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EFFECT OF COPPER AND CADMIUM CONTAMINATED DIETS ON WATER INTAKE, RUMINAL ENZYME ACTIVITIES, CHARACTERISTICS AND NUTRIENT DIGESTIBILITY IN SHEEP

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ABSTRACT

Nine healthy non-pregnant Rahmani, ewes were used in this study to evaluate the effect of copper and cadmium chloride as heavy metals on water intake, ruminal enzymes and nutrient digestibility. The animals were divided into three groups. The first group was fed 400-g concentrate mixture and 20 g DM/kg LW of hay and used as a control group. Whereas the second and third groups were fed the same diet but the concentrate was treated with $CuCl_2$ and $CdCl_2$, respectively daily just before feeding, $CuCl_2$ or $CdCl_2$ solution (5-mmol/L) was sprayed on the weighed amount of concentrate (400 g/h/d). Diets were fed once daily. Drinking water significantly (P? 0.05) increased in the ewes received copper or cadmium contaminated diet. Copper and cadmium decreased significantly (P?0.01) urease activity, whereas no significant differences were observed in the case of alanine aminotransferase (ALT), aspartate aminotransfarase (AST), and gamma-glutamyl transferase (GGT) activities. The activity of all ruminal enzymes studied was significantly affected by cadmium treatment; both ALT and AST activities were significantly increased (P?0.01) while the GGT activities were significantly decreased (P?0.05).Ruminal ammonia concentration was significantly decreased (P?0.05) by copper or cadmium chloride treatment. Lower values were obtained in the copper treated diet animals, but without significant difference (P?0.05) with cadmium treated diet. Ruminal pH and total VFA concentrations were unaffected (P?0.05) by treating the diets with both heavy metals. Copper or cadmium treated diets had decreased significantly (P?0.05) branched-chain VFAs (isobutyric and isovaleric acids), acetic and butyric acids concentrations and had no significant effect on propionic and valeric acids. Treating diets with heavy metals (copper or cadmium) resulted in reduction in the digestibility coefficients of all nutrient components except nitrogen free extract. Results of the present study indicating that, in sheep, eating diets contaminated with copper or cadmium (5-mmol) could increase water consumption, negatively affect several ruminal enzymes activity and nutrient fermentation.

Keywords: copper, cadmium, water intake, ruminal enzymes, VFA, digestibility, sheep.

INTRODUCTION

Wastes produced from factories and cars have many heavy metals that could contaminate air, soil, and waterways. Heavy metals such as copper and mercury have altered the agricultural environment. Jencik et al. (2001) reported that soil and plant biomass sample analyses from localities situated maximally 10 km from the copper and formerly mercury producing factories showed significant soil and biomass contamination by mercury, lead, cadmium, copper, and zinc ions. Magnesium-processing industry pollutes its surrounding by magnesium fly ash that may increase the risk of oversupply of this ion for exposed animals.

Copper is an essential element required for the goat and other species for a number of biochemical functions. Copper stimulates growth in swine and alters lipid metabolism in steer (Engle and Spears 2000). Results of Odenkirchen et al. (1994) showed that 2 g CuSO4 per animal per day is recommended as the maximum dose for cattle to overcome copper deficiencies. Ingestion quantities of Cu slightly higher than required may cause accumulation in the tissues and cause haemolysis. Sheep diets containing over 15 mg Cu/kg air-dry feed can cause copper poisoning (Hartmans, 1975), and it is difficult for feed compounders to keep values consistently below this limit. Problems can also arise from excessive consumption of copper containing salt licks or the unwise use of any copper supplementation. Chronic copper poisoning rarely occurs in grazing sheep under natural conditions (Wiener et al., 1978). Sheep are more sensitive to high copper supplementation than other farm animals (Allen and Gawthorne, 1987).

Cadmium is an essential microelement. Cadmium deficiency in ruminants leads to depression of milk production, growth retardation in young animals, muscle weakness, and reluctant to move or even death. Increased concentration of cadmium in the body is toxic. The toxic effect of cadmium arises from its ability to bind to protein thiol groups. Its toxicity depends on dosage, route of administration, form, length of exposure and animal species (Pribilincova et al., 1995).

However, many heavy metals could negatively affect ruminal enzyme activities (Faixova, and Faix, 2002; and Spears and Hatfield, 1978), ammonia nitrogen (Pal et al., 1998), and microbial protein synthesis (Legath et al 1990) in the rumen fluid. Heavy metals could also determine changes in digestibility of feed and volatile fatty acids production in the rumen but available data are sparse (Cacava et al., 1993).

The objective of the present study was to determine whether addition of copper and cadmium as heavy metals (causing air, soil, and biomass contamination in industrially exposed areas) in diets of sheep would affect: 1)- water consumption, 2)- ruminal enzyme activities 3)- ruminal characteristics (VFA, pH and ammonia-nitrogen concentration), and 4)- digestibility

MATERIALS AND METHODS

Animals and diets

Nine healthy non-pregnant, non-lactating Rahmani ewes were used. All animals were born in the same flock and were between 2 and 3 years of age at the start of the experiment. Average live weight of the animals was 49.5±3.20 kg LW. Each animal was fitted with a permanent rumen cannula (45-mm internal diameter) about 3 month before the start of the experiment. All animals were housed in individual pens and had free access to water.

The animals were divided into three groups (three animals in each group). The first group was fed on 400 g concentrate mixture (29% cotton seed meal, 37% ground maize, 30% wheat bran, 3% limestone and 1% salt (NaCl)) and 20 g DM/kg LW of hay and used as a control group, whereas the second and third groups received the same diet but the concentrate was

treated with $CuCl_2$ and $CdCl_2$, respectively. One hundred milliliter of 5-mmol/L $CuCl_2$ or $CdCl_2$ solution was sprayed on the weighed amount of concentrate (400 g/h/d) and completely mixed. The concentrate mixture was treated daily just before fed to animals and introduced first then followed by weighed amount of hay. Chemical analyses of hay and concentrate mixture are shown in *Table (1)*.

Drinking water and feed intake

Data mentioned in this study are the mean values of water and feed intake recorded along all the days of the experiments. Drinking water (L/kg DMI) was recorded by measuring the amount of water added daily to restore the initial level of water in the drinker. Daily feed intake (g DM/h/d) was determined by the difference between the DM of feed introduced and refused.

Table 1: Chemical composition of roughage and concentrate mixture used (on dry matter basis %).

Item	OM	CP	EE	NFE	NDF	ADF	CEL	HCEL
Hay	91.17	13.15	2.60	41.43	57.45	35.28	30.81	22.17
Concentrate mixture[1]	95.80	14.00	3.98	71.40	33.77	19.45	11.02	14.32

[1]**Concentrate mixture contains: 29% cotton seed meal, 37% ground maize, 30% wheat bran, 3% limestone and 1% salt (NaCl).**

Ruminal enzyme activities

Determination of ruminal enzymes concentrations was carried out according to the method described by Faixova and Faix, (2002). The ruminal fluid was collected from each animal and strained through four layers of cheesecloth. Copper and cadmium were separately added to 10 ml of rumen fluid so that their final concentrations were 5 mmol/L. After the addition of metals, each mixture was shaken and incubated for 30 min at 37 °C prior to assaying enzymatic activity in the rumen fluid with or without heavy metal. Alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase and urease activity were determined by the calorimetric methods using spectrophotometric kits.

Ruminal characteristics

On two consecutive days, rumen samples were collected through the rumen cannula of each sheep at 0, 1, 3, 5, 7, 9, 12 and 24 h after feeding. Rumen fluid was strained through four layers of cheesecloth and its pH determined immediately. One ml of rumen fluid was added to one ml of deproteinizing solution (10% of metaphosphoric acid and 0.06% crotonic acid; w/v) for volatile fatty acids (VFAs) determination. Twenty milliliter of rumen fluid were acidified with twenty milliliter 0.2 mol/HCl for ammonia nitrogen determination. All samples were stored at -20 °C until analyses were undertaken.

Digestibility

Digestibility trial consisted of 15 days as a preliminary period followed by 7 days as a collection period. During this period, animals were housed separately in metabolic crates fitted with mesh floor followed by sloped-netted separators for accurately collecting and separating feces from urine. Samples from the feeds were taken during the collection period, dried and ground for the chemical analysis. Feces were collected over 24-h period and representative samples (10%) were dried, bulked, and milled for analysis.

Chemical analysis

Dry matter in feeds and fecal samples were determined by drying at 105 °C until constant weight. Ash was determined by incinerating samples in a muffle furnace at 500-550 °C. Nitrogen was determined according to the Association of Official Analytical Chemists (AOAC, 1980). Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (CEL) and lignin analysis were carried out according to Goering and Van Soest (1970). Ammonia concentration was determined according to the methods of McDonald et al., (1960). VFAs were determined on centrifuged samples by Gas Chromatography as described by Ottenstein and Bartly (1971).

Statistical analysis

Statistical analysis of data was carried out according to Steel and Torrie (1980). Significant differences were determined by Duncan's multiple-range test (Duncan, 1955) using the Statistical Analysis System (1993) SAS computer program.

RESULTS AND DISCUSSION

Drinking water and feed intake

Treating sheep diet with Cu or Cd increased significantly (P?0.05) the volume of water consumed and non-significantly (P?0.05) decreased daily feed intake (*Table 2*).

Table 2: Drinking water (L/kg DMI/h/d) and feed intake (g DM/h/d) of sheep fed diet contaminated with copper (Cu) or cadmium (Cd) at the level of 5 mmol/L.

Item	Diets			Sig.
	Control	Cu	Cd	
Drinking water	5.01 ^{b}	5.82 ^{ab}	6.70 ^{a}	*
Feed intake	1337	1308	1297	NS

a,b; means in the same row with different superscripts are significantly different at (P?0.05). Sig.: Level of significant, NS: no significant.

Salinity of diets used may be the mean factor, which influence on water requirement in sheep. We are proposed that increased water consumption could be due to the effect of heavy metals added to the diet on the osmolarity of animal mouth. Increased mouth osmolarity may increase the necessity of animals to consume larger amounts of water to avoid the saline taste felt in the mouth. Precipitate the soluble proteins of saliva by heavy metals (Haurowitz, 1950) may be another factor which cause an astringent bitterness of taste felt in the animal mouth and increase the water intake to reduce this taste. Our results are in agreement with the observation of Wilson, (1978), who reported that sheep grazing mineralized forage, e.g., *Atriplex*-dominated desert ranges, require considerably more water than normal. The non-significant decrease in feed intake by treating diets with heavy metals, may be attributed to the bitter taste of the feed treated with minerals (Wilson, 1978).

Ruminal enzyme activities

Data presented in *Table 3* shows the effect of copper and cadmium on the activity of several enzymes of rumen fluid.

In comparison with control animals, copper found to decrease significantly (P?0.01) urease activity, whereas no significant differences were observed in the case of ALP, AST, and GGT activities. On the other hand, cadmium significantly affected the activity of all ruminal enzymes studied. It had stimulated ALT (P?0.05) and AST (P?0.01) activities and significantly decreased GGT (P?0.01) and urease (P?0.001) activities.

Table 3. Ruminal enzyme[1] activities of sheep fed diet contaminated with copper (Cu) or cadmium (Cd) at the level of 5 mmol/L.

Enzyme	Diets			sig.
	Control	Cu	Cd	
ALT, (µmol/L)	0.62 ^{b}	0.64 ^{b}	0.73 ^{a}	*
AST, (µmol/L)	1.08 ^{b}	1.15 ^{b}	1.40 ^{a}	**
GGT, (µmol/L)	1.32 ^{a}	1.44 ^{a}	1.15 ^{b}	*
Urease, (µg/dL)	13.70 ^{a}	3.05 ^{b}	3.15 ^{b}	**

[1]influence of copper and cadmium on alanine aminotransferase (ALT); aspartate aminotransfarase (AST); gamma-glutamyl transferase (GGT) and urease activities in rumem fluid of sheep. Each of the data points is an average of three measurements (n=3). a,b; means in the same row with different superscripts are significantly different , Sig.: Level of significant, NS: not significance, *= (P?0.05); **= (P?0.01).

Results of the present study indicate that heavy metals could affect several rumen enzymes. This is on the same line with the results of Garcia-Gomez et al., (2000) who reported that copper and cadmium effect is enzyme-specific. Also working with *Prevotella ruminicola,* an organism playing a prominent role in the breakdown of peptides in the rumen,Wallace and McKain (1996) reported that copper, chromium, and mercury, decreased the breakdown of peptides to 15, 15 and 5% of control activity, whereas cobalt, manganese and zinc stimulated activity by 189, 30 and 26%, respectively. The principal source of air, soil and water contamination by copper is caused by copper and iron manufacture. Cadmium gets into the environment from iron foundries. Cadmium and copper, released into atmosphere, finally accumulate in the soil and water and enter the food chain.

Cadmium and copper were found to decrease ruminal urease activity to about 22.3 and 23% of control activity, respectively (*Table 3*). Similar results were reported by Spears and Hatfield (1978) who reported that copper and cadmium ions inhibited, in_*vitro*, urease activity in ewes ruminal fluid whereas barium nickel and manganese were of stimulatory effect. Also Fahmy *et al.* (1998) described the effectiveness of heavy metals (Hg_2^+>Cu_2^+>Zn_2^+>Ni_2^+>Co_2) as inhibitors of the camel rumen urease at the concentration of 0.005 mM.

It is well known that Rumen urease hydrolyzes feed and endogenous urea to a form of nitrogen that can be used by most rumen microorganisms. Reducing the rate of ammonia nitrogen release from dietary urea would decrease its utilization in ruminants (Ludden *et al.* 2000a; Musalia *et al.* 2000

and Prasad *et al.* 1999). But the rumen microflora may often be capable of adapting to chronic administration of urease inhibitors, thereby limiting its practical use in improving the utilization of dietary urea (Ludden *et al.* 2000b).

On the other hand and as can be seen from *Table3* cadmium significantly increased the ALT and AST activity in the ruminal fluid. This stimulatory effect of cadmium on both transaminase activities might be a result of damage to the bacterial membrane by cadmium that could lead to release of enzymes tested into the rumen fluid Faixova, and Faix, 2002)

Ruminal characterization

Ruminal ammonia concentration, pH, and total and individual volatile fatty acids (VFA) data were shown in *Table 4.*

Table 4. Ruminal characteristics‡ of sheep fed diet contaminated with copper (Cu) or cadmium (Cd) at level of 5 mmol/L.

Item	Diets			Sig.
	Control	Cu	Cd	
NH3-N, mg/L	151.99 [a]	139.12 [b]	144.77 [ab]	*
pH	6.70	6.71	6.60	NS
Total VFA, mmol/L	76.89	75.78	73.72	NS
Individual VFA, mmol/L:				
Acetic acid	57.11 [a]	55.29 [a]	51.84 [b]	*
Propionic acid	13.98	14.36	14.84	NS
Butyric acid	4.82 [b]	4.89 [a]	5.68 [a]	*
Isobutyric acid	1.08 [a]	0.88 [b]	0.59 [b]	*
Valeric acid	0.65	0.80	0.79	NS
Isovaleric acid	1.29 [a]	1.18 [a]	0.35 [b]	*
Total isoacids	2.45 [a]	2.07 [a]	0.94 [b]	*
Acetic, Propionic ratio	4.13	3.92	3.66	NS

‡Data are average values for ruminal fluid samples taken at zero time (before feeding), 1, 3, 5, 7, 9, 12 and 24 h after feeding.

a,b; means in the same row with different superscripts significantly differ at (P?0.05). Sig.: Level of significant, NS: no significant.

A significant decrease (P?0.05) was observed in ammonia concentration by treated diets with copper or cadmium chloride. Lower values were obtained in copper treated diet without significant difference (P?0.05) with cadmium treated diet. According to the changes in ruminal ammonia concentration along the times of sampling after feeding. It was observed that the highest value (188 mg/L, approximately) for all treatments was at 3 h and then dropped to 101 at 9 h and 110 at 7 h mg/L, for copper and cadmium diets respectively (*Figure 1*). This negative effect of heavy metals on ammonia concentration could be due to the capacity of metals used to inhibit the activity of proteolytic enzymes in the rumen. Similar observation was reported by Karr et al., (1991) who suggested that heavy metals salts might inactivate proteolytic enzymes of selected ruminal bacteria. Rumen microbes can synthesize enough amino acids and peptides from the inorganic nitrogen in ammonia or other nitrogen source and carbon skeletons and sulphur precursors. Ammonia assimilation by rumen microbes depends on rumen pH (Veth et al. 1999), rumen ammonia concentration (Mehrez *et al.* 1977) and ruminal ammonia-assimilating enzyme activity.

Several ammonia-assimilation reactions by rumen bacteria are known. Enzyme glutamate dehydrogenase plays an important role in maintaining the balance between ammonia- and α-amino-nitrogen of the rumen. Alanine represents the amino acid found in the highest concentration in the intracellular pool of free amino acid rumen bacteria. Rumen bacteria possess effective mechanisms for alanine synthesis from ammonia (e.g., alanine dehydrogenase and alanine aminotransferase). Both alanine aminotransferase and aspartate aminotransferase belong to the most common transaminases in the rumen. Glutamate concentration by rumen bacteria depends on gamma-glutamyltransferase activity. This enzyme plays an important role in some peptide and amino acid transfers through the rumen wall and in the formation of an intracellular pool of glutamate as well.

Ruminal pH and total VFA concentrations were unaffected (P?0.05) by treating the diets with both heavy metals. The behaviour of changes in total and individual VFAs concentration along all times of sampling after feeding are shown in *Figure 2*. Treating diets with copper or cadmium had decreased significantly (P?0.05) branched-chain VFAs concentration such as isobutyric and isovaleric acids and acetic and butyric acid concentrations in the rumen of sheep. This effect could be due to the negative effect of heavy metals on ruminal proteolytic enzymes that affect OM and CP digestion (Cecava *et al.*, 1993). Cadmium had more negative effect than copper on ruminl VFAs concentrations. Molar proportions of propionic and valeric acids were not affected (P?0.05) by the treatments.

Increasing metal salts in feed could increase the dilution rate of the rumen fluid phase and negatively affect the protozoal population (Shawkat et al., 1985). It, therefore, decrease the digestion in the fore stomach which is later compensated for at least partially, in the lower tract (Potter et al., 1972 and Hemsley et al., 1975). Furthermore, salt intake and the associated changes in the rumen dilution rate influence the pattern of microbial fermentation (Hudgson and Thomas, 1975).

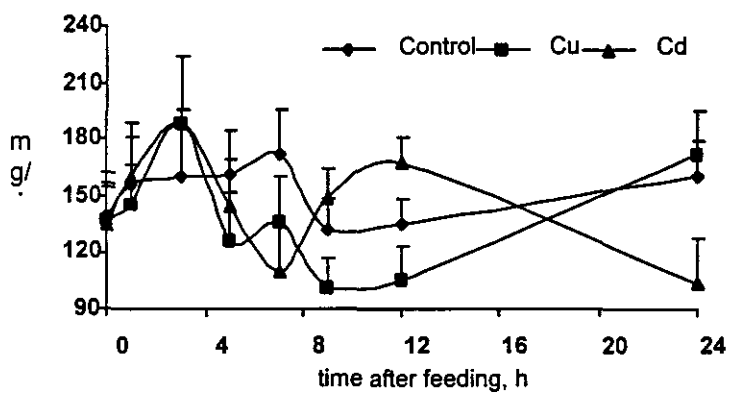

Figure 1: Ammonia concentration of experimental diets fed to sheep.

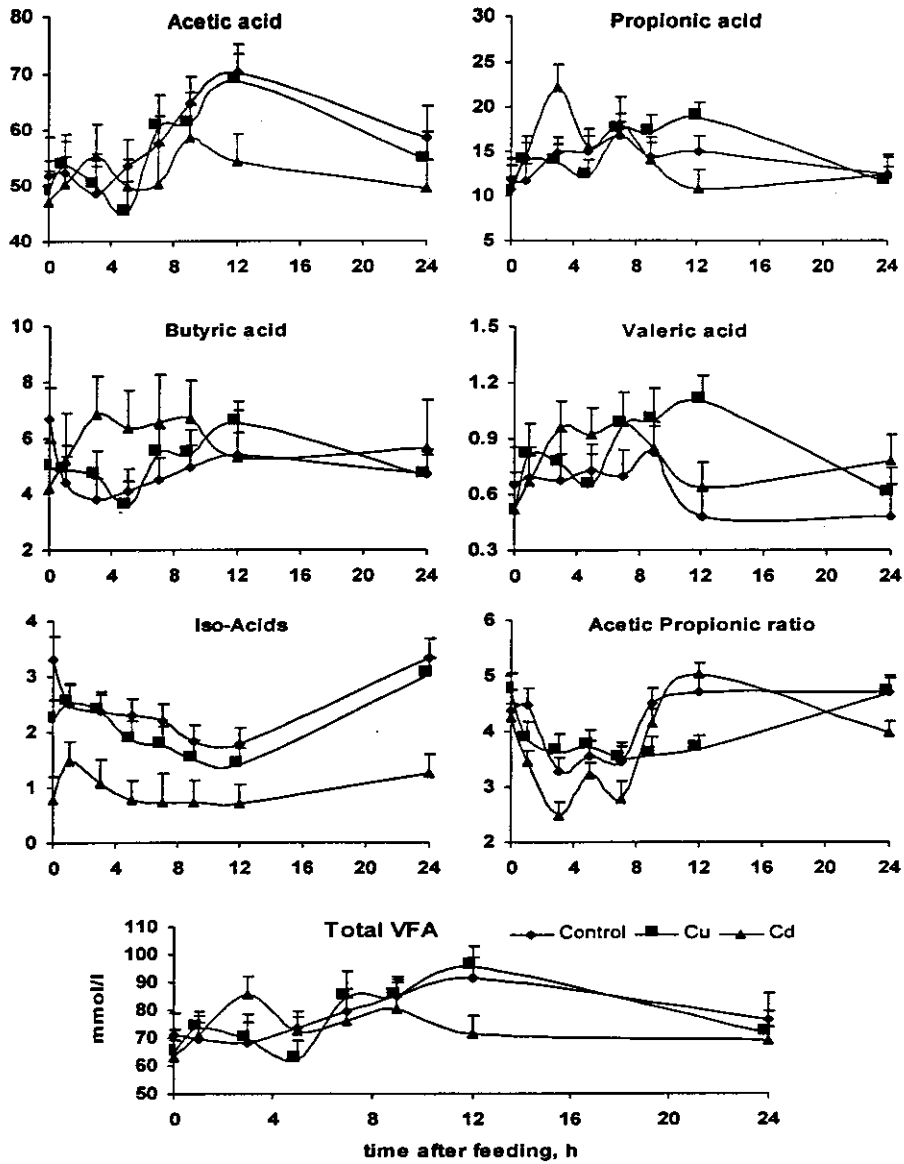

Figure 2: Total and individual volatile fatty acids (VFA) concentrations (mmol/L) in the rumen of sheep fed the experimental diets after different times of feeding.

Digestibility

Treating diets with heavy metals (copper or cadmium) appeared to reduce the digestibility coefficients of all nutrient components except nitrogen free extract (*Table 5*).

Reducing feed digestibility by treating diets with heavy metals could be as a consequence to different factors. One of this, is inhibition effect on the growth of ruminal microorganisms (Salem and Gohar, unpublished data) and inhibition of ruminal enzymes and ruminal fermentation of feed. Another factor may be the interaction of heavy metals with nutrient components which, therefore, lead to unavailability of nutrient OM for ruminal microorganism's degradation. Similar results were observed by Squires (1973) who found that heavy metal negatively affected nutrient digestion. Decreased the digestibility coefficients of both crude protein and crude fiber was also reported (Cecava, et al., 1993 and Sooud et al., 1988). This was attributed to that excessive level of metals, which may counteract each other at higher concentrations leading to their unavailability for rumen microorganisms. This reaction is thought to result in the formation of highly stable compounds (insoluble compounds) that cannot be digested and absorbed (Allen and Gawthorne, 1987).

Table 5: Digestibility coefficients (%) of sheep fed diet contaminated with copper (Cu) or cadmium (Cd) at the level of 5 mmol/L.

Item	Diets			sig.
	Control	Cu	Cd	
Dry matter	82.10 [a]	79.45 [b]	72.04 [c]	*
Organic matter	80.01 [a]	77.20 [a]	68.50 [b]	*
Crude protein	65.05 [a]	61.41 [b]	62.66 [b]	*
Ether extract	49.90 [a]	48.18 [a]	46.25 [b]	*
Nitrogen free extract	61.10	60.24	59.01	NS
Fiber fractions:				
Natural detergent fiber	56.38 [a]	49.67 [c]	51.46 [b]	*
Acid detergent fiber	54.29 [a]	49.29 [b]	49.53 [b]	*
Cellulose	61.26 [a]	57.02 [b]	58.21 [b]	*
Hemicellulose	60.61 [a]	50.41 [c]	54.75 [b]	*

a,b,c; means in the same row with different superscripts are significantly different at (P?0.05). Sig.: Level of significance, NS: no significant.

CONCLUSION

Our results presented in this study indicated that, exposure of sheep to the heavy metals dose used had increased the amount of water consumed and negatively affected several ruminal enzymes and nutrient fermentation. Copper and cadmium dose used played an important role in altering the feed rumen fermentation and its digestibility.

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REFERENCES

Allen, J. D. and J. M. Gawthorne (1987). Involvement of the solid phase rumen digesta in the interaction between copper, molybdenum and sulfur in sheep. *Br. Journal of Nutrition* 58:265.

A.O.A.C. (1980). Official Methods for Analysis. 13th ed. Washington, DC.

Cecava, M. J.; L. Deana and J. E. Parker (1993). Effect of zinc-treated soybean meal on ruminal fermentation and intestinal amino acid flows in steers fed corn silage-based diet. *Journal of Animal Science* 71:3423-3431.

Duncan, D. B. (1955). Multiple range and multiple F-test. *Biometrics*, 11:1-42.

Engle, T.E. and J. W. Spears (2000). Effect of dietary copper concentration and source on performance and copper status of growing and finishing steers. *Journal of Animal Science* 78: 2446-2451

Faixova, Z. and S. Faix, (2002). Influence of Metal Ions on Ruminal Enzyme Activities *Acta Veterinary BRNO* 71: 451–455.

Fahmy, A. S.; N. Y. Abadir and B. M. Abd-Alla (1998). Purification and characterization of urease from the ruminal fluid of camel (*Camelus dromedarius*). *Journal. Camel Pract. Res.* 5: 143-155.

Garcia-Gomez, F.; P. A. Williams; A. Aumaitre; B. D. Lee and J. K. Ha (2000). Magnesium metabolism in ruminant animals and its relationship to other inorganic elements. Proceedings of the 2000 International Symposium: Recent Advances in Animal Nutrition, Seoul, Korea, 20-22 April 2000. *Asian-Australian Journal of Animal Science* 13: 158-170

Goering, H. K. and P. J. Van Soest, (1970). Forage fiber analysis (apparatus reagents, procedures and some applications). Agricultural handbook, pp. 379. Agricultural Research Service, United States Department of Agriculture, Washington, DC.

Hartmans, J. (1975). The frequency of occurrence of copper poisoning and the role of sheep concentrates in merit inquiry. *Tijdscbrift Diergeneeskunde* 100:379-382.

Haurowitz, F. (1950). Chemistry and Biology of Proteins. Academic Press, New York.

Hemsly, J. A.; J. P. Hogan and R. H. Welson (1975). Effect of high intake of sodium chloride on the utilization of protein concentrate by sheep. II. Digestion and absorption of organic matter and electrolytes. *Australian Journal of Agriculture Research* 26:715.

Hudgson, J. C. and G. F. Thomas (1975). A relationship between the molar proporation of the propionic acid and the clearance rate of the liquid phase in the rumen of sheep. *British Journal of Nutrition* 33:447.

Jencik, F.; J. Bires; P. Bindas; Z. Dankovcikova; F. Lesnik; R. Mariscakova; N. Ucekaj and J. Buleca (2001). Transport of risky mineral elements from the soil into the plant biomass II. Xth scientific symposium, Hradok, 13-14 December 2001. Proceedings of scientific symposium On Ecology in Selected Agglomerations of Jelsava-Lubenik and Central Spis, 41-45.

Karr, K. J.; K. A. Dawson and G. E. Mitchell (1991). Inhibitory effects of zinc on the growth and proteolytic activity of selected strains of ruminal bacteria. p. 27, Beef Cattle Res., University of Kentucky, Lexington.

Legath, J.; P. Kacmar, and J. Tomas (1990). Changes in the amino acid concentrations of bacteria adhering to the ruminal epithelium of sheep after the oral administration of extremely low doses of mercury. *Vet. Med. Praha* 35:657-665.

Ludden, P. A.; D. L. Harmon; B. T. Larson and D. E. Axe (2000a). Influence of the novel urease inhibitor N-(nbutyl) thiophosphoric triamide on ruminant nitrogen metabolism: I. In vitro urea kinetics and substrate digestion. *Journal of Animal Science* 78: 181-187.

Ludden, P. A.; D. L. Harmon; G. B. Huntington; B. T. Larson and D. E. Axe (2000b). Influence of the novel urease inhibitor N-(n-butyl)thiophosphoric triamide on ruminal nitrogen metabolism: II. Ruminal nitrogen metabolism, diet digestibility, and nitrogen balance in lambs. *Journal of Animal Science* 78: 188-198.

McDonald, P.; A. C. Stirling; A. R. Henderson; W. A. Dewar; G. H. Stark; W. G. Davie; H. T. Macpherson; A. M. Reid and J. Salter (1960). Studies on ensilage Technical bulletin no. 24, Edinburgh School of Agriculture, pp. 1-83.

Mehrez, Z.; E. R. ?rskov and I. McDonald (1977). Rates of rumen fermentation in relation to ammonia concentration. *British Journal of Nutrition* 38: 433-441.

Musalia, L. M.; S. Anandan; V. R. B. Sastry and D. K. Agrawal (2000). Urea treated neem (*Azadirachta indica A.juss*) seed kernel cake as a protein supplement for lambs. *Small Anim Res* 35: 107-116.

Odenkirchen, S.; M. Holterschinken and H. Scholtz (1994). Auswirkungen oral zu verabreichender Therapeutika auf Fermentationsvorgange im Pansensaff ruminierender Rinder (in vitro). 6. *Mitteilung: Kupfersulfat. Dtsche Tierarzt Wchst* 101: 16-18

Ottenstein, D. M. and D. A. Bartly (1971). Separation of free acids C_2-C_5 in diluted aqueous solution column technology. *Journal of Chromatographic Science* 9:673-681.

Pal, D. T.; D. N. Kamra; N. N. Pathak and G. S. Bisht (1998). Influence of rumen ciliate protozoa and protein level on rumen fermentation, enzyme activities and blood metabolites in crossbred cattle calves. *Journal of Applied Animal Research* 13:153-159

Potter, B. J.; D. J. Walker and W. W. Forrest (1972). Changes in intra ruminal function of sheep when drinking saline water. *British Journal of Nutrition* 28:796.

Prasad, K.; D. S. Sahu; I. S. Nagwal and K. Prasad (1999). Effect of defaunation and two levels of protein on rumen fermentation pattern and enzyme activity in crossbred bullocks. *Industry Journal of Animal Nutrition* 16: 248-251.

Pribilincova, J.; M. Maretta; I. Janotikova and E. Marettova (1995). The effect of cadmium treatment on breeding hens and cocks and early viability of their chickens. *Vet Med-Czech* 40: 353-357.

SAS institute INC. (1993). SAS Companion for the Microsoft Windows Environment, Version 6. SAS Inc., Cary, NC, USA.

Shawkat,S. M.; M. F. A. Farid and M. H. Ahmed (1985). Effect of drinking well water in sheep. II. Rumen function and the rumen protozoa population. *1st International Congress of Animal Production In Arid Zones.* 7-14 Sept., Damascus, Syria.

Sooud, A. O.; M. A. El-Ashry and H. M. Kandil (1988). Preliminary observations of the effect of water and feed deprivation on nutrient utilization in camels offered fresh or saline water. *International symposium on the constraints and possibilities of production in the dry sub tropics*, Cairo, Egypt, 5-7 Nov.

Spears, J. W. and E. E. Hatfield (1978). Nickel for ruminants. I. Influence of dietary nickel on ruminal urease activity. *Journal of Animal Science* 47: 1345-1350.

Squires, V. P. (1973) A review of Australian experience with saline drinking for sheep. *Proceeding of the symposium on Animal and Water relations.* Kimberly, Idaho, p.133.

Steel, R. G. D. and J. H. Torrie (1980). Principles and Procedures of Statistics. 2nd ed. McGraw-Hill International.

Veth, M. J. D. E.; E. S. D. E. Kolver; M. J. Veth and D. Cottle (1999). Pasture digestion in response to change in ruminal pH. 59th conference, Holy Cross College, Mosgiel, 28 June-1 July 1999. *Proceedings of the New Zealand Society of Animal Production* 59: 66-69.

Wallace, R. J.; N. McKain (1996). Influence of 1,10 phenanthroline and its analogues, other chelators and transition metal ions on dipeptidase activity of the rumen bacterium, *Prevotella ruminicola. Journal Applied of Bacteriology* 81: 42-47.

Wiener, G.; N. F. Suttle; A. C. Field; J. G. Herbert and J. A. Woolliams (1978). Breed differences in copper metabolism in sheep. *Journal of Agriculture Science, Cambridge* 91:433-441.

Wilson, A. D. (1978). Water Requirements of sheep. In: Water in Rangelands, pp. 178-189, K.M.W.Howes, ed., CSIRO, Melbourne.

تأثير العلائق الملوثة بالنحاس و الكادميوم على الماء المتناول و نشاط إنزيمات وصفات الكرش و هضم الغذاء في الأغنام

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استخدم في هذه الدراسة ٩ من الأغنام الرحمانى لإجراء تقيم لتأثير المعاملة بكلور يد النحاس و كلوريد الكادميوم كمعادن ثقيلة على كمية الماء المتناول و إنزيمات و نشاط الكرش و كذلك هضم الغذاء. تم تقسيم الحيوانات إلى ثلاث مجموعات. المجموعة الأولى غذيت على ٤٠٠ جم علف مركز بالإضافة إلى ٢٠ جم مادة جافة/كجم وزن حي من الدريس و استخدمت كمجموعة كنترول. المجموعة الثانية و الثالثة غذيت على نفس العليقة مع معاملة العلف المركز بواسطة ١٠٠ مل لكل ٤٠٠ جم علف مركز من محلول ٥ مل مول لكل لتر من كلور يد النحاس و كلوريد الكادميوم على التوالي. تم تغذية الحيوانات مرة واحدة في اليوم.

أظهرت النتائج ارتفاع معنوي (P?0.05) في كمية الماء المستهلك بمعاملة العليقة بواسطة المعادن الثقيلة. أدت المعاملة إلى انخفاض معنوي و ملحوظ (P?0.01) في نشاط إنزيم اليوريز في كرش الحيوانات. المعاملة بالكادميوم أدت إلى حدوث تغير في جميع إنزيمات الكرش التي تم دراستها. أدت المعاملة بالنحاس وكذلك الكادميوم إلى انخفاض في تركيز الامونيا في كرش الأغنام. لم يتأثر التركيز الكلى للاحماض الدهنية الطيارة و كذلك رقم الحموضة بالمعاملة بالمعادن الثقيلة و لكن أدت المعاملة إلى حدوث انخفاض معنوي (P?0.05) في تركيز حمض البيوتيريك و الأحماض الدهنية الطيارة المتشعبة مثل الايزوبيوتيريك و الايزوفاليريك. لم يكن للمعاملة أي تأثير معنوي على تركيز حمض البروبيونيك و كذلك الفاليريك. المعاملة بالمعادن الثقيلة أدت إلى انخفاض في معاملات هضم جميع مكونات الغذاء ما عدا الكربوهيدرات الذائبة.

من هذه الدراسة يمكن استنتاج أن تعريض علائق الأغنام إلى ٥ مل مول من كلوريد النحاس أو الكادميوم يمكن أن تؤدى إلى زيادة كمية الماء المستهلكة و كذلك يمكن أن تؤثر سلبيا على العديد من إنزيمات الكرش و الاستفادة من الغذاء في القناة الهضمية للاغنام.