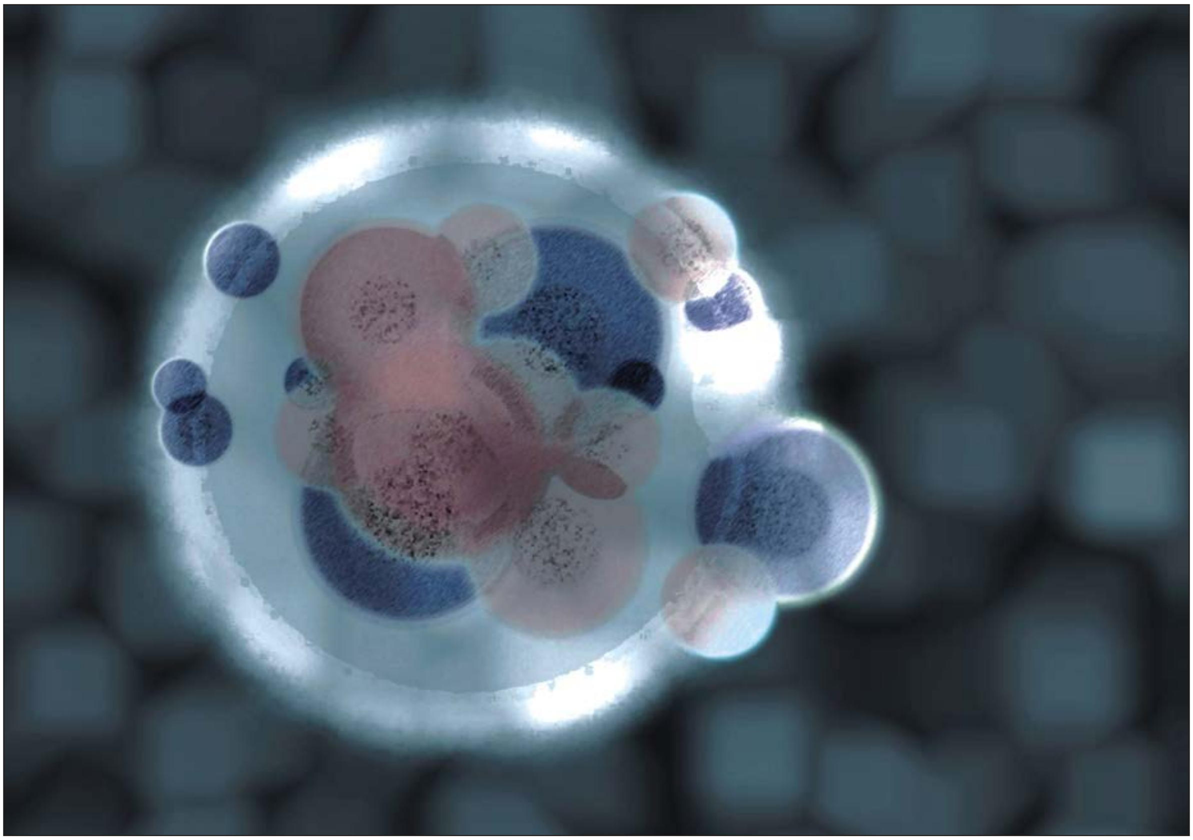

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Growth, Thermoregulation and Hematological Responses of Lambs in Relation to Age and Maternal Nutritional Supplementation

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Abstract: The maternal nutrition is a major intrinsic factor that influences intrauterine fetal growth and organogenesis. Manipulation of maternal nutrition is particularly critical during pregnancy and lactation. Therefore, evaluation the effects of maternal nutrition on growth performance and physiological responses of newborn lambs provided useful data for sheep industry. Twenty sexually mature estrus synchronized grazing ewes were assigned randomly to two equal groups, control and supplemented. The supplemented group additionally received daily 500 g/head of concentrate mixture + minerals and vitamins saltlick. The control group was maintained on grazing only. Body Weight (BW), rectal temperature (Tr) and Respiration Rate (RR) were monitored and blood samples were collected from lambs born to each group of ewes within 12 h after birth and then every 3 days for two weeks and then weekly up to weaning age of three months. At birth, BW, Tr and RR were significantly higher in lambs born to supplemented ewes. In both groups of lambs, BW, Tr and RR were significantly higher at birth compared to the values measured during the 3 months of postnatal life. The mean BW increased significantly with the advance of age while Tr and RR decreased progressively. Both groups of lambs showed a significant decrease in PCV, Hb concentration and plasma glucose level with the advance of age. The levels of serum total protein, albumin and urea decreased significantly during the first month of postnatal life in both groups and then these parameters showed gradual increase up to 3 month of age. The overall responses to concentrate supplementation of ewes was reflected on significantly higher values of parameters measured for the lambs born to supplemented ewes compared to lambs born to control groups. The findings indicate that the performance of lams can be potentiated through maternal dietary supplementation.

Key words: Lambs, maternal nutrition, growth, thermoregulation, blood indices

INTRODUCTION

The maternal nutrition is a major intrinsic factor that influences intrauterine fetal growth and organogenesis. Manipulation of maternal nutrition is particularly critical during pregnancy and lactation. Also considerable attention should be focused on post-partum period as it is critical regarding the fine-tuning shift in physiological responses of newborn lambs to extra-uterine environment. Unsupplemented grazing ewes may experience significant body weight loss and consequently fetal growth and lactation may be seriously compromised (Thomas and Kott, 1995). In addition, maternal malnutrition during gestation period is resulted in organs and tissue properties associated with their anatomical and physiological feature of the newborns which may have seriously consequences on the health status of the lambs in future (Barker, 1999; McMillen *et al*., 2001). Recent observations in developmental programming. Armitage *et al.* (2004), Wu *et al.* (2006) and Caton and Hess (2010) have suggested the concept of fetal origins of adult disease, in which it is proposed that the physiological, neuroendocrine or metabolic adaptations may occur to sustain fetal development in utero; in extrauterine life they are believed to have important pathological implications. Furthermore, animal and human studies showed that compromise of maternal nutrient supply could influence the balance between maternal and fetal needs resulting in a state of biological competition (King, 2003). The maternal nutritional status at the time of conception influences nutrient partitioning between the dam and fetus (Wallace *et al*., 1999; Wu *et al*., 2006; Caton *et al*., 2007). Significantly undernourished dam may not adapt to sustain fetal growth but rather to maintain body stores (Gao *et al*., 2008); if the dam is in a marginally deficient state, the fetus is given precedence (King, 2003).

Adequate feeding during late pregnancy and early lactation is needed in order to accelerate fetal growth

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(Dawson *et al*., 1999), mammary gland development and milk production (Swanson *et al*., 2008; Elnageeb *et al*., 2008), colostrum yield and quality (Guinan *et al*., 2005; Ocak *et al*., 2005) and ultimately lamb survival and growth rate (Elnageeb *et al*., 2008). The colostrum is rich source of energy for heat production and prevention of hypothermia in lambs (Mellor and Murray, 1986). Recent evidence indicated that maternal nutrition can alter epigenetic status of fetus which may result in developmental adaptations that modify physiological responses and postnatal growth (Wu *et al*., 2006). The blood metabolites in the newborn also were reported to be influenced by age and nutrition of the mother (Piccione *et al*., 2010, 2011). However, compromised growth in uterus is associated with immaturity, low birth weight, hypoglycemia and impaired thermoregulation (Robinson *et al*., 1994; Piper *et al*., 1996). Therefore, decreasing newborn lambs' mortality and morbidity is a major management challenge facing sheep production.

Desert sheep comprise about 60% of the sheep population in the country. They are reared within the low rainfall savannah and semi-arid zone under extensive system where the availability of food is highly depend on season. Under this harsh environment which is reflected in poor nutritional status, there was negative effects on production performance and animal welfare Nutritional strategies during different phases of the reproductive cycle may improve their reproductive performance. We have previously reported on the effects of long-term dietary supplementation on thermal and hematological responses of desert ewes (Elnageeb *et al*., 2008; Abdelatif *et al*., 2009). However, there is lack of information regarding growth and physiological responses of lambs under tropical conditions. The objective of this experiment was to evaluate the effects of long-term concentrate supplementation of Desert ewes on physiological responses of newborn lambs.

MATERIALS AND METHODS

Experimental plan: This experiment was conducted at the Sheep Breeding Unit of the University of Khartoum located in Shambat (Location: latitude 15°40' North, longitude 32°32' East and 380 above mean sea level) to evaluate the physiological performance of lambs. The lambs were delivered by 20 multiparous desert ewes assigned to two groups of 10 each (A, B). Group A ewes were allowed to graze while group B ewes additionally received concentrate supplementation (500 g/head/day of concentrate mixture+minerals and vitamins, Table 1). The lambs in both groups were allowed to suckle their dams freely until weaning at 3 months. The postnatal changes in growth rate, thermoregulation (rectal temperature, Tr; respiration rate, RR) and blood constituents of the lambs were monitored up to weaning time. The BW, Tr and RR were measured and blood samples were collected within 12 h after parturition and then every three days for two weeks postpartum, followed by weekly intervals up to the time of weaning.

Table 1: Chemical composition of the components of concentrate used in the study (g kg^{-1} DM)

Ingredients	Crushed Sorghum grain	Cotton seed cake
Dry matter	945.00	956.00
Oil	26.50	87.80
Crude protein	140.00	254.80
Fiber	29.30	241.70
NFE	784.40	351.90
Ash	22.80	36.80
Calcium	0.50	4.50
Phosphorus	3.30	3.70
ME	13.61	11.91

DM: Dry matter, NEF: Nitrogen-free extract, ME: Metabolizable energy (Cal/kg)

Body weight, thermoregulation: The lambs Body Weight (BW) was measured to nearest ±0.05 kg using a spring balance (Salter, England). The rectal temperature (Tr) of lambs was measured (±0.1°C) using certified mercury-in-glass thermometer (Wilson-Supreme, Japan). The Respiration Rate (RR) was measured visually by counting the flank movements with the aid of a stopwatch.

Collection of blood samples and analysis: A sample of 6 mL of blood was collected from the lambs by jugular venipuncture and immediately 1 mL was transferred to a capped test tube containing the anticoagulant (Na_2-EDTA). Sodium fluoride was added to inhibit the enzymatic reaction that influences glucose concentration (Kelly, 1984). The sample was centrifuged at 3000 rpm for 15 min. Hemolysis-free plasma harvested was used for glucose determination. A 1 mL of blood was also transferred to another test tube containing EDTA; the sample was used for hematological studies. The rest of the blood sample was allowed to stay for 4 h at room temperature and then centrifuged at 300 rpm. for 15 min. Hemolysis-free serum was harvested and kept in plastic vials and immediately frozen at -20°C for subsequent analysis. Hemoglobin (Hb) concentration was determined using cyano-methemoglobin method (Estridge *et al*., 2000) and the Packed Cell Volume (PCV) was determined by microhematocrit method. Serum concentrations of Total Protein (TP), Albumin (Alb) and Urea (Ur) were determined using colorimetry (King and Wootton, 1965). The plasma glucose level (Gl) was determined by enzymatic colorimetric method using a commercial kit (Randox Laboratory Ltd., London).

Statistical analysis: Standard methods of statistical analysis were adopted (SAS, 1989). The student t-test was used to compare the reproductive traits of control and supplemented groups of ewes. Student paired t-test was used to evaluate the effects of lambs age at four time points (at birth, by the end of first, second and third months of age), on body weight of lambs, thermoregulation and physiological responses. Data were analyzed by least square analysis of variance using the GLM procedure. The model included dietary supplementation. Analysis of variance (ANOVA) was used to compare the differences in the measured parameters between the two groups of lambs at the same time points.

RESULTS

A proximal chemical composition of the components of concentrate used in this study (g kg^{-1} dry matter, DM) was depicted in Table 1. Dietary supplementation has been found to improve reproductive performance of ewes (Table 2). It seem that nutritional supplementation of ewe reflected in higher conception rate of control group (60% ewes) compared to the treated (100% ewes). Significant increase ($p<0.01$) between the two groups of ewes were observed in gestation length (days), fecundity rate (%), ewes post lambing weight (kg) which were 155.7±0.30 and 43.3±1.10%, 39.7±0.16 and 46.7±.22, respectively. Furthermore, a significant higher birth body weight ($p<0.01$) was observed for the lambs born to supplemented ewes (4.87±0.2) in compared to lambs born to control group of ewes (4.15±0.2). Additionally, general changes in the responses patterns of BW, Tr, RR and blood constituents are depicted in Fig. 1-9.

Body weight (BW): The mean BW of both groups of lambs (Fig. 1) showed a progressive significant ($p<0.01$) increase with the advance of age. Also it was noted that the lambs born to supplemented ewes had significantly ($p<0.001$) higher BW compared to the lambs born to the control during 3 months of postnatal life. Lambs weaning weight for the lambs born to supplemented ewes was 19.88±0.5 kg which was significantly ($p<0.01$) higher than for the lamb born to control group of ewes (12.86±0.3).

Thermoregulation: Figure 2 shows that the lambs born to the non-supplemented ewes revealed a gradual non-significant decrease in rectal temperature (Tr) with the advance of age. The lambs born to supplemented ewes showed significantly ($p<0.05$) higher Tr (39.44±0.2°C) at birth compared to lambs born to control ewes (38.86±0.2°C). However, lambs born to supplemented ewes showed a significant ($p<0.05$) decrease in Tr during the first month of age compared to the value measured at birth. Thereafter, the gradual decrease in Tr of the lambs from supplemented ewes did not attain the level of significance. However, there was no significant difference between the two groups in Tr with the advance of age. The data depicted in Fig. 3 indicate that immediately after birth, lambs born to supplemented ewes maintained

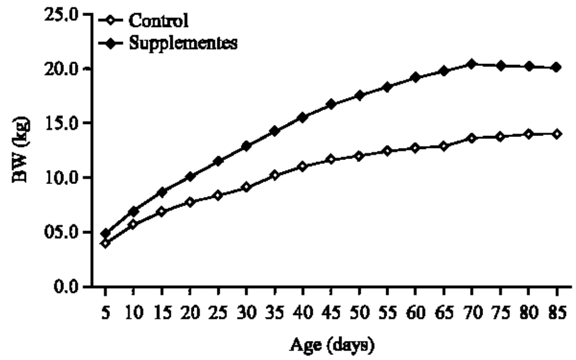

Fig. 1: Effects of dietary supplementation of ewes and age of lambs on body weight (BW) of lambs

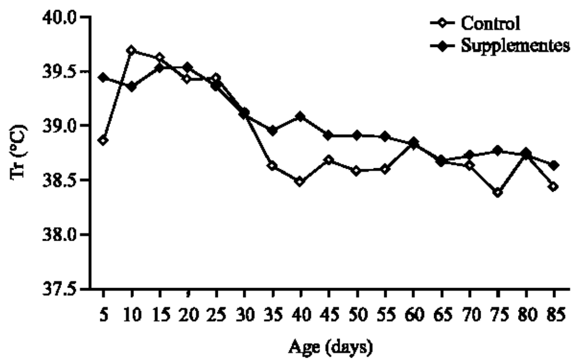

Fig. 2: Effects of dietary supplementation of ewes and age of lambs on rectal temperature (Tr) of lambs

Table 2: Effects of dietary supplementation on reproductive performance of ewes (Elnageeb *et al*., 2008)

Reproductive trait	Control ewes	Supplemented ewes
No. of responding to synchronization (%)	90	90
Mean interval end of treatment to onset of estrus (hrs)	28.3	25.2
Number of ewes inseminated	10	10
Number of ewes lambing	6	10
Conception rate (%)	60	100
Gestation length (days)	155.7±0.30b	152±0.40a
Fertility rate (%)	60	100
Fecundity rate (%)	43.3±1.10b*	51.4±1.70*
Ewes pre-lambing weight (kg)	36.3±1.37b	44.4±1.72a
Ewes post-lambing weight (kg)	39.7±0.16b*	46.7±.22a*
Lamb birth weight (kg)	4.19±0.81b**	4.87±0.62a**

Paired means within the same row bearing different superscripts are significantly different ($p<0.05$). *$p<0.01$

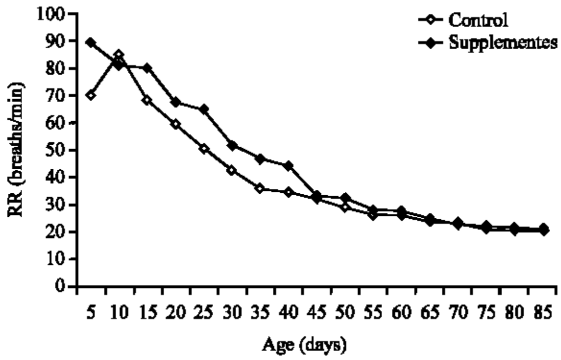

Fig. 3: Effects of dietary supplementation of ewes and age of lambs on respiration rate (RR) of lambs

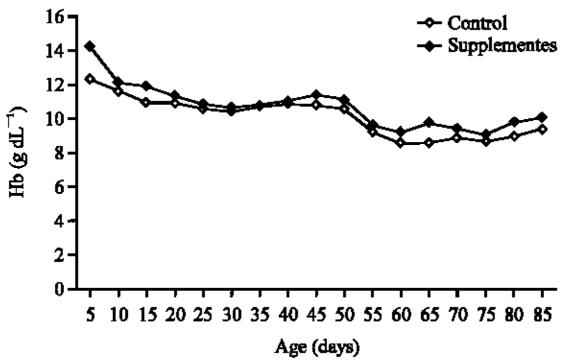

Fig. 4: Effects of dietary supplementation of ewes and age of lambs on packed cell volume (PCV) of lambs

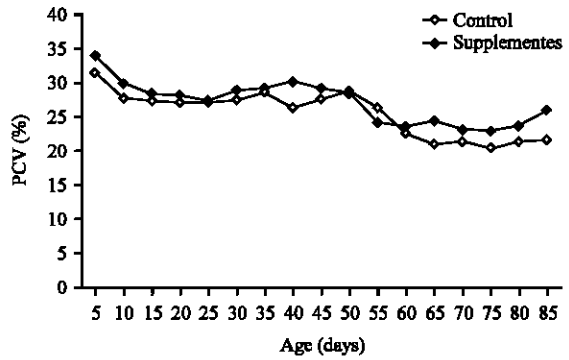

Fig. 5: Effects of dietary supplementation and age of lambs on haemoglobin (Hb) concentration of lambs

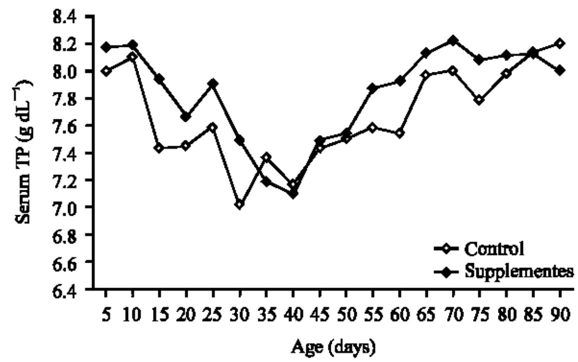

Fig. 6: Effects of dietary supplementation of ewes and age of lambs on serum total proteins (TP) of lambs

significantly ($p<0.001$) higher RR (90 breaths/min) compared to those born to the control ewes (70 breaths/min). In both groups of lambs there was a significant ($p<0.05$) decrease in RR of lambs with the advance of age.

Packed cell volume (PCV): The data show higher values of PCV (Fig. 4) at birth in both groups of lambs compared to the values measured during the 3 months. The PCV decreased significantly ($p<0.05$) with the advance of age in lambs born to the control group of ewes. The lambs from supplemented ewes showed gradual non-significant decrease in PCV. The lambs born to supplemented group of ewes showed significantly ($p<0.05$) higher PCV value (29.0±0.1.3 and 25.8±0.8%) compared to lambs from control ewes (27.5±0.8 and 21.3±0.2%) during the second and third month of age, respectively.

Hemoglobin (Hb): Figure 5 indicated that Hb concentration decreased significantly ($p<0.05$) with the advance of age in both groups of lambs. At birth, also it was noted that lambs born to supplemented ewes had significantly ($p<0.05$) higher Hb concentration compared to that of lambs born to control group ewes.

Serum total protein (TP): In both groups of lambs there was a significant ($p<0.05$) decrease in serum TP level (Fig. 6) during the first month of postnatal life compared to the values measured at birth. For both groups, TP increased significantly ($p<0.05$) in the second and third month of age compared to the values obtained in first month. There was no significant difference between the groups of lambs in the TP during 3 months of postnatal life.

Serum Albumin (Alb): The Alb level (Fig. 7) decreased significantly ($p<0.05$) in lambs born to control group ewes in the first month of postnatal life compared to birth value. Thereafter, Alb level increased significantly ($p<0.05$) in the second month and remained high in the third month of postnatal life. In contrast, there was no significant change in Alb level with advance of age in lambs born to supplemented ewes.

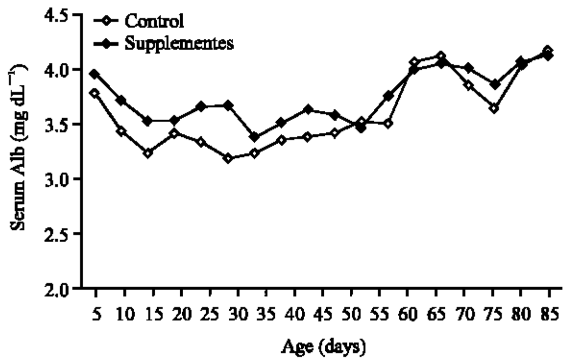

Fig. 7: Effects of dietary supplementation of ewes and age of lambs on serum albumin (Alb) levels of lambs

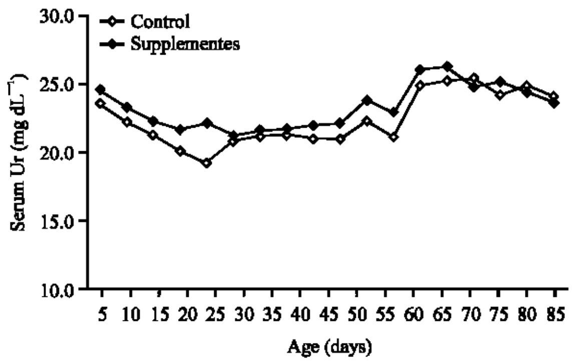

Fig. 8: Effects of dietary supplementation of ewes and age of lambs on serum urea (Ur) levels of lambs

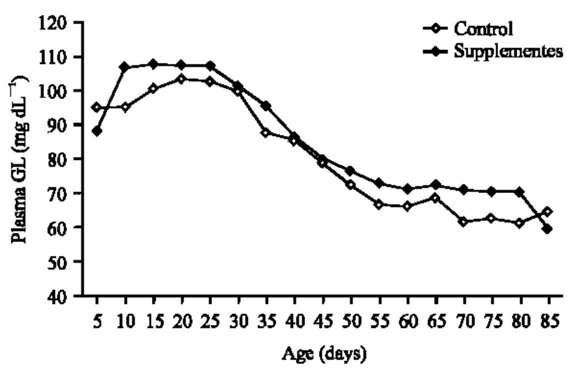

Fig. 9: Effects of dietary supplementation of ewes and age of lambs on plasma glucose (GL) levels of lambs

Serum urea (Ur): The serum Ur level (Fig. 8) showed no significant change in lambs born to the control group ewes during three months of postnatal life. In contrast, the lambs born to supplemented ewes showed a significant ($p<0.05$) decrease in serum Ur level during the first month compared to the value obtained at birth. Thereafter, the Ur level in this group increased significantly ($p<0.05$) during the second month and then it decreased slightly during the third month of postnatal life.

Plasma glucose level (GL): Figure 9 shows that in both groups of lambs there was a general progressive significant ($p<0.05$) decrease in GL level with the advance of age. The lambs born to the control ewes showed a slight gradual increase in GL level for one month after birth. Then, in this group, the GL level decreased from a high level of 88.18 to 60.42 mg dL^{-1} during three months. There was a slight increase in plasma glucose level during the first week in lambs born to supplemented group of ewes. Then the level stayed at an almost steady level for about 3 weeks before decreasing significantly ($p<0.05$) during subsequent postnatal life.

DISCUSSION

In the current study, the effects of dietary supplementation of ewes and age of the lambs on body weight (BW), thermoregulation and blood composition of the lambs have been investigated. The data generated in this study indicate that maternal nutrition had marked influence on physiological responses of lambs.

The lambs born to the supplemented ewes had significantly higher mean BW compared to lambs born to the control group. This clearly reflects the effect of feeding regimen of their dams. Similar findings were reported by Kenyon *et al.* (2011) who demonstrated that the lamb weight gain in early lactation is significantly affected by both mother Body Condition Score (BCC) and maternal nutrition. The higher nutritional status of supplemented ewes that was implemented during pregnancy and lactation accelerated the pre-weaning growth of the lambs as it influences development of the udder of ewes and consequently provision of adequate supply of milk. Increasing Crude Protein (CP) and Metabolizable Energy (ME) intake by ewes was associated with an increase in the growth rate of lambs (Hassan, 1996). A significant effect of protein levels on the lamb weaning weight was also reported (Mazzone *et al.*, 1999). However, other studies (Hall *et al.*, 1992; Murphy *et al.*, 1996; Banchero *et al.*, 2004) indicated that a short period concentrate supplementation of ewes before lambing has no significant effect on birth weight of lambs.

The results showed that, at birth, the lambs born to supplemented ewes had higher Tr and RR values compared to the lambs born to the control ewes. This may be related to the improved nutritional status of supplemented ewes. Budge *et al.* (2000) reported that increasing the quantity of food provided to ewes in late gestation promotes fetal growth and Brown Adipose Tissue (BAT) maturation, the combination of which will enhance neonatal viability. Moreover, when maternal nutrition was inadequate, the non-shivering thermogensis was increased in newborn lambs (Gate *et al*., 1999). In cattle, supplementation of fat during gestation increased heat production in newborn calves and elevated their rectal temperature (Brosh *et al*., 1998; Dietz, 2000).

The initial postnatal values of Tr and RR were lower in lambs born to non-supplemented ewes compared to values obtained for treated group. The rectal temperature, Tr (Fig. 2) and respiration rate, RR (Fig. 3) of lambs born to control ewes showed an increase during 7 days of postnatal life and attained the level of the treated lamb. This may suggested that lambs born to supplemented ewe have developed thermogenic mechanism compared to lambs born to control group. Due to reduced thermogenic capacity and relatively low energy reserve, lambs born to unsupplemented ewes attempted to maintain body temperature by increasing heat production during the first week. The higher values of Tr and RR at birth indicate that the lambs used physiological mechanisms to regulate the body temperature by increasing metabolic rate and non-shivering thermogenesis. Despite relatively warm thermal environment, the neonate lambs adapted to extra-uterine life initially by generating large quantities of heat which was indicated in the present study by higher values of Tr and RR during 7 days after birth. The findings confirm the observations of Laburn *et al*. (1992) who reported that Tr of neonatal Merino lambs peaked at 7 days of postnatal life and then declined towards adult level at the age of one month.

The PCV level (Fig. 4) and Hb concentration (Fig. 5) in lambs decreased sharply during the first two weeks after birth, stayed steady for about 5 weeks and then decreased progressively. This pattern could be related to the changes in erythropoietic activity influenced mainly by supply of Fe to their dams. Previous studies (Ullrey *et al*., 1965; Upcott *et al*., 1971) reported that the erythrocyte count declined from a high value at birth to a low level after two weeks. Furthermore, studies in neonatal calves indicated that the decreasing erythrocytic indices few weeks after birth can be considered as normal physiological development (Tennant *et al*., 1974).

In the present study, at birth, the lambs born to supplemented ewes had significantly higher value of PCV and Hb concentration. It appears that supplementation of the ewes with concentrate and salt lick during pregnancy, in which energy, protein, copper and iron were included, limited the normal physiological decrease of PCV and Hb values in the lambs during the postnatal period. Moreover, another factor involved is erythropoietin level as indicated in newborn kids (Piccione *et al*., 2006); the level of this hormone during this period of life is not adequate produced due to underdevelopment of kidneys.

The data indicate that in both groups of lambs, the levels of serum TP (Fig. 6) and Alb (Fig. 7) were significantly higher at birth and then decreased to lower values by the first month. Thereafter, the levels increased during the second and third month of postnatal life. The high serum TP level at birth could be related to the absorption of immunoglobulin from the colostrum ingested after birth. Generally, in mammals, colostrum consumption causes a temporary elevation in serum TP level (Jain, 1986) as it contains high protein concentration in ewes and goats (Swanson *et al*., 2008; Mellado *et al*., 2008). The general pattern of changes in serum TP and Alb levels after birth may be related to higher rates of protein deposition and rapid growth of the neonatal lambs associated with changes in protein metabolism and the role of liver in Alb synthesis (Thrall, 2004). The relative rates of growth and protein deposition are higher during neonatal period than at any other period of postnatal life (Goldspink and Kelly, 1984). Moreover, during the neonatal period, more rapid gain in protein mass occurs in skeletal muscle than the whole body (Young, 1970). Significantly, Reeds *et al*. (1993) reported that the rates of protein synthesis in skeletal muscle in swine are high immediately after birth and decline more rapidly during the first month of life.

The serum Urea (Ur) level (Fig. 8) in both groups of lambs, decreased progressively for 3 weeks after birth, stayed at an approximately constant level for 5 weeks and then increased to maintain higher levels during the third month. This pattern could be associated with the main nutrient available for the lambs during the postnatal life before weaning is suckled milk and nippled grasses from pasture. Preston *et al*. (1965) indicated that variations in protein intake of growing lambs could result in blood urea nitrogen level ranging from 27 to 32.9 mg dL^{-1}.

The plasma GL level in both groups of lambs (Fig. 9) was decreased significantly with the advance of age. However, for lambs born to supplemented ewes there was a moderate increase during the first week from the lower value obtained immediately after birth. The relatively lower value obtained was expected as the neonatal lambs consumed only colostrum which has relatively low lactose content (Park and Jacobson, 1993). Also in the newborn the liver begins gluconeogenesis and glycolysis during postnatal period, thus the blood GL level was elevated for 2 weeks before exhibiting a progressive decline. The progressive decline in level of GL is probably due to the shift in energy metabolism and changes in digestive products (volatile fatty acids, VFAs); which gradually replaced glucose and other sources of energy. The plasma GL level measured at 3 months of age in lambs attained the adult value. The concentration of VFAs and the development of rumen epithelium and microflora increase with the advance of age of lambs from the first week to 10 weeks of age (Zitnan *et al*., 1993). Furthermore, Lane *et al*. (2000) found that at 7 weeks of age, the intraruminal VFA concentration were elevated in lambs consuming solid feed compared to lambs given milk replacer. The authors suggested that changes in substrates oxidation from GL to butyrate are indicative of rumen metabolic maturation. The present results also indicate that the plasma GL level was higher in lambs born to supplemented ewes. This is clearly related to improved nutritional status of the ewes and the fact that more nutrients were available for the suckling lambs. Previous reports indicated that circulating fetal plasma glucose is increased with improved maternal nutrition in late gestation (Muhlhausler *et al*., 2002; Yuen *et al*., 2002) or maternal glucose infusion (Devaskar *et al*., 2002). The elevated glucose concentration also indicates that the muscle and liver glycogen content might have been higher in the lambs born to supplemented ewes. There is increasing evidence that fetuses of similar body weight and size but exposed to different maternal nutritional environments have markedly different endocrine sensitivity or tissue mRNA abundance (Heasman *et al*., 1998; Brameld *et al*., 2000; Hawkins *et al*., 2000). It is possible that postnatal secretion of, or sensitivity to, anabolic glucoregulatory hormones including insulin, growth hormone and Insulin-like Growth Factors (IGF) are programmed in utero (Rhoads *et al*., 2000; Greenwood *et al*., 2002; Gardner *et al*., 2005).

CONCLUSION

The postnatal period up to the time of weaning is critical for digestive function, metabolic adjustments and growth rate of lambs. The results presented in this study indicated that physiological responses and growth of lambs were influenced by the nutritional status of their dams. This suggested that provided the ewes are maintained in a moderate or high nutritional status during the reproductive cycle, the offspring will have better chance to withstand environmental stress and express high growth and physiological performance. Further studies warranted to explore the endocrine changes associated with metabolic adjustments in lambs under warm tropical conditions.

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