WORLD RABBIT SCIENCE 2000, VOL. 8 (4), 181-184.

# EFFECT OF LYSINE AND METHIONINE ON LIBIDO AND SEMEN CHARACTERISTICS OF BUCKS

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ABSTRACT: The aim of this experiment was to study the influence of dietary lysine and methionine levels on the libido and semen quality of bucks. Forty crossbred Hyla HYLA rabbit males (10-12 months old) were subjected to four diets (15% crude protein) differing in lysine and methionine content (diet A 0.64 and 0.58%; diet B 0.75 and 0.58%; diet C 0.64 and 0.65%; diet D 0.75 and 0.65 of lysine and methionine +cystine, respectively). Two successive ejaculates were collected once a week for 12 consecutive months. Semen evaluations of for volume, colour, pH, motility, concentration and percentage of live spermatozoa were performed every two weeks. Dietary supplementation with lysine and methionine affected few parameters and there was little variation in the values. never experienced large oscillations. Diets with higher lysine contents increased live sperm concentration from 77.1 to 78.2% in the second ejaculate and reduced the necessary interval between mating and ejaculation (sec. 20.8 vs 21.3 and 22.6 vs 23.1 for ejaculates I and II, respectively). Supplementation with methionine increased the motility of ejaculate II (72.7 vs 71.2%) and reduced libido at the first sampling (sec. 21.4 vs 20.6 sec.). No significant differences in the reproduction parameters (fertility -75.2% on average -, total born/litter and born alive itter - 7.7 alive on average) were found for does inseminated with the semen of bucks undergoing dietary treatments (100 AI /treatment).

**RÉSUMÉ : Effets de la lysine et de la méthionine sur la libido et les caractéristiques du sperme du lapin mâle.**
Le but de cette étude a été d'étudier l'influence du taux de lysine et de méthionine dans l'alimentation des lapins mâles sur leur libido et la qualité de leur sperme. Quarante mâles croisés Hyla (âgés de 10 à 12 mois) ont reçu 4 aliments (15 % de protéines brutes) dont les taux de lysine et de méthionine différaient (aliment A : 0,64 et 0,58% ; aliment B : 0,75 et 0,58 % ; aliment C : 0,64 et 0,65 % ; aliment D : 0,75 et 0,65 % de lysine et méthionine + cystine, respectivement. Deux éjaculats successifs ont été récoltés 1 fois par semaine pendant 12 mois consécutifs. L'évaluation du volume, de la couleur, du pH, de la motilité, de la concentration et du pourcentage de spermatozoïdes vivants du sperme a été effectuée toutes les deux semaines. L'addition de lysine et de méthionine dans l'aliment affecte peu de paramètres et les valeurs ne montrent jamais de grandes variations. Les aliments contenant le plus de lysine entraînent une augmentation de la concentration en spermatozoïdes vivants de 77,1 à 78,2 pour le second éjaculat et réduit l'intervalle nécessaire entre le début du comportement de saillie et l'éjaculation (20,8 sec. *vs* 21,3 et 22,6 vs 23,1 pour les éjaculats 1 et 2 respectivement). L'addition de méthionine augmente la motilité de l'éjaculat 2 (72,7 *vs* 71,2 %) mais réduit la libido au premier prélèvement ( 21,4 vs 20,6 sec). Il n'y a pas eu de différence significative enregistrée, concernant la fertilité (75,2% en moyenne), le nombre total de lapereaux nés et le nombre de nés vivants par portée (7,7), chez les lapines inséminées avec le sperme des mâles ayant reçu les aliments expérimentaux (100 IA par lot).

## INTRODUCTION

For over 20 years, artificial insemination (AI) has been used as a reproduction technique in commercial rabbit breeding (PAUFLER *et al.*, 1979; BATTAGLINI *et al.*, 1986; THEAU-CLEMENT and ROUSTAN, 1992).

Much of the rabbit breeding in Italy is conducted on intensive farms which use artificial insemination Because of the many advantages that the proper use of the technique confers in terms of management, profitability, genetics and health. Indeed, AI is a sound policy for the rapid selection of the rabbit gene pool and a rational breeding technique for maximum economic benefits from breeding stock. Obviously, to maximise AI potential, bucks must be selected for good semen quality and quantity. Increase in such production would lead to a rise in the average number of usable doses per ejaculate and thus a reduction in the male/female ratio. Quality and quantity of semen production are affected by many factors (breed, individual, health status, rate of use, environmental conditions, diet, etc.) which, though recognised to be of great importance, have received little scientific attention. This is particularly true for research into the dietary requirements of breeding males (AAVV, 1993; EL MASRY *et al.*, 1994; CASTROVILLI *et al.*, 1995; LUZI *et al.*, 1996). This research was conducted to study diets with different protein and amino-acid levels, and their effects upon rabbit semen characteristics. In an initial trial (NIZZA *et al.*, 2000) we reported results obtained with diets of different protein content. In this paper we report results arising from the use of diets differing in lysine and methionine content.

## MATERIAL AND METHODS

Forty crossbred HYLA rabbit males (10 - 12 months old) were used for 1 year to study the influence of the four diets on semen characteristics. Ingredients and chemical compositions (AOAC, 1984) of the concentrated diet are presented in Table 1. The first group (A) received *ad libutm* a pelleted basal diet. The second group (B) was fed the same basal diet supplemented with 1.1 g lysine/kg. The third group (C) received the basal diet + 0.7 g methionine/kg. The

**Table 1 : Ingredients and chemical composition of the basal diet**

| Ingredients | | |
|---|---|---|
| Wheat middling flour | % | 42.00 |
| Dehydrated lucerne meal | " | 37.00 |
| Barley | " | 10.00 |
| Beet pulp | " | 6.50 |
| Soybean meal | " | 1.70 |
| Limestone | " | 1.00 |
| Vitamin-mineral mix | " | 1.00 |
| Monocalcium phosphate | " | 0.50 |
| Salt | " | 0.20 |
| DL-methionine | " | 0.10 |
| **Chemical composition** | | |
| Crude protein | % | 15.1 |
| Crude fibre | " | 14.8 |
| ADF | " | 18.5 |
| Ash | " | 8.8 |
| Digestible energy | MJ/kg | 9.5 |
| Lysine | % | 0.64 |
| Methionine+cystine | % | 0.58 |

fourth group (D) was fed the basal diet supplemented with 1.1 g lysine and 0.7 g methionine/kg. The supplements were well mixed with barley meal and added to the concentrates throughout the trial.

Ten males were allotted at random to each of the dietsusing a computer program. Males were housed individually in wire flat-deck cages equipped with a nipple drinker and hopper trough. Males were maintained on a 12 h light-12 h dark schedule. . The experimental room was artificially heated and ventilated in order to create optimum environmental conditions ( 18±2°C).

Semen was collected by artificial vagina from each buck weekly for 12 consecutive months. Macro- and microscopic analyses of semen were performed every two weeks. Sexual activity (*libido*) was estimated as the time between introduction of the female into the male's cage and ejaculation.

Semen volume, colour, pH, motility (estimated according to ZEMJANIS, 1970), spermatozoa concentration (using a Burker chamber), percentages of live and abnormal spermatozoa were determined for each sample. Immediately after collection, the semen was transferred to a block heated at 35°C and all manipulations were conducted using warmed glassware. Two smears were prepared. One was stained with eosin-nigrosin (WEITZE and MULLER, 1991) and the other with Giemsa stain as described by WATSON (1975).

Live spermatozoa concentration (x $10^6$/ml) was estimated by counting 200 sperm cells in eosin-nigrosin stained smear. Subsequently, 200 non-stained spermatozoa were evaluated for abnormalities. Spermatozoa were divided into normal cells, spermatozoa with abnormal heads, abnormal tails, proximal protoplasmic droplets and distal protoplasmic droplets. The Giemsa-stained slide was used to evaluate the acrosomal integrity. Two hundred cells was counted and divided into cells with a normal and abnormal acrosomes. All observations throughout the experiment were made by the same observer.

Four hundred multiparous does (HYLA) were inseminated to study the influence of the semen quality on *in vivo* fertility, total kits born/litter and live kits born/litter. Does were inseminated with 20 million spermatozoa 12 days after parturition. .Data were analysed per ejaculate using the GLM procedure (SAS/STAT, 1989) to determine the effects of the different experimental groups (lysine level 0.64-0.75% and methionine level 0.58-0.65%) on libido and semen quality.

## RESULTS AND DISCUSSION

Fed intake (mean of groups: 138 g/d) was not significantly different among diets.

Ejaculates with a high urine percentage and those not followed by a second ejaculation were discarded. In all, 825 ejaculates were analysed statistically (205, 208, 200 and 212 for groups A, B, C, D, respectively). The number of ejaculates was not significantly different among groups.

Table 2 shows the physical characteristics of rabbit semen in relation to the dietary lysine and methionine contents. Variance analysis showed no significant dietary lysine x methionine interactions for the characteristics. However, supplementation with lysine

**Table 2 : Semen characteristics**

| | | Ejaculate | Lysine % | | Methionine % | | Error mean square |
|---|---|---|---|---|---|---|---|
| | | | 0.64 | 0.75 | 0.58 | 0.65 | |
| Spermatozoa | X$10^6$/ml | I | 493.5 | 499.4 | 495.2 | 497.7 | 23319 |
| Spermatozoa | X$10^6$/ml | II | 460.1 | 453.9 | 455.4 | 458.6 | 21096 |
| Volume | ml | I | 0.81 | 0.82 | 0.81 | 0.81 | 0.067 |
| Volume | ml | II | 0.77 | 0.78 | 0.78 | 0.78 | 0.062 |
| Motility | % | I | 69.5 | 70.5 | 69.4 | 70.6 | 122.9 |
| Motility | % | II | 71.4 | 72.5 | $71.2^b$ | $72.7^a$ | 121.0 |
| Live sperm | % | I | 77.6 | 78.0 | 77.8 | 77.8 | 15.9 |
| Live sperm | % | II | $77.1^b$ | $78.2^a$ | 77.3 | 78.0 | 49.7 |
| Libido | sec. | I | $21.3^a$ | $20.8^b$ | $20.6^B$ | $21.4^A$ | 9.84 |
| Libido | sec. | II | $23.1^a$ | $22.6^b$ | 22.8 | 23.0 | 9.35 |
| pH | | I | 7.26 | 7.25 | 7.24 | 7.25 | 0.70 |
| pH | | II | 7.30 | 7.30 | 7.28 | 7.27 | 0.65 |

A,B: P< 0.01; a,b: P< 0.05.

**Table 3 : Reproductive performance from inseminated does**

| Parameter | | Diets | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| Does | n | 106 | 89 | 100 | 105 |
| Fertility | % | 74.5 | 75.3 | 76.0 | 75.2 |
| Total born | n | 8.3 | 8.4 | 8.4 | 8.2 |
| Born living | n | 7.8 | 7.7 | 7.9 | 7.7 |

increased the live sperm concentration from 77.1 to 78.2% ($P < 0.05$) in the second ejaculate

Libido was higher ($P < 0.05$) in groups fed lysine supplemented diets (sec. 20.8 vs 21.3 and 22.6 vs 23.1 for I and II ejaculates, respectively). Supplementation with lysine had no effect on the other parameters considered.

Supplementation with methionine improved ($P < 0.05$) the motility of the second ejaculate (72.7 vs 71.2%) and increased ($P<0.01$) libido at the first sampling (20.6 vs 21.4 seconds). All the other parameters showed only slight differences.

It is difficult to comment on these results without further research on the effect of dietary protein and amino acids.

The protein level seems to have little effect on sperm quality. LUZI *et al.* (1996) found no significant difference in concentration and volume of semen from rabbits 19.7% and 14.5% crude protein. On the other hand, CASTROVILLI *et al.* (1995) saw a constant decrease in ejaculate volume and concentration (spermatozoa/ml) dietary protein increased (13-17-21% CP). NIZZA *et al.* (2000) reported a decrease in concentration and volume in rabbits fed 13% crude protein diets when compared to those fed 15 and 17% CP diets. They attributed the reduction in semen quality to poor food intake (135 g/d, equivalent to roughly 425 KJ of DE/kg of metabolic weight).

SCAPINELLO *et al.* (1997) used diets with 12% crude protein and 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8% methionine+cystine and found that the increase in dietary methionine levels caused a linear reduction in total semen volume and pH, but failed to influence semen quality which changed from milky to white, motility, vigour or spermatozoa concentration. The authors suggested that the lowest methionine+cystine levels studied (0.3%) are adequate to meet the sulphur amino acid requirements of male rabbits.

If the level of 0.3% methionine+cystine is sufficient to obtain good semen quality, it appears inevitable that a variation from 0.58 to 0.65 would only bring about slight differences.

In the case of lysine, albeit without the confirmation of previous research on rabbit, requirements for good semen production may well be modest. COUTO *et al.* (1998) fed heavy broiler rooster breeders diets containing 0.38, 0.43, 0.48, 0.53 and 0.58% lysine at a restricted intake of 130 g/bird daily. They that the diet with 0.38% lysine (equivalent to 494 mg/bird daily) was sufficient to meet requirements without affecting semen production, spermatozoa concentration or percentages of semen producing roosters.

An average all the samples (first and second ejaculate) gave a concentration of x $10^6$ spermatozoa/ml with a mean ejaculate volume of 0.79 ml. This quantity is very high when compared to other experiments (TACKE *et al.*, 1995; BENCHEIKH 1993;1995). However, it may be explained by the fact that only one collection per week was performed . In those trials when the cyclic production technique was not yet known, male rabbits were always used for the semen harvesting twice a week.

Numerically more spermatozoa were produced in the first ejaculates than in the second in agreement with .CASTROVILLI *et al.* (1995). These results contrast with those of THEAU-CLÉMENT *et al.* (1995) and PANELLA and CASTELLINI (1990) who observed, greater sperm production in the second ejaculate. According to THEAU-CLÉMENT *et al.* (1995) only when the males were young did the first ejaculate contain more spermatozoa than the second ejaculate.

The observed pH values are considered good and are probably the consequence of the high concentration found. BATTAGLINI (1992) reported a negative correlation ($r = -0.47$) between spermatozoa/ml and pH. The most frequent morphological defects were spermatozoa with distal or proximal protoplasmic droplets, spermatozoa with abnormal tails, and spermatozoa with abnormal heads. However, differences in the percentage (18.5) of total abnormal spermatozoa were not significant. . Also, there were no effects of dietary treatment on the incidence of acrosomal defects (14.2%).

As shown in Table 3, supplementation with lysine and methionine did not affect fertility, the number of kits born or the number of kits born alive per litter.

This work indicates that, apart from small differences in motility of live spermatozoa and libido, supplementation with lysine and methionine in the quantities examined did not appreciably modify the quality nor the fecundity of buck semen.

LAVARA *et al.* (2000) did not find any effect of vitamin (A, D, E) supplementation on the quantity and quality of semen. Supplementation of balanced diets with amino acids or vitamins may well not produce appreciable effects on characteristics of fresh semen. Further studies should be carried out to ascertain the effects of supplementation on spermatozoal survival after processing, (refrigeration, and freezing.

**Acknowledgements** : Research supported by MURST - University (ex 40%).

**Received** : May 11th, 2000
**Accepted** : December 6th, 2000

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