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BODY COMPOSITION, METABOLIC STATUS, IMMUNE RESPONSE AND INFLAMMATIONS IN RABBITS DURING LACTATION

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ABSTRACT: This experiment was carried out to investigate the influence of lactation on body composition, metabolic status, immune response and inflammation occurrence in V-line doe rabbits. A total number of 30 V-Line primiparous female rabbits were divided into two equal groups; 1st group: females were kept as non-pregnant and non-lactating (NPNL, group) and 2nd group: females were kept as lactating (L, group). Animals were fed *ad-libitum* a commercial pelleted diet contains 18.5 % crude protein, 14.5 % crude fiber, 2.5 % fat and 2730 kcal/kg digestible energy. The number of suckling kits was adjusted to 8 per litter; the kits were weaned at 30 days. Average feed intake (g) was weekly recorded. Body composition of does was determined by BIA technique using bioelectrical body composition analyzer (Quantum II) apparatus. Blood samples were collected from does through three stages of lactation period; early (within 2-3 d *post partum*), mid (14 – 15 d of suckling) and late (30 d of suckling). Blood serum was assigned for total protein, albumin, glucose and cholesterol determinations. Globulin and albumin/globulin (A/G) ratio were calculated. Concentration of T_3 hormone, Tumor Necrosis Factor – Alfa (TNF-α) and Inerleukin 2 (IL-2) were estimated by using ELISA technique. The results illustrated that feed intake of does significantly increased during lactation period. In L does, body content of humidity, protein and ash were significantly higher; in contrast, fat and energy content were significantly lower. No significant differences were found in cholesterol, glucose, albumin, A/G ratio, T_3 and TNF-α through stages of lactation. While, significant differences were obtained in total protein, globulin and IL-2 at mid and late stages compared to early stage of lactation.

In conclusion, stressed does, which suckle high number of pups (n ≥ 8) should be kept under especial nutritional care because energy balance is negative during lactation and fat stores are mobilized, especially in primiparous does.

Key Words: Rabbits-Body Composition- Lactation- Metabolic Status.

INTRODUCTION

Female rabbits in commercial farms are always gestating, suckling or both gestating and suckling at the same time; these two physiological functions, especially lactation, are very costly in term of energy. In reproductive rhythms commonly used in rabbits, where the gestation and lactation overlap, the rabbit is not able to consume enough feed to meet their needs (Pascual *et al.*, 2002; Costa *et al.*, 2004; Xiccato *et al.*, 2004 and Quevedo *et al.*, 2005). To maximize reproductive performance of does, it should not forget that ovarian activity is optimized when the animal has enough energy reserves which in turn, affect animal production in the long term (Quevedo *et al.*, 2005 and Theilgaard *et al* ., 2006).

Blood constituents have been used by many investigators as a monitor for genetic makeup, health and nutrition (Azoz and El-Kholy, 2005). In addition, hormonal and metabolite rate were considered as a good indicator for understanding the physiological requirements for efficient reproduction (Cardinali *et al*., 2009).

Thus, the aim of this study was to investigate the influence of lactation on body condition, metabolic status, immune response and inflammation occurrence in V-line doe rabbits.

MATERIALS AND METHODS

This study was carried out in the private rabbitry of 6th October city, El-Wahat road, Giza, Egypt, during the period from July up to December, 2015. A total number of 30 V-Line primiparous female rabbits aging 6 - 7 months were divided into two equal groups; 1st group: females were kept as non-pregnant and non-lactating (NPNL, group; average of body weight was 3440 g) and 2nd group: females were kept as lactating (L, group; average of weight was 3730 g). Does were housed in individual cages of commercial type provided with feeders, automatic nipple drinkers and nest-boxes. Animals were fed *ad-libitum* a commercial pelleted diet with 18.5 % crude protein, 14.5 % crude fiber and 2.5 % fat. Calculated digestible energy of the diet was 2730 kcal / kg digestible energy. All does were kept under the same managerial and hygienic conditions. After kindling the number of suckling kits were adjusted to 8 per/doe and weaned at 30 days. Feed intake (g) was weekly recorded.

Bioelectrical impedance analysis (BIA) technique was used to determine *in vivo* chemical body composition of does (humidity, g and %; protein, g and % DM; ash, g and % DM; fat, g and % DM; energy, MJ and kJ/100g DM) according to methodology and equations obtained by Pereda (2009), using bioelectrical body composition analyzer (Quantum II) apparatus.

Blood samples were collected from marginal ear vein of does into clean centrifuge tubes at different stages through lactation period; early (within 2-3 d post partum; n= 9), mid (14 – 15 d of suckling; n= 6) and late (30 d of suckling, before weaning; n= 6). Blood serum was assigned for total protein, albumin, glucose and cholesterol determinations. Methods of analysis were done according to the procedure described by the manufacturers. Globulin and albumin/globulin (A/G) ratio was also calculated.

Concentration of total T_3 hormone was determined by ELISA technique using the coated tubes kits of Immunospec Corporation, USA. Also, concentration of Tumor Necrosis Factor – Alfa (TNF-α, pg/ml) and Inerleukin 2 (IL-2, pg/ml) were estimated by using TNF-α and IL-2 ELISA kits obtained from WKEA MED SUPPLIES CORP, China.

Data were statistically analyzed using the General Linear Model Program of SAS

(2004) according to the following one way analysis model: $Y_{ij} = \mu + Tr_i + e_{ij}$
Where, Y_{ij} = any observation of j^{th} animal within i^{th} treatment, μ = overall mean, Tr_i = effect of i^{th} treatment (i: 1 - 2) and e_{ij} = experimental error. Duncan Multiple Range Test (Duncan, 1955) was used to test the level of significant differences among means.

Results

Feed intake

The results illustrated that feed intake of does significantly increased during lactation period. Total feed consumption of lactating does (L) was more than non-pregnant non-lactating does (NPNL) by about 118 % (Fig. 1, a). Moreover, lactation period (4 weeks) associated with gradually significant increase in feed intake reached at the fourth week of lactation to more than 100 % of feed intake at the first week of lactation (Fig. 1, b). The highest value of increasing feed intake was found from the 3rd week to 4th week of lactation (+ 31.7 %).

Body composition:

The results showed that body content of humidity, protein and ash (g) of L does were significantly higher than that of NPNL does (Table 1). The differences between NPNL and L does were 402.24 g (11.9 %), 21.07 g (3 %) and 8.51 g (7.2 %) in body content of humidity, protein and ash (g), respectively. In contrast, fat content (g) and energy (MJ) were significantly lower in body of L does than that of NPNL does by 97.69 g (20.6 %), 2.71 MJ (6.8 %), respectively (Table 1).

On the other hand, results obtained herein declared that body composition in percentages and grams had a similar trend whereas the body percentage of humidity, ash and protein were significant increase (+ 3.9, 0.1 and 0.2 %, respectively) in L does as compared to NPNL does (Fig. 2; a, b and c). However, significant decreases were observed in body fat (- 1.7 %) and body energy of L does (- 198.8 kJ/100g, 21.5 %) when compared with NPNL does.

Metabolic status

Blood metabolites of V-line doe rabbits throughout lactation period (early, mid and late) were presented in table (2). Values of all metabolites were within normal range of rabbits. No significant differences in cholesterol, glucose, albumin and albumin/globulin ratio were found through stages of lactation. However, significant differences were shown in total protein and globulin between mid and late stages of lactation (Table 2).

During lactation, no significant changes were obtained in T_3 concentration (Fig. 3). However, the highest level of T_3 concentration was determined at mid stage of lactation; the level was higher by about 14.3 % compared to early and late stages of lactation.

Inflammatory and immune responses

The effect of lactation stage on serum tumor necrosis factor alpha (TNF-α) concentration is shown in figure (4, a). The current investigation demonstrated that the lactation stages had no significant effects on serum TNF-α concentration. However, the highest value of TNF-α was found in the late stage of lactation (Fig. 4, a).

The effect of lactation stage on serum inetrleukin-2 (IL-2) concentration is shown in figure (4, b). The serum IL-2 levels showed significant elevation during the medium and late stages compared to early stage of lactation by about 9.9 and 8.2 %; respectively. However, the serum IL-2 concentrations did not significantly vary within the last two stages of lactation (Fig. 4, b).

DISCUSSION

Total feed intake of does significantly increased during lactation period (Fig. 1, a & b). In contrast, lactation associated with a significant decrease in body fat and energy content (Table 1 and Fig. 2, e). In this respect, Fortun-Lamothe (2006) noted that, if rabbit does keep following the intensive reproductive rhythm, with insemination every 35 or 42 days, they must meet strong nutritional needs. Indeed, lactation is very costly in terms of energy consequently energy deficit, which occurs during lactation. On the other hand, Fortun-Lamothe (2006) and Pereda *et al*. (2009) reported that during the first and second lactation, negative energy balances are produced despite the mobilization of body reserves.

The results of this study showed increasing of total protein, albumin and globulin at late stage of lactation (Table 2). Increasing in total proteins, albumin and globulin may reflect changes in the hepatic function; whereas the change in albumin level reflects the change in liver function (Azoz and El-Kholy, 2005). Jones and Bark (1979) reported that the liver is the site of albumin synthesis; meanwhile, lymphatic tissues form globulin. The decrease in A/G ratio of does seems to be due to the increase in globulin rather than the decrease in albumin. This may reflect the positive increase in the immunity through increasing the γ-globulin (More *et al.*, 1980). Furthermore, Ismail *et al.* (2002) reported that the lowest value of A/G ratio was a good indicator for increasing the immunoglobulin.

No significant difference in glucose was found through stages of lactation (Table 2). In this respect, Cardinali *et al*. (2009) reported that glucose level did not detect any significant variation in nulliparous (only gestation effect) and primiparous (overlapping of gestation and lactation) does. This can explain the homeostatic mechanisms that controlling the glycaemia. Glucose may be a good indicator of the energy balance of animals (Fortun-Lamothe, 2006). Jones and Parker (1988) found that, in rabbits, mammary gland also uses glucose for the synthesis of milk lipids. On the other hand, the blood glucose levels are even lower in rabbits, which are simultaneously gestating and lactating (Fortun, 1994).

During lactation, there were no significant changes obtained in T_3 concentration (Fig. 3), Cardinali *et al*. (2009) concluded that blood T_3 concentration decreased in primiparous does (overlapping of gestation and lactation), that may be to ascribe the energy deficit caused by milk production, responsible for intense energy mobilization. The transition from late pregnancy to lactation is a time of great physiological stress. Feed intake was decline just before parturition, this coupled with the rapid increase in energy requirements during lactogenesis. After that significant increase in feed intake was observed all over lactation period to release stored nutrients and direct them to the mammary gland. This mechanism realized body homeorhesis (Bauman and Currie, 1980).

Pregnancy and lactation are associated with immune regulation and reduced Th_1 responses (Denney *et al*., 2011). Hormonal changes during pregnancy, which persist in part during lactation, are one cause of this immune shift (Cutolo *et al*., 1995). Lactation have been shown to be associated with B-cell production and reduced inflammatory responses (Yu-Lee, 2002). However, results reported herein showed that all stages of lactation had no significant effects on serum TNF-α.

Increasing serum IL-2 during the last two stages of lactation is a good indicator for stimulating the immune response. IL-2 signals influence various lymphocyte subsets during differentiation, immune responses and homeostasis. Stimulation with IL-2 is crucial for the maintenance of regulatory T (T_{Reg}) cells (Malek , 2008) and for the differentiation of $CD4^+$ T cells into defined effector T cell subsets following antigen-mediated activation (Martins *et al*., 2008). For $CD8^+$ T cells, IL-2 signals optimize both effector T cell generation and differentiation into memory cells (Boyman *et al*., 2010). Use of IL-2 – either alone or in complex with particular neutralizing IL-2-specific antibodies - can amplify $CD8^+$ T cell responses or induce the expansion of the T_{Reg} cell population (Jin *et al*., 2008).

CONCLUSION

It can concluded that, stressed does, which suckle high number of pups (n ≥ 8) should be kept under especial nutritional care because energy balance is negative during lactation and fat stores are mobilized, especially in primiparous does.

Acknowledgements
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Table (1): Body composition of V-line lactating doe rabbits

Item	Reproductive status of doe	
	NPNL	L
Humidity, g	3372.47^{b}±24.44	3774.71^{a}±68.28
Ash, g	117.70^{b}±0.48	126.21^{a}±1.23
Protein, g	708.58^{b}±3.40	729.65^{a}±9.49
Fat, g	571.66^{a}±10.82	473.97^{b}±32.04
Energy, MJ	42.67^{a}±0.53	39.96^{b}±1.57

a, b Means with different superscripts within the same row are significantly different (P<0.05).
NPNL = non-pregnant non-lactating doe rabbits and L = lactating doe rabbits.

Table (2): Blood metabolites of V-line doe rabbits throughout stages of lactation

Item	Stages of lactation		
	Early	Mid	Late
Cholesterol, mg/dl	33.12±2.67	32.86±1.61	26.23±2.83
Glucose, mg/dl	72.62±3.81	62.70±3.55	71.19±4.95
Total protein, g/dl	6.32^{ab}±0.06	5.85^{b}±0.20	6.73^{a}±0.42
Albumin, g/dl	2.93±0.08	2.89±0.12	3.13±0.22
Globulin, g/dl	3.39^{ab}±0.04	2.96^{b}±0.09	3.60^{a}±0.34
A/G ratio	0.86±0.03	0.97±0.03	0.89±0.09

a, b Means with different superscripts within the same row are significantly different (P<0.05).

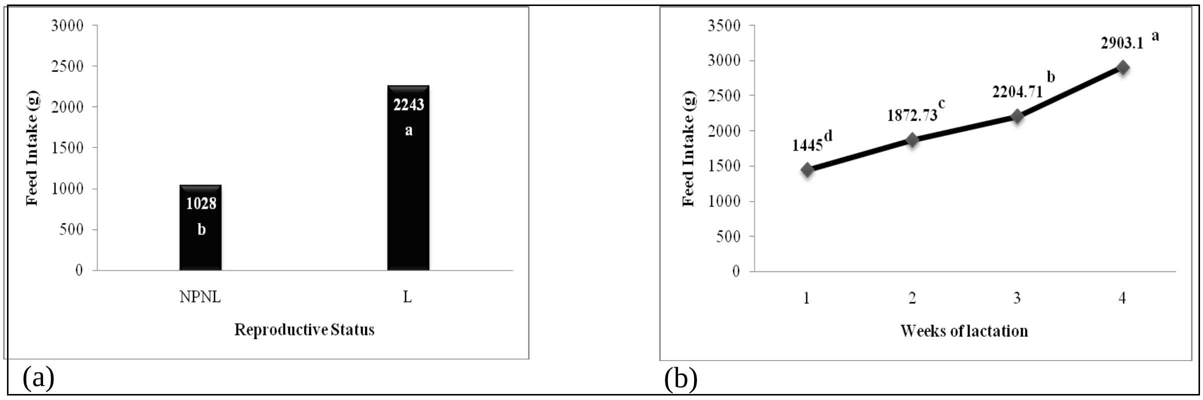


Fig. 1 (a & b). Feed intake of V-line lactating does during lactation.
a, b, c, d Means with different superscripts are significantly different (P<0.05). NPNL = non-pregnant non-lactating does and L = lactating does.

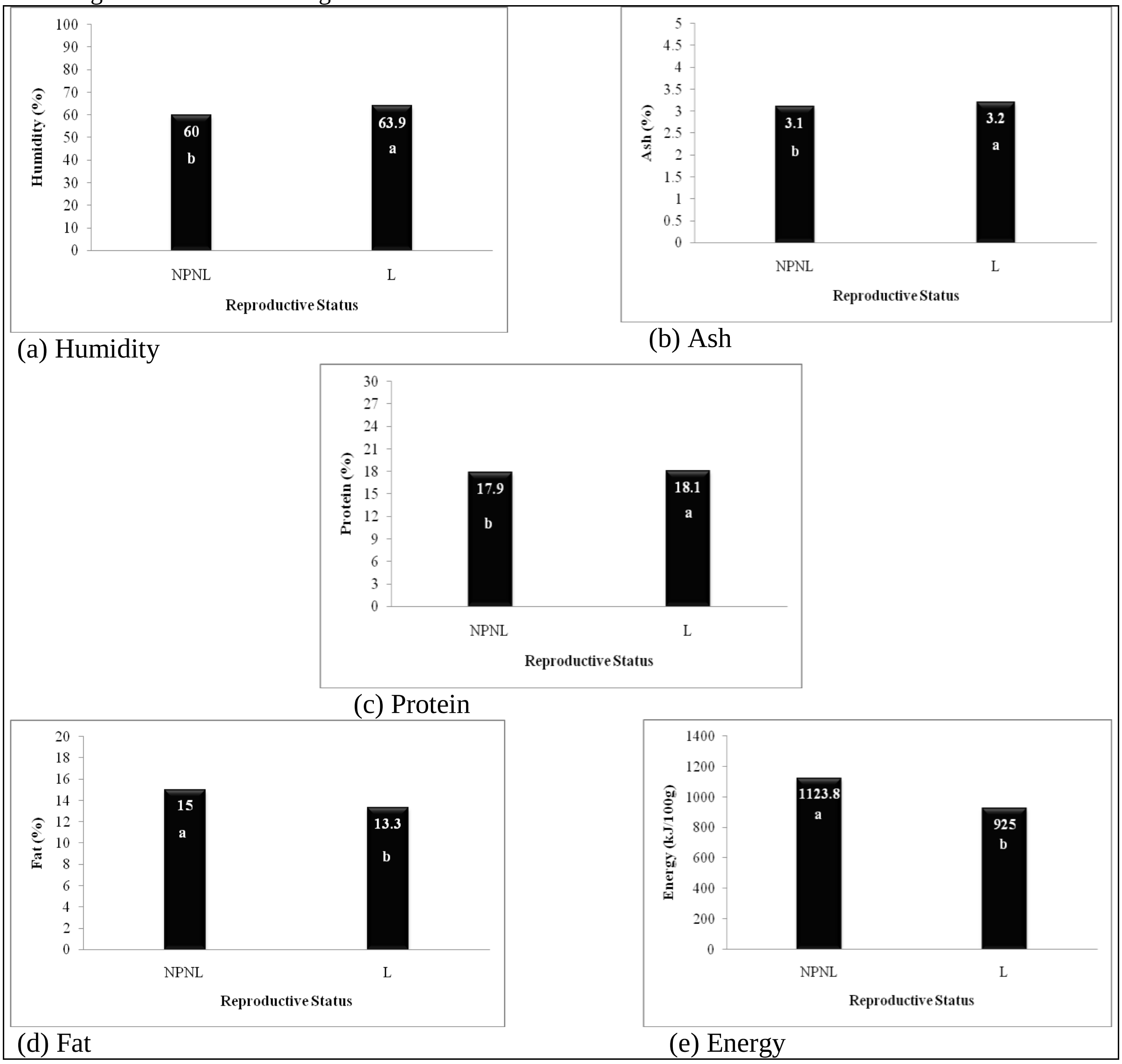


Fig. 2. Body composition of V-line lactating doe rabbits.
a, b Means with different superscripts are significantly different (P<0.05). NPNL = non-pregnant non-lactating doe rabbits and L = lactating doe rabbits.

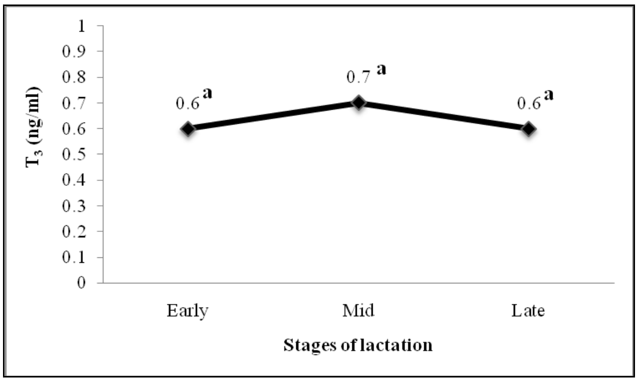
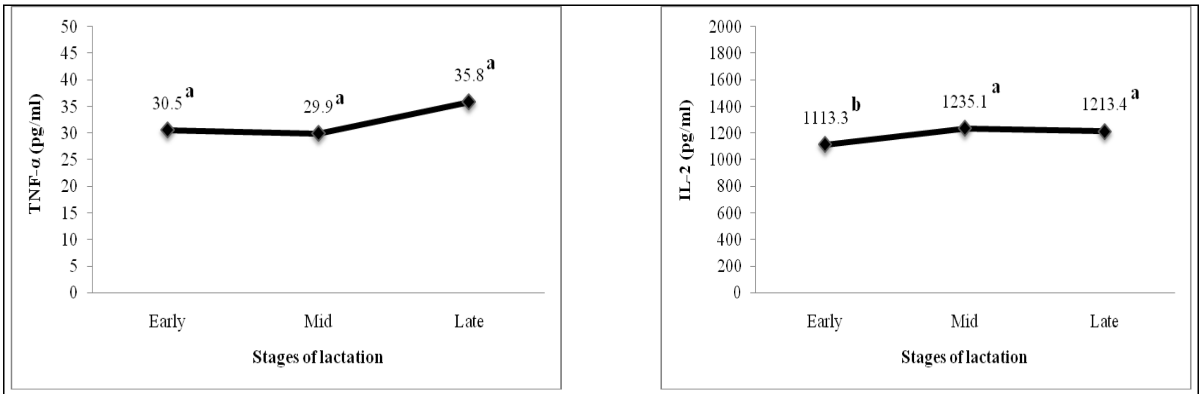


Fig. 3. T_3 concentration of V-line doe rabbits throughout stages of lactation



(a) TNF-α (b) IL-2

Fig. 4. TNF-α and IL-2 concentrations of V-line doe rabbits throughout stages of lactation

a, b, Means with different superscripts are significantly different ($P<0.05$).

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الملخص العربي

تركيب الجسم، حالة التمثيل الغذائي، الإستجابة المناعية والإلتهابات في الأرانب خلال فترة الرضاعة

محمود عبد العال محمود، أحمد عثمان عباس، أسامة جلال صقر، محمد رضا رمضان ونجوى عبد الهادي أحمد
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أُجريت هذه التجربة لدراسة تأثير الرضاعة على تركيب الجسم، حالة التمثيل الغذائي، الإستجابة المناعية وحدوث الإلتهابات في أمهات أرانب V-line. تم إستخدام عدد 30 أم من أرانب V-line (أول بطن) وتم تقسيمها إلى مجموعتين متساوتين كالتالي؛ المجموعة الأولى: الأمهات غير الحامل أو المرضع (NPNL) والمجموعة الثانية: الأمهات المرضعات (L). غُذيت الأرانب حتى الشبع على عليقة مصبعات مركزة تجارية تحتوى على 18.5% بروتين خام، 14.5% ألياف خام، 2.5% دهون و2730 كيلو كالوري طاقة مهضومة. تم توحيد عدد الخلفات لعدد 8 خلفات وتم فطامها على عمر 30 يوم.

تم تسجيل كمية العلف المأكول إسبوعياً. تم تقدير مكونات الجسم بتقنية BIA بإستخدام جهاز تقدير مكونات الجسم (Quantum II). تم سحب عينات الدم من الأمهات خلال 3 فترات من الرضاعة؛ بداية الرضاعة (خلال 2-3 يوم بعد الولادة)، منتصف الرضاعة (في اليوم 14-15 من الرضاعة) وآخر الرضاعة (في اليوم 30 من الرضاعة). تم إجراء التحليلات الكيماوية في سيرم الدم والتي شملت البروتين الكلي – الألبيومين – الجلوكوز – الكوليسترول بإستخدام الـ Kits التجارية، أيضاً تم تقدير تركيزهرمون الـ T_3 وبعض السيتوكاينز (TNF-α و IL-2) بواسطة تقنية الـ ELISA. كما تم حساب قيمة الجلوبيولين وذلك بطرح قيمة الألبيومين من قيمة البروتين الكلي، وأيضاً تم حساب نسبة الألبيومين/الجلوبيولين. وكانت أهم النتائج كالتالي:

-كانت فترة الرضاعة مصحوبة بزيادة معنوية في كمية الغذاء المأكول.

-إرتفع محتوى جسم الأمهات المرضعات (L) معنوياً من الرطوبة، البروتين والرماد. في حين إنخفض محتوى جسمها معنوياً من الدهن والطاقة.

-عدم وجود إختلافات معنوية في مستوى الكوليسترول، الجلوكوز، الألبيومين، نسبة الألبيومين/الجلوبيولين، هرمون T_3 و TNF-α بالدم خلال مراحل الرضاعة المختلفة (بداية – منتصف – آخر الرضاعة).

-أظهر تركيز كلٍ من البروتين الكلي، الجلوبيولين وIL-2 إختلافات معنوية بين منتصف وآخر الرضاعة مقارنة بالمرحلة الأولى من الرضاعة .

يُستنتج من هذه الدراسة أنه يجب وضع أمهات الأرانب (أول بطن) التي تُرضع أكثر من 8 خلفات تحت نظام غذائي خاص يجعلها لا تقع تحت إجهاد نقص ميزان الطاقة بالجسم الذي يحدث أثناء الرضاعة.

الكلمات الدالة: الأرانب، تركيب الجسم، الرضاعة، التمثيل الغذائي.