Research Article

Effect of Vitamins and Hormones during different stages of Lactation in Lohi Sheep and Beetal Goats

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Abstract | This study was conducted to evaluate the effect of vitamins and hormones during different stages of lactation in Lohi sheep and Beetal goats. The experiments were performed on 30 ewes and 30 goats between 1.5 to 5 years age group were selected from Bahadar Nagar Farm, Okara, Pakistan. Both the sheep and goats were grouped into three different lactating stages: [lac I (n=10), lac II (n=10) and lac III (n=10)]. Blood samples were collected during all production stages and serum has been preserved at -20 ^{0}C and used to evaluate the differences in vitamin E, vitamin C, Triiodothyronine (T_3), Thyroxine (T_4) and Cortisol. The statistical analysis was done by two way ANOVA and Duncan Multiple Range Test (DMRT). T_3 and T_4 showed significantly different results during various stages of lactation in Lohi sheep and Beetal goats. However, Vitamin E, Vitamin C and Cortisol did not reveal significantly different results during lactation in both ewes and does. It could be postulated that the evaluation of vitamins and hormones during various stages of lactation were good indicators for farm maintenance and production of these animals.

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Introduction

Immediately before parturition as well as during the first stage of lactation, increased mammary gland activity results in energy deficiency. Changes in metabolites and hormones occur as a result of increased metabolic demands during lactation and animals become more susceptible to a number of metabolic diseases at this stage than during other life periods compromising productivity (Ashmawy, 2015). Homeostasis is the coordinated control in metabolism of body tissues necessary to support a physiological state (Abdulkareem, 2013). Lactation period represents a critical life phase, since have to adjust metabolically to the increase in energy and nutrient requirements needed to ensure milk production (Fiore *et al.*, 2017).

Additionally increased demands for energy during early lactation would initiate oxidative reactions thus

inducing reactive oxygen species (ROS) formation (Fassaha *et al.*, 2015) and animals may face "oxidative stress". Development of ROS are counterbalanced through antioxidant defense system. The system includes enzymatic (SOD, Catalase and GSHPx) and non-enzymatic includes, Vitamin E, C, zinc, glutathione, carotene, beta carotene (Pierce *et al.*, 2004).

Vitamin E is a fat soluble antioxidant with most active homologues α-tocopherol that when react with ROS produce oxidative stress and defend the cell membranes from lipid peroxidation (Traber and Atkinson, 2007). Vitamin C or ascorbic acid is a water soluble antioxidant against ROS and defends DNA damage. It can be used in a chain breaking antioxidant which helps to inhibit the synthesis of the peroxidative process and also facilitates the recycling of glutathione and oxidized vitamin E (Chan, 1993).

During lactation, the mammary gland secretary cells utilize 80% of the circulating metabolites in the blood for milk synthesis (Karapehlivan *et al.*, 2007). Thyroid hormones play an important role in lipid metabolism and concentration of these parameters in the blood gives a clearer picture of nutritional and health status of the animal. Serum T_3 and T_4 concentrations were significantly decreased during parturition and early lactation (Fiore *et al.*, 2018) and then increased in T_3 and T_4 concentrations were observed during the late lactation. The cortisol is well-accepted indicator for stress (Schwinn *et al.*,2018) and its level varies during lactation. There is enhancement of cortisol during lactation period due to increased adrenocorticotrophic hormone that in turn causes the release of glucocorticoids from adrenal gland (ALkalby and Mohammad, 2013).

Materials and Methods

Clinically healthy Lohi sheep (n=30) and Beetal goats (n=30) aged between 1.5-5 years were selected from Livestock Production Research Institute Bahadur Nagar Farm, Okara, Pakistan . The lactating groups were divided into early, peak and low lactation stages as Lac I (30 days), Lac II (60 days) and Lac III (90 days-onwards) in both Lohi sheep and Beetal goats. Blood samples were collected and serum has been preserved at -20 ^{0}C. The analyses were done in the laboratory of Institute of Pharmacy, Physiology and Pharmacology at the University of Agriculture, Faisalabad, Pakistan.

Vitamin E (Vit. E; µmol/L) and Vitamin C (Vit. C; µmol/L)

Method of Rutkowski and Grzegorczyk (2007) was used for the evaluation of vitamin E and C. By using the following formula, the concentrations of vitamin E and vitamin C were calculated as followed:

$$Cx=\frac{Ax}{As}Cs$$

Where;

Cx: Concentration of vitamin E/C; Ax: Absorbance of the sample; As: Absorbance of the standard; Cs: Concentration of the standard solution.

Triiodothyronine (T_3: ng/mL) and Thyroxine (T_4; µg/dL)

T_3 and T_4 Elisa kit (Biocheck Inc., Foster City, CA-94404, USA, Lot No. RN - 41498) was used for evaluation of T3 and T4 concentrations. By means of using lin-log method for data reduction, concentrations of all samples were determined. Linear portions of curve were used to calibrate the readings.

Cortisol (ng/mL)

A microplate EIA kit provided by Accubind (Monobind Inc., Lake Forest, CA-92630, USA, Lot No. 3625-300) was used for quantitative evaluation of serum cortisol concentration. Linear portions of curve were used to calibrate the readings.

Statistical analysis

Data obtained was subjected to two way analysis of variance (ANOVA) technique (Steel *et al.*, 1997). Duncan Multiple Range (DMR) test was used to explain significant difference (Duncan, 1955) among various studied groups.

Results and Discussion

Results for ANOVA in Lohi sheep and Beetal goats during various stages of Lactation

Data concerning the difference between Lohi sheep and Beetal goats for vitamin E, vitamin C, Triiodothyronine (T_3) Thyroxine (T_4) and cortisol activities during various stages of lactation were analyzed by two way ANOVA and the results have been shown in Table 1. Sheep and goats groups as well as the interaction between the groups and stages did not differ significantly in Vitamin E and vitamin C. The

results for serum T_3 and T_4 concentrations revealed that both the groups pertaining to different lactation stages as well as groups × stages interaction presented statistically different results (P ≤ 0.01). However, the results concerning cortisol in Lohi sheep and Beetal goats pertaining to different lactation stages were different significantly. However, the results of groups G x S interaction did not show significant results (Table 1).

Table 1: *Analysis of variance (ANOVA) of Vitamin E, C, T_3, T4 and Cortisol of Lohi sheep and Beetal goats during various stages of Lactation.*

Parameters (F-Value)	Source of Varition		
	Groups	Stages	G x S
Vitamin E	0.125 N.S.	138.078**	0.153 N.S.
Vitamin C	0.512 N.S.	317.328**	2.177 N.S.
Triiodothyronin (T_3)	14.779**	39.751**	11.184**
Thyroxine (T_4)	287.342**	138.298**	49.798**
Cortisol	136.707**	192.347**	0.685 N.S.

***Significance = P ≤ 0.01; N.S. = Non-significant.*

Results for Mean ± SE in Lohi sheep and Beetal goats during various stages of Lactation

Vitamin E (Vit. E; µmol/L) and vitamin C (Vit. C; µmol/L)

Vitamin E levels in ewes and does were significantly similar during lactation stage-I, II and III. In sheep and goats, vitamin E concentration decreased non-significantly from lactation stage-I to II and then an increase, though non-significant was observed during stage-III. Interaction between sheep and goats groups showed significantly same results among all the stages of lactation. Overall mean vitamin E concentrations in both Lohi sheep and Beetal goats groups were significantly the same (Table 2). It had been reported that vitamin E participate in fighting against oxidative stress during postpartum and decreased in lipid peroxidation (Bouwstra *et al.*, 2008). Results of the present study are in consistent with those of Festila *et al.* (2012) had not discovered difference in vitamin E supplementation in milk production in dairy cows. Konvicná *et al.* (2015) reported a significant increase in vitamin E concentration with the advancement of lactation.

Vitamin C concentrations were significantly similar among all the stages of lactation. A non-significant increase was observed from stage-I to stage-II which decreased non-significantly during lactation stage-III in both the groups. Interaction between sheep and goats groups was significantly similar among all the stages of lactation. Overall mean Vitamin C concentrations for both sheep and goats groups were significantly the same (Table 2). The findings of present study agree with that of Ognik *et al.* (2015) who had reported that there was no change in Vitamin C concentration during various stages of lactation. Howida *et al.* (2015) concluded that vitamin C supplementation enhanced to the adverse effects of oxidative stress and improved milk quality.

Triiodothyronine (T_3;ng/mL)and Thyroxine (T_4; µg/dL)

Serum triiodothyronine concentration was observed to be significantly different (P ≤ 0.01) during stage-I in Lohi sheep. In Beetal does, significantly different results were observed in lactation stage-I and stage-II. The interaction between ewes and does manifested different results during lactation stage-I and stage-II of lactation. Overall mean T_3 concentrations were also significantly different in both Lohi sheep and Beetal goats groups (Table 2).

Serum thyroxine level was significantly different in lactation stage-I in Lohi sheep and during lactation stage-III in Beetal goats. The interaction between Lohi sheep and Beetal goats groups showed significantly different (P ≤ 0.01) results for T_4 concentration during stage-I and stage-II of lactation. Overall mean T_4 concentrations were resulted into significantly different values in Lohi sheep and Beetal goats groups (Table 2). The results of present study are in consistent with Paulikova *et al.* (2017) and Fiore *et al.* (2018), concluded a decrease in both T_3 and T_4 concentrations during lactation. These results are in consistent with resulted into decreased T_4 level during lactation.

The reason for low T_3 and T_4 could be breakdown of fat and protein in mammary tissue (Riis and Madsen, 1985). During early lactation due to limited supply of food (energy malnutrition), T_3 and T_4 levels decreased probably due to increased metabolism of T_3 and T_4 in peripheral tissues to inhibit secretary power of thyroid gland (Huszenicza, *et al.*, 2002). However, AL-kalby and Mohammad (2013) observed an increase in thyroid hormones during lactation.

Cortisol (ng/mL)

Serum cortisol level during various lactation stages exhibited significantly the same results. A non-signif-

icant decrease in cortisol concentration was observed

Table 2: *Mean ± SE of Vitamin E, Vitamin C, T_3, T_4 and Cortisol of Lohi sheep and Beetal goats during various stages of Lactation.*

PARAMETERS	Lactation Period			
	Lactation (Stage I)	Lactation (Stage II)	Lactation (Stage III)	Overall Mean
Vitamin E				
Lohi Sheep	1.40±0.01	1.29±0.02	1.51±0.01	1.40±0.02
Beetal Goats	1.40±0.01	1.30±0.02	1.51±0.01	1.40±0.02
Vitamin C				
Lohi Sheep	4.03±0.04	4.91±0.08	3.07±0.08	4.00±0.14
Beetal Goats	3.97±0.04	4.74±0.06	3.18±0.09	3.96±0.12
T_3				
Lohi Sheep	1.68±0.05 a	1.37±0.01 b	1.33±0.01 bc	1.46±0.03 A
Beetal Goats	1.45±0.05 b	1.25±0.01 c	1.39±0.02 bc	1.37±0.02 B
T_4				
Lohi Sheep	32.75±0.55 b	26.55±0.44 d	27.40±0.28 cd	28.90±0.57 B
Beetal Goats	39.30±0.52 a	37.60±0.54 a	29.15±0.41 c	35.35±0.87 A
Cortisol				
Lohi Sheep	3.53±0.09	2.58±0.08	2.02±0.06	2.71±0.12 A
Beetal Goats	2.75±0.06	1.86±0.10	1.41±0.03	2.01±0.11 B

A-B, a-d; Values in a row (a-d) or column (A-B) with different letters were significantly different (P ≤ 0.01; ANOVA, DMRT).

from stage-I of lactation to stage-III in both ewes and does. An interaction between Lohi sheep and Beetal goats groups for cortisol concentration demonstrated significantly the same results throughout all the stages of lactation. Although, statistically different results were observed for overall mean cortisol concentrations in both Lohi sheep and Beetal goats groups (Table 2).

Ghanem *et al*. (2012) and Howida *et al*. (2015) observed the same non-significant results for cortisol concentration during different stages of lactation in cows. Similarly, ALkalby and Mohammad (2013) had observed a significant decrease in cortisol concentration with the advancement of lactation.

Conclusions and Recommendations

Lactation is the physiological period of increased metabolic demand that could provoke a threat to homeostasis. Variations in vitamins and hormones revealed negative energy balance during lactation and should be consider to improve health, welfare and production of these animals.

Novelty Statement

The study was carried out in Lohi sheep and Beetal goats and the results suggested that helps the farmers for better maintenance and production of their animals and to improve their income.

Author's Contribution

Sonia Kanwal: Carried out the research work and has been written the first draft of the manuscript.
Muhammad Naeem: Supervised the research work and improved the manuscript.
Zaib Un Nisa: Provided aid during data analyses.
Rabia Farhat: Helped out during sampling process.
Zia Ur Rahman: Provided all the means to make this research possible. All authors read and proved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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