SERUM ELECTROLYTE AND MINERAL VARIATIONS DURING PREGNANCY, LACTATION AND DRY PERIODS IN BUCHI SHEEP

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The present study was designed to ascertain serum sodium, potassium, chloride, copper and zinc concentrations during pregnancy, lactation and period of Buchi sheep. A total of 60 Buchi sheep maintained at private farm in Bahawalpur District were divided into two groups (mated and not-mated). Mated ewes were identified as group M (n=30) whereas not mated ewes were recognized as group NM (n=30). Blood samples were taken from each group (M and NM) after every 3 month over 1 year period from the jugular vein. The periods were as follows: 1 = early pregnancy (October), 2 = late pregnancy (January), 3 = lactation (April), and 4 = dry season (July). Serum sodium, potassium, chloride, copper and zinc were determined on a clinical chemistry whereas copper and zinc were determined by atomic absorption spectrophotometer. The mean serum sodium, potassium, chloride concentrations varied non-significantly (P>0.05) during early to late pregnancy in group M ewes, whereas in group NM ewes, the zinc concentration differed non-significantly (P<0.05) among different periods. The results of present study showed that serum sodium, potassium, chloride concentration were almost comparable for M, NM group ewes and season, physiological state do not have any effect on these metabolites, whereas, the copper and zinc concentrations changes during pregnancy. It was suggested that while elucidating the electrolyte and mineral status, period of the year and physical status of the Buchi sheep should be taken into consideration.

Key word: sheep, serum, electrolyte, mineral, pregnancy, lactation, dry period

Sheep and goat have been used for centuries by mankind for various purposes like milk, meat, fibre, skin and even for work under different management and environmental conditions. In subsistence agriculture, common in most of the developing countries and in arid regions of the world, small holders and landless farmers keep small ruminants (sheep and goat) for sale, consumption and personal use. In Pakistan small ruminants means hard cash for small-scale farmers. Goats and sheep are an attractive option for poor farmers as these animals provide the opportunity to create value added products, such as mutton, milk, skins, fibre and manure, at low cost. Pakistan is endowed with 28.1 million of sheep which produced 36 000 tonnes milk...
for human consumption and sheep, goat produced 616 000 tonnes of mutton during 2013-14 collectively (Anonymous, 2014). Buchi is one of the important breed of sheep commonly found in Bahawalpur and Bahawalnagar Districts of Punjab, Pakistan. Wool yield per head is 4 kg/annum, and fibre diameter is 30μ. It is mainly raised for wool purpose.

Reference values are of great importance for the correct interpretation of biochemical data (Garcia et al., 2000). In animals, a large number of factors such as species, type or race, sex, age, nutritional and health status, as well as seasonal and physiological variations such as those in pregnancy and lactation can affect serum chemistry and mineral levels (Aken et al., 1991; Alonso, 2000; Garcia et al., 2000; Yokus et al., 2004; Yokus et al., 2006). The macrominerals during the dry period have an impact on sheep reproduction (Braithwaite, 1976). However, clinical signs of mineral deficiencies, imbalances, or excesses are generally not apparent until near, at, or following parturition. Macromineral needs increase because both the infant and mother are in need of them during pregnancy (Kulcu and Yur, 2003). Trace minerals participate in many important catalytic, enzymatic and structural functions of higher vertebrates, and their concentrations in mammals depend on several environmental and biological conditions (Underwood, 1971).

Metabolism of mineral substances plays a significant role in the regulation of physiological functions of the pueral period. Their concentrations in the blood circulation represent homeostatic mechanisms that are in a close relationship with neurohumoral regulation (Krajnicakova et al., 2003). On the other hand minerals are important as essential nutrients in the diet of animals. Physiological status might modify animal’s requirement to these elements (Ahmed et al., 2000). All animals require minerals (Na, K,) for growth, reproduction, lactation and performance, which often affect the specific requirement, serve as structural or catalytic components of enzymes and regulate many physiologic systems especially pregnancy and lactation (Mbassa and Poulsen, 1991; Ahmed et al., 2000; Krajnicakova et al., 2003; Liesegang et al., 2007).

In literature, many scientists have described the mineral and biochemical indicators in the blood of the sheep (Shinde et al., 1995). Nevertheless, very few authors reported about the influence of age and the reproductive status as the important requisites for the biochemical indicators interpretation in sheep (El-Sherif and Assad, 2001; Antunovic et al., 2002). In order to establish the metabolic profile of the sheep it is essential to know the influence of the reproductive status. There is meagre information regarding the minerals status in sheep during different periods of gestation. The present study was therefore designed to ascertain serum sodium, potassium, chloride, copper and zinc concentrations during pregnancy, lactation and dry period in Buchi sheep.

MATERIALS AND METHODS

Animals and management

The present study was carried out on 60 Buchi sheep during September 2011 to August 2012 at private farm in Bahawalpur District (latitude = 29° and 59°N, longitude = 73° and 19°E). All experimental ewes were in average 2 to 4 years old, healthy and in a good physical condition, free from different diseases like tuberculosis, mastitis etc. The ewes were housed in the same shed with free access to open air. The animals had free access to drinking water and common salt. Ewes were divided into two groups. The first group (n=30) was mated; the second group (n=30) was not mated. Mated ewes were identified as group M (n=30) whereas not mated ewes were recognized as group NM (n=30).

Blood sampling for mineral estimation

Blood samples were taken from each group (M and NM) after every 3 month over 1 year period from the jugular vein. The periods were as follows: 1 = early pregnancy (October), 2 = late pregnancy (January), 3 = lactation (April), and 4 = dry season (July). Ten milliliters of blood from each ewe was collected in a clean glass test tube through venipuncture of the jugular vein. After centrifugation at 3000rpm for 10 minutes, the serum was aspirated carefully with a
pipette, placed in glass vials, labeled, and stored at −20°C until analyzed. Serum sodium, potassium, and chloride were determined on a clinical chemistry analyzer (Map Lab Plus) by using standard commercial kits (Centronic GmbH-Germany) and procedures were adopted as specified by the manufacturer of kits. For copper and zinc analysis, wet digestion of samples was done by following the method of Richard (1968). Serum copper and zinc were determined by using an atomic absorption spectrophotometer (Model AA-5).

Data analysis
The mean (±SD) values for serum sodium, potassium, chloride, copper and zinc were calculated. The data was analyzed by using statistical package SPSS 13. To determine the significance among different groups, a two-way ANOVA was applied in order to compare the periods and sheep with evaluated parameters. The independent-samples t-test was done in order to compare two groups for each evaluated parameter. The comparison of the variables according to periods in each group and totally between two groups was performed by using the one-way ANOVA at 5% level of significance.

RESULTS
The mean (±SD) values for serum sodium, potassium, chloride, copper and zinc during pregnancy, lactation and dry period for group M and NM ewes are presented in Table 1 and 2, respectively.

The mean serum sodium, potassium, and chloride concentrations varied non-significantly (P>0.05) during early, late pregnancy, and during lactation, dry period in group M ewes. The concentrations of serum sodium, potassium and chloride during different periods varied non-significantly (P>0.05) in NM group ewes. There was also non-significant difference in serum sodium, potassium and chloride concentrations during different periods between group M and NM ewes.

The copper concentrations in group M ewes increased with advancing pregnancy and was significantly high (P<0.05) during late pregnancy whereas during lactation and dry period, copper concentrations dropped significantly (P<0.05). In NM group ewes, the copper concentrations varied non-significantly (P>0.05) during different periods of the year.

The zinc concentrations increased non-significantly (P>0.05) from early to late pregnancy in group M ewes. The zinc concentrations go on increasing after lambing and, compared with late pregnancy, there were significant differences (P<0.05) in zinc concentrations during lactation and dry period. In group NM ewes, the zinc concentration differed non-significantly (P>0.05) among different periods. There were non-significant (P>0.05) differences in serum copper and zinc concentrations during different periods between M and NM group ewes, except during Period-I and 2, where the M group ewes were pregnant and NM group ewes were non-pregnant.

DISCUSSION
The purpose of present study was to determine the variations of sodium, potassium, chloride, copper and zinc in the serum of mated Buchi sheep during pregnancy, lactation, and dry periods and to compare them with non-mated Buchi sheep during corresponding months of the year. It was reported that there were differences among serum metabolites during pregnancy as a result of dam and fetus requirements (Braithwaite et al., 1970; Siggurdson, 1988). Since very limited research work have been done earlier on seasonal and physiological variations on serum chemistry in sheep, so only few reports are there in literature for comparison with the present study. The serum sodium, potassium, and chloride concentrations varied non-significantly (P>0.05) during pregnancy, lactation and dry period in group M ewes and during same periods of the year in NM group ewes. Sodium and potassium play a vital role in maintaining osmotic pressure and acid-base balance as electrolyte. Both ions were found to fluctuate only slightly during pregnancy and lactation (Mbassa and Poulsen, 1991). Our findings are in agreement with Yokus et al. (2004) who reported non-significant changes in sodium, potassium and chloride concentrations in Sakiz–Ivesi crossbreed sheep during pregnancy and lactation.
The zinc concentration had a decrease during parturition. The copper concentration increased during the month of July. It is known that low concentrations of sodium increases aldosterone and this decreases the potassium level by maintaining the discharge of potassium from the tubulus. Another reason for the low concentrations of potassium in our study during the month of July might be the increased aldosterone level caused by decreased in sodium and chloride (Lippmann, 1995).

The copper concentrations in group M ewes increased with advancing pregnancy and dropped during lactation and dry period, whereas, in NM group ewes, the copper concentrations did not varied during different periods of the year. The results of present study are in agreement with other workers (Mandal et al., 1996; Yokus et al., 2004). As ewe is season-dependent poly estrus animal, so its serum estradiol concentrations increase during autumn and winter, when days shorten, regardless of whether or not they are pregnant. The elevation in serum copper concentrations during pregnancy was first observed nearly 75 years ago (Cartwright, 1950). The serum copper concentrations increase because of the elevation of ceruloplasmin synthesis, which depends on estrogens levels that increase in the pregnancy. Therefore, whether ewe is pregnant or not, it is normal not to observe any difference in serum copper concentrations. The results of present study showed that serum copper concentrations not only increase during pregnancy (October and January), but also its concentrations increases in these months that is corresponding to the pregnancy period in NM group ewes. This is due to the increased estradiol and ceruloplasmin levels. The zinc concentrations increased from early pregnancy (October) and go on increasing after lambing(during lactation and dry period) in group M ewes, whereas, in group NM ewes, the zinc concentration differed non-significantly (P>0.05) among different periods. These results corroborate with Yokus et al. (2004). The zinc concentrations were lower during pregnancy in M group ewes. There exist a correlation between plasma zinc status and events occurring during gestation and parturition (Yokus and Cakir, 2006). The zinc concentration had a tendency to decrease during parturition when copper levels are high. Drop in zinc concentrations not only increase during

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Early Pregnancy</th>
<th>Late Pregnancy</th>
<th>Lactation</th>
<th>Dry Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mmol/l)</td>
<td>149.33±2.9</td>
<td>147.6±4.02</td>
<td>147.67±3.91</td>
<td>146.21±4.07</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.91±0.56</td>
<td>4.95±0.68</td>
<td>4.88±0.11</td>
<td>4.12±0.64</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>110.41±3.12</td>
<td>111.45±3.14</td>
<td>110.17±2.49</td>
<td>109.65±2.78</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>0.92±0.15</td>
<td>1.01±0.45</td>
<td>0.93±0.43</td>
<td>0.84±0.13</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>0.67±0.31</td>
<td>0.68±0.29</td>
<td>0.77±0.16</td>
<td>0.74±0.19</td>
</tr>
</tbody>
</table>

Values sharing different superscripts in a row differed significantly (P<0.05)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Period-1 (October)</th>
<th>Period-2 (January)</th>
<th>Period-3 (April)</th>
<th>Period-4 (July)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mmol/l)</td>
<td>150.09±3.2</td>
<td>147.23±3.67</td>
<td>146.81±3.19</td>
<td>146.59±3.11</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.49±0.33</td>
<td>4.56±0.77</td>
<td>4.56±0.55</td>
<td>4.48±0.39</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>110.33±2.13</td>
<td>110.28±2.09</td>
<td>110.91±2.35</td>
<td>109.08±2.15</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>0.92±0.19</td>
<td>0.94±0.33</td>
<td>0.87±0.19</td>
<td>0.88±0.52</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>0.74±0.46</td>
<td>0.77±0.34</td>
<td>0.74±0.45</td>
<td>0.73±0.58</td>
</tr>
</tbody>
</table>

Krajnicakova et al. (2003) also reported similar findings in dairy goat at Slovakia. The concentrations of sodium, potassium and chloride were though varied non-significantly during different periods and physiological states, yet numerically low concentrations of these minerals were found in the month of July. It is known that low concentrations of sodium increases aldosterone and this decreases the potassium level by maintaining the discharge of potassium from the tubulus. Another reason for the low concentrations of potassium in our study during the month of July might be the increased aldosterone level caused by decreased in sodium and chloride (Lippmann, 1995).

The copper concentrations in group M ewes increased with advancing pregnancy and dropped during lactation and dry period, whereas, in NM group ewes, the copper concentrations did not varied during different periods of the year. The results of present study are in agreement with other workers (Mandal et al., 1996; Yokus et al., 2004). As ewe is season-dependent poly estrus animal, so its serum estradiol concentrations increase during autumn and winter, when days shorten, regardless of whether or not they are pregnant. The elevation in serum copper concentrations during pregnancy was first observed nearly 75 years ago (Cartwright, 1950). The serum copper concentrations increase because of the elevation of ceruloplasmin synthesis, which depends on estrogens levels that increase in the pregnancy. Therefore, whether ewe is pregnant or not, it is normal not to observe any difference in serum copper concentrations. The results of present study showed that serum copper concentrations not only increase during pregnancy (October and January), but also its concentrations increases in these months that is corresponding to the pregnancy period in NM group ewes. This is due to the increased estradiol and ceruloplasmin levels. The zinc concentrations increased from early pregnancy (October) and go on increasing after lambing(during lactation and dry period) in group M ewes, whereas, in group NM ewes, the zinc concentration differed non-significantly (P>0.05) among different periods. These results corroborate with Yokus et al. (2004). The zinc concentrations were lower during pregnancy in M group ewes. There exist a correlation between plasma zinc status and events occurring during gestation and parturition (Yokus and Cakir, 2006). The zinc concentration had a tendency to decrease during parturition when copper levels are high. Drop in zinc concentrations not only increase during
concentration in the present study might be the result of copper inhibition of zinc intestinal absorption (Abdel-Mageed and Oehme, 1991; Alonso, 2000).

CONCLUSION
The results of present study showed that sodium, potassium, chloride concentration were almost comparable for M, NM group ewes and season, physiological state do not have any effect on these metabolites, whereas, the copper and zinc concentrations changes during pregnancy. It is therefore suggested that while elucidating the electrolyte and mineral status, period of the year and physical status of the Buchi sheep should be taken into consideration.

REFERENCES
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