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The effect of thiamine supplementation in the diet of pregnant ewes on blood components under Upper Egypt conditions

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Abstract

This study aims to identify the effect of adding thiamine to the diets of pregnant ewes before and after birth. Two seasons were conducted to determine the effect of thiamin supplementation (vitamin B1) to the rations of pregnant ewes on some blood components. Feeding trial was conducted using forty pregnant ewes aged 2-4 years and averaged initial body weight of 35.0±0.05 kg /head. Ewes divided into four similar groups (5 for each) using the randomized complete block design for feeding trial. All animals of the experimental groups were fed on ration consisted of concentrate feed mixture (CFM), berseem hay (BH) and wheat straw (WS) at the ratio of 2: 1: 1 on DM basis, respectively. Control and tested treatments (T1), (T2) and (T3) were respectively supplemented by 0, 20, 30 or 45mg/day/head thiamin. Animals were fed according to NRC, (1985) standard for sheep. Blood sample were taken before lambing and monthly after lambing to determined blood components. The diets of ewes were starting to supplemented by thiamin fourteen weeks before lambing and ten weeks after lambing. Results indicated that feeding lactating ewes on 20, 30 or 45 mg/h/d of thiamin blood constituents indicated that the plasma total protein tended to be higher in group 1, 2, 3 compared with control before lambing. In addition, the data show that there was significant increase ($p<0.05$) in plasma total protein after lambing in the first season, in the second season the plasma total protein tended to be higher in group 2, 3 before lambing but there was significant impact ($p<0.05$) in group 1, 2 after lambing .Also, The group fed 30 mg thiamine showed significant increase ($p<0.05$) in plasma albumin before lambing in season one. Moreover, the group feed 20 mg thiamine showed significant increase ($p<0.05$) in plasma albumin in the first month after lambing. In addition, Plasma glucose was found to be higher with the supplementation different levels of thiamine in ewes. Moreover, in second month after lambing there was significant increase ($p<0.05$) in Plasma glucose concentration than control group ($p<0.01$). No significant difference between treatments in GOT, GPT and thyroid hormones (T3 and T4) concentration with slightly increases in its values in the thiamin treatments. It could be concluded that using thiamin as feed additive in ration formulation of pregnant ewes tended to improve productive performances and physiological responses as blood composition. The rate of 45 mg/h/d of thiamin supplements could be performed the best concerning the productive performance.

Keywords:
thiamine, ossimi ewes, pregnant ewes, blood components.

INTRODUCTION

Thiamine, or thiamin, is a kind of vitamin B1, it was the first B vitamin to be identified, which is why the name B1. All B vitamins serve the body to convert carbohydrates into glucose for energy production. B vitamins also help the body to metabolize fats and protein. B complex vitamins are required for healthy skin, hair, eyes, and liver. They also help the nervous system function efficient, and are necessary for optimal brain function. Vitamin B1 is important for healthy mucous membranes, digestion, muscle power, and heart health (Mc Dowell 2000). All B vitamins are water-soluble which means they are not absorbed by the body keep them. Thiamine found in both plants and animals that is essential for certain metabolic pathway. It is required by the body to produce adenosine triphosphate (ATP), which is used for energy by every cell in the body. Thiamine is the coenzyme of pyruvate dehydrogenase (PDH) and α-ketoneglutaric acid dehydrogenase (α-KGDHC) in carbohydrate metabolism (Bubber *et al.,* 2004). Thiamine pyrophosphate (TPP) is a necessary component of thiamine which a universal decarboxylase of -keto acids that plays a key role in energy metabolism in all living beings. TPP plays an important part in the pentose phosphate pathway, which is an important process for the synthesis of nucleic acids, amino acids, steroids, lipids, neurotransmitters, glutathione, and the provision of reduced NADP for numerous synthetic pathways. Thiamine deficiency leads to the accumulation of pyruvate, which is then transformed to lactate by lactate dehydrogenase (Keating, *et al.,* 2015).

MATERIALS AND METHODS

This study was carried out at the Agricultural Experiment Farm, Faculty of Agriculture, South Valley University, in Qunia Governorate in the Upper Egypt located at latitude 26°11'02.4"N and longitude 32°44'23.1"E from February, 2019, to May, 2020. The study aimed to evaluate the effect of addition vitamin B1 (thiamin) to the rations of pregnant ewes in late pregnancy in local sheep in Qena Governorate productive characteristics and some blood.

Experimental animals

A total number of forty healthy pregnant ewes local sheep on Qynia Governorate were divided randomly into four treatments (5 ewes /each), ewes aged 2-4 years and averaged initial body weight of 32.67±0.05 kg. Sheep received daily the basal diet plus thiamin at 0, 20, 30 and 45 mg/day for the control, treatments T1 and T2, T3 respectively. The control diet was, concentrate feed mixture (CFM), berseem hay (BH) and wheat straw (WS) at the ratio of 2: 1: 1 on DM basis, respectively. The animals received their requirements according to NRC, (1985). The chemical composition of feed ingredients and control diet are presented in Table (1).

Table 1 : The chemical analysis of concentrate mixture and wheat straw (on DM basis).

Sample	DM	OM	CP%	CF%	EE%	NFE%	Ash%
CFM	90.43	90.16	16.6	15.49	3.26	54.81	8.84
Wheat straw	87.30	86.34	3.26	36.01	1.42	45.65	10.79
Berseem hay	90.11	89.12	14.44	28.34	2.11	44.23	9.93

Blood sampling and analyses

Blood samples were collected from each ewe monthly. The samples were collected from five animals from each group. The samples were collected at 3 hours after eating. The blood samples were pulled from the jugular vein using 10 ml plastic disposable syringes and isolated in two tubes, approximately 2 ml was transferred to a vacuum tube containing ethylene diamine tetraacetate (EDTA). After blood plasma recovery, metabolites were analyzed using a spectrophotometer. Plasma parameters were analyzed at the Central Laboratory at Faculty of Agriculture, South Valley University, Qunia, Egypt. Plasma concentrations of total protein, albumin, globulin, ALT (SGOT), AST (SGPT), cholesterol, triglycerides, and glucose, were measured using local commercial colorimetric assay kits (Spectrum Diagnostics, Egypt). Absorbance was monitored using a spectrophotometer (the model PG Instruments Limited T80) set at a wavelength of 546 nm for all measurements except albumin which were measured at a wavelength of 623. The plasma globulin (g/dl) was determined by subtracting

concentration of albumin from total protein. And determine the concentrations of triiodothyronine T3 (ng/dl) and thyroxin T4 (ng/dl) hormones using radioimmunoassay technique.

Statistical analysis

The data were tested for normality before the statistical analysis. The data analysis was performed by one-way ANOVA using IBM SPSS Statistics 22 (IBM Corp.). The statistical model was as follows:

$$Yi = \mu + Ti + \varepsilon i,$$

Where Yi is the dependent variable, μ is the overall mean, Ti is the fixed effect of the group (4 groups), and εi is the error term.

Statistical differences between the means of groups were tested using Duncan, 1955 multiple range tests. In addition, the differences were considered significant at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Blood components

Results from Table (2, 3) indicated that thiamine addition to ewes ration was improved the total protein in group 1, 2, 3 compared with control before lambing. In addition, the data show that there was significant increase ($p<0.05$) in plasma total protein after lambing in the first season. in the second season the plasma total protein tended to be higher in group 2, 3 before lambing but there was significant impact ($p<0.05$) in group 1,2 after lambing .This result may be related to the effect of early lactation stage. The drop in total protein in early lactation is attributed to a decrease in globulin levels caused by the fast extraction of plasma immunoglobulins for colostrum production (Antunovic *et al.,* 2004).Similar results noted by Solouma *et al*. (2013). The group fed 30 mg thiamine showed significant increase ($p<0.05$) in plasma albumin before lambing in season one. Moreover, the group T1 showed significant increase ($p<0.05$) in plasma albumin in the first month after lambing but in the second month after lambing the plasma albumin decrease in all treatments. Reduced plasma albumin levels could be caused to albumin loss through the urine as a result of kidney disease or reduced liver production as a result of hepatic impairment (Al-Zuhairy and Al-Hussary, 2010). The plasma albumin was significantly increase ($p<0.05$) before lambing in the second season The Plasma globulin concentration was not affected by thiamine supplementation before lambing in season one .But, after lambing ewes fed 30mg thiamine showed significant increase ($p<0.05$) compared with control animals. Also, in the second season the Plasma globulin concentration was not affected by thiamine supplementation ($p<0.01$). In the present study, the lowest GLU concentration after lambing (1.100±0.239 mg/dL) might be due to the fact that B complex vitamins caused an increase in glucose utilization and catabolism. However, a quite large amount of B complex vitamins was needed for conversion of VFA's into energy Cole *et al*. (1982). Similary, Mostafa *et al*. (2015) observed that globulin concentration did not significantly affect by dietary thiamine in fattening Friesian calves. The data show that in the blood pregnant ewes there was significant decrease in glucose concentration in the first and second season. Low glucose levels are related to fetal growth and the mobilization of maternal glucose into fetal blood circulation in high gestation (Jacob and Vadodaria, 2001).Also, the Plasma glucose concentration was lower in the first month after lambing. Reduced blood glucose levels in suckling ewes must be accounted for as a result of continual energy loss through the milk (Pambu- Gollah *et al*., 2000). Plasma glucose was found to be higher with the supplementation different levels of thiamine in ewes. In addition, in second month after lambing there was substantial increase ($p<0.05$) in Plasma glucose concentration than control group ($p<0.01$). In season one plasma cholesterol was found to be reduced with the increasing level of thiamine in ewes. The most effective level of dietary thiamine was 45mg, such differences were significant ($p<0.05$) before and after lambing and the plasma triglycerides was significant increase before lambing ($p<0.05$) can be explain as a result of increased lipoprotein transport or a meal deficiency in energy Antunovic *et al.* (2011). The same results found by Nazifi *et al.* (2002).), but after lambing Plasma triglycerides was tend to be reduced with the increasing level of thiamine in ewes. The most effective level of dietary thiamine was 45mg .Antunovic *et al.* (2011) found that lower plasma

triglycerides levels in early lactation are related with a higher energy requirements and a negative energy balance. Similar result was noted in the second season. Dietary thiamine supplementation had no significant effect on plasma AST and ALT in both seasons. However, the concentration of plasma ALT was significantly higher ($p<0.05$) in the blood of ewes in late lactation period in the second season.

Table (2): Last square means (LSM±SE) of average blood components of different experimental treatments in season one.

Item		Control	T1	T2	T3	Sig
Before lambing	TP,g/dl	6.286±0.125	6.445±0.597	7.163±0.306	6.425±0.313	0.376
After lambing	1m	5.475±0.377^{b}	5.940±0.320^{a,b}	6.530±0.123^{a,b}	6.554±0.169^{a}	0.034
	2m	5.998±0.142^{a,b}	6.267±0.184^{a}	6.320±0.225^{a}	5.481±0.056^{b}	0.009
Before lambing	Albu ,g/dl	4.552±0.225^{a,b}	3.991±0.087^{b}	5.161±0.198^{a}	4.536±0.152^{a,b}	0.002
After lambing	1m	4.533±0.215^{a,b}	5.157±0.209^{a}	4.362±0.059^{b}	4.726±0.151^{a,b}	0.026
	2m	3.919±0.131	4.111±0.079	3.895±0.063	3.958±0.180	0.606
Before lambing	Globu, g/d	1.733±0.317	2.454±0.607	2.002±0.307	1.888±0.192	0.600
After lambing	1m	1.242±0.220^{c,b}	1.100±0.239^{c}	2.168±0.106^{a}	1.827±0.232^{a,b}	0.002
	2m	2.079±0.238	2.155±0.207	2.425±0.245	.522±0.1962	0.068
Before lambing	Gluc, mg/dl	95.877±3.81^{b}	83.575±1.980^{a}	81.323±2.23^{a}	84.370±1.69^{a}	0.005
After lambing	1m	82.448±3.376	81.224±2.899	84.625±2.523	90.476±2.678	0.152
	2m	96.611±5.841	104.848±1.545	92.272±5.010	95.999±7.047	0.243
Before lambing	Chole, mg/d	186.28±2.13^{c,b}	205.25±3.58^{a}	179.19±4.20^{c}	196.34±4.04^{a,b}	0.001
After lambing	1m	194.63±3.66^{a}	203.09±4.80^{a}	192.78±1.72^{a}	175.05±3.53^{b}	0.000
	2m	192.57±2.88	199.79±5.67	194.23±7.76	190.32±8.03	0.733
Before lambing	Trigly,mg/dl	211.32±1.64^{b}	201.20±4.50^{b}	201.44±7.70^{b}	231.32±0.85^{a}	0.001
After lambing	1m	195.80±2.00	195.00±1.73	195.80±2.00	184.40±6.11	0.090
	2m	217.10±15.46	239.51±22.02	197.58±15.97	195.36±22.28	0.494
Before lambing	ALT	16.586±1.78	15.018±1.52	17.024±1.015	15.716±1.65	0.793
After lambing	1m	12.572±1.01	12.570±1.86	16.414±1.52	12.920±0.889	0.181
	2m	15.014±3.00	13.954±1.46	15.606±1.35	14.316±1.28	0.930
Before lambing	AST	87.656±8.54	98.826±6.46	88.105±6.50	89.030±8.63	0.696
After lambing	1m	77.518±6.54	75.426±2.36	85.196±9.14	71.912±7.34	0.577
	2m	78.738±9.28	74.380±7.18	80.664±5.24	85.904±5.69	0.713
Before lambing	T3, nag/ml	1.134±0.076	1.128±0.229	0.930±0.090	0.984±0.104	0.596
After lambing	1m	1.102±0.085	1.056±0.035	1.080±0.077	1.030±0.073	0.900
	2m	1.190±0.083	1.220±0.227	1.084±0.156	1.480±0.145	0.383
Before lambing	T4, ug/dl	5.934±0.608	6.038±0.366	6.518±0.610	6.288±0.270	0.833
After lambing	1m	6.040±0.511	5.968±0.247	5.596±0.291	5.530±0.331	0.679
	2m	5.688±0.563	5.620±0.448	5.564±0.681	5.846±0.517	0.986

^{a} ,^{b} and ^{c} refer to means within the same row with different superscripts for each factor are significantly different ($p<0.05$). The animals were one of four groups: control, T1=20 mg thiamine, T2 = 30 mg thiamine and T3 = 45 mg thiamin.

Table3: Last square means (LSM±SE) of average blood components of different experimental treatments in season two.

Item		Control	T1	T2	T3	Sig
Before lambing	TP,g/dl	5.809±0.051	5.677±0.150	5.984±0.125	5.907±0.089	0.272
After lambing	1m	7.642±0.177^{a}	7.105±0.277^{a}	7.202±0.116^{a}	6.073±0.292^{b}	0.001
	2m	7.199±0.192	6.997±0.258	7.515±0.256	7.529±0.301	0.409
Before lambing	Albu ,g/dl	3.868±0.212^{b}	4.751±0.267^{a,b}	5.021±0.268^{a}	4.847±0.249^{a,b}	0.022
After lambing	1m	5.323±0.116	4.891±0.207	5.124±0.127	4.801±0.261	0.236
	2m	4.867±0.260	4.819±0.236	5.320±0.181	5.183±0.406	0.546
Before lambing	Globu, g/d	1.941±0.239	1.326±0.275	1.262±0.344	1.060±0.301	0.079
After lambing	1m	2.318±0.136	2.213±0.350	2.077±0.195	1.272±0.337	0.057
	2m	2.332±0.411	2.178±0.251	2.194±0.220	2.345±0.474	0.979
Before lambing	Gluc, mg/dl	82.90±3.71	79.15±3.63	83.63±3.71	85.69±1.04	0.558
After lambing	1m	119.55±18.66	135.20±11.84	128.12±9.38	118.13±13.70	0.802
	2m	94.68±1.96^{b}	97.80±1.84^{b}	102.58±1.63^{a,b}	115.26±6.24^{a}	0.004
Before lambing	Chole, mg/d	199.3±21.19	231.47±6.49	190.38±14.72	243.9±14.95	0.074
After lambing	1m	190.57±4.22^{b}	200.91±10.08^{a,b}	200.91±10.8^{a,b}	223.90±3.43^{a}	0.053
	2m	188.11±5.31	174.20±5.29	190.51±4.66	184.75±3.90	0.125
Before lambing	Trigly,mg/dl	151.17±16.26	138.82±14.19	145.68±7.24	130.39±8.86	0.662
After lambing	1m	190.40±1.89	191.63±2.58	193.46±4.21	184.08±3.26	0.204
	2m	202.77±1.65^{a}	191.45±4.51^{a,b}	193.08±5.61^{a,b}	184.07±4.30^{b}	0.050
Before lambing	ALT	13.970±1.65	15.714±1.23	14.666±0.890	13.270±1.18	0.581
After lambing	1m	14.666±0.890	14.316±2.02	15.018±1.79	16.856±2.16	0.280
	2m	8.292±0.338^{b}	11.350±2.02^{a,b}	12.222±1.99^{a,b}	16.100±2.05^{a}	0.046
Before lambing	AST	91.490±6.03	100.228±7.29	101.262±8.17	73.230±14.74	0.187
After lambing	1m	101.314±6.26	140.366±31.86	134.866±20.68	117.690±20.19	0.587
	2m	98.080±6.41	100.420±6.95	118.020±9.66	115.260±16.94	0.474
Before lambing	T3, nag/ml	0.798±0.097	0.898±0.078	0.958±0.124	0.944±0.0977	0.398
After lambing	1m	1.196±0.203	1.026±0.172	1.030±0.151	1.106±0.102	0.864
	2m	1.258±0.101	1.144±0.124	1.098±0.094	1.008±0.094	0.423
Before lambing	T4, ug/dl	4.494±0.856	4.816±0.326	4.686±0.535	4.618±0.277	0.980
After lambing	1m	5.802±0.122	6.814±0.838	6.424±0.461	5.874±0.369	0.482
	2m	5.406±0.431	6.152±0.660	5.184±0.541	5.110±0.856	0.655

^{a} ,^{b} and ^{c} refer to means within the same row with different superscripts for each factor are significantly different ($p<0.05$). The animals were one of four groups: control, T1=20 mg thiamine, T2 = 30 mg thiamine and T3 = 45 mg thiamine.

The increase in ALT activity, in the blood of ewes in lactation indicated an increase in hepatic metabolism. Radostits *et al.* (2007) reported that a possible cause for this condition may be that B complex vitamins cause an increase in liver and kidney metabolism. In fact, B complex vitamins stimulate fat and protein metabolism. Plasma T3 and T4 did not significantly affected by dietary treatment of different levels of thiamine in both seasons. However, the data showed Lower blood T3 concentration in pregnant ewes compared with lactating ewes. Lower levels of plasma T3 could reduce the rate of oxidation and the rate of continuous breakdown and formation of protein and fat in the most, if not all mammary tissue (Riis and Madsen, 1985). Also, Flis and Molik (2021) showed that T3 output decreases as milk yield increases, while T4 output increases as lactation continues. Thyroid hormones, as a result, have an effect on sheep milk secretion. These results are in agreement with those obtained by Capplli *et al.* (1990) found that Small differences in thyroid hormone plasma levels were found in male Wistar rats. The non-significant effect of thiamin supplementation on thyroid hormones obtained in the present are in agreement with those noted by Farahat *et al*. (2007) and El-Shanti *et al.* (2012) who tested difference levels of thiamin addition with lambs' rations.

In conclusion, this study shows that thiamine supplementation was improved blood metabolites of local sheep under the hot climate conditions in Upper Egypt and the study recommends using 45 mg/day of thiamine, which led to an improvement in blood components and improved all the studied traits.

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الملخص العربي

تأثير اضافة الثيامين في عليقة النعاج الحوامل على مكونات الدم تحت ظروف مصر العليا

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تهدف هذه الدراسة إلى التعرف على تأثير إضافة الثايمين في عليقة النعاج الحوامل قبل الولادة وبعدها. تم إجراء التجربة فى موسمين لتحديد تأثير اضافة الثيامين (فيتامين ب 1) فى علائق النعاج الحامل على بعض مكونات الدم. أجريت تجربة باستخدام اربعين نعجة حامل تتراوح أعمارهم بين 2-4 سنوات وبلغ متوسط وزن الجسم الحى 0.05 ± 35.0 كجم / رأس. تم تقسيم النعاج إلى أربع مجموعات متشابهة (10 لكل مجموعة) باستخدام تصميم القطاعات العشوائية الكاملة لتجربة التغذية. تم تغذية جميع حيوانات المجموعات التجريبية على علف مكون من خليط علف مركز (CFM) ، دريس البرسيم (BH) وتبن القمح (WS) بنسبة 2: 1: 1 على أساس DM ، على التوالي. وتمت التغية فى 4 معاملات هى الكنترول و (T1) و (T2) و (T3) على التوالي بـ 0 أو 20 أو 30 أو 45 ملغ / يوم / رأس ثيامين. تم تغذية الحيوانات وفقًا لمقررات (NRC1985) للأغنام. تم أخذ عينة الدم قبل الحمل وشهرية بعد الحمل من خمسة حيوانات فى كل مجموعة لتحديد مكونات الدم. تم اضافة الثيامين الى علائق النعاج قبل أربعة عشر أسبوعًا من الحمل وعشرة أسابيع بعد الحمل. ويمكن تلخيص النتائج المتحصل عليها إلى أن تغذية النعاج المرضعة على 20 أو 30 أو 45 ملجم / يوم من مكونات الدم بالثيامين أشارت إلى أن البروتين الكلي للبلازما كان أعلى في المجموعة 1 ، 2 ، 3 مقارنة مع مجموعةالكنترول قبل الحمل. بالإضافة إلى ذلك ، أظهرت البيانات أن هناك زيادة معنوية (P <0.05) في البروتين الكلي للبلازما بعد الحمل في الموسم الأول اما في الموسم الثاني كان البروتين الكلي للبلازما كان أعلى في المجموعة 2 ، 3 قبل الحمل ولكن كان هناك تاثير معنوي.(p<0.05) في المجموعة 1 ، 2 بعد الحمل ، كما أظهرت المجموعة التي تناولت 30 مجم ثيامين زيادة معنوية (p<0.05) في ألبومين البلازما قبل الحمل في الموسم الأول.علاوة على ذلك ، أظهرت المجموعة التي تتغذى 20 مجم ثيامين زيادة معنوية (p<0.05) في ألبومين البلازما في الشهر الأول بعد الحمل. بالإضافة إلى ذلك ، وجد أن نسبة الجلوكوز في البلازما أعلى مع إضافة مستويات مختلفة من الثيامين في النعاج. علاوة على ذلك ، في الشهر الثاني بعد الحمل كان هناك زيادة معنوية (p<0.05) في تركيز الجلوكوز في البلازما من مجموعة التحكم(p<0.01). لا يوجد فرق كبير بين العلاجات في GOT و GPT وتركيز هرمونات الغدة الدرقية (T3 و T4) مع زيادة طفيفة في قيمها في علاجات الثيامين. ويمكن التوصية بأستخدام الثيامين كمادة مضافة للأعلاف في علائق للنعاج الحامل يؤدى الى تحسين الأداء الإنتاجي والاستجابات الفسيولوجية مثل مكونات الدم. ومن النتائج توصى الدراسة باستخدام معدل 45 مجم / يوم من الثيامين قد ادى الى تحسين الاداء الانتاجى ومكونات الدم وتحسنت جميع الصفات المدروسة .