ISSN Print: 2664-6064
ISSN Online: 2664-6072
Impact Factor: RJIF 5.2
IJAN 2021; 3(2): 82-84
www.agriculturejournal.net
Received: 02-11-2021
Accepted: 08-12-2021

Naser Estuty
Libyan Authority for Scientific Research, Libyan Olive Tree Research Center, Libya

Omran Algriany
Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Corresponding Author:
Naser Estuty
Libyan Authority for Scientific Research, Libyan Olive Tree Research Center, Libya

Effect of dietary Pufa in rams on fatty acid compostion of Epidymal semen

Naser Estuty and Omran Algriany

DOI: https://doi.org/10.33545/26646064.2021.v3.i2a.106

Abstract
Mammalian spermatozoa are characterized by a high proportion of polyunsaturated fatty acids (PUFA) which play a critical role in fertilization. However, the fatty acid profile during sperm maturation remains unclear in rams. The aim of this study was to investigate the effects of feeding dietary protected fish oil (FO) and Green lipped mussel of (GLM) on lipid composition of epidymal semen. Twelve Suffolk rams with a mean age 7.0 months ± 15 days with an average live weight of 77 (±3.5) kg were divided randomly into four groups of 3 rams per group and housed individually. The rams were randomly allocated to each of foure fatty acid (FA) sources; Megalac (M), Megalac with Green lipped mussel (MGLM), Fish oil (FO) and Fish oil + Green lipped mussel (FOGLM). The concentration of C20:5 *n*-3 in the semen of rams offered diets containing fish oil with GLM were significantly greater (p=0.03) than those observed in rams receiving the Megalac diets. The most pronounced effect of feeding the fish oil diets was an increase in fatty acid concentration of C20:5 *n*-3 compared with Megalac diet.

Keywords: Fatty acid, Epidymal semen, Pufa in rams

Introduction
Sperm maturation occurs along the transit through the epididymal regions, which allows for functional and morphological changes in the spermatozoa (Fouchecourt *et al.* 2000) [5]. After these modifications, the spermatozoa stabilize its plasma membrane through structural changes in lipid profile and, eventually, acquire progressive motility (Jervis and Robaire 2001) [7] and (Amann *et al* 1993) [1]. Many studies revealed that PUFA in the diets can be incorporated into sperm cells in many animal species such as in humans (Conquer *et al.* 2000) [3]; fowls (Blesbois *et al.* 1997) [2], swine (Penny *et al*., 2000) [10] and sheep (Drokin 1999) [4]. It has been reported that the fluidity and phospholipid (PL) composition of sperm membranes changed during their maturation in the epididymis (Hall *et al.* 1991) [6]. There is also evidence that a positive correlation exists between the amounts of long chain *n*-3 PUFA in semen and an increase in the progressive motility and normality of sperm (Rooke *et al*., 2001 and Nissen and Kreysel, 1983) [11, 9]. After leaving the testes and during their transit through the epididymis, mammalian spermatozoa are known to undergo important morphological, physiological and biochemical modifications that contribute to their maturation (Voglmayr, 1975) [12]. The aim of this study was to investigate the efficacy of dietary fat sources evaluated for their susceptibility to bio hydrogenation to improving the lipid composition of epididymal semen of rams. Also, to evaluate the susceptibility to rumen bio-hydrogenation of the *n*-3 PUFA contained in the Bionovate GLM powder®.

Material and Methods
Animals managements and diets
Twelve rams with a mean age 7 months ± 15 days with an average live weight of 77 (±3.5) kg were divided randomly into four groups of 3 rams per group and housed individually. Four diets were formulated to provide a similar fat level (50 g/kg) from different fat sources as shown in Table 1. The rams were fed a 70:30 basal diet of haylage and concentrate. On a fresh weight basis, the rams consumed 1 kg of concentrate (86% DM) and 3 kg of haylage per day. The four treatment diets were prepared by supplement the basal die as follows:

Megalac: Megalac (a calcium soap of palm oil: Volac Ltd.UK) contained 36g/kg which is high in palmatic acid (C16:0), a saturated fatty acid.

Megalac + GLM: Megalac with GLM powder contained 35g/kg Megalac with 6g/kg Bionovate GLM Powder®. Bionovate GLM Powder® is produced using a unique, cold extraction process, resulting in a supplement that is high in protein and glycosaminoglycan-rich carbohydrates These characteristics of the product may confer greater resistance to rumen biohydrogenation compared to other PUFA supplements.

Fish oil: Fish oil adsorbed onto a vermiculite matrix, (Trouw UK Ltd, Nantwich, Cheshire) contained 65 g/kg protected fish oil (Trouw UK Ltd, Nantwich, Cheshire) its specification: 50% oil, C20:5 *n*-3 (165 g/kg, C22:6 *n*-3 (110 g/kg).

Fish oil+ GLM: Fish oil with GLM powder a mixture of 61g/kg protected fish oil and 6g/kg GLM. The novel fat supplement (Bionovate GLM Powder®) and protected fish oil supplied preformed long chain *n*-3 PUFA, C20:5 *n*-3 and C22:6 *n*-6.

Table 1: Raw material of experimental diets containing different fat sources and the daily intake of fatty acids.

	Megalac	Megalac +GLM	Fish oil	Fish oil +GLM
Ingredients (g/kg DM)				
Concentrate	273	272	266	264
Haylage	691	687	673	669
Megalac	36	35	0	0
GLM powder	0	6	0	6
Protected fish oil	0	0	61	61
Fatty acid intake (g/d)				
C18:0	1.6	1.7	3.2	3.7
C18:1 *n*-9	11.9	13.5	13.0	15.9
C18:1 *n*-7	0.4	0.4	2.2	2.5
C18:2 *n*-6	7.2	7.1	14.9	16.8
C18:3 *n*-3	3.2	2.9	2.2	2.6
C20:5 *n*-3	0.2	0.4	1.8	2.1
C22:6 *n*-3	n.d.	0.2	0.6	0.7
Remaining fatty acids	3.3	3.9	9.3	11.9
Total fatty acid	43.8	45.3	56.0	66.3

n.d.: not detected

Samples taken at slaughter

At the end of the experiment, rams (three rams per treatment) were fasted for 18 hours and taken to the abattoir. They were subsequently killed by electric stunning followed by exsanguination. One testis (including the epididymis) of each ram was collected and stored on ice until transported to the laboratory. After arriving at the laboratory, the testis with the epididymis attached was isolated from the scrotum. After that, epididymal semen was extracted and put in an eppendorf tube and stored at -20 °C for fatty acid analysis.

Gas liquid chromatography of fatty acid methyl esters (FAME): Fatty acid methyl esters were analyzed by gas chromatography using a Hewlett Packard HP 6890 plus GC, an Agilent 7683 series auto injector and equipped with a Varian CPSl88 fused silica capillary column (100 x 0.25mm film thickness). Helium was used as a carrier gas at a constant flow rate of 0.5/min and injection was used. The oven temperature was at 160º C then programmed to increase gradually from 160 °C to 220 °C at a rate of 1.5 °C/min, hold for 10 min then increase from 220 °C to 230 °C at a rate of 5.0 °C/min. The fatty acids were identified by comparison with a marine FAME reference mixture (Restec, Dorset, UK). Fatty acids were identified on the basis of their retention time within the capillary column. Data was collected on a Varian workstation and the % area below each of the peaks was calculated and expressed as a % of total peak area. Quantities of individual fatty acids were calculated as g/kg of total fatty acids using the peak areas.

Statistical analysis

A simple one-way Analysis of Variance Comparison of epididymal semen PUFA content between treatments was calculated by one-way ANOVAs using (Genstat 9, Lawes Agricultural Trust).

Results

Diet fatty acid composition: Fatty acid composition for supplement diets containing different fat sources are presented in Table 2. The Megalac diets contained the highest concentrations of palmitic acid C16: 0). The mean intake of C16:0 in rams offered the Megalac diet was 15.3 g/d; this was approximately two times greater than values for the fish oil and fish oil with GLM powder diets. The mean intakes of C20:5 *n*-3 were 0.2, 0.4, 1.8 and 2.1 g/d for the Megalac, Megalac with GLM powder, fish oil and fish oil with GLM powder diets respectively. The intakes of the C22: 6 *n*-3 by rams receiving the Megalac with GLM, fish oil and fish oil with GLM powder diets were on average 0.2, 0.6, and 0.7 g/d respectively, whilst their intakes were negligible for the Megalac supplemented diets

Fatty acid composition of epididymal semen

The fatty acid composition of epididymal semen is displayed in Table 3. There were no significant treatment differences in the proportion of C14:0, C16:0, C16:1, C18:0, C18:1 *n*-9, C18:2 *n*-6, C18:3 *n*-3, C20:4 *n*-6, C22:6 *n*-3, remaining fatty acids, total fatty acids, ∑SFAs, ∑MUFAs, ∑PUFAs and P/S ratio in epididymal semen fatty acids. The concentration of C20:5 *n*-3 in the semen of rams offered diets containing fish oil with GLM were significantly greater (p=0.03) than those observed in rams receiving the Megalac diets. However, the Megalac diet caused a significantly greater (p=0.02) in the *n*-6/*n*-3 ratio compared to that observed in rams receiving the fish oil and fish oil with GLM diets.

Table 2: Fatty acid composition for supplement diets containing different fat sources

Fatty Acid Concentrations (g/100g TFA)	Megalac	Megalac + GLM	Fish oil	Fish oil + GLM Fish oil + GLM
C14:0	0.9	1.0	0.5	0.5
C16:0	35.0	31.4	13.1	12.5
C16:1	0.2	0.3	1.9	1.9
C18:0	3.7	3.8	5.7	5.6
C18:1 *n*-9	27.2	30.0	23.2	23.9
C18:1 *n*-7	0.9	0.9	3.9	3.8
C18:2 *n*-6	16.4	15.6	26.6	25.5
C18:3 *n*-3	7.3	6.5	4.0	3.9
C20:4 *n*-6	0.4	0.4	0.3	0.3
C20:5 *n*-3	0.4	0.9	3.2	3.2
C22:6 *n*-3	ns	0.4	1.1	1.0

ns.: not detected

Table 3: Effects of dietary fat source on the fatty acid composition (g/100g total fatty acids) of epididymal semen from Suffolk rams (n = 3)

		Treatment				
FA composition (g/100g TFA)	**Megalac**	**Megalac + GLM**	**Fish oil**	**Fish oil + GLM**	**SED**	**(P)**
C14:0	11.1	11.6	10.8	10.1	1.77	NS
C16:0	18.3	17.7	17.5	17.0	1.61	NS
C16:1	0.1	0.1	0.1	0.1	0.04	NS
C18:0	7.4	7.5	6.7	6.0	0.55	NS
C18:1 *n*-9	1.3	1.2	1.1	1.2	0.10	NS
C18:2 *n*-6	2.3	2.2	1.6	1.7	0.33	NS
C18:3 *n*-3	0.03	0.03	0.05	0.04	0.17	NS
C20:4 *n*-6	4.1	3.8	3.1	3.6	0.49	NS
C20:5 *n*-3	0.11^{a}	0.23^{a}	0.50^{ab}	1.14^{b}	0.30	0.03
C22:6 *n*-3	34.8	37.3	37.8	38.2	2.29	NS
Remaining Fatty acids	20.2	18.4	20.6	21.0	3.12	NS

Mean values with different superscripts are significantly different. NS = not significant, SED = standard error of the difference, GLM = Green Lipped Mussel Extract

Discussion

Fatty acid composition of epididymal semen

The epididymis is the first external duct leading from the testis. The role of the epididymis in sperm maturation has been demonstrated by several studies. Changes in the membrane lipid of spermatozoa during epididymal transit have been reported on several animal species (Nikolopoulou *et al*., 1985) [8]. The results of this study are in agreement with earlier studies which had shown both that ram and bull spermatozoa lose 25-50% of their phospholipid during the epididymal transit (Scott *et al*., 1967: Poulos *et al*., 1975) [13, 14]. The most pronounced effect of feeding the fish oil diets was an increase in fatty acid concentration of C20:5 *n*-3 compared with Megalac diet.

References

1. Amann RP, Hammerstedt RH, Veeramachaneni DN. The epididymis and sperm maturation: A perspective. Reprod Fertil Dev. 1993;5(4):361-81. Epub 1993/01/01.
2. Blesbois E, Lessire M, Grasseau I, Hallouis JM, Hermier D. Effect of dietary fat on the fatty acid composition and fertilizing ability of fowl semen, Biology of Reproduction. 1997;56:1216-1220.
3. Conquer JA, Martin JB, Tummon I, Watson L, Tekpetey F. Effect of DHA supplementation on DHA status and sperm motility in asthenozoospermic males. Lipids. 2000;35:149-54.
4. Drokin SI, Vaisberg TN, Kopeika EF, Miteva KD, Pironecheva GL. Effect of cryopreservation on lipids and some physiological features of spermatozoa from rams pastured in highlands and in valleys. Cytobios journal. 1999;100:27-36.
5. Fouchecourt S, Metayer S, Locatelli A, Dacheux F, Dacheux JL. Stallion epididymal fluid proteome: qualitative and quantitative characterization; secretion and dynamic changes of major proteins. Biol Reprod. 2000;62(6):1790-803. Epub 2000/05/20.
6. Hall JC, Hadley J, Doman T. Correlation between changes in Rat sperm membrane Lipid, Protein, and the membranephysical state during epididymal maturation. Journal of Andrology. 1991;12:76-87.
7. Jervis KM, Robaire B. Dynamic changes in gene expression along the rat epididymis. Biol Reprod. 2001;65(3):696-703. Epub 2001/08/22.
8. Nikolopoulou M, Soueek DA, Vary JC. Changes in the lipid content of boar sperm plasma membranes during epididymal maturation. Biochemica Biophysica Acta. 1985;815:486-498.
9. Nissen HP, Kreysel HW. Polyunsaturated fatty acids in relation to sperm motility. Androloglia. 1983;15:264-269.
10. Penny P, Noble R, Maldjian A, Cerolini S. Potential role of lipids for the enhancement of boar fertility and fecundity. Pig News Inf. 2000;21:119N-126N.
11. Rooke JA, Shao CC, Speake BK. Effect of feeding tuna oil on the lipid composition of pig spermatozoa and *in vitro* characteristics of semen. Reproduction. 2001;121:315-322.
12. Voglmayr JK. Metabolic changes in spermatozoa during epididymal transit. In Handbook of Physiology. 1975;5:437-452.
13. Scott RL. On viewing rhetoric as epistemic. Communication Studies. 1967 Feb 1;18(1):9-17.
14. Poulos HG. Torsional response of piles. Journal of the Geotechnical Engineering Division. 1975 Oct;101(10):1019-35.