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INFLUENCE OF DIETARY PROTEIN CONTENT ON LIBIDO AND SEMEN CHARACTERISTICS BUCKS

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ABSTRACT

The aim of this experiment was to study the influence of dietary protein level and environmental conditions on the libido and semen quality of bucks. 30 crossbred Hyla rabbit males (12-15 months old) were subjected to three diets (13-15-17% crude protein) for 12 months. Two successive ejaculate were collected once a week for 12 consecutive months. Semen evaluations of volume, colour, pH, motility, concentration, percentage of live spermatozoa were performed every two weeks.

Concentration of spermatozoa (average I and II ejaculate 439 *vs* 550 *vs* 546 x 10^6 spermatozoa for groups 13-15 and 17% crude protein, respectively) and volume of ejaculates (average I and II ejaculate 0.72 *vs* 0.82 *vs* 0.82 ml for groups 13-15 and 17 CP, respectively) were significantly lower in males submitted to diet 13% crude protein. The temperature registered at the semen drawing time influenced only the spermatic concentration (average I and II ejaculate 498 *vs* 530 x 10^6 spermatozoa for groups 13-18°C and 20-26°C, respectively). The born alive of females inseminated with the semen of males fed on diet of 13% of crude protein, was significantly lower than in the other two groups (7.7 *vs* 8.2 *vs* 8.2; $P < 0.05$).

INTRODUCTION

Since the 1970's, artificial insemination (A.I.) has been introduced as a reproduction technique in commercial rabbit breeding (PAUFLER *et al*., 1979; BATTAGLINI *et al*., 1986; THEAU-CLEMENT and ROUSTAN, 1992). At present the artificial insemination is widely used in the intensive rabbitries, because of its beneficial influence on management, productivity and breeding (FACCHIN *et al.*, 1991). Consequently, is very important to control all the factors affecting reproductive performance.

As regards the males few studies have analysed the physiology of rabbit bucks and the effect of management and environmental conditions on the semen production (CASTELLINI, 1996). The use of a special male diet in order to optimise libido and semen quality, is in question.

The aim of this work was to determine the influence of dietary protein level and environmental conditions on the libido and semen quality of bucks. Moreover, semen characteristics *in vivo* (fertility rate, litter size) were studied.

MATERIAL AND METHODS

30 crossbred HYLA rabbit males (12-15 months old) were used during a period of 1 year to study the influence of the three diets on the semen characteristics. Three iso-energetic diets (9.5 MJ digestible energy (DE)/kg)were formulated (13-15 and 17% in crude protein). Their ingredients and chemical composition are reported in Table 1. Chemical analysis of the diets followed the method of the AOAC (1984) for dry matter, ash, crude protein (CP) and crude

fibre (CF), and VAN SOEST et al. (1991) for acid detergent fibre (ADF) with a termostable amylase pre-treatment. Each group of ten males was allotted at random to the diets. Males were caged individually with a controlled light (12 h of light) and temperature and humidity of the chambers were recorded continually.

Table 1 - Ingredients and chemical composition of diets

	Diets (% Crude Protein)		
Ingredients %	13	15	17
Wheat middling flour	40.00	42.00	40.00
Dehydrated lucerne meal	22.00	37.00	37.00
Lucerne hay	12.00	-	-
Beet pulp	11.70	6.50	2.30
Barley	7.90	10.00	10.00
Soybean meal	-	1.70	8.00
Molasses	3.00	-	-
Soybean oil	0.60	-	-
Limestone	1.00	1.00	1.00
Vitamin-mineral mix	1.00	1.00	1.00
Monocalcium phosphate	0.50	0.50	0.50
Salt	0.20	0.20	0.20
Lysine	0.10	0.08	-
DL-methionine	-	0.02	-
Chemical composition %			
Crude protein	13.2	15.1	17.1
Crude fibre	14.7	14.8	14.7
ADF	18.4	18.5	18.5
Ash	8.7	8.8	8.8
Digestible energy MJ/kg	9.5	9.5	9.5

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

Semen volume, colour, pH, motility estimated by using a microscope provided with a hot stages according to ZEMJONIS (1970), spermatozoa concentration (Burker chamber), percentages of live and abnormal spermatozoa were determined in each sample immediately after semen collection. 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).

Live spermatozoa concentration (x 10^6/ml) was evaluated by counting 200 sperm cells, in each of eosin-nigrosin stained smears. Afterwards another 200 white spermatozoa were evaluated to study the abnormalities. 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. 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.

Six hundred multiparous does (HYLA) were inseminated to study the influence of the semen quality on the *in vivo* fertility and total born/litter and born alive/litter.

Data were analysed using a GLM procedure (SAS/STAT, 1990) to determine the effects of the different experimental groups (crude protein level 13-15-17%) and environmental temperature (13-18 *vs* 20-26°C) on libido and semen quality.

RESULTS AND DISCUSSION

During the trial the temperature fluctuated between 13 and 26°C. Ejaculates containing much urine and those which were not followed by a second ejaculation were also discarded. In total, 572 ejaculates were analysed statistically (160, 210, 202 for groups 13, 15 and 17%, respectively). The lowest level of proteins in the diet has given a number of ejaculates significantly lower than in the other two groups.

The semen characteristics compared to the different diets and temperature are shown in Table 2. For all parameters considered no significant dietary protein content x temperature interaction appeared in the analysis of variance. The effect of temperature on characteristics of rabbit semen was observed only for concentration of spermatozoa ($P<0,01$). The highest temperatures gave more spermatozoa/ml (567.4 x 10^6 *vs* 535.8 and 492.2 x 10^6 *vs* 460.7, respectively for I and II ejaculates). These results may seem apparently in contrast with the ones reported in literature (EL MASRY et al 1994; AMIN et al., 1987; BICUDO and PASCHOAL, 1991) which report a reduction of sperms concentration and of other semen characteristics with high temperatures. But it's to point that negative effects of high temperatures occur above 30°C.

Table 2 - Semen characteristics

			Temperature (°C)		Diets (% Crude Protein)			Error mean square
	Unit	Ejaculate	13-18	20-26	13	15	17	
Spermatozoa	$x10^6$/ml	I	535.8^B	567.4^A	458.2^B	599.0^A	590.1^A	30941
Spermatozoa	$x10^6$/ml	II	460.7^B	492.2^A	420.2^B	501.3^A	505.0^A	24904
Volume	ml	I	0.81	0.79	0.72^B	0.84^A	0.83^A	0.07
Volume	ml	II	0.77	0.79	0.71^B	0.81^A	0.82^A	0.06
Motility	%	I	61.0	60.9	58.7^B	61.9^A	62.0^A	147.4
Motility	%	II	60.6	60.8	59.9	60.8	61.3	143.3
Live sperm	%	I	76.4	76.9	76.2	77.0	76.7	35.14
Live sperm	%	II	76.2	76.3	75.8	76.3	76.6	50.05
Libido	Sec.	I	19.5	19.8	20.0	19.5	19.7	13.02
Libido	Sec.	II	20.8	20.6	21.1	20.7	20.4	15.10
pH		I	7.28	7.31	7.28	7.30	7.31	0.77
pH		II	7.31	7.32	7.30	7.33	7.31	0.75

A,B: $P< 0.01$

The analysis revealed a statistical significance of protein level for some parameters. Concentration of spermatozoa and volume of ejaculates were significantly higher in groups 15 and 17% CP than in group 13% CP ($P < 0.01$). LUZI et al. (1996) in rabbit fed on 19.7% and 14.5% of crude protein diets, didn't find significant differences in concentration and volume values of the groups. Instead, CASTROVILLI et al. (1995), found a concentration (spermatozoa/ml) decrease as the diet proteins increased (13-17-21% CP) and a constant value of ejaculate volume. The results we obtained, which are in contrast with the ones of the above said authors, might be also due to the different food diet. In fact, ours rabbits ingested, on the average, 135 grams of nourishment (without significant differences in the groups), which is about 425 KJ of DE/kg of metabolic weight, a little bit higher value than the keeping needs (400 KJ of DE/kg of metabolic weight) (LEBAS, 1989).

Protein levels higher increased sperm motility in first ejaculate, but not in second ejaculate.

Sexual activity (interval necessary between the introduction of the female into the male's cage and ejaculation) followed the same overall pattern in all groups, resulting nearly the same as

the one found by LUZI *et al.* (1996) and next to the one observed by THEAU-CLÉMENT *et al.* (1995) in rabbits subjected to 8 hours of light. The differences observed on pH and on the percentage of living spermatozoa as regards as groups, were almost modest. The values observed for these two parameters seem good and they're probably the consequence of the high found concentration. BATTAGLINI (1992), in fact, reports a positive correlation between spermatozoa and motility (r = 0.68) and negative correlation between spermatozoa/ml and pH (r = -0.47).

More spermatozoa were produced in the first ejaculate than in the second, which has been observed also by CASTROVILLI *et al.* (1995). The increase was greater in the group which was fed with diet at 15% of protein. These results are in contrast with the ones of THEAU-CLÉMENT *et al.* (1995), PANELLA and CASTELLINI (1990) who observed, instead, a bigger spermatic production in the second ejaculate. According to THEAU-CLÉMENT (1995) the first ejaculate contained on average more spermatozoa than the second ejaculate only at the start, when the males were young.

On average all the samples (n.668 first ejaculate and n. 668 second ejaculate) showed a concentration of 515 x 10^6 spermatozoa/ml with an average ejaculate volume of 0.79 ml. This quantity is very high compared to other experiments, but can be explained because only one collection for week was performed (TACKLE *et al.*, 1995; BENCHEIKH 1993-1995). In the foregoing trials, in fact, when the cyclic production technique was not known yet, male rabbits were always used for the semen harvesting twice a week.

The study about spermatozoa morphological alterations pointed out the following most frequent lacks: spermatozoa with a distal protoplasm droplet, spermatozoa with a proximal protoplasm droplet, spermatozoa with an abnormal tail, spermatozoa with an abnormal head. However, differences in acrosoma defects percentage were not significant when related to dietary treatments. Also the acrosoma defects did not show significant differences due to the experimental treatment.

Parameters observed *in vivo* as regards as proteic content of the diet (fertility, total born and live born) are reported in table 3. Fertility, even being lower in 13% proteins group, didn't reach the statistical significativity. As the diet proteic contents increases, instead, the number of rabbits born per female (born rabbits total 8.4 vs 8.9 vs 9.0; living born rabbits 7.7 vs 8.2 vs 8.2, respectively for groups 13-15 gropups and 17% of crude protein).

Table 3 - Reproductive performance from inseminated does

Parameter		Diets (% Crude Protein)		
		13	15	17
Fertility	%	75.0	80.5	81.5
Total born	n	8.4 ^{b}	8.9 ^{ab}	9.0 ^{a}
Born alive	n	7.7 ^{b}	8.2 ^{a}	8.2 ^{a}

a,b : P< 0.05

CONCLUSIONS

The results of this work, indicate that, apart from a higher concentration of sperms at 20-26°C, the characteristics of the rabbit seed undergo small changes in the range of temperature between 13 and 26°C. The proteic contents of the diet, instead, has remarkably influenced the seed quality and the fertility parameters noticed in vivo, pointing out the necessity not to lower the contents under 15% of crude protein of male's diet.

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REFERENCES

AMIN S.O., EL-FOULY M.A., EL-SHOBHY H.E., EL-SHERBINY A.M., 1987. Effect of season, breed and sequence of ejaculation on some physical characteristics of rabbit semen. In: *Proc. 1st Conf. Agric. Develop. Res.*, **1**, 54-67.

A.O.A.C., 1984. Official Methods of Analysis of the Association of Official Analytical Chemists. *14th Ed. Association of Official Analytical Chemists, Washington, DC*.

BATTAGLINI M., 1992. Fecondazione artificiale, attenti al maschio. *Riv. Coniglic*. **5**, 47-51.

BATTAGLINI M., COSTANTINI F., BOITI C., CANALI C., 1986. Ormoni steroidei, performance riproduttive dei maschi e fecondazione artificiale nel coniglio. *Riv. Coniglic*., **3**, 56-59.

BENCHEIKH N., 1993. Production de sperme et fertilité du lapin male *Oryctolagus cuniculus*. Effet de la fréquence de collecte et du type génétique. *Thèse d'Etat. Ecole Nationale Agronomique de Tolouse.*

BENCHEIKH N., 1995. Effet de la fréquence de collecte de la semence sur les caractéristiques du sperme et des spermatozoïdes chez le lapin. *Ann. Zoot*., **44**, 263-279.

BICUDO S.D., PASCHOAL J.P.S., 1991. Some rabbit semen traits in spring and early summer. In: *Ann. IX Congresso Brasileiro de Repruducao Animal, Belo Horizonte, Brasil*, **2**, 457-459.

CASTELLINI C., 1996. Recent advances in rabbit artificial insemination. *6th World Rabbit Congress, Toulose*. Vol. II, 13-25.

CASTROVILLI C., TAMBURINI A., CARRARA C., 1995. Livelli proteici della dieta, linee genetiche e caratteristiche del materiale seminale nel coniglio. *49° Conv. SISVet*., 1037-1038.

EL-MASRY K.A., NASR A.S., KAMAL T.H., 1994. Influences of season and dietary supplementation with selenium and vitamin e or zinc on some blood constituents and semen quality of new zealand white rabbit males. *World Rabbit Science*, **2** (3), 79-86.

FACCHIN E., CASTELLINI C., BALLABIO R., 1991. Ciclo riproduttivo della coniglia e suo controllo farmacologico. Nota 2: la programmazione dei parti in coniglie di allevamenti intensivi con prostaglandine sintetiche (α-prostol). *Riv. Zoot. Vet*., **19** (2), 13-18.

LEBAS F., 1989. Besoins nutritionnels des lapins. *Cuni-Sciences*, **5**, 1-28.

LUZI F., MAERTENS L., MIJTEN P., PIZZI F., 1996. Effect of feeding level and dietary protein content on libido and semen characteristics of bucks. *6th World Rabbit Congress, Toulose*, Vol. II, 87-92.

PANELLA F., CASTELLINI C., 1990. Fattori ambientali e genetici che influiscono sulle caratteristiche del seme di coniglio. *Riv. Coniglic* **8**, 39-41.

PAUFLER S., SCHLOLAUT W., LANGE K., 1979. Post-partale insemination beim kaninchen mit ovulationsauslösung durch synth LH-Release hormone. *Zuchtygiene*, **14** (1), 37-42.

SAS, 1989. User's Guide Statistics. Version 6.11 *Edition, SAS Inst., Inc., Cary, NC*, USA.

TACKE S., GOTZE S., PINGEL H., 1995. Einfluβ der Häufigheit der Spermagewinnung auf die Spermaqualität der Rammler. 9. Arbeitstagung über Haltung und Krankheiten der

Kaninchen, Pelztiere vJId Heimtiere. Celle, 10-11 May 1995. *Ed Deutsche Vet. Med. Gesellschaft e. V., Giessen*, 41-49.

THEAU-CLEMENT, M., MICHEL N., ESPARBIE J., BOLET G., 1995. Effects of artificial photoperiods on sexual behaviour and sperm output in the rabbit. *Animal Science*, **60**, 143-149.

THEAU-CLEMENT, M., ROUSTAN A., 1992. A study on relationships between receptivity and lactation in the doe and their influence on reproductive performances. *J. Appl. Rabbit Res*., **15**, 412-421.

VAN SOEST P.J., ROBERTSON J.B., LEWIS B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583-3597.

ZEMJANIS R., 1970. Diagnostic and therapeutic techniques in animal reproduction. 2nd Ed., Balliere Tindall and Cox Ltd, London (ed.).

WATSON P.F., 1975. Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Veterinary Record*, **97**, 12-15.

WEITZE K.F., MULLER E., 1991. Prinzipien der Spermauntersuchung. *In: Kühstliche Besamung bei Nutzitieren. Ed. Busch W., Löhle K., Peter W., Gvstav Fischer Verlag. Jena*, 269-310.