JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2019.f307
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

March 2019
VOL 6, No. 1, PAGES 18–24

ORIGINAL ARTICLE

Productive performance, metabolic, and hematologic parameters of pregnant nulliparous rabbit does according to dietary protein level

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ABSTRACT

Objective: This study aims at investigating the productive performance, metabolic, and hematological profiles of Algerian local rabbits does during their first pregnancy and according to dietary protein content.

Materials and methods: A total of 52 nulliparous rabbit does, 3,116 ± 72.9 g live weight, were allocated individually to three groups (17/18 females per group) being on isoenergetic diets [2,600 kcal Digestible Energy (DE)/kg] that differ in their digestible protein content by 15%, 17%, and 19%, respectively, for L, M, and H diets. All these diets were provided *ad libitum*.

Results: The use of a high protein diet did not affect does weight and feed intake during the pregnancy period. Statistically, no significant difference ($p > 0.05$) was recorded in born or alive litter size and birth weight. There was no effect of diet and sampling time ($p > 0.05$) on plasma metabolites but there were significant effects of sampling time ($p < 0.0001$) on the metabolic parameters studied with prolificacy used as a covariate. Hematologic indices in pregnant rabbits were not affected by the diet exceeded the red blood cells rate that increased significantly ($p < 0.05$) with the increase in dietary protein content during the different periods of gestation.

Conclusion: The use of a high protein diet has no effect on weight during the pregnancy period. No effect of diet and sampling time on plasma metabolites and hematological profiles are recorded; however, significant effects of sampling time are recorded on the metabolic parameters.

ARTICLE HISTORY

Received September 17, 2018
Revised October 21, 2018
Accepted October 30, 2018
Published December 28, 2018

KEYWORDS

Diet; metabolism; pregnancy; protein content; rabbit doe

Introduction

Rabbit females are particularly sensitive to adequate dietary energy and protein during pregnancy. With an emphasis on nulliparous, as they are still growing and use ingested nutrients to support body maintenance and growth, as well as fetus's body and placenta, and annexes (uterus and mammary tissue) [1,2]. The same was noticed with sow by Jang et al. [3].

Rabbit does are bred when reaching 75% of adult weight and growth continues during the first cycle of reproduction. Ouhayoun [4] showed that the balances between the various components of feed, the level of energy and protein, represent factors affecting the growth. On their side, Carabaño et al. [5] showed that a significant reduction in dietary protein content could have negative effects on rabbits' growth and mortality rate during breeding.

As with other mammalian species (e.g., cows and sows), rabbit doe at this stage has not yet reached maximum voluntary dietary intake. Associated negatively with the physiological decrease of intake during last week of pregnancy [2], this state becomes a limiting factor and has implications on metabolism since the female is forced to mobilize body reserves to provide needs at the beginning of gestation (the first 21 days). rabbit does retain body protein subsequently used during rapid fetal development in the last period of gestation [1,6], in the last three days of pregnancy, perirenal fat is mobilized [7]. Manchetti et al. [8] report that the live weight gain and weight gain of the

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How to cite: Saidj D, Ainbaziz H, Iles I, Dahmani Y, Hornick JL, Moula N. Productive performance, metabolic, and hematologic parameters of pregnant nulliparous rabbit does according to dietary protein level. J Adv Vet Anim Res 2019; 6(1):18–24.

doe fat mass during gestation allow for better productive performance.

Several studies have been carried out, particularly on Algerian local population [9], by varying energy content of feed in rabbits simultaneously pregnant and lactating. The present study was conducted with the objective to evaluate effects of different dietary protein content on zootechnical, hematological, and biochemical performances of local rabbits does during their first pregnancy to assess the best dietary protein contents for best production of Algerian local rabbits.

Materials and Methods

Ethical approval

Due to the lack of animal ethics commission in Algeria, the authors followed the regulations applied in Liege University, Belgium.

Animals and management

This experiment was carried out in the experimental rabbitry of the National Veterinary School in Algiers (Algeria). A total of 52 nulliparous local rabbit does weighing 3,116 ± 72.9 gm were individually kept in galvanized cages and arranged according to flat deck system (46.5 × 62 × 29 cm height), with a closable nest-box (43 × 26 × 26 cm height). They were provided with feed and water *ad libitum* during the experiment**.** The does were first mated at the age of 4.5 to 5 months. They were randomly allotted to one of three experimental groups receiving diets containing different crude protein levels (15%, 17%, and 19% for L, M, and H groups) during gestation (Table 1). Each diet contained 2,600 kcal of Digestible Energy (DE)/kg. Formulating the three experimental diets was made so as to modify the rate of raw materials incorporated to modify the protein content. Materials used were corn grain, alfalfa meal, barley grain, soybean meal, and wheat bran.

Natural mating was performed. The does were tested for pregnancy by abdominal palpation 10 days after mating. Live weight and feed intake were recorded at mating, weekly (1st, 2nd, 3rd, and 4th week between mating and parturition), and at day 30 *post coitum*. Litters were weighed and enumerated at birth.

Blood sample collection and analyses

At mating and thereafter at weekly intervals until d28, blood samples (2–4 ml each) were collected between 07:00 and 09:00 a.m. from marginal ear vein using heparinized tubes for glucose. Cholesterol, triglycerides, total proteins, and urea determination and into Ethylenediaminetetraacetic acid (EDTA) tubes for hematology analysis. Tubes for metabolites analysis were immediately centrifuged at

Table 1. Ingredients and calculated nutritional characteristics of the experimental diets.

	Diet		
	L	M	H
Ingredients (%)			
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
Dicalcium phosphate	1.8	1.8	1.8
Premix [1]	1.0	1.0	1.0
Calculated chemical composition (%)			
Digestible Energy (kcal/kg)	2,600	2,600	2,600
Crude fiber (%)	13.9	13.9	14.0
Indigestible crude fiber (%)	11.5	11.6	11.6
Crude protein (%)	15	17	19
Méthionine-cystine (%)	0.49	0.54	0.59
Lysine (%)	0.73	0.87	1.0
Calcium (%)	1.48	1.48	1.50
Total phosphorus (%)	0.59	0.61	0.63

[1]Composition of mineral-vitamin complement: vitamin B6: 100 mg; folic acid: 200 mg. vitamin D1: 200 mg; biotin: 4 mg; cholinclorure: 18 mg; Co: 40 mg; Fe: 4,000 mg; Cu: 1,000 mg; Mn: 2,000 mg; I: 80 mg; Zn: 6,000 mg; Se: 8 mg; Mg: 26,000 mg; S: 6,800 mg. L (Low), M (Medium), and H (High).

3,000 rpm for 10 min and plasma was aliquoted and stored at −20°C until assay. Blood parameters were determined by using a spectrophotometer (LKB Novastec) and available commercial kits (SPINREACT.SA. Spain).

For hematology analysis, the measurements were total white blood cells (WBC), total red blood cells (RBC), hematocrit percentage (HCT), hemoglobin concentration (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet count (PLT). All hematologic measurements were performed using automated hematology analyzers as described by Post et al. [10].

Statistical analysis

The different results are expressed as least square mean ± SE. The data were subjected to variance analysis, using the general linear model procedure of SAS software (Statistical Analysis Systems Software 2005), to determine the effect of protein content, on the different zootechnical parameters (does weight and their feed intake during first pregnancy and pups weight at parturition). Number of pups was analyzed using a non-parametric procedure (Kruskal–Wallis tests) and expressed as means. For live weight and feed intake, the litter size at birth was used as a covariate. Biochemical and blood parameters were tested according to a mixed model for repeated measurements (Proc MIXED) [11] including the effects of the diet (2 df), the day of measurement (3 df), and the litter size (7 df). Correlation between successive measurements was modeled using a type 1-autoregressive structure. Treatment means differences were considered indicating significant differences at $p < 0.05$and tendency at $p < 0.1$.

Results and Discussion

Live weight and feed intake at pregnancy

There is no significant difference between the different groups on the rabbits does weight throughout the first gestation (Table 2) without the effect of litter size as a covariate. No significant differences were found on weekly and total voluntary feed intake along gestation period but the effect of total litter size as covariate was significant at the second week of gestation ($p = 0.047$) and tended to decrease by increasing dietary protein content at the third week ($p = 0.058$) and at the end of gestation ($p = 0.083$).

Our results do not agree with those found by Yassein et al. [12] who observed that rabbits fed on diet higher in protein before and during the first gestation ingest smaller diet amounts. On the other hand, our results are similar to those mentioned by Dias et al. [13] who found no significant difference of low fed (13%), medium (17%), and higher protein diets on does live weight. But these rabbits does were not in pregnancy; they were just before a reproduction period.

Litter size and weight at birth

No significant differences were found in litter size and weight at birth as well as live litter size and weight (Table 3). Different protein contents of diet did not affect does live weight at parturition. Xiccato et al. [14] observed in the pregnant rabbit does that a reduction of digestible protein (DP)/DE decreases litter weight and size at born while having less effect on the protein body balance. The results showed a considerable difference on total litter weight and alive litter weight at birth, which increased respectively by +7.43% and +10.3% between groups L

Table 2. Effects of the dietary protein levels on the body live weight and feed intake of nulliparous gestating rabbit does.

Parameters (g)	Diet (Dp: g/kg)			*p*-value		R^2
	L	M	H	Diet	Covariate	
Live weight at mating	3332 ± 99.8	3395 ± 102.8	3449 ± 99.6	0.710	-	0.02
Live weight at pregnancy						
1st week	3681 ± 94.8	3722 ± 97.6	3650 ± 94.6	0.870	0.910	0.01
2nd week	3860.52 ± 97.4	3791.99 ± 100.3	3826 ± 97.2	0.887	0.602	0.01
3rd week	4015 ± 103.7	3967 ± 106.8	4058 ± 103.5	0.828	0.635	0.01
4th week	4157 ± 110.8	4043 ± 114.2	4169 ± 110.6	0.687	0.980	0.01
30 days	4170 ± 121.5	4062 ± 125.2	4193 ± 121.3	0.726	0.420	0.02
Feed intake at pregnancy						
1st week	1383 ± 25.4	1345 ± 26.2	1346 ± 25.4	0.505	0.128	0.07
2nd week	1380 ± 27.5	1332 ± 28.4	1339 ± 27.5	0.428	0.047	0.10
3rd week	1455 ± 50.2	1362 ± 51.6	1424 ± 50	0.433	0.058	0.10
4th week	1244 ± 67.3	1110 ± 69.3	1291 ± 67.2	0.163	0.203	0.11
28th–30th day	449 ± 48.4	518 ± 49.8	395 ± 48.3	0.214	0.083	0.12
Total feed intake	5912 ± 145.6	5669 ± 150	5798 ± 145.3	0.516	0.889	0.03

L (Low), M (Medium), and H (High). Covariate: Total litter size at partum.

Table 3. Effects of the dietary protein level on the reproductive performance at parturition of primiparous rabbit does.

	Diet			*p*-value		R^2
	L	M	H	Diet	Cov.	
Does live weight	3681 ± 103.7	3646 ± 106.9	3668 ± 103.5	0.973	0.643	0.01
Total born per litter	5.82 ± 0.57	6.19±0.48	6.06 ± 0.26	0.85	-	0.56
Total born weight	349 ± 12.5	357±12.9	377 ± 12.5	0.261	0.0001	0.66
Born alive per litter	4.59 ± 0.67	5.50±0.50	5.24 ± 0.39	0.586	-	0.54
Total born alive weight	305 ± 25.1	324±24.3	340 ± 23.6	0.592	0.004	0.18

L (Low), M (Medium), and H (High).

and H. The effect of diet protein contents on the development of litter could occur on the longer term. Indeed. Fleming et al. [15] pointed out that feeding rabbit does in reproduction would not only affect the weight characteristics of fetuses at birth but also help to determine the levels of feed risk (digestive metabolism and reproductive problems) that the rabbit will tolerate throughout its life as well as its subsequent growth. According to Maertens et al. [16], during lactation, litters receiving milk richer in protein and in large quantities grow more rapidly, with a strong correlation (0.91) between the amount of milk produced and the growth of the young. De Blas and Wiseman [17] also observed in fattening rabbits that an exaggerated decrease in the dietary protein level decreases ileal protein flux so reduces mortality during the fattening period.

Metabolic parameters

There was no effect of diet (L, M, and H) on plasma metabolites but there were significant effects of sampling time ($p < 0.0001$) on glucose, cholesterol and triglycerides (Table 4), and on total protein (Table 5), without significant effect of prolificacy as a covariate. Our results are close to those found by Wang et al. [18] in fattening rabbits reporting that variation in dietary protein content had no significant effect on metabolic profile.

Parigi-Bini et al. [6] reported that during early gestation, rabbit increases voluntary feed consumption by 25%–50% while low fetal growth requirements, giving a positive metabolic balance allowing an increase in body reserves. On the other hand, at the end of gestation, rabbit reduces ingestion and fetal growth accelerates causing

Table 4. Effects of the dietary protein level on the energetic blood metabolic profiles of rabbit does during their first pregnancy.

Parameter	Diet			*p*-value		
	L	M	H	Diet	Day	Cov.
Glucose (mg/dl)						
Mating	123.6 ± 18.16	142 ± 31.45	158.6 ± 31.45	0.684	0.001	0.307
07 days	143.6 ± 10.48	138 ± 10.48	137.3 ± 10.48			
14 days	136.6 ± 10.5	124.7 ± 11.1	133.1 ± 11.1			
21 days	139.9 ± 10.5	114.2 ± 10.5	10.7.1 ± 11.1			
28 days	97.5 ± 10.5	101.4 ± 11.1	110.7 ± 11.9			
Cholesterol (mg/dl)						
Mating	61.5 ± 11.0	50.8 ± 19.0	25.7 ± 19.0	0.428	0.0001	0.480
07 days	62.8 ± 6.34	52.6 ± 6.34	47.2 ± 6.34			
14 days	47.8 ± 6.34	42.1 ± 6.73	51.0 ± 6.73			
21 days	23.6 ± 6.73	26.3 ± 6.34	27.2 ± 6.73			
28 days	19.2 ± 6.34	12.0 ± 6.73	18.6 ± 7.19			
Triglycerids (mg/dl)						
Mating	43.1 ± 18.3	84.5 ± 31.7	62.1 ± 31.7	0.342	0.0001	0.189
07 days	68.0 ± 10.6	71.3 ± 10.6	55.8 ± 10.6			
14 days	70.2 ± 10.6	75.9 ± 11.2	66.6 ± 11.2			
21 days	100.5 ± 10.6	118 ± 10.6	71.8 ± 11.2			
28 days	46.7 ± 10.6	33.7 ± 11.2	36.8 ± 12.0			

L (Low), M (Medium), and H (High).

Table 5. Effects of the dietary protein level on the proteic blood metabolic profiles of rabbit does during their first pregnancy.

Parameter	Diet			*p*-value		
	L	M	H	Diet	Day	Cov.
Urea (mg/dl)						
Mating	15.4 ± 6.7	31.6 ± 11.6	22.5 ± 11.6	0.413	0.106	0.385
07 days	28.6 ± 3.87	33.7 ± 3.87	33.5 ± 3.87			
14 days	26.6 ± 3.87	35.1 ± 4.10	30.8 ± 4.10			
21 days	25.1 ± 3.87	28.8 ± 3.87	25.2 ± 4.10			
28 days	22.6 ± 3.87	24.9 ± 4.10	25.4 ± 4.38			
Total protein (g/dl)						
Mating	4.46 ± 0.54	5.12 ± 0.94	3.90 ± 0.94	0.665	0.013	0.719
07 days	4.81 ± 0.31	5.12 ± 0.31	5.20 ± 0.33			
14 days	4.59 ± 0.31	5.04 ± 0.33	5.09 ± 0.33			
21 days	4.30 ± 0.31	4.84 ± 0.31	4.79 ± 0.36			
28 days	4.25 ± 0.31	4.10 ± 0.33	4.03 ± 0.36			

L (Low), M (Medium), and H (High).

Table 6. Effects of the dietary protein level on the blood count profiles of rabbit does during their first pregnancy.

Parameter	Diet			*p*-value		
	L	M	H	Diet	Day	Cov.
WBC × 10^3 (Ml)						
07 days	7.88 ± 0.62	7.80 ± 0.48	6.92 ± 0.50	0.839	0.0001	0.646
14 days	7.71 ± 0.50	7.64 ± 0.53	7.82 ± 0.53			
21 days	5.85 ± 0.53	6.67 ± 0.50	6.27 ± 0.57			
28 days	4.87 ± 0.43	5.17 ± 0.48	5.52 ± 0.50			
RBC × 10^6 (µl)						
07 days	5.90 ± 0.24	4.99 ± 0.23	5.93 ± 0.23	0.044	0.023	0.324
14 days	5.86 ± 0.24	5.97 ± 0.25	6.28 ± 0.25			
21 days	5.67 ± 0.25	5.80 ± 0.24	6.23 ± 0.27			
28 days	5.43 ± 0.21	5.52 ± 0.23	5.91 ± 0.24			
Hgb (g/dl)						
07 days	13.63 ± 0.49	12.01 ± 0.46	12.91 ± 0.46	0.712	0.0001	0.667
14 days	13.67 ± 0.49	14.01 ± 0.51	14.19 ± 0.51			
21 days	12.96 ± 0.51	13.47 ± 0.49	13.78 ± 0.55			
28 days	11.87 ± 0.42	12.27 ± 0.46	12.34 ± 0.49			
HCT (%)						
07 days	36.94 ± 1.52	36.54 ± 1.44	35.22 ± 1.44	0.261	0.016	0.922
14 days	37.01 ± 1.52	36.42 ± 1.61	38.86 ± 1.61			
21 days	35.71 ± 1.61	36.29 ± 1.52	38.31 ± 1.73			
28 days	34.15 ± 1.32	34.24 ± 1.44	35.59 ± 1.52			
MCV (fl)						
07 days	62.08 ± 1.03	61.68 ± 0.98	59.41 ± 0.98	0.111	0.490	0.020
14 days	63.33 ± 1.03	60.95 ± 1.10	61.55 ± 1.10			
21 days	63.06 ± 1.10	62.47 ± 1.03	60.30 ± 1.17			
28 days	62.99 ± 0.9	61.96 ± 0.98	60.26 ± 1.03			
MCH (pg)						
07 days	22.98 ± 0.81	25.16 ± 0.77	21.79 ± 0.77	0.052	0.059	0.584
14 days	23.32 ± 0.81	23.45 ± 0.86	22.54 ± 0.86			
21 days	22.84 ± 0.86	23.17 ± 0.81	22.11 ± 0.92			

Continued

Parameter	Diet			*p*-value		
	L	M	H	Diet	Day	Cov.
28 days	21.83 ± 0.070	22.17 ± 0.77	20.91 ± 0.81			
PLT× 10^6 (µl)						
07 days	175.00 ± 42.26	195.00 ± 40.09	194.70 ± 40.09	0.815	0.155	0.963
14 days	147.78 ± 42.26	188.37 ± 44.82	181.12 ± 44.82			
21 days	182.68 ± 44.82	189.33 ± 42.26	169.43 ± 47.91			
28 days	263.50 ± 36.59	253.50 ± 40.09	220.22 ± 42.26			

L (Low), M (Medium), and H (High).

a negative balance resulting in a mobilization of the body reserves.

Hematologic parameters

The effect of diet on hematologic parameters in rabbits, in general, is poor in literature. The Table 6 showed that hematology indices in pregnant rabbits were not affected by diet, only the number of RBC significantly increased ($p < 0.05$) with the increase in diet protein content during the different periods of gestation. Also, a trend ($p = 0.052$) for the MCH parameter throughout the experiment; MCH content tends to increase in animals in the group fed with M diet.

The parameters of blood formula vary significantly with sampling time, except for MCV and PLT. According to Bischof [19], in mammals, pregnancy is a physiological phenomenon that looks like the inflammatory response to different immune responses one. However, Melillo [20] reported that higher total leukocyte counts can be used to characterize malnutrition in rabbits. In our experiment, no abnormalities were observed. No significant effect of either the protein content of the diet or the gestation period was observed on the PLT content despite very high values at the end of gestation. In the previous study, results showed that in gestating rats, PLT increases significantly [21]. Hematological analyzes show no effect for dietary protein content on the blood hemoglobin Hgb concentration. The concentration of hemoglobin may be associated with the reduction of erythrocytes in rabbits. Wells et al. [22]showed that RBC, hemoglobin, and hematocrit decrease along pregnancy in rabbit does. The same was noticed in a pregnant period of rats [23,24], during the period of pregnancy of women [25] and with pregnant monkeys [26]. These decreases indicate that anemia was induced significantly at the end of pregnancy, associated with hematopoietic dysfunction.

Conclusion

During the first gestation, zootechnical monitoring, the metabolic and hematological profiles of the nulliparous rabbit does do not show overall effect according to the protein content of the diet. Further, this work should be continued at fattening period of the litters from does and after parturition and several lactations in rabbits does to assess the effect of experimental diets in the long term, especially during lactation or rabbits often suffers negative energy balance, and for better control metabolism to ensure good productivity in rational farming conditions.

Acknowledgments

The authors thank Zootechnical Laboratory of Higher National Veterinary School of Algiers (Algeria), Nutrition Laboratory of Liège University and Animal Production laboratory Agro-Bio Tech of Gembloux (Belgium) for diets analysis.

Conflict of Interests

The authors declare that none of them have any conflict of interest to declare.

Author Contribution

All authors have reviewed and approved the final manuscript submission.

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