



RESEARCH ARTICLE

The effect of dietary olive polyphenols on markers of inflammation and bodyweight in senior domestic cats

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Abstract

The high rates of obesity and degenerative joint disease in companion animals has resulted in a demand for dietary supplements that support joint health and reduce inflammation. Polyphenols have received considerable attention in this space, although literature in companion animals is lacking or conflicting. This study determined whether a diet supplemented with olive polyphenol extract had the potential to reduce inflammation and/or bodyweight. Eight senior domestic cats aged 11.01 ± 0.74 years (mean \pm standard error of the mean) and weighing 3.6 ± 0.3 kg (mean \pm SEM) were used for this study. The cats were fed, *ad libitum* with a complete (AAFCO) canned diet supplemented with 0.1% olive polyphenol extract for 56 days. Cats were weighed weekly and blood samples taken on day 0 (baseline), 28, and 56 of the study. Biochemistry, haematology, and cytokine (19 cytokines or chemokines) panels were run for each blood sample. While there was an initial aversion to the supplemented diet, intakes of the cats increased, and they consumed enough to meet or exceed their daily maintenance energy requirements by day 10 of the trial. On average, the cats lost approximately 8% of their starting weight over the trial, which was interesting given that feed intake exceeding maintenance energy requirements for most of the study. Whether the decrease in bodyweight was due to seasonal changes, the supplemented diet, or a combination of the two warrants further investigation. There were little to no changes to any of the blood parameters, which was surprising given that previous studies have reported reductions in pro-inflammatory cytokines following polyphenol supplementation. Perhaps a higher concentration of olive polyphenols is required to elicit the anti-inflammatory response observed in other species. A study evaluating the dose-dependent effects of dietary polyphenols on inflammatory and oxidative markers in cats would be valuable in this context.

Keywords

antioxidants – cytokines – diet supplementation – weight loss

1 Introduction

Obesity and degenerative joint disease (DJD; e.g. osteoarthritis) are becoming increasingly prevalent in companion animals worldwide (Cave *et al.*, 2012; Chiang *et al.*, 2022; Lascelles *et al.*, 2010a; Kimura *et al.*, 2020). Obesity and DJD are often closely associated and can

have a wide range of adverse effects on the health and well-being of cats. In cats, obesity has been closely linked to increased oxidative stress and an increased risk of diabetes mellitus, renal disease, hepatic lipidosis, hyperlipidaemia, and DJD (Cave *et al.*, 2012; Chiang *et al.*, 2022; Scarlett and Donoghue, 1998; Scarlett *et al.*, 1994). Degenerative joint disease in cats is primarily associated

with inflammation, joint pain, reduced physical activity, and an increased risk of weight gain (Lascelles, 2010; Lascelles and Sheilah, 2010).

At present, the primary treatment recommendations for both obesity and DJD are lifestyle changes (e.g. caloric restriction, modified feeding regimes, and limiting food intake) (Burkholder and Bauer, 1998). These changes are aimed at promoting physical activity and reducing bodyweight to alleviate pressure on joints with DJD and reduce factors that adversely affect joint health (e.g. oxidative stress and chronic inflammation; Rychel, 2010). Pharmacological treatments for companion animals with DJD are also available (e.g. non-steroidal anti-inflammatory or glucocorticoid treatments), but the long-term use of these treatments can have various side effects, many of which can be severe (Hardie, 1997). Thus, there is a clear need for research into alternative treatments for feline DJD.

In recent years, there has been a particular interest in dietary supplements that could alleviate symptoms of DJD or oxidative stress (associated with DJD and obesity) by promoting anti-inflammatory cytokines and reducing both proinflammatory cytokines and reactive oxygen species (ROS; Forman and Zhang, 2021). Dietary polyphenols (plant-based compounds containing more than one phenolic hydroxyl group, e.g. flavonoids, phenolic acids, lignans, and stilbenes) have shown promise as an alternative therapy for DJD and oxidative stress in humans (Pandey and Rizvi, 2009). Polyphenols have been found to improve joint health and function through their anti-inflammatory and antioxidant properties (Ansari *et al.*, 2020; Sung *et al.*, 2019). Polyphenols (e.g. citrus flavonoids) have been found to have lipid-lowering and hypoglycaemic properties, suggesting that they may be beneficial for weight loss (Millán-Laleona *et al.*, 2023; Williamson, 2017). However, despite the large amount of promising research in humans, few studies have investigated the effects of dietary polyphenols in companion animals. However, one study in growing puppies demonstrated antioxidant effects of catechin supplementation in a green tea extract without impacting growth rates or faecal quality (Kara *et al.*, 2016).

To the authors' knowledge, there have been only two studies investigating of dietary polyphenols on inflammation and oxidative stress in cats. Leray *et al.* (2011) and Jeusette *et al.* (2010) administered a diet supplemented with polyphenols and looked at the effects the treatment had on chronic inflammatory and oxidative markers, respectively. Jeusette *et al.* (2010) found giving a diet supplemented with citrus polyphenols to obese

cats for five months lead to decreased plasma lipid concentrations (cholesterol and triacyl-glycerides) and reduced oxidative (lower urinary F2-isoprostane concentrations). This suggested that dietary polyphenols could reduce oxidative stress in cats. Leray *et al.* (2011) found that obese cats given dietary polyphenols for eight weeks exhibited a reduction in the concentrations of plasma acute-phase proteins (APP), showing that polyphenols could target the liver to reduce the chronic inflammatory state associated with obesity. In contrast to studies in obese and/or diabetic mice (Jain *et al.*, 2009; Weisberg *et al.*, 2008), the polyphenol treatment did not affect the circulating cytokine concentrations of cats (Leray *et al.*, 2011). This was possibly due to the lower doses given (Leray *et al.*, 2011), but it is important to note that research into the use of dietary polyphenol extracts should be conducted cautiously in terms of dose determination. A toxicology study in dogs clearly emphasised the importance of careful dose selection when administering polyphenols, as high doses of dietary green tea polyphenol extracts were associated with many severe side effects, including pathology of many major organs and mortality (Kapetanovic *et al.*, 2009). Despite this, the potential beneficial effects of an appropriate dose of dietary polyphenols on chronic inflammation and oxidative stress in companion animals warrants further investigation, especially given the promising research published for humans.

This study aimed to determine whether a diet supplemented with low-dose (0.1%) olive polyphenol extract affects the serum concentrations of inflammatory markers and/or bodyweight of senior domestic cats. The hypothesis was that this low dose polyphenol extract treatment would be sufficient to reduce the levels of inflammatory markers in the cat and lead to weight loss over the eight-week study.

2 Materials and methods

Animals and animal husbandry

Eight cats from the Centre for Feline Nutrition (Massey University, Palmerston North, New Zealand) were selected for this study. Older cats were selected as inflammation and joint problems are more prominent in older cats (Lascelles *et al.*, 2010a). The cats aged from 9.6 to 15.5 years (mean \pm standard error of the mean (SEM); 11.01 \pm 0.74 years) and, at the start of the study, weighed from 2.9–5.0 kg (mean \pm SEM; 3.6 \pm 0.3 kg). The cats were housed in a single group at the Centre for

Feline Nutrition throughout the eight-week trial. Both food and water were available *ad libitum* and replaced daily. The cats were exclusively fed a canned diet supplemented with 0.1% pure olive polyphenol extract (see details below), with a daily allocation of approximately 350 g per cat per day. The total food consumption of the group was recorded daily. The quantity of supplemented food offered was increased if all food was close to being consumed over a 24 h period to ensure *ad libitum* access to food. The cats were weighed weekly throughout the trial; this was to ensure that the cats did not lose a significant amount of bodyweight as a result of consuming the diet. The American Association of Feed Control Officials (AAFCO) guidelines (2021) state that if any individual cat loses >15% of its starting bodyweight it should be removed from a trial, or if the group loses >10% of their starting bodyweight then the trial should be stopped. No animals were withdrawn from this study.

Care of the animals complied with the Animal Welfare (Cats) Code of Welfare (2010). The study design was approved by the Massey University Animal Ethics Committee (MUAEC) and given the protocol number MUAEC 21/37 prior to the commencement of the study.

Diet

The base diet was canned wet food (Chef Classic Chicken flavour; Kraft Heinz Wattie's Ltd, Hastings, New Zealand). This diet was AAFCO formulated and had been feed tested for cats. In addition, this diet was familiar and proven to be palatable to the cats at the Centre for Feline Nutrition. The initial daily food allowance was

derived from the daily maintenance energy requirements (MER) of all cats in the group: MER (kcal) = $100 \times \text{kg BW}^{0.67}$ (NRC, 2006). Based on the starting bodyweight of each of the cats, a total daily MER of 1,877 kcal (i.e. 7,852 kJ/kg) was required (Table 1). The Chef Classic Chicken diet contains 765 kcal/kg (i.e. 3,200 kJ/kg), thus the group of cats required a dietary intake of 2,454 g/day to meet MER. Therefore, the cats were given 3,160 g (four cans) of diet supplemented with 0.1% olive polyphenols daily to ensure the provision of an *ad libitum* diet. The cats did not consume all of the provided diet on any day, so all food intake values represent the voluntary food intake of the cats.

The base diet was supplemented with 0.1% (31.6 g/day) olive polyphenol extract. The olive polyphenol extract was mixed thoroughly into a fresh batch of diet each day to ensure even distribution. No study has reported dietary supplementation of feline diets with pure olive polyphenols; thus, the dose of olive polyphenols was determined from two studies that used 0.1% pure citrus polyphenols in cats for two to five months without any adverse side effects (Jeusette *et al.*, 2010; Leray *et al.*, 2011). Note that caution is needed when determining dietary polyphenol concentrations due to the toxic effects associated with high doses (Kapetanovic *et al.*, 2009).

Experimental design

The cats were fed the diet supplemented with 0.1% polyphenol extract for a total of eight weeks (56 days). Blood samples (2 ml) were collected on days 0 (baseline), 28,

TABLE 1 The maintenance energy requirements (MER) of the cats based on bodyweight (BWT) at the start of the study for a canned diet (containing 764.8 kcal/kg) mixed with olive 0.1% polyphenols¹

Cat	Initial BWT (kg)	Initial MER (kcal)
Blur	2.92	208.35
Dalu	5.00	294.13
Katy	2.90	203.99
Klara	3.22	218.68
Obi	4.40	269.89
Piri	4.21	262.02
Vilma	2.93	205.54
Zilla	3.11	213.96
Total	28.76	1,876.57 kcal
Total intake of diet in g	–	2,453.67 g

¹ Maintenance energy requirements were calculated as MER (kcal) = $100 \times \text{kg BW}^{0.67}$ (NRC, 2006).

and 56 (end) of the study using jugular venepuncture (25G needle). Local anaesthetic, Emla cream (5% lignocaine and prilocaine), was administered to the shaved sample site 30 minutes prior to blood collection. For each blood sample, half the blood volume was placed into a vacuum tube (Becton and Dickinson Co., Franklin Lakes, NJ, USA) and used for haematology and a biochemistry health screen. The other half of each blood sample was placed into a vacuum tube, left for two hours, and centrifuged at $3,000\times g$ before the serum was extracted and stored at $-80\text{ }^{\circ}\text{C}$ until a comprehensive cytokine and chemokine analysis was conducted.

Analysis of blood samples

Biochemistry health screen (Feline)

Blood samples collected on days 0, 28, and 56 were sent for biochemistry health screening. This included measurement of symmetric dimethylarginine (SDMA), creatine kinase (CK), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, total protein, albumin, globulin, urea, creatinine, phosphate, calcium, cholesterol, sodium, potassium, and chloride.

Complete blood count (differential)

Blood samples collected on days 0, 28, and 56 were sent for complete blood count (CBC) analyses. This included measurement of red blood cell concentration (RBC), haemoglobin concentration (HB), haematocrit or packed cell volume (HCT/PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet concentration (PLAT), absolute reticulocyte count (absolute retic), reticulocyte haemoglobin (rHb), and white blood cell concentration (WBC).

Cytokines and chemokines

A comprehensive cytokine and chemokine assay (Millipore Feline Cytokine Panel, 19-plx; MilliporeSigma, Burlington, MA, USA) was conducted on all blood samples (i.e. days 0, 28, and 56) by Massey University Nutrition Laboratory (School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand). The following cytokines and chemokines were assessed: apoptosis antigen 1 (i.e. Fas or CD95), Fms-like tyrosine kinase 3 ligand (Flt-3L), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma ($\text{IFN-}\gamma$), platelet-derived growth factor (PDGF-BB), keratinocytes-derived chemokine (KC or CXC-L1), stromal cell-derived factor 1 (SDF-1 or CXC-LI2), chemokine ligand 5 (CCL5 or RANTES), stem cell factor (SCF), monocyte chemoattractant protein 1 (MCP-1),

tumour necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-2, IL-4, IL-6, IL-8, IL-12, IL-13, and IL-18.

Statistical analyses

All statistical analyses were conducted using RStudio with a significance level of $P<0.05$. All data were checked for normality and outliers. The body weights of the cats at the start and end of the trial were compared using a paired t-test. In addition, a Pearson's correlation coefficient was used to examine the relationship between the mean bodyweights of the cats and time. For all blood test parameters, significant outliers were removed from the data according to Chauvenet's criterion and non-parametric data were transformed using the TransformTukey function (RCompanion Package). Repeated measures ANOVAs were then used to determine the effects of the polyphenol treatment on each of the parameters measured in serum or blood. Paired post hoc tests (paired pairwise test or paired pairwise Wilcoxon test) were used to further investigate parameters that yielded a statically significant repeated measures ANOVA. Note that some cytokine parameters were undetectable in several serum samples, so statistical comparisons were not possible.

3 Results and discussion

Bodyweight data

The mean bodyweights of the cats were negatively correlated with time (Pearson's correlation coefficient: $r^2 = 0.9$, $P<0.001$; Figure 1). The average bodyweights of the cats were 3.6 ± 0.3 kg at the start of the trial and 3.3 ± 0.3 at the end of the trial ($P=0.034$). It is worth noting that the bodyweights of the cats decreased during the five weeks prior to the trial starting and the rate did not increase after the trial start date (Figure 1), thus the decrease in bodyweight was unlikely related to the dietary polyphenol supplementation. Cats have been shown to exhibit a seasonal decrease in bodyweight the period over which this study was conducted is associated with weight loss (Bermingham *et al.*, 2013; Serisier *et al.*, 2014). However, five of the cats reached a bodyweight that was lower than their lowest bodyweight for the previous year (2020). This was likely due to the fact the older cohort of cats was selected for this trial, and cats tend to lose weight as they age. Alternatively, there may have been a decrease in voluntary food intake, since voluntary food intake also changes in cats throughout the year (Bermingham *et al.*, 2013; Serisier *et al.*, 2014).

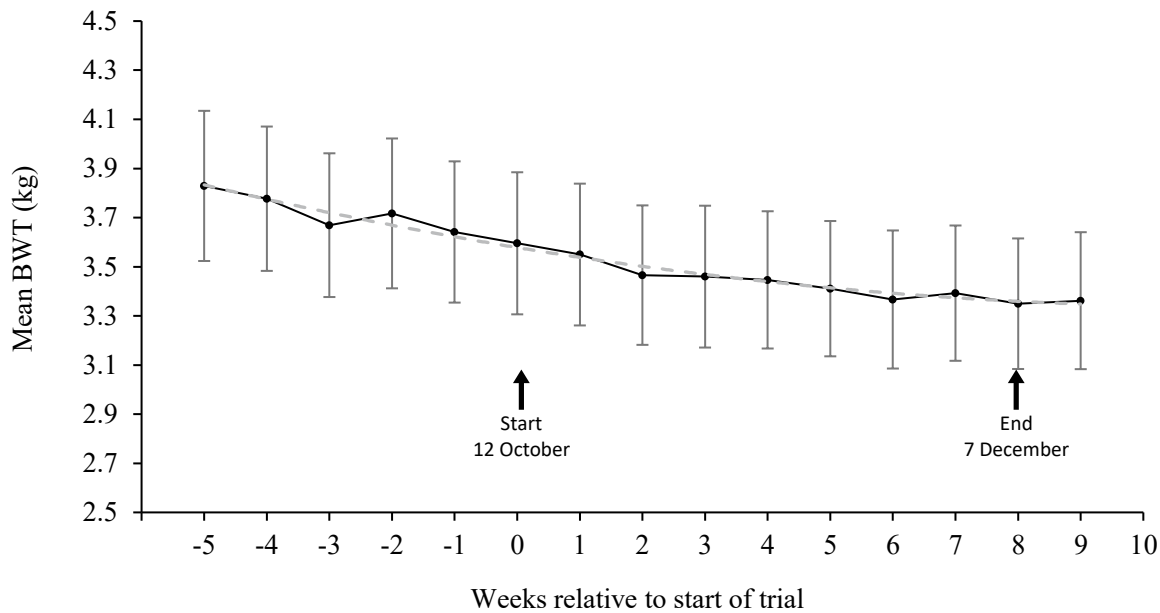


FIGURE 1 Mean \pm standard error of the mean (SEM) bodyweights of the cats ($n=8$) over time relative to the start (week 0) and end (week 8) of dietary supplementation with 0.1% olive polyphenols. The grey dashed line represents the linear regression line ($r^2=0.9$, $P<0.001$).

Food consumption

This trial was conducted over a period during which voluntary feed intake has been found to decrease in cats (i.e. during spring when day length/photoperiod is increasing) (Serisier *et al.*, 2014). However, in the present study, the feed intake of the group of cats increased

from day 0 (Start of trial) to day 56 (end of trial; $r^2 = 0.77$; Figure 2). While the initial total voluntary dietary intake was below the group daily MER requirements (Figure 2), by day 10 of the trial all feed intakes exceeded the MER for the group of cats (N.B., individual feed intakes of the cats could not be recorded due to the group housing). It

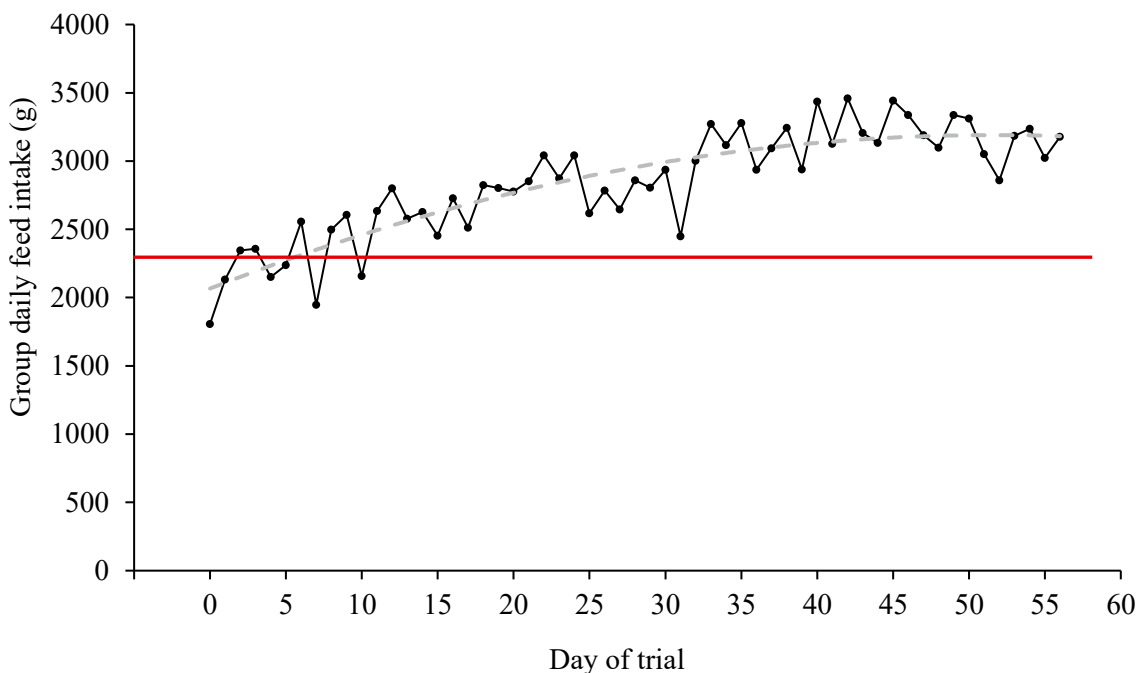


FIGURE 2 The daily voluntary feed intake of the group of cats ($n=8$) given a diet of Chef Classic chicken supplemented with 0.1% olive polyphenols. The grey line represents polynomial regression ($r^2 = 0.77$). The red line represents the daily dietary intake required to meet the maintenance energy requirements of the group of cats based on their starting bodyweights.

is unclear whether the initially low levels of voluntary food intake were due mixed Chef Classic Chicken diet (different texture and appearance associated with mixing the diet) or the polyphenol supplementation (polyphenols have been attributed to a bitter taste) (Tarragon and Moreno, 2020). A palatability trial comparing Chef Classic Chicken, mixed Chef Classic Chicken, and Chef Classic Chicken mixed with 0.1% polyphenols may help answer this question. At present, it would be advisable to progressively add (25%, 50%, 75%, and then finally 100% of diet) the Chef Classic Chicken mixed with 0.1% polyphenols to the cats existing diet over a week to minimise the chance of this depression in intake.

It was interesting to note that there was a decrease in the bodyweight of the cats despite an increase in feed intake (Figures 1 and 2). The total energy expenditure, and thus MER, of cats, is comprised primarily of basal metabolic rate and physical activity. Thus, it was possible that that physical activity of the cats increased during the present study, which led to the decrease in bodyweights of the cats despite a dietary intake exceeding the calculated MER. This would not be surprising as this trial was conducted over a period of increasing daylength and ambient temperature. Colony-housed domestic cats have previously been shown to be more active and to lose weight as daylength increases (Bermingham *et al.*, 2013; Serisier *et al.*, 2014). Alternatively, it is possible that the polyphenol treatment effectively alleviated inflammation, and in turn, increase physical activity. Indeed, treatment of DJF has previously been found to increase that physical activity of cats (Guillot *et al.*, 2013; Lascelles *et al.*, 2010b). However, this cannot be confirmed in the present study as the physical activity of the cats was not accessed.

Biochemistry health panel and haematology

General health of the cats on day 0:

On day 0, none of the cats had any biochemistry or haematology results that showed either acute or chronic illness. Renal function appeared to be normal in all cats. Three of the cats had slightly elevated SDMA (15, 16, and 19 ug/dl; normal range 0–15 ug/dl), which could be transient or indicative of early renal disease. Since SDMA concentrations were only slightly elevated and both urea and creatinine concentrations were normal, renal degeneration or disease is unlikely. In addition, markers of liver function (e.g. ALT, ALB, BR, and TP) were normal in all cats.

Red blood cells were present at normal concentrations (RBC count) and proportions (haematocrit) in all cats on day 0. In addition, the volume of RBC (MCV) and haemoglobin contents of each RBC (MCHC) of each cat were normal. The absolute reticulocyte count was low in most cats. This can indicate decreased erythropoiesis due to anaemia-causing conditions such as chronic kidney disease (Cowgill *et al.*, 2003). However, the RBC did were normal for all cats and the diet did not affect the absolute reticulocyte count or RBC of the cats. One cat had a mildly elevated WBC count (36.9×10^9 cells/l; normal range $5.5\text{--}19.5 \times 10^9$ cells/l) on day 0, which suggested that the cat may have been experiencing an infection or inflammation. The WBC count of this cat remained slightly above the normal range throughout the study. There were no other signs of illness in this individual's biochemistry or CBC panel, thus inflammation was the most likely cause.

The effect of polyphenol treatment on blood parameters

The provision of a diet supplemented with 0.1% olive polyphenols for 56 days had a minimal effect on the parameters assessed in the biochemistry health panel and haematology of the cats (Table 2). Serum urea, phosphate, and potassium concentrations were all significantly higher on day 56 than on day 0, and 28 (Table 2). All serum urea and phosphate concentration values were within the normal range for cats (Table 2). As such, the physiological significance of the increases in these parameters is unclear and should not be over-interpreted. The mean potassium concentrations on day 56 were just above the normal range (Table 2), but slightly elevated potassium alone is of minimal significance in a clinical context. There was a tendency ($P < 0.1$) for AST to increase from day 0 to 56 (Table 2), but all AST concentrations were within normal ranges.

In terms of haematology, none of the parameters in the cats differed significantly over time (Table 2), which suggested that the polyphenol treatment had no effect. The mean values of all haematology parameters were within the normal ranges throughout the study with the exception of elevated WBC in one cat and a low absolute reticulocyte count on day 0, 28, and 50 for most cats (Table 2). As mentioned previously, the low absolute reticulocyte count is unlikely to be clinically significant as RBC counts were normal for all tests.

TABLE 2 Mean \pm standard error of the mean (SEM) serum concentrations of biochemistry and haematological parameters after 0, 28 and 56 days of treatment with a Chef Classic Chicken diet supplemented with 0.1% olive polyphenols from day 0–56¹

Tests	Normal range	Unit	Day 0	Day 28	Day 56	P-value
Biochemistry						
Symmetric dimethylarginine (SDMA)	0–14	$\mu\text{g}/\text{dl}$	14.0 \pm 1.0	14.0 \pm 0.9	14.0 \pm 1.2	1.00
Creatine kinase (CK)	0–344	IU/l at 37 °C	140.8 \pm 24.7	138.8 \pm 13.5	167.6 \pm 11.5	0.29
Aspartate aminotransferase (AST)	0–66	IU/l at 37 °C	29.9 \pm 2.9	32.3 \pm 2.5	36.4 \pm 2.7	0.09
Alkaline phosphatase level test (ALP)	0–85	IU/l at 37 °C	31.1 \pm 3.8	29.9 \pm 3.6	28.4 \pm 3.5	0.59
Alanine aminotransferase (ALT)	0–100	IU/l at 37 °C	65.1 \pm 7.1	75.0 \pm 7.7	75.9 \pm 13.3	0.44
Bilirubin	0–5	$\mu\text{g}/\text{dl}$	3.9 \pm 0.1 ^a	3.0 \pm 0.0 ^b	3.4 \pm 0.2 ^a	0.04
Total protein	63–83	$\mu\text{mol}/\text{l}$	75.8 \pm 2.0	75.1 \pm 1.8	79.3 \pm 1.3	0.17
Albumin	26–40	g/l	29.6 \pm 1.0	27.5 \pm 1.1	28.9 \pm 0.9	0.61
Globulin	24–49	g/l	46.3 \pm 2.0	47.6 \pm 2.4	50.6 \pm 2.0	0.15
Albumin:globulin	0.6–1.6	ratio	0.6 \pm 0.0	0.6 \pm 0.1	0.6 \pm 0.0	0.30
Urea	5.7–12.9	mmol/l	9.6 \pm 0.8 ^a	11.5 \pm 0.5 ^a	12.1 \pm 0.6 ^b	0.04
Creatinine	70–159	$\mu\text{mol}/\text{l}$	90.0 \pm 4.7	94.9 \pm 5.7	90.0 \pm 8.7	0.98
Phosphate	1.3–5.8	mmol/l	1.3 \pm 0.1 ^a	1.3 \pm 0.1 ^a	1.5 \pm 0.0 ^b	0.01
Calcium	1.8–2.7	mmol/l	2.3 \pm 0.04	2.3 \pm 0.03	2.4 \pm 0.03	0.74
Cholesterol	1.5–6.0	mmol/l	3.2 \pm 0.2	3.1 \pm 0.2	3.3 \pm 0.3	0.82
Sodium	147–156	mmol/l	151.6 \pm 0.5	149.8 \pm 0.7	151.4 \pm 0.5	0.77
Potassium	3.5–5.0	mmol/l	4.7 \pm 0.1 ^a	4.8 \pm 0.2 ^a	5.1 \pm 0.1 ^b	0.046
Chloride	108–128	mmol/l ¹	116.8 \pm 0.8	115.5 \pm 0.9	115.6 \pm 0.5	0.27
Haematology						
Red blood cell count (RBC)	5–10	$\times 10^{12}/\text{l}$	7.6 \pm 0.3	6.8 \pm 0.4	7.2 \pm 0.3	0.37
Haemoglobin (Hb)	80–150	g/l ¹	111.8 \pm 3.8	101.6 \pm 4.5	106.7 \pm 4.1	0.39
Haematocrit (HCT)	0.24–0.45	l/l	0.4 \pm 0.01 ^a	0.3 \pm 0.01 ^b	0.3 \pm 0.02 ^{ab}	0.30
Mean corpuscular volume (MCV)	39–55	fl	46.4 \pm 0.7	44.0 \pm 0.9	45.6 \pm 0.7	0.47
Mean corpuscular haemoglobin (MCH)	13–18	Pg	14.9 \pm 0.3	15.1 \pm 0.4	14.9 \pm 0.3	0.99
Mean corpuscular haemoglobin conc.	290–360	g/l	319.8 \pm 6.2 ^a	341.7 \pm 4.0 ^b	327.0 \pm 6.7 ^a	0.39
Platelets	300–800	$\times 10^9/\text{l}$	319.7 \pm 69.2	310.3 \pm 71.6	321.6 \pm 59.5	1.00
Absolute reticulocyte count (abs. retic)	19–107	$\times 10^9/\text{l}$	13.9 \pm 2.7	16.4 \pm 2.8	13.4 \pm 2.9	0.94
Reticulocyte haemoglobin (Retic Hb)	13.2–20.8	pg	15.3 \pm 0.5	15.4 \pm 0.4	15.5 \pm 0.5	0.76
White blood cells	5.5–19.5	$\times 10^9/\text{l}$	17.3 \pm 3.0	18.9 \pm 2.7	17.8 \pm 1.4	0.27

¹ Normal ranges have been obtained from IDEXX Laboratories, Palmerston North, New Zealand. The P-values have been obtained from a repeated-measures ANOVA. Significant ($P < 0.05$) differences between day 0, 28 and 56 are indicated by different superscript letters.

Cytokines and chemokines

Reference ranges are lacking for most cytokines in domestic cats, making it difficult to determine whether the concentrations were within normal ranges. Troia *et al.* (2020) examined the concentrations of several cytokines and chemokines in the plasma of 40 clinically healthy cats, and these results are presented in Table 3. The concentrations of many cytokines (Flt-3L, IL-6, IL-8, IL-12, MCP-1, PDGF-BB, RANTES, SDF-1, IL-4, and IL-13) in the present study were markedly higher than previously reported (Troia *et al.*, 2020; Table 3). This was possible because a cohort of cats selected for this trial was older/senior (>10 years), although this was difficult

to confirm as Troia *et al.* (2020) did not report the age of the cats used. Nonetheless, several conditions that are more prominent in older cats (e.g. kidney or liver disease and joint disease) are known to increase cytokine concentrations and oxidative stress (Clarke *et al.*, 2005; Prah *et al.*, 2007). While there were no clear signs of these conditions in the biochemistry and haematology panels for the cats, it is worth noting that these parameters have a low diagnostic sensitivity and may not be able to detect such conditions until mid to late-onset. It is possible that the senior cats in this study had early onset conditions that led to these increases in cytokine concentrations, but this could not be confirmed. In any

TABLE 3 Mean \pm standard error of the mean (SEM) serum concentrations of pro-inflammatory cytokines after 0, 28 and 56 days of treatment with a diet supplemented with 0.1% olive polyphenols¹

Cytokine/Chemokine/ Growth factor	Control/references median ² (min-max) (pg/ml) ^a	Mean start (pg/ml)	Mean end (pg/ml)	P-value
Pro-inflammatory (primarily)				
Apoptosis antigen 1 (Fas or CD95)	ND	23.2 \pm 4.2	39.1 \pm 8.8	0.16
Fms-related tyrosine kinase 3 ligand (Flt-3L)	9.1 (4.0–38.6)	156.3 \pm 15.3	119.1 \pm 11.6	<0.001
Granulocyte macrophage colony- stimulating factor (GM-CSF)	ND	11.2*	15.3 \pm 6.3	ND
Interferon γ (IFN- γ)	ND	178.3 \pm 20.3	161.5 \pm 36	0.88
Interleukin 1 β (IL-1 β)	14.0 (14.0–61.7)	52.8*	ND	ND
Interleukin 2 (IL-2)	ND	13.7 \pm 1.0	10.5 \pm 2.3	ND
Interleukin 6 (IL-6)	25.0 (25.0–25.0)	189.3 \pm 36.2	210.4 \pm 44.6	0.74
Interleukin 8 (IL-8)	7.0 (7.0–7.0)	57.2 \pm 8.8	56.8 \pm 6.5	0.93
Interleukin 12p40 (IL-12p40)	107.1 (9.0–965.8)	1,068.8 \pm 144.7	1,042.9 \pm 167.7	0.78
Interleukin 18 (IL-18)	30.0 (30.0–30.0)	901.3 \pm 566.3	522.3 \pm 275.5	ND
Keratinocyte chemoattractant (KC or CXCL1)	1.0 (1.0–1.0)	28.5 \pm 6.7	38.6 \pm 7.7	0.32
Monocyte chemoattractant protein 1 (MCP-1)	220.6 (164.0–2482)	2,973.9 \pm 345.5	3,080.2 \pm 391.8	0.78
Platelet-derived growth factor BB (PDGF-BB)	198.0 (198.0–297.4)	5,499.4 \pm 595.1	5,346.5 \pm 1010.3	0.82
Chemokine ligand 5 (CCL5 or RANTES)	1.0 (1.0–11.46)	97.1 \pm 12.6	110.3 \pm 9.5	0.20
Stem cell factor (SCF)	ND	232.5 \pm 29.7	226.3 \pm 29.8	0.77
Stromal cell-derived factor 1 (SDF-1)	945.7 (248.0–2,233.0)	2,090.6 \pm 491.3	2,091.6 \pm 342.6	1.00
Tumour necrosis factor α (TNF- α)	7.0 (7.0–43.3)	33.1*	17.7 \pm 9.3	ND
Anti-inflammatory (primarily)				
Interleukin 4 (IL-4)	30.0 (30.0–363.7)	454.3 \pm 140.0	487.9 \pm 141.3	0.64
Interleukin 13 (IL-13)	7.0 (7.0–7.0)	48.2 \pm 9.4	47.5 \pm 9.9	0.96

¹ The P-value column represents the results of a paired Wilcoxon or T test. ND represents no data or insufficient data to complete a statistical comparison from the start to end of the study.

² Reference values were the results from 40 clinically healthy cats and were presented in Troia *et al.* (2020).

case, cytokine concentrations are well-known to differ with various factors (e.g. age, breed, sex, disease, etc.), hence why each cat in the present trial was used as its own control.

A comparison of the concentrations of the assessed cytokines at the start (day 0) and end (day 56) of dietary supplementation with 0.1% polyphenols is presented in Table 3. There were no clear differences in the circulating cytokine concentrations of the cats from the start to the end of the trial, except for Flt-3L, which had a tendency ($P=0.09$) to decrease over the treatment period (Table 3). However, a *post-hoc* test yielded no significant differences. The main role of Flt-3L is to promote haematopoiesis, that is, the production of new blood cells (Gilliland *et al.*, 2002). The decrease in Flt-3L could have been associated with the low absolute reticulocyte count observed in this study (Table 2). The clinical relevance of the decreased Flt-3L concentrations remains unclear and should not be overinterpreted, as it was only a statistical trend. It was worth noting that Flt-3L concentrations at the end of the study were still well above those previously reported by (Troia *et al.*, 2020). None of the other cytokines or chemokines exhibited either statistical or visual differences over time.

Overall, there were no clear and consistent differences in cytokine and chemokine concentrations as a result of dietary supplementation with 0.1% olive polyphenols (Table 3). This lack of difference was somewhat surprising, based on past literature in other species such as rats, which suggested that dietary polyphenol supplementation decreases the concentrations of several pro-inflammatory cytokines, increase the concentrations of several anti-inflammatory concentrations, and decreases reactive oxygen species (i.e. oxidative stress) (Molina *et al.*, 2015; Shen *et al.*, 2012). It may be that the dose of 0.1% of the diet was insufficient to elicit a clear response to the polyphenol treatment.

Similar concentrations (0.1%) of citrus polyphenol extract have been found to have physiological effects and decrease IFN- γ and IL-2 mRNA levels in the peripheral mononuclear blood cells of cats (Leray *et al.*, 2011). However, Leray *et al.* (2011) did not assess circulating cytokine and chemokine concentrations, as in the present study, making direct comparison difficult. It is possible that olive polyphenols are required in higher concentrations than citrus polyphenols, but this requires confirmation. Based on the present data, the dose given could be increased, although caution is needed when giving dietary polyphenols as excessive doses can result in a plethora of adverse effects including death (Kapetanovic *et al.*, 2009). In addition, there is

a risk of adversely affecting the palatability of the diet as polyphenols are considered bitter, especially given the initial aversion or low intake of the diet observed in this study. Alternatively, there may be more sensitive parameters (e.g. cytokine mRNA levels in the peripheral mononuclear blood cells or markers of oxidative stress) that could be used to investigate the effects of polyphenols in cats.

It is worth noting the Millipore Feline Cytokine Panel, which was selected as it can be used to analyse 19 cytokines from a single low-volume blood sample, did not include some of the major anti-inflammatory cytokines (e.g. IL-10 and transforming growth factor β (TGF- β)). While major pro-inflammatory cytokines were not significantly affected by the polyphenol treatment, changes in these key anti-inflammatory cytokines may have occurred and warrants further investigation. There is evidence that dietary polyphenols can decrease oxidative stress in cats, which has a number of health benefits for older cats in which oxidative stress is more prominent (Jeusette *et al.*, 2010). Thus, it may be worthwhile investigating whether dietary polyphenol supplementation decreased markers of oxidative stress of the cats (e.g. urinary isoprostane or blood reactive oxygen species concentrations); stored urine and serum samples from the present study could be used to assess this.

4 Conclusions

In conclusion, there was an initial aversion to the provision of a mixed diet supplemented with 0.1% olive polyphenols. It was unclear whether this was due to the palatability of the polyphenols or the different texture and/or appearance of a mixed diet. In any case, the cats appeared to become accustomed to this diet within 7–10 days and consumed enough of the diet to meet and exceed the MER for the group. Therefore, there may be a need to transition the cats to this diet gradually and there may be a palatability threshold for polyphenol inclusion. However, despite the high intake of the supplemented diet, the cats lost 300 g on average (8% of their starting bodyweight) during the study. This result was intriguing and this effect should be elucidated. Dietary supplementation with 0.1% olive polyphenols had little-to-no clear effects on blood biochemistry, haematology or cytokine/chemokine profiles of senior domestic cats. This was surprising, given that a diet with 0.1% citrus polyphenols has been found to have physiological effects and reduce proinflammatory cytokines in mononuclear blood cells. It may be olive polyphenols

may be required at a higher dose than citrus sources to be physiologically active or that circulating cytokine concentrations are less sensitive than cytokine mRNA expression in mononuclear blood cells. Regardless, dietary supplementation for senior cats to reduce oxidative stress and inflammation is an important area of research with increasing interest. Thus, more information regarding dietary supplementation with polyphenols is certainly needed, perhaps using (with care) a slightly higher dose of polyphenol extract. It would be worthwhile investigating the effects of dietary supplementation with polyphenols on markers of oxidative stress (e.g. reactive oxygen species or urinary isoprostanes) and anti-inflammatory cytokines (e.g. IL-10 and TGF β) in domestic cats.

Conflict of interest

The authors indicate no conflict of interest.

References

- Association of American Feed Control Officials (AAFCO), 2021. Official Publication. AAFCO Inc., Champaign, IL, USA.
- Ansari, M.Y., Ahmad, N. and Haqqi, T.M., 2020. Oxidative stress and inflammation in osteoarthritis pathogenesis: role of polyphenols. *Biomedicine and Pharmacotherapy* 129: 110452.
- Bermingham, E., Weidgraaf, K., Hekman, M., Roy, N., Tavendale, M. and Thomas, D., 2013. Seasonal and age effects on energy requirements in domestic short-hair cats (*Felis catus*) in a temperate environment. *Journal of Animal Physiology and Animal Nutrition* 97: 522–530.
- Burkholder, W. and Bauer, J., 1998. Foods and techniques for managing obesity in companion animals. *Journal of the American Veterinary Medical Association* 212: 658–662.
- Cave, N., Allan, F., Schokkenbroek, S., Metekohy, C. and Pfeiffer, D., 2012. A cross-sectional study to compare changes in the prevalence and risk factors for feline obesity between 1993 and 2007 in New Zealand. *Preventive Veterinary Medicine* 107: 121–133.
- Chiang, C.-F., Villaverde, C., Chang, W.-C., Fascetti, A.J. and Larsen, J.A., 2022. Prevalence, risk factors, and disease associations of overweight and obesity in cats that visited the Veterinary Medical Teaching Hospital at the University of California, Davis from January 2006 to December 2015. *Topics in Companion Animal Medicine* 47: 100620.
- Clarke, S., Mellor, D., Clements, D., Gemmill, T., Farrell, M., Carmichael, S. and Bennett, D., 2005. Prevalence of radiographic signs of degenerative joint disease in a hospital population of cats. *Veterinary Record* 157: 793–799.
- Cowgill, E. S., Neel, J. A., and Grindem, C. B., 2003. Clinical application of reticulocyte counts in dogs and cats. *Veterinary Clinics: Small Animal Practice* 33: 1223–1244.
- Forman, H.J. and Zhang, H., 2021. Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery* 20: 689–709.
- Gilliland, D. G., and Griffin, J. D., 2002. The roles of FLT3 in hematopoiesis and leukemia. *Blood. Journal of the American Society of Hematology* 100: 1532–1542.
- Guillot, M., Moreau, M., Heit, M., Martel-Pelletier, J., Pelletier, J. P., and Troncy, E., 2013. Characterization of osteoarthritis in cats and meloxicam efficacy using objective chronic pain evaluation tools. *Veterinary Journal* 196: 360–367.
- Hardie, E.M., 1997. Management of osteoarthritis in cats. *Veterinary Clinics of North America: Small Animal Practice* 27: 945–953.
- Jain, S.K., Rains, J., Croad, J., Larson, B. and Jones, K., 2009. Curcumin supplementation lowers TNF- α , IL-6, IL-8, and MCP-1 secretion in high glucose-treated cultured monocytes and blood levels of TNF- α , IL-6, MCP-1, glucose, and glycosylated hemoglobin in diabetic rats. *Antioxidants and Redox Signaling* 11: 241–249.
- Jeusette, I., Torre, C., Salas, A., Iraculis, N., Compagnucci, M., Romano, V. and Kirschvink, N., 2010. Effects of consuming diets containing various fats or citrus flavanones on plasma lipid and urinary F2-isoprostane concentrations in overweight cats. *American Journal of Veterinary Research* 71: 1039–1044.
- Kapetanovic, I., Crowell, J., Krishnaraj, R., Zakharov, A., Lindeblad, M. and Lyubimov, A., 2009. Exposure and toxicity of green tea polyphenols in fasted and non-fasted dogs. *Toxicology* 260: 28–36.
- Kara, K., Sarikaya, A.E. and Aktug E., 2016. Dietary supplementation of catechin: Effect on performance, fecal quality, mineral and malondialdehyde levels in Belgian Malinois puppies. *Animal Nutrition and Feed Technology* 16: 119–128.
- Kimura, T., Kimura, S., Okada, J., Suzuki, S. and Kitanaka, T., 2020. Retrospective radiographic study of degenerative joint disease in cats: Prevalence based on orthogonal radiographs. *Frontiers in Veterinary Science* 7: 138.
- Lascelles, B.D. and Sheilah, A.R., 2010. DJD-associated pain in cats: what can we do to promote patient comfort? *Journal of Feline Medicine and Surgery* 12: 200–212.
- Lascelles, B.D., 2010. Feline degenerative joint disease. *Veterinary Surgery* 39: 2–13.

- Lascelles, B.D., DePuy, V., Thomson, A., Hansen, B., Marcellin-Little, D.J., Biourge, V., and Bauer, J.E., 2010b. Evaluation of a therapeutic diet for feline degenerative joint disease. *Journal of Veterinary Internal Medicine* 24: 487–495.
- Lascelles, B.D., Henry III, J.B., Brown, J., Robertson, I., Sumrell, A.T., Simpson, W., Wheeler, S., Hansen, B.D., Zamprogno, H., and Freire, M., 2010a. Cross-sectional study of the prevalence of radiographic degenerative joint disease in domesticated cats. *Veterinary Surgery* 39: 535–544.
- Leray, V., Freuchet, B., Le Bloc'h, J., Jeusette, I., Torre, C. and Nguyen, P., 2011. Effect of citrus polyphenol-and curcumin-supplemented diet on inflammatory state in obese cats. *British Journal of Nutrition* 106: S198–S201.
- Millán-Laleona, A., Bielsa, F.J., Aranda-Cañada, E., Gómez-Rincón, C., Errea, P. and López, V., 2023. Antioxidant, anti-diabetic, and anti-obesity properties of apple pulp extracts (*Malus domestica* Bork): a comparative study of 15 local and commercial cultivars from Spain. *Biology* 12: 891.
- Molina, N., Bolin, A. and Otton, R., 2015. Green tea polyphenols change the profile of inflammatory cytokine release from lymphocytes of obese and lean rats and protect against oxidative damage. *International Immunopharmacology* 28: 985–996.
- Pandey, K.B. and Rizvi, S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity* 2: 270–278.
- Prahl, A., Guptill, L., Glickman, N.W., Tetrack, M. and Glickman, L.T., 2007. Time trends and risk factors for diabetes mellitus in cats presented to veterinary teaching hospitals. *Journal of Feline Medicine and Surgery* 9: 351–358.
- Rychel, J.K., 2010. Diagnosis and treatment of osteoarthritis. *Topics in Companion Animal Medicine* 25: 20–25.
- Scarlett, J. and Donoghue, S., 1998. Associations between body condition and disease in cats. *Journal of the American Veterinary Medical Association* 212: 1725–1731.
- Scarlett, J., Donoghue, S., Saidla, J. and Wills, J., 1994. Overweight cats: prevalence and risk factors. *International Journal of Obesity and Related Metabolic Disorders* 18: S22–28.
- Serisier, S., Feugier, A., Delmotte, S., Biourge, V. and German, A.J., 2014. Seasonal variation in the voluntary food intake of domesticated cats (*Felis catus*). *PLoS ONE* 9: e96071.
- Shen, C.-L., Cao, J.J., Dagda, R.Y., Chanjaplammoovil, S., Lu, C., Chyu, M.-C., Gao, W., Wang, J.-S. and Yeh, J.K., 2012. Green tea polyphenols benefits body composition and improves bone quality in long-term high-fat diet – induced obese rats. *Nutrition Research* 32: 448–457.
- Sung, S., Kwon, D., Um, E. and Kim, B., 2019. Could polyphenols help in the control of rheumatoid arthritis? *Molecules* 24: 1589.
- Tarragon, E. and Moreno, J.J., 2020. Polyphenols and taste 2 receptors. Physiological, pathophysiological and pharmacological implications. *Biochemical Pharmacology* 178: 114086.
- Troia, R., Mascalzoni, G., Agnoli, C., Lalonde-Paul, D., Giunti, M. and Goggs, R., 2020. Cytokine and chemokine profiling in cats with sepsis and septic shock. *Frontiers in Veterinary Science* 7: 305.
- Weisberg, S.P., Leibel, R. and Tortoriello, D.V., 2008. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabetes. *Endocrinology* 149: 3549–3558.
- Williamson, G., 2017. The role of polyphenols in modern nutrition. *Nutrition Bulletin* 42: 226–235.