

EFFECT OF DIFFERENT RUMINALLY UNDEGRADABLE PROTEIN SOURCES ON REPRODUCTIVE PERFORMANCE OF RAMS

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ABSTRACT

The present study aimed to evaluate the effect of partial replacement of 50% concentrate feed mixture protein (CFM) by either *Nigella sativa* meal protein (NSM) or fish meal protein (FM) as plant and animal protein sources, respectively, on reproductive performance of rams. A total of 15 sexually mature Rahmani x Finn rams was divided into three groups, five animals in each. The 1st group was fed berseem hay *ad libitum* along with concentrate feed mixture (Control). In the 2nd and 3rd group, 50% of CFM protein was replaced by equal amount of protein from NSM or FM, respectively. Two successive semen ejaculates were collected twice weekly for two months as a preliminary period and also throughout a main collection period of three months. Semen physical characteristics were determined and total sperm output was calculated in the collected fresh semen. Sexual behavior of each ram was evaluated over 30 minutes once monthly through the entire length of the experimental period using an induced oestral ewe. Relative testicular volume was measured and monthly blood samples were taken for testosterone determination in blood plasma. Results show that ejaculate volume increased ($P<0.05$) by about 29 and 34% in FM and NSM, respectively. Sperm cell concentration increased significantly ($P<0.05$) only in FM group by about 3% and insignificantly about 2.3% in NSM group as compared to the control group. Percentages of sperm motility, live sperm and sperm abnormality improved ($P<0.05$) in the two groups as compared to the control one. Total sperm output, normal and live sperm output significantly ($P<0.05$) increased by about 33.5, 34.8 and 36.7% in rams fed FM diet and about 36.8, 38.4 and 39.0% in rams fed NSM diet, respectively, as compared to the control rams. No significant differences were found among treated groups in the number of mounts, number of ejaculates and in the reaction time. Rams fed FM and NSM needed longer ($P<0.05$) time to be exhausted than the controls by about 15 and 22.4%, respectively. Rams fed NSM had the highest ($P<0.05$) values of testosterone concentration (1.095 ng/ml) in blood plasma, followed by FM group (0.690 ng/ml). The lowest values, however, were recorded in the control (0.588 ng/ml).

In conclusion, the obtained results indicated beneficial effects of feeding rams on NSM and FM on their reproductive performances.

Keywords: Ram, reproductive performance, *Nigella sativa* meal, fish meal.

INTRODUCTION

The *Nigella sativa* seeds (NSS) are commonly used in the Middle East as a condiment and medicine for variety of complaints (Houghton *et al.*, 1995). It is a member of *Ranunculaceae* family and is native to some parts of the Mediterranean region grows well in Egypt. Several attempts have been carried out to introduce NSS as an additive in diet of rabbits, which showed

favorable effects on weight gain and reproductive performance of doe rabbits (Nasr and Attia, 1998) and buck rabbits (Daghash *et al.*, 1999).

With the wide use of extract oils of NSS in the medicinal products in Egypt, there is a great attention towards the evaluation of *Nigella sativa* meal (NSM) as by-product in animal feeding. NSM contains high crude protein content and is considered as a good source of plant protein for ruminants (Awadalla, 1997). It could be used as a relatively good source of dietary energy and protein supplementation. It is characterized by low degradation rate in the rumen (El-Ayek, 1999) and could provide 50% from protein of concentrate feed mixture (CFM) without any reversible effects on nutrient digestibility, nutritive values and growth performance of growing lambs (El-Ayek *et al.*, 1999). This could have economical importance in reducing costs of feeding (El-Ayek *et al.*, 1998 and Gabr *et al.*, 1998).

In different studies, feeding NSM could improve reproductive performance of Egyptian buffalo cows (Youssef *et al.*, 1998) and Barki sheep (El-Ekhnawy *et al.*, 1999), but its effects on reproductive performance of male animals require to be studied. Fish meal (FM) could be used as an animal source of undegradable protein in diets of bulls. The sexual desire improve, however, semen quality did not differ significantly (Rocha *et al.*, 1995).

The present study aimed to investigate the effect of feeding two sources of dietary protein, NSM (plant protein) and FM (animal protein), as 50% replacement of CFM proteins, on semen quality and sexual desire of rams.

MATERIALS AND METHODS

The present study was carried out at Sakha Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

Fifteen healthy sexually mature 50% Rahmani x 50% Finn rams having average body weight of 60 kg were used in this study. Animals were taken from the station herd and were divided randomly into three similar groups according their live body weight, five animals in each group. Animals in the 1st group were fed commercial concentrate feed mixture (CFM) containing 15% uncorticated cotton seed meal, 45% wheat bran, 25% yellow maize, 5% soybean meal, 6% molasses, 3% limestone and 1% common salt. In CFM of the 2nd and 3rd groups 50% of protein content was replaced by the same amount of protein in fish meal (FM) and *Nigella sativa* meal (NSM) as a source of animal or plant protein (undegradable sources), respectively. The CP contents in FM and NSM were found to be partially three folds and double that of CFM, respectively (Table 1). Therefore, in FM and NSM diets, each 100 g CFM were replaced by 23 g FM for the second group and by 50 g NSM for the third group.

Beside CFM, FM and NSM diets, animals in all groups were fed clover hay (CH) *ad libitum*, however, water was available all day. The official methods A.O.A.C. (1985) were used for running the proximate chemical analysis of different feed stuffs used in this study (Table 1). All animals were kept under semi-open sheds in-group feeding and watering. Throughout an

experimental period of five months from July to December 2001, the first two months was considered as preliminary period and the others as a main period for semen collection.

Table (1): Chemical composition of different feed stuffs and CFM used in feeding animals.

Feed stuff	DM%	Chemical analysis on DM basis (%)					
		OM	CP	EE	CF	NFE	Ash
FM	93.7	82.3	43.9	10.5	24.3	3.6	17.7
NSM	91.6	92.1	28.4	12.1	6.2	45.4	7.9
CH	89.1	86.8	12.8	1.6	24.6	47.8	13.2
CFM	89.8	90.7	14.1	2.5	12.8	61.3	9.3

Semen was collected twice weekly by means of artificial vagina at 8:00 h. Seminal volume was recorded. Percentage of sperm gross motility was estimated using a microscope with a warmer stage according to Melrose and Laing (1970). Percentage of live sperm was estimated by testing one hundred sperm cells according to Hancock (1951). The percentage of abnormal spermatozoa was determined in the same smears prepared for live and dead sperm test. Two hundred spermatozoa were considered in each smear for live and dead test. Sperm acrosomal abnormalities were examined by staining films according to the method of Watson (1975). Sperm cell concentration was determined by direct count using Neubauer Haemocytometer. However, total abnormality percentage, total sperm output and normal sperm output were calculated using the following equations:

$$\text{Total abnormality \%} = \text{Abnormal sperm\%} + \text{spermatozoa having damaged acrosome\%}$$

$$\text{Total sperm output (x } 10^9/\text{ejac.)} = \text{Ejaculate volume (ml) x sperm cell concentration (x } 10^9/\text{ml)}$$

$$\text{Normal sperm output (x} 10^9/\text{ejac.)} = 100 - \text{Total abnormality\%} \times \text{Total sperm output (} 10^9/\text{ejac.)} / 100$$

During the main collection period sexual behavior of each ram was evaluated over 30 min once monthly using induced oestral ewe. Parameters of the sexual behavior involved number of mounts and reaction time, exhaustion time and number of ejaculates/exhaustion time and testosterone level. Testicular volume was determined twice monthly by palpation according to Oldham *et al.* (1978). Blood samples were collected from the Jugular vein once monthly during the main collection period and testosterone concentration in blood plasma was performed using active testosterone RIA, DSL-4000 (Lashansky *et al.*, 1991) using a commercial kit (Diagnostic System Laboratories, Inc. USA).

Data of physical semen characteristics of the main collection period were statistically analyzed by the methods of Least Square Analysis of Variance for repeated measurements according to Winer (1971) using general linear model procedures of SAS (1987). Duncan Multiple Range Test was used to test the differences among means (Duncan, 1955). The percentage values of motility, live, abnormal sperm, spermatozoa having

damaged acrosome and total sperm abnormality were adjusted to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS

Semen characteristics of rams:

Data in table 2 show that ejaculate volume increased ($P<0.05$) in rams fed FM and NSM diets by about 29 and 34%, respectively compared with those fed the control diet (CFM). Sperm cell concentration increased significantly ($P<0.05$) only in FM group by about 3% and insignificantly about 2.3% in NSM group as compared to the control group. However, the differences between FM and NSM groups were not significant.

Feeding rams on diets containing FM or NSM resulted in increasing ($P<0.05$) percentages of motility and live sperm. Percentages of sperm abnormality, spermatozoa having damaged acrosome and total sperm abnormality were almost improved ($P<0.05$) as compared to those in the control. The magnitude of improvement was almost larger for rams fed NSM than those fed FM, however, the differences between both groups were not significant (Table 2).

Table (2): Effect of dietary protein source on different physical semen characteristics of rams in different dietary groups.

Characteristics	Dietary group		
	CFM	FM	NSM
Ejaculate volume (ml)	0.65±0.03 ^b	0.84±0.03 ^a	0.87±0.04 ^a
Sperm motility (%)	83.5±0.3 ^b	84.2±0.1 ^a	84.4±0.2 ^a
Live sperm (%)	80.5±0.5 ^b	82.4±0.4 ^a	82.0±0.4 ^a
Abnormal sperm (%)	4.2±0.3 ^a	3.6±0.3 ^b	3.5±0.2 ^b
Damaged acrosome (%)	4.5±0.4 ^a	3.2±0.3 ^b	3.1±0.3 ^b
Total abnormality (%)	7.7±0.4 ^a	6.9±0.3 ^{ab}	6.6±0.3 ^b
Sperm concentration (x10 ⁹ /ml)	2.947±0.02 ^b	3.045±0.03 ^a	3.014±0.03 ^{ab}

a,b Means having different superscripts within the same row are significantly ($P<0.05$) different.

When spermatogenesis was evaluated in terms of total sperm, normal and live sperm output per each ejaculate as affected by source of dietary protein, rams fed FM or NSM showed significantly ($P<0.05$) higher values than those fed the control diet (CFM), being higher in NSM than FM group. However, the differences between the two groups were almost not significant (Fig. 1).

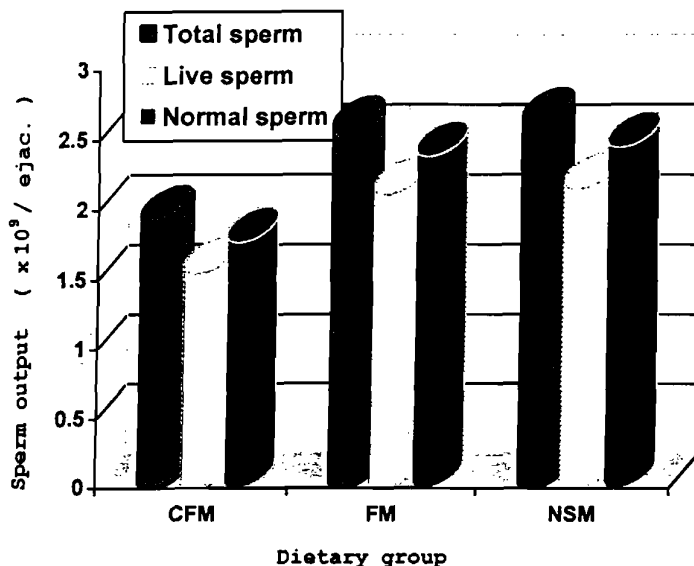


Figure (1): Total, live and normal sperm output (x 10⁹/ejac.) as affected by dietary protein source in different dietary groups.

Sexual desire of rams:

Effect of the source of dietary protein on the sexual desire of rams was studied in term of sexual behavior, testicular volume and testosterone profile of the experimental rams (Table3). The sexual behavior including number of mounts, reaction time and number of ejaculates did not differ significantly between the dietary groups. However, exhaustion time was affected ($P < 0.05$) by source of dietary protein, being longer ($P < 0.05$) by about 18% in NSM group and insignificantly longer by about 15% in FM group than that in the control group. However, the differences between FM and NSM were not significant (Table 3).

Table (3): Sexual behavior, testicular volume and testosterone profile of rams as affected by source of dietary protein.

Sexual behavior	Dietary group		
	CFM	FM	NSM
Reaction time (sec.)	47.2±1	39.6±9	65.0±15
Number of mounts	7.1±1.3	6.2±1.2	6.2±1.0
Number of ejaculates	4.0±0.	4.8±0.4	4.8±0.5
Exhaustion time (min)	10.7±0.9 ^b	12.3±0.9 ^{ab}	13.1±1.0 ^a
Testicular volume (cm ³)	336.7±3.4	337.3±5.5	339.7±7.4
Testosterone level (ng/ml)	0.588±0.07 ^c	0.690±0.05 ^b	1.095±0.08 ^a

a, b, c Means having different superscripts within the same row are significantly ($P < 0.05$) different.

Concerning the testicular volume and testosterone profile, the effect of dietary protein source was significant ($P < 0.05$) only on concentration of testosterone in blood plasma, being higher by about 86% in NSM group and by about 17% in FM group than that in the control group, which showed the lowest values (Table 3).

DISCUSSION

The present study was designed to evaluate the effects of *Nigella sativa* meal (plant source) and fish meal (animal source) as sources of dietary protein having low degradation in the rumen (El-Ayek *et al.*, 1998) on reproductive performance of rams. The significant ($P < 0.05$) increase in ejaculate volume of FM and NSM groups may indicate the beneficial effects of protein sources on the accessory sex glands to produce large volume of the seminal plasma or/and may attributed to the significant ($P < 0.05$) increase in testosterone level in blood plasma of rams fed FM or NSM diets. The accessory glands, seminal vesicles and prostate gland, are functionally controlled by testosterone and may affected by dietary protein source (Abdel-Khalek, *et al.*, 1999). In addition, Massoud *et al.* (1991) mentioned that testosterone stimulates accessory sex glands.

Improving semen quality, in terms of sperm cell concentration ($P < 0.05$) and percentages of sperm motility, live sperm and sperm abnormality ($P < 0.05$) may attributed to that NSS has a protective function against oxidant damage of spermatogonia and spermatocytes membranes through the reduction of endogenously formed hydro-peroxides of unsaturated fatty acids (Fujii, *et al.*, 1984). Also, NSS was found to have inhibiting effects on the accumulation of the toxic products and the harmful agents, non-enzymatic oxidation (Houghton *et al.*, 1995), which are responsible for damage of the cell and DNA as well as brings to irreversible loss of sperm motility due to loss of cytosolic enzyme, adenine and pyridine nucleotides (White, 1993).

Nutritionally, NSM and FM are considered as sources of undegradable plant and animal proteins, respectively (El-Ayek, 1999 and Gabr, *et al.*, 1998). The high ruminal undegradable proteins or protected proteins in diets of ruminants pass into the abomasum and subsequently increase the amino acid yield in the small intestine of the host (Beauchemin *et al.*, 1995 and Tomlinson *et al.*, 1997). Using different levels of protected protein in diets of Friesian bulls (Abdel-Khalek *et al.*, 1999) or NSS in diet of rabbit bucks (Daghash *et al.*, 1999), resulted in improvement in semen quality similar to that obtained in this study. However, Rocha *et al.* (1995) found that feeding bulls on FM diets did not affect semen quality although it is containing omega free fatty acids. However, NSM are containing high content of unsaturated fatty acids e.g., palmitic, oleic and linoleic acids (Al-Gaby, 1992). This may led to acceleration of the metabolic rate of animals fed NSM diets expressed in higher concentration of total lipids as crucial component in energy metabolism of animal (Daghash *et al.*, 1993; Youssef *et al.*, 1998 and Daghash *et al.*, 1999).

The effect of dietary protein source on sexual desire was only significant ($P < 0.05$) on level of testosterone in blood plasma, being higher for NSM than FM diets. This was almost associated with significantly ($P < 0.05$) longer exhaustion time in NSM than FM diets. Feeding Friesian bulls on different levels of dietary protected protein resulted in increasing testosterone level and improving sexual desire of bulls (Abdel-Khalek *et al.*, 1999). In accordance with the present results, Prajapatiet *et al.* (1996) reported an improvement of sexual desire of bulls fed diets containing by-pass proteins.

The significant improvement of semen quality, in association with significant increase in testosterone level of rams fed FM or NSM without any significant effect on the testicular volume may reveal that NSM or FM may affect the testicular histogenesis (Abdel-Khalek *et al.*, 2000) or spermatogenesis (Massoud *et al.*, 1991). The activity of the interstitial cells to produce testosterone was found to be controlled by metabolic rate and was related to the increase of physiological challenges in the rabbit bucks fed NSS (Daghash *et al.*, 1999).

In light on the present results, from the economical point of view NSM as a cheaper source of plant protein may have higher impacts on semen quality of rams and is considered as one of the suitable sources of undegradable dietary proteins for rams used for natural service or semen collection for AI.

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تأثير مصادر مختلفة من البروتين الغير متكسر في الكرش على الأداء التناسلي للكباش

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تهدف الدراسة المقدمة لتقييم تأثير استبدال ٥٠% من بروتين العليقة المركزة المصنعة والمحتوية على كسب القطن غير المقشور و كسب فول الصويا كمصدر للبروتين الغذائى (عليقه المقارنة) بنفس الكمية من بروتينيات ذات معدل منخفض التكسر فى الكرش من مصدر نباتى (عليقه كسب حبة البركة) أو مصدر حيوانى (عليقه مسحوق السمك) على الكفاءة التناسلية للكباش.

أستخدم فى هذه الدراسة ١٥ كباش ناضج جنسيا (رحماني × فلندى) قسمت إلى ثلاث مجاميع متقاربة فى وزن الجسم غذيت على علائق متقاربة فى مستوى الطاقة و البروتين. خلال فترة التغذية تم جمع السائل المنوى من جميع الكباش خلال الشهرين الأوليين كفترة تمهيدية ولمدة ثلاث شهور التالية كفترة أساسية. خلال فترة الجمع تم تقييم الخصائص الطبيعية للسائل المنوى (حجم القذفة- التركيز- النسبة المئوية للمووية لحركة الحيوانات المنوية- النسبة المئوية للمووية للحيوانات المنوية الحية والشاذة وذات الأكروسوم الغير طبيعى). أيضا خلال الفترة الأساسية لجمع السائل المنوى تم تقييم السلوك الجنسي مرة شهريا لكل كباش مع أخذ عينة دم لقياس تركيز هرمون الجنس الذكري (التستوستيرون) فى البلازما وقياس حجم الخصية.

أثناء فترة الدراسة تم استخلاص النتائج الأتية فى المجموعات التجريبية مقارنة بمجموعة المقارنة:

- ١- زاد حجم القذفة بمعدل ٢٩% , ٣٤% و تركيز السائل المنوى معنويا بمعدل ٣% , ٣ و ٢٣% فى الكباش المغذاة على مسحوق السمك و كسب حبة البركة على الترتيب.
- ٢- زادت النسبة المئوية للمووية لحركة الحيوانات المنوية و النسبة المئوية للمووية للحيوانات المنوية الحية و انخفضت النسبة المئوية للمووية للحيوانات المنوية الشاذة معنويا فى الكباش المغذاة على كل من مسحوق السمك و كسب حبة البركة.
- ٣- زاد محتوى كل قذفة منوية من الحيوانات المنوية كنتاج كلى وطبيعى وحى بمعدل ٣٣ و ٣٢% , ٣٤ و ٣٦% فى الكباش المغذاة على مسحوق السمك و بمعدل ٣٦ و ٣٨% , ٣٩% فى الكباش المغذاة على كسب حبة البركة.
- ٤- كان وقت استنفاد القذفات المنوية أطول معنويا فى الكباش المغذاة على مسحوق السمك و كسب حبة البركة بينما زاد مستوى هرمون التستوستيرون معنويا فقط فى الكباش المغذاة على كسب حبة البركة .
- ٥- لم يكن هناك اختلافات معنوية بين الكباش فى عدد الوثبات و وقت التجهيز الجنسي لكل قذفة و عدد القذفات اللازمة للاستفاد .

عموما فان الدراسة المقدمة توضح تحسن الأداء التناسلي للكباش المغذاة على عليقه تحتوى على كسب حبة البركة كبروتين نباتى أو مسحوق السمك كبروتين حيوانى مقارنة ببروتين كسب القطن غير المقشور و كسب فول الصويا فى العليقة المقارنة.