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EVALUATION OF SERUM ELECTROLYTE CONCENTRATIONS IN GAROLE SHEEP OF SUNDARBAN REGION

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ABSTRACT

Garole sheep is very popular for its biannual lambing and disease resistance characters. To evaluate some selected serum electrolytes (Na, K and P) ions in apparently healthy Garole sheep at Sundarban region under Shyamnagar Upazila of Satkhira district, blood samples were collected from 20 Garole sheep (8 males and 12 females) of aged about 6-48 months containing male lambs, rams, female lambs, pregnant ewes and lactating ewes according to the age, sex and physiologic status. Serum was separated and used for biochemical analysis for the measurement of target serum electrolytes. The results of the range and mean ± standard error (SE) of serum electrolytes are as follows: sodium 280-400 ppm and 342.96±7.19 ppm, potassium 30-55 ppm and 34.37 ± 0.72 ppm and phosphorus 40-90 ppm and 70.98 ±1.76 ppm respectively. The significant differences ($P<0.05$) between males and females in serum ions levels was recorded. It can be concluded that Na, K and P ion level in serum was higher in male than female and significant differences in ion level varies according to physiologic status of female Garole sheep.

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INTRODUCTION

The Garole is a prolific breed of sheep. High prolificacy in sheep carrying the Booroola gene (FecB) is the result of a mutation in bone morphogenetic protein receptor-IB (BMPR-IB) which had previously been identified in Garole sheep from the Sundarban region of West Bengal (Davis et al, 2002). There is evidence that the breed has originated from the sheep brought by the Tibetan traders and traded in the plains of Bengal during the seventeenth till the nineteenth century. Garole is a native and local sheep having distinct and separate phenotypic characters, productive performances of their own is not thoroughly characterized and established as Breed. This distinct but inadequately studied line of small sheep (locally named as Garole) is the dominant domestic species isolated so far pointed out at this extended coastal swampy Sundarban delta of West Bengal (Banerjee, 2008). The sheep Garole is very popular for its bi-annual lambing, multiple birth, grazing on aquatic weeds and grass in knee-deep water and disease resistance characters (Banerjee, 2008). They are small in size, produce rough wool, good quality skin, manure and low fat mutton. This small compact meat type animal predominantly white in color, are owned by landless and small farmers which provide principal source of income during agriculturally lean period and govern socio-economic status of the sheep farmers of this region. This sheep is the latest sensations in the world of domestic species by virtue of its prolificacy, lambing frequency, disease resistance and other extraordinary merits rarely or not even observed in other sheep breeds of the world. The involvement of oestradiol in the regulation of fluid and electrolytes balance has long been recognized (Khan, 1993). Oestradiol influences salt retention in systemic circulation and alter ion permeability in various epithelial cells. The oestrous cycle in Garole sheep is characterized by cyclic fluctuations in major ovarian steroids. During the follicular phase of oestrous cycle, oestradiol concentration increases and then declines to the luteal phase (Gaafar et al., 2005).

Blood biochemical value is an important tool for assessment of the health status of animals and this has been shown to vary even in healthy animals due to differences such as: sex, season and oestrous cycle phase (Tamukai et al., 2011; Yaqub et al., 2011). Reproductive status and sex variations in blood electrolytes have been reported in several studies (Tamukai et al., 2011; Stojevic et al., 2005). These electrolytes are very important in homeo-stasis, nerve impulse transmission, muscle contraction, ovarian steroidogenesis and the process of ovulation. The electrolytes dissolved in body fluids are not simply inert organic salts, but play a vital role in life processes. Electrolytes serve as cofactors in many enzymatically mediated metabolic reactions. Maintenance of osmotic pressure and pH of body fluids within narrow limits is essential for these biochemical reactions. There is lack of information on changes in serum biochemical parameters during the oestrous cycle in Garole sheep. The presence of the super-ovulatory gene and other important character in these sheep has seldom been seriously studied in India (Bhattacharya, 1989). Therefore the present experiment was undertaken to assess the serum electrolyte of Garole sheep and to compare the serum electrolytes according to age, sex and physiologic status of Garole sheep.

MATERIALS AND METHODS

Experimental animal and collection of sample

Blood samples were collected from 20 apparently healthy Garole sheep (Figure 1 and 2) of which 8 males were divided into 7-12 months aged as male lambs and 16-48 months as rams and 12 females were divided into female lambs 6-12 months, pregnant ewes 13-42 months and lactating ewes 18-48 months) that were selected from Shyamnagar Upazila of Satkhira district. Five milliliter (ml) blood from each individual animal was drawn from the jugular vein puncture using a 5ml syringe and blood was poured into transparent test tube without using any anticoagulant and transported to the laboratory. Serum was collected from Garole sheep reared in Shyamnagar Upazila of Satkhira district in the division of Khulna, which is situated at 22.3306°N latitudes and 89.1028° E longitudes and the laboratory work was done in the department of Physiology of Bangladesh Agricultural University, Mymensingh.

Preparation of serum

The tubes containing blood were placed in slanting position at room temperature for 1 hour. Then the clot was detached from the wall of the test tube carefully and allowed it to settle down and afterward serum was collected. Collected serum was centrifuged at 3000 rpm for 15 minutes to obtain clear serum and then stored at -20^{0}C until used.

Figure 1. Apparently Garrole

Figure 2. Blood collection from Garrole

Figure 3. Serum sample and di- acid mixture

Figure 4. White fume evolves after completion of digestion on sand bath

Figure 5. Filtering of digested samples

Biochemical analysis

Digestion of Serum for biochemical analyses

Three hundred microliter of serum was taken in 150 ml conical flask and 2 ml of di-acid mixture (HNO_3: $HClO_4$= 2:1) was added to it. It was kept overnight at room temperature by covering the mouth of the conical flask with aluminum foil paper. Then the conical flask was placed on sand bath followed by heating at temperature 200^0C for 15 minutes. After a few minutes brown fume was evolved, which indicated the starting of digestion process. Finally, white fume was seen by clearing the solution (Figure 3 and 4). At the bottom of the conical flask about 1 ml solution was noticed. Three blank solutions were also performed along with the sample solutions. After that, heating was stopped and the digested sample was cooled for 20 minutes. Then about 10 ml distilled water was added to each conical flask to avoid filtration problem. This solution was filtered (Figure 5) into a 50 ml volumetric flask and the volume was made up to the 10 ml with distilled water. The 10 ml solution was then transferred into a plastic bottle for the further utilization. The plastic bottle was stored at a room temperature.

Determination of Sodium, Potassium and Phosphorous using flame photometer

Measurement of potassium (K+) and Sodium (Na+) was carried out by using flame photometry method (George et al., 1954) and Phosphorus (P3-) was determined by using spectrophotometry method (Burns *et. al*, 1992). Sodium and potassium were determined in biological fluids by the technique of emission flame photometry and in case of phosphorus spectrophotometer method. This relies on the principle that an alkali metal salt drawn into a non-luminous flame will ionize, absorb energy from the flame and then emit light of a characteristic wavelength as the excited atoms decay to the unexcited ground state. The intensity of emission is proportional to the concentration of the element in the solution. If sprinkle table salt (NaCl) into a gas flame then it glows bright orange (KCl gives a purple color). This is the basic principle of flame photometry. A photocell detects the emitted light and converts it to a voltage, which can be recorded. Since Na+ and K+ emit light of different wavelengths (colors), by using appropriate colored filters the emission due to Na+ and K+ (and hence their concentrations) can be specifically measured in the same sample.

Determination of Sodium:

Beckman photomultiplier attachment No. 4300 was used. The wave-length knob was set at 590 rnp and the slit width at 0.01 mm. Per-cent emission was recorded following the method as described by (George et al., 1954).

Determination of Potassium:

Potassium was determined with flame emission digital flame photometer (Model:Labtronics LT65). The samples were aspirated into a gas flame. Per-cent emission was recorded following the method as described by (George et al., 1954).

Determination of Phosphorus:

Phosphorus content was determined measuring the color intensity with the help of digital spectrophotometer (Model : Labtronics LT31) at 660 nm wave length as per method (Burns et al., 1992).

RESULTS

Average concentration of sodium, potassium and phosphorus in test sera of Garole sheep are presented in Table 1 and the values of these electrolytes according to sex, age and physiologic status are presented in Table 2 and 3. Serum sodium concentration in healthy Garole sheep was found to be 342.96±7.19 ppm, ranged from 280-400 ppm. It ranged 348.19±11.27 ppm to 339.47±9.61 ppm in males and females with significant differences between them ($P<0.05$) (Table 1 and 2). On the other hand, the recorded mean value of serum potassium concentration was found to be 34.37 ± 0.72 ppm. The serum potassium was significantly

(P<0.05) higher in males 36.48±0.44 ppm than in females 32.97±0.99 ppm. The recorded mean value of serum phosphorus concentration was found to be 70.98±1.76 ppm. It was ranged 71.20±2.72 ppm in males and 70.84±2.40 ppm in females. In addition, serum phosphorus levels did not differ significantly (P<0.05) in male and females sheep (Table 2).

Table 1. Average concentration of sodium, potassium and phosphorus in sera of Garole Sheep

Electrolyte	Ranges	Mean ± Standard Error (SE)
Na (ppm)	280-400	342.96±7.19
K (ppm)	30-55	34.37 ± 0.72
P (ppm)	40-90	70.98 ±1.76

Table 2. Serum sodium, potassium and phosphorus concentrations according to sex in Garole sheep

Electrolytes	N (number)	Sex	Mean ± SE
Na (ppm)	8	Males	348.19±11.27**
	12	Females	339.47±9.61
K (ppm)	8	Male	36.48±0.44
	12	Female	32.97±0.99
P (ppm)	8	Male	71.20±2.72
	12	Female	70.84±2.40

* = Significant. (P<0.05), ** = Highly significant (P<0.01)

Table 3. Serum sodium, potassium, and phosphorus concentrations (Mean± SE) according to sex, age and physiologic status of Garole sheep

Groups		No. of sheep	Na (ppm)	K (ppm)	P (ppm)
Male	Lambs (7-12 Months)	4	346.32±14.12*	36.90±0.45*	71.88±2.61
	Rams (16-48 Months)	4	350.05±19.76**	36.05±0.77*	70.51±5.24
Female	Lambs (6-12 months)	4	345.17±15.97*	37.23±1.09**	71.25±4.87**
	Pregnant ewes (13-42 months)	4	342.17±16.72	30.57±0.54	69.15±4.35
	Lactating ewes (18-48 months)	4	331.07±21.9	31.10±0.38	72.11±4.41

* = Significant. (P<0.05), ** = Highly significant (P<0.01)

According to sex, age and physiologic status (Table 3), serum sodium concentrations were found 346.32±14.12 ppm; 350.05±19.76 ppm; 345.17±15.97 ppm; 342.17±16.72 ppm and lactating ewes 331.07±21.9 ppm in male lambs, rams, female lambs, ewes and lactating ewes respectively. The serum sodium concentration was significantly (P<0.05) lower in lactating group compared to other groups (Table 3). According to the data presented in Table 3 serum potassium concentrations in male (lambs and rams), female lambs, pregnant and lactating ewes were: 36.90±0.45 ppm, 36.05±0.77 ppm, 37.23±1.09 ppm, 30.57±0.54 ppm and 31.10±0.38 ppm, respectively. It has been shown that serum potassium concentrations in males as well as in female lambs were significantly (P<0.05) higher than those of pregnant and lactating ewes.

DISCUSSION

The study represent the measurement of serum electrolytes (Na, K and P) of Garole sheep considering various factors viz sex (male and female), age (lamb and adult), physiological status (pregnancies and lactations) and nutritional status of the animals. In general, the serum concentrations of Na, K and P measured in this work are similar to the work of many researchers (Radostits et al., 2007; Aiello, 2008; Kaneko, 2008) who reported the normal levels of serum ions in many animals including sheep. Others, such as Piccione et al. (2011) studied the seasonal concentrations of Na, K, P and Cl ions all over a year and he found variations in those levels. Relative declining of the serum ions in lactating animals could be compared with the study of Mayer and Fiechter (2012) who studied the milk constituents in sheep and they supposed that milk contains Na, K and Cl and those may be decreased by milking. Along with this weaning time is an important factor in the variations of serum electrolytes and this could be compared with the study of Lepherd et al. (2009) who described many properties related to weaned Merino lambs (9-16 weeks), they analyzed serum Na, K and P ions as well as complete blood picture, enzymes and other nutrients where they found the effect of weaning on their dams and there was an important correlation between age of the dam and times of newborns.

Food supplements were necessary for sheep nutrition to maintain certain levels of ions and to facilitate their absorption and metabolism. So that Ghanem et al. (2008) suggested that vitamin C administration to sheep and found that this orally supplemented vitamin C was also effective in alleviating stress and prevents further loss of salts and minerals. Also, Abbeddou et al. (2011) found that feeding sheep on certain agro-industrial byproducts and forages has side-effects on physicochemical properties of milk with increase the ions levels such as Na, K, Ca, Mg and P or maintain their values within normal even in milking ewes and did not affect milk or its products. On the other side, a research to Gunes et al. (2008) proposed that erythrocyte Na and K concentrations may be included in the metabolic profile testing parameters and they found positive correlation between birth rate and mean Na and K concentrations.

Another study on Garole sheep performed by Hamadeh et al. (2009) and they discussed the role of vitamin C on restricted water Garole sheep. They noticed elevated serum protein, albumin, globulin urea, creatinine, and ions (Na, K, P and Cl) concentrations in all water restricted sheep; their results were slightly near to the results obtained in the work concerning Na, K, P and Cl ions.

In contrast, a study by Michalek et al. (2010) expressed blood plasma concentrations of Na, K and P ions in goats were not significant differences in pregnant and nonpregnant goats. They implicated changes to varied levels of aldosterone and progesterone and their mutual proportions differing between the groups. Moreover, according to the presented data in Table 3 serum phosphorus concentrations in male (lambs and rams), female lambs, pregnant and lactating ewes were as follows: 71.88±2.61ppm, 70.51±5.24ppm, 71.25±4.87 ppm, and 69.15±4.35 and 72.11±4.41 ppm, respectively. It has been shown that there was no significant difference with serum phosphorus concentration among the male and female groups.

Serum Na concentrations have strong positive correlation with P in the female lambs but it was shown negative correlation with P in rams. Also the correlation recorded between K and P was strongly positive among male lambs, female lambs and lactating but not in pregnant ewes. Another negative correlation was recorded between Na and P in rams. There was certain positive correlation between Na and K levels in male lambs.

CONCLUSION

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh to evaluate of some serum electrolyte concentrations in Garole sheep of Sundarban region. The study was conducted in 20 clinically healthy Garole sheep which were selected from Shyamnagar Upazila of Satkhira district. The sheep were divided into two groups (8 males and 12 females). Then the male groups were subdivided into 7-12 months as male lambs and 16-48 months as rams and the female groups were subdivided into female lambs 6-12 months, pregnant ewes 13-42 months and lactating ewes 18-48 months. The sheep were grazed on naturally growing grass and showed no clinical signs of disease. Blood sample was collected from individual animal through the jugular vein puncture without using any anticoagulant. Then the samples were preserved, processed, transported to Department of Physiology and different analytes constituents were determined using appropriate analytical techniques.

According to sex, age, and physiologic status, serum sodium concentrations was significantly (P<0.05) lower in lactating group compared to other groups and serum potassium concentrations in males as well as in female lambs were significantly (P<0.05) higher than those of pregnant and lactating ewes . There are many factors concerning variation in serum ions viz. Na, K, and P studied in this research related to multiple factors, one of them sex, and here we found significant differences in all ions measured (values in males were higher than those in females) and this might be due to certain hormones as well as exhaustion of those ions in pregnancy and lactation in ewes. Another factor is age of the ewe and its status (number of pregnancies and lactations). There were stress factors due to multiple pregnancies and lactations affect serum levels of those ions (generally they decline with age progressing).

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