ISTANBUL UNIVERSITY CERRAHPASA

Partial Substitution of Barley by Destoned Olive-Waste Cake on Reproductive Performances in Ram

Naima HADJAB[1], Boubakeur SAFSAF[1], Hamza RAHAB[2], Redouane AISSOUS[2], Karim BENHENIA[2]

[1]Departement Sciences Vétérinaires– Laboratoire Environnement, Santé et Productions Animales – ,Universite Hadj Lakhdar Batna1, Batna, Algeria
[2]Centre de Recherche en Biotechnologie CRBt Constantine, Constantine, Algeria

Cite this article as: Hadjab, N., Safsaf, B., Rahab, H., Aissous, R., Benhenia, K. (2022). Partial substitution of barley by destoned olive-waste cake on reproductive performances in ram. *Acta Veterinaria Eurasia*, 48(2), 101-108.

ORCID IDs of the authors: N.H. 0000-0001-6631-1862, B.S. 0000-0001-8253-0635, H.R. 0000-0002-8081-7007, R.A. 0000-0002-5940-9279, K.B. 0000-0003-2205-9890.

Abstract

Destoned olive-waste cake is among the main agro-industrial by-products that can be included in the diet of farm animals. In this scope, this study was undertaken to explore the impact of substitution of barley diet by destoned olive-waste cake (DOC) on reproductive performances in rams. For this purpose, eighteen rams were divided into 3 groups of six rams each. In the first group, no substitution of barley was made (0DOC:100% barley + 0 DOC), in the second (20DOC) and third groups (40DOC) substitution of barley was of 20% and 40% DOC, respectively. After substitution, some reproductive parameters were assessed: including scrotal circumference (SC), plasma testosterone level and sperm quality (volume, concentration, motility, membrane functionality (MF) and lipid peroxidation (LPO)). The overall means of studied parameters in 20DOC and 40DOC were similar to those of control group except a significant reduction ($p \leq .05$) of LPO in DOC and a significant ($p \leq .05$) increase of SC and sperm volume in 40 DOC. The monthly follow-up revealed a significant ($p \leq .05$) progression of sperm concentration, volume, MF and a reduction of LPO in 40 DOC. In conclusion, DOC can partially substitute a barley in ram diet with a positive impact on reproductive performances particularly at 40% of substitution.

Keywords: Olive-waste cake, reproductive performances, semen quality, lipid peroxidation,ram.

Introduction

In Algeria, sheep livestock comprised 26 million heads. It is considered as the first source of meat and wool (MADR, 2014). Among these livestock, Ouled Djellal is the most dominant sheep breed (Harkat et al., 2015), which is reared mainly in an extensive system in the steppe which is characterized by arid and semi-arid climates (Bencherif, 2013; Rondia, 2006). In this system, barley is fed exclusively as a conventional energetic source for the sheep, throughout the year, especially in autumn and winter (Bourbouze, 2000; Mouhous, 2007). This high consumption of barley results in its penury and the increase of its price. This situation gets worse during drought. One of the main strategies proposed to cushion the deficit of feed is to substitute a part of the conventional resources of feed in livestock diet with non-conventional ones (Allaoui et al., 2018; Djaalab et al., 2016; Mebirouk-Boudechiche et al., 2015).

Olive-waste cake is an important agro-industrial by-product (Abdelguerfi et al., 2008; Dorbane et al., 2016) that can be used as non-conventional feed. However, it is still not exploited in livestock feeding despite its potential benefits and its large availability (Kadi et al., 2004). Several studies reported that olive waste, particularly olive cake, can substitute a part of cereal up to 40% in the diet (Benbati et al., 2016; Nefzaoui, 1991), without any harmful effect on animal health, blood parameters, carcass merits, and milk and meat quality (Branciari et al., 2015; Terramoccia et al., 2013; Tufarelli et al., 2013; Vargas-Bello-Pérez et al., 2013). Additionally, olive cake, particularly when destoned (Sadeghi et al. 2009), is an important source of vegetable oil (approximately 18–25%) (Chiofalo et al. 2004).

Ram semen is well known as a product that is very sensitive to oxidative stress particularly lipid peroxidation (LPO), a major injury that affects sperm quality and therefore the fertility outcomes (Guthrie & Welch, 2012). The major components of olive cake oil are fatty acids and antioxidants. These fatty acids are less sensitive to LPO compared to those of sperm plasma membrane (Taticchi et al., 2017).

There is emerging evidence that the incorporation of vegetable oil in the diet of animals could improve reproductive performances of animals, mainly the sperm quality and fertility (Tran et al., 2016). However, few studies had investigated the effects of olive cake

Corresponding Author: Boubakeur SAFSAF • **E-mail:** saf2_b@yahoo.fr

Received: July 28, 2021 • **Accepted:** December 20, 2021 • **Available Online Date:** March 12, 2022 • DOI: 10.5152/actavet.2022.21075

Available online at actavet.org

Copyright@Author(s) - Available online at actavet.org. Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

feeding on the reproductive performances in sheep (Dorbane et al., 2016; Kadi et al., 2004). Concerning Ouled Djellal rams, no study, to our knowledge, was performed to evaluate the impact of olive cake on their reproductive performances. Therefore, we suggested that the incorporation of olive cake in rams' diet would have a positive impact on LPO and consequently, on sperm quality.

In this context, our objective was to substitute part of the barley by destoned olive-waste cake (DOC) in the dietary regimen of Ouled Djellal rams and then investigate the effect of this substitution on the scrotal circumference, semen quality, and blood testosterone level.

Methods

Site of Study and Climate

The present study was carried out from November 2018 to March 2019 (14 weeks), during anestrus of ewes and where males were separated from females, in the experimental farm of Belgacem LAAZIZ (OuledRahmoun, Constantine, Algeria), located in the semi-arid region at longitude 6°42'19" and latitude of 36°10'58"N and an altitude between 600 m and 900 m above mean sea level. During this period, the mean environmental temperature and relative humidity were 15°C and 77%, respectively.

Animals and Experimental Design

Eighteen healthy, sexually mature Ouled Djellal rams (2–5 years old) were included in this study and were divided into three groups. The first group is taken as a control, to which no substitution has been made (0% DOC) and receive a diet composed of 1 kg of barley, 1 kg of straw, and of a mixture of minerals and vitamins (Table 1). The second and third groups received the same diet where barley was substituted with DOC at the amount of 20% and 40%, respectively (Table 1).

The rams were isolated from ewes and placed in individual pens in a well-ventilated covered building.

Diets Composition

The chemical compositions of the diets were determined (Table 1). The dry matter, organic matter, ash, crude protein, and ether extract were estimated in accordance with Association of Official Analytical Chemists (AOAC) methods (AOAC, 1997), whereas neutral and acid fiber fractions were assessed according to the method of Van Soest (Van Soest et al., 1991).

The DOC used in the current work was the crude obtained after crushing the whole fruit and extracting the olive by two phase centrifugations and then passing it through a sieve (3 mm) in a fresh form and spread on a plastic sheet for sun drying.

Scrotal and Testicular Measurements

Scrotal circumference (SC) (= scrotal perimeter), testicular length and thickness (right and left), was measured as described by Boussena et al. (2016). The SC was taken using a flexible plastic ribbon after pushing the testicle toward the depth of the scrotum. The length and thickness of the testicle (TL and TT) were taken using a digital caliper. All parameters were registered monthly before semen collection.

Semen Collection and Evaluation

Semen collection was done by electroejaculation technique because the rams were not trained to ejaculate into an artificial vagina. Ram semen was collected in a graduated tube by electro-ejaculator (ECe 320, Minitub, Tiefenbach, Germany), 2 weeks before the incorporation of DOC in the diet and at 0, 4, 8, and 12 weeks after its incorporation. Forthwith after ejaculation; volume (taken from the graduated tube), appearance, and consistency of semen were estimated. After this macroscopic evaluation, semen samples were diluted 1:1 in a pre-warmed (37°C) Tris-extender: 300 mM tris [hydroxymethyl] aminomethane, 95 mM citric acid monohydrate, 28 mM D-(+)-glucose (Mocé et al., 2010) and then transported to the laboratory of animal biotechnology at Biotechnology Research Center (CRBt, Constantine) within 30 minutes after recovery.

Sperm concentration, motility, membrane integrity, and LPO were investigated in semen samples. Sperm motility was evaluated by a computer-assisted sperm analyzer (CASA; Sperm class analyzer, SCA Microptic, S.L., Version3.2.0, Barcelona, Spain) as described by (Benhenia et al., 2018). Ten microliters of semen (diluted to 20×10^6 spermatozoa/mL in Tris-extender) sited in a pre-warmed Makler® chamber (Haifa, Israel) (37°C) were used for CASA motility analysis. Kinetic parameters were assessed at magnification ×10 for at least 200 spermatozoa (SPZ).

Those parameters were: total motility (TM, %), progressive motility (PM, %), curvilinear velocity (VCL, µm/s), straight linear velocity (VSL, µm/s), average path velocity (VAP, µm/s), linearity (LIN, %), straightness (STR, %), wobble (WOB, %), head amplitude of lateral movement (ALH, µm), and beat cross frequency (BCF, Hz). TM defines the sperm rate with VCL > 10 m/s, while PM is the percentage of sperm with VCL > 25 m/s and STR > 80% (Tamayo-Canul et al., 2011). Knowing that, TM defines the sperm rate with VCL > 10 m/s, while PM is the percentage of SPZ with VCL > 25 m/s and STR > 80% (Tamayo-Canul et al., 2011).

Table 1
Composition of Ram Diets (0DOC, 20DOC, 40DOC)

	Diets		
	0DOC	**20DOC**	**40DOC**
Mean body weight of rams (kg)	89 ($n=6$)	89.2 ($n=6$)	92 ($n=6$)
Diet composition			
Barley straw (kg/head/day)	1.00	1.00	1.00
Barley (kg/head/day)	1.00	0.80	0.60
DOC (kg/head/day)	0.00 (0%)	0.20 (20%)	0.40 (40%)
Minerals and vitamins mixture	2%	2%	2%
Dry matter intake (kg)	1.82	1.816	1.812
Chemical composition of diet			
DM (g/kg)	91.10	90.89	90.68
Organic matter (% of DM)	94.65	94.99	95.32
Crude protein (% of DS)	5.73	5.88	6.03
Grass matter (% of DM)	1.69	2.26	2.83
Mineral matter (% of DM)	3.05	3.35	3.65
Neutral detergent fiber (% of DM)	53.70	57.02	60.34
Acid detergent fiber(% of DM)	27.58	30.07	32.56
Cellulose (% of DM)	44.69	42.87	41.05
Acid detergent lignin (% of DM)	4.14	5.59	7.05

Note: DOC = destoned olive cake; DM = dry matter.

The hypo-osmotic swelling test (HOST) was used to determine the functional integrity of the sperm membrane. This HOST was performed by incubating 20 µL of sperm in 200 µL of 100 mOsM hypoosmotic solution (formed by 9 g of fructose and 4.9 g of sodium citrate in 1 L of distilled water at 37°C for 1 hour). Immediately after incubation, 10 µL of the mixture was spread on a hot slide under a coverslip (37°C) and observed at ×40 magnification. When the swollen and coiled tail of the SPZ has been observed, it indicates the SPZ has a functioning membrane.

Spermatozoa with a swollen and coiled tail were considered to have functional membranes, and so to calculate the percent of reacting SPZ, at least 200 SPZ must be taken into account (Revell & Mrode, 1993).

Sperm LPO was assessed by malondialdehyde (MDA) (final component of the LPO chain), which is quantified by thiobarbituric acid (TBA) assay (Buege & Aust, 1978). Thus, to the sonicated sperm (10^6 SPZ), 1 mL of TTH solution was added (15% trichloroacetic acid, (w/v) TBA 0.375%, and (w/v) in 0.25 N hydrochloric acid). The mixture formed was boiled for 1 hour and then cooled in an ice bath. After that, the suspension was centrifuged at 20,000 g for 15 minutes, and the supernatant was separated and measured under the absorbance of 532 nm at 25°C. The molar extinction coefficient for MDA is 1.56×10^5 $M^{-1}cm^{-1}$. The results are given in nmoles MDA/10^8 SPZ.

Blood Testosterone Level

Every month, blood samples were taken from the jugular vein in heparinized tubes. At once, the samples are placed on ice and transported to the laboratory for centrifugation at 3500g for 20 minutes. Determination of testosteronemia was obtained by using a testosterone Electrochemiluminescence kit (Roche Diagnostic, Mannheim, Germany; measuring range: 0.025–1.5 ng/mL).

Statistical Analyses

Statistical analysis was carried out using Statview V.4.02 software (Abacus Concepts Inc., Berkeley, CA, USA). Data were examined for normality. For data that were not normally distributed, logarithm and square-root transformations were used where appropriate. The effect of fixed factors, dietary group (0DOC, 20DOC, and 40DOC), month (time over which experiment was carried out, 5 months), and also interaction of diet and month, was analyzed using analysis of variance for repeated measurements. Within the same group, differences by months of reproductive parameters represent their monthly evolution. Values of each parameter were given as the mean ± standard error of the mean, and differences were examined using Fisher's test. $p < .05$ was considered significant.

Results

The differences in the results of reproductive parameters determined between the months within the same group represent the monthly evolution of each parameter by diet group.

Effect of Dietary Regimen on Scrotal Circumference

The data regarding the effect of dietary inclusion of olive cake on SC are shown in Figure 1. Scrotal circumference monthly evolution was significantly ($p \leq .02$) decreased in control and 20DOC and maintained in the 40DOC group. The results of overall means showed a significant ($p \leq .05$) improvement in SC of 40DOC compared to the control group.

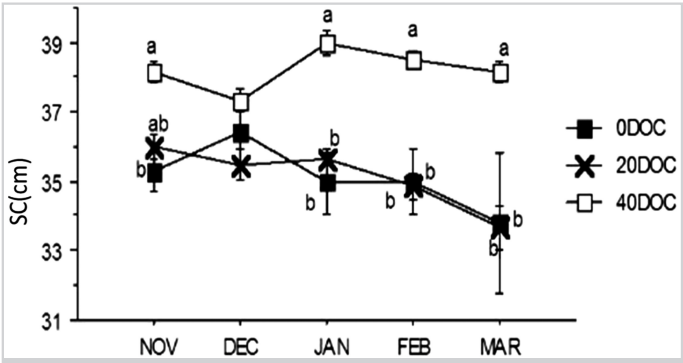

Figure 1
Mean Scrotal Circumference of Rams Received, During the Experimental Diets with 0% Destoned Olive Cake, 20% Destoned Olive Cake, and 40% Destoned Olive Cake. Different Superscripts Indicate Significant Differences ($p < .05$) Between Groups in the Same Month. DOC = Destoned Olive Cake; SC = Scrotal Circumference.

Effect of Dietary Regimen on Sperm Attributes and CASA Assessment Motility

The results of sperm concentration and volume are presented in Table 2. Regarding the volume of sperm, the 40DOC diet reveals a significant monthly progression ($p \leq .05$), inversely to the significant regression ($p \leq .05$) observed in the control. As for the sperm concentration, a significant monthly progression ($p \leq .05$) was observed in the 40DOC group. The overall means results indicated a significant improvement ($p \leq .001$) in sperm VOL of 40DOC compared to the control.

Total and progressive motility of ram sperm are shown in Figure 2. The results of TM and PM monthly kinetic revealed no significant effect of all diet groups, except a significant ($p \leq .05$) PM regression from November to January in the control group. No significant differences ($p \geq .05$) in overall means of both parameters were observed. Concerning the monthly evolution of sperm VCL, VSL, VAP, BCF, and ALH, significant ($p \leq .05$) progressions were observed in VSL of all groups and in LIN of 20DOC (DEC-JAN) ($p \leq .05$), while significant regressions ($p \leq .05$) were observed in ALH and VCL of the control group and in BCF of all groups (Table 2). No significant differences between all diet groups were noted in the overall means of all motility parameters (Table 2).

Effect of Dietary Regimen on Sperm Membrane Functionality

The values of membrane functionality (MF) are provided in Figure 3. The results of the evolution of plasma MF revealed that control diet had no significant effect on MF, while 20DOC and 40DOC had significant positive effects ($p \leq .04$; $p \leq .004$). The MF values of 40DOC were significantly ($p < .01$) increased in December. The overall results of MF revealed no significant differences between groups.

Effect of Dietary Regimen on Sperm Lipid Peroxidation

Regarding the evolution pattern of LPO, MDA, representing the degree of LPO was reduced in 20 and 40DOC group compared to the control, the significant ($p < .0001$) difference was registered in December in the 40DOC group (Figure 4). The overall results of LPO showed a significant ($p < .05$) lowest value of MDA in 40DOC group indicating high protection against LPO.

Table 2

Effect of Different Ram Diets (0DOC, 20DOC, 40DOC), Time (month), and Interaction Diet × Month on Sperm Attributes and Plasma Testosterone Level

	Volume (mL)	Concentration (SPZ $\times 10^9$)	VCL (µm/s)	VSL (µm/s)	ALH (µm)	BCF (Hz)	TESTO (ng/mL)
Overall	1.33 ± 0.06	2.04 ± 0.13	90.54 ± 2.64	57.40 ± 1.83	2.43 ± 0.23	6.96 ± 2.48	3.15 ± 0.13
Diet	*	NS	NS	NS	NS	NS	NS
0DOC	0.99 ± 0.08^{b}	1.80 ± 0.20^{a}	97.2 ± 2.64^{a}	55.1 ± 3.30^{a}	2.62 ± 0.25	7.14 ± 0.50^{a}	3.16 ± 0.25^{a}
20DOC	1.28 ± 0.10^{a,b}	1.67 ± 0.17^{a}	81.07 ± 3.13^{a}	53.70 ± 2.87^{a}	2.59 ± 0.61	7.32 ±0.46^{a}	3.72 ± 0.24^{a}
40DOC	1.67 ± 0.90^{a}	2.62 ± 0.25^{a}	94.40 ± 4.00^{a}	63.01 ± 3.13^{a}	2.11 ± 0.07	6.44 ± 0.44^{a}	2.58 ± 0.12^{a}
Month	NS	NS	**	***	***	***	**
November	1.44 ± 0.13^{a}	2.47 ± 0.30^{a}	104.27 ± 6.26^{a}	58.26 ± 4.57^{a,b}	2.66 ± 0.19^{a}	9.45 ± 1.75^{a}	3.31 ± 0.25^{a}
December	1.21 ± 0.12^{a}	1.81 ± 0.32^{a}	90.50 ± 7.20^{a}	49.27 ± 4.03^{b}	3.62 ± 1.07^{a,b}	8.20 ± 1.17^{a,b}	3.19 ± 0.35^{a}
January	1.27 ± 0.11^{a}	2.35 ± 0.18^{a}	68.85 ± 3.02^{b}	50.93 ± 3.51^{b}	1.98 ± 0.06^{b,c}	3.13 ± 0.84^{d}	2.24 ± 0.27^{b}
February	1.24 ± 0.17^{a}	1.83 ± 0.22^{a}	93.48 ± 4.27^{a}	59.37 ± 2.84^{a,b}	2.00 ± 0.09^{b,c}	6.29 ± 1.33^{c}	3.65 ± 0.24^{a}
March	1.50 ± 0.15^{a}	1.71 ± 0.37^{a}	95.58 ± 4.55^{a}	68.8 ± 3.83^{a}	1.88 ± 0.13^{c}	7.70 ± 0.74^{b}	3.36 ± 0.25^{a}
Month × Diet	***	***	***	***	***	***	***
0DOC × November	1.50 ± 0.24^{a,b,c}	2.39 ± 0.54^{a,b}	113.31 ± 16.3^{a,b}	56.32 ± 9.47^{a,b,c}	3.42 ± 0.38^{a}	9.48 ± 2.13^{a,b}	4.09 ± 0.48^{a}
0DOC × December	0.84 ± 0.10^{a,b,c,d}	1.49 ± 0.51^{a,b}	122.24 ± 11.56^{a}	55.45 ± 4.21^{a,b,c}	4.07 ± 0.74^{a}	8.49 ± 1.77^{a,b,c}	2.99 ± 0.49^{a,b,c}
0DOC × January	0.84 ± 0.18^{b,c,d}	2.55 ± 0.48^{a,b}	62.07 ± 4.92^{d}	38.16 ± 4.9^{c}	2.00 ± 0.12^{b}	3.51 ± 0.78^{f}	1.98 ± 0.50^{c,d}
0DOC × February	0.69 ± 0.08^{a,b}	1.68 ± 0.24^{a,b}	87.81 ± 6.88^{b,c,d}	55.37 ± 4.32^{a,b,c}	1.95 ± 0.09^{b}	6.07 ± 0.58^{d,e}	3.69 ± 0.53^{a,b}
0DOC × March	1.10 ± 0.17^{a}	0.90 ± 0.10^{b}	100.79 ± 10.7^{a,b,c}	70.20 ± 6.93^{a,b}	1.63 ± 0.09^{b}	8.15 ± 0.72^{b,cd}	3.04 ± 0.50^{a,b,c}
20DOC × November	1.25 ± 0.21^{a,b,c}	2.41 ± 0.58^{a,b}	88.98 ± 2.8^{b,c,d}	47.97 ± 4.50^{b,c}	2.42 ± 0.22^{b}	10.37 ± 1.57^{a}	3.34 ± 0.47^{a,b}
20DOC × December	1.33 ± 0.24^{c,d}	1.42 ± 0.26^{a,b}	67.15 ± 7.52^{d}	37.17 ± 8.24^{c}	5.06 ± 3.00^{b}	8.51 ± 0.75^{a,b,c}	3.99 ± 0.81^{a}
20DOC × January	1.58 ± 0.15^{c,d}	2.03 ± 0.25^{a,b}	73.12 ± 4.74^{c,d}	62.86 ± 5.52^{a,b}	1.93 ± 0.12^{b}	3.33 ± 0.71^{f}	3.09 ± 0.53$^{a,\,b,\,c}$
20DOC × February	1.08 ± 0.30^{d}	1.58 ± 0.28^{a,b}	89.80 ± 9.16^{b,c,d}	60.04 ± 5.90^{a,b,c}	0.24^{b}	6.92 ± 0.92^{c,d,e}	4.07 ± 0.51^{a}
20DOC × March	1.16 ± 0.21^{b,c,d}	0.90 ± 0.17^{b}	86.35 ± 5.39^{b,c,d}	60.44 ± 3.81^{a,b,c}	1.56 ± 0.13^{b}	7.46 ± 0.70^{b,c,d,e}	4.10 ± 0.42^{a}
40DOC × November	1.58 ± 0.27^{b,c,d}	2.59 ± 0.57^{a,b}	112.04 ± 9.86^{a,b}	70.17 ± 3.68^{a,b}	2.28 ± 0.22^{b}	8.52 ± 1.29^{a,b,c}	2.63 ± 0.13$^{b,\,c,\,d}$
40DOC × December	1.41 ± 0.20^{b,c,d}	2.62 ± 0.73^{a,b}	87.41 ± 6.7^{b,c,d}	56.21 ± 4.75^{a,b,c}	1.81 ± 0.14^{b}	7.65 ± 0.88^{b,c,d,e}	2.56 ± 0.32$^{b,\,c,\,d}$
40DOC × January	1.33 ± 0.16^{a,b,c}	2.50 ± 0.25^{a,b}	70.23 ± 5.70^{c,d}	49.65 ± 5.76^{b,c}	2.00 ± 0.09^{b}	2.61 ± 0.87^{f}	1.61 ± 0.06^{d}
40DOC × February	1.87 ± 0.21^{b,c,d}	2.20 ± 0.53^{a,b}	101.87 ± 5.25^{a,b,c}	63.06 ± 4.53^{a,b}	2.06 ± 0.08^{b}	5.84 ± 1.94^{e}	3.19 ± 0.05$^{a\,b,\,c}$
40DOC × March	2.18 ± 0.05^{b,c,d}	3.18 ± 0.27^{a}	100.45 ± 7.40^{a,b,c}	75.99 ± 7.85^{a}	2.40 ± 0.16^{b}	7.58 ± 0.75 ^{b,c,d,e}	2.90 ± 0.22$^{a,\,b,\,c,\,d}$

DOC = destoned olive cake; VCL = curvilinear velocity; VSL = straight linear velocity; ALH = amplitude of lateral movement of the head; BCF = beat cross frequency; TESTO = testosterone; SPZ = spermatozoa. Different superscripts a, b, c, d within the same column indicate significant differences, *$p < .05$, **$p < .01$, ***$p < .001$.

Effect of Dietary Regimen on Blood Testosterone Levels

The results of testosterone monthly evolution showed that a significant ($p \leq .01$) enhancement of testosterone from January to February for the control and 40DOC groups (Table 2). Testosterone overall means revealed no significant differences between groups (Table 2).

Discussion, Conclusion and Recommendations

The present study was undertaken to investigate the partial substitution of barley as an energetic source in the ram diet by a by-product olive cake and investigate the effects of this substitution on the scrotal circumference, sperm volume, sperm concentration, MF, LPO, and plasma testosterone levels. The latter parameters are the main indicators of ram fertility (Focşăneanu et al., 2014; Mohammed et al., 2018). In this study, the results revealed that DOC can substitute barley in the ram diet with some positive effects at 20% particularly on MF and LPO. The evident beneficial effects were observed at 40% on SC, MF, LPO, and TEST. These results could indicate an amelioration of ram fertility. More studies are required to determine the in vivo fertility of rams receiving a 40DOC diet.

In previous works, the impact of olive by-products was also investigated. In fact, El-Tarabany et al. (2018) revealed that incorporating olive cake up to 30% in the diet increased significantly the fertility of ewes. In addition, Dattilo and Congiu (1995) cited by Mioč et al. (2007) reported that an intake of 30% of olive cake in lamb concentrate results in higher daily weight gains than in those receiving a lower percentage or no dietary olive cake.

In the present study, the results indicated that DOC ensured the energetic needs of rams and there were components in DOC that may be responsible for the positive impact particularly at 40%. It was reported that the major components of olive cake oil are fatty acids,

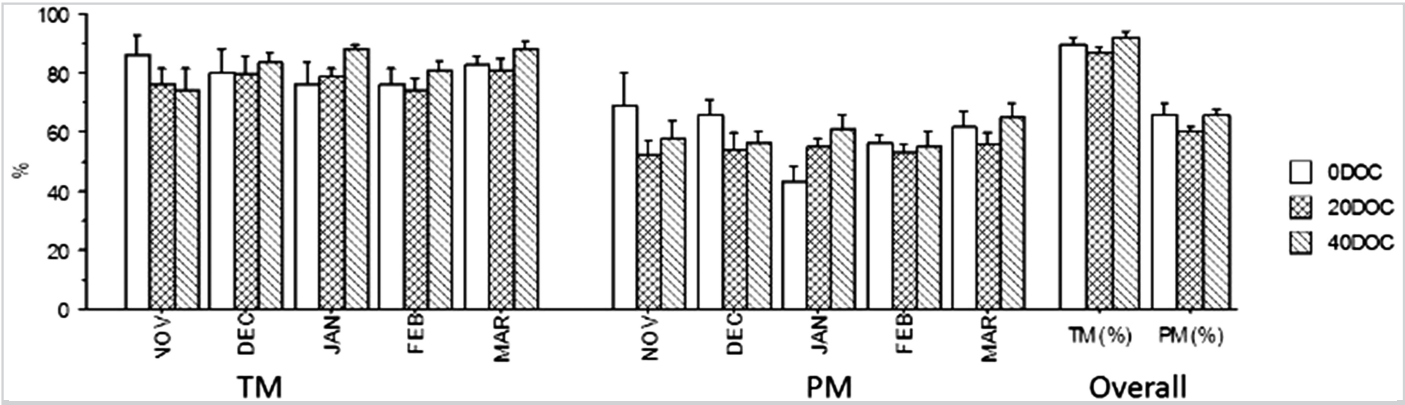

Figure 2
Mean Total Motility and Mean Progressive Motility of Sperm in Rams Received, During the Experimental Diets with 0% Destoned Olive Cake, 20% Destoned Olive Cake, and 40% Destoned Olive Cake. No Significant Differences (p < .05) Were Registered. DOC = Destoned Olive Cake; PM = Progressive Motility.

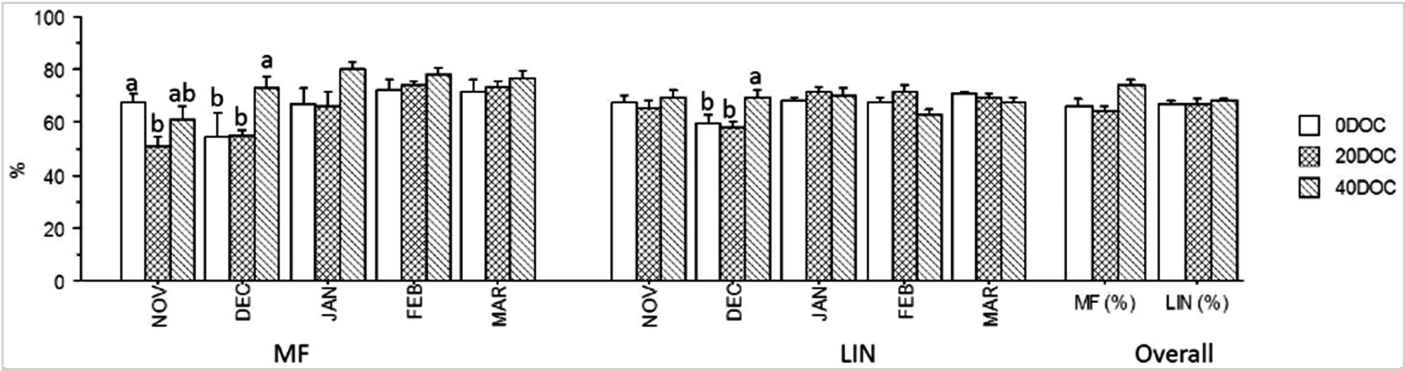

Figure 3
Mean Membrane Functionality and Mean Linearity of Sperm in Rams Received, During the Experimental Diets with 0% Destoned Olive Cake, 20% Destoned Olive Cake, and 40% Destoned Olive Cake. Different Superscripts Indicate Significant Differences, p < .05. DOC = Progressive Motility; MF = Membrane Functionality; LIN = Mean Linearity.

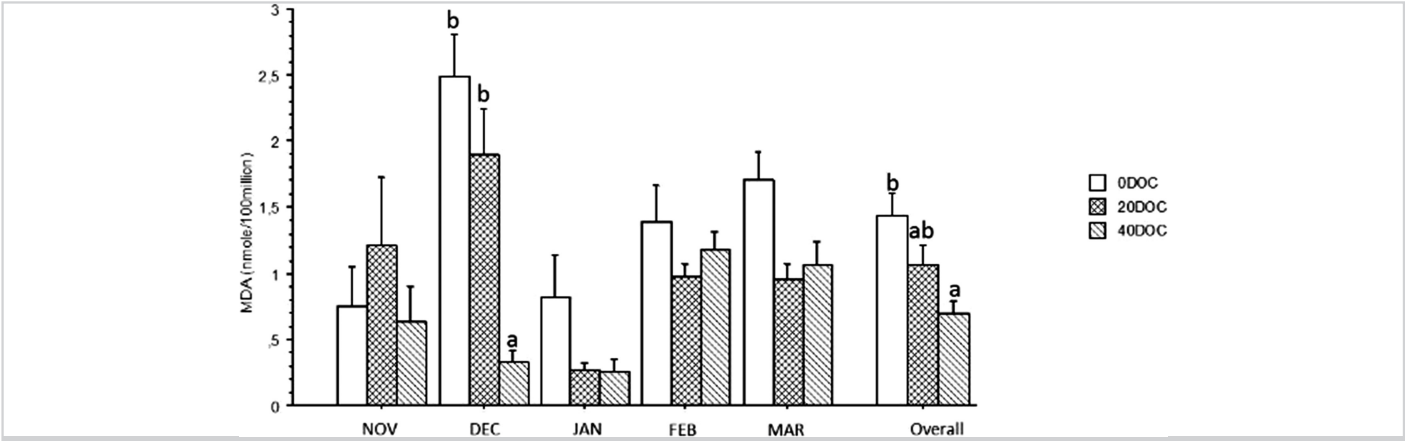

Figure 4
Mean Sperm Malondialdehyde of Rams Received, During the Experimental Diets with 0% Destoned Olive Cake, 20% Destoned Olive Cake, and 40% Destoned Olive Cake. Different Superscripts Indicate Significant Differences, p < .05. DOC = Destoned Olive Cake; MDA = Malondialdehyde.

particularly oleic, and linoleic acids (Chiofalo et al., 2004). These fatty acids could be implicated in the positive impact of olive cake on reproductive parameters, studied in our experimentation. In this context, Blache et al. (2002) and Selvaraju et al. (2012) supposed that the energetic compounds of the diet, especially fatty acids, play a key role in reproduction responses to changes in nutrition. In addition, Banihani (2017) discussed and summarized the relationship between fatty acid contents in olive oil and sperm quality by reviewing articles and reports from 1975 to 2017. He concluded that the preservation of sperm quality by olive oil is due to the improvement of gonadal function and the reduction in the degree of LPO and hence oxidative damage. In our study, the positive impact of 40DOC on MF, TEST level, sperm VOL, and LPO indicated an improvement of gonadal function that could explain the increase in the scrotal circumference.

In the current study, the significant positive effect of 40DOC on sperm quality, particularly LIN, MF, and MDA, was observed in December. This maybe due to both the weather conditions in December characterized by the shorter day length and the period of 4 weeks of receiving of 40DOC (NOV-DEC). In this respect, it is shown that short days can stimulate sexual activity in Ouled Djellal rams (Belkhiri et al., 2017; Benia et al., 2018; Esmaeili et al., 2012), and the positive effect of dietary fat supplementation may be appearing 4–8 weeks after supplementation (Esmaeili et al., 2012; Matini Behzad et al., 2014).

Knowing that oxidative stress, in particular LPO, constitutes the most important cause of disturbance of the plasma membrane of sperm and therefore a reduction in the quality and fertility of sperm (Guthrie & Welch, 2012; Wathes et al., 2007), the ram sperm is very sensitive to the LPO because of its high level in polyunsaturated fatty acids (PUFAs) (Aitken et al., 2007). In the present study, DOC, particularly at 40%, has protected ram sperm against LPO. This may be due to one or many components of olive cake. Indeed, it was reported that DOC contains antioxidants such as polyphenols and vitamin E (Antónia Nunes et al., 2018; Mojerlou & Elhamirad, 2018). These antioxidants may be involved in reducing LPO in the DOC groups. It was also revealed that monounsaturated fatty acids, particularly oleic and linoleic acids, the major components of DOC (Chiofalo et al., 2004), are less susceptible to LPO compared to poly-unsaturated fatty acids (Antónia Nunes et al., 2018; Taticchi et al., 2017). In this study, the protection against LPO may be attributed to the insert into the plasma membrane of monounsaturated fatty acids. In this context, Faraji et al. (2012) revealed that feeding olive-pulped silage enhances the quantity of unsaturated fatty acids in the plasma membrane of the sperm.

In our study, the significant protection against LPO and improvement of membrane integrity and functionality were observed 2 months after receiving DOC. In rams, this time corresponds to the spermatogenesis period during which the changes in sperm plasma membrane lipid composition occur (Esmaeili et al., 2012; Samadian et al., 2010). Further studies are required to identify if the composition of sperm plasma membrane would change after receiving olive cake in the diet.

Testosterone is the main hormone involved in male reproductive performance, particularly in sperm quality (Kishk, 2008). In the testis, testosterone is produced by Leydig cells and acts as a paracrine factor that diffuses into the seminiferous tubules to regulate spermatogenesis (Smith & Walker, 2014). Also, this steroid hormone diffuses into blood vessels to gain blood circulation (Smith & Walker, 2014). It was reported that the mean blood testosterone concentration in Ouled Djellal rams was between 1.75 ng/mL and 4.50 ng/mL (Taherti et al., 2014). Similarly, the overall blood testosterone levels of control and DOC, in the present experimentation, were between 2.24 ng/mL and 3.65 ng/mL indicating that DOC had no negative effect on plasma testosterone levels. In addition, a similar pattern of monthly evolution of control and 40DOC was also observed with high concentrations in January. In Ouled Djellal breed, many studies reported that the main factor that influenced monthly variation of testosterone is the season (Allaoui et al., 2018; Belkadi et al., 2017; Belkhiri et al., 2017). In our study, the season could be implicated in the monthly fluctuation of testosterone.

In conclusion, referring to the results obtained in this study, it might be concluded that the inclusion of 40% DOC into a concentrate-based diet of Ouled Djellal ram improves their reproductive performances after 2 months of feeding. This finding could be exploited in the sheep livestock for both reducing feeding costs and improving reproductive performances of farm animals.

Animal Welfare Statement

The protocols and procedures applied in this study were in accordance with ethics decreed by the Institutional Animal Care Committee of the National Administration of the Algerian Higher Education and Scientific Research (Ethical approval number: 98-11, Law of August 22, 1998) under the supervision of the National Council for Bioethics and Good Practical Animal Usage in Scientific Experimentations.

Ethics Committee Approval: This study was approved by Ethics committee of Institutional Animal Care Committee of the National Administration of the Algerian Higher Education and Scientific Research, (Date: August 22, 1998, Approval No: 98-11).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – B.S, N.H.; Design – B.S., N.H.; Supervision – K.B.,B.S.; Resources – N.H., K.B.; Materials – R.A., H.R.; Data Collection and/or Processing – H.R., R.A., N.H.; Analysis and/or Interpretation – N.H., K.B.; Literature Search – N.H., K.B.; Writing Manuscript – K.B., N.H.; Critical Review – B.S., K.B.

Acknowledgment: The authors are thankful to Prof Omar BOUAZIZ (director of *Laboratoire de Gestion de la Santé et Productions Animales*, Institut des Sciences Vétérinaires, Université des Frères Mentouri, Constantine) for providing electro-ejaculator and to the director and staff of the pilot Farm Belgacem BAAZIZ for providing rams that were included in this study.

Declaration of Interests: The authors declared that they have no conflict of interest.

Funding: The authors declared that this study has received no financial support.

References

Abdelguerfi, A., Laouar, M., & M'Hammedi Bouzina, M. (2008). Les production fourragères et pastorales. En *Algérie: Situation et Possibilités d'Amélioration. Revue Semestrielle, Agriculture & développement (INVA, Alger)*, 6:14–25.

Aitken, R. J., Wingate, J. K., De Iuliis, G. N., & McLaughlin, E. A. (2007). Analysis of lipid peroxidation in human spermatozoa using BODIPY C11. *Molecular Human Reproduction*, *13*(4), 203–211. [CrossRef]

Allaoui, A., Safsaf, B., Tlidjane, M., Djaalab, I., & Mansour, H. D. (2018). Effect of increasing levels of wasted date palm in concentrate diet on reproductive performance of Ouled Djellal breeding rams during flushing period. *Veterinary World*, *11*(5), 712–719. [CrossRef]

Antónia Nunes, M., Costa, A. S. G., Bessada, S., Santos, J., Puga, H., Alves, R. C., Freitas, V., & Oliveira, M. B. P. P. (2018). Olive pomace as a valuable source of bioactive compounds: A study regarding its lipid- and water-soluble components. *Science of the Total Environment*, *644*, 229–236. [CrossRef]

AOAC. (1997). *Official methods of analysis* (16th ed). Washington, DC: Association of Official Analytical Chemists.

Banihani, S. A. (2017). Semen quality as affected by olive oil. *International Journal of Food Properties*, *20*(2), 1–6. [CrossRef]

Bencherif, S. (2013). L'élevage agropastoral de la steppe algérienne dans la tourmente : enquêtes et perspectives de développement. *Mondes En Développement*, (161), 93–106. https://doi.org/10.3917/med.161.0093. Retrieved from https://www.cairn.info/revue-mondes-en-developpement-2013-1-page-93.htm.

Belkadi, S., Safsaf, B., Heleili, N., Tlidjane, M., Belkacem, L., & Oucheriah, Y. (2017). Seasonal influence on sperm parameters, scrotal measurements, and serum testosterone in Ouled Djellal breed rams in Algeria. *Veterinary World*, *10*(12), 1486–1492. [CrossRef]

Belkhiri, Y., Bouzebda-Afri, F., Bouzebda, Z., & Mouffok, C. (2017). Age and season effects on sexual parameters in mature rams used in Artificial Insemination Centre (Algeria). *Global Veterinaria*, *18*(1), 31–40.

Benbati, M., El Fatmi, A., Benjelloun, B., Mounsif, M., & Keli, A. (2016). Effet de l'incorporation des grignons d'olive dans la ration des brebis en lactation sur les performances de leurs agneaux avant et après sevrage. In M. Napoléone, H. Ben Salem, J. P. Boutonnet, A. López-Francos, & D. Gabiña (Eds.), *The value chains of Mediterranean sheep and goat products. Organisation of the industry, marketing strategies, feeding and production systems*. Zaragoza : CIHEAM, p. 231–236 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 115).

Benhenia, K., Rahab, H., Smadi, M. A., Benmakhlouf, H., Lamara, A., Idres, T., & Iguer-Ouada, M. (2018). Beneficial and harmful effects of cyclodextrin-vitamin E complex on cryopreserved ram sperm. *Animal Reproduction Science*, *195*, 266–273. [CrossRef]

Benia, A. R., Saadi, M. A., Ait-Amrane, A., Belhamiti, T. B., Selles, S. M. A., & Kaidi, R. (2018). Effect of season and age on main characteristics of sperm production in the Ouled-Djellal rams. *Livestock Research for Rural Development*, *30*(1), Article #10. Retrieved from http://www.lrrd.org/lrrd30/1/arbv30010.html.

Blache, D., Adam, C. L., & Martin, G. B. (2002). The mature male sheep: a model to study the effects of nutrition on the reproductive axis. *Reproduction (Cambridge, England) Supplement*, *59*, 219–233.

Bourbouze, A. (2000). Pastoralisme au Maghreb: La révolution silencieuse. *Fourrages*, *161*, 3–21.

Boussena, S., Bouaziz, O., Hireche, S., Derqaoui, L., Dib, A., & Moula, N. (2016). Apparition de la puberté chez les agneaux mâles de race Ouled Djellal. *Revue de Médecine Vétérinaire*, *167*, 274–282.

Branciari, R., Ranucci, D., Miraglia, D., Urbani, S., Esposto, S., & Servili, M. (2015). Effect of dietary treatment with olive oil by-product (olive cake) on physico-chemical, sensory and microbial characteristics of beef during storage. *Italian Journal of Food Safety*, *4*(4), 5496. [CrossRef]

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, *52*, 302–310. [CrossRef]

Chiofalo, B., Liotta, L., Zumbo, A., & Chiofalo, V. (2004). Administration of olive cake for ewe feeding. Effect on milk yield and composition. *Small Ruminant Research*, *55*(1–3), 169–176. [CrossRef]

Dattilo, M., & Congiu, F. (1995). Effects of olive cake on the productivity of sheep and the amino acid composition of their meat. In *Proceedings of the 7th international symposium. Protein metabolism and nutrition*, *81* (pp. 477–482). Portugal: Vale de Santarem.

Djaalab, I., Bouaziz, O., Lakhdara, N., Djaalab, H., Haffaf, S., & Allaoui, A. (2016). Effect of the ratio of incorporation of the date wastes at the end of gestation on the blood biochemical parameters in Oueld Djellal ewes. *Archives of Applied Science Research*, *8*(7), 22–26.

Dorbane, Z., Kadi, S. A., Boudouma, D., Berchiche, M., Bannelier, C., & Gidenne, T. (2016). Nutritive value of crude olive cake (*Olea europaea L.*) for growing rabbit. In *11th World Rabbit Congress* (pp. 381–384). France: Kingdao. Retrieved from https://hal.archives-ouvertes.fr/hal-01743022.

El-Tarabany, A., Mostafa, M., & Mohamed, A. (2018). Effect of dietary olive cake on reproductive and physiological traits of native pregnant ewes. *Arab Journal of Nuclear Sciences and Applications*, *51*, 1–8. [CrossRef]

Esmaeili, V., Shahverdi, A. H., Alizadeh, A. R., Alipour, H., & Chehrazi, M. (2012). Saturated, omega-6 and omega-3 dietary fatty acid effects on the characteristics of fresh, frozen- thawed semen and blood parameters in rams. *Andrologia*, *46*(1), 42–49. [CrossRef]

Faraji, Z., Zamiri, M. J., Rowghani, E., Akhlaghi, A., Jafarzadeh, M. R., Bayat, A. R., & Gholami, M. (2012). Effect of feeding olive-pulp silage on the seminal characteristics and fatty acid profile of the sperm plasma membrane in Iranian fat-tailed sheep. *Small Ruminant Research*, *105*(1–3), 216–221. [CrossRef]

Focşăneanu, V., Bogdan, L., Andrei, S., Bogdan, S., & Blaga Petrean, A. (2014). Performance of some variables used as a procedure for estimating sexual capacity (fertility) of the ram. *Bulletin UASVM Veterinary Medicine*, *71*(1), 52–58.

Guthrie, H. D., & Welch, G. R. (2012). Effects of reactive oxygen species on sperm function. *Theriogenology*, *78*(8), 1700–1708. [CrossRef]

Harkat, S., Laoun, A., Benali, R., Outayeb, D., Ferrouk, M., Maftah, A., Da Silva, A., & Lafri, M. (2015). Phenotypic characterization of the major sheep breed in Algeria. *Revue de Medecine Veterinaire*, *166*(5–6), 138–147.

Kadi, S. A., Belaidi-Gater, N., & Chebat, F. (2004). Inclusion of crude olive cake in growing rabbit's diet: effect on growth and slaughter yield. In *9th world rabbit congress*, Sep 2004. Puebla, Mexico. Hall-01743219.

Kishk, W. (2008). Interrelationship between ram plasma testosterone level and some semen characteristics. *Slovak Journal of Animal Science*, *41*(2), 67–71.

MADR (Ministry of Agriculture and Rural Development). (2014). *Agricultural statistics (areas and productions Serie B)*. Algeria, Ministry of Agriculture and Rural Development.

Matini Behzad, A., Ebrahimi, B., Alizadeh, A. R., Esmaeili, V., Dalman, A., Rashki, L., & Shahverdi, A. H. (2014). Improvement in *in vitro* fertilization rate, decrease in reactive oxygen species and spermatozoa death Incidence in rams by dietary fish oil. *Reproduction in Domestic Animals*, *49*(4), 599–605. [CrossRef]

Mebirouk-Boudechiche, L., Bouhedja, N., Boudechiche, L., & Miroud, K. (2015). Essai d'une complémentation alimentaire au flushing et au steaming de brebis Ouled Djellal par la caroube. *Archivos de Zootecnia*, *64*(248), 449–455. [CrossRef]

Mioč, B., Pavić, V., Vnučec, I., Prpić, Z., Kostelić, A., & Sušić, V. (2007). Effect of olive cake on daily gain, carcass characteristics and chemical composition of lamb meat. *Czech Journal of Animal Science*, *52*(2), 31–36. [CrossRef]

Mocé, E., Blanch, E., Tomás, C., & Graham, J. K. (2010). Use of cholesterol in sperm cryopreservation. Present moment and perspectives to future. *Repro*, *45*(2), 57–66. [CrossRef]

Mohammed, O. A., Muhyideen, K. A., Samuel, F. U., Lawal, M., Bojuwoye, O., Hassan, Z., & Chom, N. S. (2018). Relationship between testicular and epididymal biometry compared to semen quality in Yankasa rams. *Nigerian Veterinary Journal*, *39*(2), 115–123. [CrossRef]

Mojerlou, Z., & Elhamirad, A. (2018). Optimization of ultrasound-assisted extraction (UAE) of phenolic compounds from olive cake. *Journal of Food Science and Technology*, *55*(3), 977–984. [CrossRef]

Mouhous, A. (2007). Feeding of the herds of the steppe zones. Case of the zone of Hadj Mechri (Area of Laghouat), Algeria. *Livestock Research for Rural Development*, *19*(2), 1–10.

Nefzaoui, A. (1991). Valorisation des sous-produits de l'olivier. In J.-L., Tisserand, & X. Alibés (Eds.). *Fourrages et sous-produits méditerranéens*. Zaragoza : CIHEAM, 1991. p. 101–108. (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 16). Retrieved from http://om.ciheam.org/om/pdf/a16/91605051.pdf.

Revell, S. G., & Mrode, R. A. (1993). An osmotic resistance test for bovine semen. *Animal Reproduction Science*, *36*, 77–86. [CrossRef]

Rondia, P. (2006). Aperçu de l'élevage ovin en Afrique du Nord. *Filière Ovine Caprine*, *18*, 11–14.

Sadeghi, H., Teimouri Ynsari, A. and Ansari-pirsarai, Z., (2009). Effects of different olive cake by products on dry matter intake, nutrient digestibility and performance of Zel sheep. Int. J. Agric. Biol., 11: 39–43.

Samadian, F., Towhidi, A., Rezayazdi, K., & Bahreini, M. (2010). Effects of dietary n-3 fatty acids on characteristics and lipid composition of ovine sperm. *Animal*, *4*(12), 2017–2022. [CrossRef]

Selvaraju, S., Raju, P., Rao, S. B. N., Raghavendra, S., Nandi, S., Dineshkumar, D., Thayakumar, A., Parthipan, S., & Ravindra, J. P. (2012). Evaluation of maize grain and polyunsaturated fatty acid (PUFA) as energy sources for breeding rams based on hormonal, sperm functional parameters and fertility. *Reproduction, Fertility, and Development*, *24*(5), 669–678. [CrossRef]

Smith, L. B., & Walker, W. H. (2014). The regulation of spermatogenesis by androgens. *Seminars in Cell and Developmental Biology*, *30*, 2–13. [CrossRef]

Terramoccia, S., Bartocci, S.,Taticchi,A.,Di Giovanni, S., Pauselli, M., Mourvaki, E., urbani, S., & Servili, M. (2013). Use of dried stoned olive pomace in the feeding of lactating buffaloes. Effect on the quantity and quality of the milk produced.. *Asian-Australasian Journal of Animal Sciences*, *26*, 971–980. [CrossRef]

Taherti, M., Zidane, K., Aggad, H., & Kaidi, R. (2014). Sexual activity of the ram Ouled Djellal bred raised in the region of Chlef. *International Journal of Sciences: Basic and Applied Research (IJSBAR)*, *17*(2), 283–287.

Tamayo-Canul, J., Alvarez, M., López-Urueña, E., Nicolas, M., Martinez-Pastor, F., Anel, E., Anel, L., & De Paz, P. (2011). Undiluted or extended storage of ram epididymal spermatozoa as alternatives to refrigerating the whole epididymes. *Animal Reproduction Science*, *126*(1–2), 76–82. [CrossRef]

Taticchi, A., Bartocci, S., Servili, M., Di Giovanni, S., Pauselli, M., Mourvaki, E., Zilio, D. M., & Terramoccia, S. (2017). Effect on quanti-quality milk and mozzarella cheese characteristics with further increasing the level of dried stoned olive pomace in diet for lactating buffalo. *Asian-Australasian Journal of Animal Sciences*, *30*(11), 1605–1611. [CrossRef]

Terramoccia, S., Bartocci, S., Taticchi, A., Di Giovanni, S., Pauselli, M., Mourvaki, E., Urbani, S., & Servili, M. (2013). Use of dried stoned olive pomace in the feeding of lactating buffaloes. Effect on the quantity and quality of the milk produced. *Asian-Australasian Journal of Animal Sciences*, *26*, 971–980. [CrossRef]

Tran, L. V., Malla, B. A., Sharma, A. N., Kumar, S., Tyagi, N., & Tyagi, A. K. (2016). Effect of omega-3 and omega-6 polyunsaturated fatty acid enriched diet on plasma IGF-1 and testosterone concentration, puberty and semen quality in male buffalo. *Animal Reproduction Science*, *173*, 63–72. [CrossRef]

Tufarelli, V., Introna, M., Cazzato, E., Mazzei, D., & Laudadio, V. (2013). Suitability of partly destoned exhausted olive cake as by-product feed ingredient for lamb production. *Journal of Animal Science*, *91*(2), 872–877. [CrossRef]

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583–3597. [CrossRef]

Vargas-Bello-Pérez, E., Vera, R. R., Aguilar, C., Lira, R., Peña, I., & Fernández, J. (2013). Feeding olive cake to ewes improves fatty acid profile of milk and cheese. *Animal Feed Science and Technology*, *184*(1–4), 94–99. [CrossRef]

Wathes, D. C., Abayasekara, D. R. E., & Aitken, R. J. (2007). Polyunsaturated fatty acids in male and female reproduction. *Biology of Reproduction*, *77*(2), 190–201. [CrossRef]