EFFECT OF DIETARY α-LINOLENIC ACID ON THE SEMEN CHARACTERISTICS OF RABBIT BUCKS

CASTELLINI C., DAL BOSCO A., CARDINALI R.

Dipartimento di Scienze Zootecniche, 06123, Perugia, Italy

ABSTRACT

The aim of the study was to investigate the effect of detary 18:3 n3 fatty acid on the reproductive performance and oxidative status of young rabbit bucks. Twenty New Zealand White rabbit bucks (7-10 months of age) were assigned to two different diets: Control and LNA (supplemented with 5% extruded flaxseed and supranutritional level of vitamin E - 200 mg kg^{-1}). The reproductive performance and oxidative status were evaluated. The diet affected many seminal traits and the bucks of control group showed the lowest values of live cells and Eosine Exclusion Test $^{+}$, VCL, ALH and spermatozoa responsive to Hypo-osmotic Swelling Test. In conclusion this study shows that it is possible to improve the quality of rabbit spermatozoa through dietary supplementation of α-linolenic acid and the reduction of oxidative stability does not affect the reaction of spermatozoa to exogenous stimuli.

Key words: rabbit spermatozoa; oxidative status; n-3 fatty acids.

INTRODUCTION

Polyunsaturated fatty acids of n-3 series are essential for the reproductive activity representing about 30-50% of total amount in the membrane of mammal spermatozoa (POULOS *et al*., 1973) and contributing to regulate fluidity and acrosome responsiveness. Many authors (BLESBOIS *et al*., 1997; ROOKE *et al*., 2001), reported that an increase of n-3 in the sperm membrane by dietary supplementation resulted in improved spermatozoa characteristics. However, such enrichment simultaneously increases the susceptibility of spermatozoa to peroxidation, which is one of the major causes of male infertility (JONES *et al*., 1979; AITKEN *et al*., 1993). Thus, to ensure suitable sperm membrane function it is crucial to maintain an equilibrium between the level of unsaturation and the oxidative stability of semen (COMHAIRE *et al*., 2000; CASTELLINI *et al*., 2003).

The aim of the present study was to evaluate the effect of different dietary level of α-linolenic on the reproductive performance and oxidative status of rabbit bucks.

MATERIAL AND METHODS

The trial was carried out in the experimental rabbitry of the Department of Animal Science with a photoperiod of 16 hours light/day and a temperature of 18.5±1.2 °C.
Two groups of 10 New Zealand White male rabbits were used. Each group was further divided and fed *ad libitum* as follows:

- Standard diet (Control group);
- Standard diet +5% extruded flaxseed (Omegalest®, Valorex, LNA group);

Extruded flaxseed (2630 kcal DE/kg) contained about 56% of C18:3n-3 and LNA diet was supplemented with 200 IU of α-tocopheryl to guarantee a protection against the oxidative processes (CASTELLINI *et al*., 2003).

After a preliminary period of 2 months, semen collection was done weekly for 5 consecutive times using an artificial vagina.

Immediately after collection, the amount of ejaculate (mL) and the spermatozoa concentration (number of sperms mL^{-1}) were recorded by using respectively a graduated tube and an haemocytometer.

Successively, semen samples were diluted 1:2 with Tyrode's modified medium - 296 mOsm g^{-1}, pH 7.0. On aliquots of individual semen samples, kinetic and morpho-functional characteristics of spermatozoa were evaluated, whereas chemical analysis was performed on the remaining pooled portions.

The sperm membrane integrity was evaluated by a cumulative test using Eosine Exclusion Test (EET) and Hypo-osmotic Swelling Test (HOS) according to DUCCI *et al*. (2002). The test permitted the identification of four types of spermatozoa:

- sperm curled but not stained (HOS^+ EET^-);
- sperm curled and stained red (HOS^+ EET^+);
- linear sperm stained red (HOS^- EET^+);
- linear sperm not stained (HOS^- EET^-).

The sum of HOS^+ (spermatozoa reactive to hyposmotic stimuli) and EET^+ (dead spermatozoa) were also calculated.

The spontaneous and the induced acrosome reaction by Lysophosfatidil-coline (100 μg mL^{-1}) was assessed by the procedure of MENDOZA *et* al. (1992).

Chemical analyses of diets were done according to AOAC methods (1995). Fatty acids were determined on lipids extracted from samples of about 5 g in a homogeniser with 20

mL of 2:1 chloroform:methanol (FOLCH *et al*., 1957).

TBA-RS (Thiobarbituric Acid Reactive Substances) were evaluated on samples of fresh and 24h stored semen at 4°C. Briefly, the peroxidation was induced on 1 mL of semen (50 x 10^6 spermatozoa mL^{-1}) with ferrous sulphate (0.2 mM) and sodium ascorbate (1 mM) at 37 °C for 1 h using the procedure of AITKEN *et al*. (1994).

Since the collection time was not significant it was omitted from the analysis. Results are presented as least square means and pooled standard error of the means (SEM). Significance of differences was performed by a t-test (PROC GLM-SAS 1990).

RESULTS AND DISCUSSION

The chemical composition and the nutritional value of the diets (Table 1) were consistent with the standard requirements for rabbits (DE BLAS and WISEMAN, 1998).

The diets differed mainly for the fatty acid profile (Table 2): control diet showed high percentages of MUFA and PUFAn-6; whereas, as expected, LNA diet had higher level of C18:3n-3 and n-3/n-6 ratio.

The diet affected many seminal traits: the control bucks showed the lowest values of live cells and EET^-, VCL, ALH and spermatozoa responsive to HOS (Table 3).

Such results are consistent with the hypothesis, verified in other studies (CASTELLINI *et al*., 2003; CONQUER *et al*., 2000), that n-3 supplementation increases the phospholipid into spermatozoa and modifies the fatty acid profile of membrane. Presumably, this n-3 enrichment occurred mainly in the tail (CONNOR *et a*l., 1998) and thus the increase of sperm velocity and ALH would be explained by the higher flexibility of tail. The higher fluidity and functionality of tail membrane (DUCCI *et al*., 2002), is also suggested by the better reaction of LNA spermatozoa to hyposmotic solution (HOS).

The diet also affected the oxidative status of semen: LNA group, although protected by higher amount of vitamin E, showed higher TBA-RS values both on fresh and stored semen samples. However, the lower antioxidant stability of LNA group did not determine any anomalous acrosomal behaviour and did not induce any changes to the response of spermatozoa to activating stimuli.

In previous work (CASTELLINI *et al*., 2003), the supplementation of refined fish oil (2%) to rabbit bucks, beside the increase of LCP n-3 in spermatozoa membrane, resulted in a much higher TBA-RS value (+30%) and acrosome reacted sperms (+35%).

The present findings suggests that LNA is a more physiological source of n-3 fatty acids for

rabbit and that the increase of spermatozoa kinetics and functional property is obtained without severe depression of antioxidant stability of semen.

Table 1. Formulation and chemical analysis of the diets

		Control	LNA
Ingredients			
Dehydrated alfalfa meal	G kg^{-1}	350	350
Soybean meal 44%	"	130	130
Corn meal	"	230	230
Whole sunflower meal	"	80	-
Extruded flaxseed	"	-	50
Barley meal	"	150	150
Wheat bran	"	50	80
Beet molasses	"	10	10
Calcium carbonate	"	7	7
Calcium diphosphate	"	13.5	13.5
Salt	"	7	7
Dl-methionine	"	0.5	0.5
Vitamin-mineral premix †	"	10	10
α-tocopheryl-acetate	mg kg^{-1}	-	150
Analytical data			
Dry matter		89.2	89.8
Crude protein	%	17.3	17.2
Ether extract	"	5.3	5.2
Crude fibre	"	14.9	14.8
Ash	"	8.8	8.6
Digestible energy*	MJ kg^{-1}	10.9	10.6
α-tocopherol	mg kg^{-1}	85	285

† per kg diet: Vit. A 11,000 UI; Vit. D3 2,000 UI; Vit. B1 2.5 mg; Vit. B2 4 mg; Vit. B6 1.25 mg; Vit. B12 0.01 mg; α-tocopheryl acetate 50 mg; Biotine 0.06 mg; Vit. K 2.5 mg; Niacine15 mg; Folic acid 0.30 mg; D-panthotenic acid 10 mg; Choline 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg.
* Estimated according to Martens et al. (1984)

CONCLUSIONS

In conclusion this study shows that:

- it is possible to improve the functional and kinetics property of rabbit spermatozoa through dietary supplementation of α-linolenic acid;
- the reduction of oxidative stability does not produce effect on the reaction of spermatozoa to exogenous stimuli.

Table 2. Fatty acid profile of the diets (%)

	Control	LNA
SFA	19.76	18.54
MUFA	18.46	17.14
PUFA	61.78	64.32
n-6	39.27	33.42
n-3	22.51	30.90
LNA	22.20	30.55
LCPn-3	0.31	0.35
n-3/n-6	0.57	0.92

TABLE 3 Effect of dietary supplementation on libido and semen characteristics

Treatment		Control	LNA	SEM	N
Libido	sec	7	7	3	100
Volume of ejaculate	ml	0.72	0.71	0.04	100
Concentration	no.x10^6 ml^{-1}	442	386	122	100
Live cells	%	71.8[a]	81.9[b]	10.3	100
Motile cells	"	70.9	71.8	16.8	600
VCL	μm sec^{-1}	108.3[a]	123.0[b]	29.1	600
LIN	%	48.1	43.9	10.8	600
ALH	μm	3.60[a]	4.12[b]	0.4	600
Sperm curled, not stained (HOS^+ EET^-)	%	47.7[a]	56.9[b]	5.4	100
Sperm curled, stained red (HOS^+ EET^+)	"	9.9[b]	6.9[a]	1.2	100
Linear sperm, stained red (HOS^- EET^+)	"	18.3[b]	11.1[a]	2.3	100
Linear sperm, not stained (HOS^- EET^-)	"	24.1	25.0	2.9	100
Spontaneous acrosome-reacted sperm	%	16.9	17.0	4.5	100
Induced acrosome-reacted sperm	%	35.1	32.4	8.8	100
TBA-RS 0 h	nmol/MDA/$sperm^8$	2.14[a]	2.85[b]	0.2	20
TBA-RS 24 h	"	3.16[a]	3.75[b]	0.3	20

Means in the same row with no common letters are significantly different (a..b: P<0.05).

AKNOWLEDGEMENTS:

research funded by MURST ex 40%. Thanks to Marina Moschini, Giacinta Telera and Paolo Lattaioli for technical assistance.

REFERENCES

AITKEN R.J., FISHER H. (1994). Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. Bioassays, **16**, 259-267.

AITKEN R.J., HARKISS D., BUCKINGHAM D. (1993). Relationship between iron-catalysed lipid peroxidation potential and human sperm function. J. Reprod. Fertil., **98**, 257-65.

AOAC 1995. Official Methods of Analysis. 15th Ed. AOAC, Washington, DC, USA.

BLESBOIS E., LESSIRE M., GRASSEAU I., HALLOUIS J.M., HERMIER D. (1997). Effect of dietary fat on the fatty acid composition and fertilizing ability of fowl semen. Biol. Reprod., **56**, 1216-1220.

CASTELLINI C., DAL BOSCO A., MUGNAI C. (2003). Oxidative status and semen characteristics of rabbit bucks as affected by dietary vitamin E, C and n-3 fatty acids. Reprod Nutr. Dev., **43**, 41-53.

COMHAIRE FH, CHRISTOPHE AB, ZALATA AA, DHOOGE WS, MAHMOUD AMA, DEPUYDT CE. (2000). The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prost. Leukotr. Ess. Fatty Acids, **63**, 159-165

CONQUER J.A., MARTIN J.B., TUMMON I., WATSON L., TEKPETEY F. (2000). Effect of DHA supplementation on DHA status and sperm motility in asthenozoospermic males. Lipids, **35**, 149-154.

DE BLAS C., WISEMAN J. (1998). The nutrition of the rabbit. CABI Oxon, UK.

DUCCI, M., GAZZANO, A. VILLANI, C. CELA, V. ARTINI, PG., MARTELLI, F. GENAZZANI, A.R. (2002). Membrane integrity evaluation in rabbit spermatozoa. Europ. J. Ostetr. Gynec. Repr. Biol., **102**, 1, 53-56.

JONES R., MANN T., SHERINS R. (1979). Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal, properties of fatty acid peroxides, and protective action of seminal plasma. Fertil. Steril., **31**, 78-85.

FOLCH J., LEES M., SLOANE-STANLEY H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., **226**, 497-502.

MAERTENS L., MOERMANS R., DE GROOTE G. (1984). Prediction of apparent digestible energy content of commercial pelletted feeds for rabbits. J. Appl. Rabbit Res., **11**, 60-67.

MENDOZA C., CARRERAS A., MOOS J., TESARIK J. (1992). Distinction between true acrosome reaction and degenerative acrosome loss by a one-step staining method using *Pisum sativum* agglutinin, J. Repr. Fertil., **95**, 755-763.

POULOS A., DARIN-BENNET A., WHITE I.G. (1973) The phospholipid-bound fatty acids and aldehydes of mammalian spermatozoa. Comp. Biochem. **46**, 541-549.

SAS/STAT 1990. User's guide, Cary, NC, USA.

ROOKE J.A., SHAO C.C., SPEAKE B.K. (2001). Effect of feeding tuna oil on the lipid composition of pig spermatozoa and in vitro characteristics of semen. Reproduction, **121**, 315-322.