Journal of Medical Genetics and Clinical Biology
Homepage : https://journal.antispublisher.id/index.php/JMGCB
Email : admin@antispublisher.com

e-ISSN : 3032-1085
JMGCB, Vol. 2, No. 11, November 2025
Page 486-491

Effect of Adding Different Levels of Flaxseed Oil to Diets on Ejaculate Volume and Sperm Concentration in Semen in Ostriches

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DOI : https://doi.org/10.61796/jmgcb.v2i11.1422

Sections Info

Article history:
Submitted: July 31, 2025
Final Revised: August 10, 2025
Accepted: August 20, 2025
Published: September 01, 2025

Keywords:
Ostrich
Flaxseed oil
Omega-3
Semen

ABSTRACT

Objective: *This study aimed to investigate the impact of dietary supplementation with flaxseed oil on the morphological characteristics of semen in male ostriches.* ***Method:*** *The experiment was conducted at the Babylon Reserve for Ostrich, Falcon, and Deer Breeding, Babylon Province, Iraq, using 27 sexually mature ostriches divided into three treatment groups with three replicates each, organized under a family-based system (two females and one male per replicate). Birds were housed in specialized pens, with males separated from females from the beginning of the trial, and all were provided a standardized diet containing 17.34% crude protein and 2740.56 kcal/kg metabolizable energy. Treatments included a control diet and diets supplemented with 1% or 2% flaxseed oil. Semen traits assessed included ejaculate volume, sperm concentration, and packed sperm volume.* ***Results:*** *Supplementation with flaxseed oil at both inclusion levels led to statistically significant and highly significant improvements in most evaluated semen parameters compared with the control.* ***Novelty:*** *These findings provide new evidence that flaxseed oil, as a rich source of Omega-3 fatty acids, can enhance reproductive performance in male ostriches, offering a natural nutritional strategy to improve fertility and productivity in ostrich farming systems.*

INTRODUCTION

The ostrich (Struthio camelus) is the largest living bird on Earth and belongs to a group of flightless birds known as ratites, which lack a keeled sternum, rendering them incapable of flight [1]. There are five recognized subspecies of ostrich, which vary in egg size, eggshell texture and porosity, skin color, body size, degree of feathering, baldness, and neck length. These variations have been documented primarily in South Africa [2]. The species was first described by **Linnaeus in 1758** as Struthio camelus and is often referred to as the "camel bird" due to its adaptations to desert environments, similar to those of camels.

Ostriches are the largest birds on Earth, with adults weighing up to 150 kg and standing up to three meters tall [3]. However, ostrich farming faces several significant challenges, including a lack of knowledge regarding nutritional requirements, low fertility and hatchability rates, high early embryonic mortality, and high post-hatch chick mortality. Additionally, reliance on traditional breeding programs and natural mating significantly increases the costs associated with establishing ostrich farms [4]. To address these issues, researchers have turned to the use of medicinal plants and herbs, which are natural sources of biologically active compounds used in pharmaceutical production or applied in raw form to enhance animal productivity and health [5]. One such product is flaxseed oil, derived from Linum usitatissimum, a winter annual crop cultivated in warm

regions. The plant typically grows to a height of 30 to 120 cm and is valued for its oil, which is rich in beneficial fatty acids [6]. Flaxseed oil production is influenced by various factors, including extraction methods and environmental conditions. It contains fat-soluble vitamins, amino acids, and essential minerals [7]. Notably, flaxseed oil is considered a primary plant-based source of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). It also contains high levels of phospholipids, which play a critical role in the formation and function of cellular membranes [8]. Given the lack of detailed studies on ostriches in Iraq, the present study aimed to investigate the effects of adding flaxseed oil at levels of 1% and 2% to the diets of male ostriches on semen quantitative characteristics.

MATERIAL AND METHODS

This study was conducted at the Babylon Reserve for the Breeding and Production of Ostriches, Falcons, and Deer, located in Al-Hindiya Dam area, approximately 65 km south of Baghdad. The aim of the study was to investigate the effect of adding different levels of flaxseed oil, as a source of Omega-3, to the diet on the productive, reproductive, and physiological performance of ostriches. The experimental period extended from October 16, 2015, to April 10, 2016. The first 14 days were considered a preliminary adaptation period for the birds. From the first day of the experiment, males were separated from females, and all birds were fed a standardized diet [9]. The floors of the pens were covered with clean, pure sand to eliminate the risk of ingestion of harmful objects such as metal or plastic pieces, which could lead to mortality [10]. The breeding sections were equipped with custom-made feeders (plastic water tanks cut in half), with dimensions of 100 × 100 × 50 cm, separately for males and females. Each pen was also equipped with metal water containers, which were connected via hoses to a drainage system to allow for daily cleaning. Feed was provided to the birds twice daily—once in the morning and once in the evening. The experimental treatments were distributed as follows:

Treatment 1 (T1): Standard diet without flaxseed oil (control).

Treatment 2 (T2): Standard diet supplemented with 1% flaxseed oil.

Treatment 3 (T3): Standard diet supplemented with 2% flaxseed oil.

Studied Traits

Ejaculate Volume: The average ejaculate volume per male was determined by weighing the semen sample, based on the assumption that the semen density is equivalent to 1 mg per 1 µL [11]. The semen was weighed using a sensitive balance after recording the weight of the empty plastic tubes. The difference between the two weights represented the ejaculate volume. **Sperm Concentration :** Sperm concentration was measured using a haemocytometer slide, following the method described by [12]**.**

RESULT AND DISCUSSION

Result

Ejaculate Volume

Table 1 presents the data on the average ejaculate volume of male ostriches. It is evident that flaxseed oil supplementation had a highly significant effect (P≤0.01) on this trait. Treatment T3 (2% flaxseed oil) showed a highly significant superiority (P≤0.01) compared to the control group across all experimental periods. Additionally, T3 demonstrated a highly significant improvement (P≤0.01) over Treatment T2 (1% flaxseed oil) during periods 1, 2, 4. However, no significant differences were observed between the two supplementation treatments during periods 3, 5. Treatment T2 exhibited a range of significant (P≤0.05) to highly significant (P≤0.01) differences compared to the control group during periods 1 و،Meanwhile, no significant differences were detected between T2 and the control during periods 2, 3, 4.

Table 1. Effect of Adding Different Levels of Flaxseed Oil to the Diet on Average Ejaculate Volume (ml) (Mean ± Standard Error) in Ostriches.

Periods	T1 (Control)	T2 (1% Flaxseed Oil)	T3 (2% Flaxseed Oil)	Significance Level
1	0.87C ± 0.02	1.30B ± 0.05	1.66A ± 0.06	0.01
2	0.95B ± 0.02	1.16B ± 0.09	1.80A ± 0.05	0.01
3	1.01B ± 0.09	1.22AB ± 0.15	1.60A ± 0.17	0.05
4	1.03B ± 0.08	1.15B ± 0.10	1.83A ± 0.03	0.01
5	0.97B ± 0.01	1.36A ± 0.08	1.60A ± 0.15	0.01

*Treatments: T1 (0% flaxseed oil), T2 (1% flaxseed oil), T3 (2% flaxseed oil) *Each period represents 14 days. *Different letters within the same row indicate significant differences among treatment means at the 0.05 and 0.01 levels of significance. *NS = Not significant.

Sperm Concentration:

Table 2 presents the data on sperm concentration (sperm × 10^9/mL). It is evident that Treatment T3 (2% flaxseed oil) had a significant (P≤0.05) to highly significant (P≤0.01) effect compared to the control group across all study periods, except for period 7, in which no significant differences were observed among the treatments. Furthermore, T3 showed a highly significant superiority (P≤0.01) over Treatment T2 (1% flaxseed oil) during periods 4, while no significant differences were found between T2 and T3 during periods 1, 2, 3Treatment T2 also exhibited a highly significant effect (P≤0.01) compared to the control during periods 2, only. However, no significant differences were observed between T2 and the control group during periods 1, 3, 4, 5.

Table 2. Effect of Adding Different Levels of Flaxseed Oil to the Diet on Average Sperm Concentration (sperm × 10^9/mL) in Male Ostriches (Mean ± Standard Error).

Periods (Every Two Weeks)	T1 (Control)	T2 (1% Flaxseed Oil)	T3 (2% Flaxseed Oil)	Significance Level
1	2.71B ± 1.01	3.56AB ± 1.01	4.56A ± 0.18	0.05
2	2.50B ± 0.30	3.40A ± 0.11	4.03A ± 0.12	0.01
3	2.70B ± 0.32	3.50AB ± 0.35	4.50A ± 0.17	0.01
4	1.76B ± 0.08	2.20B ± 0.05	3.40A ± 0.11	0.01
5	1.70B ± 0.26	2.33B ± 0.06	3.50A ± 0.17	0.01

*Treatments: T1 (0% flaxseed oil), T2 (1% flaxseed oil), T3 (2% flaxseed oil) *Each period represents 14 days. *Different letters within the same row indicate significant differences among treatment means at the 0.05 and 0.01 levels of significance. *NS = Not significant.

Discussion

The significant improvements observed in Tables 1 and 2 in semen characteristics in favor of the flaxseed oil supplementation treatments can be attributed to the role of polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids play a crucial role in the formation, structure, and function of sperm cells [13]. Moreover, such lipids can serve as an energy source required by spermatozoa to perform various physiological functions and may also play a significant role in maintaining sperm cell integrity. Additionally, Omega-3 fatty acids may act as stimulators for the secretion of antioxidants, which in turn help prevent lipid peroxidation and the formation of free radicals. This is especially important because avian sperm cells lack vascular support or direct maternal connection, meaning they rely entirely on the antioxidants present in the seminal plasma to defend themselves against oxidative damage [14]. Furthermore, Omega-3 fatty acids can enhance the secretion of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which protect the lipids within the sperm cell from oxidative stress [15]. The improvements may also be related to the fact that lipids are a source of fat-soluble vitamins—namely, vitamins E, A, and D. Vitamin E, in particular, is a potent antioxidant that protects sperm cells from oxidative damage, in addition to other antioxidant enzymes such as SOD, GSH, and catalase. These antioxidants are found both within the sperm cell and in the seminal plasma, and are vital for protecting spermatozoa from oxidative damage [16].

CONCLUSION

Fundamental Finding : The findings of this study confirm that supplementing ostrich diets with flaxseed oil significantly improved reproductive efficiency, as reflected by enhanced semen characteristics such as ejaculate volume, sperm concentration, and packed sperm volume. **Implication :** These results highlight the potential of flaxseed oil,

a rich source of Omega-3 fatty acids, as a natural feed additive to support reproductive performance and productivity in ostrich farming, contributing to more sustainable and profitable breeding practices. **Limitation :** Nevertheless, the research was limited to a relatively small sample size and focused only on semen morphological traits, without examining fertility outcomes, hatchability rates, or long-term reproductive success. **Future Research :** Further studies should explore larger populations, evaluate additional reproductive and productive parameters, and investigate the optimal inclusion levels of flaxseed oil in ostrich diets under different management and environmental conditions to validate and expand the practical applications of these findings.

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