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STUDY ON LEVELS OF SOME BLOOD HORMONAL AND BIOCHEMICAL CONSTITUENTS DURING DIFFERENT REPRODUCTIVE STATUS IN SAIDI EWES

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ABSTRACT

The present study was conducted on 30 healthy Saidi ewes, aged 3- 10 years old, located in Malawi Research Station, APRI, Ministry of Agriculture. Blood samples were collected from ewes one month before mating (pre- mating) and from 15 ewes, out of the thirty, one month before parturition (late pregnancy) and one month after parturition (post-partum/suckling). Serum analysis was carried out to determine the influence of the reproductive status on serum hormonal levels and some blood biochemical constituents. Results showed that concentrations of serum thyroid hormones (T3 and T4) were significantly lower in ewes at the last month of pregnancy comparing with those in pre-mating and postpartum/suckling. While, significant high insulin level was recorded in pre-mating ewes than that in late pregnancy and postpartum/suckling ewes. Serum Ca, Fe and Zn concentrations were significantly affected by ewes' reproductive status. Calcium levels decreased significantly in ewes during late pregnancy and postpartum/suckling. Serum Fe concentration was higher in late pregnancy and postpartum/suckling than in pre-mating. Zn concentration was significantly low in ewes at late pregnancy and postpartum/suckling than in pre-mated ewes. Meanwhile, serum inorganic- P, K, Na and Mg levels were not affected by ewe reproductive status. In addition, the concentrations of serum total protein decreased in ewes at late pregnancy and postpartum/suckling. Also, the results showed a significant decrease in glucose level in late pregnancy.

In conclusion, monitoring the concentration of thyroid hormones, insulin and some biochemical parameters in the blood of ewes at different physiological status gives basis for the regular therapy application and carrying out the prophylactic of metabolic disturbances of ewes in the aim of reducing economy losses.

Key words: Ewes, reproductive status, thyroid hormones, insulin, minerals.

INTRODUCTION

The physiological status of the animal is one of the important factors which affects the concentrations of blood indicators that are involved in development of blood metabolic profile **(Antunovic *et al*., 2002; Roubies *et al*., 2006),** which used in assessing nutritional status and animal health **(Herdt *et al*., 2000; Antunovic *et al*., 2009)**. Moreover, for maintenance of normal health and sustaining efficient production of livestock, it is necessary to ensure adequate dietary intake of essential nutrients. In sheep, nutrients quality and quantity directly affect the high demand of reproductive functions such as expression of estrus; embryo implantation and reduction of spermatogenesis, as well as, its direct effect on animal health **(Vazquez- Armijo *et al*., 2011).** Parturition and early lactation are considered as the most critical and stressful periods of dam's life cycle because of high nutritional requirements for fetus, colostrum and milk production **(Goff and Horst, 1997; Sobiech *et al*., 2008)** where the needs for energy and minerals for milk synthesis are increased. Mineral metabolism, in particular calcium and phosphorus, undergoes a substantial change to guarantee colostrum and milk synthesis **(Yokus *et al*., 2004).** Metabolic changes during different physiological status mainly controlled

by some hormones, which cause the activation of these specific metabolic organs according to their needs **(Hatfield *et al*., 1999).** Thyroid hormones are considered to be an important indicator to their metabolic and nutritional status **(Riis and Madsen, 1985; Todini, 2007).** In addition, they maintain the homeostasis of energy and protein metabolism, thermoregulation, growth and productivity parameters **(Huszenicza *et al*., 2002).**

Saidi sheep is the oldest Egyptian breed located in Upper Egypt with fatty tail and coarse wool. They are raised mainly for lamb production in addition to wool as a secondary product. The demand for this breed increases due to its high conception rate (82- 92%, **El-Hommosi and Abdel- Hafiz, 1982**) and twining rate (1.5%; **Galal, 1987)**. Information about their blood thyroid hormones, insulin, as well as, some biochemical constituents during different reproductive stage is very important to guarantee the metabolic and nutritional needs of ewes and to reduce the mortality rates of newborns and consequently economic loss. So, the aim of this work is to determine the influence of reproductive status on these blood parameters in Saidi ewes.

MATERIALS AND METHODS

This study was carried out on 30 healthy Saidi ewes, located in Malawi Research Station, Animal Production Research Institute (APRI), Ministry of Agriculture. Ewes aged 3-10 years and of body weight ranged 30- 40 Kg. They were fed according to their physiological status **(NRC, 1985)**. Water and mineral blocks were available all the time. Ewes were naturally mated during January 2014 and watched over from mating until parturition.

Blood samples were collected from the jugular vein of the thirty ewes one month before mating (pre- mating). Then, blood samples were collected from 15 randomly selected pregnant ewes out of the thirty one month before parturition (late pregnancy) and one month after parturition (postpartum/ suckling). Serum samples were separated and stored at –20 ºC until analysis.

Quantitative determination of serum Triiodothyronine (T3), thyroxin (T4) were carried out using radioimmunoassay kits (Beckman Coulter- Czech Republic, catalog No. IM 1447 and IM3286, respectively). The assay based on competition reaction **(Meizger, 1992)** with sensitivity 0.2 ng/ml and 10.1 ng/ml for T3 and T4, respectively. The concentrations were detected by using automatic Mini-Gama counter (LKB 1275, USA).

Quantitative determinations of serum thyroid stimulating hormone (TSH) and insulin were carried out using Immunospec enzyme immune assay kits**,** catalog No. E29- 227 and E29- 072, respectively. The assay based on a solid phase ezyme- linked immunosorbent assay **(Wada *et al*., 1982 and Eastham, 1985**, respectively)

Concentrations of calcium (Ca), inorganic phosphorus (P_i), sodium (Na), magnesium (Mg), iron (Fe) and zinc (Zn) were detected in serum samples using colorimetric method (Bio-diagnostic kit) according to the procedure outlined by the manufacture. Meanwhile, potassium (K) levels were detected using turbidimetric method according to **Sunderman Jr. and Sunderman (1958).**

Total serum protein, albumin and glucose levels were measured colorimetrically by using Bio- diagnostic kit. Globulin levels were calculated by subtracting albumin from total protein levels.

The data were statistically analyzed using general linear models **(SAS, 2002)**. The significant differences between fixed items were tested using **Duncan (1955)**. Results were expressed as least square means (LSM± SE).

RESULTS AND DISCUSSION

Mean values of serum T3, T4, TSH and insulin levels in Saidi ewes at different reproductive status presented in table (1). Serum T3 and T4 levels were significantly lower ($P< 0.001$) at the last month of pregnancy (0.7± 0.16 and 20.99± 5.8 ng/ml, respectively) compared to those of pre-mating (1.9±0.16 and 57.8±4.9 ng/ml, respectively). At postpartum/suckling T4 was also significantly decreased (34.96±4.9 ng/ml). Meanwhile, no significant changes were observed for TSH level. In agreement with our results **Khaled and Illek (2012)** reported that serum T3 and T4

in Barki ewes were significantly declined in the last month of pregnancy and postpartum. They suggested that the decrease in the thyroid hormones around parturition is due to alterations in cardiac output and increase of blood volume, as reported by **Illek *et al*. (1998) and Dalvi *et al*. (1995).** In goat, **Suganya and Gomathy (2009)** reported a decline in serum T3 and T4 concentrations prior to kidding reached its lowest levels at kidding, then followed by an increase till 15 days postpartum. Similar results were reported by **Eswari *et al*. (1999)** in sheep. **Colodel *et al*. (2010)** suggested that the lower concentrations of T3 and T4 observed during gestation in comparing with non- pregnant ewes could be related to the passage of thyroid hormones through the placenta, since the ovine thyroid becomes functional only between the 6^{th} and 8^{th} weeks of embryonic life. Moreover, **Escobar (2001)** stated that the mother is the only source of T3 and T4 up to the moment that the thyroid tissue becomes active in the fetus and plays its role in organogenesis and in the development of placenta. Meanwhile, **Okab *et al*. (1993)** reported that plasma T3 and T4 levels of sheep were lower during postpartum – suckling period with respect to gestation period.

Significant increase in insulin concentration recorded in pre-mating ewes (10.7±0.6 µIU/ml) than that in late pregnancy (6.7± 0.6 µIU/ml) and postpartum/suckling (7.2± 0.6 µIU/ml). Our results agree with that of **Khan and Ludri (2002) and Suganya and Gomathy (2009)** who reported a decrease in insulin levels in goats during gestation till kidding compared to non–pregnant one, which remained low till 10 days postpartum then increased. **Vernon *et al*. (1981)** stated that the fall in insulin levels is associated with a concomitant decrease in the insulin receptors of the adiposities which is responsible for mobilization during late pregnancy. While, during postpartum period hypoinsulinemia is attributed to the continued mobilization during lactation as insulin is being removed by the mammary gland **(Williamson, 1980)**. In contrast, **Antunovic *et al*. (2011)** reported significant high concentrations of insulin in pregnant ewes in relation to not pregnant.

Results in table (2) show the mean serum levels of some biochemical constituents in Saidi ewes at different reproductive status. Calcium level decreased significantly ($P< 0.001$) in ewes at late pregnancy and postpartum/suckling (10.6± 0.34 and 12.04± 0.34 mg/dl, respectively) vs. (13.3± 0.34 mg/dl) at pre-mating. Over the pregnancy the needs for Ca are increased in parallel with the increase of Ca absorption in the intestines **(Yano et al., 1991). Baumgartner and Pernthaner (1994)** reported lower Ca levels in pregnant ewes than in lactating ones. **Antunovic *et al*. (2004)** detected statistically much higher concentrations of Ca in the blood of the non-pregnant ewes in comparison to the lactating ones. They attributed that to risks associated with parturition hypocalcemia in ruminants as reported by **Kaneko (1997).** Moreover**, Yokus *et al* (2004)** observed that Ca levels decreased during gestation period then increased at lactation period in sheep. **Araz (2013)** reported a decrease in Ca levels in Meriz goats during late pregnancy and lactation comparing to the pre-mating period, which could be attributed to the increase demand for mineralization of fetal skeleton, as reported by **Gawish and El- Shaer (2006).** Furthermore, no significant changes were observed in serum P_i, K, Na and Mg levels (Table 2). **Baumgartner and Pernthaner (1994) and Khaled and Illek (2012)** observed that the concentrations of Ca, P_i, Mg in plasma of ewes remained within the normal physiological ranges during different stages of reproduction. In our study, although no significant changes were detected in Na levels, high levels were recorded in ewes at late pregnancy and postpartum/ suckling comparing with pre- mating. **Azab and Abdel- Maksoud (1999)** recorded a significant increase in Na levels at the 3^{rd} and 4^{th} weeks postpartum with a decrease in K during late pregnancy. While, **Antunovic *et al*. (2002)** reported an increase in the concentration of Na in pregnant and lactating ewes as well as, a high level of K at the end of gestation.

Table 1: Serum hormonal levels during different reproductive status of Saidi ewes. (n=15)

Parameters	Physiological status		
	Pre- mating	Last month of pregnancy	One month after parturition (postpartum/ suckling)
T3 (ng/ml)	1.9±0.16[a]	0.7±0.16[b]	1.8±0.17 [a]
T4 (ng/ml)	57.8±4.9[a]	20.99±5.8[b]	34.96±4.9[b]
TSH (µIU/ml)	2.3± 0.3[a]	2.4± 0.3[a]	1.9± 0.3[a]
Insulin (µIU/ml)	10.7±0.6[a]	6.7±0.6[b]	7.2±0.6[b]

Data are expressed as LSM±SE

Means in the same raw with different superscripts are significantly different at ($p<0.001$).

Table 2: Serum electrolytes during different reproductive status of Saidi ewes. (n=15)

Parameters	Physiological status		
	Pre- mating	Last month of pregnancy	One month after parturition (postpartum/ suckling))
Ca (mg/ dl)	13.3±0.34[a]	10.6±0.34[c]	12.04±0.34[b]
P_i (mg/dl)	3.01±0.28[a]	3.02±0.28[a]	3.00±0.28[a]
K (nmol/l)	5.1±0.08[a]	4.98±0.08[a]	4.9±0.08[a]
Na (nmol/l)	82.99±5.5[a]	87.50±5.5[a]	87.90±5.7[a]
Mg (nmol/l)	1.20± 0.09[a]	1.10± 0.09[a]	1.03±0.09[a]
Fe (µg/dl)	114.07± 18.4[b]	234.43±18.4[a]	200.98±19.03[a]
Zn (µg/dl)	256.89± 11.2[a]	190.95± 11.2[b]	218.35± 11.2 [b]

Data are expressed as LSM±SE. Means in the same raw with different superscripts are significantly different at ($p<0.001$) except for Fe ($p<0.02$).

Mean values of Fe concentration were significantly higher ($P\leq 0.02$) in ewes at late-pregnancy (234.43±18.4 µ/dl) and postpartum/ suckling (200.98±19.03 µ/dl) in contrast to non-pregnant ones (114.07± 18.4 µ/dl, Table 2). **Gurdogan *et al.* (2006)** recorded a gradual increase in serum Fe levels during pregnancy. Also, **Tainturier *et al.* (1984) and Dar *et al.* (2014)** reported significantly high Fe levels in pregnant ewes. However, **Sema *et al.* (2009)** observed low serum Fe concentration in early pregnancy in the Awassi ewes. In goat, **Araz (2013)** recorded decrease in plasma Fe during late pregnancy, which could be related to the great demand for this element by the fetus and/or the increase of adrenocortical hormones during late pregnancy **(Swenson and Reece, 1993).**

Serum Zn level was significantly low ($P< 0.001$) in Saidi ewes in late pregnancy and post-partum suckling compared to pre- mating (Table 2). In agreement, **Master and Fels (1980)** reported a decrease in serum Zn levels in late pregnancy. **Kadzere *et al.* (1996)** and **Ahmed *et al* (2001)** recorded the highest level of Zn after parturition and during lactation in goats. On the contrary, **Khaled and Illek (2012)** observed an increase in Zn levels in late pregnancy and post- partum in ewes. The decrease in serum zinc in late pregnancy could be related to haemodilulation, as reported by **Elnageeb and Abdelatif (2010)** in desert ewes.

Mean level of serum total protein (table, 3) decreased significantly ($P< 0.001$) in ewes during late pregnancy and postpartum/ suckling (6.60± 0.17 and 6.30± 0.17 g/dl, respectively) than in pre- mating (6.90± 0.17 g/dl). While, no significant differences were detected in serum albumin and globulin concentrations. **Brozostowski *et al.* (1996), Balikci *et al.* (2007) and Sema *et al.* (2009)** recorded a decrease in total protein levels in days 150 and 120 of gestation, respectively in ewes. The decrease in serum total protein in late pregnancy may be due to that the fetus synthesizes all its proteins from the amino acids derived from mother, where the growth of the fetus increases exponentially reaching a maximum level (especially in muscles) during late pregnancy **(Jainudee and Hafez (1994);**

Brozostowski *et al*. (1996); Balikci *et al*. (2007); Sema *et al*. (2009); Safsaf *et al*. (2012).

Moreover, it could attributed to the rapid extraction of immunoglobulin from plasma during the last few months of pregnancy, when colostrum formed in the mammary gland, as well as the increase needs to proteins for the fetus development **(Castillo *et al*., 1999, Kaneko, 1997 and Antunovic *et al*., 2004).** The lower levels of serum total protein at postpartum/suckling may be attributed to the passive transfer of maternal protein via colostrum from dams to offspring **(Antunovic *et al*., 2004 and Teleb *et al*., 2009).** In contrast, **Yokus *et al*. (2006)** observed no significant changes in total protein levels within the different reproductive status**.** While **Karapehlivan *et al*. (2007) and Antunovic *et al*. (2011)** reported significantly high concentrations of total protein and albumin in pregnant ewes compared to not pregnant.

Serum glucose level in Saidi ewes was significantly lowers (P< 0.001) at the last month of pregnancy than that in pre-mating and postpartum/suckling (Table, 3). Similarly, **Antunovic *et al*. (2004 and 2011)** reported low levels of glucose in blood of pregnant ewes in relation to not pregnant. They suggested that low glucose levels in high pregnancy are associated with fetus development and mobilization of maternal glucose to fetal blood circulation, which was supported with insulin concentration (**Hamadeh *et al*., 1996; Castillo *et al*., 1999 and Jacob and Vadodaria**, **2001)**. They also, observed a decrease in blood glucose concentration in lactating ewes which considered a result of constant energy loss in the milk.

In conclusion, this study showed a significant effect of ewe reproductive status on serum levels of thyroid hormones (T3 and T4), insulin, Ca, Fe, Zn, total protein and glucose. So, monitoring the concentrations of some biochemical parameters, minerals, thyroid hormones and insulin in the blood of Saidi ewes at different reproductive status, when need for energy increase in the ewes gives basis for the regular therapy application to be carried out for prophylactic the metabolic disturbances of ewes aiming to reduce mortality rates of newborn and consequently economy losses. Results also, suggest that supplementation with Ca and glucose source (molasses) to Saidi ewes ration during pregnancy and lactation should be recommended. Further study using more sampling frequency during pregnancy and lactation period recommended to set a platform for much details and wider scales investigation.

Table 3: Serum levels of some biochemical parameters during different reproductive status of Saidi ewes. (n=15)

Parameters	Physiological status		
	Pre- mating	Last month of pregnancy	One month after parturition (postpartum/ suckling)
T.P (g/ dl)	6.90± 0.17^{a}	6.60±0.17^{ab}	6.30± 0.17^{b}
Albumin (g/ dl)	4.30± 0.28^{a}	3.70± 0.28^{a}	3.90± 0.28^{a}
Globulin (g/ dl)	2.60± 0.3^{a}	2.60± 0.3^{a}	2.70± 0.3^{a}
Glucose (mg/ dl)	62.80± 3.05^{a}	38.20± 3.05^{b}	58.95± 3.05^{a}

Data are expressed as LSM±SE

Means in the same raw with different superscripts are significantly different at (p<0.001)

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الملخص العربى

دراسة مستويات بعض الهرمونات و المكونات البيوكيميائية فى الدم خلال مراحل تناسلية مختلفة فى النعاج الصعيدى

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أجريت هذه الدراسه على عدد ٣٠ من النعاج الصعيدى السليمة صحياً، تتراوح أعمارها بين ٢- ١٠ سنوات و الموجودة بمحطة البحوث بملوى- معهد بحوث الإنتاج الحيوانى- وزارة الزراعة. تم جمع عينات دم من هذه النعاج قبل التلقيح بشهر، ثم من عدد ١٥ نعجة من الثلاثين قبل الولادة بشهر (آخر مرحلة الحمل) و شهر بعد الولادة (خلال مرحلة الرضاعة).

أجريت التحاليل على سيرم الدم لتحديد مدى تأثير المراحل التناسلية المختلفة للنعاج على مستوى بعض الهرمونات و المكونات البيوكيميائية بالدم.

أظهرت النتائج إن مستويات هرمونات الغدة الدرقية (T3 and T4) فى السيرم كانت أقل معنويا فى النعاج فى آخر شهر من الحمل بالمقارنة بمستواها قبل التلقيح وبعد الولادة (خلال فترة الرضاعة). بينما تم تسجيل أرتفاع معنوى فى مستوى الأنسولين فى النعاج قبل التلقيح والولادة عنه فى النعاج فى آخر مرحلة الحمل و بعد الولادة (مرحلة الرضاعة).

كان للحالة التناسلية للنعاج تأثير معنوى على تركيزات كل من الكالسيوم و الحديد و الزنك فى السيرم. حيث كان مستوى الكالسيوم منخفضاً فى النعاج خلال مرحلة الحمل الأخيرة و بعد الولادة (مرحلة الرضاعة). و كان تركيز الحديد فى السيرم أعلى معنوياً فى النعاج خلال مرحلة الحمل الأخيرة و بعد الولادة (مرحلة الرضاعة) عنه قبل التلقيح. و كان مستوى الزنك فى السيرم أقل معنوياً فى النعاج خلال مرحلة الحمل الأخيرة و بعد الولادة (مرحلة الرضاعة) عنه قبل التلقيح. بينما لم تتأثر مستويات كل من الفوسفات الغير عضوى و البوتاسيوم و الصوديوم و الماغنسيوم بالحالة التناسلية للنعاج.

بالإضافة إلى إن تركيز البروتين الكلى فى السيرم كان منخفضاً معنويا فى النعاج خلال مرحلة الحمل الأخيرة و بعد الولادة (مرحلة الرضاعة). كما أظهرت النتائج أيضا إنخفاضاً معنوياً فى مستوى الجلوكوز فى سيرم النعاج خلال مرحلة الحمل الأخيرة.

في الختام، رصد تركيز هرمونات الغدة الدرقية، والأنسولين وبعض القياسات البيوكيميائية في دم النعاج خلال المراحل التناسلية المختلفة يعطي الأساس لتنفيذ العلاج بانتظام و تنفيذ إجراء وقائى من اضطرابات التمثيل الغذائي للنعاج بهدف تقليل الخسائر الاقتصادية.