EFFECT OF FEEDING LEVEL AND DIETARY PROTEIN CONTENT ON LIBIDO AND SEMEN CHARACTERISTICS OF BUCKS[1]

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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 4777g. *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; CAVALCHINI *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

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(12h of light, temperature between 16 and 22°C). Males of the restricted groups were progressively restricted fed between 16 and 25 weeks of age. After 25 weeks, the restricted males received only their maintenance requirement. (400 KJ ME $kg^{-0.75}$) (PARTRIDGE *et al.*, 1989; MAERTENS, 1992b, PARIGI-BINI *et al.*, 1992).

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 (WEITZE 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 Giemsa stained slide was used to evaluate the integrity of the acrosoma. A total of 200 cells was counted and divided in cells with a normal acrosoma and cells with a defect acrosoma. Data were analysed using a GLM procedure (SAS/STAT, 1990) to determine the effects of the different experimental groups (feeding and protein level) adjusted according to animal age and genetic relationship (brother effect), on *libido* and semen quality. A multivariate ANOVA for repeated measures was developed for the data related to acrosoma and live:dead ratio.

Table 1 : Ingredients and chemical composition of diets

Ingredients (%)	Diet High Protein	Diet Low Protein
	HP	LP
Alfalfa 16	23	14.9
Wheat	-	11.2
Wheat shorts	35	35
Cassava meal	5.2	8.0
Sunflower 24	15	9
Soybean meal	5.9	-
Corn gluten	8.0	2.6
Flax chaff	-	11.1
Animal fat	1.0	1.0
Molasses cane	4.0	4.0
Min./vit. mix	2.5	2.5
L-Lysine Hcl	-	0.10
DL-Methionine	-	0.11
$CaCo_3$	0.4	0.46
Salt	-	0.03
Composition (%)		
Crude protein	19.71	14.51
Crude fiber	16.50	16.02
Crude fat	3.90	4.20
ME (MJ)	9.46	9.48
Ca*	1.00	1.03
P (total)*	0.76	0.70
Lysine*	0.76	0.64
Methionine+cystine*	0.62	0.58

* Calculated values

RESULTS AND DISCUSSION

Feed intake and live weight development

Feeding 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 weight. 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

	sp/ml ($x10^6$)	sp/ejac. ($x10^6$)	time (seconds)	*libido* (scale)	volume (ml)	motility (scale)	mo,ing score	colour (scale)	pH
Feeding level	482.2a	452.6a	21.03a	3.76a	0.96a	3.45a	3.88a	1.60a	7.31a
(restricted)	±13.4	±18.3	±1.19	±0.03	±0.05	±0.08	±0.09	±0.03	±0.01
Feeding level	471.2a	584.6b	15.62b	3.87b	1.30b	3.47a	4.00a	1.64a	7.36b
(*ad lib*)	±14.8	±20.3	±1.21	±0.03	±0.06	±0.08	±0.09	±0.04	±0.02
Protein level	482.8a	525.8a	18.02a	3.82a	1.13a	3.5 la	4.01a	1.58a	7.30a
(HP)	±14.3	±19.6	±1.14	±0.02	±0.03	±0.07	±0.08	±0.04	±0.01
Protein level	470.6a	511.5a	18.65a	3.81a	1.12a	3.42a	3. 87a	1.66a	7.3 7b
(LP)	±14.2	±19.4	±1.23	±0.02	±0.02	±0.08	±0.09	±0.04	±0.02

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

	sp/ml ($x10^6$)	sp/ejac. ($x10^6$)	time (seconds)	libido (scale)	volume (ml)	motility (scale)	mo,ing score	colour (scale)	pH
Age	***	***	***	***	***	***	***	NS	***
Protein level	NS	NS	NS	NS	NS	NS	NS	NS	NS
Group of 4 brother	***	***	NS	*	***	*	NS	***	***
(protein level)									
restr./*ad lib.*	NS	***	NS	*	***	NS	NS	NS	NS
Group of 4 brother	***	***	***	***	***	*	NS	***	***
(restr./*ad lib*)									
Age*level	NS	NS	NS	NS	NS	NS	NS	NS	NS
Age*protein	NS	NS	NS	NS	NS	NS	NS	NS	NS

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

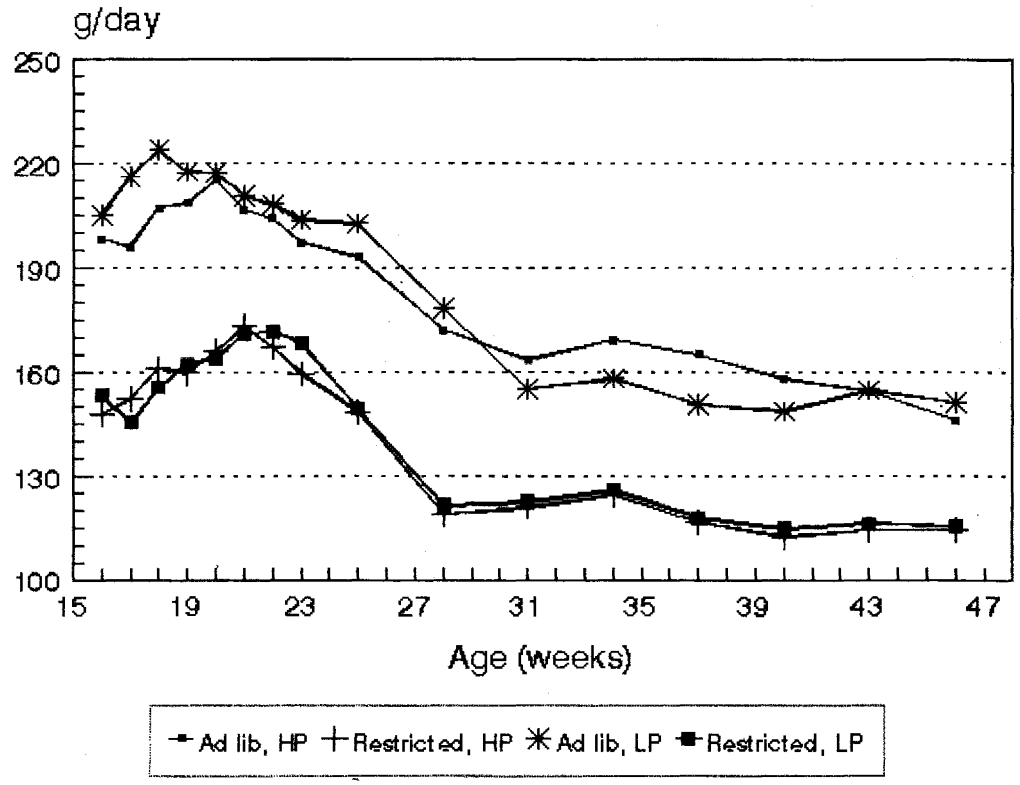


Fig. 2. Development of live weight of males between 16 and 47 weeks of age

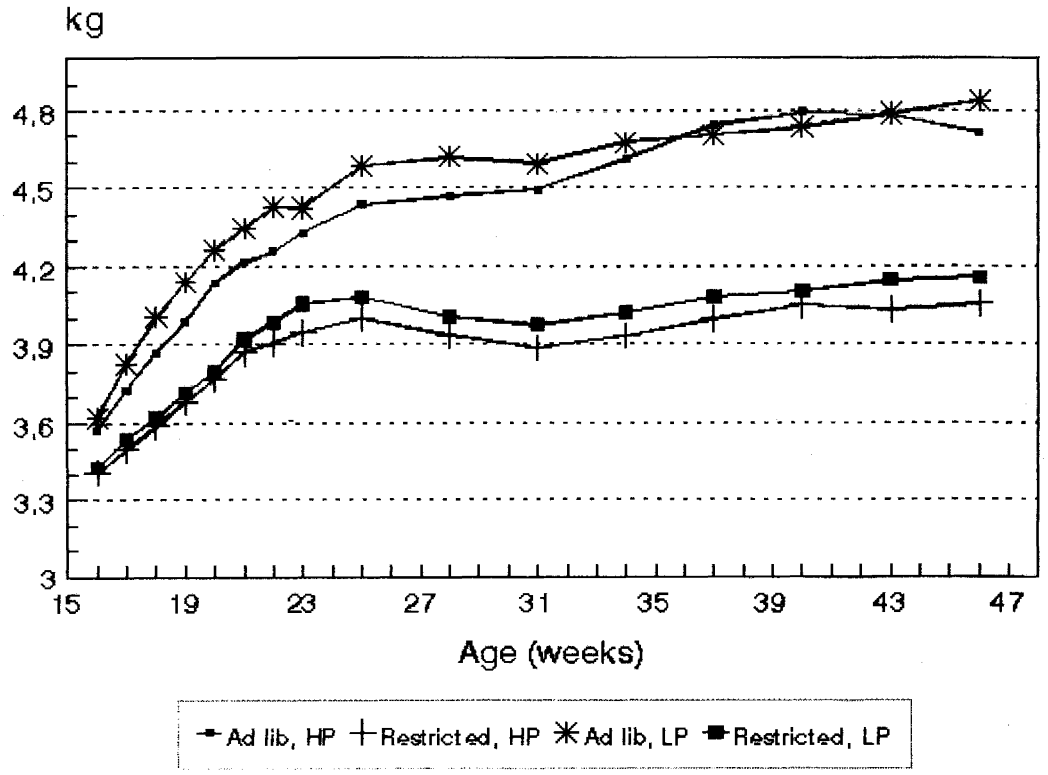


Figure 3. Spermatozoa concentration according to the diet (millions/ml)

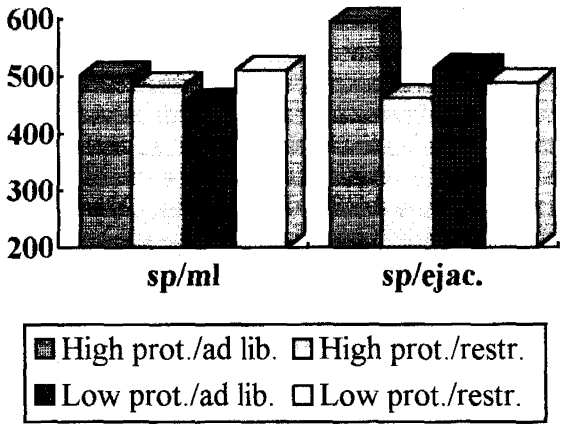


Figure 4. Volume (ml) and motility (score) according to the diets

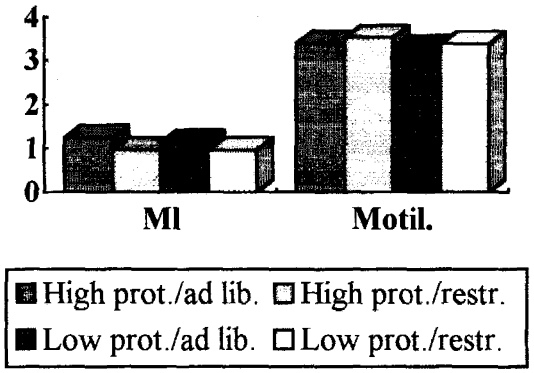
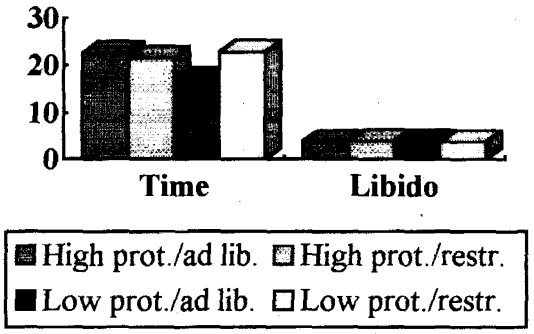


Figure 5. Time (seconds) and libido (score) according to the diets



Percentage of acrosoma defect and live: dead ratio

On average the live-dead ratio was 19.5%. Most frequent defects were: spermazoa 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.

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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* razionato, mantenendo costanti i fabbisogni energetici) sulla *libido* e sulla qualità del seme nei maschi riproduttori della specie cunicola. 96 maschi riproduttori ibridi, 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 soggetiva 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 spermatiche 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 stabilizzato intorno ai 4 kg, mentre i maschi alimentati *ad libitum* hanno ottenuto un peso media 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'effeto 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 al 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.