

# EFFECTS OF DIETARY PROTEIN CONTENT ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF UNSELECTED RABBIT DOES AND THEIR LITTERS DURING FIRST TWO LACTATIONS

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↳Supporting Information

**ABSTRACT:** The aim of the present study was to evaluate the influence of different dietary protein levels on the productive performance of unselected rabbit does and their litters during their first two lactations. For this purpose, fifty-two nulliparous rabbit does, 4.5 months of age and live weight of  $3115 \pm 71$  g, were divided into three groups (17 or 18 females per group), kept in individual cages and each group received only one of the three experimental diets. These diets were iso-energetic (10.8 MJ DE/kg), but with increasing levels of crude protein (CP): 15%, 17% and 19 % for the low (L), medium (M) or high (H) diets, respectively. Breeding was carried out by natural copulation using 6 males of 5-6 months of age and  $2865 \pm 21$  g initial weight, controlled semi-intensive lactation and weaning at 35 days after birth. Female body weight, feed intake, milk production, litter size and weight were monitored at birth and weekly after parturition during the first two lactations. The protein intake of the rabbits increased with the amount of protein in the diet (L vs. M: +12.2%; L vs. H: +18.8%;  $p < 0.001$ ), without any effect on milk production and feed intake. Milk production was unaffected by parity. Throughout the pre-weaning period, litter size and weight and maternal mortality were unaffected by dietary protein level. Dietary protein level had no effect on live weight, birth to weaning weight gain, milk production or feed intake during the first two consecutive lactations of rabbit does.

**Keywords:** Feed Intake, Litter parameters, Milk production, Protein content, Unselected rabbit does, Weight gain.

## INTRODUCTION

The main cost in raising rabbits is feed, which accounts for more than 70% of the total production cost. Rabbits does are highly fertility and can be simultaneously be pregnant and suckling. The digestible protein consumed by rabbit does hence could be used both for body growth, milk production and fetal growth. A negative protein balance can result if the does are pregnant and lactating simultaneously, especially if the feed intake does not match demand (Parigi-Bini et al., 1992; Martínez-Paredes et al., 2022).

Previous studies have reported the significant effect of energy balance on rabbit does production (Xiccato, 1996; Xiccato et al., 1999), as well as the requirement for milk, in late pregnancy, body fat, energy and protein requirements also become relevant because of the development of the pregnant uterus and the foetal protein turnover (Arias-Alvarez et al., 2009). Saidj et al. (2016) showed that there was no effect of the energy content of the feed neither on their weight or milk production of local Algerian does, nor on their litter. However, a significant decrease in feed intake in females fed most energetic diet was recorded. The protein content of the diet has a significant impact on the cost of feeding, which is the highest cost item in rabbit production - up to 70%. Diets with lower protein content allow for a significant reduction in feed costs and in excretion of nitrogen (Maertens, 1999). According to Mathika (2023), rabbits are likely to have a variable response to the level of protein in their diet. Recently, Martínez-Paredes et al. (2022) suggested that an excess of CP may increase the rate of stillbirth at first parturition.

Therefore, the aim of the present work was to study the influence of different diet protein level on productive performances of local unselected rabbit does and their litters during their two first lactations.

## MATERIALS AND METHODS

### Ethical consideration

The experiment was carried out in accordance with the guidelines for Experimental Animals approved by the Algerian Association of Animal Experimentation Sciences (58 AASEA: N° 45/DGLPAG/DVA/SDA/19).

### Animals and experimental design

A total of fifty-two (52) nulliparous local unselected rabbit does, 4.5 months old and  $3115 \pm 71$  g weight with variable phenotype, were individually weighed and subsequently kept in individual galvanized cages, disposed on flat deck system

and received one of the three experimental diets. Reproduction was performed by natural mating using six males (2 per group) of 5 to 6 months old and  $2865 \pm 21$  g initial weight with semi-intensive rhythm (mating between 10 to 12 days post-partum). The pregnancy diagnosis was made by abdominal palpation 10 days post coitum. Each cage was provided of nest box with a gate to isolate the females of their litters. Each group made up of 17 or 18 females and 2 males. All animals were subject to the same breeding conditions. Reproduction rhythm was semi-intensive, and the kits weaning done at 35 days postpartum. The live weight of does, feed intake, milk production and litters enumeration and weight were controlled at birth and weekly after partum during the two first lactations. The nest boxes were always closed except for the moment of lactation; the does were subjected to control nursing: they had access to their litter once a day (between 08.00 and 09.30). For milk yield measurement, differential weighing of does was performed immediately before and after suckling from parturition to 21d post-partum (Parigi-Bini et al., 1992).

### Experimental diets

Does were divided into three groups and offered one of the three diets formulated to be isoenergetics and to meet the requirements (10.8 MJ DE/kg) according to De Blas and Mateos (2020) but with different and increasing crude protein (CP) content i.e.15, 17 or 19% CP for Low (L), Mean (M) or High (H) diet respectively. The ingredients and nutritional characteristics of the three diets are shown in Table 1. Diets were provided ad libitum between parturition and weaning. The chemical analyses were performed at alimentation laboratory of Higher National Veterinary School, Algiers, Algeria, according to ISO methods and considering the recommendations proposed by the EGRAN group (EGRAN, 2001): dry matter (ISO 6496:1999), crude ash (ISO 5984:2002), crude protein (N $\times$ 6.25, Dumas method, ISO 16634-2:2009). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed at nutritional laboratory of animal production department, Liège University, Belgium, according to sequential method of Van Soest, ashless, without sodium sulphite, and using crucibles (Tecator apparatus) (AFNOR 1997, ISO 16472: 2006 and ISO 13906: 2008). The gross energy (ISO 9831:1998) was analyzed at Animal production laboratory Agro-Bio Tech de Gembloux, Belgium. The digestible energy was estimated with Allix software (2002) when the feeds were formulated.

**Table 1 - Nutritional characteristics of the experimental diets**

Ingredients (%)	Diets		
	L	M	H
Corn Grain	35.0	28.0	23.0
Alfalfa meal	44.1	43.5	43.6
Barley Grain	4.4	6.3	5.6
Soybean Meal	11.7	17.1	22.5
Wheat Bran	2.0	2.3	2.5
Di-Calcium Phosphate	1.8	1.8	1.8
Premix <sup>1</sup>	1.0	1.0	1.0
<b>Chemical composition (%)</b>			
Dry matter	89.3	89.5	89.6
Ash	7.71	10.07	8.40
Crude protein	15.5	17.5	18.9
Crude fibre	11.88	13.88	13.03
ADF	15.89	17.14	16.01
NDF	35.33	45.08	41.79
Ether extract	1.67	2.0	2.33
Gross energy (MJ/kg)	18.26	18.17	18.34
Digestible Energy (MJ/kg)	10.9	10.9	10.9

L: Low-protein content; M: Medium-protein content; H: High-protein content; <sup>1</sup> Composition of mineral-vitamin complement: vitamin B: 6.100 mg; folic acid: 200mg; vitamin D1: 200mg; biotin: 4mg; Choline chloride: 18mg; Co :40 mg; Fe: 4000 mg; Cu: 1000 mg; Mn: 2000 mg; Iodine: 80 mg; Zn: 6000 mg; Se: 8 mg; Mg: 26000mg; Sulphur: 6800mg.

### Statistical analysis

Statistical analysis of the data was performed using the Statistical Analysis Systems Software (SAS, 2005). The data were analyzed using the mixed model procedure of SAS software (SAS, 2005) to identify significant sources of variation. To test the effects on production performances, two fixed effects were considered: diet (L, M, and H) and parity (first and second). The litter size was considered as a covariate in the model for the live weight, milk yield, feed intake, and mortality rates. The least-squares means were compared using Tukey HSD tests (SAS, 2005). The number of kits was compared between groups using a non-parametric procedure (Kruskal-Wallis test) (SAS, 2005). The significance threshold was set at  $P < 0.05$ .

## RESULTS

### Live weight, feed intake and milk production of does

During the experimental period, the live weight of the does was not different regardless the protein level of the ingested feed ( $p > 0.05$ ). However, a significant difference in weight gain was noted at 21 days postpartum ( $p < 0.05$ ) and a trend towards significance in weight gain at weaning ( $p = 0.09$ ). Females that were fed the lowest protein diet tended to gain more weight (Table 2).

**Table 2** - Effect of an increase in the protein content of the diet on the body weight of rabbit does and their feed intake (g) during their first two reproductive cycles (LSM  $\pm$  SEM).

Parameters	Diets			Parity		P-value		
	L	M	H	P1	P2	Diet	Parity	Cov.
<b>Number of observations</b>	31	32	33	50	46	-	-	-
Live Weight at Parturition (g) <sup>A</sup>	3776 $\pm$ 76.7	3859 $\pm$ 74.0	3742 $\pm$ 76.4	3640 $\pm$ 62.6	3945 $\pm$ 66.3	0.53	0.002	0.06
<b>Live Weight During Lactation (g)</b>								
1 <sup>st</sup> Week <sup>B</sup>	4085 $\pm$ 92.1	4111 $\pm$ 83.5	3914 $\pm$ 87.3	4022 $\pm$ 76.7	4052 $\pm$ 76.0	0.23	0.79	0.55
2 <sup>nd</sup> Week <sup>C</sup>	4156 $\pm$ 89.1	4124 $\pm$ 78.6	3975 $\pm$ 83.8	4068 $\pm$ 72.0	4102 $\pm$ 71.1	0.28	0.75	0.46
3 <sup>rd</sup> Week <sup>D</sup>	4219 $\pm$ 94.0	4146 $\pm$ 82.9	3998 $\pm$ 88.3	4219 $\pm$ 75.8	4023 $\pm$ 74.8	0.22	0.08	0.47
4 <sup>th</sup> Week <sup>E</sup>	4280 $\pm$ 86.6	4180 $\pm$ 76.5	4054 $\pm$ 81.4	4247 $\pm$ 69.9	4095 $\pm$ 69.1	0.17	0.14	0.085
5 <sup>th</sup> Week (Weaning) <sup>F</sup>	4281 $\pm$ 92.4	4199 $\pm$ 81.6	4060 $\pm$ 86.8	4273 $\pm$ 74.6	4086 $\pm$ 73.7	0.22	0.09	0.26
Weight Gain Parturition-3 <sup>rd</sup> week <sup>D</sup>	407 $\pm$ 51.5 <sup>a</sup>	230 $\pm$ 45.5 <sup>b</sup>	328 $\pm$ 48.4 <sup>ab</sup>	491 $\pm$ 41.5	153 $\pm$ 50.0	0.04	<0.001	0.16
Weight Gain Parturition-Weaning <sup>F</sup>	467 $\pm$ 59.1 <sup>a</sup>	284 $\pm$ 52.1 <sup>b</sup>	391 $\pm$ 55.4 <sup>ab</sup>	542 $\pm$ 47.7	220 $\pm$ 47.1	0.07	<0.001	0.04
<b>Feed Intake (g)</b>								
1 <sup>st</sup> Week <sup>B</sup>	1287 $\pm$ 79.2	1414 $\pm$ 69.9	1444 $\pm$ 74.6	1364 $\pm$ 64.3	1400 $\pm$ 63.4	0.32	0.71	0.003
2 <sup>nd</sup> Week <sup>C</sup>	1831 $\pm$ 82.5	1734 $\pm$ 74.4	1810 $\pm$ 77.5	1804 $\pm$ 67.3	1780 $\pm$ 65.6	0.64	0.81	0.023
3 <sup>rd</sup> Week <sup>D</sup>	1979 $\pm$ 80.7	1943 $\pm$ 72.8	1871 $\pm$ 77.9	2067 $\pm$ 65.8	1795 $\pm$ 65.5	0.62	0.007	0.002
Total Feed Intake 21 d <sup>D</sup>	5102 $\pm$ 197.7	5088 $\pm$ 178.4	5150 $\pm$ 191.1	5234 $\pm$ 161.1	4993 $\pm$ 160.4	0.97	0.311	0.007
4 <sup>th</sup> Week <sup>E</sup>	2324 $\pm$ 91.2	2251.58 $\pm$ 82.3	2376 $\pm$ 85.6	2416 $\pm$ 74.4	2218 $\pm$ 72.5	0.57	0.07	<0.001
5 <sup>th</sup> Week (Weaning) <sup>F</sup>	2913 $\pm$ 130.5	2768 $\pm$ 117.6	2935 $\pm$ 122.3	2756 $\pm$ 106.5	2987 $\pm$ 103.8	0.57	0.14	<0.001
Total Feed Intake 35 d <sup>F</sup>	10351 $\pm$ 374.0	10090 $\pm$ 337.3	10428 $\pm$ 360.9	10414 $\pm$ 305.2	10165 $\pm$ 303.8	0.77	0.58	<0.001
Daily Feed Intake <sup>F</sup>	295.7 $\pm$ 10.7	288.3 $\pm$ 9.6	297.9 $\pm$ 10.3	298 $\pm$ 8.7	290 $\pm$ 8.7	0.77	0.58	<0.001
Daily protein intake <sup>F</sup>	44.5 $\pm$ 1.9 <sup>a</sup>	50.7 $\pm$ 1.8 <sup>b</sup>	54.8 $\pm$ 1.7 <sup>c</sup>	50.4 $\pm$ 1.6	49.5 $\pm$ 1.6	<0.001	0,70	<0.001

L: Low-protein content; M: Medium-protein content; H: High-protein content; P: parity; Cov.: covariate; SEM: standard error of the mean; a, b, c: Means with different letters on the same row between diets (Low, Medium and High) and parities (P1 and P2) differ significantly ( $P < 0.05$ ); A Covariate: Total litter size at partum; B Covariate: Total litter size at 07 days; C Covariate: Total litter size at 14 days; D Covariate: Total litter size at 21 days; E Covariate: Total litter size at 28 days; F Covariate: Total litter size at 35 days.

**Table 3 - Effect of an increase in the protein (%) content of the diet on the milk production of local rabbits does during their first two reproductive cycles (LSM ± SEM)**

Parameters (g)	Diets			Parity		P-value		
	L	M	H	P1	P2	Diet	Parity	Cov.
Number of observations	31	32	33	50	46	-	-	-
1 <sup>st</sup> Week <sup>B</sup>	703±33	739 ±31	741±32	699±28	756±27	0.664	0.174	<0.001
2 <sup>nd</sup> Week <sup>C</sup>	1083±46	1085±42	1063.±44	1062±38	1092±37	0.924	0.592	<0.001
3 <sup>rd</sup> Week <sup>D</sup>	1217±49	1300±45	1265±47	1241±41	1280±39	0.484	0.515	<0.001
Lactation <sup>D</sup>	3016±122	3132±111	3080±116	3007±100	3144±97	0.785	0.352	<0.001

L: Low-protein content; M: Medium-protein content; H: High-protein content; P: parity. Cov.: covariate; SEM: standard error of the mean; <sup>B</sup> Covariate: Total litter size at 07 days; <sup>C</sup> Covariate: Total litter size at 14 days; <sup>D</sup> Covariate: Total litter size at 21 days.

**Table 4 - Effect of increasing dietary protein (%) on rabbit litter performance over the first two reproductive cycles (LSM ± SEM)**

Parameters	Diets			Parity		P-value		
	L	M	H	P1	P2	Diet	Parity	Cov.
Number of observations	31	32	33	50	46	-	-	-
<b>Total Litter Size at</b>								
Birth	6.74	6.91	7.00	6.02	7.83	0.890	<0.001	-
Live at Birth	5.13	6.12	5.66	5.10	6.24	0.463	0.013	-
1 <sup>st</sup> Week	3.77	4.85	4.44	3.50	5.30	0.322	0.001	-
2 <sup>nd</sup> Week	3.71	4.76	4.34	3.42	5.22	0.361	0.001	-
3 <sup>rd</sup> Week	3.71	4.76	4.31	3.42	5.20	0.359	0.001	-
4 <sup>th</sup> Week	3.71	4.76	4.28	3.40	5.20	0.353	0.001	-
5 <sup>th</sup> Week (Weaning)	3.65	4.76	4.25	3.36	5.17	0.320	0.001	-
<b>Mortality (%) at</b>								
Birth <sup>A</sup>	25.2±5.28	11.8±5.10	18.1±5.17	16±4.34	20.77±4.55	0.193	0.457	0.800
Birth to Weaning <sup>E</sup>	31.1±7.06	24.8±6.49	30.7±6.82	34.9±5.39	22.85±5.71	0.990	0.823	<0.001
<b>Total litter weight (g) at</b>								
Birth <sup>A</sup>	394±11.2	420 ±10.8	392±10.9	394±9.1	410±9.6	0.142	0.253	<0.001
Live at Birth <sup>A</sup>	313±21.2 <sup>a</sup>	382±19.9 <sup>b</sup>	359±20.9 <sup>ab</sup>	346±17.2	356±18.1	0.062	0.705	<0.001
1 <sup>st</sup> Week <sup>B</sup>	658±25.8	674±22.7	685±24.2	648±20.8	698±20.6	0.748	0.107	<0.001
2 <sup>nd</sup> Week <sup>C</sup>	1216±49.5	1172±43.3	1157±47	1132±40	1231±38.9	0.675	0.091	<0.001
3 <sup>rd</sup> Week <sup>D</sup>	1717±69.5	1724±61.2	1694±66.4	1665±56.4	1759±54.9	0.942	0.254	<0.001
4 <sup>th</sup> Week <sup>E</sup>	2640±110.8	2573±97.6	2591±105.8	2528±90.1	2675±87.6	0.900	0.265	<0.001
5 <sup>th</sup> Week (Weaning) <sup>F</sup>	3835±149.2	3579±131.3	3786±142.3	3584±121.3	3883±118.1	0.380	0.091	<0.001

L: Low-protein content; M: Medium-protein content; H: High-protein content; P: parity; Cov.: covariate. SEM: standard error of the mean; a,b,c, Means with different letters on the same row between diets (Low, Medium and High) and parities (P1 and P2) differ significantly (P<0.05); <sup>A</sup> Covariate: Total litter size at partum; <sup>B</sup> Covariate: Total litter size at 07 days; <sup>C</sup> Covariate: Total litter size at 14 days; <sup>D</sup> Covariate: Total litter size at 21 days.; <sup>E</sup> Covariate: Total litter size at 28 days; <sup>F</sup> Covariate: Total litter size at 35 days

Of the total number of 52 nulliparous rabbits, 100% of the females was receptive. Also, the fertility rate found in the trial was 100%. No effect of parity on the weight of does was observed ( $p>0.05$ ). However, parity's effect on weight gain was significant ( $p<0.001$ ). During the experiment, no significant interaction was detected between the dietary protein content and the parity on live weight and weight gain of the does ( $p>0.05$ ). There was no effect of litter size at weaning, used as a covariate, on the weight of does between parturition and weaning ( $p>0.05$ ). On the other hand, the effect of the covariate on the weight gain of does was significant ( $p<0.05$ ) over the same period. The protein content of the diet did not influence the milk yield produced ( $p>0.05$ ). The effect of parity on milk production was not significant, although the quantity produced in the second lactation was higher by +5.4% (Table 3).

#### Litter size and weight

Regardless of the feed distributed, litter size, litter weight and nest mortality showed no significant difference between birth and weaning ( $p>0.05$ ), except for a trend towards significance in live litter weight at birth ( $p=0.06$ ); live litters fed the M feed were significantly heavier than those fed the lower protein L feed (Table 4). The effect of parity on litter size was significant ( $p<0.05$ ) between parturition and weaning. Litters from second pregnancy were larger than those from the first pregnancy. On the other hand, there was no significant difference due to parity on still birth, nest mortality and litter weight at pre-weaning ( $p>0.05$ ). However, a statistical trend ( $p=0.09$ ) was found on litter weight at weaning. The effect of litter size under the dam was highly significant ( $p<0.001$ ) and taken into account as a covariate to eliminate its effect on litter weight.

## DISCUSSION

The choice of this topic was motivated by the availability of a unique feed for rabbits in the local market, which is distributed to all rabbits of different sex and/or physiological stages. This work has already been initiated on local nulliparous pregnant rabbits from the same population (Saidj et al., 2019); the results showed that variations in the protein content of the feed had no effect neither on the weights and intakes of the females during their first management, nor on their metabolic profile. However, the bibliography showed a considerable variation in the nutritional needs of does according to their production and reproduction cycles (Parigi-Bini and Xiccato, 1993; Pascual et al., 2003, Maertens and Coudert, 2006; De Blas and Mateos, 2020). Of the total number of nulliparous rabbits, the receptivity was higher than that found by Ilés (2015) on the same population, estimated at 69.2%, but on a greater number of observations (253). Also, the fertility rate found was higher than that measured by Fellous et al. (2012) on the same local population which was 85.9% in first parity. These same authors emphasize that among the local population, the fertility rate decreased with parity. In the second parity during the present experiment, females were presented to male at the 10th day post-partum and all the females were receptive. These results showed that the experimental breeding conditions were adequate with natural reproduction and without the use of synthetic hormones, but a number of does reduce.

#### Live weight, feed intake and milk production of does

The use of high protein diets doesn't show effect on local unselected rabbit doe's weight at partum and between partum and weaning, and consecutively on does' weight gain during lactation. Does given H diet showed significantly higher protein intake a day at lactation (58.08 g for H group vs. 52.94 g for M group vs. 44.34g for L group) ( $p<0.01$ ), but no difference was detected in the feed intake a day between partum and weaning (294.5g for L group vs 311.1g for M group vs. 305.8 g for H group), the digestible energy intake a day and their milk yield. Brun and Lebas (1997) showed that the use of feed with high protein does not affect prolificacy. In this work, litter size and weight at partum and at weaning were not affected by the diets but the effect of litter size on milk production during 21 days post-partum was observed in all the three diets ( $p<0.001$ ). Saidj et al. (2021) reported, on the same rabbit population and with the same diets, that lactation peak occurs at different times (16th, 20th and 20th day PP at first lactation and 15th, 17th and 20th days post-partum at second lactation for L, M and H groups respectively) and lactation peak tended to occur later.

Sanchez et al. (1985) reported that between 21 and 28 days of lactation, live weight was lower in rabbits fed a diet containing 17.5% CP than those fed a diet with 19 or 20.5% CP. According to Odi (1990), a decrease in body weight of rabbits could be expected at 21 days post-partum. Partridge et al. (1983) point out that the mobilization of body reserves starts at about 11 days and continues at the time of peak milk production between 18 and 21 days PP, coinciding with a period of catabolism of the body. This variation in female body weight at this time of reproduction is not always evident (Odi, 1990). The gradual increase in female body weight throughout the trial is not found by Sakr (2012) who observed a decrease in female rabbit body weight between the first parturition and day 25 postpartum. The results obtained in the present study showed that the female rabbits completed their growth progressively while ensuring their gestation and lactation simultaneously.

In growing rabbits, Ouhayoun and Cheriet (1983) observed that for a given feed energy concentration, the variation of protein content (17.2 and 13.8%) had no significant effect on the growth rate and the slaughter yield, which is also found in growing piglets. Also, in growing piglets, Quiniou et al. (1994) did not notice any difference in the protein content of the feed (17.8, 15.5 and 13.6% BW) on weight gain and carcass characteristics. However, carcass fatness was reduced in rabbits fed the highest protein diets (Ouhayoun and Cheriet, 1983). On the other hand, Ouhayoun and Dalmas (1983)

found a significant increase in weight at 11 weeks of age and overall growth rate with increasing feed protein level (10.4, 13.8, and 17.2%).

According to Renouf et al. (2009), in fattening rabbits, the decrease in diet crude protein level from 16% to 14.5% had no effect on live weight, feed intake or animal mortality. However, with the highest phosphorus level, the decrease in protein level tended to reduce feed intake, while the opposite effect was observed with the lower phosphorus level.

In rabbits fed diets containing different protein levels (13.5%, 17.5% and 21% CP/kg DM), Partridge and Allan (1982) recorded a decrease in feed intake in females fed the lowest protein diet (13.5%) and an increase in milk production with an increase in the protein content of the diet, without any change in the chemical composition of the milk; in contrast to The present study results which show no effect of the dietary protein content on milk performance or feed intake of does.

In the present trial, the protein intake of rabbits increased with the protein level of the diet (L vs. M: +12.2%; L vs. H: +18.8%;  $p < 0.001$ ) without affecting milk production. The present results corroborate those found in pigs. Indeed, studies have shown the absence of a significant influence of a severe protein ration on milk production and litter growth (Revell et al., 1998; Mejia-Guadarrama et al., 2002). Jang et al. (2014) tested on pregnant sows, 4 feeds with different protein contents (11%, 13%, 15% and 17%). The results showed no significant difference in the amount of milk produced, although the daily feed intake did not vary significantly during lactation, leading to a higher protein intake in sows fed the higher protein diet (+38% protein intake between the 11 and 17% CP diets).

On the other hand, amino acids can vary milk production. In fact, in rabbits, Taboada et al. (1994) observed a positive and significant effect of the lysine content of the feed on milk production, with a better result in females fed the 0.82% lysine feed compared to the 0.68% one, knowing that the protein content of the different feeds is 18% CP. In sows and rats, an intake of amino acids in the diet, during a period of growth when the mammary cells are still multiplying, stimulates the proliferation of these cells and consequently, increases the amount of milk produced (Knight and Peaker, 1984; Jansen and Binard, 1991; Luise et al., 2023). This difference in milk production is thought to be related to dietary amino acid intake and is not due to hormonal secretion, as lysine and methionine do not stimulate galactopoietic hormone secretion to any great extent (Kuhara et al., 1991). According to Rulquin (1992), in dairy cows, supplementation with certain amino acids such as methionine and lysine (with a 14% crude protein feed), increased milk production at the beginning of lactation. However, in mid-lactation, no increase in milk production was observed. Partridge and Allan (1982) found no change in the chemical composition of milk in rabbits fed diets with different protein levels (13.5%, 17.5% and 21%). On the other hand, in dairy cows, Rulquin (1992) has shown that the composition of milk, and more specifically the composition of milk proteins, can be improved by a complementary supply of certain amino acids such as methionine and lysine (14% of crude protein); during the beginning and middle of lactation, an increase in protein content is noticed.

This physiological phenomenon could be explained, in part, by the availability of the different amino acids that make up milk at the mammary level. In pregnant sows, Jang et al. (2014) using 4 feeds of different protein levels (11%, 13%, 15% and 17%) observed no difference in the chemical composition of colostrum (at day 01 postpartum), nor milk at day 21 of lactation. On the other hand, Zhang et al. (2011) reported a positive and linear effect of the lysine level of iso-protein feeds (13% crude protein and lysine levels of 0.46, 0.56, 0.65 and 0.74%) on the protein level of the colostrum (+5%), knowing that the females concerned ingested the same amount for the different groups.

In the current study, there was no effect of parity on milk production. These results do not corroborate those found by Xiccato et al. (2004) who determined an increase in milk production by 8% and 10% respectively of the rabbits in the second and third litter compared to the first, while equalizing the litters involved in the experiment. In this work, litter equalization was not performed. However, litter size, which had a highly significant effect ( $p < 0.001$ ), was taken into account as a covariate to eliminate its effect on milk production.

Litter size has a strong influence on milk production during the two lactations studied ( $p < 0.001$ ). Indeed, according to Zerrouki et al. (2005), the milk production of the local population increases with the number of young until reaching 7 kits / litter where a production plateau is reached. Chibah-Ait Bouziad et al. (2015) confirm that regardless of the litter size at birth and/or the genetic type of the females, the increase in milk production per day and per 21 days follows the number of kits breastfed, even if they are adopted, without any relation to the number of kits at birth. In addition, the increase in milk production following the number of lactations is known in mammals, which is due to various influencing factors such as physiology, weight and maturity of the udder. In other species as prolific as the rabbit (such as sows and rats), litter size and/or parity are directly correlated to milk production with the growth of the mammary gland during lactation, which would cause a direct effect on the intensity of the mother's milk production (Farmer and Palin, 2005; Hurley, 2001).

#### **Litter size and weight**

The results obtained herein are similar to those of Brun and Lebas (1997) who showed that in crossbred rabbits (2066 X 1077) an increase in dietary protein from 14.9% to 20.6% DM had no effect on litter size at birth or weaning. Likewise, Partridge and Allan (1982) did not find that weaned litter size varied with dietary protein levels (13.5, 17.5 and 21 %). However, Brun and Lebas (1997) reported that the highest protein diet increased the average weight of a young rabbit at weaning (29 days) by 8.2% and the average litter size by 6.5%, in contrast to the lack of effect in the present

trial. Also, in the long term, female rabbits receiving the lower protein diet weaned more litters than females receiving the high crude protein diet (Brun and Lebas, 1997).

In Rex rabbits, Ren et al. (2004) recommend the use of a diet with 10.5 MJ/Kg of digestible energy and 17.5% crude protein for pregnant rabbits, which is favorable for improving the size and weight of total and live litters at birth. On the other hand, these same authors indicate that in lactating rabbits, the crude protein content should reach 19.5% for an energy content of 10.7 MJ/Kg, in order to improve the size and weight of litters at weaning. As for the effect of parity on prolificacy and litter size traits, the obtained results corroborate those obtained by Zerrouki et al. (2005) on the same population concerning litter size at birth. However, these authors did not find an effect of litter size under the dam, contrary to the present work results which show a significant effect of parity on litter size during lactation ( $p < 0.05$ ).

Furthermore, the positive effect of parity on litter weight at birth increased with the order of parity, with higher individual weanling weights in multiparous animals (Zerrouki et al., 2005). In crossbred rabbits (New Zealand X Californian), Xiccato et al. (2004) found no significant difference in litter size regardless of parity. On the other hand, litter weight at birth, at weaning and weight gain during the birth-weaning period increased significantly with parity (+7.7%, +10.3% and 6.7% respectively for the parameters mentioned).

## CONCLUSION

The local unselected rabbit does don't require high level of protein to meet the needs of reproduction and production. Similarly, the size and weight of live litters and the mortality rate under the dam are not affected by the protein content of the diet throughout the pre-weaning period. Although there are no overall effects of the protein content of the feed on the zootechnical parameters of the rabbits, the females gained more weight by receiving more proteins, which can have a positive effect on the longevity of their reproductive career, thus avoiding negative and harmful energy balance on their production career.

## DECLARATIONS

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### Authors' contribution

All authors contributed equally to the study.

### Availability of data

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

### Consent to publish

All authors have reviewed and approved the final manuscript for publication.

### Competing interests

The authors declare no competing interests in this research and publication.

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