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Effect of Vit. E Supplementation to Pregnant Ewes Ration on Some Blood Biochemical Components in Ewes and Their Offspring's

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ABSTRACT

Thirty six pregnant Rahmani ewes with an avg. 33.7 kg LBW and 3 years old age were used to investigate the effect of vitamin E daily supplementation to pregnant ewes at late gestation and early lactation on the productive performance and some biochemical blood constituents of treated ewes and their offspring's. Experimental animals were randomly assigned (28 days prepartum) to three nutritional groups; the 1st one served as a control (nil vitamin E supplement). The 2nd and 3rd groups were daily and individually administrated 400 IU of α-tocopherol acetate (vitamin E), 28 days prepartum (T1) and for 28 days pre and 28 days postpartum (T2). Experimental animals were offered their daily requirements during pregnancy and early lactation according to (NRC recommendations, 1985). Blood samples were routinely withdrawn from ewes and their offspring's to assess some blood measurements i.e. (GSH-Px), LDH, ALT, AST, Total protein and globulin fractions. Results obtained indicated that, Vitamin E supplementation to pregnant ewes during late gestation led to affect significantly ($p<0.05$) ewes and lambs (GSH-Px) enzyme activity, increase ($p<0.05$) serum total protein and globulin concentration value in favor of the two supplemented ewes groups and the corresponding offspring's. However, non-significant differences ($p<0.05$) in LDH enzyme concentration was detected. Liver enzyme activity i.e. ALT and AST indicated significant differences ($p<0.05$), indicating higher ($p<0.05$) values for both the two supplemented groups in comparison with the control ewes group and their lambs.

Keywords: Vitamin E, ALT, AST, Glutathione peroxidase , lactic acid dehydrogenase, total protein and globulin

INTRODUCTION

Vitamin E is the generic name for a series of fat soluble compounds called tocopherols and tocotrienols (NRC, 2001). Vitamin E naturally occurs in feedstuffs as α-tocopherol and is the most biologically active form of vitamin E as well (NRC, 1996; 2001). According to (Hill *et al*., 1990, 1993; Chatterjee *et al*., 2003) vitamin E is not stored for a long time in the body; the high plasma or serum tocopherol levels can be used to determine the vitamin E status of an animal. Selenium is an important trace mineral, acting in synergism with vitamin E and other anti-oxidative agents such as Cu and Zn, inhibits the oxidation of membrane fat polyunsaturated acids and DNA by oxygen radicals, produced during aerobic metabolism (Florence, 1995). Vitamin E (α-tocopherol) and selenium (Se) have complementary role, as antioxidants, in the protection of cells against the damaging effects of lipid peroxides and free radicals produced during normal metabolism. The multiple functions of both nutrients, at cellular and molecular levels, extend beyond antioxidant protection, as their inclusion in the diet at concentrations above requirements is associated with variable improvements in sheep performance and immune function (Rooke *et al*., 2004). Dietary vitamin E requirements for sheep are not clearly defined. The NRC (1985) recommends 10 to 70 IU of vitamin E/kg diet, which appears too based on levels to prevent white muscle disease. Vitamin E supplementation at higher level than the recommended has been shown to enhance cellular (Cipriano *et al*., 1982) and humoral immune response (Samanta *et al*., 2006) in calves. More importantly, supplementation of vitamin E at higher levels than those currently recommended appears to be more safe (Meydani and Hafek, 1992; Bendich, 1993). The aim of the present study was to justify the effective role of Vitamin. E supplement as an antioxidant and positive immune factor to pregnant ewes rations on some blood biochemical components in ewes and their offspring's serum blood.

MATERIALS AND METHODS

The present study was carried out during the period from (September, 2012 to April 2013) at the experimental farm station belongs to Animal Production Department, Faculty of Agric. Al-Azhar University, Nasr city, Cairo, Egypt.

Animals feeding and management:

Thirty six pregnant Rahmani ewes with an average live body weight 37.7 kg and 3 years old age were randomly assigned into three nutritional groups during late gestation (4 weeks prepartum). The first group served as a control (C), nil Vitamin E supplement. The second group (T_1) was orally and daily administrated Vit. E (1g of Rovimix E-40 %, 400 IU of α-tocopherol acetate; Roche Vitamins, Parsippany, NJ) in capsulated form during late gestation (28 days before the expected lambing date). The third group (T_2) was orally and individually administrated Vit. E capsules for 28 days just before the expected lambing date and lasted for another 28 days postpartum (early lactation period). Experimental animals were housed in semi–opened pens, offered their daily requirements during pregnancy and lactation, according to NRC recommendations (1985). Concentrate feed mixture (14 % CP and 60 % TDN) + green berseem (*Trifolium alexandrinum),* were offered to pregnant and lactating ewes in two equal meals at 9.00 am and 3.00 pm (Table 1), while fresh drinking water was freely available all over the day time.

Table 1. Chemical composition of feedstuffs on (DM basis %)

Items	DM	OM	CP	CF	EE	Ash	NFE	Vitamin E mg/100ml
Pelleted CFM	90.4	84.3	13.4	14.2	3.9	15.7	52.8	6.04
Berseem	11.6	84.5	19.3	25.4	2.9	15.5	36.9	2.71

Different experimental groups were fasted weighed at the start of the study for two consecutive times and at biweekly intervals thereafter, until eight weeks after lambing. Ewes and lambs of each group

were weighed at birth, while newborn lambs were ear tagged and births data were recorded.

Blood Samples:

Blood samples were withdrawn from ewes via jugular vein, one week after Vit. E administration and at biweekly intervals thereafter, until the end of the study (8 weeks lactation period). Blood samples of 33 newborn lambs via jugular vein were also withdrawn 7 days after birth and at biweekly intervals thereafter, until weaning age (8 weeks old age).

Blood measurements:

Glutathione peroxidase enzyme (GSH-Px) was assessed in ewes and lambs whole blood (U/g Hb) according to Habig *et al.*, (1974); lactate dehydrogenase LDH (U/L), according to Friedman and young (2001); ALT and AST (U/L), according to Reitman and Frankel (1957); total protein (g/dl) according to Gornal *et al.*, (1949) and Globulin (g/dl), according to Doumas *et al.*, (1981) and Drupt (1974). LDH, ALT, AST, Total protein, and Globulin were being assessed in lambs, pregnant and lactating ewes serum blood before lambing (4 weeks prepartum), at lambing and 7 weeks postpartum. Vitamin E (α-Tocopherol) concentrations (mg) in ewes rations (concentrate feed mix) and green berseem (*Trifolium alexandrinum*) were also assessed in the (Central Laboratories of RCFF, Central Agric. Research Laboratories, Ministry of Agric., Giza).

Statistical analysis:

Data were statistically analyzed using GLM procedures using the statistical package software SAS version 9.1.3 (SAS Institute Inc., 2002, Cary, NC., USA). The data were analyzed by fitting effects of treatment (3 nutritional groups), effects of period (28 days prepartum & 28 days postpartum) and two-way interactions between treatment and period. Differences between means were tested for significances using the L.S.D test, according to Duncan (1955) at the pre-set level of 5%. The statistical model for the trial was as follows:

$$Y_{ij} = \mu + T_i + R_j + \varepsilon_{ij}$$

Where Y_{ij} is the observation of the parameter measured, μ is the overall mean, T_i is the effect of dietary treatment, R_j is the effect of period and ε_{ij} is the random error term.

RESULS AND DISCUSSION

Effect of vit. E supplementation to pregnant & lactating ewes on glutathione peroxidase enzyme activity in ewes blood.

Glutathione peroxidase (GSH-Px) enzyme activity in whole blood, plasma/ serum and liver, is widely used by diagnostic laboratories to predict the Se status of animals, (Gerloff, 1992).

Results obtained in (Table 2) showed glutathione peroxidase (GSH-Px) enzyme activity in whole blood of pregnant ewes at late gestation. Figures obtained after one week of vitamin E administration, indicated significant differences among groups in favor of T1 (4 wks administration) and T2 (8 wks administration) in compare with the control ewes (nil. vitamin E Supplement).

Table 2. Effect of vit. E supplementation on glutathione peroxidase (GSH-Px) activities in the whole blood of pregnant ewes at late gestation and early lactation for 7 wks (U/g Hb)

Items	Time of measuring	Experimental rations			Overall mean for time
		Cont. (1)	T1(2)	T2(3)	
GSH-Px at late gestation period (U/g Hb)	Mean	637.2^{b} ±71.0	2083.1^{a} ±845.7	1820.5^{a} ±972.4	
GSH-Px after lambing (U/g Hb)	1st week	151.38	1121.19	3474.23	1582.27^{b} ± 390.36
	3rd week	314.01	3907.74	2320.84	2180.86^{ab} ± 399.99
	5th week	1799.68	4265.80	3779.76	3281.17^{a} ± 390.36
	7th week	1489.96	2526.58	2499.87	2172.14^{ab} ± 390.36
	Overall mean	938.76^{b} ± 333.81	2955.33^{a} ±340.17	3018.7^{a} ±346.41	

a,b and c small letters; means with different superscripts in the same row and column indicated significant differences at (p< 0.05).

Both of T_1 and T_2 showed higher ($p<0.05$) values, but without significant difference between them. According to Hill *et al.*, (1990 and 1993), rapid increase in plasma – α to chopherol levels has been detected after vitamin E supplementation. After one week post-partum, both the two supplemented groups still maintaining higher ($p<0.05$), (GSH-Px) values, being as high as 7 folds for T_1 to 23 times (T_2) than that of the control group *i.e.* 1121.19 and 3474.23 *vs.* 151.38 U/g Hb, for T_1, T_2 and the control groups, respectively. From the 1st week post- partum and up to the 5th week, different experimental groups, including the control one, tended to exhibit linear (GSH-Px) increase values to reach the peak of activity by the 5th week. However, both the two supplemented groups indicated in general, higher ($p<0.05$) values in compare with the control group allover five weeks (GSH-Px) assessment. Similar results were reported by Reddy *et al.*, (1987) who pointed out that serum concentration of α – to copherol reached their respective peaks at 4 weeks and then declined. Two weeks later, meanwhile by the 7th week, different experimental groups tended to record lower ($p<0.05$) values, however, both of the two supplemented groups still maintaining higher ($p<0.05$) values in compare with the control group. According to Chatterjee *et al.*, (2003) vitamin E is not stored for a long time in the body and that the high plasma or serum to chopherol levels can be used to determine the vitamin E status of animals. GSH-Px concentration might be used as an indicator to vitamin E status of an animal.

On the light of the present results, it could be concluded, that supplementing pregnant ewes with 400 IU daily of vitamin E supplement for only 4 weeks prepartum or 4 weeks pre and another 4 weeks postpartum led to increase ($p<0.05$) GSH-Px activity in whole blood of supplemented ewes. The enzyme

activity tended to have normal distribution shape, since it showed lower values 1582.27 (U/g Hb) at the 1st week postpartum, increased linearly with the advance in time of administration to reach its peak by the 5th week 3281.17 (U/g Hb) and tended to decline ($p<0.05$) to lower values *i.e.* 2172.14 (U/g Hb) by the 7th week postpartum. This result might lead to suggest that, there were maybe some hormonal and/or biological mechanisms interfere by time to keep GSH-Px enzyme activity at a constant value. It was also worthy to point out to an obvious observation, since supplementing pregnant ewes by an additional daily supply of vitamin E during early lactation didn't lead to more positive activity of GSH-Px enzyme. It was evident, lack of significancy between both of T_1 and T_2 neither during late pregnancy nor at early lactation, due to the additional supply of daily vitamin E for more longer period (4 weeks postpartum). A result which might lead to suggest to the more importance of vitamin E administration during late gestation, and that there were not a necessity to more vitamin E provision during the early lactation period, since it didn't lead to more positive influences in ewes of T_2 group.

Glutathione enzyme activity in whole blood of newborn lambs.

Results of (Table 3) revealed (GSH-Px) activity in whole blood of newborn lambs during 8 weeks assessment. Data obtained indicated significant differences among different lambs groups in favor of treated group ones in compare with the control lambs group.

Table 3. Effect of vit. E supplementation on glutathione peroxidase (GSH-Px) activities in the whole blood of newborn lambs (U/g Hb)

Items	Time of measuring	Experimental rations Cont. (1)	T1 (2)	T2 (3)	Overall mean for time
GSH-Px for lambs (U/g Hb)	1st week	1521.31	3230.06	5022.23	3257.87[a] ± 318.60
	4th week	468.18	1429.90	4825.86	2241.3[b] ± 243.50
	8th week	240.43	2328.81	4957.09	2508.8[ab] ± 328.40
	Overall mean	743.31[c] ±313.88	2329.6[b] ±303.91	4935.1[a] ±303.91	

a,b and c small letters; means with different superscripts in the same row and column indicated significant differences at ($p< 0.05$).

At one-week old age, T_1 and T_2 exhibited two and three folds concentration of (GSH-Px) in compare with lambs born to the control dams *i.e.* 1521.31, 3230.06 and 5022.23 for control, T_1 and T_2, receptively. At more advanced ages, 4 weeks old, (GSH-Px) activity tended to decrease ($p<0.05$) and linearly to more lower ($p<0.05$) values at 8 weeks old age. However, both the two treated groups exhibited as a common phenomenon, higher ($p<0.05$) values *i.e.* 2329.6 and 4935.1 (U/g Hb) in compare with only 743.31 (U/g Hb) for lambs born to the control dams (nil vitamin E supplement), respectively. It was of interest to note that lambs born to T_2 ewes (8 wks vitamin E supplement) exhibited higher ($p <0.05$), (GSH-Px) values in compare with both the control and T_1, reaching to as high as twice that of T_1 and 7 times that of the control, respectively.

On the light of the present results, it was concluded that supplementing the basal ration of pregnant ewes with vitamin E during pregnancy and early lactation (4 wks postpartum) led to increase ($p<0.05$), GSH-Px level and activity in newborn lambs blood and hence, provides more positive immunity to newborn lambs. The higher expression of (GSH-Px) activity in ewes and their lambs in whole blood might indicate the maternal status of selinoproteins in the diet, the matter which might lead in turn to improve the anti-oxidant status of experimental animals by reducing the level of reactive oxygen species, (Thannickal and Fanburg, 2000). According to McDowell *et al.*, (1996), newborn are susceptible to vitamin E deficiency and, due to the negligible amount of vitamin E crossing to the fetus in uterus, it is important that colostrum would supply the lamb with sufficient amounts of vitamin E. And from an economic point of view, supplementing dams for only four weeks prepartum at late gestation seemed to be proper enough to meet ewes and their offspring's requirements, and an excessive drugs for more longer time (4weeks post partum) tended to have lower influences but higher costs. However, and according to (Cipriano *et al.*, 1982), vitamin E supplementation at higher levels to more longer periods than that recommended has been shown to enhance cellular and humeral immune response. On the other hand, Bendich (1993) pointed out to that supplementation of vitamin E at higher levels or periods than those currently recommended appeared to be more safe.

Effect of vit. E supplementation to pregnant and lactating ewes on LDH, ALT and AST concentrations in serum blood of ewes.

Results presented in (Table 4) showed the effect of vitamin E supplement during (4 weeks prepartum, T_1) and (4 weeks pre and another 4 weeks postpartum, T_2) on lactic acid dehydrogenase enzyme (LDH) concentration in ewes serum blood. According to Friedman and Young (2001), lactate dehydrogenase is present in all cells of the body, but its higher concentrations are found in liver, heart, kidneys, skeletal muscles and erythrocytes. Total LDH concentration in serum or plasma are being increased in animals suffered from liver disorders, renal disease, myocardial infections, progressive muscular dystrophy and almost any cause of hemolysis. Data obtained in (Table 4) indicated insignificant differences among different dam groups, due to vitamin E supplementation. Figures obtained were 0.57, 0.60 and 0.54 (U/L) for the control, T_1 and T_2 , respectively. Similar results were reported by Schingoethe *et al.*, (1978), who pointed out to negative correlationship between vitamin E dietary supplement and blood LDH enzyme activity ($r =-14$). On the other hand, LDH enzyme concentration tended to decline ($p <0.05$) from the 1st week after lambing *i.e.* 0.60 U /L to 0.54 U/L at the 7th week of lactation. On the light of the present results, it was concluded that different ewes groups were in healthy condition and did not suffer from any liver or renal disorders.

Table 4. Effect of vit. E supplementation on Lactate Dehydrogenase (LDH), (ALT) and (AST) concentrations in serum of ewes during (7 wks) experimental period.

Items	Time of measuring	Experimental rations			Overall mean for time
		Cont. (1)	T1(2)	T2(3)	
LDH (U/L)	1st week	0.59	0.65	0.55	$0.60^{a}\pm0.02$
	3rd week	0.56	0.59	0.51	$0.55^{ab}\pm0.02$
	5th week	0.60	0.59	0.54	$0.58^{ab}\pm0.01$
	7th week	0.52	0.54	0.55	$0.54^{b}\pm0.03$
	Overall mean	$0.57^{ab}\pm$ 0.02	$0.59^{a}\pm$ 0.02	$0.54^{ab}\pm$ 0.01	
ALT (U/L)	1st week	0.65	0.72	0.82	$0.73^{b}\pm0.03$
	3rd week	0.74	0.72	0.85	$0.77^{ab}\pm0.03$
	5th week	0.63	0.90	0.83	$0.79^{ab}\pm0.04$
	7th week	0.67	1.01	0.80	$0.83^{a}\pm0.04$
	Overall mean	$0.67^{b}\pm$ 0.02	$0.84^{a}\pm$ 0.04	$0.83^{a}\pm$ 0.03	
AST (U/L)	1st week	0.59	0.54	0.64	$0.59^{c}\pm0.02$
	3rd week	0.61	0.60	0.70	$0.64^{bc}\pm0.03$
	5th week	0.67	0.71	0.78	$0.72^{a}\pm0.03$
	7th week	0.60	0.71	0.81	$0.71^{ab}\pm0.03$
	Overall mean	$0.62^{b}\pm$ 0.02	$0.64^{b}\pm$ 0.02	$0.73^{a}\pm$ 0.02	

a,b and c small letters; means with different superscripts in the same row and column indicated significant differences at ($p< 0.05$).

(1) Nil Vit. E supplement
(2) 400 IU oral Vit. E supplement /h/d (28 d prepartum)
(3) 400 IU oral Vit. E supplement /h/d (28 d pre-and 28 d postpartum)

On the other hand, lack of significancy at the earlier time of enzyme assay, *i.e.* from one week postpartum (0.60) to as low as (0.58 U/L) at the 5th week of early lactation might lead to suggest that, normal values of LDH ranged between as high as (0.65 U/L) to as low as (0.51 U/L), since experimental animals groups all over the period of the study didn't exhibit any negative influences or suffered from renal or liver disorders. Moreover, it was of interest to note that supplementing pregnant ewes at late gestation and with another excessive dose during lactation, didn't lead to significant LDH values, which in turn led to point out to the negative role of vitamin E administration on LDH concentration, hence there were no need to support pregnant ewes with an additional supply of vitamin E, especially when they were offered green berseem during pregnancy and lactation. It was assumed that green berseem might provide pregnant and lactating ewes with daily vitamin E requirements (Table 2) and there was no need to an additional supply of vitamin E, in condition of available and natural source of vitamin E (green berseem). Data presented in (Table 4) showed the effect of vitamin E supplement to pregnant ewes and lactating ones on ALT and AST (U/L) enzymes as biological indicators of liver function. As shown, both of T_1 and T_2 ewes indicated higher ($p<0.05$) ALT values in compare with the control group dams. And since, different ewes groups didn't suffer from any negative influences in performance during lactation or any liver disorders; it was assumed that the normal enzyme activity of ALT enzyme ranged between (0.67 to 0.84 U/L). It was also noticed a linear ($p<0.05$) increase in ALT concentrations with the advance of time of measuring which ranged between 0.73 at 1st week of assay to 0.83 U/L at 7th week, the matter which might confirm our previous observation about normal fluctuation in enzyme level, but within the normal ranges of enzyme activity. As for AST enzyme activity, it was detected significant differences among different dam groups in enzyme concentration, T_2 recorded higher ($p<0.05$) value (0.73 U/L) in compare with both of the control (0.62) and T_1 (0.64 U/L), respectively. However, and irrespective of significancy among groups, and due to the real vision; different ewes in the study didn't suffer from any liver disorders nor exhibiting any negative rearing performance. On the light of the present results, it was suggested that such significant values in both ALT and AST enzymes activity didn't represent nothing rather than normal and biological fluctuates within the normal ranges of enzyme level in healthy animals.

Lactic acid dehydrogenase; (ALT and AST) liver enzyme activity in serum blood of newborn lambs.

Results obtained in (Table 5) illustrate the effect of vitamin E supplementation on LDH, ALT and AST in serum of lambs born to different experimental groups during early lactation (8 weeks rearing period). As shown, different lambs exhibited insignificant differences in LDH concentration. Figures of enzyme activity in serum of lambs didn't differ from their corresponding dam groups (Table 4) and ranged between 0.55 for T_2 to 0.61 U/L for both of the control and T_1, respectively. On the other hand, time of measuring did not lead to any significant differences due time of assay; however LDH concentration tended to decline insignificantly from 0.57 at the 1st week to 0.59 U/L by the 8th week of assay. Similar trend was also noticed like that of their dams (Table 4).

Table 5. Effect of vit. E supplementation on Lactate Dehydrogenase (LDH), (ALT) and (AST) concentrations in serum of lambs during (8 wks) experimental period.

Items	Time of measuring	Experimental rations			Overall mean for time
		Cont. (1)	T1(2)	T2(3)	
LDH (U/L)	1st week	0.58	0.54	0.58	0.57±0.01
	4th week	0.58	0.73	0.53	0.61±0.06
	8th week	0.67	0.55	0.54	0.59±0.03
	Overall mean	0.61± 0.02	0.61± 0.07	0.55± 0.02	
ALT (U/L)	1st week	0.77	0.91	0.96	0.88±0.03
	4th week	0.87	1.15	0.81	0.94±0.06
	8th week	0.80	1.22	0.83	0.95±0.08
	Overall mean	$0.81^{b}\pm$ 0.04	$1.09^{a}\pm$ 0.08	$0.87^{b}\pm$ 0.03	
AST (U/L)	1st week	0.65	0.84	0.82	0.77±0.04
	4th week	0.59	1.10	0.70	0.80±0.08
	8th week	0.64	1.04	0.86	0.85±0.08
	Overall mean	$0.63^{b}\pm$ 0.03	$0.99^{a}\pm$ 0.10	$0.79^{b}\pm$ 0.03	

a and b, means with different small letter in the same row and column indicated significant differences at ($p< 0.05$).

(1) Nil Vit. E supplement
(2) 400 IU oral Vit. E supplement /h/d (28 d prepartum)
(3) 400 IU oral Vit. E supplement /h/d (28 d pre-and 28 d postpartum)

As for ALT and AST enzyme, (Table 5), it was observed significant AST values ($p<0.05$) among different lambs groups in enzyme concentration like that of their dams groups, but at higher values. Figures obtained were as high as (1.09) for T_1, declined to (0.81 U/L) for the control group. Values obtained pointed out

to insignificant difference between both of the control (0.81 and T_2 lambs groups 0.87 U/L), respectively. It was of interest to point out to similar ALT concentration trend between lambs concentration values and the corresponding ones of their dam groups (Table 4), since T_1 reordered the higher ($p<0.05$) values followed by T_2, 0.87 and later was the control group (0.81 U/L). Such result might lead to assume a positive correlationship between both of the offspring's and the corresponding ALT concentration values in the serum of their respective dams. Results of the present trail might suggest a positive correlationship between both of lambs and their corresponding dams.

On the other side, data presented in (Table 5) didn't lead to any significant differences among different periods of ALT assay. However, values recorded pointed out to insignificant increase in ALT concentrations with the advanced time of measuring, *i.e.* 0.88 at 1st week increased to 0.95 (U/L) by the 8th week of assay, indicating higher values in compare with their corresponding dams. Such higher values in ALT concentration in lambs blood, might assume normal biological plasma difference due to animal age.

As for AST enzyme activity in blood serum of different lambs groups during 8 weeks (early lactation period), it was noticed significant differences in AST concentrations among different experimental groups. T_1 lambs group showed higher ($p<0.05$) AST concentration (0.99 U/L), followed by T_2 (0.79 U/L) and without significant difference with the control group (0.63 U/L), respectively. Time of measuring didn't lead to any significant differences due to time of measuring; however AST concentrations tended to exhibit insignificant increase values with the advanced time of AST assessment, but at lower rates in compare with the corresponding ALT rates.

It was worthy to note that both of ALT and AST enzymes exhibited linear significant or insignificant increase ($p<0.05$) for both dam groups (Table 4) and their corresponding lambs groups, (Table 5) with the advance of time of measuring. The second obvious observation concerning liver enzymes activity, was that lambs tended to exhibit higher ($p<0.05$) ALT and AST enzyme values in compare with the corresponding figures of their dams. Such result might lead to assume that newborn lambs in their early life, either suffered somehow from negative environmental stress, due to incomplete immune system functions, or /and it was mainly refereed to normal biological differences due to an animal age .

Effect of vit. E supplementation to pregnant and lactating ewes on total protein and globulin concentrations in serum blood of experimental ewes and their offspring's.

As shown in (Table 6), there were significant differences among different experimental groups in total protein concentrations in serum of ewes. T_2, (8 weeks supplemented group) indicated higher ($p<0.05$) total protein value (10.75 g/dl) in compare with (8.02) and (6.66 g/dl) for both of T_1 supplemented group and the control ewes, respectively.

Table 6. Effect of vit. E supplementation on total protein and globulin concentrations in serum of ewes during (7 wks) experimental period.

Items	Time of measuring	Experimental rations			Overall mean for time
		Cont. (1)	T1(2)	T2(3)	
Total Protein (g/dl)	1st week	7.02	7.42	11.41	$8.62^{ab}\pm0.52$
	3rd week	6.95	5.91	11.88	$8.25^{ab}\pm0.62$
	5th week	6.67	8.87	12.77	$9.44^{a}\pm0.72$
	7th week	5.98	10.07	6.95	$7.67^{b}\pm0.43$
	Overall mean	$6.66^{c}\pm$ 0.16	$8.02^{b}\pm$ 0.40	$10.75^{a}\pm$ 0.62	
Globulin (g/dl)	1st week	1.91	3.15	7.68	$4.25^{b}\pm0.61$
	3rd week	3.02	1.52	7.62	$4.05^{b}\pm0.63$
	5th week	3.48	5.54	8.34	$5.79^{a}\pm0.67$
	7th week	2.52	6.71	2.30	$3.84^{b}\pm0.48$
	Overall mean	$2.73^{c}\pm$ 0.21	$4.23^{b}\pm$ 0.44	$6.49^{a}\pm$ 0.65	

a,b and c, means with different small letter in the same row and column indicated significant differences at ($p< 0.05$).
(1) Nil Vit. E supplement
(2) 400 IU oral Vit. E supplement /h/d (28 d prepartum)
(3) 400 IU oral Vit. E supplement /h/d (28 d pre-and 28 d postpartum)

Similar findings were reported by El-Shahat and Abdel Monem (2011) who claimed that, Baladi ewes supplemented with 50 mg vitamin E plus 0.3 mg Se /kg diet at 2 weeks before mating and extended through pregnancy till lambing resulted in a significant ($p<0.05$) increase in total serum protein and globulin. Similar results are shown in (Table 7) of the newborn lambs during (8 weeks assessment) with only one exception, that both the two supplemented groups showed higher ($p<0.05$) values in compare with the control group, and without significant difference between both the two supplemented lambs groups. In both of the control ewes and their offspring's, they tended to exhibit lower ($p<0.05$) total protein values, respectively. Such results might lead to assume a positive correlationship between both of ewes and their offspring's, in one hand. On the other hand, it could be suggested that oral administration of vitamin E to pregnant ewes during late gestation and early lactation led to significant positive effects on raising the total protein values in serum of ewes and their corresponding offspring's; (Tables 6 and 7).

Table 7. Effect of vit. E supplementation on total protein and globulin concentrations in serum of lambs during (8 wks) rearing period.

Items	Time of measuring	Experimental rations			Overall mean for time
		Cont. (1)	T1(2)	T2(3)	
Total Protein (g/dl)	1st week	6.46	9.60	11.25	9.10±0.56
	4th week	5.76	9.25	10.99	8.67±0.71
	8th week	6.23	8.80	8.86	7.96±0.55
	Overall mean	$6.15^{b}\pm$ 0.15	$9.22^{a}\pm$ 0.35	$10.37^{a}\pm$ 0.75	
Globulin (g/dl)	1st week	1.01	6.47	6.90	4.79±0.71
	4th week	1.99	6.02	5.67	4.56±0.74
	8th week	2.13	6.27	3.50	3.97±0.70
	Overall mean	$1.71^{b}\pm$ 0.25	$6.25^{a}\pm$ 0.40	$5.36^{a}\pm$ 0.90	

a and b, means with different small letter in the same row and column indicated significant differences at ($p< 0.05$).
(1) Nil Vit. E supplement
(2) 400 IU oral Vit. E supplement /h/d (28 d prepartum)
(3) 400 IU oral Vit. E supplement /h/d (28 d pre-and 28 d postpartum)

On the other hand, globulin concentration values (Tables 6 and 7) showed a similar trend, like that of total protein direction. As shown, both of the two supplemented ewes groups exhibited higher ($p<0.05$) globulin values in serum blood, while the control ewes (nil vitamin E supplement) and their offspring's showed the lower ($p<0.05$) globulin values *i.e.* (2.73 g/dl for dams and 1.71 g/dl for their lambs), respectively. Globulin is a proteinous compound derived from total protein and might lead in turn to biological biosynthesis of immunoglobulin fractions, responsible on both of ewes and lambs immunity. On basis of such obvious scientific fact, it was expected to higher ($p<0.05$) positive immunity for both the two vitamin E supplemented ewes groups and their offspring's in compare with the corresponding ewes and lambs control ones. According to, Hamam and Abou-Zeina (2007) and El-Shahat and Abdel Monem (2011) injection or administration of both of vitamin E plus Se, in Baladi ewes, increased significantly the concentrations of natural antioxidants (αtocopherol and glutathione peroxidase) in blood of sheep, hence ensures that they had an adequate amount of immune globulins, concluding that both nutrients should be administrated to sheep in order to improve their immune competence. As for the effect of time of measuring on the concentrations of both of total protein and globulin values in different ewes groups. It was detected significant total protein and globulin concentrations in serum blood of ewes during 7 weeks assessment period.

As a general trend, it was noticed gradual significant decrease ($p<0.05$) in both of ewes total protein and globulin (g/dl) with the advance of time of measuring, which ranged between 8.62 at the 1st week to 7.67 g/dl for total protein at the 7th week. While, it declined from 4.25 to 3.84 g/dl for globulin values during the same period, (Table 6). The effect of time of assay for both total protein and globulin for the corresponding offspring's of such dam groups, indicated similar decline trend, but without significant differences among weeks of assessment. Total protein in reared lambs declined insignificantly from 9.10 g/dl at the 1st week to 7.96 at the 7th week, while serum globulin values declined from 4.79 to 3.97 g/dl during the same period, in the same order.

On the light of the present results, it was concluded that assessment of some blood biochemical components could be used as indicators to animals health and productivity. And although, supplementing Rahmani ewes with daily oral administration of Vit. E capsules (400 IU/Se) at late gestation and early lactation didn't lead to any positive influences on ewes and their offspring's productivity, (Salama *et al.*, 2015). However, the biochemical assessment of some blood biochemical components indicated significant relationships between ewes and their progenies. The matter which might lead to suggest that; assessment of some enzyme and blood constituents, could be used by diagnostic laboratories as a simple technique and guideline to predict farm animals health status to somehow and signs of productivity .

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تأثير إضافة فيتامين هـ لعلائق النعاج الحوامل على بعض المكونات البيوكيميائية لمكونات الدم في الأمهات والنتاج

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إستخدم في هذه الدراسة ٣٠ نعجه رحماني بمتوسط وزن ٣٧.٧ كجم ومتوسط عمر ٣ سنوات، بهدف تقييم تأثير إستخدام فيتامين E على صورة (α-tochopherol acetate) بمعدل ٤٠٠ وحده دوليه للرأس/ يوميا خلال نهاية فترة الحمل (٤ اسابيع) وأول موسم الحليب (٨ أسابيع) على الكفاءة الإنتاجية للأمهات ونتاجها. تم تقسيم ٣٠ نعجه رحماني في أخر فترة الحمل (٤ أسابيع قبل موسم الولادة المتوقع) إلى ٣ مجاميع غذائية بمعدل ١٠ نعاج لكل مجموعه – حيث غذيت المجموعة على أساس إحتياجاتها الغذائية القياسية في آخر الحمل وبدون أية إضافات غذائية واستخدمت كمجموعه مقارنه ، بينما تم تجريع النعاج الحوامل للمجموعة الثانية بكبسولات فيتامين E وبمعدل (٤٠٠ وحده دوليه للرأس / يوميا) ولمدة ٤ أسابيع قبل ميعاد الولادة المنتظر كمجموعة معاملة أولى ، بينما تم تجريع النعاج الحوامل لمجموعة المعاملة الثانية بكبسولات فيتامين E (٤٠٠ وحده دوليه للرأس / يوميا) لمدة ٤ أسابيع قبل الولادة و ٤ أسابيع أخرى في بداية موسم الحليب واعتبرت كمجموعة معامله ثانيه. أخذت عينات دم من الأمهات بعد أسبوع من المعاملة بالفيتامين واستمرت على ذلك على مدى ٨ أسابيع لاحقه، وكذا تم أخذ عينات من دم المواليد بعد أسبوع من الولادة واستمر أخذ العينات بصفه دوريه وبمعدل كل أسبوعين للأمهات والمواليد لاحقا حتى نهاية فترة التجربة. أجريت قياسات مختلفة على دم الأمهات والمواليد بغرض التعرف على بعض مقاييس الدم ذات الدلالة والمرتبطة بتدعيم الأمهات بفيتامين E ، وقد شملت مقاييس الدم التعرف على تركيز إنزيم الجلوتاثيون بيروكسيديز (C-SH-Px) في صورة الدم الكاملة، وكذا نشاط إنزيم اللاكتيك أسيد ديهيدروجينز (LDH) ، تقدير نسبة البروتين الكلى، ومستوى الجلوبيولين في السيرم وكذا إنزيمات الكبد للأمهات والحملان تحت ظروف التجربة. وقد أظهرت النتائج المتحصل عليها أن تدعيم النعاج المعاملة يوميا بفيتامين E (بمعدل ٤٠٠ وحده دوليه للرأس / يوميا) قد أدى إلى زيادة نشاط إنزيم (G-SH-Px) معنويا في دم النعاج المعاملة وأبنائها لكلا المجموعتين عند مستوى ٥ % ، وإن لم تظهر أي اختلافات معنوية بين المجاميع سواء الأمهات أو الأبناء في درجة ونشاط تركيز إنزيم (LDH). أدى إستخدام فيتامين E أيضا كإضافات في علائق المعاملتين الثانية والثالثة إلى إرتفاع تركيز البروتين الكلى في سيرم دم النعاج المعاملة ونتاجها وإن لم تكن الفروق معنوية ، بينما إرتفع مستوى تركيز جزيئات الجلوبيولين في سيرم دم النعاج المدعومة بالفيتامين وأبنائها مقارنة بمجموعة الكنترول والنتاج المولود لها وإن كان التأثير معنويا عند مستوى ٥ % ، بطول فترة الدراسة واختلاف أسابيع التقدير. أظهرت قياسات نشاط و تركيز إنزيمات الكبد وجود فروق معنويه بين المجاميع و ظهر نفس الإتجاه بين الأبناء المولودة للمجاميع المختلفة مقارنة بانخفاض ملحوظ في نشاط إنزيمات الكبد في سيرم دم نعاج مجموعة المقارنة وأبناءها بالمقارنة مع قرينتها من نعاج ومواليد المعاملات. أنه بناءً على النتائج المتحصل عليها، فإنه يمكن استخلاص أن تقدير بعض المكونات الحيوية للدم ، يمكن الإعتماد عليها كدلائل إرشادية يمكن من خلالها التعرف على الحالة الصحية والإنتاجية للحيوانات الزراعية إلى حد كبير، وأنه بالرغم من أنه لم يثبت في دراسة سابقة (Salama *et al.*, 2015) أي مردود إيجابي لتجريع الأمهات في أخر الحمل وأول الحليب بكبسولات فيتامين (هـ) بمعدل ٤٠٠ وحدة دولية للرأس في اليوم على الكفاءة الإنتاجية للنعاج ونتاجها ، فإن تقدير بعض إنزيمات الدم ومكوناته البيولوجية معمليا ، قد أشارت إلى وجود علاقات وثيقة ومعنوية ما بين تركيزاتها في دم النعاج ونتاجها ، الأمر الذي يمكن من خلاله استخدامها كدلائل إرشادية مبسطة يمكن من خلالها التنبؤ والتعرف على الحالة الصحية والإنتاجية للحيوانات الزراعية.