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EL-MADAWY, Azza
Sheep and Goat Research Department, Animal Production Research Institute, Agricultural Research Center

EL-SHARAWY, Mohamed
Animal Production Department, Faculty of Agriculture, Kafrelsheikh University

ALI, Mohamed
Animal Production Department, Faculty of Agriculture, Kafrelsheikh University

HAFEZ, Youssef
Sheep and Goat Research Department, Animal Production Research Institute, Agricultural Research Center

他

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Effect of Unsaturated Fatty Acids Supplementation on Productive and Reproductive Performance of Ram Lambs

Azza EL–MADAWY[1], Mohamed EL–SHARAWY[2], Mohamed ALI[2], Youssef HAFEZ[1], Nobuhiko YAMAUCHI[3] and Abd El–Salam METWALLY[2]*

Laboratory of Reproductive Physiology and Biotechnology, Department of Animal and Marine Bioresource Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 819–0395, Japan
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The objective of this study was to determine the effect of dietary unsaturated fatty acids supplementation on productive and reproductive performance of ram lambs. In this study, 15 crossbred ram lambs (½Finnish × ½Rhamni) at 7 months of age with an average live body weight of 24.3±1.4 kg were used and divided into three groups (5 lambs each). The first group was fed the control diet (G1). While, fish oil was added to the control diet at level of 1.5 and 3% of total dry matter (DM) intake for G2 and G3, respectively. Results indicated that the average live body weight of ram lambs at the end of the experimental period was 34.2; 37 and 37 kg for G1(control), G2 and G3, respectively ($P<0.05$). Also, average daily gain of ram lambs was the highest level ($P<0.05$) in G3 (93.3 g/day) followed by G2 (90.3 g/day) and G1 (73.3 g/day). The scrotal circumferences of ram lambs significantly increased ($P<0.05$) in G2 and G3 (27.0 and 27.3 cm, respectively) by given dietary fish oil than control diet (24.5 cm). Body growth weight in ram lambs at 1st ejaculation was 33.4, 36.0 and 35.4 kg for control, G2 and G3, respectively ($P<0.05$). Testosterone concentration of ram lambs was recorded the highest level ($P<0.05$) in G3 (5.028 ng/ml) followed by G2 (2.542 ng/ml) and G1 (0.664 ng/ml). In conclusion, addition of fish oil at rate of 1.5 and 3% to growing lambs improved semen characteristics and had beneficial effects on productive and reproductive performances.

Key words: Ram Lambs; Semen Characteristics; Fish oil; Growth performances

INTRODUCTION

Among nutrients, lipids have a crucial role in male fertility since they can be consumed as a source of energy and additionally they are critical components of spermatozoa membranes (Santos *et al*., 2008). Fatty acids such as n–3 and n–6 polyunsaturated fatty acids (PUFA) are critical nutrients, used to improve male reproductive performance through modification of fatty acid profile and maintenance of sperm membrane integrity, especially under cold shock or cryopreservation condition. Also, PUFA provide the precursors for prostaglandin synthesis and can modulate the expression patterns of many key enzymes involved in both prostaglandin and steroid metabolism (Tran *et al*., 2017).

Animals are unable to synthesize of the n–3 and n–6 fatty acids in their body as they do not have sufficient amount of fatty acid denaturize enzymes, therefore these fatty acids need to be taken by dietary sources (Wathes *et al*. 2007). The Semen quality plays a vital role in bringing higher farm economic profit through the enhancement of conception rate and total annual number of animals born for each herd and it is influenced by many factors such as genetics, management, environment and nutrition (Khoshvaght *et al*., 2016). There is emerging evidence that dietary polyunsaturated fatty acids (PUFA) may act as specific regulators of some reproductive processes through modification of fatty acid profile and maintenance of sperm membrane integrity, especially under cold shock or cryopreservation condition (Moallem *et al*., 2015). The fatty acid composition, metabolism of spermatozoa and Sertoli of the sperm plasma membrane are the major determinant of the mobility characteristics and viability (Hammerstedt *et al*., 1990). PUFAs account for nearly 60% of the phospholipids that bind in the total fatty acid of cells and in sperm (Lenzi *et al*., 1996 and Castellano *et al*., 2011). In particular, the diets that rich in omega–3 or omega–6 affect phospholipid fatty acid composition; maintaining sperm membrane fluidity and motility, assembling enzymes involved in mammalian sperm–egg interaction, gives the resistance to physicochemical modifications of the head during capacitation and fertilization (Rooke *et al*., 2001; Safarinejad *et al*., 2010).

In rams, omega–3 has a better effect on sperm quality compared to the sources of saturated fatty acids and omega–6 for improving the sperm quality of post–thaw ram semen, due to the effective incorporation of DHA into cell membrane phospholipids and consequent cold shock prevention (Esmeaili *et al*., 2014). Testis development and spermatogenesis depend on many gonadal hormones, so the prostaglandin synthesis and steroidogenesis via the direct influence on steroid acute regulator (StAR) and cytochrome P450, which play critical roles in regulating steroid synthesis (Gulliver *et al*., 2012 and Feng *et al*., 2015).

[1] Sheep and Goat Research Department, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt
[2] Animal Production Department, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
[3] Department of Animal and Marine Bioresource Science, Faculty of Agriculture, Kyushu University, Fukuoka 819–0395, Japan
* Correspondence author: Abd El–Salam M. METWALLY (E–mail: metwally_un@yahoo.com)

The objective of this study was to investigate the influence of fish oil on some productive and reproductive performance of ram lambs.

MATERIALS AND METHODS

This study was conducted at Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Egypt and the experimental work was conducted at Sakha Experimental Station, Animal Production Research Institute, Ministry of Agriculture from September, 2017 to June, 2018.

Animals and Management

At seven month of age, 15 crossbred ram lambs (½Finnish × ½Rhamni) with an average live body weight of 24.3±1.4 kg were randomly divided into three groups (5 from each group). Male lambs were fed separately in group feeding. The first group (control) was fed a basal diet consisting of concentrate fed mixture (CFM) plus berseem hay, while in group 2 and 3, fish oil was added to the basal diet at the rate of 1.5 and 3% of total DM intake, respectively. All lambs were weight at biweekly intervals till the end of the experimental period and daily gain was calculated. Feed intake was adjusted every two weeks according to the changes in animal body weight status according to NRC (1985). Feedstuffs were offered in two almost equal meals at 8.0 am and 4.0 pm. Fresh water was available for all the day round.

Blood samples

Blood samples were collected from 5 lambs for each group. Blood sampling started from 7 to 16 months of age every two weeks during the experimental period. All blood samples were collected from the jugular vein into lithium heparinized vacutainer tubes. Tubes were immediately centrifuged for 15 min. at 3000 rpm. Plasma was harvested and stored at –20°C for later analysis.

Reproductive performance

Male ram lambs in the experimental three groups were subjected to detect changes in sexual behavior, once every 10 days interval during the period from 7 months of age till the onset puberty (first successful ejaculate with motile sperm). To ensure the availability of at least two ewes in estrus at each time of libido test, five ewes were subjected to estrus synchronization by intramuscular injection of 25 mg progesterone (Lutone, Misr Co., for Pharm Ind. SAA, Cairo) for five successive days followed by a signal injection of 5 mg estradiol benzoate (Folone, Misr Co, for Pharm Ind. SAA, Cairo) 24 hours after the last progesterone injection. Treated ewes were subjected to estrus detection 24– 48 hours after last hormonal injection using intact ram. Treatment for estrus synchronization was planned at a time suitable for the time of libido test. Age, weight, scrotal circumference and testosterone concentration were determined at 1st mounting, mounting with erection (1st penile protrusion) and puberty.

Scrotal circumference

Scrotal circumference was measured and recorded once every two weeks from 7 up to 16 months of age according to Hahn *et al.* (1969), scrotal circumference was measured for each ram lambs by a flexible plastic tape around the greatest diameter of the scrotum.

Semen collection and quality evaluation

Semen was collected by using an artificial vagina once per week at puberty. During the time of collection, ram lambs were sexually stimulated by allowing two false mounts followed by 5 minutes restrain. After collection semen was maintained at 37°C and immediately transferred to the laboratory for semen evaluation which the following parameters: Semen volume was measured directly in milliliter to the nearest of 0.1 ml using a graduated collecting tube. Sperm motility was assayed directly after collection by placing a drop of fresh semen on a microscopic slide covered with a cover slip and examined under a high power microscope at a magnification× 200. During examination the temperature was held at 37°C by means of a stage warmer attached to the microscope. Initial gross motility of raw semen (without extension) was estimated on a percentage score basis as described by Melrose and Laing (1970).

Percentage of live spermatozoa was estimated by counting one hundred sperm cells. Percentage of abnormal spermatozoa was determined according to Hancock (1951) in the same smears prepared for live and dead sperm test two hundred spermatozoa were counted in each smear for abnormal sperm count.

Sperm cell concentration ($\times 10^9$ml) was counted microscopically using Neubauer Haemocytometer after a 1:200 dilutions with a 3% (w/v) Nacl solution. The total sperm output (volume ×concentration) per ejaculate was calculated by multiplying sperm cell concentration by ejaculate volume.

Testosterone concentration (ng/ml)

Testosterone assay was conducted by radioimmunoassay (RIA) method. Direct RIA technique was performed for serum testosterone determination. Pantex 333 kits (1125) were used to measure the level of testosterone. Type of testosterone assayed were (a) total testosterone (direct, extraction, coated tubes) and (b) free testosterone. It is well known that total testosterone in plasma include free testosterone and that bound to 1pound to sex steroid hormone binding globulin (SHBG), albumin corticosteroid binding globulin (GBG). According to the instructions of the producing company (pantex, Santa Monica) the solvents used in this assay break the protein binding during extraction process. The standard curve of testosterone ranged between 0.1 and 25.6 ng/ml.

Statistical Analysis

Data obtained in this study were subjected to statistical analysis using One–way adapted by software (SPSS, version 20.0) for user's guide. Duncan (1955) Multiple range test of SPSS programmer was done to determine

the degree of significance among the means at ($P<0.05$).

RESTLTS AND DISCUSSION

Effect of fish oil addition on ram lamb's productive performance:

Average live body weight of growing lambs:

Average live body weight of ram lambs at successive months of the experimental period as affected by fish oil addition are presented in Table (1).

The present results revealed that the average monthly live body weight of all experimental groups was tended to be gradually increased with advancing animals ages (Table, 1). However, it is of interest to note that the value of live body weight of group 2 and 3 were tended to be significantly higher ($P<0.05$) than the control group through all measuring time.

Table 1. Average live body weight (kg) of ram lambs fed the different experimental diets

Age (month)	Live body weight (kg)		
	Group 1 (Control)	Group 2	Group 3
7	24.2±2.7	24.2±1.4	24.6±3.5
8	26.4±2.8	27.4±1.8	27.8±3.4
9	29.0±3.0	30.2±2.6	30.8±3.2
10	31.0±3.0	33.6±2.4	32.8±3.8
11	33.4±2.4^{b}	36.0±2.6^{a}	35.4±3.5^{a}
12	35.0±2.7^{b}	38.4±2.2^{a}	38.4±4.2^{a}
13	37.4±2.4^{b}	41.0±2.3^{a}	40.8±3.9^{a}
14	40.0±2.3^{b}	43.8±2.7^{a}	43.6±4.0^{a}
15	42.2±2.0^{b}	46.8±2.6^{a}	46.4±4.1^{a}
16	44.0±2.2^{b}	48.6±2.3^{a}	49.8±4.5^{a}
Overall mean	34.2±2.5^{b}	37.0±2.2^{a}	37.0±3.8^{a}

a, b Means within the same raw with different superscripts are significantly different at ($P<0.05$).

It was observed that the differences in average live body weights among different groups were not significant until the age of 10th, thereafter a significant differences were found among different groups from 11th month of age to the end (16th) of the experiment. The experimental data cleared that the highest value noted in the G2 followed by G3 without significantly affect between them while the lowest value recorded in G1 with a significantly affect with other groups.

Moreover, at the 16th month, live body weight of ram lambs fed 1.5% (G2) and 3% (G3) fish oil increased about 10.4% and 13.1% than the control group, respectively. Similar trend obtained herein have been observed by El–Badawy (2008).

Robinson *et al*. (1999) found that the fish oil increased the growth rate of early weaned lambs and the inclusion of fishmeal at level of 3.25 g/kg of body weight and increased live weight gain by about 32%. These production responses reflect the low rumen degradability of ruminant fishmeal proteins and their high content of essential amino acids which are readily digested in the small intestine (Marinova *et al*. 2005).

In addition to El–Hag *et al*. (1985) noticed that live body weight gain in goats was improved by the inclusion of 10% tallow in the diet.

Lough *et al*. (1994) fed rams rations based on 77% forage supplemented with 10.7% palm oil, showed that supplemented dietary oil lead to decrease significantly dry matter intake ($P<0.01$) compared with control diet, despite the average daily gain was not significantly affected by added oil.

2– Average daily gain:

Average daily weight gain changes of ram lambs throughout the experimental months are presented in Table (2). The differences in an average daily weight gain among G_1, G_2 and G3 were statistically ($P<0.05$) significant.

Table 2. Effect of the experimental diets on daily weight gain (gm) of lambs

Age (month)	Daily weight gain (gm)		
	Group 1 (Control)	Group 2	Group 3
7–8	73.3±16.3^{b}	106.6±16.3^{a}	106.6±22.6^{a}
8–9	86.6± 8.1^{b}	93.3±26.6^{ab}	100.0±22.1^{a}
9–10	66.6± 0.0^{b}	113.3± 8.1^{a}	66.6±26.6^{b}
10–11	80.0±24.4	80.0±26.6	86.6±13.3
11–12	53.3±12.4^{b}	80.0±20.0^{ab}	100.0±22.1^{a}
12–13	80.0±18.2	86.6±12.4	80.0±30.9
13–14	86.6± 8.1	93.3±24.9	93.3±19.4
14–15	73.3±10.5^{b}	100.0± 8.1^{a}	93.3± 6.6^{ab}
15–16	60.0±10.5^{b}	60.0±12.4^{b}	113.3±19.4^{a}

a, b Means within the same raw with different superscripts are significantly different at ($P<0.05$).

The overall daily weight gain of ram lambs was the highest ($P<0.05$) value for lambs fed 3% fish oil (93.3 g) followed by that fed 1.5% fish oil (90.3 g), while the control group had the lowest average daily gain value (73.3 g).

Average daily weight gain of ram lambs fed 1.5% (G_2) and 3% (G_3) fish oil was heavier by about 23.1% and 27.2% when compared to the control group, respectively. In the same way, Lough *et al*. (1993), who found a significant increase ($P<0.05$) in average daily gain by feeding palm oil of the rams.

Also, Marinova *et al*. (2005) found that addition of fish oil to kids led to increase on average daily gain and feed utilization with fat have been related to its energy density. Additionally, the present results are in agreement with other studies that have compared linseed with fish oil in sheep (Demirel, 2000). In contrast, Cooper *et al*. (2004) found that no effect of oil supplements on growth performance of cows. However, Clary *et al*. (1993) stated that 4% tallow tended to increase average daily gain but the differences were not significant in steer diets.

Marinova *et al*. (2007) observed that the lambs received diets supplemented with 2.5% fish oil of wet weight of concentrate for 28 days at 16.62 kg initial weight did not induce significant changes in the average daily gain of the lambs (209.10 vs 197.80 g/d).

Okukpe *et al*. (2011) found that the average weight gain in goats fed control diet, 500, 1000 and 1500 mg of omega–3 fatty acid was 83.34; 74.41; 83.33 and 89.29 g/day, respectively. This may be attributed to improve the ability to use nutrients from food and convert them into muscle protein and hence improve daily weight gain. Ferreira *et al*. (2014) noted that the fish oil did not affect the variation of the ewe's body weight and the average daily weight gain ranged between 246 and 284 g/day over the experimental period.

3– Scrotal circumferences:

The present results revealed that the average monthly Scrotal circumferences of all experimental groups was tended to be gradually increased significantly ($P<0.05$) with advancing animals ages (Table, 3). However, it is noted that the value of scrotal circumference of group2 and group3 were tended to be significantly higher ($P<0.05$) than the control group throughout the experimental period except at the start of the study.

Table 3. Average scrotal circumferences (cm) of ram lambs fed the different experimental diets

Age (month)	Scrotal circumferences (cm)		
	Group 1 (Control)	Group 2	Group 3
7	19.8±3.2	19.8±2.1	20.1±1.1
8	21.0±3.3^{b}	21.8±2.1^{b}	23.6±1.2^{a}
9	20.6±2.7^{c}	22.4±2.0^{b}	24.6±1.2^{a}
10	21.4±2.1^{b}	25.2±1.2^{a}	26.0±1.3^{a}
11	23.4±1.5^{b}	27.6±1.1^{a}	27.8±1.2^{a}
12	26.0±1.4^{b}	28.0±0.7^{a}	28.2±1.3^{a}
13	27.4±1.6^{b}	29.6±1.0^{a}	30.0±1.5^{a}
14	27.8±0.8^{b}	31.2±1.1^{a}	30.0±1.1^{a}
15	29.0±1.0^{b}	31.6±0.8^{a}	31.4±1.2^{a}
16	29.4±0.7^{b}	32.8±0.8^{a}	31.6±1.5^{ab}

a, b, c Means within the same raw with different superscripts are significantly different at ($P<0.05$).

The present results are in agreement with those reported by Sutama and Edey (1986) and El–Badawy (2008). Also, the increase nutrients intake was associated with increase in testicular weight (size) and secretary output of accessory sex glands (Cupps, 1993).

The testicular growth of rams is partly independent on changes in gonadotrophin releasing hormones (GnRH) secretion. But, the testicular growth persists on LH and FSH secretion. So, the gonadal and gonadotropin response are correlated over time (Hotzel *et al*., 1997). The effect of nutrition on hormone secretion lead to regulate the testicular growth (Perez–Claring *et al*., 1998 and El–Saidy *et al*., 2004).

Effect of fish oil addition on reproductive performance of ram–lambs:

The reproductive performance of all the experimental animals was studied from 7 months of age up to the first ejaculation (puberty). Average age, body weight, scrotal circumferences and serum testosterone concentration at the first incidence of each of these elements were recorded for each tested groups (Table, 4).

1– Sexual behaviour:

The present data revealed that the mean age at 1st ejaculation was 342.0±22.4, 348.0±11.1 and 351.0±27.6 days for the treated groups, respectively and the differences among different groups were significantly juried at 1st ejaculation.

The present study cleared that the age at puberty is later than those reported by El–Badawy (2003), who reported that crossbred ram lambs (½Finnish × ½Rhamni) reached the puberty and first collected ejaculate at age between 248 and 266 days. Also, in crossbred ram lambs (½Finnish × ½Rhamni), El–Saidy *et al*. (2004) found earlier age at 1st mounting with erection and 1st ejaculation being 153.2 and 180.5 days, respectively. These differences were mainly related to breed of ram lambs, season of lambing, type of nutrition and other management factors.

There are a significantly affect ($P<0.05$) of the average live body weight and scrotal circumferences between different groups on the at 1st erection and at 1st ejaculation and it was 33.4, 36.0 and 35.4 kg for G_1, G_2 and G_3, respectively (Table, 4).

Whereas, the average scrotal circumferences was reached the highest value at first ejaculation (puberty) in both G2 and G3 without significant effect between them but the lowest value recorded in G1.

In general, testicular growth increased with increasing the live body weight more than age in all treated groups, which confirmed the findings of El–Saidy *et al*. (2004) and El–Badawy (2008).

Adibmoradi *et al*. (2012) observed that fish oil diet significantly elevated ($P<0.05$) the testicular growth measurements (circumference, volume, width and length). Perez–Claring *et al*. (1998) concluded that improved nutrition accelerated the testicular growth and a transit increase occurred pulsatile secretion.

Many studies carried out on diets supplemented with PUFA lead to improve the testis development and spermatogenesis in a variety of livestock species (Tran *et al*., 2017).

Tran *et al*. (2016) found that the age of puberty and scrotal circumference significantly increased by dietary fat effect ($P<0.05$) of which n–3PUFA enriched diet Linseed oil (CaLFA) had the largest influence as compared to other diets prilled fat (PFA) and calcium salt from soybean (CaSFA).

2– Testosterone concentrations (ng/ml):

Differences among different groups were significant

for the blood testosterone concentration at 1st ejaculation (Table, 4). Whereas, the blood testosterone concentration was 0.664; 2.542 and 5.028 (ng/ml) in G1; G2 and G3, respectively at the first ejaculation.

The present values of testosterone concentration in blood plasma of all groups at puberty are within the ranges which reported by Schanbacher and Crouse (1980) in Suffolk ram lambs (0.800–3.400 ng/ml), 0.380–6.79 in crossbred (Finnish × Rhamni) (El–Badawy, 2008).

Testosterone concentration rise at the 1st ejaculation may be due to the strength of the hormone secretion at the initiation of puberty and sexual development of the animals. Thus, a negative feedback by gonadal steroid including testosterone is known to be an integral component of the hypothalmo–pituitary–gonadal axis in males and serves to modulate the release of gonadotropins by the anterior pituitary gland (D'Occhio *et al*., 1982). It is well established that androgens are essential for most stages of spermatogenesis particularly meiosis (Mann and Clutwak–Mann, 1981).

On the other hand, Esmaeili *et al*. (2014) reported that the rams that received fish oil had the highest total testosterone concentrations (11.3 ng/ml) versus 10.8 for sunflower oil as well as improved free testosterone concentration with fish oil supplementation.

Moreover, feeding rams with fish oil possibly affected phospholipids composition in plasma membranes of the testes, altered the expression and affinity of gonadotropin receptors and influenced the rate of testosterone synthesis (Speake *et al*., 2003).

3– Semen characteristics:

Overall mean of ejaculate volume (ml), initial motility %, live sperm (%), abnormal spermatozoa (%), sperm concentration ($\times 10^9$/ml) and sperm output ($\times 10^9$/ejaculate) of ram lambs in the three tested groups are shown in Table (5).

All tested semen characteristics of ram lambs at 1st ejaculation were significant affect. On the other hand, these parameters recorded the highest value in G2 followed by G3 and G1, approximately.

The present results of semen quality (at first ejaculate) are in agreement with those reported by El–Badawy (2008), who found that dietary fish oil supplementation led to improve semen characteristics of pubertal ram lambs at 1st ejaculation by complete development of the sex organs.

Moreover, Jafaroghi *et al*. (2014) and Dolatpanah *et al*. (2008) found that a dietary fish oil of goats had a positive significantly effect on semen volume; sperm motility and sperm concentration/ml compared with the control group. This result is mainly a function of n–3 fatty acids feeding may be effective on the sexual glands excretions the synthesis and secretion of seminal plasma.

This is result mainly attributed to the high concentration of PUFAs in fish oil that can incorporate in the sperm lipids (Cerolini *et al*., 2006) and may be cause alteration in the fluidity and flexibility of the sperm membrane (Conquer *et al*., 2000).

In goats, supplementing diets with 2.5% fish oil during the non–breeding season caused only numerical improvement in semen characteristics (El–Shamaa, 2002). Speake *et al*. (2003) suggested that DHA (doco-

Table 4. Reproductive performance of ram lambs fed the different experimental diets at 1st ejaculation (Puberty):

Characters	Experimental groups		
	Group 1 (Control)	Group 2	Group 3
Age (day)	342.0 ±22.4^{b}	348.0 ±11.1^{a}	351.0 ±27.6^{a}
Body weight (kg)	33.4 ± 1.0^{b}	36.0 ± 1.9^{a}	35.4 ± 1.8^{ab}
Scrotal circumference (cm)	23.4 ± 0.4^{b}	27.6 ± 0.5^{a}	27.8 ± 2.1^{a}
Testosterone concentration (ng/ml)	0.66± 0.33^{b}	2.54± 0.81^{b}	5.03± 0.82^{a}

a, b Means within the same raw with different superscripts are significantly different at (P<0.05).

Table 5. Semen characteristics of pubertal ram lambs at 1st ejaculation as affected by the experimental diets

Semen characteristics	Treatment		
	Group 1 (Control)	Group 2	Group 3
Ejaculate volume (ml)	0.32±0.04^{c}	0.52±0.10^{a}	0.44±0.07^{b}
Sperm motility (%)	70.0 ±0.02^{b}	75.0 ±0.03^{a}	66.0 ±0.09^{c}
Live sperm (%)	65.0 ±0.04^{b}	67.8 ±0.08^{b}	70.6 ±0.04^{a}
Abnormal sperm (%)	15.0 ±0.02^{a}	14.8 ±0.01^{a}	9.4 ±0.01^{b}
Sperm concentration ($\times 10^9$/ml)	1.95±0.10^{b}	2.36±0.12^{ab}	2.46±0.19^{a}
Sperm output ($\times 10^9$/ejaculate)	0.63±0.09^{b}	1.20±0.20^{a}	1.09±0.22^{a}

a, b, c Means within the same raw with different superscripts are significantly different at (P<0.05).

sahexaenoic acid) may increase the flexibility and compressibility of the sperm tail and hence, improve the ability of the lipid bilayer to tolerate the stress of flagellar movement.

4– Semen characteristics:

1–4– Ejaculate volume (ml):

Means Ejaculate volume (ml) of ram lambs in different groups during the experimental collected period are presented in Table (6). The effects of dietary treatments and months were significant at ($P<0.05$).

Overall mean of semen Ejaculate volume significantly at ($P<0.05$) increased in G2 (0.75 ml) and G3 (0.84 ml) as compared to the control group (0.64 ml) in Table (6).

These findings are in agreement with those reported by Drokin *et al*. (1999) and Rooke *et al*. (2001), who found that the ejaculate volume increased ($P<0.05$) in rams fed rations supplemented with fish oil compared with those fed the control diet. Also, Habibi *et al*. (2016) and Esmaeili *et al*. (2014) found that effect of addition of fish oil was a significant effect on semen volume ($P<0.05$).

2–4– Percentage of sperm motility:

Sperm motility Percentage of ram lambs in different tested groups during the period from 10 to 15 months of age is presented in Table (6). Sperm motility Percentage was affected significantly ($P<0.05$) by both different dietary treatment and age.

Percentage of sperm motility was recorded the highest value (81.1%) in G3 followed by in G2 (79.5%), while the control group showed the lowest sperm motility Percentage (77.7%). These changes of sperm motility Percentage may be attributed to testicular and epididymal development especially during the period of early sexual development. Similar observations were reported by Cupps (1993) and Perez–Claring *et al*. (1998). Also, Conquer *et al*. (2000) found that the addition of fish oil with decosahexaenoie acids in diets ram lambs led to improve sperm motility than non–addition of fish oil. Rege *et al*. (2000) reported that the sperm motility a gradual improvement with the increasing age and reach the better value at 12 months of age and more.

Esmaeili *et al*. (2014) reported that the interaction between diets and days in fish oil treatment ($P<0.01$) were significant for the total motility of fresh sperm versus other groups. Omega–3 fatty acids, in particular

Table 6. Semen characteristics for ram lambs as affected by the experimental diets at different ages

Groups	Age (months)						
	10	11	12	13	14	15	Overall mean
				Ejaculate volume (ml)			
Control	0.43±0.10	0.45±0.03^{b}	0.50±0.11^{b}	0.71±0.06^{b}	0.87±0.13^{b}	0.90 ±0.13^{b}	0.64±0.09^{b}
Group 2	0.43±0.13	0.55±0.03^{ab}	0.60±0.06^{ab}	0.80±0.04^{a}	0.95±0.06^{a}	1.20 ±0.05^{a}	0.75±0.62^{ab}
Group 3	0.45±0.06	0.66±0.04a^{a}	0.83±0.03^{a}	0.85±0.16^{a}	0.95±0.06^{a}	1.35 ±0.15^{a}	0.85±0.08^{a}
				Sperm motility (%)			
Control	73.7 ±1.2^{b}	74.2 ±1.2	76.7 ±1.2^{b}	79.0 ±2.0^{b}	80.2 ±1.2^{b}	82.7 ±1.2^{b}	77.7 ±1.3^{b}
Group 2	75.0 ±0.0^{a}	75.2 ±1.2	78.7 ±2.3^{ab}	80.2 ±1.2^{b}	82.2 ±1.2^{b}	85.7 ±1.2^{a}	79.5 ±1.2^{ab}
Group 3	76.2 ±2.3^{a}	76.0 ±1.0	80.0 ±1.0^{a}	83.0 ±2.0^{a}	85.5 ±1.4^{a}	86.2 ±2.3^{a}	81.1 ±1.3^{a}
				Live sperm (%)			
Control	68.5 ±4.4	69.5 ±0.6^{b}	73.7 ±1.1^{b}	75.0 ±1.8	75.7 ±0.7^{b}	77.7 ±1.3^{b}	73.3 ±1.6^{b}
Group 2	67.0 ±2.8	72.7 ±0.8^{a}	75.5 ±1.9^{a}	76.7 ±2.9	78.7 ±3.1^{ab}	80.7 ±1.7^{a}	75.2 ±2.2^{a}
Group 3	67.2 ±0.4	73.2 ±1.7^{a}	76.5 ±2.3^{a}	77.5 ±2.3	80.5 ±1.1^{a}	81.7 ±3.1^{a}	76.1 ±1.8^{a}
				Abnormal sperm (%)			
Control	14.2 ±3.4	13.0 ±0.4^{a}	13.7 ±1.2^{a}	10.7 ±0.8	9.0 ±0.9^{a}	9.1 ±1.1^{a}	11.6 ±1.3^{a}
Group 2	14.0 ±0.4	10.7 ±0.8^{b}	11.5 ±0.8^{b}	10.5 ±0.6	9.2 ±0.8^{a}	8.1 ±0.6^{b}	10.6 ±0.7^{ab}
Group 3	13.9 ±2.7	11.0 ±0.4^{b}	11.7 ±1.2^{b}	9.2 ±1.7	7.5 ±0.6^{b}	7.2 ±1.0^{c}	10.0 ±1.3^{b}
				Sperm concentration ($\times10^{9}$/ml)			
Control	1.82±0.03^{b}	1.86±0.03^{b}	2.14±0.08^{c}	2.39±0.06^{b}	2.47±0.09^{b}	2.96 ±0.08^{b}	2.27±0.36^{b}
Group 2	2.15±0.02^{a}	2.32±0.03^{a}	2.54±0.05^{a}	2.64±0.07^{a}	2.69±0.08^{a}	2.87 ±0.05^{b}	2.53±0.05^{a}
Group 3	2.05±0.07^{a}	2.13±0.46^{a}	2.37±0.04^{b}	2.46±0.05^{a}	2.52±0.02a^{a}	3.11 ±0.10^{a}	2.44±0.07^{a}
				Sperm output ($\times10^{9}$/ejaculate)			
Control	0.77±0.17^{b}	0.84±0.06^{b}	1.07±0.22^{b}	1.70±0.17^{b}	2.15±0.37^{b}	2.66 ±0.30^{b}	1.53±0.22^{b}
Group 2	0.91±0.27^{a}	1.27±0.06^{a}	1.52±0.19^{ab}	2.11±0.10^{a}	2.56±0.25^{a}	3.44 ±0.15^{ab}	1.97±0.17^{b}
Group 3	0.92±0.16^{a}	1.41±0.19^{a}	1.97±0.06^{a}	2.09±0.36^{a}	2.39±0.15^{ab}	4.191±0.14^{a}	2.16±0.18^{a}

a, b, c Means within the same raw with different superscripts are significantly different at ($P<0.05$).

decosahexaenoie acids (DHA C22:6 n–3), are important for sperm membrane integrity, sperm motility and viability, as cold sensitivity (Robinson *et al*., 2006).

Aksoy *et al*. (2006) found that FAs are important for sperm membrane integrity motility and viability, as well as cold sensitivity. Feeding fish oil increased the proportion of spermatozoa with progressive motility and with a normal a acrosome score and reduced the proportion of spermatozoa with abnormal morphologies (Rooke *et al*., 2001).

Gholami *et al*. (2010) reported that the motility of fresh semen was improved in bulls supplemented with dietary DHA but there was no improvement detected in frozen–thawed semen in the same study.

3–4– Percentage of live sperm:

Means of live sperm percentage of ram lambs in different dietary groups at successive age are presented in Table (6). Live sperm Percentage was affected significantly by dietary treatment and age. Generally, values of percent of live sperm were gradually increased as age advanced up to 15 months.

Overall mean of live sperm percentage was significantly ($P<0.05$) higher in semen of rams fed both two levels of fish oil addition (G2 and G3) when compared with those fed control diet. These results are in agreement with those reported by Conquer *et al*. (2000), who found that the addition of fish oil with diets of ram lambs led to improve the sperm livability percentage than control diet without addition. Also Jafaroghli *et al*. (2014) reported that feeding fishmeal and vitamin C improved the percentage of live sperm ($P<0.01$).

On the other hand, the changes in sperm livability Percentage had affected by age of ram lambs and this result supported with the results obtained by Rege *et al*. (2000), who found marked decrease in proportion of dead spermatozoa in semen with age progress.

Samadian *et al*. (2010) found that the dietary omega–3 fatty acids have been shown to improve sperm parameters in rams. Also, Esmaeili *et al*. (2014) showed that feeding a diet supplying 35 g fish oil and 50 IU vitamin E per day improved the quality of fresh and frozen ram semen as compared with the diets containing palm oil or sunflower oil with vitamin E.

Gulliver *et al*. (2012) reported that omega–3 fatty acids, especially Docosahexaenoic acid (DHA), improve the sperm quality and protect the sperm against stress conditions by maintaining the membrane integrity and sperm viability. Also, Khan *et al*. (2015) found that feeding of flaxseed (FL–L) as source of n–3 fatty acids had good positive effect on the bovine bull semen quality parameters.

4–4– Percentage of Abnormal sperm:

Overall mean of abnormal sperm percentage in semen of ram lambs in the different tested groups during the experimental period from the 10^{th} to 15^{th} months of age are presented in Table (6). The dietary treatments and age were significantly at ($P<0.05$) effect on the abnormal sperm percentage.

These results are in agreement with those given by Drokin *et al*. (1999), who found that addition of fatty acids in diets of rams led to decrease sperm abnormality percentage when compared with the control group. Also, feeding fish oil reduced the proportion of spermatozoa with abnormal morphologies (Rooke *et al*.,2001).

However, increasing sperm abnormality percentage up to 13.5–15.0% at the early collection months was also observed by Kumi– Diaka *et al*. (1985), who found that the average morphological sperm abnormalities at puberty was 25.2 % in subtropical ram lambs.

In the same way, Jafaroghli *et al*. (2014) reported that feeding ram with fish oil and vitamin C led to decrease in proportion abnormal sperm to record 21.49%, while the highest percentage of abnormal sperm was calculated in the control group (25.2%). Habibi *et al*. (2016) found that the dietary rams supplemented with fish oil or vitamin E produced a lower percentage of abnormal sperm.

5–4–Sperm concentration ($\times10^9$/ml):

Means of sperm concentration in semen of ram lambs in different tested diets during the period form the 10^{th} to 15^{th} month of age are presented in Table (6). Sperm concentration was increased significantly ($P<0.05$) by dietary supplemented with fish oil and with progress of age.

The overall mean of sperm concentration was increased significantly ($P<0.05$) in G2 (2.533×10^9/ml) and G3 (2.438×10^9/ml) as compared to the control group (2.271×10^9/ml).

So, the change in the nutrition may be led to profound responses in testicular size and therefore the rate of production of spermatozoa. In the same trend, these changes may be attributed to the change in the size of the somniferous tubules and in the efficiency of spermatogenesis. Nissen and kreysel (1983) found that sperm concentration in ram semen was higher by feeding animals on fish oil addition than control diet. Also, Drokin *et al*. (1999) and Rooke *et al*. (2001) who found that sperm concentration improvement by addition fish oil for diets of rams.

Additionally, Esmaeili *et al*. (2014) reported that there was a significant effect for sperm concentration from the n–3 FA dietary supplemented rams compared to the control group.

6–4–Total sperm output ($\times10^9$/ejaculate):

Means of total sperm output/ejaculate in semen of ram lambs in different tested diets during the study period form the 10^{th} to 15^{th} month of age are presented in Table (6). The date cleared that the total sperm output /ejaculate was affected significantly ($P<0.05$) by both treatments and age.

Sperm output /ejaculate is calculated from the semen volume/ ejaculate and sperm concentration/ml. Therefore, both items contribute to the progressive increase in sperm output /ejaculate as shown in the previous result whereas the volume of ejaculate and, its concentration improved in the tested diets with fish oil

and age progress.

Total sperm output/ejaculate is very important factor affecting male fertility and depends on both the ejaculate volume and sperm concentration. Factors affecting the ejaculate volume can be also affect the sperm concentration and in turn total sperm output/ejaculate which has important role in male fertility. These findings are in agreement with those reported by Sutama and Edey (1986) and Coulter *et al*. (1997).

The highest total sperm output/ejaculate was obtained with rams fed fish oil at level 3% (G3) (2.160×10^9 sperm/ ejaculate), followed by the rams received diet containing 2% fish oil (G2) (1.969×10^9 sperm/ ejaculate), while the control group showed the lowest values (1.530×10^9 sperm/ ejaculate). The same trend was obtained by Rege *et al*. (2000), who found the pronounced increase in total sperm output by age advancing from 6 up to 12 months of age. Additionally, Esmaeili *et al*. (2014) reported that the maximum sperm output in fish oil meal was achieved about 7.59×10^9 when compared to palm oil 5.39×10^9.

CONCLUSION

On the light of the foregoing results, it could be concluded that fish oil addition at rate of 1.5 and 3% to ram lambs had beneficial effects on productive and reproductive performance.

AUTHOR CONTRIBUTIONS

1) Study conception and design: Azza EL–MADAWY, M. ALI, Y. HAFEZ, and A. METWALLY.

2) Acquisition of data: Azza EL–MADAWY, M. EL–SHARAWY, M. ALI, Y. HAFEZ1, and A. METWALLY.

3) Analysis and interpretation of data: Azza, EL–MADAWY, M. EL–SHARAWY, Y. H. HAFEZ, N. YAMAUCHI

4) Drafting of manuscript: M. EL–SHARAWY, M. ALI, N. YAMAUCHI and A. METWALLY

5) Critical revision: M. EL–SHARAWY, M. ALI, N. YAMAUCHI and A. METWALLY

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