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Blood Constituents in Cycling, Gestating and Lactating Desert Ewes (*Ovis aries*) in Relation to Dietary Supplementation

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Abstract: The long term responses in blood parameters were evaluated during normal reproduction cycle, pregnancy and lactation of desert ewes receiving supplement feeding. Twenty sexually mature desert ewes were divided into two equal groups, control and supplemented. Both groups were kept on grazing residues of harvested field of sorghum. The supplemented group received daily 500g of concentrate mixture rich in energy and protein (crushed sorghum grain and cottonseed cake). In both groups, oestrus was synchronized by hormonal method and the ewes were inseminated naturally. Pregnancy was diagnosed by ultra-sound technique. The haemoglobin concentration (Hb) increased significantly with the advance of pregnancy in both groups of ewes. The packed cell volume (PCV) decreased with the advance of pregnancy, increased at parturition and decreased thereafter with the progress of lactation. The supplemented ewes maintained higher values of these indices during pregnancy and lactation. The serum levels of total protein (Tp) and albumin (Alb) decreased during early-and mid-pregnancy, while levels of serum urea and plasma glucose increased significantly with the advance of pregnancy. At parturition serum (Tp), (Alb) and urea (Ur) and plasma glucose level (Gl) increased in both groups. The serum (Ur) and plasma (Gl) increased gradually with the advance of lactation in both groups. Dietary supplementation increased significantly serum concentrations of (Tp), (Alb), (Ur) and plasma (Gl) level during pregnancy and lactation. Maintaining concentrate supplement enhanced the plane of nutrition of the ewes during normal cycling, pregnancy and lactation. This evidence would provide means for evaluation of the physiological responses and their implications in productive traits of desert ewes.

Key words: Ewes · Pregnancy · Lactation · Dietary supplementation · Blood constituents

INTRODUCTION

Several metabolic criteria were studied in sheep of various genetic background, as challenged by feeding requirements versus reproductive status. The concentrations of blood metabolites are parameters particularly monitored in several of these investigations [1, 2]. Related studies indicated that plasma glucose level, serum total protein, albumin and urea may be influenced by supplemental feeding of pregnant and lactating ewes [3, 4, 5, 6].

Desert sheep are prolific, remarkably reproductive and may be distinctively prone to metabolic disorders. These criteria entail seasonality of reproduction [7] and influence by of level of nutrition [8]. These responses may justify studies on physiological characteristics in relation to nutrition. It is desirable to look into the changes in blood constituents and metabolic profiles that influence the reproductive and nutritional status and most appropriately predict performance associated with metabolic insufficiency [9, 10, 6].

The objective of this study was to evaluate the effects of long term supplementation with concentrate, rich in energy and protein, on haematological indices and blood metabolites during stages of pregnancy and lactation in desert ewes.

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MATERIALS AND METHODS

Experimental Animals: Twenty desert ewes selected from the breeding stock of Khartoum University Farm (Location: latitude 15°40′ North, Longitude 32°32′ East and 380 m above mean sea level) were used in this study during the period January 2006-January, 2007. The ewes were 2.5-3.0 years old with average body weight of 35.19±3.60 kg at the beginning of the study. The experimental groups (designated A and B) were evenly matched regarding weight age and physiological status.

The animals were apparently healthy, as monitored according to the standard procedures. Before commencement of the experiment, anthelmentic (Ivomec, Anupco-England: 1.0 ml/50 kg BW) and prophylactic antimicrobial treatment (Sulphadiamedin pyrimidine, Richter-Pharma, Austria: 1.0 ml/10 kg BW) were given. The ewes were ear-tagged and housed in open shed with adequate ventilation and watering and provided with appropriate facilities for feeding.

Feeding Regimen: Both groups of animals (A and B) were kept on grazing residues of the harvested fields. Group B, considered as the treatment group, further received supplement feed of concentrate mixture. All animals were daily put on pasture for 6 hours (from 8:00 am to 2:00 pm.) on sorghum residues (*Sorghum bicolor*) and grasses (*Cyndon dacylon*). The animals in group B were individually penned and offered supplements of concentrate mixture composed of high energy and rich protein sources. Each animal received daily (at 7:00 am) allowance of 250 grams of crushed sorghum grains plus 250 grams of cottonseed cake, in a mixture form. Table 1 shows the chemical composition of the components of the concentrate diet. The treated group also received salt and vitamins offered in block form (macro-mineral, micro-mineral and vitamin D).

Synchronization of Oestrus and Detection of Pregnancy: Synchronization of oestrus in the ewes was performed by hormonal method [11]. Pregnancy was detected by ultra-sound techniques (standard device 550 Trioga PIE Medica-Netherlands) after one month following natural inseminations; 17 ewes were found pregnant, 7 in group A and 10 in group B.

Collection of Blood Samples and Analysis: 6 ml of whole blood samples were collected from the external jugular vein and immediately 1.0 ml was transferred to a capped test tube containing anti-coagulant (Na_2-EDTA).

Table 1: The chemical composition of the components of concentrate diet (g/kg DM)

Ingredients	Crushed sorghum grain	Cotton seed cake (DM)
Dry matter	945.00	956.00
Oil	26.50	87.80
Crude protein	140.00	254.80
Crude fibre	29.30	241.70
Nitrogen free extract (NFE)	784.40	351.90
Ash	22.80	63.80
Calcium	0.51	4.50
Phosphorus	3.30	3.70
Metabolizalbe energy(Cal/kg)	13.61	11.91

Fluoride was added to the sample tubes kept for glucose determination. Blood samples were centrifuged (3000 r.p.m for 15 min.) and haemolysis-free plasma samples were used for glucose determinations. Other portions of blood samples containing EDTA were used for the haematological measurements. The rest of the blood samples were allowed to stay for 4 hours at room temperature and then centrifuged to collect serum. Haemolysis-free serum was transferred to clean plastic vials and immediately frozen at -20°C for subsequent analysis.

Haemoglobin (Hb) concentration was determined using the cyano-methaemoglobin method [12] and the packed cell volume (PCV) was determined by the microhaematocrit method. Serum concentrations of total protein (Tp), albumin (Alb.) and urea (Ur) were determined using a spectrophotometer [13]. The glucose level (Gl) was determined by the enzymatic colorimetric method using commercially available kit (Randox Laboratory Ltd., London).

Statistical Analysis: Standard methods of statistical analysis were adopted [14]. Analysis of variance test (ANOVA) was used to evaluate the effects of physiological state on blood constituents. The ewes were compared at the end of each period of the experiment, the initial (1N), mating (MT), early-(EP), mid-(MP) and late-(LP) pregnancy and at parturition (PR) periods. After lambing, the two groups were compared in the first, second and third month of lactation (L1; L2 and L3, respectively). The student (t) test was used to compare the control and supplemented group at each of the physiological states.

RESULTS

Packed Cell Volume (PCV): The data depicted in Fig. 1 show that the values obtained from the supplemented and

control groups, during early flushing, after mating and during gestation and lactation periods were significantly lower (P<0.05) compared to value measured during the initial period. There was progressive significant decrease (P<0.05) in (PCV) during early flushing and early and mid-gestation, in both groups. The (PCV) increased significantly (P<0.05) to higher values at parturition. There was a significant (P<0.05) decrease in (PCV) during the first and second month of lactation compared to pre-partum values in both groups. During the third month of lactation, the control ewes showed a significant (P<0.05) decrease in (PCV) level and the supplemented group showed a slight decrease. The supplemented ewes showed significantly (P<0.001) higher (PCV) values at gestation, parturition and lactation phases as compared to respective control group values.

Haemoglobin Concentration (Hb): The (Hb) concentrations presented in Fig. 2 indicate no significant change in the values obtained during the initial, early flushing and after mating periods. In the gestating supplemented ewes, the (Hb) concentration increased (P<0.05) with the advance of pregnancy and until parturition. The pregnant control group showed a slight insignificant and gradual increase in (Hb) concentration. The (Hb) concentration decreased (P<0.05) after parturition and during the first month of lactation compared to prepartum values. Higher (P<0.05) concentrations of (Hb) were revealed by the supplemented group during the stages of gestation and lactation.

Serum Total Protein: The effects of dietary supplementation on the concentration of serum total protein (Tp) are shown in Fig. 3. The (Tp) level in the supplemented group decreased (P<0.05) after mating. However, both groups showed progressive decrease (P<0.05) in the concentration of (Tp)during early flushing and after mating as compared to their initial values. The extent of this decrease in values was gradual for the supplemented group during the early flushing, but did not attain the level of significance. The control group showed increase (P<0.05) in the concentration of (Tp) compared to the values of the early flushing period and reached higher values at parturition. The supplemented group showed increase (P<0.05) during the early and mid-gestation, followed by a decrease in (Tp) values during late-pregnancy and a rise at parturition time. There was no significant change in (Tp) level with the advance of lactation. However, the supplemented group showed increase (P<0.05) in (Tp) values during the third month of lactation. The lactation (Tp) values were slightly higher compared to values obtained during gestation. The overall effect of feed supplement (during early flushing, after mating and during gestation) was reflected in higher values (P<0.05) in (Tp) levels compared to the respective control group.

Serum Albumin: The values in Fig. 4 show that there was increase (P<0.05) in albumin (Alb) level during early flushing and after mating in the control group as compared to the values obtained during the initial period. The (Alb) level of the supplemented group decreased (P<0.05) after mating as compared to the initial values. The supplemented group showed no significant change in (Alb) values during early flushing period and the values obtained were higher (P<0.05) when compared to the control group. During gestation, for control group there was increase (P<0.05) during mid-gestation, In contrast, the supplemented group showed decrease (P<0.05) in the (Alb) level with the advance of gestation. At parturition, both groups showed increase (P<0.05) in (Alb) level as compared to the respective values obtained during late gestation. There was no significant change in (Alb) levels with the advance of lactation. However, the supplemented ewes showed increase (P<0.05) in the level of serum (Alb) during the third month of lactation. The (Alb) levels were higher (P<0.05) in the supplemented ewes as compared to the control in physiological states including early flushing, mating, early gestation and up to the third month of lactation.

Serum Urea: Fig. 5 indicates that there was no significant difference in serum urea (Ur) between the groups in early flushing and mating periods. However, serum (Ur) levels measured during early flushing and mating periods were lower (P<0.05) when compared to the initial values. Higher (P<0.05) serum (Ur) values were shown for mid-gestation in both groups. At parturition, there was no significant change in (Ur) level of the control group. The supplemented group exhibited increase (P<0.05) in serum (Ur) values in comparison to values obtained during late gestation. With the advance of lactation, serum (Ur) expressed no significant change in the control group and the supplemented group showed an increase (P<0.05) in the third month of lactation. Also it was observed that (Ur) level was significantly higher (P<0.05) in supplemented ewes as compared to the control only during mid-gestation.

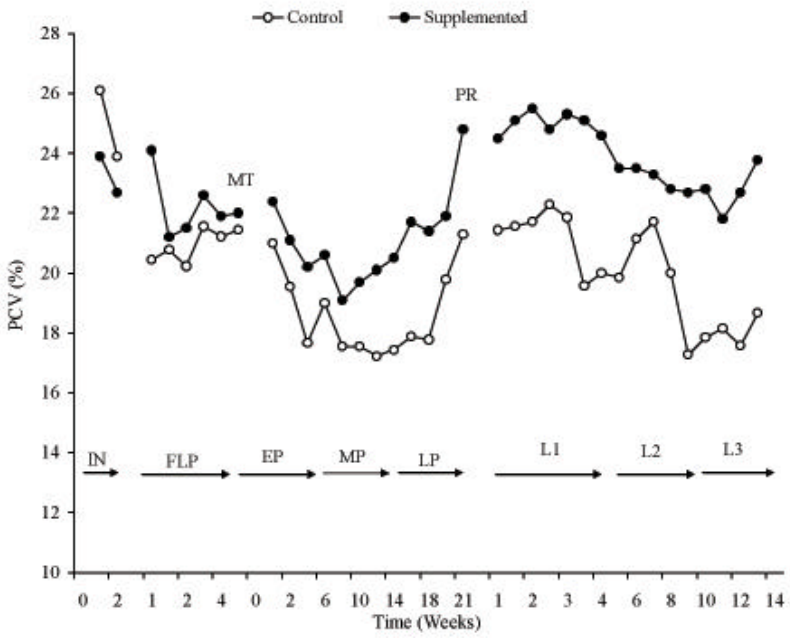

Fig. 1: Effect of dietary supplementation during different physiological states on packed cell volume (PCV) of desert ewes

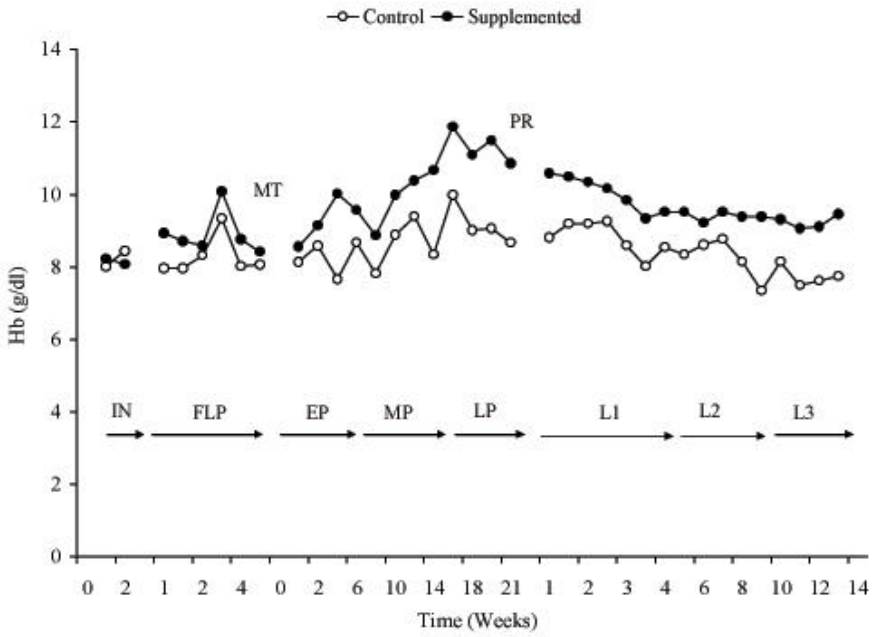

Fig. 2: Effect of dietary supplementation during different physiological states on haemoglobin (Hb) concentration of desert ewes

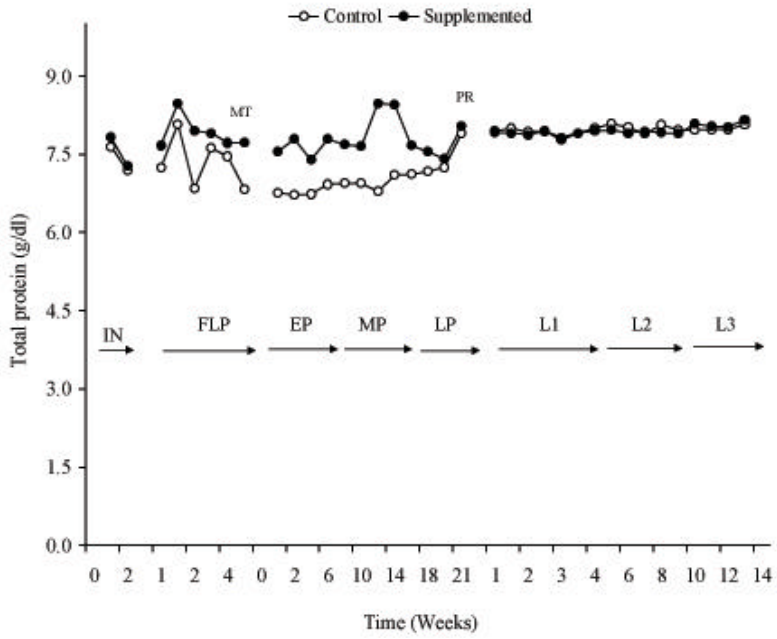

Fig. 3: Effect of dietary supplementation during different physiological states on serum total protein (TP) of desert ewes

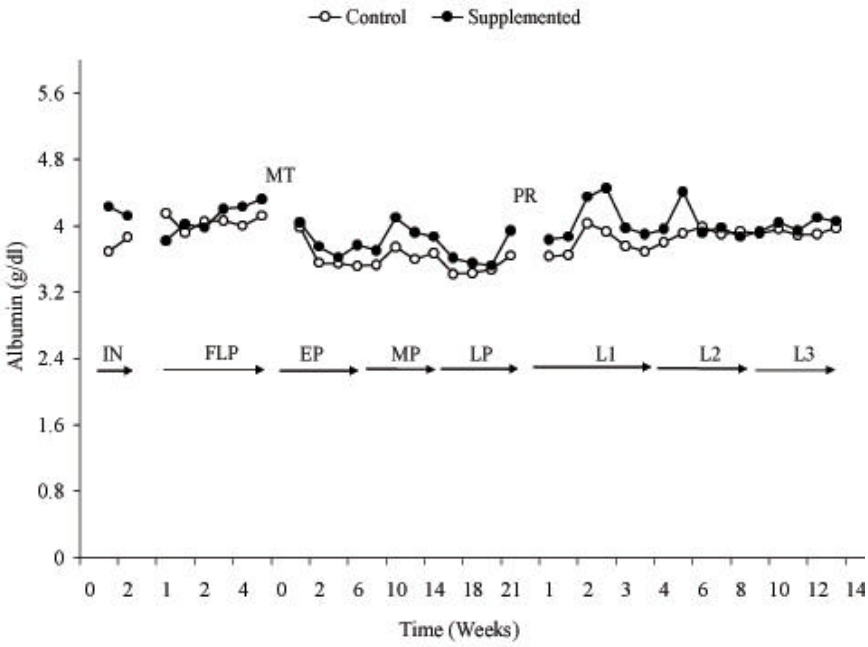

Fig. 4: Effect of dietary supplementation during different physiological states on serum albumin level (Alb) of desert ewes

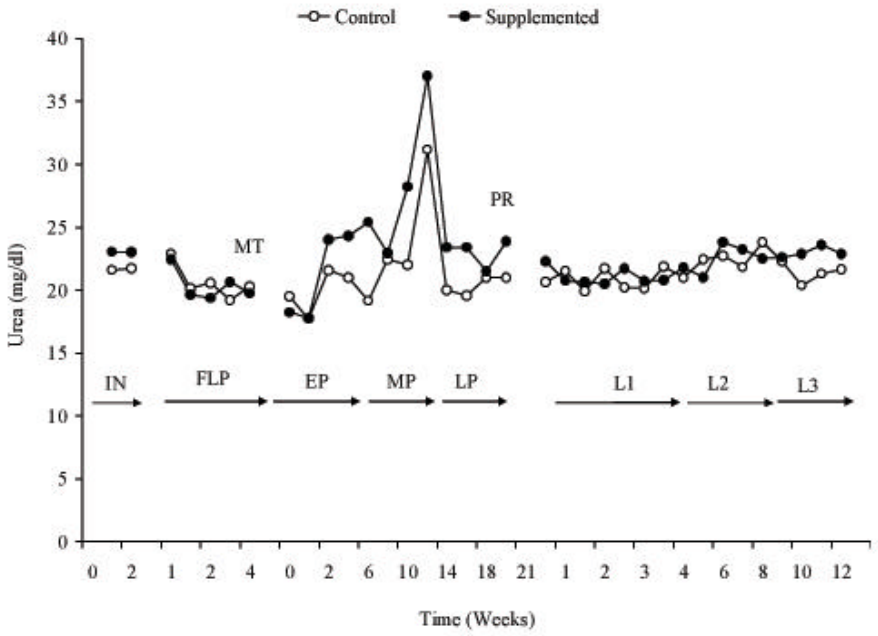

Fig. 5: Effect of dietary supplementation during different physiological states on serum urea (Ur) level of desert ewes

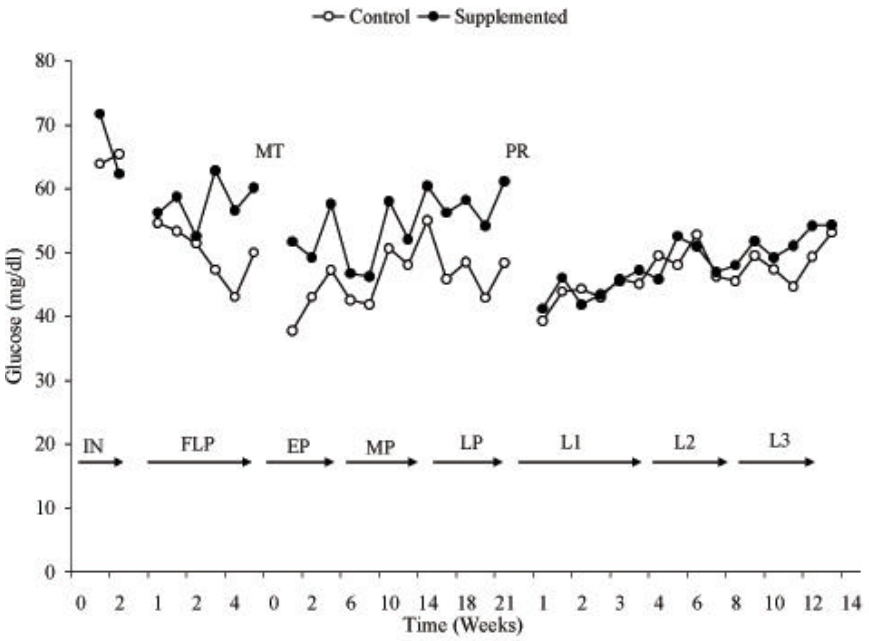

Fig. 6: Effect of dietary supplementation during different physiological states on plasma glucose level (Gl) of desert ewes

Plasma Glucose: The results presented in Fig. 6 show that in both the control and the supplemented group plasma glucose levels (Gl) were lower ($P<0.05$) during early flushing period and after mating when compared to the initial values. Gestating ewes, in both groups, showed (Gl) levels which were lower ($P<0.05$) compared to the initial values. These values reached higher levels by mid-gestation and then decreased during late gestation. At parturition, the (Gl) was significantly higher ($P<0.05$) in both groups. During lactation, the control group showed decrease ($P<0.05$) in (Gl) in the first month and an increase ($P<0.05$) during the second month of lactation when compared to the values obtained before and at parturition. For the supplemented ewes, there was a significant ($P<0.05$) decrease in plasma glucose during the first and second month of lactation compared to values measured before and at parturition. The supplemented group maintained significantly ($P<0.001$) higher plasma glucose level compared to the control during early flushing, after mating, at mid-gestation and parturition.

DISCUSSION

This study covers some major blood components that constitute data obtained for long continuous periods of supplemental feeding and physiological states that indicate coverage of a wide-range of the metabolic profiles for the desert ewes. The metabolic profile is indicative of and enhanced by supplementary feeding under different physiological states. The parameters studied have practical implications for the gestating and lactating sheep.

Extended concentrate feeding improved the plane of nutrition of ewes. The monitoring of the physiological responses would provide detailed account that facilitates more critical evaluation of nutritional status of desert ewes.

The Gestational Responses: Pregnancy had marked influence on the (PCV) level in the present study. The decrease in (PCV) values during pregnancy (Fig. 1) could be attributed to haemodilution and is supported by previous studies associating gestation to an increase in blood volume and haemodilution [15, 16]. The observed decrease in (PCV) also agrees with the results reported for gestating Barki ewes [17] and the findings for Probstheida breed pigmy goat [10]. The higher (PCV) values during late gestation and at parturition reflect the need for excess erythrocytes to transport oxygen to meet the enhanced metabolism. An associated increase in the rate of oxygen consumption and metabolic heat production was previously reported for the pregnant Merino ewes [18]. However, pregnancy in sheep resulted in a 31% greater red cell volume in mid-gestation, but no significant change in plasma or blood volume [19].

Gestation was also associated with increase in (Hb) concentration of the supplemented ewes and only lately in the controls (Fig. 2). These changes coincide with higher demand for oxygen and the requirements of higher metabolic rate for pregnancy. During pregnancy and near term, the uterine blood flow was shown to increase significantly in ewes [20, 19]. Increases in foetal weight are highly correlated with linear increase in placental blood flow and logarithmic increases in uterine uptake of oxygen and glucose [21]. The observed increase in (Hb) concentration during pregnancy in the present study confirms the findings in Merino ewes [22] and supports the observations in Barki ewes [23].

The concomitant increases in both PCV and (Hb) concentrations may be attributed to the availability of certain nutrients needed for the formation of red blood cells. This may be most demanding in the last trimester of pregnancy for supply of nutrients and blood to the foetus and may increase many fold to meet the requirements of the dam under nutritional stress [24].

The observed decline in serum total protein (Tp) during pregnancy (Fig. 3) is comparable to the values obtained during the initial and early flushing period and it is likely to be related to decreases in the albumin fraction in serum. These findings confirm the results reported in previous studies in sheep [17, 25] and cattle [26]. The decrease in the level of serum (Tp)during pregnancy could be attributed partly to haemodilution. The blood and plasma volume increased gradually during pregnancy in Finnish Landrace x Dorset Horn ewes and the increase was influenced by litter size [15]. Also, it could be related to the provision of amino acids and proteins for the foetus by the mother for the purpose of growth and development. The increase in the rate of delivery of amino acids from the maternal blood lowered the serum protein level. The gradual elevation in serum (Tp) level during late gestation in ewes may reflect the high capacity to compensate the loss in plasma protein. This observation indicates the importance of dietary supplementation during this critical period for rebuilding of the body protein of the gestating ewes [27].

The decrease in serum albumin (Alb) level during gestation (Fig. 4) could be attributed to haemodilution; It has suggested [15] that the half-life of (Alb) might be reduced and the rate of its synthesis limited by diversion

of amino acids to gravid uterus to sustain foetal growth. The increase in serum (Tp) and (Alb) with supplementation of gestating ewes reflects improvement in the nutritional status of the animals. These results are in agreement with previous findings in sheep [28, 4].

The level of serum urea (Ur) during advanced pregnancy (Fig. 5) is clearly reflecting increase in protein catabolism [29] and an increase in urea synthesis. The marked elevation in urea level during mid-gestation is apparently associated with increase in the formation of embryo tissues. Also, it is probable that protein catabolism and high demand for energy by the pregnant ewes during the mid-gestation led to an increase in (Ur) level to an extent above the ability of the kidneys to eliminate excess amounts from plasma [30]. Moreover, the (Ur) level is considered as an index of protein intake and urea recycling in the pregnant ewe [4]. From studies on Targhee ewes during mid-gestation. It was reported [31] that the serum urea level could be related to forage intake and consequently crude protein consumption.

The higher serum urea level at mid-pregnancy confirms previous observations in sheep [28, 31, 25]. The non-significant effects of supplementation of soybean and blood meals on level of urea was attributed [32] to the fact that protein supplement source is only contributing a small proportion of the total dietary crude protein that escaped rumen fermentation or degradation. Moreover, it has been reported that higher blood urea nitrogen may be associated with lower nitrogen intake due to recycling of urea in the ruminant animal [33].

The relative increase in plasma glucose level (Gl) during the advanced stages of pregnancy (Fig. 6) indicates that the size of the foetus induces change on maternal carbohydrate metabolism [34] as glucose is a major limiting nutrient of foetal growth [35]. The increase in (Gl) level could be associated with decline in maternal insulin concentration with the advance of pregnancy [36]. The reduction in insulin secretion during pregnancy minimizes peripheral glucose utilization and maximizes glucose extraction of the uterus [37]. It could be associated with increase in the rate of secretion of glucocorticoids and adrenaline and stimulation of glycogenolysis in the liver [38]. The mobilization of amino acids from body protein pool by adrenocorticoids is associated with an increase in the rate of hepatic deamination and conversion of certain resultant á-ketoacids to glucose. The marked increase in plasma (Gl) level at parturition in the present study could be attributed to the release of the mother from foetal glucose demands, or it may reflect an urgent requirement of the mother for readily available energy. Similar increase in blood (Gl) was reported at parturition in ewes [17].

The rise in (Gl) during pregnancy is also likely to be related to an increase in the rate of glucose production and utilization. Gluconeogenesis from propionate was reported to be more efficient during pregnancy and was significantly affected by plane of nutrition in Merino ewes [39] and the efficiency increased with the advance of pregnancy [40]. Hene the feed supplementation in the present study may have resulted in high level of rumenally degradable starch and crude protein that yielded more propionic acid and amino acids that stimulated glucose production. The present findings in desert ewes are consistent with the observations made in sheep [41] and goats [42].

The Lactation Responses: The observed postpartum rise in (PCV) and (Hb) (Fig. 1, 2) values may be related to mobilization of erythroytes sequestered in the spleen [17] which is considered an adaptive mechanism that improves the blood capacity for oxygen transport to meet the higher metabolic demands of parturition and colostrum production. Also it could be related to the relief of blood from the effect of haemodilution which usually occurs during gestation. These observations are consistent with previous findings reported in sheep [23] and Danish Landrace dairy goats [43].

The postpartum maintenance of relatively high (PCV) and (Hb) values shown in the present study may indicate that during lactation, the availability of nutrients is for erythrocyte production and (Hb) synthesis. Erythropoiesis was reported to be affected by the level of feeding in sheep [44].

The relatively higher level of serum (Tp) obtained during lactation (Fig. 3), compared to gestation values may indicate a higher protein demand during the period of pregnancy. This observation is in agreement with the findings in sheep [6] and Danish Landrace goats [43]. The higher serum (Tp) level in supplemented ewes is clearly related to availability of nutrients.

The lactation responses of serum (Alb) levels (Fig. 4) are apparently related to lower rate of protein catabolism during lactation compared to that during pregnancy. The high need of the ewes for protein during pregnancy has been noted [45]. Moreover, other workers [46, 47] indicated that the foetus synthesizes all its protein from the amino acids derived from the mother. The higher level of serum (Alb) during lactation may be partly attributed to relief from the effect of haemodilution which usually occurs during pregnancy. The relatively higher serum

(Alb) level observed during lactation in the present study is in agreement with the findings for lactating Barki ewes [23] and for Wurtemberg ewes [6].

The lactation response of serum (Ur) level (Fig. 5) is a good indicator of protein metabolism and intake in dairy ewes [5]. The gradual increase in (Ur) level with the advance of lactation in the present study is presumably related to increase in feed intake associated with higher nutrient demands for lactation. An increase in feed intake after lambing associated with expansion of rumen to meet the increase in requirements of lactation has been reported [48]. The maintenance of high (Ur) level of lactating desert ewes may also be related to increase in the activity of the thyroid gland during the period of advanced lactation as reported in crossbred Blackface ewes [45]. Thyroid hormones stimulate oxygen consumption, protein metabolism and milk yield. An increase in the level of thyroid hormones might be considered as indicator for tissue protein catabolism [49].

The rise in serum (Ur) level in the control group during the second and third month of lactation to values almost matching the level in supplemented ewes could be related to urea recycling to supplement milk synthesis. Moreover, it has been reported that serum (Ur) level is indicative of tissue protein catabolism and is influenced by urea recycling [4]. Levels of 23-90% of serum urea could be recycled through the saliva, with higher values associated with lower nitrogen intake in ruminants [33].

During early lactation both groups of ewes showed a sharp decrease in plasma (Gl) level from the high level maintained during late gestation (Fig. 6). Lactation poses a great demand for Gl, since milk contains about 90% as much sugar as blood and glycerol has to be synthesized for production of milk fat [50]. Moreover, a marked decrease in plasma (Gl) level during early lactation was reported [51] in ewes and others [52] found a sharp decrease in (Gl) level immediately after parturition. This pattern of response reflects the high metabolic demands of lactation which influence glucose kinetics. The studies on ewes [53] suggest that the increase in sensitivity of many body tissues to insulin stimulation during lactation supports milk production by increasing the amount of glucose and possibly amino acids available for the mammary glands. (Gl) is known to be the major precursor for the synthesis and secretion of lactose, which in turn dictates milk volume [54, 55]. The gradual increase in plasma (Gl) during lactation (Fig. 6) might indicate concomitant decrease in uptake and utilization of glucose by the mammary gland. As reported for Karadi ewes [56], the concentrations of milk lactose and protein decreased progressively during lactation. Furthermore, Chaiyabutr et al and coworkers [57] reported a decrease in (Gl) uptake, lactose production and milk yield during mid-and late-stage of lactation in Holestein cattle.

The lack of response of (Gl) to dietary supplementation, as indicated in the present study (Fig. 6) would probably be related to the maintenance of higher milk yield by supplemented group. Over this period of time, the supplemented ewes might have used more glucose in the process of lactose synthesis and therefore maintained blood glucose levels similar to the values reported for the control group.

CONCLUSIONS

The results indicate that pregnancy and lactation affect blood constituents of grazing sheep; the responses were modified by concentrate supplementation. The maintenance of concentrate supplementation as adopted in the current study would enhance the plane of nutrition of the ewes during normal cycling, pregnancy and lactation. This nutritional strategy would improve the reproductive capacity and lactational performance of ewes under tropical conditions. The endocrine responses and lipid profile should be investigated in future studies.

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