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Effect Of Vêo® Premium and Vitamin C Supplementation on Lipid Profile Before and During Pregnancy in Some Local Iraqi Ewes During Heat Stress

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Abstract

The present study was designed to investigate the effects of VêO® premium and vitamin C (Vit. C) supplementation on lipid profile before and during pregnancy in local Iraqi ewes during heat stress. The study was conducted in a private field in Fallujah city, west Iraq, during the period from 6/8/2019 to 8/2/2020. Twenty local Iraqi ewes were divided randomly into four equal groups (5 for each group). The first group (T_1) was given VêO® premium (4 g/ ewe/ day), the second group (T_2) was given VêO® premium (2 g/ ewe/ day), and the third group (T_3) was given Vit. C (40 mg/ ewe/ day), and the fourth group (T_4) was left without treatment, as a control group. After 14 days of treatment, vaginal sponges were inserted for 14 days and, after their withdrawal, the rams were introduced for 6 days. Blood samples were collected from the jugular vein into vacutainer tubes (10 ml) at 4 periods (periods 1 and 2 before pregnancy; periods 3 and 4 during pregnancy). Lipid profile parameters were also estimated, which includes cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and malondialdehyde (MDA). There were no significant difference in the serum levels of cholesterol, triglyceride, HDL, and LDL among the various treatments before and during pregnancy. The level of VLDL was significantly higher in T2 group as compared to the other treatments ($P \leq 0.05$) at the fourth periods. There was a significant difference in the level of MDA ($P \leq 0.05$) in treatment T3 in the first period compared with the treatments T1, T2, and T4, whereas treatment T4 showed significantly higher level ($P \leq 0.05$) in the second, third, and fourth periods as compared to treatments T1, T2, and T3.

In conclusion, different effects of VêO® premium and Vitamin C supplementation were noticed on the lipid profile before and during pregnancy in local Iraqi ewes during heat stress.

Keywords: VêO® premium, Vitamin C, lipid profile, pregnancy, heat stress, local Iraqi ewes

تأثير إضافة الفيوبريميوم وفيتامين C على مستوى الدهون قبل وخلال الحمل في النعاج العراقية المحلية خلال الإجهاد الحراري

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الخلاصة

صممت التجربة لمعرفة تأثير إضافة الفيوبريميوم وفيتامين C على مستوى الدهون قبل وخلال الحمل في النعاج العراقية المحلية. أجريت التجربة في مدينة الفلوجة للمدة من 6/ 8/ 2019 إلى 8/ 2/ 2020، استخدمت 20 نعجة قسمت عشوائياً إلى أربع مجاميع متساوية لكل مجموعة 5 نعاج المجموعة الأولى أعطيت الفيوبريميوم 4 غم/ نعجة/ يوم، المجموعة الثانية أعطيت 2 غم/ نعجة/ يوم، المجموعة الثالثة أعطيت 40 ملغم/ نعجة/ يوم، المجموعة الرابعة اعتبرت مجموعة سيطرة. بعد 14 يوم من وضع الإسفنجات المهبلية تم رفعها وإدخال الكباش لمدة 6 أيام. جمعت عينات الدم (10 مل) عن طريق الوريد الوداجي بواسطة أنبوبة خالية من مانع التخثر (الفترة 1 و2 قبل الحمل) و (3 و4 خلال مدة الحمل). تم قياس مستوى الدهون لكل من: الكوليسترول، الدهون الثلاثية، البروتين الدهني مرتفع الكثافة، البروتين الدهني منخفض الكثافة، بروتين منخفض الكثافة جدا، المالون داي الديهايد. بينت النتائج عدم وجود فروق معنوية في الكوليسترول، والدهون الثلاثية، والبروتين الدهني مرتفع الكثافة، والبروتين الدهني منخفضة الكثافة بين المعاملات قبل وخلال فترة الحمل. لوحظ ارتفاع في البروتين منخفض الكثافة جدا في المعاملة الثانية خلال المدة الرابعة. وجد ارتفاع معنوي في مستوى المالون داي الديهايد في المعاملة الثالثة ($P\leq0.05$) في المدة الأولى مقارنة بالمعاملة الأولى والثانية والرابعة، بينما تفوقت المعاملة الرابعة بصورة معنوية ($P\leq0.05$) في المدة الثانية والثالثة والرابعة على بقية المعاملات الأولى والثانية والثالثة مقارنة بالمعاملة الأولى والثانية والثالثة.

نستنتج من الدراسة تأثير إضافة الفيوبريميوم وفيتامين C الملاحظ على مستوى الدهون قبل وخلال الحمل في النعاج العراقية المحلية خلال الإجهاد الحراري.

Introduction

Heat stress is the main factor that negatively affects livestock production. It is important to identify and select animals that tolerate heat and adapt to climate change [1].

Several major problems are caused by the high temperature of the sheep environment, including the lack of appetite that results in a decrease in the amount of feed consumed. This leads to a lack of the necessary nutrients, which subsequently causes a weakness in growth as well as a decrease in production and reproductive performance [2].

Lipids are considered as hydrophobic and soluble in organic solvents. Their most important functions include energy storage, signaling, and building the structure of cell membranes. They are absorbed by the small intestine and emulsified by bile salts, while they are made from cholesterol in the liver and stored in the gallbladder [3].

Exposure to heat stress reduces total lipid and cholesterol concentrations, with a decrease in the level of blood hormones, such as growth hormone, insulin, thyroxine and aldosterone. This is associated with increases in respiratory rate and rectal and skin temperature [4, 5]. High temperature leads to an increase in the formation of free radicals of reactive oxygen species that cause oxidative damage to cells. These effects are caused by producing lipid peroxide, followed by oxidative damage to the proteins and the genetic material [6]. Lipids in the diet positively affect concentrations of cholesterol, progesterone as well as the rate of synthesis and metabolism of PGF2 α and the rates of follicle growth and pregnancy [7].

Recently, a feed additive, VêO® premium, has been produced. The purpose of using such a product is to stimulate the animal's appetite, increase the voluntary consumption of the animal, promote growth at times, stimulate the digestive processes, and increase the body's immunity. It also acts as an antioxidant and reduces fat oxidation. Adding vitamin C has a role in enhancing the functioning of the immune system and maintaining animal health. It is one of the vitamins that the body cannot manufacture, and therefore it must be taken daily from its various sources [8].

Therefore, the aim of the present study was to assess the effects of VêO® premium and Vit. C supplementation on lipid profile before and during pregnancy in local Iraqi ewes during heat stress.

Materials and Methods

The study was conducted in a private field at the College of Veterinary Medicine/ University of Fallujah, Fallujah city, West Iraq, during the period from 6/8/2019 to 8/2/2020.

Heat stress was known from the environment surrounding the animals and was measured by a special thermometer inside the field.

Twenty local Iraqi ewes aged 2-4 years were included, with body weight between 35 and 60 kg. The ewes were divided randomly into four equal groups (5 for each). The first group (T_1) was given VêO® premium (4 g/ ewe/ day), the second group (T_2) was given VêO® premium (2 g/ ewe/ day), the third group (T_3) was given Vit. C (40 mg/ ewe/ day), and the fourth group (T_4) left was without treatment, as a control group. VêO® premium (Phodé, France) ingredients included orange sweet (Vit. C), wheat flour, calcium carbonate, silicon dioxide, and Vit. E. After 14 days of treatment, vaginal sponges were inserted for 14 days and, after their withdrawal, the rams were introduced for 6 days. Blood samples were collected from the jugular vein into vacutainer tubes (10 ml) at 4 different periods (Periods 1 and 2 before pregnancy; period 3 and 4 during pregnancy). The serum was collected after centrifugation of the blood samples (3000 RPM for 15 minutes) and stored under -20°C until the time of the assays. Lipid profile measurements included the following:

Cholesterol concentrations were quantitatively determined using the kit provided by Agappe Diagnostice Company, Switzerland [9]. Triglycerides [10] and HDL [11]were measured by a kit provided by Biomerieux Company, France. LDL was calculated by the equation LDL= cholesterol-HDL+ vLDL. VLDL was calculated by dividing the level of triglycerides by 5 [12]. Used the method of (Witte *et al*., 1970) [13] to estimate Malondialdehyde (MDA) in the blood serum. Statistical analysis was performed using the General Linear Model (GLM) procedure in the SPSS program (version 25) to examine the influence of groups on the tested parameters. Differences among means were compared using the Least Significant Differences (LSD) test according to Steel and Torrie [14].

Results and Discussion

- Effects of VêO® premium and Vit. C supplementation on cholesterol level

The effects of VêO® Premium and Vit. C on cholesterol level in the blood of treated ewes are illustrated in Table-1. There was no significant difference in total cholesterol concentration between the treatments before and during pregnancy. However, a significant difference ($P \leq 0.05$) was observed between in treatment group and the other treatments.

Table 1- Effects of VêO® premium and vitamin C supplementation on serum cholesterol level (mg/dl) before and during pregnancy in local Iraqi ewes

Treatment	Periods			
	Before pregnancy		During pregnancy	
	1	2	3	4
T1	84.89 ± 3.17 b	93.96 ± 2.92 ab	100.85 ± 5.85 a	104.85 ± 8.32 a
T2	91.85 ± 0.83 b	92.82 ± 5.90 b	100.45 ± 2.93 ab	109.19 ± 6.08 a
T3	84.14 ± 4.47 b	96.05 ± 4.35 ab	98.84 ± 2.49 a	104.49 ± 6.00 a
T4	79.62 ± 3.29 b	85.33 ± 2.86 b	102.36 ± 3.36 a	101.41 ± 4.17 a

*The different small letters refer to significant differences between different periods (raw) at ($P \leq 0.05$)

Heat stress lowers cholesterol levels, due to the increased degradation of fatty acids to produce energy due to a decrease in the concentration of glucose in the blood [15]. The decrease in cholesterol concentration is caused by its dilution due to an increase in total body water or a decrease in the concentration of acetate, which is the primary raw material for cholesterol synthesis. An increase in the level of the glucocorticoid hormone in heat-stressed animals causes low cholesterol in the blood [16, 17].

Cholesterol was reported to be transferred to the ovarian follicles for the synthesis of steroid hormones through some genetic expressions [18]. Synthesis of progesterone occurs in all steroidogenic organs, including the placenta, by the transfer of cholesterol from the outer mitochondrial membrane to the inner membrane, where it is converted into pregnenolone by the side-chain cleavage of the enzyme cytochrome P-450 [19]. Reactive oxygen species inhibit steroidogenesis by blocking cholesterol transport into mitochondria of luteal cells [20]. The administration of vit. C increases progesterone and oestradiol synthesis, mediated by enhanced expression of the side-chain cleavage of the steroidogenic enzymes P450 cholesterol, 3ß-hydroxysteroid dehydrogenase type 1, and aromatase [21]. Yusuf *et al.* [22] reported that supplemented vit. C with a dose of 600 mg/ kg increased cholesterol concentration in the serum of goats. Supplemention of vit. E leads to an increase in the levels of cholesterol and androgenic and estrogenic hormones [23, 24]. The increased concentration of vitamin E in the corpus luteum due to cholesterol absorption during steroid synthesis. [25]. Calcium, which is a component of VeO® Premium, stimulates steroidogenesis by enhancing the transfer of cholesterol and the conversion of pregnenolone to progesterone [26, 27].

- **Effects of VêO® premium and Vit. C supplementation on triglyceride level**

The effects of VêO® Premium and Vit. C on Triglycerides are shown in Table-2. There was no significant difference in triglyceride levels between the treatments before and during pregnancy.

Table 2- Effects of VêO® premium and Vit. C supplementation on serum triglyceride levels (mg/dl) before and during pregnancy in local Iraqi ewes

Treatment	Periods			
	Before pregnancy		During pregnancy	
	1	2	3	4
T1	86.35 ± 1.98	72.28 ± 2.68	81.16 ± 3.18	79.36 ± 3.44
T2	85.29 ± 3.03	82.96 ± 6.45	84.27 ± 4.55	90.14 ± 7.36
T3	79.17 ± 1.09	79.64 ± 1.43	80.62 ± 2.90	83.38 ± 7.96
T4	78.70 ± 3.26	76.91 ± 0.69	76.77 ± 2.39	73.93 ± 1.93

Heat stress affects the decrease in triglycerides level during the period of estrus cycle, which is clearer in the luteal phase due to the acceleration in body fat catabolism and lipid mobilization [28, 29]. Reduced levels of vit. C result in the accumulation of triglycerides in the blood [30]

- **Effects of VêO® premium and Vit. C supplementation on HDL** level

The effects of VêO® Premium and Vit. C on serum HDL levels are presented in Table-3. There was no significant difference in HDL concentration between the treatments before and during pregnancy. However, a significant difference (P≤0.05) was observed between in treatment group.

Table 3- Effects of VêO® premium and Vit, C supplementation on serum HDL (mg/dl) levels before and during pregnancy in local Iraqi ewes

Treatment	Periods			
	Before pregnancy		During pregnancy	
	1	2	3	4
T1	45.82 ± 1.79 b	50.85 ± 3.28 b	55.50 ± 4.09 b	68.80 ± 12.09 a
T2	45.90 ± 1.61 b	55.23 ± 4.43 ab	57.74 ± 2.30 ab	67.18 ± 4.20 a
T3	47.30 ± 4.08 b	61.33 ± 4.44 ab	59.18 ± 4.16 ab	66.56 ± 5.55 a
T4	41.82 ± 2.53 c	49.67 ± 4.51 bc	60.27 ± 1.64 ab	66.16 ± 3.77 a

*The different small letters refer to significant differences between different periods (raw) at (P≤0.05)

The damage in biological macromolecules due to the accumulation of ROS, which is an indication of oxidative stress, results in differences in the level HDL [31].

An increase in vitamin E increases HDL by increasing the steroidogenic activity of the corpus luteum [32, 33].

- **Effects of VêO® premium and Vit. C supplementation on LDL** level

The effects of VêO® Premium and Vit. C on serum LDL levels are shown in Table-4. There was no significant difference in LDL levels between the treatments before and during pregnancy, and no significant difference (P≤0.05) was observed between one treatment, but there are statistical differences between the treatments

Table 4- The effects of VêO® premium and Vit C supplementation on serum LDL levels (mg/dl) before and during pregnancy in local Iraqi ewes

Treatment	Periods			
	Before pregnancy		During pregnancy	
	1	2	3	4
T1	21.81 ± 1.53	28.66 ± 2.24	29.15 ± 2.69	19.83 ± 4.74
T2	28.90 ± 1.84	20.99 ± 5.30	25.86 ± 2.43	23.98 ± 3.35
T3	21.00 ± 3.38	18.80 ± 3.43	23.54 ± 4.47	21.26 ± 2.17
T4	22.06 ± 2.23	20.41 ± 1.93	26.74 ± 2.14	20.47 ± 2.04

The synthesis of steroid hormones occurs on the smooth endoplasmic reticulum and in the adrenal mitochondria. Cholesterol is the precursor of all steroid hormones and is present as LDL in the plasma [34].

Vit. E, which is one of the components of VêO® Premium, is released from the liver with LDL, and it maintains the protection from LDL oxidation in cooperation with Vit. C and other substances [23, 35, 36].

- **Effects of VêO® premium and Vit. C supplementation on VLDL level**

No significant differences were noticed in serum VLDL levels over the study periods. . However, the level in T2 group was significantly higher compared with the other treatment (P≤0.05) during the fourth periods (Table-5).

Table 5- The effects of VêO® premium and Vit, C supplementation on serum VLDL level (mg/dl) before and during pregnancy in local Iraqi ewes

Treatment	Periods			
	Before pregnancy		During pregnancy	
	1	2	3	4
T1	17.27 ± 0.40	14.45 ± 0.53	16.20 ± 0.63	16.23 ± 0.44 AB
T2	17.05 ± 0.61	16.59 ± 1.29	16.85 ± 0.91	18.02 ± 1.47 A
T3	15.83 ± 0.22	15.92 ± 0.29	16.12 ± 0.58	16.67 ± 1.59 AB
T4	15.74 ± 0.65	15.38 ± 0.14	15.35 ± 0.48	14.78 ± 0.39 B

*The different capital letters refer to significant differences between different treated groups (column) at (P≤0.05)

Higher levels of VLDL are associated with increased estrogen levels. Heat stress causes the accumulation of free radicals, which in turn damages the biological molecules, leading to an increase the level of HDA [31, 37, 38]. Vitamin E works consistently with metabolic functions. The function of vit. E is to protect cells from oxidative stress by converting oxygen of water and fat into non-interactive situations [39].

Vit. E prevents the oxidation in the membranes that are under cellular oxidative damage to lipid by reducing the formation of hydrogen peroxide, thus maintaining membrane integrity and reducing oxidative stress [40].

- **Effects of VêO® premium and Vit. C supplementation on MDA** level

Table-6 shows that there was a significant difference (P≤0.05) in the serum level of MDA in treatment T3 in the first period compared with the treatments T1, T2, and T4. Whereas, treatment T4 showed a significantly higher level (P≤0.05) in the second, third, and fourth periods of the experiment as compared to treatments T1, T2, and T3.

Table 6- The effects of VêO® premium and Vit C supplementation on serum MDA levels (mmol/ l) before and during pregnancy in local Iraqi ewes.

Treatment	Periods			
	Before pregnancy		During pregnancy	
	1	2	3	4
T1	0.052 ± 0.006 C	0.096 ± 0.002 B	0.070 ± 0.002 B	0.023 ± 0.005 B
T2	0.062 ± 0.006 BC	0.081 ± 0.014 B	0.036 ± 0.003 B	0.049 ± 0.011 B
T3	0.208 ± 0.090 A	0.125 ± 0.018 AB	0.026 ± 0.006 B	0.018 ± 0.002 B
T4	0.125 ± 0.027 B	0.169 ± 0.011 A	0.252 ± 0.014 A	0.250 ± 0.045 A

*The different capital letters refer to significant differences between different treated groups (column) at (P≤0.05)

Malondialdehyde is a biomarker of lipid peroxidation and protein damage. MDA is a decomposition product of peroxidized polyunsaturated fatty acids [41, 42]. MDA level increases during the estrous cycle and in pregnant animals during the period of oxidative stress, especially during steroidogenesis due to the high metabolism of the placenta and the formation of steroids [29, 43]. The decrease in the levels of antioxidant vitamins in the plasma and the increase in MDA concentration during reproduction are results of steroid formation and the cyclical activities of the ovaries. Thus, reducing antioxidant vitamins can cause lipid oxidation in red blood cells, which leads to higher MDA [44]. The beginning of cyclic ovarian activity during the reproductive season and early pregnancy leads to oxidative stress and thus an increased demand for antioxidant vitamins in genital tissues [45]. Antioxidants reduce MDA and between sugars and proteins to form glycoproteins.
Vit. C inhibits the process of protein glycation and prevents the binding of glycosylation of proteins, thus lowering the level of MDA [22].
In conclusion, different effects VêO® premium and Vitamin C supplementation were noticed on some lipid profile parameters before and during pregnancy in local Iraqi ewes exposed to heat stress.

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