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Haemato-biochemical and endocrine profiling of north western Himalayan Gaddi sheep during various physiological/reproductive phases

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Abstract

The study was aimed to provide baseline data regarding haemato-biochemical and endocrine profiling of Gaddi sheep found in north western Himalayan region of Himachal Pradesh, India. Each random sample was collected from 45 Gaddi sheep reared in government sheep breeding farm Tal, Hamirpur, India, during various reproductive phases viz. anestrus, breeding season and post partum period. Haematology revealed significantly higher ($P<0.05$) RBC as well as haematocrit values in pregnant animals ($n=23$) during breeding season than during other reproductive phases. The number of platelets were significantly lower ($P<0.05$) and MCH, MCHC values were statistically higher ($P<0.05$) during postpartum period than during other reproductive phases. Blood biochemistry revealed significantly higher ($P<0.05$) concentrations of plasma cholesterol (83.98 ± 3.68 mg/dl), plasma calcium (71.06 ± 1.52 mg/l), magnesium (18.21 ± 0.53 mg/l), potassium (5.10 ± 0.13 mEq/l) and significantly lower ($P<0.05$) concentrations of plasma total protein (5.75 ± 0.31 gm/dl), globulin (3.04 ± 0.29 gm/dl) and sodium (138.83 ± 1.83 mEq/l) during postpartum period in comparison to other reproductive phases. Endocrine profile revealed significantly higher ($P<0.05$) serum estrogen (60.97 ± 1.24 pg/ml) and T4 (6.0 ± 0.27 µg/ml) concentrations during postpartum phase. Similarly, significantly higher ($P<0.05$) serum progesterone (5.16 ± 0.76 ng/ml) as well as TSH (0.70 ± 0.14 µg/ml) concentration were recorded during pregnancy. From the study it can be concluded that physiological status significantly affects the blood metabolic and endocrine profile in Gaddi sheep.

Keywords: Biochemistry, Endocrinology, Gaddi sheep, Haematology.

Introduction

Gaddi sheep is well recognized dual purpose breed of Himachal Pradesh, India. It is well adapted to migration and is least susceptible to many of known diseases occurring in exotic sheep. Although, sufficient literature is available on the haemato-biochemical and endocrine profiles of exotic sheep breeds like Merino (Jelinek *et al.*, 1986), Bighorn (Borjesson *et al.*, 2000), Württemberg (Antunovic *et al.*, 2002), Sakiz-Ivesi (Yokus *et al.*, 2004) Comisana (Piccione *et al.*, 2009) and Tsigai (Antunovic *et al.*, 2011) during different reproductive/physiological phases but literature on Gaddi sheep is scarce.

Blood metabolic profile (BMP) is a set of diagnostic procedures that are based on determining the various indicators in the blood of animals (Van Saun, 2000). The most common indicators in the blood of animals used in the preparation of BMP are biochemical and haematological parameters. Pregnancy and lactation are physiological stages considered to modify metabolism in animals (Krajnicakova *et al.*, 2003; Iriadam, 2007). Pregnancy and lactation, especially in the early stages, are very demanding physiological state of the organism when nutritional requirements are increased (Goff and Horst, 1997). During lactation the mammary gland secretory cells utilize 80% of the circulating metabolites

in the blood for milk synthesis (Karapehliyan *et al.*, 2007). Moreover, metabolism of mineral substances plays a significant role in regulation of physiological function of the puerperal period.

The aim of present study was to determine the influence of physiological status on BMP and to generate baseline data of haemato-biochemical and endocrine profile of north western Himalayan Gaddi sheep.

Materials and Methods

Forty five healthy adult Gaddi ewes (2-5 years old) reared in government sheep breeding farm Tal, Hamirpur Himachal Pradesh, India (31.63° N, 76.58° E) were undertaken for study. Ewes were kept in stables as well as grazed and were fed 300 g of concentrate feed (40% maize, 25% GNC, 15% wheat bran, 13% DORB, 5% molasses, 1% minerals and 1% salt), 300 g of meadow hay and *ad libitum* water daily. Male segregation was practiced. Random blood samples were collected aseptically from the jugular vein. Each collection was aliquoted into 3 parts (2 ml whole blood in 3% dipotassium EDTA for haematology, 5 ml whole blood in heparinized polyvinyl vials for plasma separation and 5 ml in similar vials without anticoagulant for serum separation). The plasma was separated by centrifugation of the heparinized blood at 3000 r.p.m for 10 minutes. The blood plasma and serum

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were stored at - 20°C in 2 ml capacity micro-centrifuge tubes in duplicate, pending analysis. The random blood samples were collected during different reproductive/physiological phases viz. anestrus (one month prior to mating; in July), breeding season (one month after mating; in October) and postpartum/early lactation (one month after lambing; in February). Haematological parameters (Hb, PCV, TEC, TLC, MCV, MCH, MCHC and PLT) were done using haematology analyser BC 2800 Vet, Mindray, Shanghai as per the manufacturer's instructions. This instrument uses two independent measurement methods, viz. the impedance method for determining the WBC, RBC and platelet data and the colorimetric method for determining the haemoglobin concentration. Blood biochemical parameters (Total Protein, Cholesterol, Albumin, Globulin and Albumin Globulin ratio) using Agappe Diagnostic kits in Automated Biochemistry Analyzer 5010 (Boehringer Mannheim) at wavelengths 546 nm for protein and albumin and 492 nm for cholesterol in stored plasma samples. Hormonal assays (E2, P4, T3, T4 and TSH) were done from stored serum samples (at - 20°C) using Lumax™ Model 4101 Chemiluminescence Immuno Assay (CLIA) Strip Reader (Monobind, Inc. USA) as per manufacturer's instructions. Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K) and Chlorine (Cl) were estimated from plasma using atomic absorption spectrophotometer (Perkin Elmer AAS 400). The wavelengths used for estimation of Ca, Mg, Na, K and Cl were 422.7 nm, 285.2 nm, 324.8 nm, 213.9 nm and 240.7 nm respectively. Statistical analysis was done by "t-test" using SAS (2002).

Results

The haemato-biochemical and endocrine profile of Gaddi sheep during different reproductive phases has been tabulated in Table 1, 2, 3 and 4 respectively. Haematology of Gaddi sheep revealed significantly higher ($P<0.05$) RBC as well as haematocrit values during pregnancy than other reproductive phases. The number of platelets were significantly lower ($P<0.05$) and MCH and MCHC values were significantly higher ($P<0.05$) during postpartum period than during other reproductive phases.

Analysis of blood biochemical parameters revealed that plasma cholesterol concentration was significantly higher during post partum/early lactation phase than other phases. Total plasma protein and globulin concentration were significantly lower during post partum phase than during other reproductive phases. Albumin to Globulin ratio was lowest during pregnancy confirming the strong immune status of individual ewe during that phase.

Study of mineral analysis revealed that plasma Ca, Mg and K concentrations were significantly higher ($P<0.05$) during post partum/early lactation phase than during other reproductive phases. Contrarily plasma

Table 1. Hematological profile of Gaddi sheep during various reproductive phases.

Reproductive phase	W.B.C. ($\times 10^9/L$)	R.B.C. ($\times 10^{12/L}$)	H.G.B. (g/dl)	H.C.T. (%)	M.C.V. (fl)	M.C.H (pg)	MCHC (g/dl)	RDW (%)	PLT ($\times 10^9/L$)	MPV (fl)	PDW	PCT (%)
Anestrus (n=45)	10.43±0.45	9.75±0.22	9.52±0.17	30.03±0.56 ^b	32.81±1.57	9.68±0.17 ^a	32.72±0.76 ^a	16.90±0.20 ^a	411.73±27.43 ^a	4.24±0.06	15.39±0.08 ^a	0.17±0.01
Breeding season (n=45)												
Pregnant (n=23)	9.62±1.15	11.43±0.38 ^a	10.96±0.42	34.49±0.91 ^a	30.34±0.61	9.54±0.23 ^a	31.64±0.63 ^a	16.99±0.29 ^a	391.58±35.68 ^a	4.13±0.09	15.28±0.09 ^a	0.16±0.02
Non pregnant (n=22)	11.73±5.65	9.90±0.97	10.04±0.75	31.92±1.95	29.43±0.74	9.09±0.38 ^a	31.12±1.21 ^a	16.57±0.29 ^a	502.73±65.75 ^a	4.18±0.08	15.42±0.07 ^a	0.22±0.03 ^a
Postpartum (n=23)	7.72±1.10	9.18±0.42 ^b	10.86±0.39	28.86±0.97 ^b	31.70±0.66	12.16±0.42 ^b	36.66±0.62 ^b	15.37±0.25 ^b	199.94±25.85 ^b	4.08±0.08	16.08±0.12 ^b	0.12±0.02 ^b

Means with different superscripts within a column differs significantly ($P<0.05$).

Table 2. Biochemical profile of Gaddi sheep during various reproductive phases.

Reproductive Phases	Biochemical profile				
	Cholesterol (mg/dl)	Total Protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Alb: Glob
Anestrus (n=45)	59.54±2.42 ^a	6.86±0.14 ^a	3.00±0.09	3.87±0.16 ^a	0.77
Breeding season (n=45)					
Pregnant (n=23)	71.1±3.25 ^a	7.72±0.23 ^a	3.00±0.09	4.75±0.22 ^a	0.63
Non pregnant (n=22)	63.05±2.99 ^a	7.02±0.37 ^a	3.13±0.22	4.05±0.42 ^a	0.77
Post partum (n=23)	83.98±3.68 ^b	5.75±0.31 ^b	3.14±0.09	3.04±0.29 ^b	1.03

Means with different superscripts within a column differs significantly ($P<0.05$).

Table 3. Mineral profile of Gaddi sheep during various reproductive phases.

Reproductive phases	Mineral profile				
	Ca (mg/l)	Mg (mg/l)	Na (mEq/l)	K (mEq/l)	Cl (mg/dl)
Anestrus (n=45)	53.46±2.79 ^a	11.69±0.80 ^a	145.60±1.76 ^b	4.23±0.09 ^a	119.28±2.77
Breeding season (n=45)					
Pregnant (n=23)	58.89±2.34	14.41±1.28	149.65±1.39 ^b	3.99±0.08 ^a	127.52±6.20
Non pregnant (n=22)	62.87±2.90	14.79±1.02 ^b	150.57±1.63 ^b	4.21±0.14 ^a	133.82±6.79
Post Partum (n=23)	71.06±1.52 ^b	18.21±0.53 ^b	138.83±1.83 ^a	5.10±0.13 ^b	123.70±3.83

Means with different superscripts within a column differs significantly ($P<0.05$).

Table 4. Hormonal profile of Gaddi sheep during various reproductive phases.

Reproductive phase	Reproductive hormones				
	E2 (pg/ml)	P4 (ng/ml)	T3 (µg/ml)	T4 (µg/ml)	TSH (µg/ml)
Anoestrus (n=45)	45.65±2.51 ^a	1.44±0.22 ^a	1.63±0.24	4.11±0.36 ^b	0.54±0.03 ^{ab}
Breeding season (n=45)					
Pregnant (n=23)	49.29±2.43 ^a	5.16±0.76 ^b	2.04±0.30	4.80±0.48 ^{ab}	0.70±0.14 ^a
Non- pregnant (n=22)	46.57±3.93 ^a	1.57±0.34 ^a	1.46±0.33	3.47±0.44 ^b	0.50±0.05 ^{ab}
Post-partum (n=23)	60.97±1.24 ^b	0.95±0.21 ^a	2.04±0.18	6.0±0.02 ^a	0.24±0.10 ^b

Means with different superscripts within a column differs significantly ($P<0.05$).

sodium concentration was significantly lower ($P<0.05$) during post partum phase than other phases.

Endocrinological profile of Gaddi sheep revealed significantly higher ($P<0.05$) concentrations of estradiol and T4 during postpartum phase in comparison to other reproductive phases. Likewise significantly higher ($P<0.05$) progesterone (P4) concentrations were observed during pregnancy. Contrarily significantly lower ($P<0.05$) TSH concentrations were observed during postpartum phase.

Discussion

Significantly higher ($P<0.05$) haemoglobin and haematocrit concentration in the pregnant ewes are probably due to increased demand for oxygen and the requirements of higher metabolic rate for pregnancy. This rise may also be associated with increased RBC volume during pregnancy. These findings are in agreement with the earlier reports (El-Sherif and Assad, 2001; Antunovic *et al.*, 2011).

The RBCs and PCV subsequently decreased during early lactation/postpartum phase, which might be attributed to the hemodilution effect resulting from an increase in plasma volume and/or the increasing water mobilization to mammary gland through the vascular system (El-Sherif and Assad, 2001; Bamerny, 2013). Whereas Mohammed *et al.* (2014) reported high haemoglobin and PCV values during lactations than pregnancy. MCH and MCHC were significantly higher ($P<0.05$) during the postpartum phase. Similar findings were earlier reported for MCH concentrations by Antunovic *et al.* (2011). Significantly lower ($P<0.05$) concentrations of platelets were recorded during postpartum phase in this study.

Total Plasma proteins showed a decreasing trend from breeding and anestrus phase to postpartum/early lactation phase which might be due to the preparation of reproductive system during pregnancy (growth of uterus) which requires large quantity of protein

during pregnancy (Vihan and Rai, 1983). The decrease of protein concentration after parturition is due to using protein for colostrum production (Williams and Millar, 1979). Similar results were also reported by Juma *et al.* (2010), Antunovic *et al.* (2011), Bamerny (2013) in goats and Boudebza *et al.* (2014). However, Baumgartner and Pernthaner (1994) observed no effect of physiological stage on total protein. Similar decreasing trend were also recorded for globulin (Antunovic *et al.*, 2011; Bamerny, 2013) during postpartum/early lactation phase which might be due to accumulation of proteins especially immunoglobulins (Ig) which occurs as a preparatory step for the bulk secretion of Ig into colostrum after lambing (El-Sherif and Assad, 2001; Karapehliyan *et al.*, 2007; Kaneko *et al.*, 2008). Globulin might also be used for formation of milk protein (casein). Albumin globulin ratio was also recorded highest during the postpartum phase indicating the immune status of individual animal. Significantly higher ($P<0.05$) levels of cholesterol were recorded during postpartum phase in present study which might be due to its role in ovarian steroidogenesis. Similar results were also reported by Krajnicakova *et al.* (2003) and Piccione *et al.* (2009).

Significantly higher ($P<0.05$) concentrations of Ca, Mg and K were recorded during postpartum/early lactation phase in present study. Ca and Mg are required for normal skeletal development and also very good amount is secreted in the milk along with blood. Similar trends were observed by Antunovic *et al.* (2011) who found higher concentrations of Ca during postpartum phase. Reverse trends were observed by Gawish and El-Shaer (2006) and Bamerny (2013). Significantly lower ($P<0.05$) concentrations of Mg were observed by Bamerny (2013) during postpartum in goats. No change was observed in Cl concentrations in our study. Whereas significantly higher ($P<0.05$) levels of Cl were observed during lactation by Antunovic *et al.* (2011). Changes in the concentrations of electrolytes in blood of ewes, particularly during the lactation were mainly associated with increased requirements for intensive growth of fetus in high pregnancy due to increased synthesis of milk in lactation (Kaneko *et al.*, 2008). Significantly lower ($P<0.05$) concentrations of Na were recorded in our study which corresponds to results by Antunovic *et al.* (2011) whereas Bamerny (2013) reported no variation in Na concentrations during different physiological phases in goats.

Significantly higher ($P<0.05$) estradiol concentration during postpartum/early lactation phase in present study were suggestive of initiation of ovarian activity. Likewise significantly higher ($P<0.05$) progesterone concentration during the pregnancy can be attributed to the presence of Cl verum on ovary. T3 hormone concentrations were approximately similar during pregnancy as well as postpartum. Significantly higher

($P<0.05$) T4 concentration was recorded during postpartum/early lactation phase. Contrary results with regards to T3 concentration were observed by Hart *et al.* (1978), Karapehliyan *et al.* (2007) and Antunovic *et al.* (2011). Lower blood T3 concentration could reduce the rate of oxidation and the rate of continuous breakdown and formation of protein and fat in the most, if not all, mammary tissue (Riis and Madsen, 1985). Todini *et al.* (2007) reported increased thyroid activity and blood hormone levels during gravidity in all mammalian species. The thyroid hormones maintain the homeostasis of energy and protein metabolism, thermoregulation, growth and productivity parameters (Huszenicza *et al.*, 2002). Thyroid hormones have an important role to play in the development of the mammary gland, the synthesis and release of important milk proteins such as α -lactalbumin (Ramos *et al.*, 2000). Pezzi *et al.* (2003) reported lower blood T4 and T3 concentrations at the initiation of lactation which increase during lactation and subsequently decrease during the dry period. Significantly higher ($P<0.05$) TSH concentrations were recorded in present study during pregnancy.

In conclusions effect of physiological status was significantly manifested on the BMP and concentrations of hormones (thyroid hormones). Increased metabolic activities due to lactation significantly affected certain biochemical parameters in Gaddi sheep. This study warrants further well planned intensive investigation of these blood metabolic as well as hormonal profile during different physiological stages especially peripartum period.

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