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Liver copper and serum ceruloplasmin concentrations in hyperketonemic pregnant ewes

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ABSTRACT

This study has accomplished due to evaluate the effect of induction of subclinical pregnancy toxemia (PT) and subsequent hyperketonemia in ewes on serum ceruloplasmin and liver copper concentrations. The experiment was performed on five pregnant native ewes aged 3-4 years, 45-50 kg weight with BCS of 3.5-4 on a 0-5 scale. The pregnancy of these ewes were confirmed with ultrasound examination. Blood and biopsy samples were taken from the jugular vein and 12th ICS, respectively before the induction of subclinical pregnancy toxemia by food deprivation and after that (serum β-hydroxybutyrate concentrations>0.8mmol/l) when ewes suffering subclinical PT. Serum BHBA concentrations of ewes after induction of subclinical PT were significantly higher than before the induction (P<0.01). Serum glucose concentrations of induced hyperketonemic ewes were significantly lower than before the induction of hyperketonemia (P<0.01). Serum ceruloplasmin concentrations of ewes after the induction of subclinical PT were significantly higher than before the induction (P<0.01), but liver copper concentrations showed significant decrease after the induction of subclinical PT (P<0.05). It is concluded that circumstances such as hyperketonemia, can increase ceruloplasmin concentrations and decrease liver copper concentrations. Although evaluation of serum ceruloplasmin concentrations in ewes is a routine procedure for estimation of copper status, its levels should be evaluated with caution during late pregnancy.

Keywords: liver copper, ceruloplasmin, hyperketonemic ewe

INTRODUCTION

The laboratory evaluation of the copper status of farm animals is complex because the biochemical values are often difficult to interpret and to correlate with the clinical state of the animal. However, Evaluations of serum ceruloplasmin (Cp) along with liver copper (Cu) concentrations in pregnant ewes are normally used for estimation of the copper status of the animal [1]. Although Cu is an essential micronutrient normally subject to effective homeostatic control, excess dietary intake can be toxic in some circumstances [2]. Some researchers extrapolate that Cu levels will fall during the final stages of pregnancy , but needs to more accurately map the fall to better determine the timing of proactive sampling and treatment. Since copper deficiency can be an important factor for lamb survival to weaning, there is a need to characterize the dynamics of Cu levels in the pregnant ewes [3]. On the other hand, one of the major disorder of pregnant ewes is energy deficiency and subsequent hyperketonemia. Long-term energy deficiency causes mobilization in the body's fat deposits and an increase in ketone bodies, especially

acetoactate and β-hydroxybutyrate (BHBA), relative to energy metabolism [4-5]. It is reported that some circumstances such as pregnancy, inflammation and hormones could affect on ceruloplasmin concentrations [6-7] that may leading to provide the incorrect estimation of Cu status especially in pregnant ewes. The aim of this study was to determine the effects of hyperketonemia after induction of subclinical pregnancy toxemia(PT) in pregnant ewes on serum ceruloplasmin and liver copper concentrations.

MATERIALS AND METHODS

Animals and sample collection: The present experiment was performed after receiving approval from the Ethics Committee of the faculty of Veterinary Medicine, Science and Research branch, Islamic Azad University.

Five pregnant Iranian native ewes aged 3-4 years, 45-50 kg weight with body condition score (BCS) of 3.5-4 on a 0-5 scale [8] were selected. Animals had regular vaccination and deworming programs. Health of animals were examined according to clinical and hematological evaluation before and during the investigation. Age of gestation was confirmed by ultrasound examination using 5 MHz trans-abdominal probe (CUS2000, Carewell, China) on day 90 after mating.

Selected ewes were transferred to the experimental farm, keep separated under the same environmental conditions. All animals were fed on 500 grams concentrate, 1 kg of alfa alfa and 1 kg wheat straw. (concentrate contained corn (40%), barley (25%), wheat bran (25%), soybean meal (5%) and vitamin-salt mixture (5%).

From day 120 of gestation, blood samples were taken from the jugular veins into sterile blood tubes without additive. Liver specimens were obtained by biopsies through 11th intercostal spaces. An appropriate area was clipped and surgically prepared, A topical antiseptic was applied and after injection of 10 ml of lidocaine hydrochloride 2 %(Pasteur inst.Iran) the biopsy needle(Bard Magnum, MN1210, Crawley, UK) was inserted into abdominal cavity at the site of local anesthesia. A point of liver periphery was identified and grasped firmly. Liver samples stored in -80 C and experiments followed in continue. The levels of serum BHBA, glucose, ceruloplasmin and liver copper were measured.

Feed deprivation was started for induction of subclinical pregnancy toxemia(PT) in ewes and continued to full cessation on day 3 until serum β-hydroxybutyrate concentrations reached levels greater than cut point(0.8 mmol/L) during daily measurements. The blood samples were taken daily and were sent to laboratory. On day 3-4 all experimental ewes had hyperketonemia with serum BHBA concentrations greater than 0.8 mmol/L. At this time, the second liver biopsies were performed (subclinical ketosis). specimens were sent to laboratory for measurement of liver Cu before and after the induction of PT. None of the sheep developed any complication following the biopsy and were in good general health. Serum ceruloplasmin and glucose concentrations were also measured in the blood samples. We compared seum cooncentrations of BHBA, glucose and ceruloplasmin along with liver copper concentrations, before and after the induction of subclinical PT.

BHBA and glucose assays:
Serum concentration of BHBA was determined using a kinetic enzymatic method in the commercial kits of Runbut (Randox, UK).
Serum glucose concentration was measured by spectrophotometry using a commercially available kits (Farasamed, Iran).

Serum ceruloplasmin and liver copper assays:
Serum Ceruloplasmin concentration was analyzed on an automatic analyzer (BT-1500, Italy) using commercial kit (Minineph, UK).

The copper content of liver specimens was determined by the method of Alcock [9] using spectrophotometry flame atomic absorbtion (Shimadzu-AA-670).

Statistical analysis:
Statistical analysis was performed using the IBM- SPSS program version 20. Data checked for normal distribution, by using the Kolmogoroff-Smirnoff test. The paired T- test was used to examine the differences between parameters

in normal and experimentally hyperketonemic ewes. All data were expressed as the mean±SD. All differences were considered to be statistically significant at the P<0.05 level of confidence.

RESULTS

Table 1 shows the serum biochemical and liver copper concentration in normal and experimentally hyperketonemic ewes. BHBA concentrations in normal ewes were significantly lower compared to experimental ewes (P<0.01). There was significant (P<0.01) decreases in glucose concentration after induction of hyperketonemia. Serum concentrations of ceruloplasmin in hyperketonemic ewes were significantly higher than normal ewes (P<0.01) but liver Cu concentrations after induction of hyperketonemia were significantly lower after induction of subclinical PT(P<0.05).

Table1. Mean ± SD of serum BHBA, glucose, calcium, ceruloplasmin and liver copper concentrations in normal and experimentally hyperketonemic ewes

parameters	Normal ewes	Hyperketonemic ewes	P value
BHBA(mmol/L)	0.43 ±0.07	1.00 ±0.07	P<0.01
Glucose(mg/dl)	49.40 ±4.21	29.80 ±4.76	P<0.01
ceruloplasmin (mg/dl)	11.80 ±0.83	15.80 ±1.30	P<0.01
Liver copper(µmol/kg)	4612.40 ±1765.38	2391.40±1360.27	P<0.05

DISCUSSION

The objective of this study was to evaluate the effect of induced hyperketonemia by short fasting on serum ceruloplasmin and liver copper concentrations in ewes.

All experimental ewes had higher BHBA and lower glucose concentration after induction of hyperketonemia by food deprivation.

BHBA concentration is a golden marker for diagnosis of PT and/or ketosis in ewes [10]. Hyperketonemia and hypoglycemia are common biomarkers for PT [11]. It is indicated that low glucose and high BHBA (the major ketone body of ruminants) plasma concentration occurred during pregnancy toxemia, and there is a significant negative correlation between ketone bodies and glucose [12]. Results of the present study showed that food deprivation and consequently hyperketonemia tend to increase the Cp concentrations of late gestation in ewes. Similarly, Gursel et al. (2010) experienced ewes fed deficient energy during late pregnancy and resulted that serum Cp levels were significantly higher in the pregnant normal energy group than in the non-pregnant normal energy group and were significantly lower in the pregnant normal energy group than in the pregnant deficient energy group [7]. A possible reason for this event is stress condition that increase Serum Cp concentrations [13-14]. The reference range of serum Cp in sheep is 4.5-10 mg/dl [1], while our results showed high Cp levels either befor and after induction of PT which have been related to the pregnancy per se. Mc Ardle (1995) reported that serum levels of Cp can markedly rise during pregnancy [15]. Daszynaska et al. (1968) have shown the increasing the serum Cp and Cu levels in pregnant cows [16]. Karp et al. (1986) stated that the increasing of serum Cp concentrations in pregnancy can be attributed to estrogen, and treatment of animals with estrogen had same effect on ceruloplasmin concentration [17].

Mas and Sarkar (1992) showed that the increase of maternal Cp during pregnancy reflects a requirements for copper transport to fetus [18]. Al –Qudah (2011) demonstrated that oxidative stress and lipid peroxidation are involved in the development and complications of pregnancy toxemia. An association between hyperketonemia and lipid peroxidation, suggesting that ketonemia is a risk factor for lipid peroxidation and oxidative stress in ewes affected with PT [19]. These results are also in accordance with observations of other investigators showed that normal pregnancy is associated with the increase in oxidative stress and lipid peroxidation [20]. Our results showed significant increase in Cp after induction of subclinical PT. This study also indicated a significant decrease in liver Copper concentration (P<0.05).

Copper (Cu) is a component of a variety of intracellular and extrecellular enzymes, including cytochrome oxidase, lysyl oxidase, superoxide dismutase, tyrosinase and ceruloplasmin [1,21]. Excess Cu beyond immediate requirements is stored as the complex form in methalothionein, mainly in the liver. Most plasma Cu is present as a component of ceruloplasmin (Cp). Cp is synthesized in the liver where it receives six Cu atoms, and then is secreted into plasma(Tom, 1993) [22]. Cp is an acute phase protein and acts as an antioxidant through ferroxidase activity [6,23]. Antioxidants play an important role in preventing free radical damage [6,23]. The decrease in liver Cu concentrations may be associated with increase in Cp synthesis in the liver, where it receives six cu atom [22]. The synthesis of Cp during an infection increases the Cu requirements [24]. Richardson and Hodgson (2005) extrapolated falling of Cu levels during the final stages of pregnancy [3].

In conclusion, Although evaluation of serum ceruloplasmin concentrations in ewes is a routine method for estimation of copper status, its measurements should be evaluated with caution during late pregnancy. Significant rising of serum Cp concentrations in this study relates to combination of dietary deficiency, late pregnancy, lipid peroxidation, stress and acute phase responses.

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