

The impact of 5% flaxseed supplementation on sex hormones, antioxidant status, and blood profile in female spotted deer during the breeding season

YOUSSEF MESFER ALHARBI¹ , AHMAD IBRAHEEM ALNUQAYDAN² ,
ABDEL KADER AHMAD ZAKI^{1,3*} 

¹Department of Medical Biosciences, College of Veterinary Medicine,
Qassim University, Buraidah, Saudi Arabia

²Zoo Supervisor, King Khalid Military City Under the Ministry of Defense,
Hafar Al-Batin, Saudi Arabia

³Department of Physiology, Faculty of Veterinary Medicine, Cairo University,
Giza Square, Egypt

*Corresponding author: dr.abd.el.kader@hotmail.com

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Abstract: Phytoestrogens, such as those found in flaxseed, can mimic oestrogen and influence animal physiology. For spotted deer, an economically significant species in Saudi Arabia, information on how phytoestrogen-rich diets affect reproductive health and overall physiology is limited, particularly in captive zoo settings. This work aimed to clarify the effects of a 5% flaxseed dietary supplement on the sex hormone profile, antioxidant capacity, haematological parameters, and histological features of female spotted deer during the onset of the breeding season. Twelve female deer were separated into two groups: a control group fed a standard diet of alfalfa and pellets, and an experimental group receiving the same diet supplemented with 5% flaxseed for six weeks. Weekly measurements included body weight and feed intake. Blood samples were analysed for sex hormones (oestradiol, testosterone), sex hormone-binding globulin (SHBG), antioxidant enzymes, oxidative stress markers, complete blood count (CBC), and biochemical parameters. Ovarian and reproductive tract histology was also examined. Flaxseed supplementation significantly increased serum oestradiol (E2) levels and SHBG ($P < 0.05$), reducing the Free Androgen Index (FAI). It also significantly enhanced antioxidant enzyme activities (SOD and CAT). Haematologically, it increased red blood cell count but induced microcytic hypochromic erythropoiesis, suggesting possible iron chelation. A notable increase in eosinophils was observed. Histologically, reproductive tissues showed enhanced development in the flaxseed group. Incorporating 5% flaxseed into the diet of female spotted deer induces a significant oestrogenic shift, improves antioxidant defence, and enhances reproductive tissue morphology. However, it may also cause iron deficiency-like haematological changes and a potential allergic response. This suggests that flaxseed is a potent dietary tool for modulating reproduction in captive deer, but its application requires careful management to mitigate adverse effects.

Keywords: antioxidants; *Axis axis*; captive wildlife; erythropoiesis; lignans; oestradiol; phytoestrogens

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Spotted deer are economically significant in Saudi Arabia, but information on the physiology and reproductive management of this species remains limited. Phytoestrogens are plant-derived compounds found in a variety of diets, with flax seeds being the most common. Sutrisno (2022) mentioned that phytoestrogens have several health benefits, including a lower incidence of osteoporosis, breast cancer, and heart disease; nevertheless, many are also categorised as hormonal disruptors. Phytoestrogens originate from nonsteroidal phenolic chemical substances found in plants. Krizova et al. (2019) evaluate the genetic potential of alfalfa, soybean, and cowpea, three significant legume crops, to provide high-quality feed and fodder for animals. Kulkarni et al. (2018) examine how climate change affects forage quality and discuss initiatives to improve plant tolerance to abiotic stress. Abdelrazek et al. (2019) showed how isoflavones, as a hormone replacement therapy (HRT), affected parameters related to bone health and immunity of deer. They concluded that treatment with soy isoflavones promoted bone ER α and reduced oxidative stress, thereby normalising nearly all metabolic and immunological markers to levels similar to the sham group. In oestrogen deprivation, isoflavones appeared to be a good HRT since they reduced hunger, weight gain, pro-inflammatory markers, lipid profile, and bone turnover. Soybeans contain peptides with a variety of biological actions, including antioxidant and anti-inflammatory properties, making them a promising source (Chatterjee et al. 2018).

Phytoestrogens are plant-derived compounds that can mimic or modulate the action of oestrogen in the body. In animals like deer, these compounds can affect their reproductive health, growth, and development. To optimise the health and well-being of deer, a balanced approach to dietary supplementation is crucial. While phytoestrogens can offer potential benefits, it is important to avoid excessive intake and to monitor the long-term effects of these compounds. Research is needed to determine optimal phytoestrogen intake levels for deer and to assess the potential risks and benefits of different dietary strategies. By understanding the complex interactions between phytoestrogens and animal physiology, we can develop targeted feeding strategies to promote deer health and reproductive performance. Therefore, this study aims to explore the impact of a specific diet incorporating flaxseed (5%) on the health of deer, as well as the influence

of pellets and alfalfa on deer kept in a zoo setting, focusing on their sex hormones, antioxidant capacity, and blood parameters during the onset of the breeding season.

MATERIAL AND METHODS

Ethical approval

All the study methods have been approved by the Animal Care and Use Committee of the Experimental Animal Centre at Qassim University (No. 24-08-17).

Area and animals

The K.K.M.C. zoo is located in King Khalid Military City, near Hafar al-Batin Governorate, KSA. From September 2023 to January 2024, oestrous female spotted deer (chital, *Axis axis*) displayed subtle receptive behaviours. These included lifting the tail, urinating, and allowing males to approach, which typically resulted in mating. Twelve female spotted deer, aged four to five years, were housed in two cages (Figure 1). The



Figure 1. Spotted deer of experimental groups in the zoo

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animals were selected from a larger managed herd of 25 adult females based on their age, good health status, and absence of any previous reproductive disorders. They were randomly assigned to two groups ($n = 6$ per group) to ensure a balanced distribution of body weights and ages between groups. The zoo's cage is 25 by 5 m, or 125 square meters. The total area is estimated at 20.8 m² (223.9 ft²) per deer. Spotted deer and feed intake were weighed weekly for six weeks using Gahndz scales and Compact Balances EK-I EW-I Series, respectively, and expressed in kg. The animals were fed a meticulously balanced diet composed of pellets and alfalfa. The pellet composition is illustrated in Table 1. Feed intake was calculated as the difference between the amount of food fed weekly and the amount remaining. The deer were fed a balanced diet of 4.2 kg per deer daily, including 75% alfalfa and 25% pellets. The animals were fed in two groups of 6 individuals each. Group 1 served as the control group and was fed pellets and alfalfa in the zoo, while group 2 was fed the same basal diet with an additional 5% flaxseed supplement. Alfalfa was supplied by the Almerzm Agricultural Company and stored in a warehouse. The deer were given

3.15 kg/deer in two periods: morning and evening. Pellets were supplied by the Concentrated Feed Factory (Qassim, KSA) and stored in a warehouse. Deer received 1.05 kg/deer in two periods: morning and evening. Khairat Specialty Company supplied flax seeds, which were mixed into the pellets at 5% of the total feed (0.21 kg).

High-performance liquid chromatography (HPLC)

Following a method adapted from Granato et al. (1983), feed samples (flax seeds, alfalfa, and pellets) were prepared for HPLC analysis. After washing the feed samples, the compounds of interest (daidzein, genistein, and coumestrol) were extracted by agitating the tissue in DMSO. This extract was then clarified by centrifugation and filtration. The analysis was conducted using an Agilent 1200 Series HPLC system, managed by ChemStation software, and employed a Zorbax Extend-C18 reversed-phase column for chromatographic separation. All feed samples were analysed in triplicate ($n = 3$).

Table 1. Nutritional composition and ingredients of the pelleted feed

Components	Components percent
Metabolisable energy	2 700 kcal/kg
Moisture	8.5%
Crude protein	13%
Crude fibre	14%
Crude fat	3%
Vitamins	Vit A 1 300 IU/kg, Vit D3 35 IU/kg, Vit E 30 mg/kg
Sodium	0.4%
Potassium	9 500 ppm
Copper	14 ppm
Selenium	0.3 ppm
Magnesium	0.25%
Starch	25%
Other components	calcium 1.38% – phosphorus 0.55%
Ingredients	soybean meals, maize, barley, wheat bran, phosphate dehydrate granular, vitamins and minerals, sodium chloride, cal- cium carbonate

Sampling

To minimise stress, blood samples were collected from the jugular vein of the deer every two weeks over a 6-week period using a standardised procedure. A total of 10 ml of blood was drawn aseptically during each collection. For serum analysis, 5 ml of blood was placed in a vacuum tube without anticoagulant. This sample was allowed to clot at room temperature (20–25 °C) for 30 min, then centrifuged to separate the serum. The serum samples were then stored at –80 °C until further analysis for chemical components, hormones, and antioxidant enzyme activity. The remaining 5 ml was collected in a vacuum tube containing the anticoagulant Na₂ EDTA for haematological analysis, which was performed within 24 h of collection to ensure cell viability.

Haematological parameters were processed in the last week of the experiment using an automated haematologic counter for veterinary use (ABX MICROS ESV 60; Horiba Medical, France). The parameters include red blood cell counts; haemoglobin; haematocrit; blood indices; mean platelet volume; platelet

count; total and differential leucocyte count. Blood biochemical parameters were processed in the last week of the experiment, using the automatic veterinary biochemical analyser Skyla VB1 with a specialised software (Tianliang Medical Equipment Co., Ltd., New Taipei City, Taiwan). The evaluated parameters were glucose, total protein, albumin, globulin, alkaline phosphatase, alanine transaminase, urea, creatinine, calcium, phosphate, sodium, potassium, total bilirubin, amylase, and blood urea nitrogen.

Hormone levels, including total testosterone (TT), oestradiol (E2), and sex hormone-binding globulin (SHBG), were assessed in the first, third, and sixth weeks. TT and E2 were measured using solid-phase competitive ELISA kits (Monocent, REF. EL1-1263 and EL1-1254, respectively). The TT assay had an intra-assay coefficient of variation (CV) below 3.8% and an inter-assay CV below 6.7%, with a detection range of 3–19.5 ng/ml and a sensitivity of 0.07 ng/ml. For E2, the sensitivity was 8.7 pg/ml, with intra- and inter-assay CVs of 9.2% and 8.5%, respectively, and a detection range of 21.23–571.44 pg/ml. SHBG was quantified using a sandwich ELISA kit (REF SL1576Hu; Sunlong Biotech Co., Ltd, Hangzhou, P.R. China), with intra- and inter-assay CVs below 10.5% and 12.3%, respectively; an assay range of 0.3–20 ng/ml; and a sensitivity of 0.05 ng/ml. The free androgen index (FAI) was determined as $(TT / SHBG) \times 100$, based on concentrations in nanomoles per litre, rendering the index unitless (Fraser and Sohrabi 2014). Free testosterone (FT) and bioavailable testosterone (BioT) were calculated using the online tool available at <https://www.issam.ch/freetesto.htm>.

In the final week of the experiment, antioxidant enzyme activity was evaluated via colorimetric assays using a spectrophotometer. Malondialdehyde (MDA; Elabscience, CAT. E-BC-K025-S) was measured at 532 nm, catalase (CAT, CAT. CA 2517; Bio Diagnostic, Giza, Egypt) at 510 nm, glutathione peroxidase (GPx, CAT. GP 2524; Bio Diagnostic, Giza, Egypt) at 340 nm, and superoxide dismutase (SOD, CAT. SD 2521; Bio Diagnostic, Giza, Egypt) at 560 nm.

Histology

At the end of the experiment, we obtained permission to slaughter one deer from each group for histology. Tissue samples from each group were

preserved using 10% neutral buffered formalin (NBF). We performed the histological procedure as described by Suvarna et al. (2018).

Statistical analysis

Data are presented as mean $\pm$ standard error (SE). We assessed the normality of the data distribution using the Shapiro-Wilk test. All data were normally distributed ($P > 0.05$), justifying the use of parametric statistical tests. For longitudinal comparisons (within-group changes from Week 1 to Week 6), a one-way ANOVA was performed, followed by post-hoc analysis using Tukey's test. For cross-sectional comparisons (between-group differences at each week), an independent Student's *t*-test was used. Statistical significance was defined as $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS AND DISCUSSION

Chromatography (Figure 2 and Table 2) reveals significant differences in phytoestrogen content among deer feeds. Flax seeds contain the highest concentrations of daidzein, genistein, and coumestrol, followed by alfalfa, with pellet feed containing the lowest levels. These compounds can influence deer physiology, including reproduction and health. Because of their significantly lower phytoestrogen content, pellets are suggested as the safest option for long-term feeding without disrupting hormonal balance, while flax seeds, though highly oestrogenic, also provide other beneficial nutrients. The significant drop in coumestrol levels in pellets makes them the safest option in terms of oestrogenic content, suggesting they are most appropriate for long-term feeding without influencing the deer's hormonal balance (Bennetau-Pelissero 2018). Additionally, forage legumes can reduce the risk of toxicosis associated with consuming certain grass species, such as tall fescue or *Phalaris*. This benefit arises from increased pasture species diversity, which offers livestock a broader selection for competitive grazing and helps mitigate the intake of potentially toxic grasses (Melchior and Myer 2018). Flax seeds are renowned for their high lignan content, a class of phytoestrogens distinct from isoflavones. While they contain minimal amounts of daidzein and genistein, flax seeds are particularly rich in lignans such

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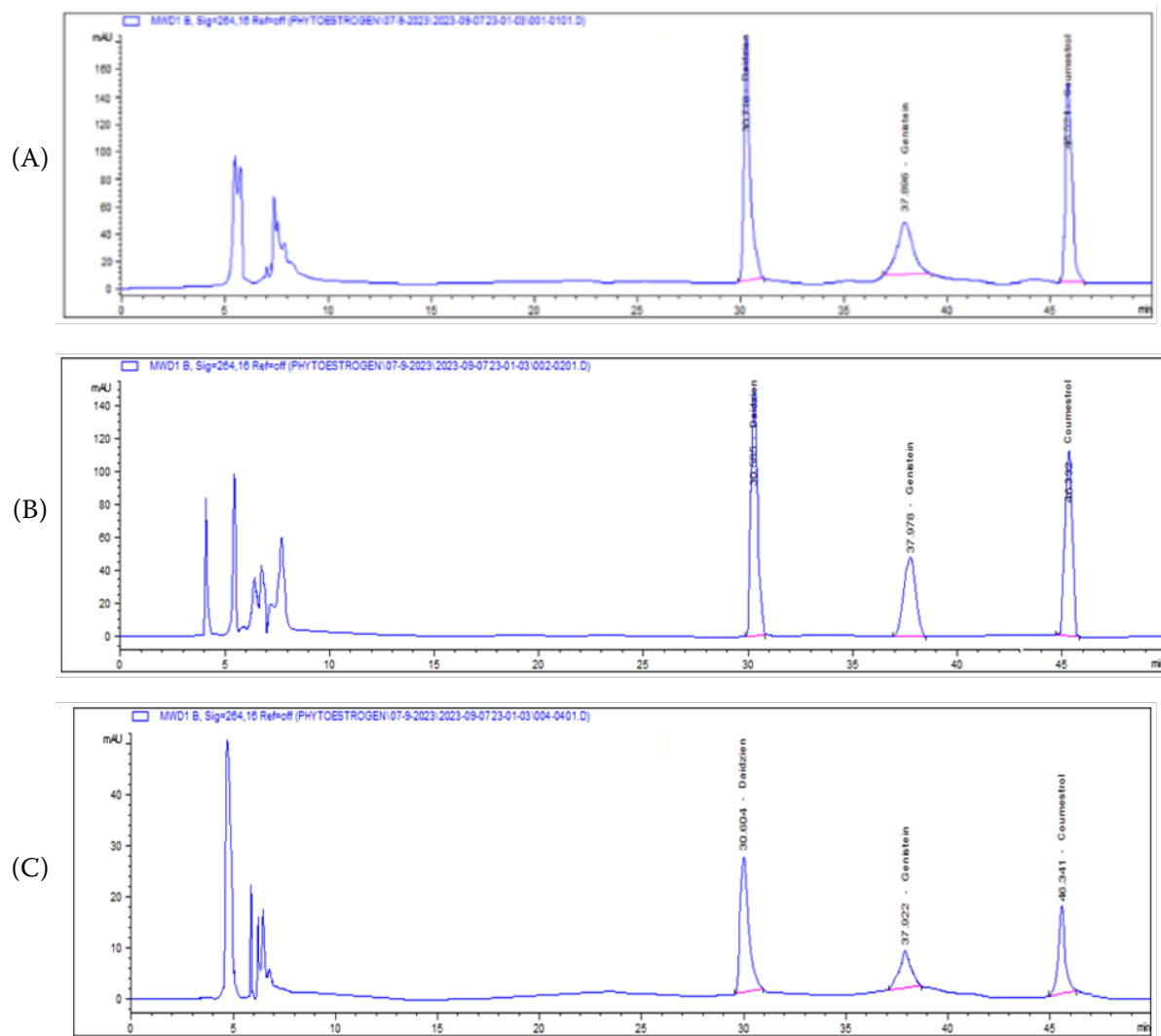


Figure 2. Chromatography curves of flax seeds (A), pellets (B), and alfalfa (C)

Table 2. High-performance liquid chromatography analysis of deer feed samples

Feed	Phytoestrogens	Amount (µg/g tissue)
Flax seeds	daidzein	1 152
	genistein	628
	coumestrol	892
Alfalfa	daidzein	473
	genistein	290
	coumestrol	264
Pellets	daidzein	179
	genistein	120
	coumestrol	103

as secoisolariciresinol. These lignans are metabolised in the human body to enterolignans, which exhibit oestrogenic activity. Although phytoestro-

gens are not considered essential for plant survival, they contribute to plant protection by facilitating interactions between plants or between plants and microbial communities (Karki et al. 2020).

Table 3 presents the mean initial and final weights, as well as the mean weight gain, for two groups over six weeks. In the zoo, over six weeks, the control group exhibited steady weight gains (average ~2.89 kg/week), peaking in Week 3 (4.02 ± 0.68 kg), while the flaxseed 5% group showed higher initial gains in Week 1 (3.85 ± 0.63 kg) but declined sharply by Week 5 (1.58 ± 0.74 kg), resulting in a marginally lower average (~2.74 kg/week); final weights favoured the control group (55.64 ± 1.29 kg vs group 2's 54.14 ± 1.29 kg), suggesting flaxseed supplementation did not consistently enhance growth, though both groups achieved comparable total gains (~16–17 kg), with control group consist-

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Table 3. Weekly mean initial weights (kg); mean final weights (kg) and mean weight gain (kg) of female spotted deer reared in the zoo after the addition of 5% flaxseed to the diet for six weeks from the onset of breeding

Groups	Week	Mean initial weights (kg)	Mean final weights (kg)	Mean weight gain (kg)
Control (alfalfa+ pellets)	W1	39.28 ± 1.12	41.79 ± 1.22	2.51 ± 0.63
	W2	41.42 ± 1.06	43.75 ± 1.13	2.33 ± 0.61
	W3	43.35 ± 1.03	47.37 ± 1.32	4.02 ± 0.68
	W4	47.79 ± 1.13	49.87 ± 1.20	2.08 ± 0.69
	W5	49.34 ± 1.17	52.91 ± 1.28	3.57 ± 0.74
	W6	52.81 ± 1.24	55.64 ± 1.18	2.83 ± 0.76
Flaxseed 5%	W1	37.34 ± 1.01	41.19 ± 1.26	3.85 ± 0.31
	W2	41.56 ± 1.11	43.75 ± 1.12	2.19 ± 0.17
	W3	43.36 ± 1.26	46.96 ± 1.09	3.60 ± 0.33
	W4	46.32 ± 1.18	49.11 ± 1.20	2.79 ± 0.41
	W5	49.68 ± 1.33	51.26 ± 1.18	1.58 ± 0.66
	W6	51.74 ± 1.16	54.14 ± 1.23	2.40 ± 0.15

Data are presented as mean ± standard error, $n = 6$ per group. Statistical comparisons were made within groups across weeks using one-way ANOVA and between groups at each time point using an independent Student's *t*-test

ency contrasting the 5% flaxseed group's variability, potentially due to dietary adaptation or unmeasured environmental factors.

Table 4 compares feed intake for spotted deer housed in a zoo over six weeks. Both the control group and the 5% flaxseed group received identical weekly feed supplies (176.4 kg), with the control group demonstrating a progressive increase in feed intake (from 171.79 kg in W1 to 174.27 kg in W6) alongside steadily decreasing feed remains (4.61 kg to 2.13 kg), while flaxseed 5% group began with slightly lower intake (171.08 kg in W1) but gradually approached the control group's lev-

els by W6 (173.65 kg), ultimately revealing minimal differences in average weekly consumption (control group: ~173.06 kg vs flaxseed 5% group: ~172.50 kg) and equally efficient feed utilisation ($\leq 3.2\%$ remains weekly), indicating that flaxseed supplementation had a negligible impact on intake patterns, with both groups achieving near-identical consumption trends by the study's conclusion.

Table 5 presents the results of a nutritional intervention study investigating the effects of a 5% flaxseed dietary supplement on the serum sex hormone profile of spotted deer reared in a zoo setting. The measured parameters provide a com-

Table 4. Weekly feed intake of female spotted deer reared in the zoo after the addition of 5% flaxseed to the diet for six weeks from the onset of breeding

Groups	Weeks	Feed supply/week (kg)	Feed remains (kg)	Feed intake (kg)
Control (alfalfa+ pellets)	W1	176.4	4.61	171.79
	W2	176.4	4.28	172.12
	W3	176.4	3.72	172.68
	W4	176.4	2.85	173.55
	W5	176.4	2.46	173.94
	W6	176.4	2.13	174.27
Flaxseed 5%	W1	176.4	5.32	171.08
	W2	176.4	4.79	171.61
	W3	176.4	4.15	172.25
	W4	176.4	3.43	172.97
	W5	176.4	2.96	173.44
	W6	176.4	2.75	173.65

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Table 5. Sex hormone profile in the serum of female spotted deer reared in the zoo after the addition of 5% flaxseed to the diet for six weeks from the onset of breeding

Parameters	Groups	Week 1	Week 3	Week 6	<i>P</i> value
TT (ng/ml)	alfalfa + pellets	16.04 ± 1.23	12.04 ± 1.84	10.3 ± 1.55	0.119 7
	flaxseed 5%	18.62 ± 1.90	17.23 ± 4.98	14.37 ± 3.43	0.802 2
SHBG (ng/ml)	alfalfa + pellets	3.237 ± 0.835	5.127 ± 0.587	4.858 ± 0.546	0.153 5
	flaxseed 5%	2.372 ± 0.296	3.61 ± 0.343*	3.461 ± 0.106* ^b	0.012 8
FAI	alfalfa + pellets	437.1 ± 58.3	449.5 ± 56.8	440.2 ± 51.3	0.986 4
	flaxseed 5%	313.2 ± 79.5	214.6 ± 19.7	214.6 ± 19	0.376 2
FT (ng/ml)	alfalfa + pellets	0.434 ± 0.02	0.494 ± 0.04	0.402 ± 0.014	0.200 1
	flaxseed 5%	0.324 ± 0.091 5	0.310 5 ± 0.08	0.324 ± 0.091 5	0.991 4
TT/FT Ratio	alfalfa + pellets	41.56 ± 3.49	30.75 ± 5.06	37.92 ± 5.26	0.405 6
	flaxseed 5%	30.29 ± 2.37	28.14 ± 3.77	34.40 ± 4.64	0.550 3
BioT (ng/ml)	alfalfa + pellets	10.18 ± 0.42	11.55 ± 0.95	9.425 ± 0.335	0.197 5
	flaxseed 5%	7.62 ± 2.15	10.8 ± 1.71	7.28 ± 1.81	0.452 0
E2 (pg/ml)	alfalfa + pellets	130.93 ± 3.72	157.41 ± 6.46**	117.06 ± 4.44	0.027 4
	flaxseed 5%	184.8 ± 37.9 ^a	219.5 ± 12.91 ^c	227.4 ± 22.6 ^c	0.609 7

*,**Values in the same item with marks differ from the first-week value in the same group at $P < 0.05$ and $P < 0.01$, respectively. ^{a-c}At the same week, values in the same item with letters differ at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Mean ± standard error

BioT = bioavailable testosterone; E2 = oestradiol; FAI = free androgen index; FT = free testosterone; SHBG = sex hormone-binding globulin; TT = total testosterone

prehensive overview of the hypothalamic-pituitary-gonadal axis activity, including TT, E2, SHBG, FT, FAI, the TT/FT ratio, and BioT. Starting with TT, there were no significant differences between the two groups at any individual time point. The most striking results were observed in the E2 levels. The control group showed a significant change over time ($P = 0.027 4$), with a peak at Week 3 (157.41 pg/ml) before dropping below baseline by W6 (117.06 pg/ml). In stark contrast, the flaxseed group started with a markedly higher baseline (184.8 pg/ml, $P < 0.05$, indicating a significant difference from the control at that week) and showed a substantial, sustained increase, reaching 227.4 pg/ml by W6. The within-group change for the flaxseed group was not statistically significant, likely due to high individual variability (large standard error at W1), but the between-group differences at Ws3 and 6 are highly significant ($P < 0.001$).

SHBG data show interesting dynamics. The control group's SHBG levels increased from W1 to W3 and remained elevated, though this change was not significant ($P = 0.153 5$). The flaxseed group started with lower SHBG than the control (2.372 vs 3.237 ng/ml) and also increased sig-

nificantly over time ($P = 0.012 8$), with its value at W6 (3.461 ng/ml) significantly different from its own baseline ($P < 0.001$).

The FAI, a calculated ratio (TT/SHBG) that estimates free androgen activity, reveals a clear divergence between the groups. The control group's FAI remained stable around 440 throughout the study. The flaxseed group, however, started with a lower FAI (313.2) and showed a pronounced numerical decrease to 214.6 by W3, which was maintained at Week 6. Although the P -value suggests this within-group trend was not statistically significant, the magnitude of the change is physiologically noteworthy. Conversely, direct measurements of FT showed no significant changes within or between either group over the six weeks. Both groups maintained stable FT levels. The TT/FT Ratio and BioT, which provide alternative estimates of the fraction of testosterone not bound to SHBG, also showed no statistically significant changes in either group over time, mirroring the stability seen in the FT measurements. The primary and most significant finding of this study is the profound elevation of serum E2 in deer supplemented with 5% flaxseed. This effect can be almost certainly attributed to the

high concentration of lignans, specifically secoisolariciresinol diglucoside (SDG), found in flaxseed. SDG is converted by gut microbiota into the mammalian lignans enterodiol and enterolactone, which are phytoestrogens-plant-derived compounds with structural similarity to 17β -oestradiol. These phytoestrogens can exert oestrogenic effects by binding to oestrogen receptors ($ER\alpha$ and $ER\beta$) in various tissues (Lecomte et al. 2017; Gorzkiewicz et al. 2021). The sustained rise in E2 levels in the flaxseed group, contrasted with the fluctuating levels in the control group, strongly suggests a direct oestrogenic effect of the flaxseed supplementation. This is a well-documented phenomenon in other species, including livestock and humans, where flaxseed consumption has been shown to influence oestrogen metabolism and increase serum levels of SHBG (Musazadeh et al. 2023).

The statistically significant increase in SHBG in the flaxseed group aligns with this established mechanism. Oestrogens are a primary stimulator of SHBG synthesis in the liver. The elevated E2 levels in the flaxseed-fed deer likely triggered an upregulation of SHBG production (Musazadeh et al. 2023). This increase in SHBG has crucial implications for androgen bioavailability. TT circulates bound primarily to SHBG (with high affinity) and albumin (with low affinity); only the unbound (free) fraction is biologically active. The $FAI = TT / SHBG$ is a surrogate marker for this free fraction. The numerical halving of the FAI in the flaxseed group, from 313.2 to 214.6, indicates a substantial reduction in the bioavailable androgen pool, directly resulting from the rise in SHBG, even though TT levels remained relatively unchanged.

This creates an apparent paradox: despite stable TT and FT (as directly measured), the hormonal milieu shifted dramatically towards a more oestrogenic and less androgenic environment. The stability of directly measured FT, despite rising SHBG, is intriguing and may point to compensatory mechanisms within the HPG axis or limitations in the assay's sensitivity. However, the overall physiological signal is clear: the flaxseed diet promoted an oestrogenic state. In the context of the breeding season, this could have significant implications. Oestradiol is not solely a female hormone; it plays a critical role also in male reproduction, including regulating libido, erectile function, and feedback on the HPG axis. However, excessively high levels of E2 can be detrimental, potentially

leading to suppressed gonadotropin release and subsequent inhibition of testosterone synthesis (Yuan et al. 2023).

This study has direct implications for the management and welfare of captive deer populations. Zoos often face challenges with suboptimal reproductive performance in captive animals. Dietary manipulation is a key tool to improve health and reproduction. The results suggest that flaxseed supplementation could be a powerful dietary intervention to manipulate sex hormone profiles. For species where a heightened oestrogenic environment is desirable such as in females to promote oestrus cycles or in certain scenarios to manage aggressive, testosterone-driven behaviours in males flaxseed could be highly beneficial (Yuan et al. 2023). The reduction in FAI, indicative of lower bioactive androgen, might correlate with reduced aggression, a common management issue in captive herd dynamics, particularly during the breeding season.

However, this intervention must be applied with caution. The dramatic hormonal shift could disrupt the natural, delicate balance of breeding-season physiology. Furthermore, the long-term effects of such a phytoestrogen-rich diet on overall health, including potential impacts on the prostate and other hormone-sensitive tissues, are not known for this species and warrant further investigation.

While highly informative, this study has several limitations. The high standard errors, particularly in the flaxseed group for parameters like TT and E2, indicate substantial individual variation in response to the supplement. This could be due to age, dominance rank, or individual differences in gut microbiota that convert flaxseed lignans. A larger sample size would help confirm the statistical significance of the observed trends, especially for the FAI. Furthermore, the study lasted only six weeks. Longer-term studies are needed to determine whether these effects are sustained, whether the HPG axis adapts, and to monitor any long-term health consequences.

In summary, the data demonstrate that the addition of 5% flaxseed to the diet of female spotted deer over a six-week period during the onset of breeding induces a significant shift in their sex hormone profile. The most profound effect is a substantial increase in serum E2 levels, coupled with a significant rise in SHBG. This combination results in a notable reduction in the FAI, signalling a decrease in BioT and an overall shift towards a more oestrogenic

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endocrine environment. This effect is mechanistically explained by the phytoestrogenic properties of flaxseed lignans. For zoo management, this presents a potential non-invasive tool to modulate animal physiology and behaviour. However, this power must be wielded carefully, with a full understanding of the potential to both enhance and disrupt natural reproductive cycles. This study provides a strong foundation for further research into targeted nutritional strategies to improve the welfare and reproductive health of captive wildlife.

The results in Table 6 indicate a clear positive effect of flaxseed supplementation on the antioxidant profile. The flaxseed group exhibited significantly higher activity of two crucial antioxidant enzymes: CAT (325.0 ± 21.6 U/l vs 228.6 ± 24.1 U/l in the control, $P < 0.05$) and SOD (16.461 ± 0.650 U/ml vs 13.219 ± 0.616 U/ml in the control, $P < 0.05$). The significantly elevated activities of CAT and SOD in the flaxseed-supplemented group point towards an enhanced endogenous antioxidant defence system. This effect can be directly attributed to the unique biochemical composition of flaxseed, which is rich in several bioactive compounds with known antioxidant properties. The most prominent of these are alpha-linolenic acid (ALA, an omega-3 fatty acid), lignans (particularly secoisolariciresinol diglucoside - SDG), and dietary fibre. This enhancement works through two mechanisms: direct scavenging and indirect upregulation. Firstly, the lignans and other phenolic compounds in flaxseed act as direct scavengers of reactive oxygen species (ROS), donating electrons to neutralise free radicals like superoxide anions and hydroxyl radicals (Huang et al. 2024). By directly neutralising these radicals, the body's primary antioxidant enzymes may experience less substrate burden, but the data suggest a more active role. Secondly, and more importantly, these bioactive compounds are known to activate the nuclear factor erythroid 2-related factor 2 (Nrf2)

pathway. Nrf2 is a master regulator of the cellular antioxidant response. Under oxidative stress, Nrf2 promotes the transcription of genes encoding antioxidant enzymes (Obeme-Nmom et al. 2024). The lignans and ALA in flaxseed have been shown to act as Nrf2 activators. This upregulation of the body's own defence machinery is a sustainable and powerful way to combat oxidative stress, as it provides a constant supply of protective enzymes rather than a one-time scavenging effect. This proposed mechanism explains why these enzymes are more active in the supplemented group. CAT primarily located in peroxisomes, converts H_2O_2 into water and oxygen. GPx, which utilises glutathione as a cofactor, reduces H_2O_2 and organic hydroperoxides to water and corresponding alcohols, respectively, thereby protecting cell membranes from lipid peroxidation (Pei et al. 2023).

MDA is a terminal product of the peroxidation of polyunsaturated fatty acids (PUFAs) in cell membranes and is a reliable marker of oxidative damage to lipids. The lower MDA level in the flaxseed group, although not statistically significant in this study, aligns with the expected outcome of a bolstered antioxidant system. The fact that MDA levels did not increase and even trended downward underscores the potent protective effect of the accompanying antioxidants (lignans, etc.) within the flaxseed. The antioxidant components effectively protect the dietary lipids and, by extension, the animal's own cellular lipids from oxidative damage.

For zoo-managed species like the spotted deer, managing oxidative stress is critical to animal health and welfare. Captivity can introduce stressors not found in the wild, such as altered diets, limited space, and artificial social structures, which can contribute to elevated oxidative stress. This chronic state can impair immune function, reduce fertility, and accelerate aging. The onset of the breeding season is a period of heightened metabolic and physio-

Table 6. Antioxidant enzyme profile in the serum of spotted deer reared in the zoo after the addition of 5% flaxseed to the diet for six weeks from the onset of breeding

Groups	CAT(U/l)	GPx (mU/ml)	SOD (U/ml)	MDA (nmol/ml)
Control (alfalfa + pellets)	228.6 ± 24.1	$3\ 636.8 \pm 39.3$	13.219 ± 0.616	0.995 ± 0.02
Flaxseed 5%	325.0 ± 21.6^a	$3\ 681 \pm 12.9$	16.461 ± 0.650^a	0.958 ± 0.03
<i>t</i> -value	2.98	1.07	3.62	1.03

^aValues measure the difference between groups for the same enzyme at $P < 0.05$. Mean $\pm$ standard error. The *t*-value represents the *t*-statistic from the independent Student's *t*-test

CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase

logical activity, which can increase ROS generation. Therefore, the timing of this intervention is crucial. By enhancing the antioxidant capacity through flaxseed supplementation, zoo managers can potentially mitigate the negative impacts of captivity-induced and reproduction-related stress.

Enhanced antioxidant status has been directly linked to improved reproductive outcomes in both males and females. In males, reduced oxidative stress is associated with higher sperm quality, motility, and DNA integrity (Yuan et al. 2023). In females, flaxseed supplementation creates a more favourable physiological state for reproduction: a hormonally balanced environment supported by a robust antioxidant defence system. Flaxseed is a renowned functional feed ingredient, rich in alpha-linolenic acid (ALA, an omega-3 fatty acid), lignans (e.g., secoisolariciresinol diglucoside – SDG), and dietary fibre, all of which are known

to modulate physiology, metabolism, and immunity (Wang et al. 2025).

The most significant change in haematology (Table 7) was the marked increase in RBC count in the flaxseed group ($12.533 \pm 0.05 \times 10^6/\mu\text{l}$ vs $10.920 \pm 0.51 \times 10^6/\mu\text{l}$). This suggests stimulation of erythropoiesis. The breeding season imposes increased energetic and oxygen demands due to courtship behaviours and physiological changes. Enhanced erythropoiesis may be an adaptive response to this demand. Omega-3 fatty acids, like ALA in flaxseed, can influence bone marrow metabolism. They are incorporated into cell membranes, increasing membrane fluidity and potentially facilitating cell division and differentiation (Al-Madhagy et al. 2023). Furthermore, the anti-inflammatory effects of ALA and lignans may create a more favourable bone marrow microenvironment by reducing inflammatory cytokines that can suppress erythro-

Table 7. Complete blood count (CBC) of spotted deer reared in the zoo after the addition of 5% flaxseed to the diet for six weeks from the onset of breeding

Items	Groups		<i>t</i> -value
	control (alfalfa + pellets)	flaxseed 5%	
RBC ($10^{12}/\text{l}$)	10.92 ± 0.51	12.53 ± 0.05^a	3.10
HGB (g/l)	122.7 ± 11.7	128.1 ± 6.1	0.41
HCT (%)	22.00 ± 3.17	25.63 ± 1.56	1.02
MCV (fl)	48.33 ± 1.28	44 ± 0.96	2.69
MCH (pg)	16.53 ± 0.44	15.03 ± 0.26	2.90
MCHC (g/l)	343.0 ± 0.9	325.0 ± 8.8	2.01
RDW (%)	19.33 ± 0.60	17.5 ± 0.29	2.75
PLT ($10^9/\text{l}$)	550 ± 143	513 ± 122	0.20
MPV (fl)	6.90 ± 1.90	7.68 ± 2.41	0.25
WBC ($10^9/\text{l}$)	7.15 ± 0.73	7.46 ± 0.21	0.41
LYM ($10^9/\text{l}$)	51.27 ± 9.88	37.3 ± 1.64	0.35
MON ($10^9/\text{l}$)	1.60 ± 0.09	1.67 ± 0.07	1.80
GRA (%)	54.77 ± 7.82	61.23 ± 1.58	0.81
EOS (%)	1.53 ± 0.05	1.47 ± 0.07	0.70
LYM ($10^9/\text{l}$)	1.13 ± 0.06	1.1 ± 0.07	1.57
MON ($10^9/\text{l}$)	0.09 ± 0.02	0.05 ± 0.01	0.46
GRA ($10^9/\text{l}$)	1.90 ± 0.16	1.83 ± 0.16	0.28
EOS ($10^9/\text{l}$)	0.06 ± 0.01	0.28 ± 0.08	2.48

^aValues indicate differences between groups for the same enzyme at $P < 0.05$. Mean $\pm$ standard error. The *t*-value represents the *t*-statistic from the independent Student's *t*-test

EOS = eosinophils; GRA = granulocytes; HCT = haematocrit; HGB = haemoglobin; LYM = lymphocytes; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; MON = monocytes; MPV = mean platelet volume; PLT = platelets; RBC = red blood cell; RDW = red cell distribution width; WBC = white blood cells

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poiesis (Jang et al. 2022). However, this quantitative increase was paired with qualitative changes indicating microcytic hypochromic erythropoiesis. Significant decreases in MCV, MCH, and MCHC are a classic haematological signature of iron-deficient erythropoiesis (Roccaforte et al. 2024). This paradox increased cell production but deficient haemoglobinisation can be explained by the antinutritional factors in flaxseed. Flaxseed contains phytic acid, a potent mineral chelator, particularly of iron and zinc, which can significantly reduce their intestinal absorption (Kausar et al. 2024). The bone marrow, stimulated to produce more cells, does so with an inadequate iron supply, resulting in smaller cells with less haemoglobin. The decrease in RDW indicates this new cell population is uniformly small, supporting a systematic, diet-induced shift rather than a variable deficiency state.

The stable total WBC count indicates no overall systemic inflammatory or immunosuppressive effect. The proportional shift within the differential counts, a decrease in LYM% and an increase in GRA%, without changes in absolute counts, suggests an immunomodulatory effect rather than outright suppression or activation. PUFAs from flaxseed are known to be incorporated into immune cell membranes, influencing eicosanoid production. Omega-3 PUFAs generally promote the synthesis of less inflammatory eicosanoids, which can subtly alter immune cell trafficking and function (Mititelu et al. 2024). This can sometimes manifest as a shift in differential counts without altering the total WBC.

The most alarming change was the fourfold increase in the absolute EOS (0.28 vs $0.066 \times 10^3/\mu\text{l}$). Eosinophilia is typically associated with parasitic infections, allergic diseases, or certain autoimmune conditions (Weaver et al. 2024). While a coincidental parasitic infection cannot be ruled out, a dietary cause is plausible. Although rare, flaxseed can be an allergen in humans and potentially to other animals (Mota et al. 2024). The introduction of a novel protein source could incite a Th2-type immune response, characterised by interleukin-5 (IL-5) production, which is the primary cytokine driving eosinophil production, differentiation, and activation. This finding necessitates immediate investigation, as sustained eosinophilia can lead to tissue damage and inflammation.

The lack of change in PLT count and MPV indicates that flaxseed supplementation at this dosage did not affect thrombopoiesis or platelet activation.

Omega-3 fatty acids are often associated with mild anti-platelet effects in humans; however, this effect is species-specific and may not be pronounced in deer at this supplementation level (Koutsaliaris et al. 2024).

The significant increase in total protein (TP; 64.0 vs 60.0 g/l), with non-significant rises in ALB and GLOB, points towards an improved metabolic protein status. This could be due to better nitrogen retention or enhanced protein synthesis. The stability of liver enzymes (ALT, ALP) and TBIL is a crucial finding, confirming that the 5% flaxseed diet was not hepatotoxic (Parikh et al. 2024). The slight, non-significant decrease in ALT might even suggest improved hepatic cellular integrity.

The substantial elevation in GLU (926.7 vs 693.3 mg/l) is a major metabolic consequence. The breeding season is a period of high energy demand, and this hyperglycemia may provide readily available fuel. The mechanism is likely multifactorial. The high fibre content of flaxseed can modulate post-prandial glucose absorption, but the sustained elevation suggests a more systemic effect. Stress, mediated by cortisol, can induce hyperglycemia. The breeding season itself is a stressor, and the flaxseed diet, while beneficial in some respects, may have inadvertently modulated the hypothalamic-pituitary-adrenal (HPA) axis. Alternatively, changes in insulin sensitivity or gluconeogenic pathways could be involved, a known effect of omega-3 fatty acids in some models (Werida et al. 2023). The parameters present a complex but ultimately positive picture. The significantly lower creatinine level (10.2 vs 11.6 mg/l) strongly indicates reduced muscle catabolism, as creatinine is a breakdown product of creatine phosphate in muscle (Avila et al. 2025). This suggests the animals on the flaxseed diet were in a better anabolic state, which is highly desirable during the metabolically costly breeding season. The parallel decrease in urea (463.0 vs 504.3 mg/l) supports improved nitrogen utilization and reduced protein catabolism. The decrease in BUN seems to be related to the timing of blood collection, dietary protein content, or hydration status. The overall trend (lower Urea and Creatinine) strongly indicates no renal impairment and possibly improved metabolic efficiency.

The stability of Na, K, Ca, and PHOS is critical. It demonstrates that the dietary modification did not disrupt electrolyte homeostasis or mineral balance, which is essential for fundamental physiologi-

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cal processes including nerve conduction, muscle contraction, and bone metabolism (Table 8).

Finally, the 5% flaxseed supplementation elicited a multifaceted physiological response in spotted deer. The benefits are significant: enhanced erythropoiesis, improved protein status, reduced muscle catabolism, and increased energy availability as glucose. These effects are likely mediated by flaxseed's unique nutritional profile, particularly its omega-3 fatty acids and lignans. This analysis, supported by physiological principles and scientific literature, shows that while flaxseed holds great promise as a functional feed to enhance breeding readiness in captive deer, its application must be carefully managed to mitigate its anti-nutritional and potential immunogenic effects.

Histology

Research on deer reproductive morphology has been established in species like *Rusa* (Mahre et al. 2016), white-tailed (Gastal et al. 2017; Green et al. 2017), and pampas deer (Ungerfeld

et al. 2008). Histological analysis of deer fed a control diet (pellets and alfalfa) shows normal ovarian function, evidenced by structures like antral follicles and a regressed corpus albicans, indicating regular ovulation cycles (Figure 3). The addition of 5% flaxseed, a source of phytoestrogens, did not disrupt core ovarian function, as mature follicles and corpora lutea were still present. The corpus luteum is vital for producing progesterone and facilitating reproductive cycles, with noted species-specific variations in deer compared to cattle (Kawate et al. 2000; Kim 2021). In deer, particularly Sika deer, ovarian morphology and function during oestrus and pregnancy show distinct differences compared to domesticated cattle. For instance, corpus luteum function in deer differs from that in cattle, especially during oestrus and pregnancy, suggesting species-specific adaptations in reproductive physiology (Perez et al. 2013; Kim et al. 2014). Recent studies by (Lee et al. 2023) revealed that the composition and structure of the corpus luteum in Sika deer are not as clearly defined as in Water deer. These morphological and functional differences may also influence the Sika

Table 8. Biochemical parameters of spotted deer reared in the zoo after addition of 5% flaxseed to the diet for six weeks from the onset of breeding

Items	Groups		<i>t</i> -value
	control (alfalfa + pellets)	flaxseed 5%	
ALB (g/l)	36.0 ± 1.8	38.3 ± 1.0	1.10
TP (g/l)	60.0 ± 1.3	64.0 ± 0.7	2.65
GLU (mg/l)	693.3 ± 52.5	926.7 ± 80.1	2.42
ALP (U/l)	222.8 ± 60.3	182.8 ± 15.0	0.64
ALT (U/l)	55.33 ± 1.65	51.00 ± 1.59	1.89
TBIL (mg/l)	6.7 ± 0.5	5.3 ± 0.8	1.31
AMY (U/l)	211.33 ± 9.59	203.33 ± 0.21	0.83
BUN (mg/l)	235.7 ± 5.1	216.3 ± 7.5	2.12
CREA (mg/l)	11.6 ± 0.6	10.2 ± 0.1	1.97
Ca (mg/l)	19.0 ± 4.5	21.1 ± 0.5	0.45
PHOS (mg/l)	10.7 ± 1.8	10.5 ± 0.2	0.06
Na (mmol/l)	136.00 ± 0.96	135.67 ± 0.55	0.29
K (mmol/l)	5.76 ± 0.18	5.73 ± 0.07	0.16
GLOB (g/l)	24.0 ± 2.9	25.6 ± 1.7	0.49
UREA (mg/l)	504.3 ± 11.0	463.0 ± 15.9	2.13

Mean ± standard error. The *t*-value represents the *t*-statistic from the independent Student's *t*-test

ALB = albumin; ALP = alkaline phosphatase; ALT = alanine transaminase; AMY = amylase; BUN = blood urea nitrogen; Ca = calcium; CREA = creatinine; GLOB = globulin; GLU = glucose; K = potassium; Na = sodium; PHOS = phosphate; TBIL = total bilirubin; TP = total protein; UREA = urea

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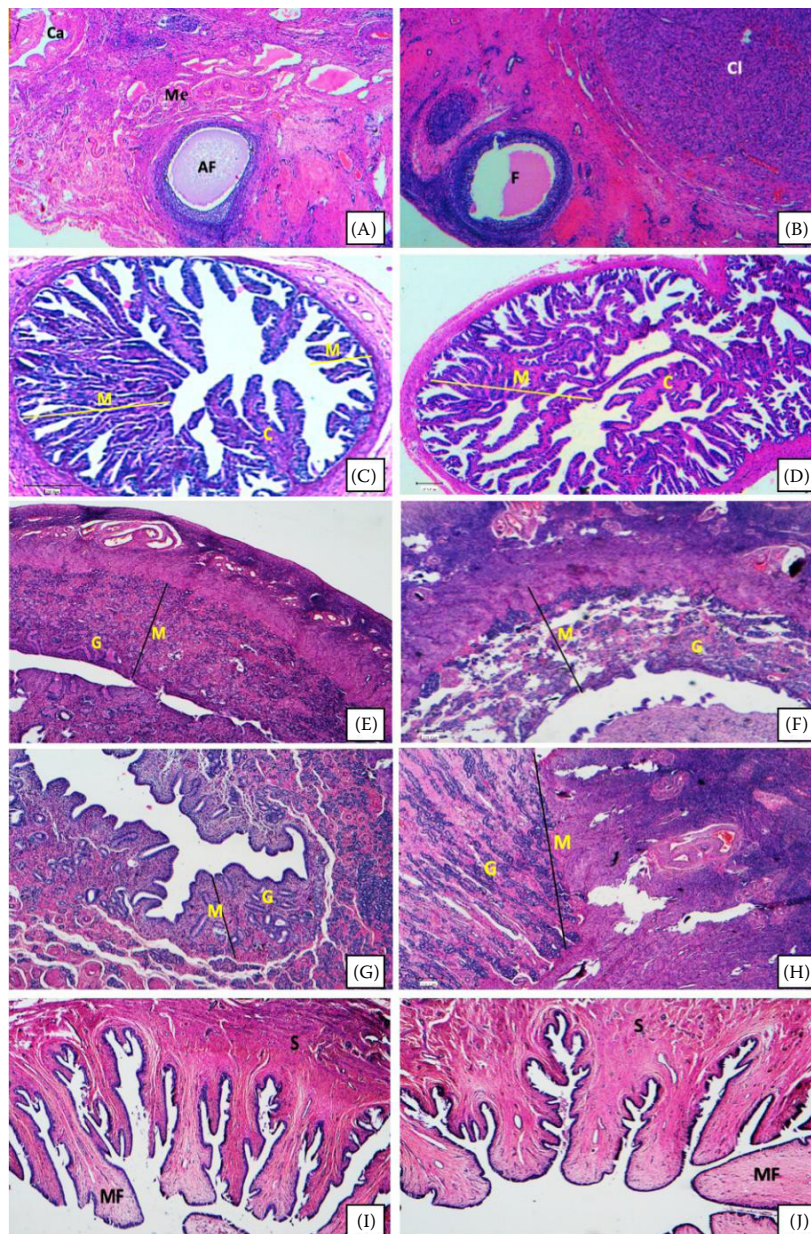


Figure 3. Photomicrograph of reproductive organs of zoo-reared spotted deer fed 5% flaxseed

(A) Photomicrograph of the deer ovary from the control group showing an extended medulla, one antral follicle, and a regressed corpus albicans. (B) Photomicrograph of the deer ovary from the flaxseed group showing that the cortex contains a mature follicle and corpus luteum. (C) Photomicrograph of the deer fallopian tube from the control group, showing that the mucosa is relatively thin with scanty connective tissue and slight folds in many regions. (D) Photomicrograph of the deer fallopian tube from the flaxseed group, showing that the mucosa is relatively thick with increased connective tissue and mucosal folds. (E) Photomicrograph of the deer uterine horn from the control group, showing that the mucosa has blood vessels and some uterine glands. (F) Photomicrograph of the deer uterine horn from the flaxseed group, showing that the mucosa has blood vessels and increased uterine glands. (G) Photomicrograph of the deer uterus from the control group; the mucosa appears with blood vessels and some uterine glands. (H) Photomicrograph of the deer uterus from the flaxseed group; the mucosa appears with blood vessels and increased uterine glands. (I) Photomicrograph of the deer cervix from the control group showing thin mucosal folds and a submucosal layer of smooth muscle and collagenous fibers. (J) Photomicrograph of the deer cervix from the flaxseed group showing thick mucosal folds and submucosal smooth muscle. H&E, $\times 40$ AF = antral follicle; C = connective tissue; Ca = corpus albicans; Cl = corpus luteum; F = follicle; G = uterine glands; M = mucosa; Me = medulla, MF = mucosal folds; S = smooth muscle

deer ovary's ability to support and maintain pregnancy, highlighting the complex and varied nature of reproductive mechanisms across species.

Flaxseed supplementation appeared to stimulate other reproductive tissues. The fallopian tubes showed thicker mucosa and more prominent folds, potentially enhancing ovum transport. In the uterine horns, the flaxseed diet was associated with increased vascularisation, glandular development, and mucosal thickness, suggesting an improved environment for implantation compared to the inactive, anoestrus-like state seen in the control group. Similar subtle, stimulatory changes were noted in the cervical mucosal folds. While the histology of cervids like the red brocket deer is documented, data on *Rusa timorensis* remains limited. The findings suggest that both diets adequately support reproductive health, but the flaxseed diet may enhance the functional morphology of the uterus and oviducts, likely due to the influence of its phytoestrogenic compounds.

This work highlights the potential impact of dietary phytoestrogens on deer health and physiology, particularly reproductive function and hormone regulation. However, it is crucial to consider the potential risks associated with excessive phytoestrogen intake, such as hormonal imbalances and adverse health effects. Therefore, a balanced approach to dietary supplementation is essential to optimise the benefits while minimising potential risks. Furthermore, the sample size ($n = 6$ per group), while typical for studies on captive wildlife in zoo settings, is a limitation of this study. This small sample size may have contributed to the high standard errors observed in some parameters and limited the statistical power to detect subtle but biologically significant differences. Future studies with larger cohorts are necessary to confirm and extend these findings.

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Conflict of interest

The authors declare no conflict of interest.

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