clean water throughout the study. Eligibility required a normal clinical examination by a veterinarian before enrolment; no dog was excluded, all 24 completed the trial and every dog contributed to every outcome, so there was no attrition and no missing animal-level value. The study is reported in line with the ARRIVE 2.0 guidelines [16].

Diets and Feeding

Dogs were allocated to three groups of eight by drawing lots (simple randomisation) by an investigator not involved in feeding or sampling; the resulting groups were then checked for balance in body weight (29.9±2.8, 31.4±3.7 and 30.9±3.7 kg; P=0.68), sex and pen location. The groups received the basal diet without MSM (control), or the same diet top-dressed with 4 g (MSM4) or 8 g (MSM8) MSM per head daily. The supplement was pharmaceutical-grade methylsulfonylmethane (purity 99%; ZAG Kimya, Türkiye; product code ZK.101678.1000) supplied as a pure crystalline powder without carrier or excipient; the achieved doses were 127 (range 107-148) and 259 (216-300) mg/kg body weight per day. Daily portions were weighed into coded containers each week and mixed onto the daily ration immediately before feeding. The MSM was added on top of the full basal allowance and did not displace any food, so intake of the basal diet and of its energy and nutrients was identical across groups. Dogs were fed once daily at the same time, and the daily allowance was calculated to meet the maintenance energy requirement of kennel-housed adult dogs with low activity (metabolisable energy = body weight × 0.75 × 105 kcal; mean 1370 kcal/day) [17-19]. Consumption was checked at every feeding and any refusal was to be weighed back; the ration was consumed completely by every dog on every day, so no refusals or spillage were recorded. Because MSM was top-dressed as a visible powder and no placebo powder was given to the control group, the feeding staff could not be blinded to the presence of a supplement. Faecal scoring, sample processing and all laboratory analyses, however, were performed by personnel unaware of group allocation, and samples were identified by dog number only. The ingredient and analysed chemical composition of the basal diet are presented in *Table 1*. The feeding trial lasted 30 days, including an initial adaptation period, and serum and haematological samples were obtained on days 0 and 30.

Chemical Analysis

Experimental feed and faeces were analysed for dry matter, crude ash, crude protein (CP), acid-hydrolysed ether extract (EE) and crude fibre (CF) according to the

Table 1. Ingredient and chemical composition of the basal diet

Item	Value
Ingredient, %	
Meat-and-bone meal	13.0
Poultry meal	14.0
Maize	25.3
Brewer's yeast	5.0
Rice	23.45
Wheat middlings	9.0
Vitamin-mineral premix	0.25
Poultry fat	10.0
Analysed composition (dry-matter basis unless stated; single batch)	
Dry matter, % as-fed	88.0
Crude ash, % DM	11.6
Crude fat, % DM	7.2
Crude fibre, % DM	5.8
Crude protein, % DM	22.9
Nitrogen-free extract, % DM (calculated)	52.5
Metabolisable energy, kcal/kg DM (calculated)*	3002

** Metabolisable energy calculated on a dry-matter basis. DM: dry matter*

official methods of AOAC [20]; starch was determined by the same reference. Nitrogen-free extract (NFE) and the metabolisable energy (ME) of the diet were then calculated from the analytical results using the following equations [17]:

$$NFE\ (\%) = 100 - (CP\% + EE\% + CF\% + moisture\% + crude\ ash\%)$$

$$ME\ (kcal/kg) = [(5.7 \times CP \times 10) + (9.4 \times EE \times 10) + (4.1 \times (NFE \times 10 + CF \times 10))] \times (91.2 - (1.43 \times CF)) / 100 - (1.04 \times CP \times 10)$$

Digestibility Trial

Apparent total-tract digestibility was determined using acid-insoluble ash (AIA) as an internal indigestible marker [21,22]. All faeces voided by each dog over the final five days of the trial were collected quantitatively, weighed immediately after defecation and stored at −20°C in individually labelled bags. The faeces of each dog were then thawed, pooled, homogenised, dried at 55°C to constant weight, ground to pass a 1-mm screen and stored at −20°C until analysis. For AIA determination, samples were ashed at 600°C, boiled in 2 N hydrochloric acid, filtered, re-ashed and weighed [20]. Digestibility was calculated as follows:

$$Dry\text{-}matter\ digestibility\ (\%) = 100 - 100 \times (AIA\ diet / AIA\ faeces)$$

$$Nutrient\ digestibility\ (\%) = 100 - 100 \times [(AIA\ diet \times nutrient\ faeces) / (AIA\ faeces \times Nutrient\ diet)]$$

Organic matter was calculated as 100 − crude ash, and apparent digestibility coefficients were computed for dry matter (DMD), organic matter (OMD), crude protein (CPD) and crude fat (CFD).

Blood Sampling and Analyses

Blood was collected from each dog on the first (day 0) and last (day 30) day of the trial, in the morning after a 12-h fast, from the cephalic or saphenous vein under aseptic conditions. Blood for serum was drawn into plain (additive-free) tubes and, in a separate draw, into K_3EDTA tubes for haematology; the serum tube was always filled first to avoid anticoagulant carry-over. Serum was obtained by centrifugation (3000 rpm, 10 min) after 30 min at room temperature and stored at −20°C until analysis [23]. Serum from both sampling days was analysed in a single batch by a commercial veterinary diagnostic laboratory (Institute MG, Konya, Türkiye) with an automated chemistry analyser operated according to the laboratory's standard procedures; the storage interval between the day-30 sampling and analysis was two months. The serum variables measured were blood urea nitrogen (BUN), creatinine, glucose, total cholesterol, triglyceride, total protein, albumin, aspartateaminotransferase (AST), alanineaminotransferase (ALT), gamma-glutamyltransferase (GGT), amylase, calcium, phosphorus and magnesium. A complete blood count, including erythrocyte and leukocyte indices and platelet variables, was performed with an automated haematology analyser. Reference intervals were those supplied by the laboratory, taken from the MSD Veterinary Manual (2024 edition), and were used only for descriptive interpretation [24].

Faecal Consistency Score and Faecal pH

During the collection period, faecal consistency was independently scored immediately after defecation by four trained observers blinded to treatment using a five-point scale [25], defined as: 1 = liquid formless; 2 = soft, poorly formed; 3 = soft, formed and moist, leaving a mark on the ground; 4 = well-formed and firm, not leaving a mark; 5 = hard and dry. On this scale, scores of 3-4 are generally regarded as desirable, whereas values near 2.5 lie at the soft end of the range. The mean of the observers' scores over the scoring days was used for each dog. Inter-observer agreement, assessed on 12 dogs scored on three consecutive days by all four observers (36 samples), was good: the intraclass correlation coefficient was 0.73 for a single observer (ICC(2,1)) and 0.92 for the mean of the four observers (ICC(2,4)). Faecal pH was measured in fresh faeces collected during the same period using a calibrated pH meter, and the mean of duplicate readings per sample was recorded.

Faecal Short-Chain Fatty Acids and Ammonia

During the last four days of the trial a fresh faecal sample was collected from each dog immediately after

defecation on each day, always in the morning within the same time window after the previous day's meal. The four daily samples of each dog were pooled into one composite per dog, which was analysed in duplicate, so that one dog-level value entered the analysis and the dog was the experimental unit for all faecal variables. Two grams of faeces were mixed with 5 mL of 2 M hydrochloric acid and stored at −20°C until analysis [26]. After thawing, the acidified suspension was clarified by centrifugation and the supernatant was diluted 1:10 (v/v) with deionised water before analysis (dilution factor D = 10). Acetate, propionate and butyrate were quantified by gas chromatography (6890N, Agilent Technologies, USA) equipped with a split injector, a flame-ionisation detector and a capillary column (30 m × 320 µm × 1.00 µm; Agilent J&W). The oven programme was 80°C for 1 min, 80-120°C at 20°C/min, then 230°C for 3 min; injector temperature 250°C, injection volume 1 µL, split ratio 10:1; detector temperature 250°C; carrier gas hydrogen (purity ≥99.998%) at 30 mL/min with air at 300 mL/min [27]. A certified short-chain fatty acid mixture (acetic, propionic, isobutyric, butyric, isovaleric, valeric and n-heptanoic acids; 99.5% purity; Chem Service, West Chester, USA) was used as the quantitative external standard [28]. Each extract was injected in duplicate and the mean of the two injections was used for the individual dog; the between-injection coefficient of variation was below 5%. Valeric, isocaproic and hexanoic acids were above the limit of detection in only 1, 1 and 4 of the 24 dogs, respectively, and were not quantified; no substitution of zeros was needed for the three major acids, which were detected in every sample. Ammonia nitrogen (NH_3-N) was determined in the same diluted extracts, in duplicate, by the phenol-hypochlorite method of Weatherburn [29] (UV Mini 1240, Shimadzu, Japan; absorbance at 625 nm) against an external ammonium standard curve [30]. Concentrations were converted from the extract to a faecal dry-matter basis for each dog, as in previous canine studies [26,31], with the equation C (µmol/g DM) = C_extract (mmol/L) × V_extract (mL) × D/m_DM (g). V_extract is the 5 mL of acid plus the water in the 2 g faecal aliquot, calculated from the individual faecal dry-matter content (mean 6.02 mL). D = 10 is the dilution factor. m_DM is the dry mass of the aliquot (2 g × dry-matter fraction; mean 0.98 g). SCFA and ammonia-N are reported as µmol/g DM (1 µmol NH_3-N = 14.007 µg N). Total SCFA was calculated as the sum of acetate, propionate and butyrate; the proportion of each acid in this total and the acetate-to-propionate (A:P) ratio were then derived.

Statistical Analysis

The primary outcomes, fixed before the trial, were the apparent digestibility coefficients of dry matter, organic matter, crude protein and crude fat. Serum biochemical and haematological variables and faecal consistency and pH were secondary outcomes, and faecal SCFA and ammonia were exploratory. With eight dogs per group the study had 80% power ($\alpha = 0.05$) to detect a large effect only (Cohen's f = 0.68 among three groups, or d = 1.5 between two groups), equivalent to an 11.6-percentage-point difference in dry-matter digestibility or 3.7 points in crude-fat digestibility at the observed pooled standard deviations. Smaller true differences could not have been detected reliably. Data were analysed with SPSS (v26.0; IBM Corp., Armonk, NY, USA). Normality was assessed with the Shapiro-Wilk test together with visual inspection of Q-Q plots and skewness/kurtosis values, and homogeneity of variance with Levene's test. For variables measured on both days (serum biochemistry and haematology), among-group differences at day 30 were assessed by one-way analysis of covariance (ANCOVA) using the corresponding day-0 value as a covariate, thereby adjusting for baseline; equality of groups at baseline was confirmed by one-way ANOVA. The homogeneity-of-regression-slopes assumption (group × baseline interaction) was tested for every variable and held in all cases except BUN, eosinophil percentage, platelet count and plateletcrit, none of which showed a treatment effect; for these four the ANCOVA result is reported descriptively. Baseline-adjusted (least-squares) means with 95% confidence intervals were derived from these models and are reported in the text and in *Fig. 1* for the variables that differed; the tables show raw day-30 means ± SD for descriptive purposes together with the ANCOVA P values. Pairwise comparisons after a significant ANCOVA used Bonferroni-adjusted contrasts of the adjusted means. Within-group day-0 versus day-30 comparisons are not reported, because they do not address the treatment question. End-of-trial variables were compared by one-way ANOVA followed by the Tukey HSD test when parametric assumptions were met (digestibility coefficients, faecal pH, acetate, propionate, acetate and propionate proportions, A:P ratio and ammonia-N), or by the Kruskal-Wallis test with Dunn's post-hoc test and Bonferroni correction otherwise (faecal consistency score, butyrate, total SCFA and butyrate proportion). In addition, because the three treatments represented equally spaced, ordered MSM levels (0, 4 and 8 g/head/day), prespecified orthogonal polynomial contrasts were used to test linear and quadratic dose–response trends. These were estimated from the same linear model (on the baseline-adjusted estimates for ANCOVA outcomes, with standard errors from the model residual variance) and are reported for all variables, including those whose omnibus test was non-parametric, as a descriptive trend statistic. No adjustment was made for the number of outcomes tested; results with P values close to 0.05 are therefore regarded as exploratory and hypothesis-generating rather than confirmatory. No value was missing and no outlier was removed. Exact P values are reported throughout. Significance was set at $P<0.05$, and results are presented as mean±standard deviation.

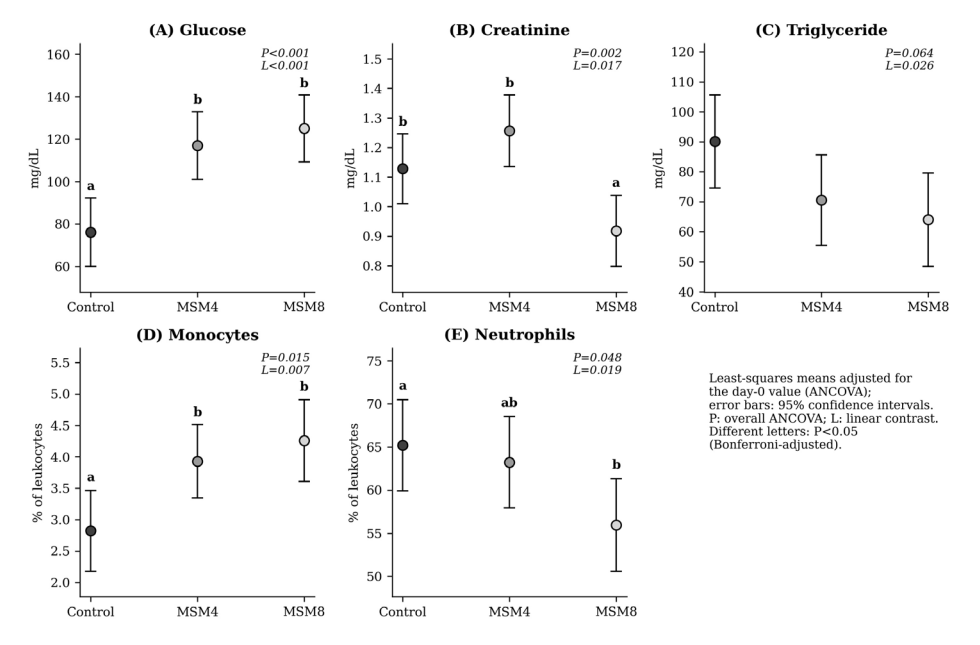

Fig 1. Baseline-adjusted (ANCOVA) means with 95% confidence intervals of serum glucose **(A)**, creatinine **(B)** and triglyceride **(C)** and of the relative monocyte **(D)** and neutrophil **(E)** proportions at day 30 in dogs fed diets supplemented with methylsulfonylmethane (MSM; n = 8 per group). Points are least-squares means adjusted for the day-0 value; error bars are 95% confidence intervals. Different letters indicate P<0.05 (Bonferroni-adjusted pairwise contrasts of adjusted means); P values of the overall ANCOVA and of the linear contrast are given in each panel

Results

Nutrient Digestibility

Apparent total-tract digestibility coefficients are shown in *Table 2*. MSM did not significantly affect the digestibility of dry matter, organic matter, crude protein or crude fat (P>0.05), although mean values were numerically higher in both MSM groups for all four fractions, the highest being recorded in MSM4. Body weight, re-measured on day 30, did not change in any group, and the ration was consumed completely throughout.

Serum Biochemistry

Day-30 serum biochemical values are presented in *Table 3* (raw means; baseline-adjusted means with 95% CI for glucose, creatinine and triglyceride in *Fig. 1)*; groups did not differ at baseline for any analyte (day-0 ANOVA, P>0.05). Serum glucose and creatinine differed among groups at the end of the trial (ANCOVA P<0.001 and P=0.002). Glucose increased with MSM level in a significant linear dose–response (adjusted means 76, 117 and 125 mg/dL; linear P<0.001), whereas creatinine was lowest in MSM8 (adjusted 1.13, 1.26 and 0.92 mg/dL) and described a significant quadratic pattern (P=0.004). Triglyceride decreased with dose and, although the overall group effect was not significant (P=0.064), the decline was significant as a linear contrast (P=0.026). Liver enzymes, amylase, total protein, albumin, cholesterol, calcium, phosphorus and magnesium did not differ among groups (P>0.05); GGT showed a borderline linear trend (overall P=0.094; linear P=0.050).

Haematology

Haematological findings are summarised in *Table 4*. The leukocyte differential is reported both as relative

Table 2. *Apparent total-tract nutrient digestibility (%) of dogs fed diets supplemented with methylsulfonylmethane (MSM)*

Item	Control	MSM4	MSM8	Combined P-values	Contrast Analyses	
					L	Q
Dry matter	81.60±11.17	85.20±3.79	82.88±6.29	0.833	0.743	0.385
Organic matter	88.13±7.09	90.40±2.44	88.95±4.18	0.785	0.743	0.397
Crude protein	81.71±10.48	86.37±3.29	83.78±5.29	0.508	0.564	0.247
Crude fat	94.55±3.12	95.60±2.28	95.52±1.58	0.645	0.454	0.603

Values are mean±standard deviation (n = 8 per group). Control: no MSM; MSM4: 4 g MSM/head/day; MSM8: 8 g MSM/head/day. Combined P values: overall group effect (one-way ANOVA or Kruskal-Wallis); Contrast analyses: P values for the linear (L) and quadratic (Q) orthogonal polynomial contrasts across MSM dose

Table 3. *Serum biochemical variables (day 30) of dogs fed diets supplemented with methylsulfonylmethane (MSM)*

Parameter (unit)	Control	MSM4	MSM8	Combined P-values	Contrast Analyses	
					L	Q
BUN (mg/dL)	10.64±3.63	13.60±12.69	12.75±2.80	0.930	0.708	0.960
Creatinine (mg/dL)	1.12±0.11[b]	1.29±0.25[b]	0.89±0.14[a]	0.002	0.017	0.004
Glucose (mg/dL)	77.75±35.84[a]	115.62±7.31[b]	124.62±8.26[b]	<0.001	<0.001	0.096
Cholesterol (mg/dL)	161.0±99.7	122.8±71.1	158.1±69.7	0.537	0.965	0.271
Triglyceride (mg/dL)	91.88±32.11	70.62±14.86	62.12±4.12	0.064	0.026	0.473
Total protein (g/dL)	3.93±1.74	4.07±2.57	5.01±1.08	0.662	0.490	0.566
Albumin (g/dL)	2.44±0.92	2.16±0.79	1.91±0.37	0.370	0.174	0.769
AST (U/L)	10.00±4.99	11.50±6.65	12.88±4.22	0.599	0.649	0.387
ALT (U/L)	49.75±20.00	46.38±11.35	47.00±8.82	0.930	0.899	0.724
GGT (U/L)	5.75±3.11	5.62±1.77	7.38±2.07	0.094	0.050	0.339
Amylase (U/L)	926.8±217.5	879.0±191.5	794.3±166.7	0.442	0.210	0.847
Calcium (mg/dL)	6.14±2.61	6.09±2.28	7.80±1.14	0.251	0.248	0.241
Phosphorus (mg/dL)	4.97±0.88	4.58±0.87	5.33±0.82	0.259	0.832	0.107
Magnesium (mg/dL)	2.00±0.49	1.79±0.72	1.55±0.30	0.160	0.060	0.737

Values are raw day-30 mean±standard deviation (n = 8), shown for descriptive purposes; P values derive from the baseline-adjusted (ANCOVA) models. Combined p values: overall group effect from analysis of covariance adjusted for the day-0 baseline; Contrast analyses: P values for the linear (L) and quadratic (Q) polynomial contrasts across MSM dose, computed on the baseline-adjusted estimates. Means within a row with different superscript letters differ (P<0.05, Bonferroni-adjusted pairwise contrasts of adjusted means). The homogeneity-of-slopes assumption did not hold for BUN; its ANCOVA result is descriptive. Total protein, albumin and calcium were below the reference interval in all groups at both sampling days and are not interpreted in absolute terms (see Discussion)

Table 4. *Haematological variables (day 30) of dogs fed diets supplemented with methylsulfonylmethane (MSM)*

Parameter (unit)	Control	MSM4	MSM8	Combined P-values	Contrast Analyses	
					L	Q
WBC (10^9/L)	6.87±1.47	6.58±1.42	6.43±1.44	0.739	0.442	0.955
Neutrophils (10^9/L)	4.48±0.95	4.22±1.22	3.61±1.17	0.325	0.147	0.733
Lymphocytes (10^9/L)	2.11±0.68	1.94±0.49	2.22±0.57	0.675	0.442	0.632
Monocytes (10^9/L)	0.17±0.05	0.26±0.09	0.29±0.09	0.054	0.026	0.425
Eosinophils (10^9/L)	0.09±0.04	0.16±0.08	0.30±0.24	0.225	0.234	0.278
Neutrophils (%)	65.54±4.99[a]	63.64±7.45[ab]	55.24±9.05[b]	0.048	0.019	0.405
Lymphocytes (%)	30.46±4.89	29.98±7.12	34.66±5.71	0.249	0.166	0.352
Monocytes (%)	2.58±0.74[a]	3.91±1.00[b]	4.53±0.76[b]	0.015	0.007	0.270
Eosinophils (%)	1.38±0.44	2.41±1.02	5.49±4.94	0.220	0.101	0.701
RBC (10^{12}/L)	7.26±1.37	6.32±0.94	6.97±0.76	0.144	0.948	0.053
Haemoglobin (g/dL)	17.39±3.22	15.40±2.40	16.99±2.04	0.234	0.939	0.093
Haematocrit (%)	47.79±8.06	42.64±6.37	46.81±5.46	0.196	0.979	0.075
MCV (fL)	66.03±3.30	67.49±3.61	67.11±2.92	0.135	0.615	0.052
MCHC (g/dL)	36.33±0.72	36.16±0.54	36.26±0.40	0.873	0.755	0.690
Platelets (10^9/L)	247.8±66.5	221.4±45.5	234.1±97.7	0.902	0.689	0.857
MPV (fL)	9.80±1.12[ab]	10.21±0.71[a]	9.46±0.97[b]	0.032	0.031	0.122

Values are raw day-30 mean±standard deviation (n = 8), shown for descriptive purposes; P values derive from the baseline-adjusted (ANCOVA) models. Leukocyte differentials are given as relative proportions (compositional; sum ≈ 100%) and as absolute counts. Combined p values: overall group effect from analysis of covariance adjusted for the day-0 baseline; Contrast analyses: P values for the linear (L) and quadratic (Q) polynomial contrasts across MSM dose, computed on the baseline-adjusted estimates. Means within a row with different superscript letters differ (P<0.05, Bonferroni-adjusted pairwise contrasts of adjusted means); for MPV the omnibus test was significant but only MSM8 versus MSM4 remained significant after adjustment. The homogeneity-of-slopes assumption did not hold for eosinophil %, platelet count and plateletcrit; their ANCOVA results are descriptive

proportions, which are compositional and sum to approximately 100%, and as absolute counts. The relative monocyte proportion increased with MSM and was higher in both MSM groups than in the control (P=0.015), rising linearly with dose, while the relative neutrophil proportion decreased and was lower in MSM8 (P=0.048). Absolute monocyte counts showed a linear trend only (overall P=0.054; linear P=0.026) and absolute neutrophil counts did not differ (P=0.33). Mean platelet volume differed overall (ANCOVA P=0.032; linear P=0.031); after Bonferroni adjustment MSM8 differed from MSM4 (P=0.048) but not from the control (P=0.092). Eosinophils followed similar numerical trends that were not significant after adjustment for baseline, reflecting a higher eosinophil proportion already present in MSM8 at day 0. The overwhelming majority of erythrocyte, leukocyte and platelet indices remained within the reference intervals for dogs.

Faecal Consistency and pH

Faecal consistency score and faecal pH are shown in *Table 5*. Neither variable differed among groups (P>0.05). Mean faecal consistency scores were similar across groups (2.46-2.73 on the five-point scale) and faecal pH was close to 6.5 in all groups. Thus, MSM supplementation did not alter stool quality or the faecal acidity of the dogs.

Faecal Short-Chain Fatty Acids and Ammonia

Faecal SCFA concentrations, the derived fermentation profile and faecal ammonia are presented in *Table 6* and *Fig. 2*. Acetate was the predominant acid in all groups. Faecal acetate was lower in MSM8 than in the control (29.1 vs 62.1 µmol/g DM; overall P=0.021, Tukey) and declined linearly with dose (P=0.011). Propionate differed among groups overall (P=0.045) and showed a linear decline (P=0.024), although no single pairwise comparison reached significance after Tukey adjustment. Butyrate and total SCFA did not differ in the overall Kruskal-Wallis test (P=0.073 and P=0.052) but both showed a significant linear contrast (P=0.017 and P=0.011).

The relative composition of the SCFA pool changed in parallel: the share of propionate rose from 28.3% to 37.2% of total SCFA (overall P=0.082; linear P=0.046) while the shares of acetate and butyrate fell, and the acetate-to-propionate ratio decreased from 2.48 to 1.69 (overall P=0.059; linear P=0.027). Because the absolute propionate concentration also decreased, this represents propionate forming a larger proportion of a smaller measured SCFA pool rather than an increase in propionate. Valeric, isocaproic and hexanoic acids were below the detection limit in the great majority of samples (detected in 1, 1 and 4 of 24 dogs, respectively) and were not analysed statistically.

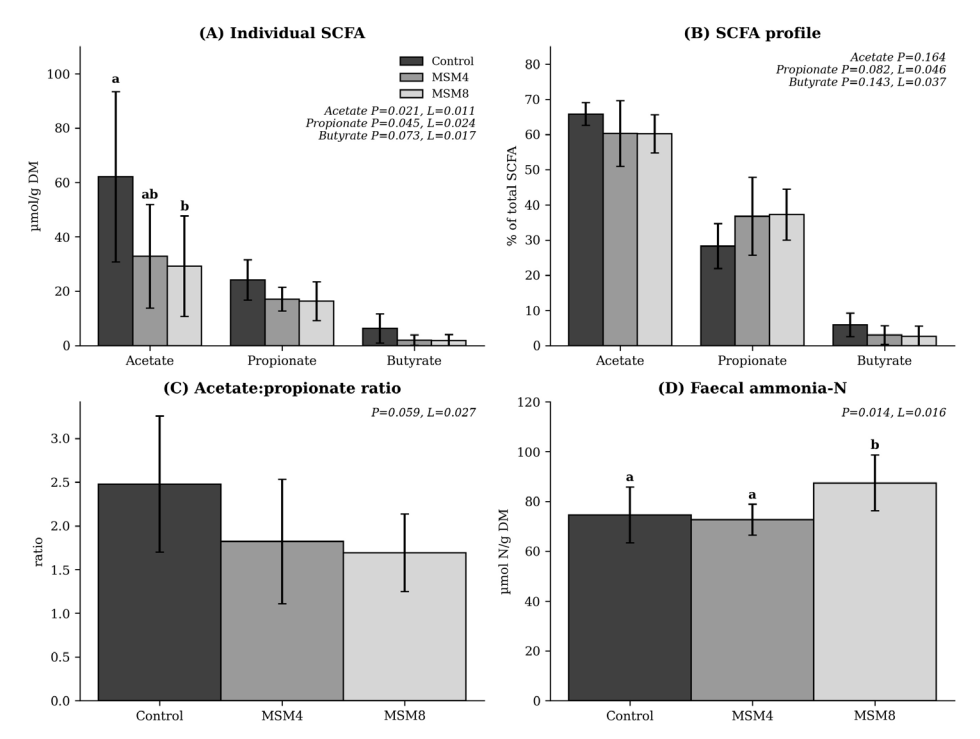

Fig 2. Faecal short-chain fatty acid (SCFA) concentrations (A), SCFA profile (B), acetate-to-propionate ratio (C) and faecal ammonia nitrogen (D) at the end of the trial in dogs fed diets supplemented with methylsulfonylmethane (MSM; n = 8 per group). Bars are raw means and error bars are standard deviations, expressed per g faecal dry matter. Different letters above bars indicate P<0.05 (Tukey HSD); P values of the overall test and of the linear contrast are given in each panel

Table 5. *Faecal consistency score and faecal pH of dogs fed diets supplemented with methylsulfonylmethane (MSM)*

Item	Control	MSM4	MSM8	Combined P- values	Contrast Analyses	
					L	Q
Faecal consistency score (1–5)	2.73±0.30	2.46±0.34	2.65±0.44	0.350	0.653	0.172
Faecal pH	6.59±0.21	6.53±0.15	6.60±0.18	0.698	0.946	0.403

Values are mean±standard deviation (n = 8). Faecal consistency was scored on a five-point scale (1 = liquid/formless; 2 = soft, poorly formed; 3 = soft, formed and moist; 4 = well-formed, firm; 5 = hard and dry). Combined P values: overall group effect (one-way ANOVA); Contrast analyses: linear (L) and quadratic (Q) polynomial contrasts across MSM dose. Scores of 3-4 are generally regarded as desirable on this scale; the observed means (about 2.5) lie at the soft end of the range. Faecal score was compared by Kruskal-Wallis and faecal pH by one-way ANOVA

Table 6. *Faecal short-chain fatty acid concentrations, fermentation profile and ammonia nitrogen of dogs fed diets supplemented with methylsulfonylmethane (MSM)*

Item	Control	MSM4	MSM8	Combined P- values	Contrast Analyses	
					L	Q
Acetate (µmol/g DM)	62.1±31.3[a]	32.8±19.1[ab]	29.1±18.5[b]	0.021	0.011	0.227
Propionate (µmol/g DM)	24.1±7.4	17.0±4.4	16.3±7.1	0.045	0.024	0.264
Butyrate (µmol/g DM)	6.35±5.38	1.98±1.95	1.78±2.20	0.073	0.017	0.188
Total SCFA (µmol/g DM)	92.6±42.5	51.9±24.9	47.3±27.5	0.052	0.011	0.214
Acetate (% of total)	65.85±3.24	60.30±9.36	60.18±5.45	0.164	0.097	0.347
Propionate (% of total)	28.27±6.36	36.72±11.07	37.23±7.24	0.082	0.046	0.292
Butyrate (% of total)	5.88±3.36	2.98±2.62	2.58±2.86	0.143	0.037	0.340
Acetate: propionate	2.48±0.78	1.82±0.71	1.69±0.44	0.059	0.027	0.367
Ammonia-N (µmol/g DM)	74.6±11.2[a]	72.7±6.2[a]	87.4±11.2[b]	0.014	0.016	0.065

Values are mean±standard deviation (n = 8), expressed per gram of faecal dry matter after conversion from the acidified extract (see Methods); ammonia-N is given as µmol N per g DM (× 14.007 = µg N/g DM). Total SCFA is the sum of acetate, propionate and butyrate; proportions are calculated on this total. Combined P values: overall group effect by one-way ANOVA (acetate, propionate, acetate %, propionate %, A:P ratio, ammonia-N) or Kruskal–Wallis (butyrate, total SCFA, butyrate %); Contrast analyses: P values for the linear (L) and quadratic (Q) polynomial contrasts across MSM dose. Means within a row with different superscript letters differ (P<0.05; Tukey HSD). Propionate differed overall (P=0.045) but no pairwise comparison reached significance after Tukey adjustment

Faecal dry-matter content did not differ among groups (P=0.500), indicating that the concentration differences were not a consequence of differences in faecal moisture.

Faecal ammonia-N moved in the opposite direction to the SCFA: it was higher in MSM8 (87.4 µmol/g DM, equivalent to 1224 µg N/g DM) than in either the control (74.6) or MSM4 (72.7 µmol/g DM) (overall P=0.014, Tukey) and increased linearly with dose (P=0.016).

Discussion

To the authors' knowledge, this is among the first studies to characterise the effects of dietary MSM on apparent nutrient digestibility together with serum biochemistry, haematology, faecal quality and hindgut fermentation in dogs. In this small 30-day study, MSM at 4 or 8 g/head/day was not associated with detectable differences in nutrient digestibility, faecal consistency or faecal pH, within the limits of a design that could detect only large effects. Several clinical-pathology variables and faecal metabolites differed among groups or showed dose-related trends: faecal acetate and total SCFA were lower and faecal ammonia-N higher at the highest dose, and serum glucose, creatinine and the leukocyte differential also changed. Whether any of these shifts is biologically important cannot be decided from the present data, and they are discussed below as observations requiring confirmation rather than as established effects.

The absence of significant digestibility differences agrees with recent canine and feline work, in which MSM did not change feed intake or faecal output in poodles [1] and did not alter growth in Ragdoll kittens [12], whereas it improved nutrient digestibility in broilers [10]. The poultry result should not be extrapolated to dogs, whose digestive physiology and diet differ substantially. The numerical values obtained here were slightly higher in the supplemented groups for all four fractions, but the extruded diet was already highly digestible, leaving limited scope for improvement, and the between-animal variation typical of client-quality dogs reduced statistical power. Absence of a significant difference must not be read as evidence of equivalence: with eight dogs per group only differences of roughly 12 percentage points in dry-matter digestibility, or about 4 points in crude-fat digestibility, would have been detected reliably, and no equivalence margin was predefined. Any digestibility benefit of MSM

would plausibly act through support of mucosal integrity rather than a direct enzymatic effect [9], a mechanism proposed from rodent work that remains untested in dogs and is more likely to become apparent under digestive challenge than in healthy dogs. The coefficients obtained here fall in the same range as those reported previously for Golden Retrievers fed extruded diets [23,32], indicating that the acid-insoluble ash method [21,22] behaved consistently in this setting, although the present design does not itself validate the marker.

Faecal quality is a key acceptability criterion for a functional pet-food ingredient, and neither consistency score nor pH was affected, although the mean scores of about 2.5 lay at the soft end of the scale in all groups, including the control. This should be kept in mind when the absence of a treatment effect is interpreted. Faecal SCFA are increasingly used as a readout of hindgut fermentation in dogs, where they respond to the type and amount of fermentable substrate reaching the colon [33-36]. Faecal concentrations are, however, the net result of microbial production, mucosal absorption, transit time and dilution, so a lower faecal concentration may reflect reduced production, increased absorption or both. The present data therefore describe a change in measured faecal concentrations, not a demonstrated change in colonic production. Acetate and total SCFA fell as dose rose, every acid showed a linear contrast, and faecal dry matter did not differ among groups, so dilution by a wetter stool is excluded. This contrasts with Ragdoll kittens fed 0.2-0.4% MSM for 65 days, in which faecal SCFA were unaffected [12], and with laying hens, in which dietary MSM did not alter the proportions of ileal SCFA [6]. The inclusion rates used here were higher (about 0.8 and 1.5% of the daily ration as fed), and whether the faecal SCFA response is dose-dependent or specific to dogs cannot be determined from the present data. The higher proportion of propionate should be interpreted accordingly: absolute propionate also declined (24.1 to 16.3 µmol/g DM), so propionate simply formed a larger share of a smaller pool, and the lower acetate-to-propionate ratio was driven mainly by the larger fall in acetate. One hypothesis for such a pattern is that MSM-derived sulfur reaching the colon supports sulfate-reducing bacteria, which compete with acetogens for hydrogen; MSM has also been reported to modify the canine and feline faecal microbiota [1,12]. Neither sulfur species, sulfide, microbiota composition nor hydrogen metabolism was measured here, however, and this explanation remains untested speculation.

Faecal ammonia-N was higher at 8 g/head/day while the SCFA were lower. Ammonia is a product of microbial protein breakdown and, in dogs, rises when more protein reaches the colon and falls when fermentable carbohydrate increases [37]; in canine faecal microbiota fermented *in vitro,* SCFA are used as markers of saccharolytic and ammonium and branched-chain fatty acids as markers of proteolytic fermentation [38]. The present pattern is therefore consistent with, but does not demonstrate, a relative shift from saccharolytic towards proteolytic activity: faecal ammonia also depends on mucosal absorption and on the nitrogen reaching the colon, and apparent crude-protein digestibility did not change. Faecal pH did not differ among groups, which may reflect the modest size of these shifts relative to the buffering capacity of canine faeces. The ammonia difference was about 17% above the control, all groups remained within a narrow range, and no clinical sign of large-bowel disturbance was observed over 30 days. The absolute concentrations measured here (acetate 29-62, total SCFA 47-93 µmol/g DM; ammonia-N 73-87 µmol/g DM) are of the same order as those obtained with the same extraction and chromatographic procedure in a previous canine trial [23], but lower than values reported by other laboratories using different extraction, dilution and calibration schemes, for example 167 µmol/g DM for acetate, 288 for total SCFA and 187 for ammonia in healthy adult dogs fed a low-fibre control diet [31]. Butyrate also formed a smaller share of the SCFA pool here (3-6%) than in that study (about 14%) [31], although similarly low faecal butyrate (about 3 µmol/g DM) has been reported in dogs fed extruded diets and analysed with the same chromatographic procedure [39]. Between-laboratory comparison of absolute faecal SCFA concentrations is therefore not straightforward, and the fermentation findings reported here should be confirmed with fully documented and validated analytical methods; the within-study comparison among groups, analysed under identical conditions, is not affected by this limitation. Whether a lower faecal SCFA concentration is beneficial or unfavourable is not settled by these data: butyrate is the principal energy source for colonocytes, but faecal concentrations reflect what escapes absorption rather than what is produced.

Among the serum variables, glucose rose with MSM dose and remained higher after adjustment for baseline (adjusted means 76, 117 and 125 mg/dL), the two MSM groups lying at the upper limit of the laboratory reference interval (76-119 mg/dL). A comparable rise was reported in feed-restricted laying hens [13]; whether the present finding reflects altered glucose handling, a sampling or fasting artefact, or chance in a small study cannot be decided here, and it warrants targeted follow-up with fasting insulin and fructosamine. Creatinine was lowest in MSM8 (adjusted 0.92 versus 1.13 and 1.26 mg/dL) with a quadratic pattern; all group means lay within the reference interval (0.5-1.7 mg/dL) and no other renal analyte changed, so a renal effect is not indicated, but the difference is statistically robust and should not be

dismissed [40]. Triglyceride fell linearly, consistent with reports of improved hepatic lipid handling in rodents [41,42], although it rose in hens [13], so the lipid response is species-dependent and the canine effect is unconfirmed. Liver enzymes did not differ; in a 30-day study this is compatible with, but does not establish, hepatic safety [15], and the antioxidant and cytoprotective actions attributed to MSM [3,4,6,43] have been shown in rodents and *in vitro* rather than in dogs.

Serum total protein, albumin and calcium lay below the laboratory reference interval in every group and did so already at day 0, before supplementation; none differed among groups or with dose, so the pattern is independent of MSM and indicates a uniform analytical offset affecting the whole batch rather than a biological change. Sampling order and tube type, anticoagulant carry-over, dilution and analyser calibration are recognised pre-analytical and analytical sources of such systematic shifts in canine clinical chemistry, and chelation by EDTA in particular lowers measured calcium [44]. Serum here was drawn into plain tubes before the EDTA tube and separated before freezing, and the dogs showed no oedema, weight loss or other sign of hypoproteinaemia or hypocalcaemia. These three analytes are therefore interpreted only comparatively among groups, which were sampled and analysed under identical conditions, and not against the reference interval.

The clearest haematological change was a dose-dependent rise in the relative monocyte proportion with a lower relative neutrophil proportion in MSM8. Because the differential percentages are compositional, a lower neutrophil share arises arithmetically when the monocyte share rises; the absolute monocyte count showed only a linear trend and the absolute neutrophil count did not differ, so the change is small in absolute terms. These shifts are compatible with the immunomodulatory activity of MSM, which regulates monocyte and macrophage inflammatory signalling, including inhibition of nuclear factor-κB and inflammasome pathways [8,9,11], and a recent 30-day MSM trial in horses likewise modified exercise-responsive inflammatory gene expression in skeletal muscle [45]. These are, however, rodent, *in vitro* and equine observations. Immune modulation has not been demonstrated in dogs, and a differential count alone cannot establish it. Values remained largely within reference intervals and no leukocytosis, left shift or anaemia developed. Mean platelet volume was lower in MSM8 than in MSM4 after Bonferroni adjustment but did not differ from the control, and the difference (about 1 fL) is within analytical variation. The apparent rise in eosinophils did not survive adjustment for baseline, since eosinophil proportions were already higher in MSM8 at day 0, which is why the day-0 value was used as a covariate.

This study has limitations. The trial lasted 30 days in a single breed, a restricted age range and at two fixed daily doses, in healthy adult dogs housed under kennel conditions. It therefore cannot establish safety for other breeds, for longer use, for clinical populations or for routine commercial use, and it was powered to detect only large effects. Adverse events were monitored by daily clinical observation of appetite, demeanour, vomiting and faeces, and none was recorded; however, no structured adverse-event instrument was used and the feeding staff were not blinded; body weight, re-measured on day 30, was unchanged in all groups. Faecal SCFA and ammonia were measured on a single four-day composite sample per dog, and branched-chain fatty acids, microbiota composition and systemic antioxidant status were not determined. The absolute faecal metabolite concentrations also depend on the extraction, dilution and calibration scheme, and formal recovery and limit-of-quantification validation of the SCFA and ammonia assays was not performed; these values therefore require confirmation.

In conclusion, in this small 30-day study of healthy adult Golden Retrievers, supplementation with 4 or 8 g MSM per dog per day was not associated with detectable differences in apparent total-tract digestibility or faecal consistency. Several clinical-pathology and faecal metabolite measures differed or showed dose trends, but their biological significance is uncertain. The faecal SCFA and ammonia findings require confirmation with fully documented and validated analytical methods; these data do not establish changes in microbial composition or fermentation flux.

Declarations

Availability of Data and Materials: The datasets generated and analysed during the current study are available from the corresponding author (ZSİ) on reasonable request.

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Declaration of Generative Artificial Intelligence (AI): The authors declare that generative AI and AI-assisted technologies were not used in the design or conduct of the study, in the collection or analysis of the data, or in the creation of the tables and figures. AI-assisted tools were used only to improve the readability and language of the manuscript; the authors reviewed and edited the text and take full responsibility for the content of the publication.

Ethical Compliance Declaration: The authors declare that the study was conducted in accordance with all applicable animal welfare and research ethics principles, institutional and national/international

regulations, and the requirements of the relevant ethics committees and funding/supporting institutions, where applicable. The authors accept full responsibility for the ethical integrity of the study.

Authors' Contributions: ZSİ: Conceptualisation and hypothesis of the study, diet formulation, conduct of the feeding trial, statistical analysis, writing of the original draft and final decision. HDA and OK: Study design, diet formulation, supervision, assessment of the results and critical revision of the manuscript. EY: Laboratory analyses of faecal short-chain fatty acids and ammonia, interpretation of the fermentation data. BY and DŞ: Follow-up of the experimental procedure, sample collection, digestibility and blood analyses. All authors read and approved the final manuscript.

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