Theriogenology Insight: 4(1):1-7. April, 2014

DOI Number: 10.5958/j.2277-3371.4.1.001

Studies on Certain Serum Metabolites in non Pregnant and Pregnant Bannur Ewes

P.M. Gururaj, T. Veena, M. Narayana Swamy*, M.R. Jayashankar[1] P.T. Ramesh[2] and N.B. Shridhar[3]

Department of Veterinary Physiology, [1]Department of Animal Genetics and Breeding, [2]Department of Veterinary Medicine, [3]Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hebbal, Bangalore -560 024, India

Abstract

A study was undertaken with the objective of making an insight in to the changes with respect to the level of certain metabolites in non pregnant and pregnant Bannur ewes which are indicative of their nutritional status and physiological well being. Eighteen Bannur ewes maintained under identical managemental conditions in a semi-intensive rearing farm which were of two to four years of age are categorized in to three groups, comprising of six animals in each group, such as non pregnant (Group I), early pregnant (Group II, at 20 to 35 days of pregnancy) and late pregnant (Group III, at 105 to 120 days of pregnancy) ewes based upon ultrasound imaging technique. The metabolites such as serum levels of β-hydroxybutyrate, glucose, total protein, blood urea nitrogen and lipid profile components were determined at 0, 7 and 15th day of sample collection. The biochemical parameters did not vary significantly ($P>0.05$) between the groups except for the glucose levels which were significantly ($P<0.05$) lower in Group III compared to Group I and Group II. It was concluded that, all the parameters studied in Bannur breed of ewes during non pregnancy, early pregnancy and late pregnancy were within the normal reference range described for sheep and it was suggestive that none of the ewes in the present study suffered from any kind of metabolic disorders.

Keywords: *Bannur ewes, Non pregnant, Early pregnant, Late pregnant, β-hydroxybutyrate*

Introduction

Sheep husbandry is having a vital role in meat and wool production. Bannur breed of sheep (Mandya sheep) is an important and prominent indigenous breed of Karnataka state and one of the best mutton breed of India, distributed predominantly in Mandya district and also in neighbouring districts such as Bangalore Rural, Mysore, Tumkur and Kolar districts (Govindaiah *et al.,* 2006).

Most of the physiological processes in the animal body involve transport of metabolites by the blood; it is therefore helpful in detecting changes in types or rates of biochemical processes related to growth or productivity by measuring changes in levels of certain blood constituents (Bowden, 1971). It is

*Corresponding Author: mns263@yahoo.com
MS Received: 14th January, 2014
MS Accepted: 15th March, 2014

important to know the metabolic profile of pregnant ewes that include biochemical indicators depicting the nutritional status. This understanding is necessary to prevent health disorders in pregnant ewes that may lead to production and reproductive disturbances. The levels of serum β-hydroxybutyrate and glucose are the most common metabolites used to assess the energy status of ruminants. Blood urea nitrogen (BUN) and total protein are the biochemical constituents related to protein metabolism. Lipid components are also important in the assessment of nutritional status of the animals. The understanding of the normal values would be a useful index in the determination of physiological aspects in non-pregnant and pregnant ewes (Khatun *et al.,* 2011). Since, there was paucity of information on the changes in the metabolism of sheep during early and late pregnancy which serve as an indicator of nutritional status, physiological well being and metabolic disorders, if any, and also that could be comparable with the metabolism of non pregnancy state in Bannur ewes, the present study was undertaken.

Materials and Methods

The present study was conducted in Bannur ewes maintained at the Instructional Livestock Farm Complex, Veterinary College, Bangalore. All the animals were maintained under semi-intensive rearing system.

A real time B-mode ultrasound scanner equipped with a 3.5 MHz sector probe (Honda 2000 Co. Ltd., Japan) was used for detecting the status of pregnancy and non pregnancy by ultrasound technique. A total of eighteen sheep of two to four years of age were selected and were equally divided into Group I (non pregnant ewes), Group II (early pregnant ewes) and Group III (late pregnant ewes) comprising of six ewes in each group. Based on the measurement of embryonic vesicle and ultrasound features, ewes were assigned to non pregnant, early pregnant (20 to 35 days) (Plate 1) and late pregnant (105 to120 days) (Plate 2) groups as described by Soroori *et al.* (2007).

Approximately 10 ml of blood was collected by jugular venipuncture from all the ewes in each group at seven day interval *i.e.* on day 0 (day of first sampling), 7 (day of second sampling) and 15 (day of third sampling) of the study using disposable syringes and 18 G x 1 ½ inch sterile needles, prior to feeding in the morning with proper aseptic measures. Blood samples collected were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 3000 rpm at 30^{o}C for 20 min and stored at -20^{o}C until analyzed.

The β-hydroxybutyrate content in the serum was assayed immediately by using commercially available reagent kits (DiaSys Diagnostic Systems, Germany) based on the method described by Williamson *et al*. (1962) with a digital meter (Optium Xceed, Abbott, Mumbai). The serum levels of glucose, total protein

and blood urea nitrogen were analyzed by using kits obtained from Span Diagnostics Ltd., Surat, India, which were based on Glucose oxidase - peroxidase (GOD - POD) method (Trinder, 1969), biuret method (Gornall, 1949) and urease method (Young, 1990), respectively. The total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides levels were assayed by using reagent kits (Span Diagnostics Ltd., Surat, India) as described by Burtis and Ashwood (1999). Low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were calculated according to the methods of Bergmeyer (1985) and Warnick *et al*. (1983), respectively, as indicated below:

LDL cholesterol = Cholesterol – [HDL cholesterol + VLDL cholesterol]

VLDL cholesterol = Triglycerides ÷ 5

The average of the three repetitive samples, *i.e*. samples collected on day 0, 7 and 15 from each animal was considered for the statistical analysis. Data were analyzed to know the differences between the different serum components among various groups, with the aid of computerized statistical software, GraphPad Prism (San Diego, USA, 2010) by application of one-way ANOVA with Bonferroni's post test that compares all pairs of columns.

Results and Discussion

The mean ± values of β-ydroxybutyrate, glucose, total protein, blood urea nitrogen and lipid profile components are presented in Table 1.

The non significant (P>0.05) variation of beta-hydroxy butyrate (BHB) levels between different groups was in agreement with the observations made by Firat and Ozpinar (2002) who recorded similar BHB values at different stages of pregnancy in ewes. Bell *et al.* (1989) reported BHB levels of 0.4

Table 1: Mean ± SE values of biochemical parameters in different groups of Bannur ewes (n=6)

Parameters	Non pregnant (Group I)	Early pregnant (Group II)	Late pregnant (Group III)
β-hydroxybutyrate (mmol/L)	0.41 ± 0.05	0.43 ± 0.08	0.45 ± 0.01
Glucose (mg/dl)	55.67 ± 0.14[a]	54.96 ± 0.17[a]	49.17 ± 0.21[b]
Protein (g/dl)	6.84 ± 0.53	6.75 ± 0.49	6.63 ± 0.43
Blood urea nitrogen (mg/dl)	17.29 ± 0.05	17.13 ± 0.10	17.05 ± 0.13
Total cholesterol (mg/dl)	85.99 ± 0.30	85.84 ± 0.64	89.67 ± 1.27
HDL- cholesterol (mg/dl)	44.31 ± 0.72	46.37 ± 1.11	46.53 ± 0.80
Triglycerides (mg/dl)	17.18 ± 0.24	17.69 ± 0.21	18.33 ± 0.33
LDL- cholesterol (mg/dl)	38.24 ± 0.84	35.94 ± 1.70	39.47 ± 0.73
VLDL- cholesterol (mg/dl)	3.43 ± 0.04	3.53 ± 0.04	3.66 ± 0.06

Values in the same row with different superscripts differ significantly (P<0.05)

mmol/L before day 120 and 0.5 mmol/L on the day 120 in the pregnant ewes. Significantly higher concentrations of BHB were observed in pregnancy toxemia (Scott *et al.,* 1995; Balikci *et al.,* 2009 and Hefnawy *et al.,* 2011) in late pregnant ewes which was attributed to energy deficiency. Phiri *et al.* (2007) opined that the nutritional status of the animal reflects the value of BHB and if the concentration of glucose decreases the BHB increases. Long term starvation, energy deficiency in nutrition and poor quality hay are the factors causing hyperketonemia (Moghaddam and Hassanpour, 2008). In the present study, it was concluded that the BHB concentration of non pregnant, early pregnant and late pregnant Bannur ewes were within the physiological limits and none of the ewes suffered from subclinical pregnancy toxaemia which is a metabolic disorder.

The significantly ($P<0.05$) reduced levels of glucose in late pregnant ewes compared to early pregnant and non pregnant ewes were in agreement with the observations of Antunovic *et al.* (2004). Several authors have described the significantly reduced glucose levels in pregnant ewes of different sheep breeds (Firat and Ozpinar, 1996; Takarkhede *et al.,* 1999; Seidal *et al.,* 2006; Mogaddam and Hassanpour, 2008; Hafez *et al.,* 2010; Taghipour *et al.,* 2010; Antunovic *et al.,* 2011) which is attributed to more glucose usage by the developing fetus. However, Obese *et al.* (1994) and Firat and Ozpinar (2002) did not find any significant changes in the glucose levels between the different reproductive stages. In contrary, increased glucose content was recorded in late pregnant ewes (El-Sherif and Assad, 2001; Husted *et al.,* 2008 and Khatun *et al.,* 2011) which were attributed to decreased insulin responsiveness during late pregnancy. In the present study, it was opined that the decreased serum glucose levels was due to glucose utilization by the fetus for its development.

The non significant ($P>0.05$) variations in the total protein levels were recorded in the present study. However, Antunovic *et al.* (2004) observed statistically higher concentration of total protein in non pregnant than pregnant sheep. Significant decrease in total protein values during different stages of pregnancy in ewes were also observed (El-Sherif and Assad 2001; Antunovic *et al.,* 2011). In contrary, some researchers have reported significantly increased concentration of total serum protein during pregnancy in sheep (Takarkhede *et al.,* 1999; Khatun *et al.,* 2011).

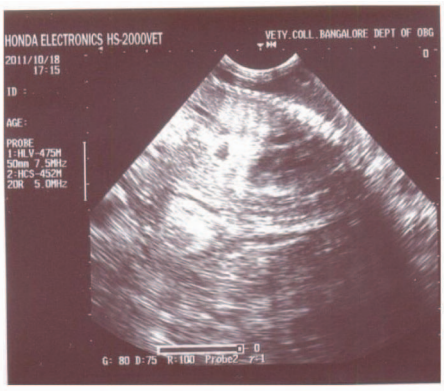
The findings for blood urea nitrogen (BUN) in the present study were in agreement with the observation made by Baumgartner and Pernthaner (1994) where they found non-significant difference between early and non pregnant ewes. But, a significant difference was reported between non pregnant and late pregnant Karakul ewes. Similar observations were also made by Seidal *et al.* (2006) and

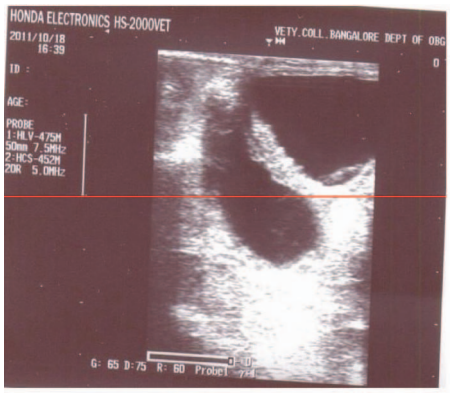
Antunovic *et al.* (2011) in sheep. Firat and Ozpinar (1996) found lower levels of blood urea nitrogen in late pregnant ewes compared to early and non pregnant ones. However, significant decrease in the levels of BUN concentration during late pregnancy were also reported by Husted *et al.* (2008), Antunovic *et al.* (2004) and Taghipour *et al.* (2010) in which they attributed to the decreased feed intake due to stress and hormonal changes during lambing. On the other hand, a significant increase in BUN levels were reported by El-Sheriff and Assad (2001) and Khatun *et al.* (2011) in different breeds of sheep which was attributed to increased levels of cortisol near parturition which caused increased catabolism of proteins. High plasma levels of BUN occurred in the ewes suffering from poor nutrition in last period of pregnancy (Balikci *et al.,* 2009).

Antunovic *et al.* (2004) opined that an increased level of cholesterol in circulation was due to stress during pregnancy. However, a significant decrease in the levels of cholesterol and triglycerides had been reported by Khatun *et al.* (2011) wherein the decreased levels of these lipid parameters were attributed for increased usage of cholesterol towards ovarian steroidogenesis.

The non-significant differences of HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol in the present study were in agreement with the findings of Antunovic *et al.* (2011). But, numerically slight increase in the concentration of these in the blood of late pregnant ewes could be due to consequence of transport of lipoproteins into the circulation from the liver.

In conclusion, in the present study, it was opined that all the parameters studied for Bannur ewes such as serum levels of â-hydroxybutyrate, glucose, total protein, blood urea nitrogen and lipid profile components such as total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and VLDL cholesterol were within the normal reference range described for sheep, suggesting that





either non pregnant or pregnant ewes did not suffer from any type of metabolic disorder.

References

Antunovic, Z., Novoselec, J., Sauerwein, H., Speranda, M., Vegara, M. and Pavic, V., 2011. Blood metabolic profile and some hormones concentration in ewes during different physiological status. *Bulgarian J. Agri. Sci.,* **17**: 687-695.

Antunovic, Z., Speranda, M. and Steiner, Z., 2004. The influence of age and the reproductive status to the blood indicators of the ewes. *Arch. Tierz. Dummerstorf.,* **47**(3): 265-273.

Balikci, E., Yildiz, A. and Gurdogan, F., 2009. Investigation on some biochemical and clinical parameters for pregnancy toxemia in Akkaraman ewes. *J. Anim. Vet. Adv.,* **8**(7): 1268-1273.

Baumgartner, W. and Pernthaner, A., 1994. Influence of age, season and pregnancy upon blood parameters in Austrian Karakul sheep. *Small Ruminant Res*., 13: 147-151.

Bell, A. W., Mcbridge, T. M. and Tall, D. H., 1989. Chronic heat stress and prenatal development in sheep: conceptus growth and maternal plasma hormones and metabolites. *J. Anim. Sci.,* **67:** 3289-3299.

Bergmeyer, H. U., 1985. Methods of enzymatic analysis. *Edn.* 3rd, (8):154-160.

Bowden, D. M., 1971. Nonesterified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants: A review. *Can. J. Anim. Sci.,* **51**(1): 1-13.

Burtis, C. A. and Ashwood, E. R., 1999. Tietz Textbook of Clinical Chemistry. *Edn.* 3rd., W.B. Saunders Company, Philadelphia., *pp.* 809-861.

EL-Sherif, M. M. and Assad, F., 2001. Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi arid conditions. *Small Ruminant Res.,* **40**: 269-277.

Firat, A. and Ozpinar, A., 1996. The study of changes in some blood parameters (Glucose, Urea, Bilirubin, AST) during and after pregnancy in association with nutritional conditions and litter size in ewes. *Turk. J. Vet. Anim. Sci.,* **20**: 387-393.

Firat, A. and Ozpinar, A., 2002. Metabolic profile of pre-pregnancy, pregnancy and early lactation in multiple lambing Sakiz ewes. 1. Changes in plasma glucose, â-hydroxybutyrate and cortisol levels. *Ann. Nutr. Metab.,* **46**(2): 57-61.

Gornall, A., 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem*., **177**: 751.

Govindaiah, M. G., Jayashankar, M. R. and Jain, A., 2006. Final report on network project on survey, evaluation and characterization of Mandya sheep breed. *pp* 4-7.

Hafez, A. E. F., Mahfouz, M. K. and Maksoud, H. A. A., 2010. Biochemical Changes in glutathione redox system and glucose regulation in late pregnant Ossimi ewes. *J. Anim. Sci.,* **6**(11): 742-748.

Hefnawy, A.E., Shousha, S. and Youssef, S., 2011. Hematobiochemical profile of pregnant and experimentally pregnancy toxemic goats. *J. Basic Appl. Chem.*, **1**(8): 65-69.

Husted, S. M., Nielsen, M. O., Blache, D. and Ingvartsen, K. L., 2008. Glucose homeostasis and metabolic adaptation in the pregnant and lactating sheep are affected by the level of nutrition previously provided during her late fetal life. *Dom. Anim. Endocrinol.,* **34**: 419–431.

Khatun, A., Wani, G. M., Bhat, J. I. A., Choudhury, A. R. and Khan, M. Z., 2011. Biochemical indices in sheep during different stages of pregnancy. *Asian J. Anim. Vet. Adv.,* **6**: 175-181.

Moghaddam, G. and Hassanpour, A., 2008. Comparison of blood serum glucose, beta hydroxybutyric acid, blood urea nitrogen and calcium concentrations in pregnant and lambed ewes. *J. Anim. Vet. Adv.,* **7**(3): 308-311.

Obese, E. Y., Tuah, A. K., Okai, D. B., Adomako, D., Frempong, E. B. and Said, A. N., 1994. Effect of diet and stages of pregnancy on blood chemistry and hematology of Djallonke sheep fed diets containing various levels of cocoa pod husk. *Ghana J. Biochem. Mol. Biol.,* **2**: 44-51.

Phiri, I.K., Phiri, A.M. and Harrison, J.S., 2007. The serum glucose and b-hydroxybutyrate levels in sheep with experimental *Fasciola hepatica* and *Fasciola gigantica* infection. *Vet. Parasitol.,* **143** :287–293.

Scott, P., Sargison, N., Penny, C., Pirie, R. and Kelly, J., 1995. Cerebrospinal fluid and plasma glucose concentration of ovine pregnancy toxemia cases, inappetant ewes and normal ewes during late gestation. *Br. Vet. J.*, **151** (1): 39-44.

Seidal, H., Novotay, J. and Kovac, C., 2006. Selected biochemical indices in sheep during pregnancy and after parturition. *Bull. Vet. Inst. Pulway.*, **50**:167-170.

Soroori, S., Veshkini., A. and Tajik., P. 2007. Assessment of the gestational age in Chall fetus by measuring the embryonic vesicle, biparietal diameter and crown rump length. *J. Vet. Med.,* **62**(2): 1-5.

Taghipour, B., Seifi, H. A., Mohri, M., Farzaneh, N. and Naserian, A., 2010. Variations of energy related biochemical metabolites during periparturition period in fat-tailed Baloochi breed sheep. *Iranian J. Vet. Sci. Tech.,* **2**(2): 85-92.

Takarkhede, R. C., Gondane. V. S., Kolte. A. Y. and Kekhate., 1999. Biochemical profile during different phases of reproduction in ewes in comparison to rams. *Indian Vet. J.*, **76**: 205-207.

Trinder, P., 1969. Determination of Glucose in blood using Glucose Oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.,* **6**:24-25.

Warnick, G.R., Benderson, V. and Albers, N. 1983. Selected Methods. *Clinic. Chem*. **10**, 91-104.

Williamson, D. H., Mellanby, J. and Krebs, H. A., 1962. Enzymic determination of Beta hydroxybutyric acid and acetoacetic acid in blood. *Biochem. J.,* **82:** 90-96.

Young, D.S., 1990. Effects of drugs on Clinical Laboratory Tests. *3rd Edn.*, AACC press, Washington, DC., pp 5.