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Copper, Manganese, Zinc, Iron and Calcium in Fetal Tissue of Baladi Goats at Northern of Jordan

Mutassim M. Abdelrahman
Department of Animal Production, Faculty of Agriculture, JoMu'tah University, Karak, Jordan

Abstract: Endemic deficiencies of minerals can be known by determining the concentration of these minerals in fetal tissues at different stages of gestation. Liver, kidney and bone samples were collected from fetuses of does (n=32), raised under extensive system, during routine slaughter operation at Irbid slaughterhouse at northern of Jordan. The fetuses' age were determined by crown to rump length measurements. Fetuses were grouped into three categories by periods: period 1, d 30 to 50, period 2, d 51 to 100 and period 3, d 101 to 150. All tissue samples were prepared and analyzed for Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe) and Calcium (Ca) by AAS. Selenium (Se) concentrations in fetal liver were also analyzed by AAS. Copper, Mn and Zn concentration in fetal liver and kidney were significantly lower ($P<0.05$) at late gestation compared with early and mid gestation, but liver Fe at late gestation was significantly higher ($P<0.05$) than at first two stages. Iron, Cu, Mn and Zn concentrations in fetal bone were significantly low ($P<0.05$) at late gestation. Selenium concentrations in fetal liver were significantly increased ($P<0.05$) with progress of gestation. Liver Fe concentrations were negatively correlated with kidney Fe ($r= -0.60$; $P<0.01$). Moreover, liver Cu concentrations were negatively correlated with liver Mn ($r= -0.50$; $P<0.05$). In conclusion, fetal liver Se and Fe storage was high and adequate during late gestation. On the other hand, Cu, Mn and Zn deficiencies and abnormalities of the newborn can be expected because of low fetal tissue reserve of these elements during late gestation.

Key words: Goats, fetus, tissues, minerals, and gestation

Introduction

Goats and sheep are the main livestock species in the middle east as well as in Jordan.Goat and sheep population in Jordan were estimated to be 631 thousands and 1.58 million heads, respectively (MOA, 1999). The goat along with sheep were raised in mixed herds and allowed extensive grazing on the natural pastures where they graze or browse. The nutrition of goats during the grazing season depends completely on grazing poor quality pasture and cereal stubble. Therefore, grazing may not provide does with enough nutrients to satisfy their minimal needs especially during pregnancy. Gestation is the most critical period that greatly influences the fetal growth and development especially in case of undernutrition (Ribnson, 1999). Copper, Mn, Zn, and Fe are often the most limiting elements for the fetus and neonate for normal development. Deficiencies of these elements impair fetal growth and can cause death (Underwood, 1977). Copper play an essential role in the normal development of central nervous system (CNS) of the embryonic lamb (Underwood, 1977; Mertz, 1987). Likewise, deficiencies of Mn and Zn in the dam result in subsequent deficiencies in the fetus and neonate (Hidiroglou *et al.*, 1981; Mertz, 1987). Zinc deficiency during pregnancy result in malformation of nervous system and reduced embryonic development (Dreosti *et al.*, 1972). Finch *et al.*, (1983) reported a severe anemia in pregnant animals and abnormalities development of fetuses with Fe deficiency.

Goats appear to be different in the metabolism of many trace elements from cattle and sheep, eg. Iodine, Iron, Copper and Molybdenum, but few studies exist involving goats. Kids appear to be born with very low iron and other mineral stores and in early need of supplementation which can not provided by their does's milk (Van Horn and Haenlein, 1984)

Fetal tissues can be used to assess the nutritional status of the dam and determine the endemic deficiencies of minerals. No studies are available regarding the accumulation of Cu, Mn, Zn and Fe in fetal tissues of baladi goats under the extensive system at northern of Jordan. Therefore, the present study was conducted to monitor the developmental changes in the deposition of Cu, Mn, Zn, Fe and Ca in fetal tissues of grazing baladi does at different stages of gestation. In addition, to determine the concentrations of Cu, Mn, Zn, Fe and Ca in blood serum of baladi does and their newborn kids at parturition.

Materials and Methods

Thirty-two pregnant baladi does were raised under the extensive system at the northern part of Jordan. The does were slaughtered at different stages of pregnancy. The fetuses were collected, and crown to rump length measurements (Arthur *et al.*, 1989) were used to estimate fetal age; fetuses were grouped into three categories by periods: period 1, d 30 to 50, period 2, d 51 to 100, period 3, d 101 to 150. Liver, kidney, and rib samples were collected immediately from fetuses at the slaughterhouse. Fifteen baladi does were selected randomly from flock raised under the same extensive system at the northern part of Jordan. At parturition, blood samples were collected from these does and their kids.

Liver samples were wet ashed in a tertiary acid mixture (perchloric, nitric, and sulfuric acids) for analysis of Cu, Mn, Zn, Fe and Se. Kidney and rib samples were dry-ashed at 600°C. Kidney and ribs samples were diluted after ashing by using 0.1 N Hydrochloric acid. Blood samples were centrifuge at 3000 rpm/ 15 minutes to separate serum. Serum was treated with Trichloroacetic acid (TCA) to participate the protein. Blood and fetal liver, kidney and rib samples were analyzed for Cu, Mn, Zn, Ca and Fe by atomic absorption spectrophotometry (AAS). Moreover, only liver was analyzed for selenium by AAS too.

Statistical analysis: The general linear model procedure of SPSS® (version 7.5) was used to analyze the data for a complete randomized design. Fetal tissue Cu, Zn, Mn, Ca, Fe and liver Se were used as dependent variables, and stage of gestation was the independent variable. Means were compared using the protected least significant difference (LSD) test of SPSS®. Significance was declared at ($P<0.05$) unless noted otherwise. Pearson correlation procedures of SPSS® were used to correlate the concentration of minerals among fetal tissues.

Results and Discussion

Trace element concentrations in fetal liver, kidney and bone: Copper concentrations in fetal liver were significantly lower ($P<0.05$) at late gestation compared to early and mid gestation Table 1. The lower concentrations of fetal liver Cu during late gestation may happened because of rapid growth of fetus and its organs. Ruminants are known for their capacity for hepatic storage of Cu which represents 72% of total body Cu (Mertz, 1987). Changes in Cu concentrations in fetal liver with advancing gestation were reported by Suttle (1987) and Gooneratne and Christensen (1989a). Abdelrahman and Kincaid (1993) did not fin any significant change in fetal liver Cu with the age of the fetus in the bovine. The Cu concentration normally is higher in fetal liver than in adults (Pryor, 1964). Fetal liver stores of Cu are very important in the neonate because bovine milk is a poor source of Cu (.10 to .88 μg/kg). In fetal kidney, Cu concentration decreased significantly ($P<0.05$) with the progress of gestation Table 2. This result agreed with Abdelrahman and Kincaid (1993) who reported a significant decrease in kidney Cu with advance in gestation in bovine.

Manganese in fetal liver and kidney were significantly lower ($P<0.05$) at late gestation compared with early and mid gestation Tables 1 and 2. Little Mn storage exists in tissues of calves born from dams with adequate Mn intakes (Mertz, 1987). These support the importance of Mn supplementation to pregnant goats and other ruminants at late gestation to avoid any metabolic problems and abnormal growth by fetuses and newborns. Hansard (1972) found that Mn supplementation of ewes increased Mn transfer through the placenta to the fetus. In the bovine, Howes and Dyer (1971) and Rojas et al. (1965) reported that Mn supplementation to pregnant cows increased the Mn concentrations in the liver and muscles of newborn calves compared with that in calves born from cows with adequate or low concentrations of dietary Mn. Although the bovine fetus stores Mn efficiently (Hidiroglou *et al.*, 1980) in the liver, their concentration is relatively low compared to other minerals such as Zn and Fe. Moreover, Abdelrahman and Kincaid (1993) did not detect any significant change in the concentrations of Mn in fetal liver and kidney with fetus age in the bovine, which disagreed with our findings in this study.

Zinc concentrations in fetal liver and kidney decreased significantly ($P<0.05$) with stage of gestation Tables 1 and 2. These findings agreed with the results of Abdelrahman and Kincaid (1993) who reported a significant decrease in fetal liver and kidney Zn with progress of gestation. A fetal liver Zn dropped down from 1110 to 716 ug/g at early and late gestation respectively. Kidney Zn also reduced from 115.2 to 100.2 ug/g DM, respectively. Others (Widdowson *et al.*, 1974) have reported a rapid decrease in hepatic Zn with fetal age in several species. Gooneratne and Christensen (1989b) reported a significant decline in fetal kidney Zn after the first trimester but found that fetal liver Zn was increased by the third trimester, which agreed with our findings in this study.

Table 1: The concentration of Cu, Mn, Zn, and Fe in caprine fetal liver at different stages of gestation

Stage of gestation		Cu		Mn		Zn		Fe	
d	n	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
		µg/gofDM							
30-50	13	71.7^{a}	4.9	526.7^{a}	22.2	665.0^{a}	19.0	1285^{a}	264
51-100	10	78.3^{b}	1.3	718.3^{a}	12.1	8250^{b}	60.0	1825^{b}	71.5
101-150	9	58.3^{c}	0.7	276.7^{b}	13.6	512.0^{c}	22.0	2600^{c}	83.0

^{a,b,c} Within a column, means followed by different superscripts differ ($P<0.05$)

Table 2: The concentration of Cu, Mn, Zn and Fe in caprine fetal kidney at different stages of gestation

Stage of gestation		Cu		Mn		Zn		Fe	
d	n	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
		µg/gofDM							
30-50	13	58.3^{a}	11.0	752^{a}	46.0	448.3^{a}	33.4	637^{a}	87.0
51-100	10	33.3^{b}	4.9	665^{a}	78.0	275.0^{b}	42.6	7.5^{a}	75.0
101-150	9	37.0^{ab}	5.1	355^{b}	21.4	105.0^{c}	11.2	390^{b}	8.9

^{a,b,c} Within a column, means followed by different superscripts differ ($P<0.05$)

Table 3: The concentration of Cu, Mn, Zn and Fe in caprine fetal bone at different stages of gestation

Stage of gestation		Cu		Mn		Zn		Fe	
d	n	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
		µg/gofDM							
30-50	13	75.0^{a}	16.8	671.7^{a}	63.0	146.7^{a}	10.5	765.6^{a}	162
51-100	10	30.0^{b}	6.3	666.7^{a}	84.0	225.0^{b}	29.4	414.7^{b}	64.3
101-150	9	20.0^{b}	3.7	361.7^{b}	41.9	141.7^{a}	47.7	176.7^{c}	18.6

^{a,b,c} Within a column, means followed by different superscripts differ ($P<0.05$)

Table 4: The effect of stage of gestation on the ash percentages of liver, kidney and bone

Stage of gestation		Liver		Kidney		Bone	
		$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
d	n	%ofDM					
30-50	13	1.48^{a}	0.07	2.95^{a}	0.06	7.52^{a}	0.4
51-100	10	1.24^{b}	0.05	1.89^{b}	0.17	9.84^{b}	0.5
101-150	9	1.67^{c}	0.01	1.34^{c}	0.09	30.23^{c}	0.9

^{a,b,c} Within a column, means followed by different superscripts differ ($P<0.05$)

Table 5: The effect of stage of gestation on the Calcium concentration in caprine fetal bone, kidney and liver

Stage of gestation		Liver		Kidney		Bone	
		$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
d	n	%ofDM					
30-50	13	0.55^{a}	0.05	2.12^{a}	0.21	30.45^{a}	2.39
51-100	10	0.46^{ab}	0.08	1.07^{b}	0.18	34.98^{a}	0.81
101-150	9	0.35^{b}	0.02	0.35^{c}	0.01	50.13^{b}	1.55

^{a,b,c} Within a column, means followed by different superscripts differ ($P<0.05$)

Table 6: Correlation matrix between Cu, Ca, Mn, Zn and Fe concentrations in liver and kidney of caprine fetuses

	L Cu	L Ca	L Mn	L Zn	L Fe	K Cu	K Ca	K Mn	K Zn	K Fe
L Cu	....									
L Ca	0.04	....								
L Mn	-0.5*	-0.43	....							
L Zn	0.09	0.00	0.03	....						
L Fe	-0.05	-0.27	0.45	0.19	....					
K Cu	0.01	0.40	-0.12	-0.37	-0.24	....				
K Ca	0.19	0.70**	-0.47	-0.23	-0.67**	0.42	....			
K Mn	0.05	0.59**	-0.35	0.16	-0.65**	0.40	0.77**	....		
K Zn	0.26	0.75**	-0.56*	-1.7	0.70**	0.55*	0.92**	0.73**	....	
K Fe	0.05	0.53	-0.49*	0.40	-0.60**	0.07	0.39	0.60**	0.47*	....

[1]Total number of fetuses = 32. *Correlation is significant at the 0.05 leve.
** Correlation is significant at the 0.01 level.

Table 7: The concentration of Cu, Mn, Zn, Fe and Ca in blood serum of does and their kids at parturition

Element	Doe*		Kid		Adequate level**
	x̄	SD	x̄	SD	
Cu, (μg/ml)	0.71	0.17	0.66	0.11	0.70 - 2.00
Mn, (μg/ml)	1.58	0.45	3.43	0.50	0.80 - 5.10
Zn, (μg/ml)	0.77	0.19	0.71	0.21	0.90 - 1.20
Fe, (μg/ml)	1.45	0.12	1.87	0.22	0.70 - 1.96
Ca, (mg/dl)	8.18	1.22	5.91	0.21	8.00 - 11.0

*Average concentration of 20 baladi does at parturition **Range of adequate levels of elements

Milk Zn may be too low to meet the needs of the newborn for growth and development. The concentration of Zn in bovine colostrum exceeds 15 μg/ml but is only 3 to 5 μg/ml in milk (Kincaid and Cronrath, 1992). Similar Zn concentrations are reported for caprine colostrum and milk (13 and 5 μg/ml, respectively) and for ovine colostrum and milk (14 and 1 μg/ml) (Mertz, 1987). These results reflect the importance of adequate tissue reserves of Zn for the newborn to prevent possible development of Zn deficiencies (Mills *et al.*, 1979).

Iron concentrations in fetal liver increased significantly (P<0.05) with the progress of fetal age. It was increased from 1285 ug/g DM at early gestation to 2600 ug/g DM at late gestation Table 1. In contrast, Hoskins and Hansard (1964) reported a decrease in placental transfer of Fe appears to occur between mid and late gestation in the pregnant ewes. Ralis and Pansteriadis (1987) found a continuos increase in Fe concentration in fetal liver throughout pregnancy. On the other hand, Fe concentrations in fetal kidney and bone showed a significant drop down (P<0.05) with the progress of gestation Table 2 and 3.

Bone Mn, Fe, Cu and Zn concentrations decreased significantly (P<0.05) with gestation Table 3. This result disagreed with the findings of Abdelrahman et al., (unpublished data). Moreover, kidney and liver Ca concentrations were significantly decreased (*P*<0.05), but increased in bone with gestational progress Table 5.

Dry matter percentage of fetal liver, kidney and bone significantly increased between periods 1 and 3 Table 4. Moreover, ash percentage in fetal liver and bone were significantly higher (P<0.05) at late gestation compared to early and mid gestation (1.67 vs 1.48 and 1.24%, respectively; 30.23 vs 7.52 and 9.84%, respectively), but were significantly higher (P<0.05) in fetal kidney and bones during late gestation Table 5.

A significant increase (P<0.05) were detected in liver Se with progress of gestation from 223.3 μg/g DM at early gestation to 346.8 μg/g at mid gestation and 523.3 μg/g DM at late gestation.

Correlation between tissue elements concentrations: Minerals may interact with other minerals, with other nutrients, or with nonnutrients (Lucille *et al.*, 1983). These interactions can occur in the diet, in the digestive tract, or in tissues and cells. The interactions may be antagonistic or synergistic. Understanding these interactions can help prevent mineral deficiencies (Lucille *et al.*, 1983). Potential synergistic interactions are direct interactions during the synthesis of structural proteins, competition between elements for the active sites on an enzyme, or activation of some enzyme systems. Mineral antagonisms in tissues occur often, in direct interaction between elements, competition between ions at an active site, or competition for a common transport site or ligand (Hill, 1976).

In this study, correlation was positive between fetal liver Ca and kidney Ca ($r=.70$; $P<0.01$). Fetal liver was negatively correlated with liver Mn ($r=-0.5$: $P<0.05$). A high positive correlation was observed between Kidney Zn and kidney Mn ($r= 0.73$; $P<0.01$) and kidney Fe concentrations were positively correlated with kidney Fe ($r=0.47$: $P<0.01$) and with kidney Cu ($r= 0.55$; $P<0.05$: Table 6.

Because deficiencies in trace elements are most often expressed in the young, tissue reserves of trace elements are important to the survival and growth of kids. Although blood and diet samples can be obtained fairly easily for analysis in most confinement operations, this is not the case in most areas of the world where extensive grazing of goats is practiced. Because many goats are pregnant at slaughter, fetal tissues offer a valuable resource to assess trace element nutrition in local herds and to determine possible endemic deficiencies. If fetal tissues are to be used to assess the nutritional status of the dam, the effect of gestational age on the concentration of minerals in fetal tissues must be known.

Concentration of Cu, Mn, Zn, Fe and Ca in blood serum of Baladi goat and their kids: Table 7. shows the concentration of Cu, Mn, Zn, Fe and Ca in blood serum of does and their newborn kids, at parturition, raised under the traditional extensive system. In addition, the adequate levels of these elements are according to Pulse (1991). The concentration of Mn and Fe in the blood of does and the newborn kids were fallen within the adequate levels, but Cu and Zn concentrations were marginally deficient compared with the standard adequate levels as shown in Table 7. Moreover, Ca concentrations in blood serum of does were considered adequate, but deficient in the blood serum of their kids. These findings suggest the idea of sufficient intake of Fe, Mn and Se by does and the proper transfer of these elements to their fetuses through the placenta. On the other hand, fetuses face a high demand for Cu, Zn and Ca for normal fast growth and development during late gestation, which didn't cover by does through the placenta and low tissues reserve. This inadequate transfer of these elements through the doe's placenta might be caused as a result of insufficient dietary intake of these elements by does during late gestation to face the high demand by fetuses.

Conclusions

Results reported here in suggest adequate intake of Fe and Se by grazing pregnant baladi goats that lead to high reserve in fetal liver Se and Fe at late gestation. A lower Cu, Zn and Mn concentrations in fetal liver during late gestation possibly reflect the high needs for these minerals by fetuses at late gestation for the synthesis of many metallo-enzymes and fetal growth. More studies are recommended to develop a supplementation program for improving newborn health by avoiding mineral deficiencies.

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