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Effect OF Dietary Alpha-Linolenic Acid On Reproductive Performance And The Semen Characteristics Of Rabbit Males

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ABSTRACT

This study was carried out in the Animal Production Experimental farm Faculty of Agriculture, South Valley University, during the period from January to April 2018. The aim of the present study was to evaluate the effect of different dietary level of a-linolenic on the semen characteristics of the male rabbits of New Zealand White. The aim of the present study was to evaluate the effect of different dietary level of a-linolenic on the semen characteristics of the male rabbits of New Zealand White. Used Thirty New Zealand White rabbit males were divided into three groups. First group; males were fed control ration ,second group males were fed tested one ration ; (supplemented with 4% extruded flaxseed) and third group males were fed tested ration two; (supplemented with 6% extruded flaxseed). The reproductive performances and semen characteristics of the male rabbits of New Zealand White were evaluated . The diet affected many seminal traits and the males of control group showed the lowest values of live cells and spermatozoa concentration. The linoleic (18:3, n-3) fatty acids in the diet (supplemented with 4 % or 6 % extruded flaxseed) was to increased significantly their levels within the spermatozoa and to have extensive beneficial effects on parameters of spermatozoa function and therefore male fertility in the New Zealand White rabbit males .

KEYWORDS: Reproductive performance; rabbit spermatozoa; n-3 fatty acids.

1. INTRODUCTION

There is an overwhelming preponderance of linoleic (18:3, n-3) in proprietary feeds of domestic farm animals. Other fatty acids of the n-6 series are notable by their virtual absence. That such a predominance of linoleic acid may not always be wholly beneficial to the well-being and health of the animal through effects upon tissue fatty acid composition and aspects of metabolism is now being asked. With such a high-profile presence of long chain polyunsaturated fatty acids of the n-3 series in mammalian sperm lipids, it is suggested that the alteration of the current fatty acid profile of animal proprietary feeds towards increasing levels of acids of the n-3 series may be highly relevant to the ontogeny of the characteristic fatty acid profiles and subsequent function of the sperm. The need exists to extend the life of semen for a fresh delivery service and enhance the ability to maintain spermatozoa function during the following cryoscopic storage in all farm animal species. All animal species' spermatozoa have high concentrations of polyunsaturated phospholipids. In mammalian species e.g. the bull, buffalo, ram and rabbit males the substantial level of polyunsaturated present is characteristically dominated by the alpha-linolenic acid (18:3, n-3) series .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). The lipid composition of the spermatozoan membrane may be a major determinant of motility, cold sensitivity and a wide selection of factors associated with overall viability within fresh ejaculates or stored ejaculates maintained at –196 c degree for artificial insemination. 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). Flaxseed oil are quite a few different herbs that males can take to increase their fertility. One such herb which plays a role in promoting male fertility is flaxseed oil. The ingredients within flaxseed oil help

to keep the sperm healthy and may also help with regard to male impotence. The aim of the present study was to evaluate the effect of different dietary level of a-linolenic on the semen characteristics of the male rabbits of New Zealand White.

2. MATERIALS AND METHODS

This study was carried out in the Animal Production Experimental farm Faculty of Agriculture, South Valley University, during the period from January to April 2018.

2.1. Animals :-

A total of 30 male rabbits of New Zealand White breed were used. After weaning (at four weeks of age), the rabbits were housed in individual cages (with a photoperiod of 16 hours light: 9 hours dark and temperatures ranging from (18.6 c to 2 4.4 c) in the same room, receiving ration and water ad libitum .

2.2. Diets :-

Animals are fed ad libitum with commercial rabbit pellets. Three groups of 10 New Zealand White male rabbits were used. Each group was further divided and fed ad libitum as follows: T1 standard diet (Control group);

T2 standard diet+4 % extruded flaxseed(Second group fed tested one ration);

T3 standard diet + 6% extruded flaxseed (Third group fed tested two ration) ;

Extruded flaxseed (2630 kcal DE/kg) contained about 56% of C18:3n-3 (Castellini et al., 2003) as described in Table1.Chemical analyses of diets were done according to A.O.A.C. methods (2015). 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).

2.3.Semen collection and analysis

At five months of age males started the training period with artificial vagina. One ejaculated was collected per male and week. At six months of age they started production period. During7 weeks, weekly two ejaculates per male and week were collected, with an interval of 30 minutes between them. All ejaculates were stored at 37.° C in a water bath until evaluation, non-later than 15 minutes after collection. Volume and pH of the ejaculate were determined by using a graduated tube and a pH-meter 507 Crison, respectively. Immediately after collection, the amount of ejaculate (ml) and the spermatozoa concentration (number of sperms ml) were recorded by using respectively a graduated tube and an haemocytometer . Mass motility (Mm) was assessed in a subjective scale from1to 5, using aliquots (10.l) of the raw semen and a light microscope (Nikon) at x10.

2.4.Statistical analyses

Analysis of variance was carried out using SAS (2011), and the treatment means (spermatozoa mass volume and volume of ejaculate) compared by the test of Duncan (1955).

3. RESULTS AND DISCUSSION

The chemical composition and the nutritional value of the diets (Table 2) 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, Treatment diet had higher level of C18:3n-3 and n-3/n-6 ratio. The diet affected many seminal traits: the control males showed the lowest values of live cells and spermatozoa concentration (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 al., 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 males, beside the increase of LCP n-3 in spermatozoa membrane, resulted in a much higher TBA-RS value (+30%) and acrosome reacted sperms (+35%).Although in the case of the male rabbits these was a marked difference in the levels attainable within the spermatozoa of the long chain n-3 polyunsaturates, nevertheless effects on spermatozoa parameters were very positive. 22:6 (n-3) is an extensively available fatty acid (Castellini et al., 2003) That such a predominance of linoleic acid may not always be wholly beneficial to the well-being and health of the animal through effects upon tissue fatty acid

Table 1. Formulation and chemical composition of the diets (g/kg)

Ingredients %	Control	tested one ration	tested two ration
Alfalfa hay %	342	342	342
Soybean meal (44% CP) "	125	125	125
Corn meal "	225	225	225
Whole sunflower meal "	70	30	10
Extruded flaxseed "	-	40	60
Barley meal "	140	140	140
Wheat bran "	50	50	50
Beet molasses "	12	12	12
Calcium carbonate "	13.72	13.72	13.72
Calcium diphosphate "	6.71	6.71	6.71
Sodium chloride "	5	5	5
Dl-methionine "	0.57	0.57	0.57
Vitamin-mineral premix*"	10	10	10
Total	1000	1000	1000
Calculated chemical composition of the diets .			
Nutrient or energy	Percentage or kcal/kg		
Dry matter %	89.2	89.8	89.5
Crude protein "	17.3	17.2	17.00
Ether extract "	5.3	5.2	5.3
Crude fibre "	14.9	14.8	14.8
Ash "	8.8	8.6	8.7
Digestible energy kcal/kg	2.610	2600	2600
Digestible energy** MJ kg-1	10.9	10.6	10.7

* 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; Alpha-tocopheryl acetate 50 mg; Biotine 0.06 mg; Vit. K 2.5 mg; Niacine15 mg; Folic acid 0.30 mg; Dpanthotenic 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)

Tables 2. Fatty acid profile of the diets % .

	Alpha linolenic acid level		
Items	0.0 control	4% extruded flaxseed	6% extruded flaxseed
SFA	19.89± 0.76a	19.22 ±0.68a	19.01 ± 0.76a
MUFA	18.46± 0.28a	17.81 ±0.34ab	17.14 ± 0.31b
PUFA	61.78± 0.49c	62.90 ±0.19b	64.22 ± 0.10a
Linoleic(18:2, n-6)	39.27± 0.38a	35.73 0.39b	33.42 ± 0.39c
Alpha linolenic (18:3, n-3)	22.51± 0.68c	27.26 ±0.57b	30.82 ± 0.42a
n-3/n-6	0.56± 0.02b	0.76 ±0.02b	00.97 ± 0.03a

a,b means in the same row followed by the same letter are not significantly different (p>0.05) .

Tables 3. Effect of dietary Alpha linolenic acid (18:3, n-3) supplementation semen characteristics of the male rabbits of New Zealand White.

	Alpha linolenic acid level		
Items	0.0 control	4% extruded flaxseed	6% extruded flaxseed
Ejaculate volume (ml)	0.63± 0.01a	0.61± 0.01a	0.60± 0.01a
Concentration /ml ($x10^6$)	442.0±8.50b	401.0±5.77a	384.0± 5.79a
Semen motility (%)	70.81±0.81a	71.59 ±1.25a	71.18±0.90a
Live cells	71.80±0.67b	81.90 ±0.89a	84.10 ±1.06a

a,b means in the same row followed by the same letter are not significantly different (p>0.05) .

composition and aspects of metabolism is now being asked. With such a high-profile presence of long chain polyunsaturated fatty acids of the n-3 series in mammalian sperm lipids, it is suggested that the

alteration of the current fatty acid profile of animal proprietary feeds towards increasing levels of acids of the n-3 series may be highly relevant to the ontogeny of the characteristic fatty acid profiles and subsequent function of the sperm (Ducci et al., 2002). 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.

4. CONCLUSIONS

The inclusion of the Alpha linolenic (18:3, n-3) fatty acids (supplemented with 4 % or 6 % extruded flaxseed) in the diet was to increase significantly their levels within the spermatozoa and to have extensive beneficial effects on parameters of spermatozoa function and therefore male fertility in the New Zealand White rabbit males.

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الملخص العربى

تأثير حمض اللينولينيك فى الغذاء على الاداء التناسلى وصفات السائل المنوى فى ذكور الارانب

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أجريت هذه الدراسة بمزرعة الإنتاج الحيواني كلية الزراعة جامعة جنوب الوادي بقنا خلال من شهر يناير الى أبريل ٢٠١٤ لدراسة تأثير حمض اللينولينلك على صفات السائل المنوي في ذكور الأرانب واستخدم في هذه الدراسة ثلاثون من ذكور الأرانب (النيوزيلندي) ووزعت الحيوانات عشوائيا تبعا لأوزانها وأعمارها في ثلاثة مجموعات متساوية عشرة في كل مجموعة. الأولى مقارنة حيث غذيت فيها الحيوانات على العليقة الأساسية، الثانية غذيت الحيوانات على العليقة الأساسية+ ٤% زيت بذرة الكتان أما الثالثة فغذيت الحيوانات على العليقة الأساسية + ٦% زيت بذرة الكتان وتم جمع السائل المنوي مرة كل أسبوع طوال فترة التجربة من كل حيوان على حدة بمعدل قذفتين فى كل مرة. وأجريت الاختبارات الطبيعية على قذفات السائل المنوى لدراسة خصائصه(الحجم–الحيوية–التركيز –الحى والميت). واتضح من الدراسة عدم وجود فروق معنوية فى حجم القذفة بين مجموعتى المعاملة ومجموعة الكنترول أو بين مجموعتى المعاملة. أما بالنسبة لتركيز الحيوانات المنوية/مل فى السائل المنوى كانت الفروق معنوية عند مستوى معنوية ٥ % بين مجموعتى المعاملة ومجموعة المقارنة. أما نسبة الحى والميت من الحيوانات المنوية كانت الفروق بين مجموعتى المعاملة ومجموعة المقارنة معنوية عند مستوى معنوية ٥% ولم يكن هنالك فروق معنوية بين استخدام مستوى٤% زيت بذرة الكتان و٦% زيت بذرة الكتان . من هذه النتائج يتضح أن اضافة زيت بذرة الكتان الى عليقة ذكور الارانب النيوزيلندى ألابيض سواء عند مستوى٤% أو ٦% قد أدى الى تحسين الصفات الطبيعية للسائل المنوى لذكور الارانب النيوزيلندى ألابيض التى تمت دراستها فى هذا البحث.