EFFECT OF DIETARY PROTIEN LEVEL ON REPRODUCTIVE PERFORMANCE AND SOME PHYSIOLOGICAL PARAMETERS OF NEW-ZEALAND WHITE RABBIT DOES EXPOSED TO SHORT FEED RESTRICTION PERIOD

HODA A. SHABAAN, E.M. ABDEL-KAFY AND T. A. EL-AASAR

Animal Production Research Institute, ARC, Ministry of Agriculture, Dokki, Giza

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Abstract

This study aimed to investigate the effect of three levels of dietary protein on reproductive performance of rabbit does exposed to short feed restriction period before the first insemination. Thirty NZW rabbit does, average live body weight of 2347g and about 17 weeks of age, were used. All does were fed restrictively 75% of ad-libitum consumption for 15 days from 17 to 19 weeks of age. The rabbit does were fed a basal diet containing 16.4% crude protein (CP) and 2510 DE Kcal./Kg diet. Three diets were formulated to provide 16.4, 17.6 or 18.9% CP, respectively. At the beginning of 20 weeks of age rabbit does (10 does per level) were randomly assigned to one of the three experimental diets (low, medium or high CP%). At mid pregnancy, three does from each CP level were slaughtered to evaluate genital weight and histopathological studies. Besides, blood samples were also collected to determine some hematological and hormonal parameters.

Results showed that conception rate and the number of services per conception were improved in the medium CP level group compared with the other levels. The values of litter size and weight at birth were the highest in the high CP level. The RBCs counts were increased by feeding low CP level. The LH and FSH concentrations increased, while T_3 decreased in medium CP level.

The histopathological observations indicated that there were more primary and growing follicles near the outer surface in tuinca albuginea in ovaries of rabbit does fed medium CP level. Also, the uterine wall of this group had gravid endometrium with rich blood vascular supply in serosal layer. It is concluded that before mating, rabbit does fed diet with 17.6% CP flushing post short feed restriction improved sexual maturity, ovarian activity and decreased fetal mortality.

INTRODUCTION

Protein is the essential nutrient to build or rebuild the rabbit bodies. The higher or lower levels of protein in diets of rabbit does lead to a bad effect on the reproductive function (Lebas *et al.*, 1997). So, the rabbit does at the first insemination (nulliparous) need to obtain all nutrient requirements to avoid the deficiency of protein in the diets. Concentration of FSH significantly increases from 14 weeks to 17 and 20 weeks, respectively. LH concentration decreases ($p<0.05$) at the age of 17 weeks than at 14 or 20 weeks (Diaz *et al.,* 1991).

Nutritional flushing followed by a restricted feeding period in nulliparous rabbit does was reported to improve the reproductive performance of rabbits (Gosalvez *et al.*, 1994). Also, Manchisi *et al.* (1990) observed that the restricted feeding (about 80% of ad-libtum) significantly increased the number of young born in nulliparous New- Zealand White rabbits does. The feeding restrictively (about 50% of ad-Libitum) had a negative influence ($P<0.01$) on the body gain and feed intake in the does during pre-puberty from 95 to 117 days of age.

Therefore, the aim of the present study was to determine the optimal dietary protein level of the rabbit does exposed to short feed restriction before first insemination.

MATERIALS AND METHODS

This study was carried out in a private rabbits Farm in Giza Governorate (Abo El-Nomrous city), during the period from April to June 2005. The laboratory work was carried out in Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

Thirty NZW rabbit does of about 17 weeks of age and an average live weight of 2347g, were fed restrictively 75% of ad-libtum consumption on a diet containing 16.4% CP and 2510 DE Kcal./Kg diet for 2 weeks from 17 to 19 weeks of age. At the 20^{th} week of age, rabbit does were fed ad-libitum and randomly divided into three experimental diets (10 does each). In the first group, rabbit does were fed diet containing 16.4% crude protein and 2510 DE Kcal./ Kg diet. The second group was fed diet containing 17.6% crude protein and 2549 DE Kcal./Kg diet, and the third group was fed diet containing 18.9% protein and 2585 DE Kcal./Kg diet.

The rabbit does were weighed at 20 weeks of age (after feed restriction) as an initial weight. For natural mating, 6 bucks average weight 3000-3500g and 6 -7 months of age were used. The does were transferred to the buck's cage at the time of service. Mating of females was executed twice by bucks of proven fertility. Pregnancy was diagnosed by abdominal palpation at 10-14 days after service. Those failed to conceive were immediately re-mated. Body weight at mating, feed intake, conception rate (%), number of services per conception, litter size at birth, born alive and dead and litter weight at birth (g) were recorded.

All the experimental animals were reared under the same management, and the ambient temperature was 23-35^{0}C. Blood samples were withdrawn from ear vein of seven experimental does from each treatment before mating, at the mid and the end of pregnancy. Plasma samples were prepared by centrifugation (3000-rpm for 15 minutes) and stored at -20^{o}C until the determination of blood hormones. Quantitative measurement of T_3 in plasma was carried out by DSL-3100-USA ACTIVETM Triiodothyronine (T_3) coated tube radioimmunoassay kits. Hematological parameters were determined by auto cell counter for hematological cell parameters (AL Vet. 300) made in Germany. At the mid of pregnancy, three does from each group were randomly slaughtered to evaluate fetus, genital organs weight and histopathological study for ovary, uterus and placenta.

Data were statistically analyzed using SAS (1996) according to the following model. Means were compared (P<0.05) using Duncan's multiple range test (Duncan, 1955).

$Y_{ij} = \mu + G_i + e_{ij}$, Where: Y_{ij} = an observation of the ij^{th} rabbits, μ = overall means, common element to all observations, G_j= effect of the i^{th} group and e_{ij}= random error component assumed to be normally distributed.

RESULTS AND DISCUSSION

Growth performance

Average body weight, daily weight gain and feed intake NZW rabbit does as affected by CP levels are shown in Table 1. Body weight gain and daily gain were insignificantly increased by 31.4 and 14.3%, respectively, in the does fed diet with 17.6% CP, while, it was insignificantly decreased by 2.88 and 18.0%, respectively, in those fed diet containing 18.4% CP, as compared with rabbit does fed diet with 16.4% CP. This observation was in agreement with the finding of Gad Alla *et al.* (2002), who found that rabbit does fed diet containing 18% CP improved insignificantly in body weight and body weight gain. Feed intake insignificantly decreased from 298 to 260g/d as the CP level increased from 16.4 to 18.9% in rabbit diets. Similar, results were obtained by Carregal and Nikuma (1980).

Table 1. Average body weight, feed intake and daily weight gain of NZW rabbit does as affected by CP level .

Treatment	Levels of CP (%)		
Traits	Low (16.4)	Medium (17.6)	High (18.9)
Initial weight (g)	2535 ± 54.7	2446±54.7	2499±52.1
Mating weight (g)	2847±23.8	2870±23.8	2802±27.7
Body weight gain (g)	312	410	303
Daily gain (g)	18.9±3.5	21.6±3.5	15.5±3.3
Feed intake (g/doe/day)	298±14.4	286±14.2	260±14.1

Reproductive performance

Averages of some reproductive traits of NZW rabbit does as affected by CP levels are shown in Table 2. The post-treatment maturation period in the high CP level group was the least compared with other CP levels (16.2 vs 20.1 and 18.0 days) for the lower and medium levels, respectively. However, there were no significant differences among the studied CP levels. The results showed that the significant highest conception rate was found in rabbit does fed diet with 17.6% CP as compared with those fed the other two CP levels. Similar findings were found by Khalifa (2006), who reported that prepubertal short feeding program is associated with higher conception rate. This may be due to the better effect of feed restriction and flushing before the first insemination on ovarian function, and consequently, ovulation rate. Controlled feed restriction can increase conception rate that may influence the reproductive performance by number of mechanisms, including central effects on gonadotrophin secretion and (or) local effects of ovarian function. Peltoniemi *et al.* (1997) reported that the increase in conception rate of nulliparous feed-restricted does could be due to LH hormone secretion, and this was related to metabolic changes in early pregnancy. The present study confirmed that increase in LH concentration which was higher in 17.6 and 18.9% CP levels than 16.4% CP (Fig. 2) supports the pervious findings.

Number of services per conception was insignificantly affected by CP level, but, the highest number of services per conception was observed in 16.4% CP level. No significant differences were observed in both litter size of rabbits born alive and dead (Table 2). Similar findings were obtained by Hussein *et al.* (1999).

Litter weight at birth in the high CP level group was insignificantly improved by 12.7% in rabbit does fed diet with 18.9% CP as compared with those fed diet containing 16.4% and 17.6% CP. This improvement may be attributed to the established anabolic drive to maternal tissue synthesis, which is maintained at the expense of gradually evolving nutrient requirements of the gravid uterus (Wallace *et al.*, 2004). In the rabbit, a protein named blastokinin (uteroglobulin) can influence blastocyst formation from morula. Moreover, uterine secretions, including protein, provide an optimal environment for survival and capacity of spermatozoa and promoting cleavage of the early blastocyst before implantation. In addition, blood proteins are also necessary for milk synthesis during lactation and a precursor of placental lactogen hormone (Hafez and Hafez, 2000).

Table. 2. Averages of some reproductive traits of NZW rabbit does as affected by CP levels.

Treatment	Levels of CP (%)		
Traits	Low (16.4)	Medium(17.6)	High (18.9)
Post treatment maturation (days)	20.1±3.3	18.0±3.3	16.2±3.2
Conception rate (%)	70.0^{b}	90.0^{a}	79.0^{b}
No. of service per conception	1.3±0.1	1.0±0.1	1.2±0.1
Litter size born alive	4.9±1.2	4.8±1.2	5.5±1.1
Litter size born dead	0.1±0.2	0.0	0.3±0.2
Litter weight at birth (g)	252±63.8	272±63.8	284±60.8

a, b Means within raw with different superscripts are significantly different ($P\leq0.05$).

Hematological parameters

Some hematological parameters in NZW rabbit does as affected by CP level are shown in Table 3. Values of hematological parameters are within normal range, the means of red blood cells, white blood cells, hemoglobin and hematocrit were 4.84±0.5 (X 10^6), 7.25±2.3 (X10^3), 11.6±1.1 (g/100ml) and 36.3±3.2 (%) for NZW rabbit adults, respectively, as reported by Steven *et al.* (1974). There were no significant differences in hematological parameters at all CP levels. All studied hematological parameters, except red blood cells, were higher in high CP level as compared to the other CP levels, whereas, red blood cells were higher in the low CP level.

Table. 3. Averages of some hematological parameters of NZW rabbit does as affected by CP level.

Treatment / Traits	*Levels of CP*		
	Low (16.4)	Medium (17.6)	High (18.9)
Red blood cells (x10^6/ml)	6.52±0.79	5.00±0.75	5.17±1.12
White blood cells (x10^3/ml)	4.61±0.50	4.83±0.48	5.55±0.71
Hemoglobin (g/100ml)	13.68±0.55	12.48±0.52	14.28±0.78
Hematocrit (%)	35.84±2.60	36.80±2.45	40.48±3.67

Blood levels of thyroidal (T_3) and gonadotrophic (LH and FSH) hormones

There were no significant differences in plasma T_3 hormone concentration between the three CP levels. However, the value of plasma T_3 was lower in high CP level group as compared to the other groups (Fig. 1). This may be due to time of sampling related to higher thyroid activity as the does were at their sexual maturity before and during pregnancy. This may suggest the higher demand for thyroid hormones during these periods, especially, when dietary protein level was lower. Our results support this idea, where T_3 level was lower in does fed the higher CP level diets. It is postulated that the endogenous anabolic and catabolic hormones in plasma, may depress the thyroid function measured as a decrease of free T_3, when the dietary protein level was optimum. The rise of these hormones associated with increase in protein level, and hence, those related to protein metabolism could explain and support our findings, which are in close agreement with results by Buttery and Lindsay (1980).

There were no significant differences in plasma LH concentration in the three CP levels, but, the highest concentration was obtained in medium CP level compared with the low and high levels as shown in Fig. 2.

There were no significant differences in plasma FSH hormone among the three CP levels. The highest value of FSH concentration was observed in the medium CP level before mating, at 7 and 14 days of pregnancy. Furthermore, FSH concentration decreased with increasing the days of pregnancy as shown in Fig. 3. These results may indicate that there are many mature follicles in second parity in medium CP level. Similar findings were reported by El-Ashry *et al.* (1989), who observed a decrease in the plasma FSH level of rabbit does during the second week of pregnancy. There were cooperative interaction between the hormone (LH or FSH) and its receptor. This interaction describes the change in affinity of receptors for the hormone with changes in the concentration of hormone, where in control protein, some of these receptors lost their affinity. The administration of the hormones that might be used for purposes of inducing protein metabolism and increasing tissue and body protein gain in the whole organism may enhance this process as reported by Buttery and Lindsay (1980).

Number and weight of fetus

Number and weight of fetuses are presented in Table 4. The average of the fetus weight was heavier in the medium and high CP levels than that in the low CP level. This may be due, not only to increasing the number of fetus in these CP levels, but also to the live body weight of does in the medium CP level which were heavier than the other groups (Table 1). This may support the well known idea about the great association between fetal growth and the developmental growth and health of parents during the adult life. These results are in agreement with Wallace *et al.* (2004), who reported that maternal nutrient intakes were influencing the growth of the placenta and uterus during early and mid gestation period. In this concern, our results indicate that the ovary weight and uterus with fetus weight were higher in does fed medium and high CP diet. This increase is related to the higher concentration of gonadotrophic hormones (FSH and LH) in the present study.

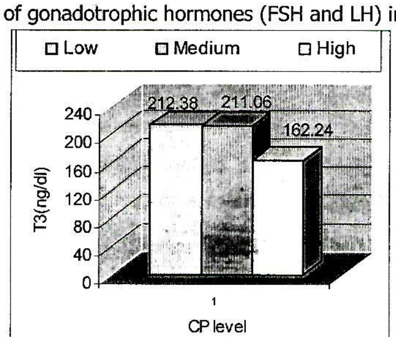

Fig. 1. The effect of CP levels on T3 concentration

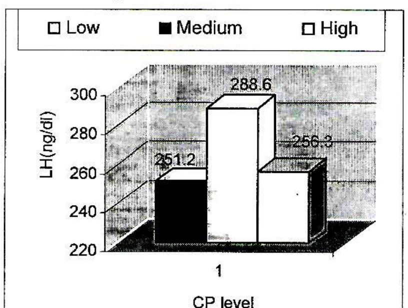

Fig. 2. The effect of CP levels on LH concentration

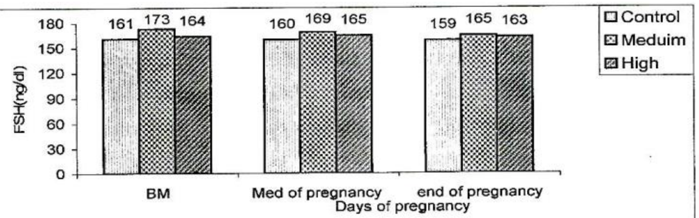

Fig (3) The effect of CP levels on FSH concentation

(BM: Before Mating)

Table. 4. Average values of genital weight of NZW rabbit does as affected by CP level

Treatment	Levels of CP			
Organs	Low (16.4)	Medium (17.6)	High (18.9)	**±SE**
Ovary weight (g)	0.26^{c}	0.27^{b}	0.28^{a}	0.05
Uterus & fetus weight (g)	23.9	26.6	31.34	9.87
No. of fetus	3.00	3.75	4.00	1.28
Fetus weight (g)	0.52	0.62	0.58	0.17
Placenta weight (g)	0.80	0.89	0.98	0.31

a, b, c Means within raws with different superscripts are significantly different ($P \leq 0.05$).

Histopathology of ovary, uterus and placenta

In medium CP level group, primary and growing follicles were noticed near the outer surface in the tuinca albuginea and germinal epithelial layers associated with the inter-follicular tissue in between (Plate 1), while, the corpus luteum occupied a wide area (Plate 2). In low CP level group, there were different stages of growing follicles with inter follicular tissue in between (Plate 3), with appearance of deep eosinophilic area of the corpus luteum (Plate 4). In high CP level group, numerous growing follicles were observed with interfolicular tissue in between (Plate 5), associated with appearance of the corpus luteum (Plate 6). These results are in agreement with the observation of El-Aik (2007), who reported that dietary protein level could affect ovarian, and hence, follicular growth of rabbit does. These results may be due to high vascular system in cells of the theca-interna as reported by Buttery and Lindsay (1980), who found that plasma proteins would be able to pass between these cells causing proliferation of ovarian tissues.

Histopathological findings of uterus as affected by revealed CP level, a gravid endometrium and well developed myometrium layer are shown (Plate 7). Low CP level sections showed oedema in the last area of myometrium (Plate 8), and severe oedema in the serosal layer (Plate 9). It is likely that the uterine uptake of protein was closely related to the amount of fetal tissue during the gestation period. Similar conclusion was suggested by the results of Buttery and Lindsay (1980) for protein uptake by normal or under nourished guinea-pig fetuses. They studied protein uptake as individual amino acids, and found a positive relationship between the uterine proteins and the endometrial wall of uterus.

The highest weight of the ovary may be attributed to increased FSH and LH hormones concentrations, which affected follicles development and growth of ovary and increased the number of growing and mature ova (Hafez and Hafez, 2000).

Concerning the placenta histopathology in medium CP level group, the trophospongium with the fibrinoid material was observed (Plate 10), followed by a labyrinth layer and trophoblasts (Plate 11). In the low and high CP level groups, the trophospongium with the fibrinoid material were detected (Plates 12 and 13). The labyrinthine with trophoblasts also were detected in low and high Cp levels, respectively, (Plates 14 and 15). These observations agree with those by Wallace *et al.* (2004), and may be explained by the high rate of protein turnover and amino acids uptake of placenta (Young *et al.*, 1979). It appears that the low protein level may increase the rate of protein degradation because the decreased substrates of protein influences the machinery of degradation (Buttery and Lindsay, 1980).

Thus, it could be concluded that, before mating, the rabbit does fed diet with 17.6% CP flushing post short feed restriction improved sexual maturity, ovarian activity and decreased fetal mortality.

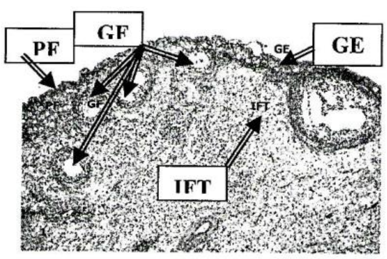

Plate 1: Ovary of rabbit in medium flushing group. Primary (PH) and growing follicles (GF) neat to the tunica albuginea and germinal epithelium (GE) of the surface with appearance of interfollicular tissue (IFT) in between (X16)

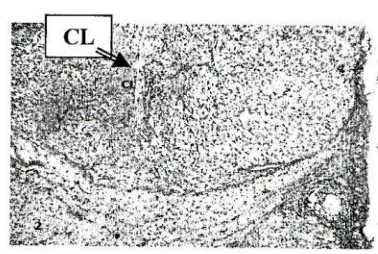

Plate 2. Ovary of rabbit in medium flushing group. Showing the corpus luteum (CL) in a wide area of ovarian tissue (X16)

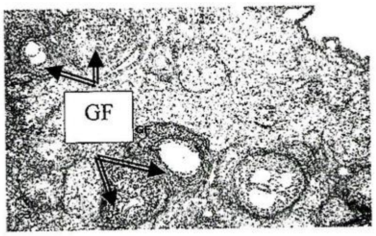

Plate 3. Ovary of rabbit in low flushing group. Showing different stage of growing follicles (GF) and interfollicular tissue (X16)

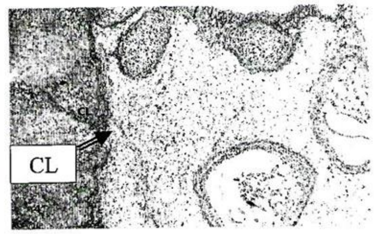

Plate 4. Ovary of rabbit in low flushing group. Showing the corpus luteum (CL) in a wide area (X16)

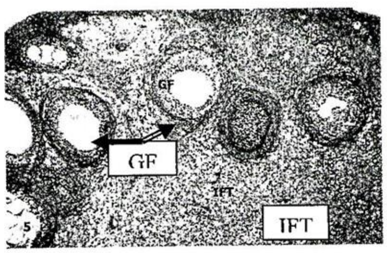

Plate 5. Ovary of rabbit in high flushing group. Showing the growing follicles (GF) with interfollicular tissue (IFT) in between(X16)

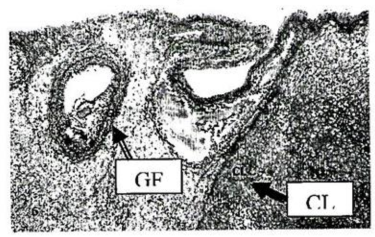

Plate 6. Ovary of rabbit in high flushing group. Showing a wide area of corpus luteum (CL) and some growing follicle (GF) (X16)

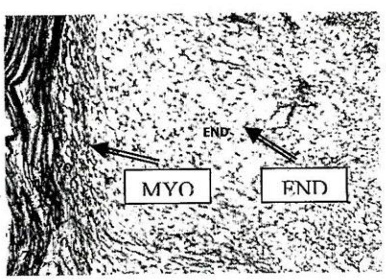

Plate 7. Uterus of rabbit in medium flushing group. Showing the gravid endometrium(END) and a part of myometrium (MYO) (X40)

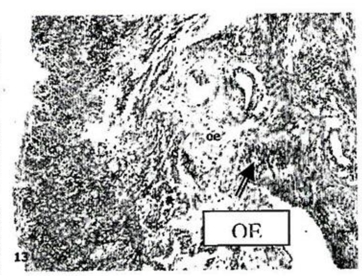

Plate 8. Uterus of rabbit in low flushing group. Showing oedema (OE) in the lost area of myometrium (X40)

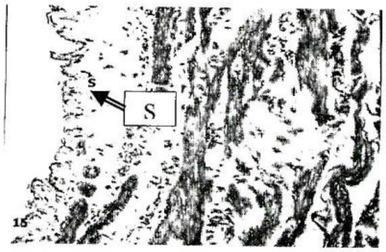

Plate 9. Uterus of rabbit in low flushing group. Showing severe oedema in the serosal (S) layer (X40)

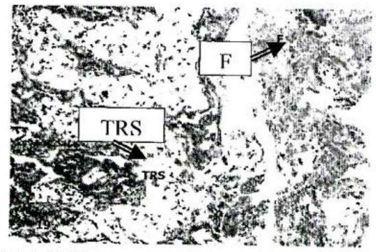

Plate 10. Placenta of rabbit in medium flushing group. Showing the fibrinoid material (F) with underlying trophospongium (TRS) (X40)

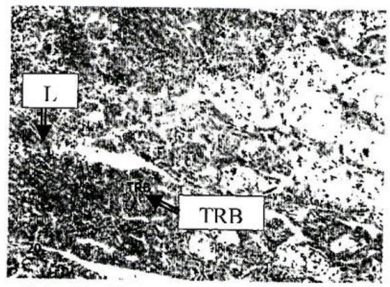

Plate 11. Placenta of rabbit in meduim flushing group. Showing the labyrinth (L) with Trophoblasts (TRB) (X40)

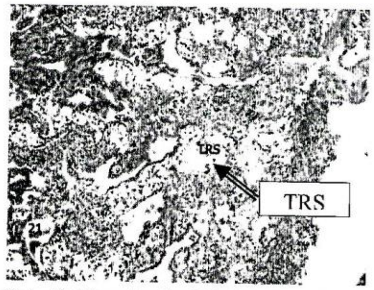

Plate 12. Placenta of rabbit in low flushing group. Showing trophospongium (TRS) with fibrinoid (F) material (X40)

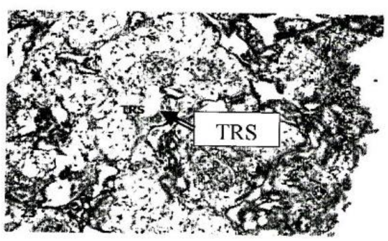

Plate 13. Placenta of rabbit in high flushing group. Showing the trophospongium (TRS) with fibrinoid (F) material were detected (X40)

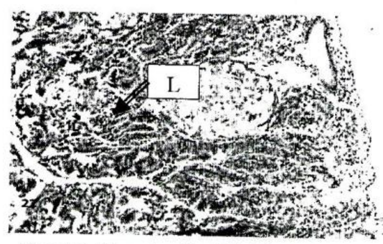

Plate 14. Placenta of rabbit in low flushing group. Showing the laybyrinth (L) with trophoblast (TRS) (X40)

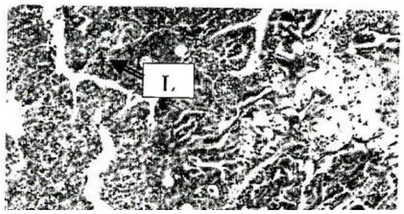

Plate 15. Placenta of rabbit in high flushing group. Showing the laybyrinth (L) with trophoblast (X40)

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تأثير مستوي بروتين العليقة علي الأداء الانتاجي وبعض الصفات الفسيولوجية لامهات الأرانب النيوزيلندي الابيض التي تعرضت إلي تحديد غذائي لفترة قصيرة

هدي عبد الرؤوف شعبان ،السيد محفوظ عبد الكافي ، طارق عبد الغفار الأعصر

معهد بحوث الإنتاج الحيواني – مركز البحوث الزراعية – وزارة الزراعة – الدقى – جيزة

أستهدف هذا البحث دراسة تأثير مستوي البروتين في العليقة علي الأداء الانتاجي وبعض الصفات الفسيولوجية لامهات الأرانب التي تعرضت إلي تحديد غذائي لفترة قصيرة قبل التلقيح الأول. أستخدم في هذا البحث ثلاثون أنثي نيوزيلندي أبيض بمتوسط وزن ٢٣٤٧جم وعمر حوالي ١٧ أسبوع حيث تم تحديد الغذاء لها بنسبة ٧٥ % من العلف المأكول في الحالة الطبيعية وذلك لمدة أسبوعين (من عمر ١٧ الى١٩ أسبوع). كان مستوي بروتين العليقة المستخدمة هو١٦,٤% و ٢٥١٠ كيلو كالورى طاقة مهضومة . تم عمل ثلاث علائق مختلفة في مستوي البروتين تحتوي علي ١٦,٤% ، ١٧,٦%، ١٨,٩% وتم تغذية الامهات علي هذه العلائق خلال فترة الحمل. في منتصف فترة الحمل تم ذبح ثلاث إناث عشوائيا من كل مجموعة لتقدير وزن الأعضاء التناسلية ودراستها هستوباثولوجيا كما أخذت عينات من الدم لتقدير بعض الهرمونات وكذلك صورة الدم (%الهيموجلوبين وعدد كرات الدم).

وتتلخص أهم النتائج فيما يلي:- لوحظ تحسن في نسبة الخصوبة و عدد التلقيحات اللازمة لحدوث الحمل في المجموعة المغذاه علي المستوي المتوسط من البروتين مقارنة بالمجموعات الأخرى . لوحظ ارتفاع عدد ووزن المواليد عند الميلاد في الاناث المغذاه علي المستوي العالي من البروتين مقارنة بالمجموعات المغذاه علي المستويات المتوسطة والمنخفضة من البروتين. هناك زيادة في عدد كرات الدم الحمراء في المستوي المنخفض من البروتين وكذلك انخفض هرمون الدرقية مع زيادة في كل من هرموني LH, FSH في المستوي المتوسط من البروتين. لم يكن هناك أي تأثيرات معنوية علي وزن الأعضاء التناسلية ولكن يزداد الوزن بزيادة مستوي البروتين في العلف. أوضح الفحص الهستوباثولوجى للمبيض وجود تحسن مع زيادة مستوي البروتين وحيث لوحظ زيادة في عدد الحويصلات المبيضية الأولية والنامية القريبة من سطح الغشاء السطحي للمبيض. كما تحسنت الطبقة الجرثومية والمصلية وكذلك عدد الأجسام الصفراء في المجموعة المغذاه علي مستوي البروتين العالي وكذلك وجد أن الطبقةّ المبطنة للرحم (الاندوميتريم) وأيضا الطبقة التالية لها ذات سمك مرتفع، كما أن الطبقة العضلية كانت غنية بالأوعية الدموية التي تمد الرحم بالدم. ومن النتائج السابقة توصي الدراسة باستخدام فترة تحديدغذائي قصيرة (١٥ يوم) قبل تلقيح الإناث ثم عملية دفع غذائي باستخدام عليقة بها ١٧,٦% بروتين خام وذلك لتحسين الصفات الانتاجية والتناسلية لأمهات الأرانب.