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EFFECT OF VITAMIN A SUPPLEMENTATION ON SOME PHYSIOLOGICAL REACTIONS OF EWES AND THEIR MALE LAMBS DURING SUCKLING PERIOD

(With 4 Tables)

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تأثير الإمداد بفيتامين أ على بعض الإستجابات الفسيولوجية للنعاج وحملانها الذكور أثناء فترة الرضاعة

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أجريت هذه الدراسة على عدد ٢٠ من النعاج (١/٢ أوسيمى x ١/٢ كيوس) بمتوسط وزن ٥١,٢٥ ± ١,٠٥ كجم وذلك لتقييم تأثير إمداد هذه النعاج بفيتامين أ بدءا من الإسبوعين الأخيرين من فترة الحمل وخلال فترة الرضاعة على بعض الإستجابات الفسيولوجية لهذه النعاج ولحملانها الرضيعة من الذكور. قسمت النعاج عشوائيا إلى مجموعتين متساويتين الأولى للمقارنه (الكنترول) بينما أعطيت نعاج المجموعة الثانية فيتامين أ عن طريق الفم بمعدل ٥٠٠,٠٠٠ وحدة دولية/ رأس/إسبوعيا. وقد تم تقدير بعض المقاييس الفسيولوجية والهيماتولوجية وبعض مكونات البلازما. وقد أظهرت النتائج أن الحملان المولودة للنعاج المعاملة بفيتامين أ سجلت قيم أعلى معنويا فى كل من متوسطات وزن الجسم ومعدلات الزيادة اليومية فى الوزن ، تركيز هيموجلوبين الدم ، محتوى البلازما من البروتينات الكلية والجلوبيولين وفيتامين أ وذلك مقارنة بالحملان المولودة للنعاج الكنترول. معاملة النعاج بفيتامين أ لم يكن له تأثير معنوى على درجة حرارة المستقيم ، معدل التنفس ومعدل النبض. سجلت النعاج المعاملة بفيتامين أ قيما معنوية فى محتوى البلازما من البروتينات الكلية ، الألبيومين ، الجلوبيولين وكذلك فيتامين أ مقارنة بالنعاج الكنترول. أظهرت النعاج المعاملة بفيتامين أ وكذلك حملانها زيادة معنوية فى نسبة كرات الدم البيضاء التصنيفية لليمفوسيت وإنخفاض معنوى فى نسبة الكرات المتعادلة وكان ذلك مصحوبا بزيادة معنوية فى العدد الكلى لكرات الدم البيضاء فى النعاج المعاملةبفيتامين أ. نستنج من هذه الدراسة أن إمداد النعاج بفيتامين أ أدى إلى تحسين معدل النمو وبعض خصائص القدرة المناعية لحملانها الذكور كنتيجة لإستجاباتها الفسيولوجية الإيجابية.

SUMMARY

Twenty ewes (1/2 Ossimi X 1/2 Chios) averaged 51.25 ± 1.05 kg were used in this study to evaluate the effect of vitamin A supplementation to ewes at 2 weeks late gestation and during suckling period on some physiological reactions of these ewes and their male lambs. The ewes were randomly divided into two equal groups (ten ewes in each) of similar initial body weights. The first group served as control while the second group received oral administration of vitamin A (retinol) with 50.000 IU/head/weekly. Some physiological and hematological parameters as well as plamsa constituents were measured. The results showed that lambs born to vitamin A-supplemented ewes recorded higher (P<0.01) averages of body weight and daily gain than those born to control ewes. Vitamin A-supplementation to ewes had no significant effect on rectal temperature, respiration rate and pulse rate either for ewes or their lambs. There was a significant (P<0.01) increase in blood Hb concentrations by 8.73 % and a slight increase in PCV by 3.7 % for lambs from vitamin A-supplemented ewes compared with those from control ewes. Ewes received vitamin A had higher (P<0.01) concentrations of plasma total protein, albumin, globulin and vitamin A than those of control ewes. Lambs from ewes received vitamin A had greater concentartions of plasma total protein, globulin (P<0.01) and vitamin A (P<0.05) than those from control ewes. Total count of white blood cells (WBCs) was increased (P<0.01) for ewes received vitamin A, showing a marked increase in lymphocyte percentages and a decrease in neutrophils compared with control ewes (P<0.01). Lambs from ewes received vitamin A showed a significant (P<0.01) increase in lymphocytes and a decrease (P<0.05) in neutrophils with an insignificant higher total WBCs count than those lambs from control ewes. These results declare that vitamin A supplementation to ewes at late pregnancy and during suckling period improved performance and some immune response of their male lambs as a result of favorable signs in their physiological reactions.

Key Words : *Vitamin A, Ewes, Suckling lambs, Physiological Reactions.*

INTRODUCTION

Vitamin A involved in a number of physiological functions in animals. It is essential for stimulation of growth, proper development of skeletal tissue, normal reproduction and maintaining the integrity of epithelial tissues (Pond *et al.*, 1995). Consequently, vitamin A deficiency results in clinical signs such as metabolic disorderes, growth retardation, bone malformation and degeneration of reproductive organs (Weber, 1983 and Pond *et al.*, 1995). On the other hand, immunity and animal health are impaired by inadequacies in vitamin A and ß-carotene which are required for antioxidant defence (Chew, 1987). They improved immune response to decrease infectious disease incidences (Chew *et al.*, 1982). Thus, vitamin A deficiency results in compromised immune responsiveness and increased prediposition to disease and such observation should be helpful in preventive health programmes (Cravens and Vaden, 1994). Therefore, the amounts of vitamin A and ß-carotene needed for immuno-enhancement is higher than the suggested required amounts by NRC (Nockles and Blair, 1996).

Vitamin A supplementation is essential for ewes during late pregnancy and suckling periods since, in addition to night blindness, its deficiency may result in lambs being born weak, malformed or dead at birth (McDonald *et al.*, 1987). In the same regard, additional amounts of vitamin A should be added per day to compensate for what is produced in ewes milk (NRC, 1985). Furthermore, shortage of green fodder resources during summer led to the lack of vitamin A.

Vitamin A supplementation to pregnant buffaloes resulted in improving their calves weight gain (El-Barody *et al.*, 1993). In sheep, however, informations considering the effect of vitamin A given to ewes on their lamb performance and related physiological reactions are limited. Therefore, the present work oriented to evaluate the mechanistic aspects through which ewe-vitamin A supplementation, at 2 weeks late gestation and during suckling period, might influence the performance of their lambs by monitoring the responses of some blood parameters and specific plasma constituents as well as some immune indices such as total and differential cell counts of leucocytes.

MATERIAL and METHODS

Twenty pregnant ewes (1/2 Ossimi X 1/2 Chios) averaged 51.25 ± 1.05 kg were used in this experiment during the months from August to Novembcrat at the Farm of Animal Prod. Dept., Fac. of Agric., Minia Univ. The ewes were randomly divided into two equal groups (ten ewes in each) of similar initial body weights. The first group served as control while the second group received oral administration of vitamin A (retinol) with 50.000 IU/head/weekly (140 IU/kg live weight/day). Ewes were supplemented with vitamin A starting at 2 weeks late gestation and during suckling period. The ewes were fed on diet to cover their nutrient requierments according to their live body weight (NRC, 1985). They were fed on concentrate mixure and bean straw. The concentrate mixure contained 30% yellow corn, 30% wheat bran, 31% rice bran, 6% cotton seed meal, 2% limestone and 1 % common salt in which the calculated feeding values were 66.7% TDN and 13.74% crude protein. The calculated concentartion of ß-carotene in the concentrate mixure was 1.5 mg/kg DM. The NRC requierments for ewes in late gestation and during suckling period are 147 µg/kg live weight/day for ß-carotene and 100 IU/kg live weight/day for vitamin A with consideration of suckling twins. Feed was offered twice a day at 8 a.m and 2 p.m and mineral blocks and drinking water were available to the animals all times. The experimental animals were apparently healthy and proved to be free from internal and external parasites.

Data were collected in the morning before animals access to feed or water. Body weights of ewes in control and vitamin A supplemented groups were recorded at starting vitamin A administration and at biweekly thereafter. Body weights of male lambs born to ewes in each group were recorded within 24 hours from birth and then at 2 weeks intervals during suckling period. Averages of daily gain of lambs were calculated. Measurements of rectal temperature (R.T, °C), respiratory rate (R.R, Res/min.) and pulse rate (P.R, Pulse/min.) were recorded biweekly for ewes and their lambs at 8-9 a.m. The averages of ambient temperature and relative humidity during the experimental period were 24.7 °C and 65 % respectively at 8-9 a.m.

After parturition and at 2 weeks intervals, blood samples were collected form ewes and their male lambs in each group at 8.30 a.m. Heparinized blood samples (5 ml) were used for some hematological parameters as hemoglobin, packed cell volume, red blood cell and white blood cell counts. Stained blood smears with Lieshman's stain were prepared for the differential white blood cell count (Dacie and Lewis, 1991). Plasma samples were obtained and stored at -20°C utill assayed for biochemical analysis. Plasma vitamin A concentrations were determined using the method described by Neeld and Pearson (1963). Plasma total protein, albumin, total lipids and glucose were measured spectrophotometrically using standard test kits supplied from Bio-Merieux (Marcy-1, Etolie Charbonnieres- Les Bains, France) and Bio-Analytics kits (USA). Globulin was calculated mathematically by substracting the difference betwen total protein and albumin.

The data were analyzed by least square means analysis of variance using General Linear Models (GLM) procedure of the statistical analysis system (SAS, 1992). The model used to analyze the different traits studied for ewes or lambs was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where : Y_{ij}= ij^{th} observation, μ = population mean; T_i = effect of i^{th} treatments (1=control and 2= vitamin A treatment and eij=random error particular to the ij^{th} observation and assumed to be indepently and randomly distributed (0, $\acute{O}^2$). Duncan's Multiple Range test was used to detect differences between means of the experimental groups (Duncan, 1955).

RESULTS

The differences in averages of body weight (BW) were not significant between ewes received vitamin A and those of control (Table 1). However, averages of BW tended to increase for ewes received vitamin A by 4.95, 4.4 and 5.88% at 4, 8 and 10 weeks postpartum respectively when compared to control ewes. Lambs born to vitamin A-supplemented ewes recorded higher (P<0.01) averages of BW by 8.6, 11.2 and 13.3% at 4, 8 and 10 weeks of age respectively than those born to control ewes.

Table 1 : Averages of body weight of ewes as affected by vitamin A supplementation. (mean ± SEM).

(Body weight, kg)*	Ewes		% change	± SEM
	Control	Vitamin A suupl.		
At starting	50.92	51.58	1.37	1.05
4 weeks postpartum	43..83	46.00	4.95	0.93
8 weeks postpartum	47.25	49.33	4.40	0.98
10 weeks postpartum	49.00	51.88	5.88	0.72

* The differences were not significant.

Averages of daily gain were greater ($P<0.05$ and $P<0.01$) by 8.7, 15.8 and 21.4 % at periods of birth-4 , 4-8 and 8-10 weeks of age respectively for lambs from vitamin A-supplemented ewes than those from control ewes (Table 2).

Table 2: Averages of body weght and daily gain as affeced by ewe-vitamin A supplementation (mean ± SEM).

Parameters	Lambs of control ewes	Lambs of Vit. A suupl. ewes	± SEM	Sig.
Body weght (kg) ;				
At birth	2.91	2.92	0.72	NS
4 weeks	7.67 b	8.33 a	0.66	**
8 weeks	12.76 b	14.19 a	0.85	**
10 weeks	15.24 b	17.27 a	0.75	**
Daily gain (g/day)				
Birth-4 weeks	159.02 b	172.87 a	2.14	*
4 - 8 weeks	169.33 b	196.10 a	1.85	**
8 - 10 weeks	165.55 b	200.90 a	2.53	**

a,b means within the same row having different superscripts significantly different (* $P<0.05$, ** $P<0.01$). NS=not significant.

Vitamin A supplementation to ewes had no significant effect on rectal temperature (R.T, °C), respiratory rate (R.R, Res/min.) and pulse rate (P.R, Pulse/min.) either for ewes or their lambs (Table 3). Although blood hematological parameters (RBCs, Hb and PCV) of ewes or their lambs did not significantly affected by vitamin A supplementation to ewes, there was a significant ($P<0.01$) increase by 8.73 % in blood Hb concentrations and a slight increase by 3.7 % in PCV for lambs born to vitamin A supplemented ewes compared with those from control ewes. Ewes received vitamin A supplementation had higher ($P<0.01$) concentrations of plasma total protein, albumin and globulin by 20.5, 12.3 and 27% than those of control ewes. Concentrations of plasma vitamin A increased ($P<0.05$) by 22.6% for vitamin A-supplemented ewes compared with control ewes. Lambs born to ewes received vitamin A had higher concentrations of plasma total protein, globulin ($P<0.01$) and vitamin A ($P<0.05$) by 13.3, 20.0 and 17 % respectively than those born to control ewes. Although the differences in plasma concentrations of total lipids and glucose for ewes and plasma albumin, total lipids and glucose for lambs were not significant due to vitamin A supplementation to ewes, these plasma factors tended to increase for vitamin A-supplemented ewes and their lambs (Table 3).

Table 3: Effect of ewe-vitamin A supplementation on the thermo-cardio-respirstory reactions, hematological parameters and plasma constituents of ewes and their lambs (means ± SEM).

Parameters	Ewes		± SEM	Lambs of Cont. ewes	Lambs of Vit. A Suppl. ewes	± SEM
	Control	Vit. A suppl.				
R.T (° C)	39.23	39.33	0.04	39.66	39.74	0.03
R.R (rate/min.)	45.56	46.25	1.14	61.83	65.19	1.32
P.R (pulse/min)	73.61	74.72	0.76	86.53	89.44	0.89
Bood parameters						
RBC ($x10^6/mm^3$)	10.23	10.50	0.08	12.04	12.05	0.12
Hb (g/dl)	10.93	11.20	0.12	11.80 b	12.83 a	0.15
PCV (%)	30.42	30.52	0.35	33.75	35.00	0.44
Plasma constituents						
Total protein (g/dl)	7.32 b	8.82 a	0.24	6.97 b	7.90 a	0.17
Albumin (g/dl)	3.25 b	3.65 a	0.08	3.07	3.22	0.06
Globulin (g/dl)	4.07 b	5.17 a	0.17	3.90 b	4.68 a	0.13
Total lipids (mg/dl)	176.1	183.9	3.70	112.2	120.0	7.05
Glucose (mg/dl)	53.70	55.75	1.13	70.56	76.11	2.36
Vitamin A (μg/dl)	14.56 b	17.85 a	0.73	13.20 b	15.45 a	0.45

a,b means within the same row having different superscripts significantly different ($P<0.05$ and $P<0.01$).

The total count of WBCs was increased ($P<0.01$) by 19.6 % for ewes received vitamin A, showing a marked increase ($P<0.01$) in lymphocytes and a decrease ($P<0.01$) in neutrophils percentage compared with those ewes of control (Table 4). Lambs of ewes received vitamin A showed a significant ($P<0.01$) increase in lymphocytes and a decrease ($P<0.05$) in neutrophils with an insignificant higher total WBCs count as compared to lambs from control ewes.

Table 4: Effects of ewe-vitamin A supplementation on total white blood cell counts and its differencial cell percentages (mean ± SEM).

Parameters	Ewes		± SEM	Lambs of Cont. ewes	Lambs of Vit. A Suppl. ewes	± SEM
	Control	Vit. A suppl.				
Total WBC ($x10^3/mm^3$)	7.85 b	9.39 a	0.15	8.6	9.20	0.17
Neutrophils (%)	30.0 a	21.28 b	0.98	28.61 a	22.42 b	0.69
Eosinophils (%)	4.0	3.92	0.15	4.78	4.64	0.16
Basophils (%)	1.0	1.0	0.00	1.0	1.0	0.00
Lymphocyes (%)	60.0 b	69.0 a	1.12	59.55 b	66.00 a	0.96
Monocytes (%)	5.0	4.8	0.14	6.06	5.94	0.24

a,b means within the same row having different superscripts significantly different ($P<0.05$ and $P<0.01$).

DISCUSSION

In the current study, vitamin A supplementation to ewes (50.000 IU/head/week) at 2 weeks late gestation and during suckling period improved averages of body weight and daily gain of their male lambs from birth up to weaning. In the same respect, ewe lambs received oral administration of vitamin A (100.000 IU/ 2 weeks) exhibited a higher growth response (Bruns and Webb, 1990). These beneficial effects of vitamin A on sheep performance are in accordance with earlier study working on other ruminants. El-Barody *et al.* (1993) found that vitamin A supplementation to pregnant buffaloes resulted in improving their calves weight gain, suggesting that the increasing of milk yield and vitamin A concentrations in milk of vitamin A supplemented-buffaloes may resulted in improving their calves performance. Vitamin A is required for normal epithelial cells, normal bone growth and remodeling (Pond *et al.*, 1995), in addition to its major function in normal metabolism to preserve the stability, structural integrity and the normal permeability of cell and subcellular membranes (Harper et al., 1979) by which it has positive effects on tissue biosynthesis and growth responses (Chew, 1993 and El-Masry *et al.*, 1998).

The significant increase in blood Hb concentrations and a tendecy to increase PCV for lambs born to ewes received vitamin A may signify a case of active metabloism and biological oxidation on the cellular base (Frandson, 1986) that lead to availability of metabolites required for tissue growth. However, its deficiency leads to atrophy of all epithelial cells but the important effects are limited to those types of epithelial tissues which have a secretory as well as covering function. Vitamin A participates in the metabolism of the body in a much more genaralized way (Harper *et al.*, 1979). In this respect, vitamin A intake affects thyroid function and its deficiency reduces thyroxin secretion whereas thyroxin stimulates conversion of carotenoids to vitamin A (Pond *et al.*, 1995). This finding might revealed that vitamin A supplementation is a metabolic requirement for higher thyriod activity reflecting the improvment gained in growth performance of lambs born to vitamin A-supplemented ewes which attained in the present study. On the other hand, vitamin A deficiency may associated with altered Fe metabolism, including reduced plasma Fe (Pond *et al.*, 1995).

In the present study, some plasma metabolite concentrations were changed up either for ewes received vitamin A or their lambs. These treated ewes and their lambs exhibited higher levels of plasma total protein and its fractions (albumin and globulin). This response may be physiologically useful in controlling the osmotic pressure and flow of water between blood and tissue fluids (Kobeisy *et al.*, 1997). Elevated plasma globulin observed for vitamin A-supplemented ewes and their lambs agrees with El-Masry *et al.* (1998) who noticed an increase in globulin fractions for growing calves supplemented with vitamin A, but they failed to show a significant increase in plasma total protein or albumin. Also, the significant increases in plasma concentrations of vitamin A in ewes received vitamin A or their lambs are in harmony with similar trend observed in other ruminants as dairy cows (Mehrez, 1989) and buffaloes (El-Barody *et al.*, 1993). These increases in plasma vitamin A may associated to the increase in total protein since vitamin A is transported from the liver to peripheral tissues as free retinol bound to a specific protein (retinol-binding protein, RBP). So, it is indicated that when there may be a reduction in serum total protein, it may be observed that vitamin A levels are low (Harper *et al.*, 1979). In the same respect, protein deficiency causes reduced plasma vitamin A concentrations which will be reduced as a result from reduced transport of vitamin A from liver because of reduced serum albumin, the carrier protein for vitamin A in blood (Pond *et al.*, 1995). It is noteworthy that the RBP thought to prevent toxic effects of vitamin A on biological membranes (Goodman, 1984). Such significant changes in plasma factors in addition to a tendency to increase plasma total lipids and glucose for ewes received vitamin A and their lambs may account for the trend towards higher performance for these lambs. Metabolites concentrations in plasma represent a buffering state for metabolic synthesis and catabolism end products (Swenson, 1984).

In this study, the increase in total WBCs accompanied with elevated lymphocyte and a reduction in neutrophil populations for ewes receiving vitamin A or their lambs show a useful response of these animals to vitamin A which may improve their adaptability against adverse envioronmental conditions. These results in sheep support some earlier studies dealt with the effect of vitamin A on the immune status of animal. In cows, supplementation with ß-carotene or vitamin A enhanced the immune function and proliferation of their blood lymphocytes (Michal *et al.*, 1994). Also, *in vitro* study on lactating cattle showed that vitamin A enhanced the function and proliferation of lymphocytes (Tjoelker *et al.*, 1988b), meanwhile it had null or negative effects on neutrophil function since its phagocytosis was suppressed as documented by Tjoelker *et al*. (1988a) who noticed that these immune responses to vitamin A influenced by the physiological state of animal. In addition, in vivo studies showed that vitamin A enhanced the mitogen-induced lymphocyte proliferation in rats, mice and human (Dennert, 1984). However, Jensen and Eberhart (1981) failed to show such effects in vitamin A supplemented mice. With respect to lymphocytes function, they are the cell principally involved in the immune responses of animal since they stimulate the immunoglobulins formation that circulate in the blood and capable of combining with and neutralizing the harmful effects of the inducing substances. In addition, the various regions of the immune system are interconnected by an orderly traffic of lymphocytes (Fawcett, 1986).

Although the exact mechanisms by which vitamin A and ß-carotene exert its protective effect have not been fully defined, this protective function of vitamin A may be mediated stimulating immunoglobulin synthesis, stimulating naturall killer cells and cytotoxic T-lymphocytes (Dennert, 1984) and enahncing resistance to intracellular pathogens (Chew, 1993). Recently, Nonnecke *et al*. (2000) observed that vitamin A influences broad aspects of leucocyte function in terms of DNA synthesis and increased mitogen-stimulated mononuclear leucocytes, suggesting that the bioavailability of this vitamin may alter immune competency and disease susceptability of newborn calves .

In the present study, vitamin A supplementation to ewes appears to provide for additional increases in plasma concentrations of vitamin A either for treated ewes or their lambs. These animals also showed an increase in total leucocyte count and lymphocyte populations. In this regard, it has reported that optimal plasma concentrations of vitamin A exist for leucocyte function (Eicher *et al*., 1994). Since a well-known function of vitamin A is maintenance of functional integrity of epithelial tissues, this functional state of epithelium will regulate transfer of immunoglobulins which reflect on immune status. Thus, insufficiency of vitamin A may impair functionl integrity of epithelial tissues and resulted in invation of mastitis organism by which the transfer of immunoglobulin are decreased, therefore, a sufficient intake of vitamin A guarantees the normal function of epithelial tissues, which improve the defenses of the mammary gland against infections in dairy cows and their calves (Chew, 1987).

The present results declare that vitamin A supplemention to ewes at 2 weeks late gestation and during suckling period improved growth performance and some immune respones of their male lambs. This useful effect of vitamin A for these ewes and their lambs was accomapnied with favorable signs in their physiological reactions.

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