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-Ekhawy 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.

<table>
<thead>
<tr>
<th>Feed stuff</th>
<th>DM%</th>
<th>OM</th>
<th>CP</th>
<th>EE</th>
<th>CF</th>
<th>NFE</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
<td>93.7</td>
<td>82.3</td>
<td>43.9</td>
<td>10.5</td>
<td>24.3</td>
<td>3.6</td>
<td>17.7</td>
</tr>
<tr>
<td>NSM</td>
<td>91.6</td>
<td>92.1</td>
<td>28.4</td>
<td>12.1</td>
<td>6.2</td>
<td>45.4</td>
<td>7.9</td>
</tr>
<tr>
<td>CH</td>
<td>89.1</td>
<td>86.8</td>
<td>12.8</td>
<td>1.6</td>
<td>24.6</td>
<td>47.8</td>
<td>13.2</td>
</tr>
<tr>
<td>CFM</td>
<td>89.8</td>
<td>90.7</td>
<td>14.1</td>
<td>2.5</td>
<td>12.8</td>
<td>61.3</td>
<td>9.3</td>
</tr>
</tbody>
</table>

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:

Total abnormality % = Abnormal sperm% + spermatozoa having damaged acrosome%

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

Normal sperm output (x10^9/ejac.) = 100 - Total abnormality% x Total sperm output (10^9/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.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Dietary group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFM</td>
<td>FM</td>
<td>NSM</td>
</tr>
<tr>
<td>Ejaculate volume (ml)</td>
<td>0.65±0.03b</td>
<td>0.84±0.03a</td>
<td>0.87±0.04a</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>83.5±0.3b</td>
<td>84.2±0.1a</td>
<td>84.4±0.2a</td>
</tr>
<tr>
<td>Live sperm (%)</td>
<td>80.5±0.5b</td>
<td>82.4±0.4a</td>
<td>82.0±0.4a</td>
</tr>
<tr>
<td>Abnormal sperm (%)</td>
<td>4.2±0.3b</td>
<td>3.6±0.3b</td>
<td>3.5±0.2b</td>
</tr>
<tr>
<td>Damaged acrosome (%)</td>
<td>4.5±0.4b</td>
<td>3.2±0.3b</td>
<td>3.1±0.3b</td>
</tr>
<tr>
<td>Total abnormality (%)</td>
<td>7.7±0.4a</td>
<td>6.9±0.3ab</td>
<td>6.6±0.3b</td>
</tr>
<tr>
<td>Sperm concentration (x10^6/ml)</td>
<td>2.947±0.02b</td>
<td>3.045±0.03a</td>
<td>3.014±0.03ab</td>
</tr>
</tbody>
</table>

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 \((x 10^9/\text{ejac.})\) 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 terms of sexual behavior, testicular volume and testosterone profile of the experimental rams (Table 3). 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.**

<table>
<thead>
<tr>
<th>Sexual behavior</th>
<th>Dietary group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFM</td>
</tr>
<tr>
<td>Reaction time (sec.)</td>
<td>47.2±1</td>
</tr>
<tr>
<td>Number of mounts</td>
<td>7.1±1.3</td>
</tr>
<tr>
<td>Number of ejaculates</td>
<td>4.0±0.0</td>
</tr>
<tr>
<td>Exhaustion time (min)</td>
<td>10.7±0.9b</td>
</tr>
<tr>
<td>Testicular volume (cm³)</td>
<td>336.7±3.4</td>
</tr>
<tr>
<td>Testosterone level (ng/ml)</td>
<td>0.588±0.07c</td>
</tr>
</tbody>
</table>

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 (Beauchmin *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, Prajapat 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 has 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.

REFERENCES


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تأثير مصادر مختلفة من البروتينات الغير متكرر في الكربس على الأداء التناسلي
للكلاب

مصطفى الحيبري - بدر الصعيدي - عبد الخالق السيد - و عمان أبو عمرو

1- قسم الإنتاج الحيوي - كلية الزراعة - جامعة المنصورة
2- مهندس الانتاج الحيوي - الدقي - الجهرة

تهديف الدراسة المقدمة لتقدير تأثير استبدال 60% من بروتينات المكونة للجوع حبة البركة (الطية المقرنة) بأغذية متكاملة ملتزمة بالكبد على كربس القطن غير المتكرر و كربس فول الصويا كمصادر البروتينات الخاذية.

استخدمت هذه الدراسة 30 كلب ناضج جنسيًا مثليًا في وزن الجسم غذي معتاد على عائلة مشاركة في مستوى الطاقة و البروتين. خلال فترة الدراسة تم جمع السائل المنوي من جميع الكبار خلال الستة أشهر الأولى ككفاءة من قدرة الجوع. تم تقديم الطعام المذكور (لحم الخنزير، البقول، الأرز، الفاكهة، الخضروات) خلال فترة الأسابيع الأولى من الدراسة. في تلك الأسابيع، تم قياس السائل المنوي من كل كلب بعد إتمام كل فحص. تم قياس السائل المنوي بعد إنهاء كل فحص.

1- فحص للكلاب، و يتم قياس كربس الخنزير، و كربس البقول، و كربس الأرز، و كربس الفاكهة، و كربس الخضروات.

2- خلال فترة الدراسة، تم قياس السائل المنوي من كل كلب بعد إتمام كل فحص. في كلراد، تم قياس كربس الخنزير، و كربس البقول، و كربس الأرز، و كربس الخضروات.

3- كان وقت استعمال الخيارات المتفرقة من السائل المنوي، و تم قياس كربس الخنزير، و كربس البقول، و كربس الأرز، و كربس الخضروات.

4- كان وقت استعمال الخيارات المتفرقة من السائل المنوي، و تم قياس كربس الخنزير، و كربس البقول، و كربس الأرز، و كربس الخضروات.

5- لم يكن هناك اختلافات معنوية بين كلاب، و بعض الهرمونات ككبدية جيني، و بعض الهرمونات ككبدية جيني، و بعض الهرمونات ككبدية جيني، و بعض الهرمونات ككبدية جيني.

عوامل الدراسة المقدمة توضح تحسن الأداء التناسلي للكلاب، و كربس الفول الصويا، و كربس كربس البقول، و كربس كربس الخضروات، و كربس كربس الهرمونات، و كربس كربس الهرمونات، و كربس كربس الهرمونات.