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A cross-over dietary intervention in captive cheetahs (*Acinonyx jubatus*): Investigating the effects of glycine supplementation on blood parameters

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Abstract

Captive cheetahs are prone to unusual diseases which may be attributed to their high muscle meat, collagen deficient captive diet. Glycine is a simple amino acid that is abundant in collagen rich tissues and has many physiological functions, specifically in collagen synthesis and in the conjugation of detrimental by-products produced during gut bacterial fermentation. Therefore, the aim of this study was to investigate the effect of a 4 week glycine supplementation on the body measurements, haematology and serum blood parameters of 10 captive cheetahs using a randomised controlled cross-over design. This approach has not yet been used to investigate the effect of diet in captive cheetahs. Cheetahs were randomly assigned to a control diet (horse meat only) or a glycine diet (30 g glycine per 1 kg meat) for 4 weeks before being crossed over. Blood was collected at baseline and after each intervention. The glycine diet resulted in a decreased serum albumin, alkaline phosphatase and total calcium concentration and increases in eosinophils and basophils counts compared to the control diet. Body weight also decreased on the glycine diet which may be due to increased β-oxidation and fat loss. This was the first study to investigate the effect of glycine supplementation, which resulted in slight body and blood changes, in captive cheetahs using a cross-over design and this approach should be utilised for future dietary studies.

KEYWORDS
amino acid, captivity, exotic felid, nutrition

1 | INTRODUCTION

The cheetah (*Acinonyx jubatus*) is a vulnerable, unique felid that is highly specialised for the rapid pursuit of prey, reaching speeds of 29 m/s (104 km/h), and is the last remaining species of the genus *Acinonyx* (Durant et al., 2015; Hayward et al., 2006; Sharp, 1997). Adult cheetahs typically weigh between 25 and 65 kg, with males being larger for many morphometric measures compared to females (Marker & Dickman, 2003). Historically, cheetahs occupied a wide variety of habitats, but they now only occur in 9% of their historic range with an estimated 7100 adult and adolescent cheetahs distributed across 33 global population groups (Durant et al., 2017). In captivity, cheetahs are prone to an array of unusual diseases such as chronic lymphoplasmacytic gastritis,

glomerulosclerosis, adrenal cortical hyperplasia, splenic lymphocytic depletion and veno-occlusive disease, which are not observed in their free-roaming counterparts (Munson, 1993; Munson et al., 1999). Various aspects of captivity, including stress, diet, age and lack of physical activity may be contributing to these diseases in captive cheetahs (Gillis-Germitsch et al., 2017; Mitchell et al., 2018; Munson et al., 2005).

Cheetahs are efficient hunters that generally pursue small to medium sized prey and are obligate carnivores that, in the wild, consume bones, tendons and cartilage on a regular basis (Depauw et al., 2013; Hayward et al., 2006). In captive facilities, however, a raw meat diet is the most popular, followed by a commercially prepared and carcass diet (Whitehouse-Tedd et al., 2015). There have been a number of studies investigating the nutritional composition of the various diets that captive cheetahs are fed to elucidate the potential cause of the high disease rate in captivity (Bechert et al., 2002; Depauw, Bosch, et al., 2012; Depauw, Hesta, et al., 2012; Depauw et al., 2013; Lane et al., 2012; Setchell et al., 1987; Vester et al., 2008). These studies have highlighted aspects such as high protein and incomplete protein digestion, low nutrient diversity and increased substrate fermentation by gut bacteria as potential contributors.

Glycine is the simplest amino acid and is traditionally classified as 'nutritionally non-essential' as it is synthesised endogenously (Wang et al., 2013). Glycine plays a key role in the metabolism, cytoprotection, immune response, growth and development of many mammals (Wang et al., 2013). Among its many physiological functions, the most notable is its role in the synthesis of collagen, where glycine molecules are incorporated into every third position of the collagen triple helix (Li & Wu, 2018). Collagen provides the strength, rigidity and flexibility of connective tissue found in bones, skin, cartilage and blood vessels, and is therefore crucial in the growth, development and health of all animals (Li & Wu, 2018). Chronic glycine shortage could lead to suboptimal growth, impaired immune response and other adverse metabolic effects (Wang et al., 2013). Animal studies have used up to 3% glycine supplementation while clinical studies in humans have ranged between 3 g per day and 0.8 g/kg body weight per day with no adverse side effects (McCarty et al., 2018; Petzke et al., 1986). The effects of dietary glycine supplementation has been studied in vivo in rats, pigs, chickens, fish, rabbits and humans with an array of benefits including controlling metabolic syndrome and diabetes, inhibiting atherosclerosis, reducing oxidative damage, improving sleep quality, collagen synthesis and promoting a healthy and optimum metabolism (Badenhorst et al., 2014; Imenshahidi & Hossenzadeh, 2022; Li & Wu, 2018; McCarty et al., 2018; Wang et al., 2014). Additionally, glycine supplementation has been shown to increase serum and plasma glycine concentration in humans and pigs, indicating absorption into the body (Gannon et al., 2002; Wang et al., 2014).

Cheetahs are prone to a variety of unusual gastrointestinal diseases in captivity which may be associated with their high muscle meat diet. This excess muscle protein in the diet or incomplete protein digestion can lead to bacterial fermentation in the intestine producing detrimental by-products such as ammonia, indolic and phenolic compounds (Depauw et al., 2013; Vester et al., 2008). Importantly glycine plays a vital role in the conjugation of these compounds to allow their urinary excretion (Badenhorst et al., 2014). Indeed, Tordiffe et al. (2017) showed that a third of the 30 most abundant organic acids excreted in the urine of cheetahs are associated with phenylalanine and tyrosine-relatedglycine conjugates. This suggests that cheetahs have a substantial metabolic need for glycine, due to the high excretion of glycine conjugates, which may not be met through endogenous synthesis or conventional captive diets, which have lower collagen content, as has been found in other species (Meléndez-Hevia et al., 2009). Anecdotal evidence from a glycine supplementation pilot study conducted on five cheetahs with severe gastritis, suggested that the addition of glycine to their diet improved their gastritis scores and serum creatinine concentrations (Tordiffe, unpublished). However, this was an uncontrolled trial and the metabolic implications of glycine supplementation in captive cheetahs were still unknown and warranted further investigation. Furthermore, randomised controlled cross-over trials are considered the strongest study design in biomedicine and pharmacology, yet these study designs have rarely been used in captive animals, including cheetahs, and may prove to be a beneficial approach to further understand the effect of different diets (Elbourne et al., 2002). It is for the above reasoning that the present study was primarily undertaken.

Although diet has been investigated in a limited number of studies in captive cheetahs, the effect of glycine supplementation has not been investigated in this population. Therefore, the aims of this study were to investigate the effect of a 4 week glycine supplementation on the body measurements, haematology and serum blood parameters of 10 captive cheetahs using a randomised controlled cross-over design. Haematology and serum biochemistry have been analysed in both captive and free-ranging cheetahs in several studies which provide a valuable reference point for comparison (Bechert et al., 2002; Depauw, Hesta, et al., 2012; Hawkey & Hart, 1986; Hudson-Lamb et al., 2016). Therefore, investigating these blood parameters may provide an initial clinical indication of the effect of glycine in cheetahs before investigating specific metabolic effects through more comprehensive techniques.

Research Highlights

- Four week glycine diet in captive cheetahs caused body weight and blood changes.
- First to explore this in exotic felids, important when dietary collagen is limited.
- Randomised controlled cross-over design is a useful method to study diets in captivity.

2 | METHOD

2.1 | Study design and animals

The study was designed as a randomised cross-over dietary intervention on 10 captive cheetahs. Five young male and five female cheetahs housed at Cango Wildlife Ranch in Oudtshoorn, Western Cape, South Africa were included. The males and females had an average age of 4.2 ± 1.3 and 3.2 ± 1.1 years, respectively, with an age range of 2–5 years. At the time of the study, all animals were healthy with no clinical signs of gastritis or renal failure which were inclusion criteria. The animals were housed in medium to large enclosures (400–1350 m^2) away from the public in a rural setting.

2.2 | Diet and feeding intervention

Before the start of the study, all animals received a varied diet containing a combination of horse or donkey muscle mince (approximately 80% of total diet), organ mince, shredded skin, bones with meat, a vitamin and mineral predator supplement, calcium mixture and iodated salt. To standardise the diet across the 10 cheetahs, a 3 week habituation period was implemented before the first baseline sample collection (T1). All animals were fed the control diet during this 3 week period which consisted of 1 fasting day per week. Cheetahs received only horse muscle mince with 10 g of vitamin and mineral supplement for every 1 kg mince (Panthera supplement; WildCat Nutrition). The following vitamins and minerals were contained per 10 g of supplement: 3 g calcium, 0.67 g phosphorous, 4 mg copper, 1.5 mg manganese, 0.34 g sodium chloride, 5000 IU vitamin A, 5 mg vitamin B_1 (thiamin), 2.5 mg vitamin B_2 (riboflavin), 10 mg vitamin B_5 (pantothenic acid), 100 µg vitamin B_7 (biotin), 800 µg vitamin B_9 (folic acid), 125 mg vitamin C, 100 IU vitamin D_3, and 50 mg vitamin E. The nutritional breakdown of horse muscle meat has previously been described by Badiani et al. (1997) with an approximate 19.8 g protein, 6.6 g lipids and an array of vitamins and minerals per 100 g. The supplement was mixed into the meat. The standardisation was implemented to control the glycine intake of the cheetahs as collagen contains high concentrations of glycine (skin and bones), but glycine is relatively low in meat. Therefore, a meat only diet was fed during the habituation and control phase. Baseline data and samples were collected after the 3 week habituation period.

Following the first sample collection (T1), cheetahs were randomly assigned to a control (no glycine) or intervention group (glycine supplemented). Due to unforeseen logistical challenges, the groups were uneven with six cheetahs in Group 1 (control diet) and four cheetahs in Group 2 (glycine diet). Those on the control diet continued with the habituation diet, whereas the cheetahs in the intervention group were fed an additional 30 g of glycine powder per 1 kg meat (Glycine supplement; WildCat Nutrition). Cheetahs remained on these diets for 4 weeks, with 1 fasting day per week, whereafter the second sample collection occurred (T2). All animals then underwent a 2-week washout period where they were fed the habituation diet to negate any effects caused by the glycine supplementation and return the animals to baseline. The groups were then crossed-over and fed the corresponding diet for another 4 weeks. The third sample collection (T3) occurred after the 4 weeks. The length of time for each phase of the study was determined based on available time and funding with 4 weeks deemed to be an appropriate period of supplementation as previous studies in humans, pigs and rodents were conducted within a range of 2–16 weeks (Imenshahidi & Hossenzadeh, 2022; McCarty et al., 2018; Petzke et al., 1986; Wang et al., 2014). An overview of the study is presented in Figure 1.

2.3 | Immobilisation

The cheetahs were immobilised at each sampling time point via intramuscular hand injection with 30 µg/kg medetomidine hydrochloride (10 mg/mL, Medetomidine; Kyron Laboratories Pty Ltd) in combination with 1 mg/kg zolazepam/tiletamine (100 mg/mL, Zoletil®; Virbac). Once sedated, the cheetahs were transported to the medical centre and maintained under general anaesthesia with 1%–2% isoflurane in oxygen for the duration of the collection period, which lasted approximately 1 h. Throughout the sedation period, the standard anaesthetic and cardiovascular parameters of the cheetahs were monitored. Once all the samples were collected, the sedation was reversed with 5 mg/kg intramuscular atipamezole (5 mg/mL, Antisedan®; Pfizer). Cheetahs were monitored regularly throughout the day and the subsequent day.

2.4 | Sample collection and storage

At the medical centre, the cheetahs were weighed, body length and height measured while the cheetah was in lateral recumbency. Length was measured from the occiput at the caudal edge of the skull to the base of the tail, whereas height was measured from the dorsal rim of the scapula to the metacarpal pad. Length and height were only measured during the first sampling (T1). The size index of each cheetah was calculated by multiplying the body length with the shoulder height. The size index was divided by weight to determine the body mass index (BMI) of the cheetahs as described by Kirberger and Tordiffe (2016).

Blood was collected from the jugular vein using an 18 G needle and 20 mL syringe, whereafter it was divided and transferred to an EDTA and serum tube. The fresh blood was handled with care to avoid haemolysis of the red blood cells. The whole blood in the EDTA tube was used for haematological analysis on site. After analysis, whole blood was stored at −20°C. The blood in the serum tube was allowed to clot for 30 min at room temperature and subsequently

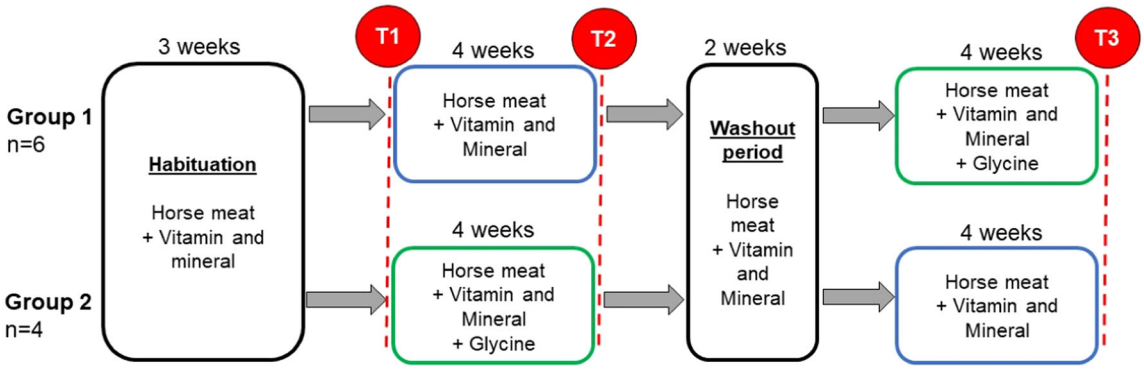

FIGURE 1 The cross-over study design for 10 captive cheetahs. T1, first sample collection; T2, second sample collection; T3, third sample collection. [Color figure can be viewed at wileyonlinelibrary.com]

centrifuged for 10 min at 6000 rpm. The supernatant was transferred to a new microtube and stored at −20°C until analysis.

2.5 | Blood analyses

Whole blood was analysed on site using the Abaxis Vetscan HM5 haematology system set to the cat species setting. The white blood cell (WBC) count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC), red blood cell distribution width (RDW), platelet count and percentage, mean platelet volume (MPV) and platelet distribution width (PDW) were determined.

Routine serum biochemistry analyses were performed and the concentration of the following parameters were determined using the Cobas Integra® 400 plus analyser (Roche): total serum protein (TSP), albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, phosphate, triglycerides and total calcium. Sodium, potassium and chloride were measured using the Rapidpoint® 500 System (Siemens Ltd) and specific electrolyte assay cartridges.

2.6 | Statistical analysis

Statistical calculations were performed using GraphPad Prism 5 version 5.03 (GraphPad Software). Values are expressed as mean ± standard deviation and normality was confirmed within diet groups using the Shapiro–Wilks normality test. As the study design divided the animals into Group 1 with six animals and Group 2 with four animals, a nonparametric Mann–Whitney *U* test was performed to identify if there was any difference between these two groups for all measured variables. There was no difference between groups and, therefore, groups were combined under each diet, that is, a combined baseline, control and glycine supplemented diet. A repeated measures one-way ANOVA with a Tukey post hoc test was used to compare the three groups. Significance was set at $p < .05$.

3 | RESULTS

3.1 | Diet and feeding

The control and glycine supplemented diet had no adverse effect on the cheetahs and all food offered was consumed throughout the study. The average amount of meat, vitamin and mineral supplement, and glycine supplement consumed is reported in Table 1. The amount of food each animal consumed remained fairly consistent throughout the study with three males, from Group 2, having a 200 g meat increase as the study progressed (12.5% average increase).

3.2 | Body measurements

The weight and body measurements of the study cheetahs are reported in Table 2. Male and female data have been shown separately, however statistical difference was only investigated between the different diets as groups were matched which also reduced any confounding effects caused by sex. The average weight at baseline and on the control diet were 40.2 ± 4.5 and 39.5 ± 4.0 kg, respectively. Weight significantly decreased to 38.8 ± 4.4 kg from baseline on the glycine supplemented diet. At baseline, the BMI of the cheetahs were 52.2 ± 3.6 kg/m^2 which was similar to the control diet at 51.2 ± 2.9 kg/m.^{2} The glycine diet had a significantly lower BMI of 50.3 ± 3.5 kg/m^2 compared to the baseline. All the parameters fell within the healthy ranges reported by Kirberger and Tordiffe (2016).

3.3 | Haematology

The whole blood haematology data are shown in Table 3. Neutrophils contributed the most to the WBC count while eosinophils increased significantly from baseline (0.15 ± 0.07 × 10^9/L) to the control diet (0.26 ± 0.08 × 10^9/L) and the glycine diet (0.25 ± 0.09 × 10^9/L). Similarly, the basophil count also increased from the baseline diet

($0.01 \pm 0.01 \times 10^9$/L) to the control and glycine supplemented diets with 0.02×10^9/L, albeit remaining the lowest count of the WBCs. The RBC count and the various parameters remained similar across the diets—haemoglobin, haematocrit, MCV, MCH and RDW. The platelet count and parameters, including MPV and volume in the blood (PCT), remained similar across the diets. The PDW significantly increased from baseline (42.5 ± 0.3%) to the glycine supplemented diet (42.8 ± 0.5%), with the control diet at 42.6 ± 0.5%. All the haematological data across the diets fell within the reference ranges.

TABLE 1 Average daily amount of meat, vitamin and mineral supplement, and glycine supplement for male ($n = 5$) and female ($n = 5$) cheetahs during the habituation, control and glycine intervention periods.

	Horse muscle meat (kg)	Vitamin and mineral supplement (g)	Glycine supplement (g)
Habituation (3 weeks)			
Male	1.7 ± 0.3	17.4 ± 2.5	0
Female	1.8 ± 0.3	18.0 ± 2.6	0
Control diet (4 weeks)			
Male	1.9 ± 0.2	18.6 ± 1.5	0
Female	1.8 ± 0.3	18.0 ± 2.6	0
Glycine diet (4 weeks)			
Male	1.8 ± 0.3	17.8 ± 2.6	53.4 ± 7.8
Female	1.8 ± 0.3	18.0 ± 2.6	54.0 ± 7.6

Note: Data are expressed as mean ± SD.

3.4 | Serum biochemistry

The serum biochemistry data of the 10 captive cheetahs are shown in Table 4. The TSP and, therefore, the albumin concentration was significantly different from the baseline (TSP: 63.5 ± 2.8 g/L; albumin: 42.7 ± 1.8 g/L) to the control diet (TSP: 61.9 ± 3.4 g/L; albumin: 40.6 ± 2.1 g/L) and glycine diet (TSP: 60.9 ± 3.1 g/L; albumin: 40.5 ± 1.6 g/L). ALP, an enzyme predominantly synthesised in the liver, was significantly higher at baseline (14.5 ± 9.4 U/L) compared to the control diet (11.2 ± 6.7 U/L) and glycine supplemented diet (10.2 ± 5.7 U/L). Potassium, on the other hand, was higher in the control diet (4.2 ± 0.1 mmol/L) compared to the baseline (3.9 ± 0.3 mmol/L). The total calcium concentration (bound and free) was higher at baseline (2.66 ± 0.06 mmol/L) compared to the glycine supplemented diet (2.59 ± 0.05 mmol/L), with the control diet at 2.61 ± 0.09 mmol/L. ALT, urea, creatinine, sodium, phosphate, chloride and triglyceride concentrations remained constant across the three groups. Most of the parameters, including those that were significantly different, in Table 4 fell within the indicated reference ranges, but ALP activity was noticeably lower in the current study compared to what was reported by Bechert et al. (2002).

TABLE 2 Body weight and measurements of male ($n = 5$) and female ($n = 5$) captive cheetahs at baseline, control and glycine diet.

	Baseline		Control diet		Glycine diet		
	Mean	SD	Mean	SD	Mean	SD	Reference range
Weight (kg)	40.2[a]	4.5	39.5	4.0	38.8[a]	4.4	
Female	37.5	2.6	36.8	2.3	36.5	2.3	30.9–44.1
Male	43.0	4.6	42.2	3.6	41.1	4.9	33.6–50.6
Body length (m)							
Female	1.00	0.04	—	—	—	—	0.92–1.01
Male	1.03	0.06	—	—	—	—	0.93–1.10
Shoulder height (m)							
Female	0.74	0.02	—	—	—	—	0.64–0.73
Male	0.77	0.02	—	—	—	—	0.68–0.77
Size index							
Female	0.74	0.04	—	—	—	—	0.61–0.72
Male	0.80	0.06	—	—	—	—	0.63–0.84
Body mass index	52.2[a]	3.6	51.2	2.9	50.3[a]	3.5	
Female	50.6	1.9	49.7	1.1	49.3	0.8	45.9–66.4
Male	53.7	4.4	52.7	3.5	51.3	5.0	42.9–63.1

[a]$p < .05$ between combined male and female baseline and no glycine groups; — indicates data was not measured. Size index calculated as body length x shoulder height. Body mass index calculated as weight/size index. Reference range from Kirberger and Tordiffe (2016).

TABLE 3 Haematology data of 10 captive cheetahs at baseline, with the control and glycine diet.

Parameters	Baseline		Control diet		Glycine diet		Previous literature	
	Mean	SD	Mean	SD	Mean	SD	Bechert	Hawkey
WBC (x10^9/L)	11.12	3.24	11.30	4.19	11.31	2.81	7.6–16.2	5.9–12.0
Lymphocytes	2.44	1.51	1.90	0.47	1.88	0.31	0.9–3.1	0.9–2.7
Monocytes	0.55	0.28	0.63	0.37	0.54	0.30	0.07–0.64	0–0.3
Neutrophils	7.97	2.45	8.49	4.09	8.62	2.74	5.4–12.9	5–9.4
Eosinophils	0.15[a,b]	0.07	0.26[a]	0.08	0.25[b]	0.09	0.09–0.81	0.0–1.4
Basophils	0.01[a,b]	0.01	0.02[a]	0.00	0.02[b]	0.01		0
RBC (x10^{12}/L)	8.72	0.45	8.54	0.44	8.33	0.59	5.7–10.3	6–8.3
Haemoglobin (g/L)	160.00	8.46	155.80	8.72	153.60	8.81	102–195	116–166
Haematocrit (%)	48.43	2.06	47.47	1.74	46.51	2.57	29.3–58.7	
MCV (fL)	55.60	1.71	55.60	1.71	55.90	1.73	46.1–58.1	50–62
MCH (pg)	18.38	0.87	18.23	0.69	18.46	0.50	15.9–20.0	18.4–21.8
MCHC (g/L)	330.60	13.90	327.70	7.39	330.30	6.07	328–348	315–369
RDWc (%)	19.59	0.50	19.80	0.49	19.48	0.35		
RDWs (fL)	40.55	1.83	40.92	1.32	40.54	1.40		
Platelets (x10^9/L)	406.00	123.58	395.60	78.47	429.00	89.09	243–475	
MPV (fL)	12.60	1.30	12.61	0.71	12.54	0.81		
PCT (%)	0.50	0.14	0.50	0.09	0.53	0.08		
PDWc (%)	42.53[b]	0.31	42.57	0.49	42.84[b]	0.50		
PDWs (fL)	26.21	1.32	26.27	1.48	26.81	1.19		

Abbreviations: MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PCT, platelet volume in blood; PDW, platelet distribution width; RBC, red blood cells; RDW, red blood cell distribution width; WBC, white blood cells.

Reference range from Bechert et al. (2002) and Hawkey and Hart (1986).

[a]$p < .05$ between baseline and control groups.

[b]$p < .05$ between baseline and glycine groups.

4 | DISCUSSION

4.1 | Body measurements

There was a decrease in the average body weight of the cheetahs from baseline to the glycine supplemented diet, and this was likely due to a reduction in the body weight of the males as the females remained fairly consistent in weight. This change in weight also led to a corresponding decrease in BMI from the baseline to the glycine supplemented diet. Although the weight of the animals decreased, they were still well within a healthy range, such as what was provided by Kirberger and Tordiffe (2016). The amount of meat consumed by each animal remained constant throughout the study, with a marginal increase during the glycine phase with ±100 g meat per feed. This increase was determined by the designated carers who deemed it necessary due to apparent hunger based on the cheetah's behaviour. Throughout the study, the standard feeding procedures of Cango Wildlife Ranch were followed, particularly the time of day when food was provided, number of days and the amount of meat provided. The standard feeding procedures were determined by the appropriate staff based on the cheetah's feeding history, appetite and behavioural response to the food. Therefore, the weight loss is not due to a decrease in food intake.

A potential contributor to the weight loss is the study diet itself, which would be suboptimal. Before the study, the animals received a combination of organ mince, skin and bones which would provide them with a greater array of nutrients more aligned to their carnivorous diet in the wild. However, to investigate the effects of the glycine alone, it was necessary to remove all other potential sources of glycine, such as collagen in bones and skin (Li & Wu, 2018). While body weight appeared to decrease from the baseline to the control diet, it was not significantly different, indicating that the glycine supplemented diet potentially played a larger role in weight loss. In healthy humans supplemented with 3.6–5.4 g glycine, the concentration of plasma glycine increased fourfold within 40 min and remained elevated for 2 h, indicating rapid absorption of glycine (Gannon et al., 2002). Glycine also constantly stimulated the release of glucagon (which increases blood glucose), but in contrast, when glycine was ingested with glucose, this reduced the plasma glucose concentration by half. The authors speculated that the oral ingestion of

TABLE 4 Serum data of 10 captive cheetahs at baseline, control diet and glycine diet.

	Baseline		Control diet		Glycine diet		Previous literature		
	Mean	SD	Mean	SD	Mean	SD	Bechert	Hudson-Lamb	Depauw
Total serum protein (g/L)	63.54[a,b]	2.84	61.87[a]	3.37	60.93[b]	3.14	60–76		69 ± 1
Albumin (g/L)	42.65[a,b]	1.81	40.56[a]	2.08	40.51[b]	1.59	35–43		40 ± 1
Globulins (g/L)	20.91	2.13	21.31	2.76	20.44	2.56	24–33		29 ± 1
ALT (U/L)	79.19	23.78	74.37	17.80	71.96	18.06	66–198		69 ± 13
ALP (U/L)	14.50[a,b]	9.44	11.22[a]	6.68	10.23[b]	5.65	22–30		
Urea (mmol/L)	15.12	3.15	14.29	2.23	14.73	2.38		7.4–22.9	16.1 ± 2.3
Creatinine (μmol/L)	190.50	29.30	182.90	20.19	184.80	26.93	176–389	114–276	180 ± 23
Na (mmol/L)	155.58	0.68	155.27	1.01	155.09	0.47		148–160	157 ± 1
K (mmol/L)	3.97[a]	0.25	4.22[a]	0.11	4.07	0.17		3.8–5.2	4.2 ± 0.1
Cl (mmol/L)	121.14	1.33	121.93	1.78	121.69	1.45		116–129	122 ± 2
Phos (mmol/L)	1.65	0.23	1.66	0.24	1.72	0.28			1.9 ± 0.3
Triglycerides (mmol/L)	0.53	0.12	0.54	0.12	0.47	0.12			0.59 ± 0.16
Ca (T) (mmol/L)	2.66[b]	0.06	2.61	0.09	2.59[b]	0.05			2.7 ± 0.1

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; Ca(T), total calcium; Cl, chloride; K, potassium; Na, sodium; Phos, phosphate.

[a]$p < .05$ between baseline and no glycine groups.

[b]$p < .05$ between baseline and glycine groups. Reference range from Bechert et al. (2002), Hudson-Lamb et al. (2016) and Depauw, Hesta, et al. (2012).

glycine stimulates the secretion of a gut hormone that works with insulin to reduce glucose circulation and inhibits the effect of glucagon despite the increased concentration (Gannon et al., 2002). In sucrose fed rats (which promotes intra-abdominal fat accumulation), glycine supplementation reduced the amount of intra-abdominal fat, blood pressure, adipocyte size, increased β-oxidation and the concentration of various nonesterified fatty acids (e.g., palmitic acid, arachidonic acid) returned to control levels (El Hafidi et al., 2004). This forms part of the increasing evidence base of the beneficial glycine supplementation effects in the potential treatment of obesity and type 2 diabetes (Imenshahidi & Hossenzadeh, 2022; Wang et al., 2013). While glucose and insulin concentrations were not measured in the serum of the captive cheetahs, the effect of glycine on this aspect of metabolism may have contributed to their weight loss through the loss of fat and increased β-oxidation (El Hafidi et al., 2004). Although the cheetahs included in this study were healthy weight, and insulin resistance and obesity are not an apparent problem with captive cheetahs, this indicates a potential role of glycine in energy metabolism which has been found in previous studies in different species. However, other factors such as environmental or seasonal variation may have also contributed to the weight loss over the study period which spanned from late winter to late spring.

4.2 | Haematology

The WBC measured forms part of the innate immune system and provides the front line, rapid response to foreign microbes—the innate and acquired immune system are dependent on the availability of certain amino acids for the synthesis of proteins and polypeptides (Li et al., 2007). Therefore, dietary protein deficiencies compromise the immune system and its ability to function effectively. Glycine is an effective anti-inflammatory and immunomodulatory agent, although the mechanisms of its action is poorly understood (Zhong et al., 2003). However, the seemingly slight increase in WBC in the control and glycine supplemented diet compared to baseline, largely driven by the increase in the granulocytes (neutrophils, basophils and eosinophils), is likely due to the suboptimum nature of the diet as highlighted above. Additionally, the similarity between the control and glycine groups may indicate that an aspect other than glycine, such as a different component of collagen, is driving this WBC increase from baseline. This increase may lead to a greater immune response to counter the effects of potential infections caused by malnutrition (Li et al., 2007). The immune cell range in the current study is similar to what was found in Bechert et al. (2002) in which the cheetahs were also fed a variety of supplemented meat over a 12 month period, confirming that the cheetahs from the present study were within a normal immune response range.

Glycine is essential in the synthesis of porphyrins in which eight molecules of glycine are required for the synthesis of one haem group (Meléndez-Hevia et al., 2009). In humans, an estimated 240 mg/day glycine is consumed in the production of porphyrins which are mostly used for the haem of haemoglobin or myoglobin (Meléndez-Hevia et al., 2009). In this study, there was no difference in the RBC count or related parameters in response to the glycine supplement. As the average RBC, haemoglobin and haematocrit values were in the higher range of what was found in the reference literature, this may indicate that the healthy cheetahs in this study

were already optimal and that the additional glycine was used in the synthesis of other metabolites, such as purines or creatine.

4.3 | Serum biochemistry

There was a decrease in the TSP driven by the decrease in albumin from baseline to the control and glycine supplemented diets. Although the meat protein content was the same throughout, before the start of the study, the cheetahs potentially received a more diverse source of amino acids. The potential reduction in the dietary protein source, and therefore amino acids, may have led to a reduction in the serum albumin synthesis rate which was observed in rats fed high or low protein diets—with very little change to the catabolism rate until a critical level was reached (Kirsch et al., 1968). In human patients, a positive correlation was found between body composition and serum protein and albumin (Forse & Shizgal, 1980). The authors concluded that although nutritional status is an important determinant of serum albumin concentration, there are other factors such as malnutrition and malabsorption that may be contributing factors. Therefore, the decrease in TSP and albumin is likely linked to the weight loss of the cheetah and the suboptimum diet, with the supplementation of glycine not providing the variety of amino acids required to maintain the serum protein levels. This was also found with the haematology data and highlights that another aspect of the prestudy diet, which contained higher collagen concentrations, may be contributing more significantly to these parameters than glycine alone.

There was a significant decrease in ALP concentration from baseline to the control and glycine supplemented diet. Although not significant, the same trend was observed with ALT. Both are predominantly liver enzymes that will leak into the bloodstream when there is liver injury or damage (Fernandez & Kidney, 2007). Therefore, a decrease in these enzymes may indicate improved liver function or less damage in response to the control and glycine supplemented diet. However, there are various physiological factors that could increase ALP activity—such as age, pregnancy, lactation and a high fat diet, although these factors are not apparent in the current study (Fernandez & Kidney, 2007). Interestingly, the ALP concentration in the study by Bechert et al. (2002) was higher than what was found in the current study. This result might be partly due to older cheetahs being included in that study or due to the higher fat content of the commercial diets. The generally lower ALP and ALT found in the current study may simply indicate that these cheetahs are indeed young and healthy.

The total serum calcium concentration was significantly higher at baseline compared to the glycine supplemented diet. This decrease may be directly attributed to the lower albumin concentration found in glycine supplemented cheetahs, which would lead to less bound calcium in the serum. Throughout the study, no bones were provided to the cheetahs which would conventionally be a major source of calcium and highlights the importance of calcium supplementation for captive cheetahs fed exclusively meat based diets (Depauw, Hesta, et al., 2012). The vitamin and mineral supplement provided to the cheetahs in this study had sufficient levels of calcium and would unlikely contribute to the calcium decrease. Different combinations of immobilisation drugs have been shown to cause hypertension and hypoxaemia with the zolazapam–tiletamine–medetomidine combination causing elevated blood calcium, potassium and glucose concentrations (Buck et al., 2022). This drug combination was used in the current study and may have attributed to the differences found in the serum biochemistry parameters, but is highly unlikely, as the same drug combinations were used during all three immobilisation events.

4.4 | Limitations and future directions

The uneven distribution of the cheetahs as well as the varied length of the control diet between groups is a clear limitation of the study. However, due to logistical challenges there was no suitable alternative to this approach. The habituation period was implemented to standardise the diets for all cheetahs and it was anticipated that the 3 week habituation would also lead to a collagen and glycine deficiency which likely did not occur. This indicates that the cheetah's diet before the start of the study had much higher levels of collagen and glycine than was anticipated. Ideally, samples could have been collected before the habituation period which would have been a better representation of a collagen diet. As no intervention studies of this nature have been conducted in cheetahs, the length of the diet intervention (i.e., 4 weeks) and the amount of glycine (i.e., 30 g per kg meat) was deemed suitable based on studies in rodents, pigs and humans and fell within the time and funding constraints of the study. The study design and feeding intervention limitations highlighted in this study may provide an important guideline for future dietary intervention studies in exotic felids. In future studies, a carcass diet would be a better indicator of an optimum diet obtained in the wild. This approach may also clearly elucidate whether it is indeed glycine or another component of collagen that is influencing the metabolic response. Future studies could also investigate the effect of glycine over an extended period of time (such as 6 months) or the effects in a cheetah population with gastrointestinal or other diseases, as the current study was performed using a healthy and young cohort. Therefore, the clinical body and blood parameters measured in this study are indicative of a healthy cohort's response to glycine supplementation and future studies should investigate these changes in a diseased population or utilise more specific measures.

5 | CONCLUSION

A 4 week glycine supplementation in captive cheetahs resulted in decreased body weight, albumin, ALP and total calcium concentration and an increased eosinophil and basophil count. The changes in the serum and haematology parameters are likely linked to the extended duration on a suboptimum diet with a lower variety of amino acids,

although the decrease in ALP concentration indicates a potential improvement in liver function. The decrease in body weight on the glycine diet may be due to increased β-oxidation and fat loss. Therefore, glycine supplementation resulted in slight body and blood changes in captive cheetahs, although investigating more acute markers of metabolic changes may be a better indicator of the effect of glycine, which is particularly important when dietary collagen is limited. This study provides baseline clinical data on the effect of glycine supplementation and is the first study to investigate this effect in an exotic felid. Additionally, the use of a randomised controlled cross-over intervention may be a useful and reliable method to investigate the effect of various diets in captivity with the current study providing a potential guideline for future intervention studies.

AUTHOR CONTRIBUTIONS

Conceptualisation and design: All authors. *Sample collection and processing*: Adrian S. W. Tordiffe and Kathryn M. van Boom. *Sample analysis*: Kathryn M. van Boom. *Data analysis and interpretation*: All authors. *Writing*: Kathryn M. van Boom. *Reviewing and editing of manuscript*: All authors.

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CONFLICTS OF INTEREST STATEMENT

A. S. W. Tordiffe is the Director of WildCat Nutrition, Pretoria, South Africa that provided the Panthera vitamin and mineral supplement and Glycine supplement used in this study. The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the Animal Research Ethics Committees of the Faculty of Veterinary Science, University of Pretoria (REC231-19) and the University of the Western Cape (AR20/3/4). Sampling was conducted under the standing Threatened or Protected Species (TOPS) permit for the Faculty of the Veterinary Science (reference number: S02559). A Department of Agriculture, Forestry's and Fisheries Section 20 permit was also granted for the undertaking of this research (reference number: 12/11/1/7).

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REFERENCES

Badenhorst, C. P. S., Erasmus, E., Van Der Sluis, R., Nortje, C., & Van Dijk, A. A. (2014). A new perspective on the importance of glycine conjugation in the metabolism of aromatic acids. *Drug Metabolism Reviews*, *46*, 343–361. https://doi.org/10.3109/03602532.2014.908903

Badiani, A., Nanni, N., Gatta, P. P., Tolomelli, B., & Manfredini, M. (1997). Nutrient profile of horse meat. *Journal of Food Composition and Analysis*, *10*, 254–269. https://doi.org/10.1006/jfca.1997.0540

Bechert, U., Mortenson, J., Dierenfeld, E. S., Cheeke, P., Keller, M., Holick, M., Chen, T. C., & Rogers, Q. (2002). Diet composition and blood values of captive cheetahs (*Acinonyx jubatus*) fed either supplemented meat or commercial food preparations. *Journal of Zoo and Wildlife Medicine*, *33*, 16–28. https://doi.org/10.1638/1042-7260(2002)033[0016:DCABVO]2.0.CO;2

Buck, R., Tordiffe, A., & Zeiler, G. (2022). Ketamine-medetomidine compared to tiletamine-zolazepam-medetomidine for immobilisation of semi-captive cheetahs (*Acinonyx jubatus*). *Journal of the South African Veterinary Association*, *93*, 25–31. https://doi.org/10.36303/JSAVA.2022.93.1.489

Depauw, S., Bosch, G., Hesta, M., Whitehouse-Tedd, K., Hendriks, W. H., Kaandorp, J., & Janssens, G. P. J. (2012). Fermentation of animal components in strict carnivores: A comparative study with cheetah fecal inoculum. *Journal of Animal Science*, *90*, 2540–2548. https://doi.org/10.2527/jas.2011-4377

Depauw, S., Hesta, M., Whitehouse-Tedd, K., Stagegaard, J., Buyse, J., & Janssens, G. P. J. (2012). Blood values of adult captive cheetahs (*Acinonyx jubatus*) fed either supplemented beef or whole rabbit carcasses. *Zoo Biology*, *31*, 629–641. https://doi.org/10.1002/zoo.20427

Depauw, S., Hesta, M., Whitehouse-Tedd, K., Vanhaecke, L., Verbrugghe, A., & Janssens, G. P. J. (2013). Animal fibre: The forgotten nutrient in strict carnivores? First insights in the cheetah: Dietary animal fibre in the cheetah. *Journal of Animal Physiology and Animal Nutrition*, *97*, 146–154. https://doi.org/10.1111/j.1439-0396.2011.01252.x

Durant, S. M., Groom, R., Ipavec, A., Mitchell, N., & Khalatbari, L. (2022). *Acinonyx jubatus*. The IUCN red list of threatened species 2022. e.T219A124366642. https://doi.org/10.2305/IUCN.UK.2022-1.RLTS.T219A124366642.en

Durant, S. M., Mitchell, N., Groom, R., Pettorelli, N., Ipavec, A., Jacobson, A. P., Woodroffe, R., Böhm, M., Hunter, L. T. B., Becker, M. S., Broekhuis, F., Bashir, S., Andresen, L., Aschenborn, O., Beddiaf, M., Belbachir, F., Belbachir-Bazi, A., Berbash, A., Brandao de Matos Machado, I., … Young-Overton, K. (2017). The global decline of cheetah *Acinonyx jubatus* and what it means for conservation. *Proceedings of the National Academy of Sciences*, *114*, 528–533. https://doi.org/10.1073/pnas.1611122114

Elbourne, D. R., Altman, D. G., Higgins, J. P., Curtin, F., Worthington, H. V., & Vail, A. (2002). Meta-analyses involving cross-over trials: Methodological issues. *International Journal of Epidemiology*, *31*, 140–149. https://doi.org/10.1093/ije/31.1.140

Fernandez, N. J., & Kidney, B. A. (2007). Alkaline phosphatase: Beyond the liver. *Veterinary Clinical Pathology*, *36*, 223–233. https://doi.org/10.1111/j.1939-165X.2007.tb00216.x

Forse, R. A., & Shizgal, H. M. (1980). Serum albumin and nutritional status. *Journal of Parenteral and Enteral Nutrition*, *4*, 450–454. https://doi.org/10.1177/014860718000400503

Gannon, M. C., Nuttall, J. A., & Nuttall, F. Q. (2002). The metabolic response to ingested glycine. *The American Journal of Clinical Nutrition*, *76*, 1302–1307. https://doi.org/10.1093/ajcn/76.6.1302

Gillis-Germitsch, N., Vybiral, P. R., Codron, D., Clauss, M., Kotze, A., & Mitchell, E. P. (2017). Intrinsic factors, adrenal gland morphology, and disease burden in captive cheetahs (*Acinonyx jubatus*) in South Africa: Adrenal morphology and disease in cheetahs. *Zoo Biology*, *36*, 40–49. https://doi.org/10.1002/zoo.21341

El Hafidi, M. E., Pérez, I., Zamora, J., Soto, V., Carvajal-Sandoval, G., & Baños, G. (2004). Glycine intake decreases plasma free fatty acids, adipose cell size, and blood pressure in sucrose-fed rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *287*, R1387–R1393. https://doi.org/10.1152/ajpregu.00159.2004

Hawkey, C. M., & Hart, M. G. (1986). Haematological reference values for adult pumas, lions, tigers, leopards, jaguars and cheetahs. *Research in Veterinary Science*, *41*, 268–269. https://doi.org/10.1016/s0034-5288(18)30611-8

Hayward, M. W., Hofmeyr, M., O'Brien, J., & Kerley, G. I. H. (2006). Prey preferences of the cheetah (*Acinonyx jubatus*) (Felidae: Carnivora): Morphological limitations or the need to capture rapidly consumable prey before kleptoparasites arrive? *Journal of Zoology*, *270*, 615–627. https://doi.org/10.1111/j.1469-7998.2006.00184.x

Hudson-Lamb, G. C., Schoeman, J. P., Hooijberg, E. H., Heinrich, S. K., & Tordiffe, A. S. W. (2016). Reference intervals for selected serum biochemistry analytes in cheetahs (Acinonyx jubatus) *Journal of the South African Veterinary Association*, *81*(1). https://doi.org/10.4102/jsava.v87i1.1316

Imenshahidi, M., & Hossenzadeh, H. (2022). Effects of glycine on metabolic syndrome components: A review. *Journal of Endocrinological Investigation*, *45*, 927–939. https://doi.org/10.1007/s40618-021-01720-3

Kirberger, R. M., & Tordiffe, A. S. W. (2016). Ultrasonographic adrenal gland findings in healthy semi-captive cheetahs (*Acinonyx jubatus*): Cheetah adrenal gland ultrasonography. *Zoo Biology*, *35*, 260–268. https://doi.org/10.1002/zoo.21285

Kirsch, R., Frith, L., Black, E., & Hoffenberg, R. (1968). Regulation of albumin synthesis and catabolism by alteration of dietary protein. *Nature*, *217*, 578–579. https://doi.org/10.1038/217578a0

Lane, E. P., Miller, S., Lobetti, R., Caldwell, P., Bertschinger, H. J., Burroughs, R., Kotze, A., & van Dyk, A. (2012). Effect of diet on the incidence of and mortality owing to gastritis and renal disease in captive cheetahs (*Acinonyx jubatus*) in South Africa: Diet and disease in captive cheetahs. *Zoo Biology*, *31*, 669–682. https://doi.org/10.1002/zoo.20431

Li, P., & Wu, G. (2018). Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. *Amino Acids*, *50*, 29–38. https://doi.org/10.1007/s00726-017-2490-6

Li, P., Yin, Y. L., Li, D., Woo Kim, S., & Wu, G. (2007). Amino acids and immune function. *British Journal of Nutrition*, *98*, 237–252. https://doi.org/10.1017/S000711450769936X

Marker, L. L., & Dickman, A. J. (2003). Morphology, physical condition, and growth of the cheetah (*Acinonyx jubatus* jubatus). *Journal of Mammalogy*, *84*, 840–850. https://doi.org/10.1644/BRB-036

McCarty, M. F., O'keefe, J. H., & DiNicolantonio, J. J. (2018). Dietary glycine is rate-limiting for glutathione synthesis and may have broad potential for health protection. *The Ochsner Journal*, *18*, 81–87. https://doi.org/10.1043/TOJ-17-0022

Meléndez-Hevia, E., De Paz-Lugo, P., Cornish-Bowden, A., & Cárdenas, M. L. (2009). A weak link in metabolism: The metabolic capacity for glycine biosynthesis does not satisfy the need for collagen synthesis. *Journal of Biosciences*, *34*, 853–872. https://doi.org/10.1007/s12038-009-0100-9

Mitchell, E. P., Prozesky, L., & Lawrence, J. (2018). A new perspective on the pathogenesis of chronic renal disease in captive cheetahs (*Acinonyx jubatus*). *PLoS One*, *13*, e0194114. https://doi.org/10.1371/journal.pone.0194114

Munson, L. (1993). Diseases of captive cheetahs (*Acinonyx jubatus*): Results of the cheetah research council pathology survey, 1989–1992. *Zoo Biology*, *12*, 105–124. https://doi.org/10.1002/zoo.1430120110

Munson, L., Nesbit, J. W., Meltzer, D. G., Colly, L. P., Bolton, L., & Kriek, N. P. (1999). Diseases of captive cheetahs (*Acinonyx jubatus* jubatus) in South Africa: A 20-year retrospective survey. *Journal of Zoo and Wildlife Medicine: Official Publication of the American Association of Zoo Veterinarians*, *30*, 342–347.

Munson, L., Terio, K. A., Worley, M., Jago, M., Bagot-Smith, A., & Marker, L. (2005). Extrinsic factors significantly affect patterns of disease in free-ranging and captive cheetah (*Acinonyx jubatus*) populations. *Journal of Wildlife Diseases*, *41*, 542–548. https://doi.org/10.7589/0090-3558-41.3.542

Petzke, K. J., Albrecht, V., & Przybilski, H. (1986). The influence of high glycine diets on the activity of glycine-catabolizing enzymes and on glycine catabolism in rats. *The Journal of Nutrition*, *116*, 742–750. https://doi.org/10.1093/jn/116.5.742

Setchell, K. D. R., Gosselin, S. J., Welsh, M. B., Johnston, J. O., Balistreri, W. F., Kramer, L. W., Dresser, B. L., & Tarr, M. J. (1987). Dietary estrogens—A probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology*, *93*, 225–233. https://doi.org/10.1016/0016-5085(87)91006-7

Sharp, N. C. C. (1997). Timed running speed of a cheetah (*Acinonyx jubatus*). *Journal of Zoology*, *241*, 493–494. https://doi.org/10.1111/j.1469-7998.1997.tb04840.x

Tordiffe, A. S. W., van Reenen, M., Reyers, F., & Mienie, L. J. (2017). Gas chromatography-mass spectrometry profiles of urinary organic acids in healthy captive cheetahs (*Acinonyx jubatus*). *Journal of Chromatography B*, *1049–1050*, 8–15. https://doi.org/10.1016/j.jchromb.2017.02.018

Vester, B. M., Burke, S. L., Dikeman, C. L., Simmons, L. G., & Swanson, K. S. (2008). Nutrient digestibility and fecal characteristics are different among captive exotic felids fed a beef-based raw diet. *Zoo Biology*, *27*, 126–136. https://doi.org/10.1002/zoo.20172

Wang, W., Dai, Z., Wu, Z., Lin, G., Jia, S., Hu, S., Dahanayaka, S., & Wu, G. (2014). Glycine is a nutritionally essential amino acid for maximal growth of milk-fed young pigs. *Amino Acids*, *46*, 2037–2045. https://doi.org/10.1007/s00726-014-1758-3

Wang, W., Wu, Z., Dai, Z., Yang, Y., Wang, J., & Wu, G. (2013). Glycine metabolism in animals and humans: Implications for nutrition and health. *Amino Acids*, *45*, 463–477. https://doi.org/10.1007/s00726-013-1493-1

Whitehouse-Tedd, K. M., Lefebvre, S. L., & Janssens, G. P. J. (2015). Dietary factors associated with faecal consistency and other indicators of gastrointestinal health in the captive cheetah (*Acinonyx jubatus*). *PLoS One*, *10*, e0120903. https://doi.org/10.1371/journal.pone.0120903

Zhong, Z., Wheeler, M. D., Li, X., Froh, M., Schemmer, P., Yin, M., Bunzendaul, H., Bradford, B., & Lemasters, J. J. (2003). L-glycine: A novel antiinflammatory, immunomodulatory, and cytoprotective agent. *Current Opinion in Clinical Nutrition and Metabolic Care*, *6*, 229–240. https://doi.org/10.1097/00075197-200303000-00013

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