

EFFECT OF SUPPLEMENTATION OF OMEGA-3 FATTY ACIDS ON BLOOD PARAMETERS AND SEMEN QUALITY OF FRIESIAN BULLS

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Abstract: The aim of this work was to evaluate the effects of flaxseed oil supplementation as a source of omega-3 fatty acids (OFA) on some blood parameters, semen quality and testosterone level in male Frisian bull. A total of 30 Frisian bulls (14.2 ± 0.16 month of age and 265±15 kg body weight) were divided into three equal groups according to age and live body weight. Bulls in the 1st group (G1) were supplemented with 2% dry matter intake (DMI) flaxseed oil as a source of OFA, while those in 2nd group were supplemented with 4% DMI flaxseed oil as a source of OFA at 14 to 21 months of age (G2). Bulls in the 3rd group were fed a basal diet and considered as a control group (G3). The obtained results revealed that OFA administrated animals had a significant ($P < 0.05$) inducing effect on serum levels of total protein, globulin, glucose, high density lipoprotein (HDL) and triglyceride compared to the control group. No significant changes were noticed in albumin levels among the three groups. However, the levels of total cholesterol, urea and low density lipoprotein (LDL) were significantly lower in bulls received OFA than the control group. Omega-3 fatty acids treatment has a significant positive effect on the semen characteristics and lower abnormality in G2 and G3 than in G1. G2 and G3 also showed a significant higher intact spermatozoa cell membrane than in G3 by about 9.68 and 10.84%, respectively. Omega-3 treatment significantly increased blood testosterone levels to be 23.78% in G2 and 31.82% in G3 higher than that of the control. In conclusion, dietary supplementation with omega-3 in male Frisian ration improved semen quality and reproductive potentiality as well as testosterone level.

Key words: Frisian bull; omega-3 fatty acids; testosterone and semen quality

Introduction

Bull fertility has a high economic importance in cattle artificial fertilization (AI) industry as semen high quality is crucial for successful AI. Flaxseed oils are an excellent as a source of α -linolenic acid, a member of the omega-3 fatty acids (OFA) (1). The fraction of

flaxseed oil is approximately 0.55 omega-3 α -linolenic acid (2). OFAs are able to lower the risks of some diseases (1, 3). Alpha linolenic acid, an essential OFA, is a precursor of eicosapentaenoic acid (EPA), which in turn is a precursor for the formation of eicosanoids. Eicosanoids are hormone-like compounds that play an essential role in immunity faction. Some

study reported that EPA can elongate further to docosahexanoic acid (DHA), an OFA that is essential for cell membrane integrity and brain health (4).

Prostaglandins (PG) may play an important role in reproductive performance, especially semen quality (5). Arachidonic acid is a subsequent production of PG and is involved on synthesis of the steroid hormone (6). Total sperm number (7) and sperm motility (8) were improved following administration of fish oil to boars. On the other hand, semen characteristics were negatively affected after treatment with omega-6 in humans (9). High concentrations of polyunsaturated fatty acid (PUFA) in sperm membranes may improve semen quality after supplementation of long-chain omega-3 (5).

The aim of this work was to study the effect of OFA on some blood parameters, testosterone level and semen quality in Friesian bull.

Materials and methods

The experiment was performed at Sakha Experimental Station, Kafr-Elsheikh Governorate located in the Northern part of the Nile delta, at Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt.

Animals

A total number of 30 Friesian bulls averaging 14.2 ± 0.16 month of age and 265 ± 15 kg body weight were used in this study. The bulls were randomly classified into three groups (10 each), according to their body weight and age. Bulls in the 1st group (G1) were supplemented with 2% DMI flaxseed oil as a source of OFA, while the bulls in the 2nd group were supplemented with 4% DMI flaxseed oil (G2). Bulls in the 3rd group were fed a basal diet and considered as a control group (G3). All bulls were judged as free of diseases and physical defects genitalia. The experimental animals were kept freely under semi-open sheds and were fed according to the recommendations of Animal Production Research Institute (APRI, 10) throughout the experimental period.

Feeding system and management

The concentrate feed mixture (CFM) was used in bull feeding in all groups. It was composed of 37.5% yellow corn, 20% soybean meal, 15% corn gluten, 22.5% wheat bran, 3% molasses, 0.5% premix (one kg of premix contained 3.3×10^6 IU vit. A; 3.3 g vit. E; 3.3×10^6 IU vit. D3; 0.33 g vit. K; 0.33 g vit. B1; 1.33 g vit. B2; 6.67 g vit. B5; 0.50 g vit. B6; 3.3 g vit. B12; 3.3 g vit. pantothenic acid; 0.33 g folic acid; 16.67mg Biotin; 166.67 g Cholin; 1g Copper; 10 g Iron; 13.3g Mn; 15 g Zn; 0.1 g iodine; 0.03 g Se and carrier CaCO_3 to 1kg) and 1.5% common salt.

Bulls in all groups were fed equal amounts of diet containing CFM, rice straw and fresh berseem (during winter season) or berseem hay (during summer season) according to the recommendation of the APRI, (10). Allowances for growing dairy bulls based on live body weight. Chemical analysis of representative monthly samples of foodstuffs was analyzed for CP, CF, EE, NFE and ash on DM basis according to the official methods of the A.O.A.C (11). Chemical composition of CFM, rice straw, fresh berseem and berseem hay used in feeding bulls in both groups is shown in Table (1).

Experimental procedures

Throughout the experimental period, semen samples were collected on all animals twice weekly using an artificial vagina at 18 months of age bulls at time of collection up to 21 months of age. After semen collection, each ejaculate was evaluated for volume, sperm concentration ($\times 10^6/\text{ml}$) mass motility (%), live sperm (%), sperm abnormality (%) and sperm concentration/ejaculate ($\times 10^6/\text{ml}$). As well as, sperm cell concentration was directly evaluated according to Barth (12).

The integrity of plasma membrane for the fresh spermatozoa was examined using hypo-osmotic swelling (HOS) test (13). The HOS solution at a concentration of 100 mOsm/kg was prepared by mixing 0.49 g of sodium citrate and 0.9 g fructose with 100 ml distilled water. In brief, 250 μl of diluted semen were added to

1 ml of the pre-warmed HOS solution and incubated for 60 min at 37°C. A volume of 5 µl from each sample was put on clean and warm, microscope slide and were examined at x400. Spermatozoa were counted 200 per sample and the indicative of intact plasma membrane were also determined.

Blood sampling

Blood samples were monthly collected during the experimental period in clean test tubes via the jugular vein from all the experimental bulls and were centrifuged at 3000 rpm for 10 minutes. The obtained serum was kept at -20°C until determination of testosterone concentration.

Testosterone assay

Total and free serum testosterone assay was conducted by radio immune assay method (RIA) using Pontex 335 kit (I^{125}). Total testosterone included free testosterone and that bound to sex steroid binding globulin hormone, albumin, corticosteroid binding globulin (CBG). The standard curve of testosterone ranged between 0.1 and 25.6 ng/ml.

Biochemical assays

Serum biochemical parameters (total protein, globulin, glucose, cholesterol, triglyceride, HDL and LDL) were done using commercial kits (Diagnostic System Laboratories, Inc., USA) and as previously described (14-16).

Statistical analysis

Statistical analyses of data were carried out applying the package of Snedecor and Cochran (17). A factorial design (3 groups x ages) was used and the statistical model was:

$$Y_{ijk} = U + A_i + B_j + AB_{ij} + e_{ijk}.$$

Where:

Y_{ijk} = Observed values;

U = Overall mean;

A_i = group;

B_j = age;

AB_{ij} = Interaction due group x age;

e_{ijk} = Random error

The significant differences among means were tested using Duncan Multiple Range Test. Correlation analysis was carried out using computer programmer of SAS system. The percentage values of semen characteristics were tested by arcsine transformation so the means were presented after recalculated from the transformed values to percentages.

Results and discussion

Serum biochemical parameters in blood

Flaxseed oil is essential polyunsaturated fatty acids work as constituent of many enzymes which involved of majority of metabolic pathways also was important for metabolism of protein and growth of organ and immunity response. In the current study, flaxseed oil (as a source of OFA) supplementation to Friesian bulls improved the serum content of total protein and lipid profile and immunity markers (Table 2). The addition of flaxseed oil to the bulls ration, significantly ($P < 0.05$) increased serum HDL, albumin and globulin, and decreased the LDL, cholesterol, TG and blood urea as compared to the control. These results agree with those of other studies where flaxseed oil was reported to reduce total lipids concentration in calves' blood serum (18-22).

Some studies suggested different ways by which PUFA can alter cholesterol concentration (23, 24). The synthesis of cholesterol is known to be increased with high PUFA intake (23, 24). However, in the current study, the lowering of serum cholesterol with supplementation of flaxseed oil as source of PUFAs could be attributed to the upregulation of LDL receptors (25) and/or the cholesterol redistribution between tissue pools and plasma (23). Flaxseed oil had high percentage of α -linolenic acid about 55% of oil's total fatty acids (26, 27). The diets which are rich in OFA decrease aggregation of platelet also, blood triglycerides and levels of cholesterol, blood clots formation, also, show both antithrombotic and anti-inflammatory effects (28, 29).

Table 1: Chemical analysis of different feedstuffs (on dry matter basis) used in feeding bulls

Item	Chemical composition (%) (on DM basis)			
	CFM	Rice Straw	Fresh Berseem	Berseem Hay
Dry matter, DM	90.42	89.24	15.26	88.23
Organic matter, OM	90.24	83.22	86.15	88.58
Crude protein, CP	16.04	1.59	14.71	14.41
Crude fiber, CF	10.96	37.21	24.9	24.67
Other extract, EE	4.91	1.47	2.90	6.04
Nitrogen free extract, NFE	56.38	42.85	43.64	43.16
Ash	9.76	16.78	13.85	11.42

Table 2: Concentration of biochemical parameters in serum as affected by flaxseed oil supplementation

Item	Experimental group			±MSE
	G1	G2	G3	
Total protein (g/100 ml)	7.68 ^a	7.55 ^a	7.23 ^b	0.03
Albumin (g/100 ml)	3.62	3.67	3.51	0.02
Globulin (g/100 ml)	4.06 ^a	3.89 ^{ab}	3.72 ^b	0.01
Glucose (mg/100ml)	69.87 ^a	71.03 ^a	61.66 ^b	2.54
Total cholesterol (mg/100ml)	166.2 ^b	163.1 ^b	187.3 ^a	3.12
High density lipoprotein (mg/100ml)	99.50 ^b	102.4 ^a	77.39 ^c	3.80
Low density lipoprotein (mg/100ml)	67.33 ^b	61.39 ^b	93.99 ^a	4.62
Triglyceride (mg/100ml)	37.03 ^a	38.01 ^a	28.9 ^b	0.85
Urea-N (mg/dl)	26.45 ^b	24.75 ^c	31.56 ^a	2.12

G1 and G2: Bulls received flaxseed oil 2 and 4%/kg DMI, respectively. G3: Control

Data in the raw followed by different letters are significant at P<0.05.

Table 3: Semen quality in Friesian bulls as affected by flaxseed oil supplementation

Item	Experimental group			±MSE
	G1	G2	G3	
Ejaculate volume (ml)	4.13 ^a	4.24 ^a	3.74 ^b	0.03
Sperm cell concentration (x 10 ⁶ /ml)	1.372 ^a	1.444 ^a	1.138 ^b	0.02
Mass motility (%)	75.76 ^a	79.17 ^a	68.06 ^b	0.01
Live sperm (%)	77.00 ^a	81.17 ^a	71.17 ^b	1.85
Sperm abnormality (%)	10.06 ^b	9.39 ^b	13.67 ^a	3.12
Sperm cell concentration per ejaculate (x10 ⁶ /ml)	5.776 ^a	6.240 ^a	4.261 ^b	0.32
Hypo-osmotic swelling test (%)	53.94 ^a	54.51 ^a	49.18 ^b	1.24

G1 and G2: Bulls received flaxseed oil 2 and 4%/kg DMI, respectively. G3: Control

Data in the raw followed by different letters are significant at P<0.05.

Table 4: Concentration of testosterone hormone(ng/ml) in blood serum as affected by flaxseed oil supplementation

Time (month)	Experimental group			±MSE	Overall means
	G1	G2	G3		
15	1.507	1.960	1.189	0.12	1.552 ^c
16	2.340	2.633	2.328	0.10	2.434 ^{ab}
17	2.474	2.263	2.023	0.12	2.253 ^b
18	2.323	2.339	1.949	0.11	2.204 ^b
19	2.541	3.084	2.082	0.11	2.569 ^a
20	2.732	2.860	1.753	0.15	2.448 ^a
21	2.736	2.860	2.121	0.14	2.572 ^a
Overall means	2.774 ^a	2.954 ^a	2.241 ^b	0.13	

G1 and G2: Cows received flaxseed oil 2 and 4%/kg DMI, respectively. G3: Control

Data in the raw followed by different letters are significant at P<0.05.

Semen quality

In the current study, flaxseed oil as a source of omega-3 fatty acids treatment significantly ($P < 0.05$) increased ejaculate volume, sperm cell concentrations, mass motility (%), live sperm (%) and sperm cell concentrations per ejaculate (1×10^6 ml) in G2 and G3 than in G1 (Table 3). Moreover, sperm abnormality (%) was significantly lower in treated groups than in the control group by 26.41% in G1 and 31.31% in G2, respectively. This improvement in all semen characters could be attributed to flaxseed oil supplementation with its high content of linoleic and linolenic acids as good antioxidants. Supplementation of flaxseed oil also produced a great improvement of all semen characters of rams (31). Moreover, different ratios of omega-3/omega-6 PUFA were reported to improve semen characteristic by elevating omega-3/omega-6 PUFA that increased sperm concentration and motility and to reduce the deformity rate of the sperm (7). In the rats diet appropriate ratio of omega-3/omega-6 PUFA improved semen quality and changes in hormone metabolism due to improving reproductive performance (32). The progressive motility was reported to be higher in frozen-thawed semen in the flaxseed oil treated group than in the fish oil group (33). Dietary supplemented with PUFA improved reproductive performance, development of testis, spermatogenesis, sperm of motility and viability in fresh or freeze semen sample in ruminant (34).

In the HOS test, incubation of sperm in hypo-osmotic media is necessary to estimate the plasma membrane covering the principle piece (35, 36). In the current study HOS test show that flaxseed oil supplementation to bulls significantly ($P < 0.05$) increased the resistance of the sperm covering membrane to the hypo osmotic challenge in both G1 and G2 compared to the control G3 by about 9.68 and 10.84%, respectively. The incorporation of DHA may be increased with Omega-3 treatment in the principle piece, facilitating sperm membrane stability against hypo-osmotic media (37).

The improvement of semen quality may be related to the supplementation with flaxseed oil

with its PUFA the important molecules that serve as a source of energy and are critical components of the physical and functional structure of cells (38). Addition of OFA to animals diets improved sperm characteristics (39), increased sperm density and concentration per ejaculate (40, 41).

Testosterone concentrations

Testosterone is the key player in spermatogenesis and development reproductive tract in male (49). In this study, Friesian bulls serum testosterone concentrations were significantly increased by flaxseed oil treatments in G2 and G1 by 31.82 and 23.78% as compared to bulls in control (G3) (Table 4). The results agree with some study stated that omega-3 and 6 PUF may affect metabolism of important reproductive hormones. Testosterone concentration significantly increase in bulls supplemented with omega-3 may be due to the adequate amount of unsaturated fatty acids such as linoleic and linolenic acids. These unsaturated fatty acids especially linolenic could be converted or involved in the synthesis of cholesterol which is considered the precursor materials for testosterone synthesis (43). It was indicated that spermatogenesis and steroidogenesis in the avian testis are increased with the omega-3 diets and this improvement dependent on the increase levels of FSH, LH and testosterone. However, O'Donnell *et al.* (45) reported that the concentrations of reproductive hormones and testosterone were positively higher related to presence of some important fatty acids and quality and morphology of sperm.

Conclusion

Dietary supplementation with omega-3 in male Friesian ration improved semen quality, testosterone level, lipid profile and immune function. Therefore the addition of flaxseed oil as a source of omega-3 is recommended to improve male animals semen quality and reproductively.

Conflict of interest

The authors declare that they have no conflict of interest.

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