EFFECT OF FEEDING LEVEL AND DIETARY PROTEIN CONTENT ON LIBIDO AND SEMEN CHARACTERISTICS OF BUCKS

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Abstract - Using a 2 factorial design, the effect of dietary protein level (19.7% vs 14.5%) and feeding level (ad libitum vs restricted at maintenance energy requirement) was investigated on libido and semen quality in bucks. 96 young hybrid males were allotted to 4 experimental groups at the age of 15 weeks. From the age of 22 weeks, sperm collections were performed for 6 months. Judgement of libido (subjective scale 0-4) and time to collect semen (in seconds) were determined weekly. In total 1040 semen samples were judged. Semen evaluations of volume, colour, pH, motility (scale 0-6) concentration (Burker chamber) were performed every two weeks. At the end of the experimental period, sperm of all males was investigated for percentage of acrosoma aberrations (normal or defect) and live-dead ratio.

Ad libitum fed males showed an intake which was 40-50g/d higher (or 20-25%) than their restricted brothers during the whole experimental period. Live weight of restricted males stabilised around 4kg while the ad libitum fed ones had an average final weight of 47.77g. Ad libitum fed males showed a decreasing feed intake trend with increasing age (week 21: 217g/d; week 47: 149g/d). The feeding level influenced (P<0.05) libido, sperm output ejaculate, but semen quality seemed to be unaffected. The effects of the dietary protein level were limited both on feed intake and sperm characteristics. Age as well as genetic relationship (brother groups) were shown to have significant (P<0.01) effects on all the variables studied. A multivariate ANOVA for repeated measures was developed on acrosoma (normal or defect), aberrations and live-dead ratio percentages, but differences due to the experimental factors were not significant.

INTRODUCTION

Since the late eighties, artificial insemination has been used as a reproduction technique in commercial rabbit breeding. Nowadays, in the main rabbit meat producing countries (Italy, France, Spain), artificial insemination combined with an adapted management technique (cyclization) are widespread. Consequently, a lot of insemination centres have recently been established with several hundred males. The use of a special male diet in order to optimise libido and semen quality, is in question. However, there is little literature concerning possible dietary effects and for other meat producing species too, bibliography is scarce. (BROWN et al., 1983, SEXTON et al., 1989; CAVALCHI HI et al., 1993)

The purpose of the present experiment was to study if the dietary protein level and the feeding level have an influence on the libido and semen quality of bucks. Moreover, genetic and age related effects were studied.

MATERIAL AND METHODS

The experiment was performed at the "Rijksstation voor Kleinveeteelt" (Merelbeke, Belgium) between August 94 - April 95. Males were end products of the pure lines of the Institute (MAERTENS, 1992a). In total 24 groups (replicates) of 4 brothers were allotted to the 4 experimental groups. Two iso-energetic diets (9.5 MJ ME/kg) were formulated and prepared at the Institute (MAERTENS, 1992b). They mainly differed in protein content. Synthetic lysine and methionine were added to the low protein diet to avoid deficiencies (Table 1). Using a two factorial scheme, each group of four brothers was allotted at random to the diets: low protein diet (LP) and high protein diet (HP) or feeding level (ad libitum vs restricted). From the age of 15 weeks on, they received the experimental diets and were reared in individual cages under normal environmental conditions.

1 Parts of this paper were presented at the «XLIX Convegno della Società Italiana delle Scienze Veterinarie », Parma (Italy), 27-30 September, 1995
In order to maintain some final growth, they received an extra amount of feed corresponding to 200 and 100 KJ ME kg \(^{-0.75}\), respectively between 16 to 20 and 20 to 25 weeks of age. From the age of 22 weeks, semen was collected weekly for 6 consecutive months. In total 1040 samples were judged. Libido was judged both subjective (behaviour scale 1 to 4) as the time between the introduction of the female and collection of the ejaculate. Macro-microscopic analyses of semen like volume, colour, pH, motility (subjective scale from 0 to 6), concentration (Burker chamber) were performed bi-weekly (LUZI et al., 1993; PIZZI et al., 1993). At the end of the experimental period, all males were judged on the percentage of acrosoma aberrations (normal or defect) and live:dead ratio in their semen. Immediately after collection of the ejaculate the semen was transferred to a heated block at 35°C and all manipulations were conducted using warmed glassware. Two smears were prepared. One was stained with eosin-nigrosin (WITZEL and MULLER, 1991) and the other with Giemsa stain as described by WATSON (1975). On the first slide the live:dead-ratio and the sperm morphology was evaluated. The percentage of white cells (living) and red cells (dead) was first of all determined by counting 200 spermatozoa. Afterwards another 200 white spermatozoa were evaluated to study the gross morphology aberrations. The spermatozoa were divided into normal cells, spermatozoa with an abnormal head, with an abnormal tail, with a proximal protoplasm droplet and with a distal protoplasm droplet. When more than 1 abnormality was present, only the most important was noted. The morphology was evaluated. The percentage of white cells (living) and red cells (dead) was first of all determined by counting 200 spermatozoa.

RESULTS AND DISCUSSION

Feed intake and live weight development

Feed intake data and corresponding live weight (LW) development are shown in Figures 1 and 2. Feed intake was not significantly different between diets. The difference between ad libitum fed males and restricted ones was always between 40 and 50g or 20 to 25% of the ad libitum intake. Feed intake pattern of non restricted males showed a decreasing trend (a drop of nearly 30%) with increasing age. At the age of 16 weeks, feed intake was on average 201.5±29.9 g. It increased till the age of 21 weeks (216.6±34.3 g) but from then on feed intake dropped to 148.5±25.4 g at 47 weeks. Restricted males received a quantity related to their metabolic requirement. On average they received between 114g (1.10 MJ ME/d) and 125g (1.20 MJ ME/d) daily from the age of 25 weeks. LW of ad libitum fed males increased rapidly between 15 and 25 weeks, afterwards the increase was limited to approximately 15 g per week and a mean final weight of 4777±484 g was observed at the age of 47 weeks. Males fed the low protein diet ad libitum were heavier (not significant) in the first half of the experimental period due to the higher feed intake. Initial feed restriction, even at a level of 1.5 times energy
maintenance requirement caused LW stagnation for one week. Afterwards males increased LW till they received only their maintenance energy requirement (from week 25 off). LW stabilised around 4 kg and an overall difference (P<0.001) of 671 g was observed with the ad libitum fed males at the end of the experimental period. However, after the 8 months experimental period, no difference in number of surviving males or sore hocks was observed among experimental groups.

**Libido and semen characteristics**

The semen characteristics compared to the different diets are shown in Figures 3, 4 and 5. The analysis revealed a statistical significance of the age of the males on all the variables which is in agreement with PANELLA and CASTELLINI (1989) and TACKE et al. (1995). Males fed ad libitum showed significant (P<0.01) increased volume ejaculate, spermatozoa per ejaculate and better values for both parameters used to appreciate males' libido (Table 2). However, their concentration (spermatozoa/ml) was comparable with the restricted ones and with the exception of the pH, semen quality was not statistically different. Only a slight effect on the initial pH was observed. Much more pronounced (P<0.001) was the effect of group (brothers) within feeding level and within protein level. This genetic effect was observed for all parameters considered (Table 3). The only significant interaction (P<0.01) was found between feeding level and protein level for libido and output of spermatozoa ejaculate suggesting that ad libitum fed males ask for a low protein diet only to reduce the collection time, but the highest semen concentration is achieved with high protein/ad libitum diet.

On average all samples (n=1040) showed a concentration of 472x10^6 spermatozoa/ml with a average ejaculate volume of 1.14 ml. This quantity is quite high compared to other experiments but can be explained because only one collection per week was performed (TACKLE et al., 1995). Because the difference in semen volume and corresponding spermatozoa per ejaculate was significant (P<0.01), a severe feed restriction cannot be recommended for young males.

### Table 2: Least Square Means and Standard Errors of the semen characteristics according to the dietary treatments

<table>
<thead>
<tr>
<th></th>
<th>sp/ml (x10^6)</th>
<th>sp/ejac. (x10^6)</th>
<th>time (seconds)</th>
<th>libido (scale)</th>
<th>volume (ml)</th>
<th>motility (scale)</th>
<th>mobility score</th>
<th>colour (scale)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding level (restricted)</td>
<td>482.2a</td>
<td>452.6a</td>
<td>±13.4</td>
<td>±18.3</td>
<td>±0.03</td>
<td>±0.05</td>
<td>±0.06</td>
<td>±0.03</td>
<td>±0.03</td>
</tr>
<tr>
<td>Feeding level (ad lib)</td>
<td>471.2a</td>
<td>584.6b</td>
<td>±14.8</td>
<td>±20.3</td>
<td>±0.06</td>
<td>±0.08</td>
<td>±0.06</td>
<td>±0.04</td>
<td>±0.02</td>
</tr>
<tr>
<td>Protein level (HP)</td>
<td>482.8a</td>
<td>525.8a</td>
<td>±14.3</td>
<td>±19.6</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.07</td>
<td>±0.04</td>
<td>±0.01</td>
</tr>
<tr>
<td>Protein level (LP)</td>
<td>470.6a</td>
<td>511.5a</td>
<td>±14.2</td>
<td>±19.4</td>
<td>±0.02</td>
<td>±0.06</td>
<td>±0.08</td>
<td>±0.04</td>
<td>±0.02</td>
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</tbody>
</table>

Mean values within a column with different letters are significantly different (P<0.01).

### Table 3: Effects of the considered sources of variation on the semen characteristics

<table>
<thead>
<tr>
<th></th>
<th>sp/ml (x10^6)</th>
<th>sp/ejac. (x10^6)</th>
<th>time (seconds)</th>
<th>libido (scale)</th>
<th>volume (ml)</th>
<th>motility (scale)</th>
<th>mobility score</th>
<th>colour (scale)</th>
<th>pH</th>
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<tr>
<td>Age</td>
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<td>***</td>
<td>***</td>
<td>***</td>
<td>NS</td>
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<td>Protein level</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Group of 4 brother (protein level)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>***</td>
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<tr>
<td>restr./ad lib.</td>
<td>NS</td>
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<td>Group of 4 brother (restr./ad lib)</td>
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<td>Age*level</td>
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<td>Age*protein</td>
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BS = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001
Fig. 1. Feed intake (g/d) of males between 16 and 47 weeks of age

![Graph showing feed intake (g/d) for males between 16 and 47 weeks of age.]

Fig. 2. Development of live weight of males between 16 and 47 weeks of age

![Graph showing development of live weight for males between 16 and 47 weeks of age.]
Figure 3. Spermatozoa concentration according to the diet (millions/ml)

![Bar graph showing spermatozoa concentration](image)

- High prot./ad lib. □ High prot./restr.
- Low prot./ad lib. □ Low prot./restr.

Figure 4. Volume (ml) and motility (score) according to the diets

![Bar graph showing volume and motility](image)

- High prot./ad lib. □ High prot./restr.
- Low prot./ad lib. □ Low prot./restr.

Figure 5. Time (seconds) and libido (score) according to the diets

![Bar graph showing time and libido](image)

- High prot./ad lib. □ High prot./restr.
- Low prot./ad lib. □ Low prot./restr.
Percentage of acrosoma defect and live: dead ratio

On average the live-dead ratio was 19.5%. Most frequent defects were: spermatozoa with a distal protoplasm droplet (6.5%), spermatozoa with a proximal protoplasm droplet (3%), spermatozoa with an abnormal tail (3%), spermatozoa with an abnormal head (7%). However, differences in acrosoma defects percentage were not significant when related to the dietary treatments. Also the acrosoma defects, which was on average of 5.5% did not show significant differences due to the experimental treatment. Therefore, because all the males were of the same age and housed under homogeneous environmental conditions, it is possible to conclude that the tested feeding level and the dietary protein level do not influence these semen characteristics.

Acknowledgements - Research was donated with "Ministero Dell'Università, Ricerca Scientifica e Tecnologica" - Roma (40% and 60%) and "World Rabbit Science Association" (Belgium Branch) funds. Further we would like to thank A. VERMEULEN and C. SAELENS for their skilful technical help and R. LEMMENS for the care of the males.

REFERENCES


Riassunto - Utilizzando un disegno bi-fattoriale, si è studiato l'effetto del livello proteico nella dieta (19,7% vs 14,5%) e del livello alimentare (ad libitum vs razionario, mantenendo costanti i fabbisogni energetici) sulla libido e sulla qualità del seme nei maschi riproduttori della specie cunicola. 96 maschi riproduttori liberi, sono stati suddivisi in 4 gruppi sperimentali all'età di 15 settimane. Dall'età di 22 settimane, per un periodo di 6 mesi, si sono iniziati i prelievi del seme (n=1040); ogni settimana sono stati determinati la libido (scala soggettiva da 0 a 4) ed il tempo di raccolta del seme (in secondi). Il volume, il colore, il pH, la motilità (scala soggettiva da 0 a 6), la concentrazione (camera di Burker) sono stati determinati ogni 2 settimane. Durante l'ultimo periodo della prova, si sono inoltre analizzate la percentuale degli acrosomi intatti, le aberrazioni e le cellule spermate vive/morte. I maschi alimentati ad libitum hanno evidenziato, durante tutto il periodo sperimentale, una assunzione di alimento che è stata più alta in media di 40-50 g/die (o del 20-25%) rispetto a quelli con restrizione alimentare. Il peso vivo dei maschi alimentati in modo ristretto si è stabilito intorno ai 4 kg, mentre i maschi alimentati ad libitum hanno ottenuto un peso medio finale di 4.777 g. Gli animali alimentati ad libitum hanno mostrato una diminuzione dell'assunzione di alimento all'aumentare dell'età (a 21 settimane 217 g/die; a 47 settimane 149 g/die). Il livello alimentare ha influenzato (P<0,05) la libido, la quantità di spermatozoi per eiaculato ma non la qualità del seme. Gli effetti del livello proteico della dieta sono stati abbastanza limitati, sia sull'assunzione di alimento, sia sulla caratteristiche del seme. L'età e l'effetto genetico (gruppi di fratelli) hanno dimostrato effetti significativi (P<0,01) su tutte le variabili studiate. Infine, per quanto riguarda l'analisi dei dati relativi alla percentuale di acrosomi intatti, alle aberrazioni e a rapporto degli spermatozoi vivi e morti in relazione alle diete somministrate, si è utilizzata una ANOVA multivariata per misure ripetute, non si sono riscontrate differenze statisticamente significative.