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EFFECTS OF DIETARY ENERGY AND PROTEIN CONTENT ON LIBIDO AND SEMEN CHARACTERISTICS OF BUCKS

PAPADOMICHELAKIS G.[1], FEGEROS K.[1], XYLOURI-FRANGIADAKI E.[2], PAPADOPOULOS G.[1]

[1]Lab. of Animal Nutrition, Faculty of Animal Sciences
[2]Lab. of Anatomy & Physiology of Farm Animals, Faculty of Animal Sciences,
Agricultural University of Athens,
75, Iera Odos Str., 11855, Athens, Greece.
e-mail: efxil@aua.gr

ABSTRACT

The effects of dietary digestible energy (10 vs. 12 MJ DE kg-1) and protein content (15 vs. 19%) on libido, semen characteristics and testosterone level, in male rabbits were studied, using a 2x2 factorial design. Thirty-two New Zealand White X California bucks weighing 3.9 kg, allotted randomly into 4 experimental groups, were used at the age of 22 weeks. From the age of 29 months, semen collections were performed weekly, over a 9 week period. Evaluations of libido (scale 0-4), time to collect semen (sec), sperm concentration and motility (Makler Chamber), fructose in seminal plasma (hexokinase method), serum testosterone (RIA), live sperms, morphology abnormalities and acrosomal integrity were performed once a week. Data were analyzed using GLM Procedures (SAS/STAT, 1990).

Males fed on high-energy diets showed lower feed intake (10-20 g/day) than the low energy fed ones. DE intake was not different between groups, while groups that consumed high DE diets had higher (300 g) final live weight ($P<0.05$). All groups showed a decreasing feed and energy intake, with increasing age and environmental temperature. The energy level influenced libido, sperm morphology, semen volume ($P<0.01$), pH ($P<0.05$) and total spermatozoa per ejaculate ($P<0.001$). Total live sperm cells, motility, fructose concentration and serum testosterone were not affected by the dietary treatments.

INTRODUCTION

The intensive rabbit production requires the increasing use of high performance reproduction stock. Several efforts have taken place, since the late eighties, to optimize the reproductive function of male and female rabbits, such as adapted management techniques (cyclization) combined with the use of artificial insemination (Luzi *et al*., 1996).

Semen quality improvement has become an important part of these efforts, since artificial insemination has spread worldwide. Semen quality is influenced by a lot of factors such as buck management (Lopez *et al*., 1996), ejaculation frequency (Bodnar *et al*., 1996), environment (Xylouri et al., 1998), nutrition etc. However the literature concerning the dietary effect on semen traits is scarce. Luzi *et al*. (1996) showed that restriction of feeding level is not recommended for young males. However the effect of dietary energy content on semen is not proved, as in other animal species, like bulls (Coulter *et al*., 1984).

The present work was conducted to determine the effects of dietary energy and protein content on libido and semen characteristics of bucks.

MATERIAL AND METHODS

Animals- Thirty-two New Zealand White X Californian male rabbits, at the age of 22 weeks, were used. Animals were allotted randomly in to four experimental groups of 8 animals per group. They were kept indoors, in individual wire cages, under natural environmental conditions and a 16-8h light-dark schedule.

Diets- Using a 2x2 experimental design, four diets with two levels of energy (10 vs.12 MJ

DE/kg) and protein (15 vs. 19%) were formulated and prepared (Kalaisakis,1981; Lebas, 1988). From the age of 22 weeks on, the 4 experimental groups consumed the following pelleted (7 mm diameter) diets *ad libitum*: A (Low energy, Low protein- LL), B (Low energy, High protein- LH), C (High energy, Low protein- HL) and D (High energy, High protein- HH), respectively. Synthetic lysine and methionine were added to the low protein diets, to avoid deficiencies (Table 1). The energy digestibility of the diets was checked in a subsidiary digestibility trial. Eight males per diet were used and collected during 8 days.

Libido, semen and testosterone evaluation- From the age of 29 weeks, semen was collected weekly, for 9 consecutive weeks. The following traits were evaluated:

Libido: it was determined subjectively (behavior scale 0 to 4) and as the time between the introduction of the female in the cage and collection of the ejaculate (Luzi *et al.*, 1996).

Sperm concentration and motility: the number of spermatozoa/ml and of the forward moving spermatozoa were counted using Makler counting chamber, diluting a small quantity of semen (30 μl) with normal saline solution (1:4). Every sample was examined under a light microscope, on which a digital video camera was adapted. Images were recorded in videotape. Motility was evaluated according to a point system from 0 to 5: 0=0%, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80% and 5=81-100% (Bodnar *et al.*, 1996).

Table 1. Ingredients and chemical composition of experimental diets.

Ingredients (%)	A (LL)	B (LH)	C (HL)	D (HH)
Alfalfa hay	26.0	26.0	32.5	31.0
Wheat straw	15.0	15.0	2.0	2.0
Sunflower meal	9.0	9.0	9.0	9.0
Corn	7.5	3.5	48.5	36.3
Barley	34.0	25.0	-	-
Soybean meal	5.5	18.5	5.0	18.6
Min./Vit. mix.	1.7	2.0	1.6	2.0
L-Lysine	0.2	-	0.2	-
DL-Methionine	0.1	-	0.1	-
$CaCO_3$	0.5	0.5	0.5	0.5
NaCl	0.5	0.5	0.6	0.6
Composition (%)				
Dry matter	90.1	90.2	89.5	89.8
Crude protein	15.2	19.1	14.5	19.0
Crude fiber	12.7	13.6	9.0	10.2
Ether extract	2.1	2.0	2.6	2.3
Digestible energy (MJ/kg)*	11.1	11.4	12.7	12.6

* According to the digestibility trial

Living spermatozoa and sperm morphology: they were evaluated in a smear stained with Eosin- Nigrosin. A total of 200 sperm cells were counted and the percentage of living (white) and dead (red) cells was calculated. Morphological abnormalities were recorded in a total count of 200 living cells.

Acrosomal integrity: smears stained with May-Grunwald-Giemsa were used to count a total of 200 cells with normal and defected acrosoma and the percentage of the spermatozoa with normal acrosoma was calculated. The percentage of the living cells and those with normal morphology was evaluated with a point system (0-5), like motility's.

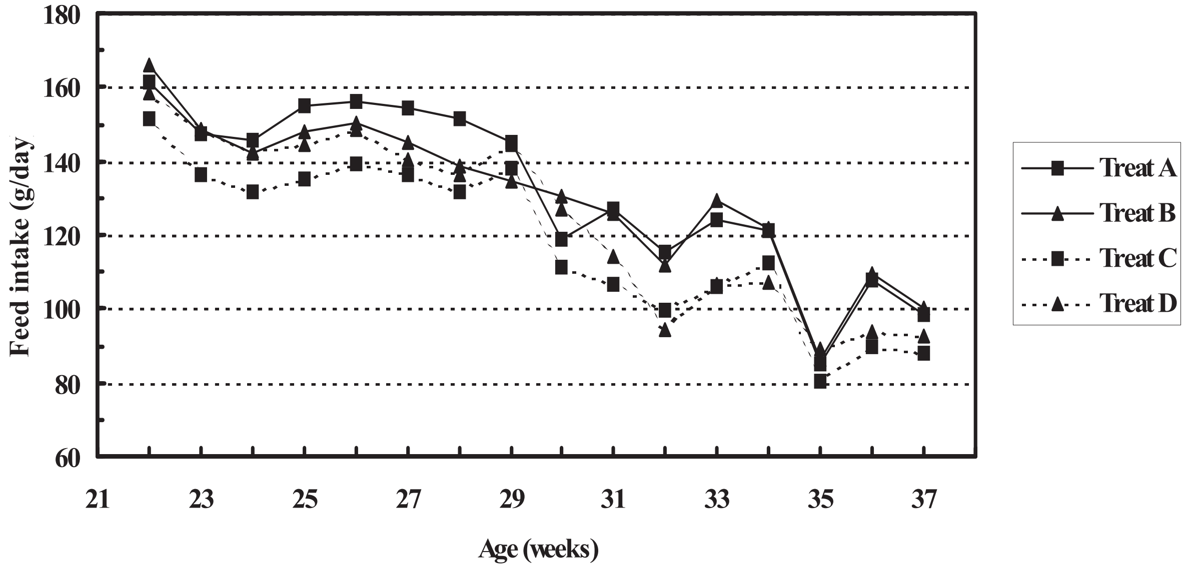
Fructose: the fructose concentration in seminal plasma was determined, according to the hexokinase method, using the Boehringer-Mannheim Test combination kit.

Testosterone: blood samples were collected, once a week during the experimental period and serum testosterone level was determined, using RIA.

Statistical analysis- Data were analyzed using the GLM procedures of SAS (1990), to determine the effects of energy and protein content on semen traits, as well as on feed intake and live weight development.

RESULTS AND DISCUSSION

Feed intake and live weight development



Dietary energy and protein content did not affect the feed and DE intake significantly. However, the groups fed on high-energy diets, showed a slightly higher feed and energy intake on average, than the ones fed on low energy diets. This difference was between 10 and 20 g/day and 0.02 to 0.1 MJ DE/day, respectively. This fact indicates that the ad libitum fed rabbits regulate *ad se* feed intake according to the dietary energy level.

Figure 1. Feed intake of animals during the experiment.

Both feed and energy intake showed a decreasing trend, for all the groups during the experiment, from 160 to 100 g/day and 1.9 to 1.1 MJ DE/day, respectively. This is due to increasing age for the first seven weeks, considering that, the environmental temperature was always between 17 ^{0}C and 24.8 ^{0}C. The average feed intake was 140 g/day at the age of 29 weeks, but from that point on, it dropped to 100g at 37 weeks old. The environmental temperature (summer time) during the last 9 weeks of the experiment, showed a high increase from 24 ^{0}C to 34 ^{0}C. That caused a decrease of feed and energy intake due to both age and temperature increasing. This fact is explained when referring to the 35th week, where the temperature exceeded 34 ^{o}C (Figure 1).

The live weight development of the experimental groups was limited during the experiment (Figure 2). The males fed on high-energy diets were heavier and reached a live weight of 4.4 kg on average. A significant difference of 300g was observed ($P<0.05$), comparing to the low energy fed males, which stabilized at around 4.1 kg, at the end of the experiment.

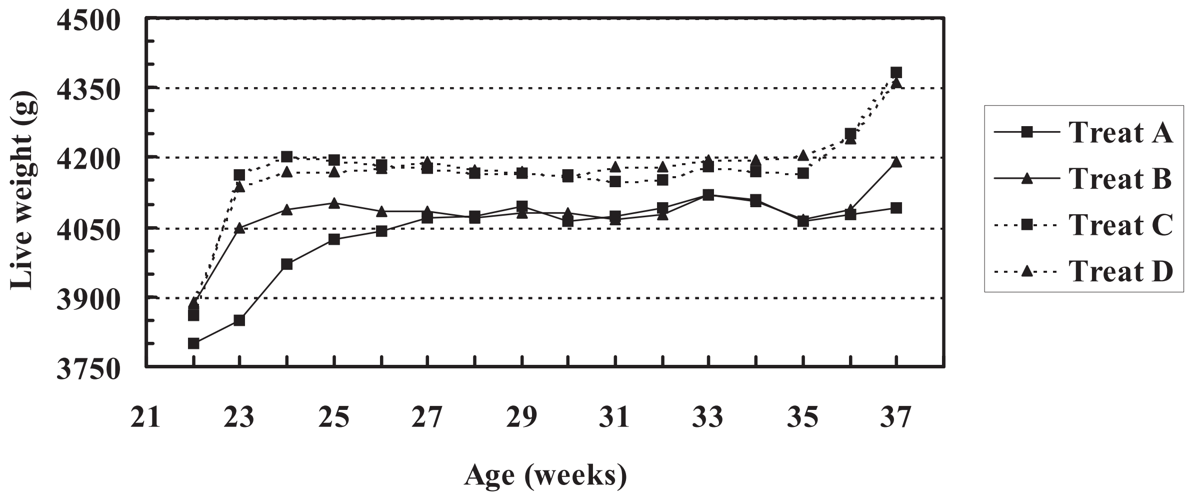
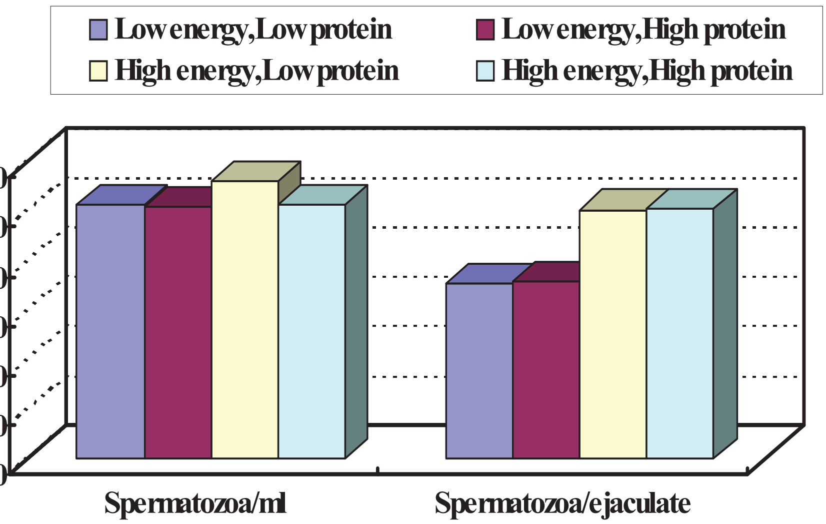


Figure 2. Live weight development of experimental animals.

Libido, semen traits and testosterone level



The semen traits compared to the different dietary treatments are shown in Figures 3, 4 and 5. The statistical analysis did not reveal any effect of the age of experimental animals, on all the variables studied. Similar research gave different results (Luzi *et al*., 1996). This may be due to the fact that they evaluated semen in younger bucks than we did (start at 22 weeks of age), considering that, rabbit semen traits are improved progressively with increasing age until 32 to 36 weeks old (Facchin *et al.,* 1993).

Figure 3. Sperm density (x 10^6/ml) according to dietary treatments

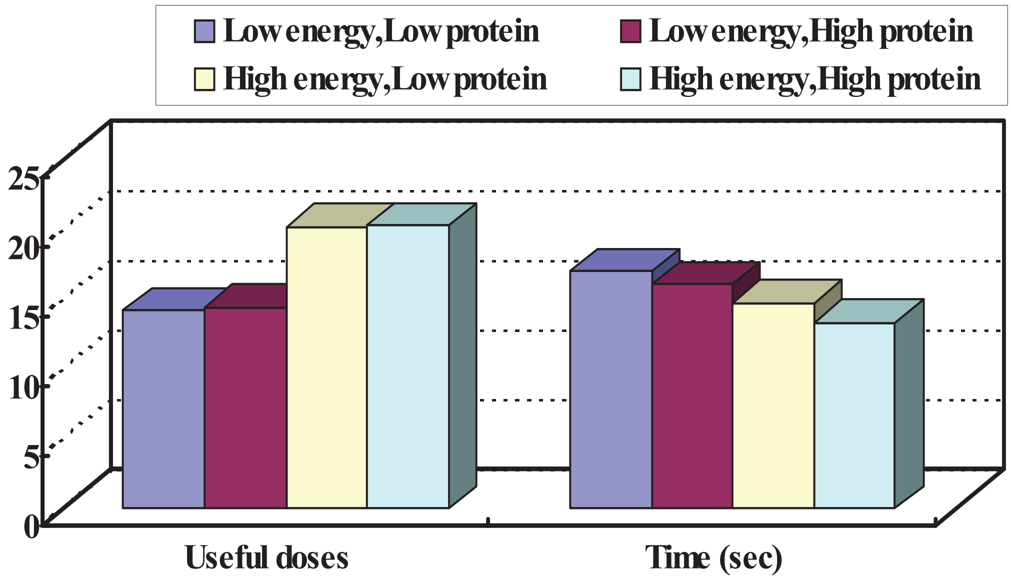
Males fed on high energy diets had significantly increased semen volume (P<0.01), spermatozoa per ejaculate (P<0.001), sperm morphology (P<0.01) and better values for libido both subjectively (scale 0-4) and objectively, as the time to collect semen (P<0.01). The effect of energy level was more pronounced on number of spermatozoa per ejaculate, than the other semen traits studied (Table 2).

The high dietary energy content also resulted in significantly higher number of doses for insemination (Table 2 and Figure 4). Doses were calculated according to Lopez, 25 millions spermatozoa per dose (Lopez et al., 1996).

Table 2. Effect of dietary treatments on semen characteristics (least square means ± SEM)

	Sp/ml x10[6]	Sp/ejac x10[6]	Time (sec)	Libido (0-4)	Vol. (ml)	pH	Motil. (0-5)	Morph (0-5)	Fruct. (g/l)	Doses
Energy level (Low)	513.0 ±11.4	358.3 ±13.4	16.6 ±0.3	3.72 ±0.03	0.74 ±0.02	7.33 ±0.04	4.20 ±0.06	4.19 ±0.06	1.27 ±0.08	14.3 ±0.54
Energy level (High)	539.4 ±11.4	505.9 ±13.3	14.1 ±0.3	3.88 ±0.03	0.93 ±0.02	7.12 ±0.04	4.32 ±0.06	4.67 ±0.06	1.37 ±0.07	20.2 ±0.53
P	NS	***	**	**	**	*	NS	**	NS	***
Protein level (Low)	539.1 ±11.3	430.5 ±13.2	15.9 ±0.3	3.76 ±0.03	0.81 ±0.02	7.17 ±0.04	4.28 ±0.06	4.48 ±0.06	1.33 ±0.07	17.2 ±0.53
Protein level (High)	512.9 ±11.5	433.7 ±13.5	14.7 ±0.3	3.83 ±0.03	0.83 ±0.02	7.27 ±0.04	4.24 ±0.06	4.38 ±0.06	1.31 ±0.08	17.4 ±0.54
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS: Non significant, *: P<0.05, * *: P<0.01, ***: P<0.001



The most frequent morphological defects were: spermatozoa with an abnormal tail and head, while spermatozoa with distal or proximal protoplasm droplet rarely occurred. The dietary energy level influenced the pH of semen (P<0.05), but no significant effect was found on the number of spermatozoa per ml, motility, vitality or acrosomal integrity.

Figure 4. Number of doses and time to collect semen according to dietary treatments

The dietary treatments did not affect fructose concentration in seminal plasma and testosterone level in blood serum. Effect of dietary protein level or any significant interaction between energy and protein level was not found in this research. However, it seems that better values are achieved with high energy/high protein diet, for all the parameters studied. All semen samples evaluated in this study had a concentration of $526x10^6$ spermatozoa per ml, on average, with a mean volume of 0.82 ml.

The fact that, the high energy level of the diets resulted in higher semen volume, corresponding spermatozoa per ejaculate and better morphology, shows that rations with high DE level are recommended for bucks. However, there is a need for further research, in order to determine the mechanism of energy influence on semen traits, since no differences were found on feed and energy intake between the treatments.

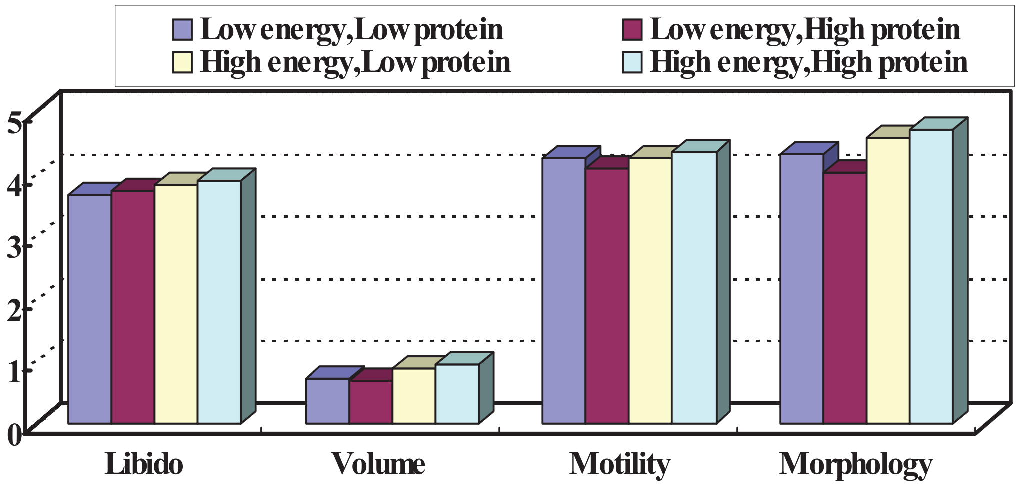


Figure 5. Libido, motility, morphology (scale) and volume (ml) according to the diets

Acknowledgements

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