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Haemato-biochemical safety of mineral and vitamin supplementation and the influence of serum zinc on seminal quality and glutathione peroxidase in infertile male dogs

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Abstract

Mineral and vitamin supplementation is increasingly used to manage male infertility in dogs, yet its systemic safety and the specific contribution of serum zinc to seminal outcomes remain poorly defined. Six client-owned infertile male dogs were supplemented with a mineral - vitamin preparation for 90 days. Haemato-biochemical parameters - total erythrocyte count, total leukocyte count, platelet count, packed cell volume, haemoglobin, serum alanine aminotransferase (SGPT) and creatinine - were measured on day 0 and day 90, and the influence of serum zinc concentration on sperm viability, sperm concentration and serum glutathione peroxidase (GPx) was examined by linear regression. All haemato-biochemical parameters remained within the normal canine reference range and did not differ significantly between day 0 and day 90, indicating that prolonged supplementation was haematologically, hepatically and renally safe. Serum zinc concentration was a significant positive determinant of sperm viability (day 90; $p = 0.033$), sperm concentration (day 60; $p = 0.042$) and GPx activity (day 60; $p = 0.028$). It is concluded that the supplementation regimen is systemically safe and that serum zinc is an important determinant of seminal quality and antioxidant status in infertile male dogs.

Keywords: Dog, glutathione peroxidase, haemato-biochemical, infertility, zinc

Introduction

Diminishing reproductive performance in dogs is a growing concern for breeders and small-animal practitioners, and the male partner is frequently implicated when an altered semen picture is present. In most cases the cause of poor semen quality remains idiopathic, and nutritional deficiencies of macro- and micro-minerals and vitamins are among the reversible contributors (Kumaresan *et al.*, 2020) [4]. Among the trace elements, zinc is of particular interest because it acts as a cofactor for antioxidant enzymes, stabilises the sperm plasma membrane and participates in spermatogenesis, and dietary zinc, selenium and vitamin E have been reported to improve canine semen quality (Ebisch *et al.*, 2006; Alonge *et al.*, 2019) [3, 1]. Supplementation with mineral - vitamin complexes is therefore widely recommended, but two questions are seldom addressed together. First, the systemic safety of prolonged supplementation - its effect on the haemogram and on hepatic and renal markers - is rarely documented. Second, although semen quality improves during supplementation, the extent to which the change is driven by serum zinc status has not been quantified. The efficacy of the present regimen on seminal attributes, serum mineral profile, antioxidant enzymes and testosterone in the same animals has been reported separately (Mujawar *et al.*, 2024) [7]. The present study addresses the previously unreported haemato-biochemical safety of the regimen and the quantitative relationship between serum zinc and sperm viability, sperm concentration and glutathione peroxidase activity in infertile male dogs.

Materials and Methods

The study was conducted on six client-owned male dogs, aged 2 - 8 years, presented to the

small-animal outpatient unit of the Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Hebbal, Bengaluru, with a history of failure to impregnate different bitches on three or more matings. After physical examination, testicular and prostatic ultrasonography and a baseline seminogram, dogs found to be sub-fertile/infertile were enrolled. Each dog received a mineral - vitamin tablet preparation (containing Ca, P, Mg, Fe, Zn, Cu, Se, vitamins A, D3, E, K, B-complex and C, choline and nicotinamide) at two tablets/day for large breeds and one tablet/day for small breeds for 90 days.

Blood was collected from the cephalic or saphenous vein on day 0 (before supplementation) and on day 90. Haematological parameters (total erythrocyte count, total leukocyte count, platelet count, packed cell volume and haemoglobin) and serum biochemical markers (alanine aminotransferase, SGPT; and creatinine) were estimated by standard automated methods. Serum zinc was estimated by the colorimetric method (Mindray BS-120 analyser). Semen was collected by digital manipulation on days 0, 30, 60 and 90; sperm viability was assessed by eosin - nigrosin staining and sperm concentration by direct counting with a Neubauer haemocytometer. Serum glutathione peroxidase (GPx) was measured using a canine GPx ELISA kit (PUREGENE Laboratories, Delhi).

Data are expressed as mean $\pm$ standard error ($n = 6$). Haemato-biochemical values on day 0 and day 90 were compared by paired analysis. To determine the influence of zinc status on reproductive and antioxidant outcomes, serum zinc concentration was entered as the independent variable in a linear regression against sperm viability, sperm concentration and GPx (dependent variables) at days 30, 60 and 90. A probability of $p < 0.05$ was considered significant. Statistical analyses were performed using SPSS v20.

As the study used client-owned animals presented for clinical management, non-invasive sampling was performed with the informed consent of the owners and in accordance with institutional norms for the ethical use of animals.

Disclosure

The seminal attributes, serum mineral profile, antioxidant enzymes and testosterone responses of these six animals to the same 90-day supplementation have been reported separately (Mujawar *et al.*, 2024) [7]. To avoid redundancy, those descriptive outcomes are not re-tabulated here; the present paper reports the previously unpublished haemato-biochemical data and the regression of serum zinc on seminal and antioxidant outcomes.

Results and Discussion

Haemato-biochemical safety. The haemato-biochemical parameters recorded before and after supplementation are presented in Table 1. Total erythrocyte count (5.44 ± 0.44 vs $5.56 \pm 0.39 \times 10^6/\mu\text{L}$), total leukocyte count (11.07 ± 1.04 vs $11.02 \pm 1.03 \times 10^3/\mu\text{L}$), platelet count, packed cell volume (49.65 ± 1.46 vs $48.28 \pm 2.04\%$) and haemoglobin (12.73 ± 1.11 vs 12.78 ± 0.97 g/dL) remained essentially unchanged and within the normal canine reference range throughout. Serum SGPT (58.43 ± 6.79 vs 58.08 ± 6.81 U/L) and creatinine (0.97 ± 0.10 vs 0.87 ± 0.08 mg/dL) likewise showed no significant change. The stability of the erythron and leucon indicates the absence of any haemotoxic or marrow-suppressive effect, while the unchanged SGPT and creatinine indicate that the 90-day regimen imposed no hepatic or renal burden. Prolonged mineral - vitamin supplementation at the doses used can therefore be regarded

as systemically safe in dogs.

Serum zinc as a determinant of seminal quality and antioxidant status. The linear-regression relationships of serum zinc with sperm viability, sperm concentration and GPx are summarised in Table 2. Serum zinc concentration was a significant positive determinant of sperm viability by day 90 (regression coefficient $\beta = 3.00 \pm 0.93$; $p = 0.033$), of sperm concentration by day 60 ($\beta = 4.27 \pm 1.44$; $p = 0.042$) and of GPx activity by day 60 ($\beta = 1.00 \pm 0.29$; $p = 0.028$). The positive coefficients indicate that, as circulating zinc rose during supplementation, sperm viability, sperm concentration and antioxidant (GPx) activity increased proportionately. The emergence of significance during the middle-to-late supplementation period is consistent with the length of the canine spermatogenic cycle, so that the reproductive benefit of an improved zinc status becomes measurable only after a spermatogenic wave has been completed.

These findings agree with reports that zinc, selenium and vitamin E supplementation improves sperm quality in dogs of lowered fertility (Domosławska *et al.*, 2015; Alonge *et al.*, 2019) [2, 1] and that folic acid and zinc influence sperm characteristics in the male (Ebisch *et al.*, 2006; Wong *et al.*, 2001) [3, 8]. Mechanistically, zinc is a structural and catalytic cofactor for antioxidant enzymes and contributes to the stability of the sperm plasma membrane; the significant zinc - GPx relationship observed here supports the view that a large part of the antioxidant protection conferred by supplementation is zinc-dependent (Lenzi *et al.*, 2002; Michael *et al.*, 2007) [5, 6]. The serum zinc concentrations recorded were within the range reported for healthy dogs (Zentrichová *et al.*, 2022) [9]. Taken together, the data identify serum zinc as a measurable determinant of both the fertilising quality of semen and its antioxidant defence in infertile dogs.

Table 1: Haemato-biochemical parameters (mean $\pm$ SE) before (day 0) and after (day 90) mineral - vitamin supplementation in infertile male dogs ($n = 6$)

Parameter	Day 0	Day 90
Total erythrocyte count ($\times 10^6/\mu\text{L}$)	5.44 ± 0.44	5.56 ± 0.39
Total leukocyte count ($\times 10^3/\mu\text{L}$)	11.07 ± 1.04	11.02 ± 1.03
Platelet count ($\times 10^5/\mu\text{L}$)	2.51 ± 0.25	2.28 ± 0.24
Packed cell volume (%)	49.65 ± 1.46	48.28 ± 2.04
Haemoglobin (g/dL)	12.73 ± 1.11	12.78 ± 0.97
SGPT (U/L)	58.43 ± 6.79	58.08 ± 6.81
Creatinine (mg/dL)	0.97 ± 0.10	0.87 ± 0.08

Differences between day 0 and day 90 were non-significant ($p > 0.05$). Reference intervals for the dog were not exceeded for any parameter.

Table 2: Linear regression of serum zinc concentration (independent variable) on sperm viability, sperm concentration and glutathione peroxidase (dependent variables) in supplemented infertile male dogs ($n = 6$)

Dependent variable	Day	Regression coefficient ($\beta \pm \text{SE}$)	p-value
Sperm viability (%)	30	2.18 ± 0.50	0.012*
	60	1.44 ± 1.16	0.218
	90	3.00 ± 0.93	0.033*
Sperm concentration ($\times 10^6/\text{mL}$)	30	4.71 ± 2.87	0.177
	60	4.27 ± 1.44	0.042*
	90	2.53 ± 1.54	0.171
Glutathione peroxidase (ng/mL)	30	0.64 ± 0.51	0.283
	60	1.00 ± 0.29	0.028*
	90	0.24 ± 1.45	0.569

* Significant at $p < 0.05$. Serum zinc was entered as the independent (predictor) variable in each model.

Conclusion

Ninety days of mineral - vitamin supplementation in infertile male dogs produced no adverse change in haematological, hepatic or renal parameters, confirming that the regimen is systemically safe. Serum zinc concentration emerged as a significant positive determinant of sperm viability, sperm concentration and glutathione peroxidase activity, indicating that zinc status is central to the seminal and antioxidant benefits of supplementation. Zinc-inclusive mineral - vitamin supplementation can therefore be recommended as a safe and rational adjunct in the management of canine male sub-fertility, although confirmation in a larger population is warranted.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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