Influence of in vitro addition of metal ions salts on rumen fermentation parameters and selected ruminal enzymes activity in sheep and goats

Mousa, S.A

Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University. Giza, 12211, Egypt.
sdiamond_201011@yahoo.com

Abstract: Rumen fluid was collected from 10 sheep and 10 goats to study effect of in vitro addition of $MgCl_2$, $CuCl_2$ and $ZnCl_2$ in concentration (5mmol/L) on total volatile fatty acids (TVFAs), ammonia concentration and total protozoa count and activity of ruminal alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and glutamate dehydrogenase (GDH) with or without salts after shaken and incubation for 30 min at 37c°. The results showed that $MgCl_2$ significantly increase ($p\leq0.001$) of ammonia and significantly decrease ($p\leq0.01$) of total protozoa count in sheep with significantly decrease($p\leq0.01$) of TVFAs, ($p\leq0.001$) of total protozoa count and significantly increase ($p\leq0.01$) of ammonia in goats. It stimulated significantly ($p\leq0.001$) both GGT and GDH activity in sheep and goats. $CuCl_2$ significantly increase ($p\leq0.001$) of ammonia, significantly decrease ($p\leq0.001$) of total protozoa count in sheep with significantly decrease ($p\leq0.01$) of ammonia and total protozoa count in goats also it significantly inhibited ($p\leq0.001$) GDH activity in both sheep and goats. $ZnCl_2$ significantly decrease ($p\leq0.001$) of TVFAs and total protozoa count in sheep with significantly increase ($p\leq0.001$) of ammonia and significantly decrease ($p\leq0.001$) of total protozoa count in goats. Also it significantly stimulated ($p\leq0.001$). ALT, AST and GDH activity with significantly inhibited ($p\leq0.001$) GGT activity in both sheep and goats. In conclusion in vitro addition of heavy metal salts have a serious effect on both rumen fermentation properties and rumen enzymes activity which sequencelly will be reflect on animal health and productivity.
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Key words: Metal salts, Rumen fermentation, Ruminal enzyme, Sheep, Goats

1. Introduction:

Environmental pollution is one of major worldwide problem that affect human health and livestock productivity. Heavy metals consider the main causes of environmental toxicity in farm animals (Rajoganapathy et al, 2011) as it contaminate water, food and air with induction of harmful hazard on animals by affecting hormone system, biological function and growth of body tissue (Teresa et al, 1997; Bojkovski et al, 2010). Digestion in ruminant is depending on ruminal microbes which have ability to synthesis enough amino acids and peptides from the inorganic nitrogen in ammonia (Faixova et al, 2002) that supply 70 to 100% of amino acid requirement in the form of microbial protein and also have ability to synthesis70 to 85% of energy requirement in the form of short chain fatty acids (Thirumalesh et al, 2013). 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.

Glutamate dehydrogenase (GDH) plays a great role in balance between ammonia and α amino nitrogen of the rumen. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have an important job in process of transamination in the rumen. Gamma glutamyl transferase (GGT) plays an essential role in transferring of some amino acids and peptides through the ruminal wall and formation of an intracellular pool of glutamate (Faixova et al, 2002).

Many of papers have dealt with effect of different metal ions salts on ruminal enzymes activity but not on rumen fermentation parameters, So this study was aimed to clarifying the effect of in vitro addition of various metal ions salts as magnesium, copper and zinc chloride that produce air, soil and water biomass contamination in industrial exposed areas on ruminal fermentation parameters and selected ruminal enzyme activity.

2. Materials and methods:

Animals

Ten adult apparent healthy sheep and ten adult apparent healthy goats aged between 2-5 year, weighting between 30-35kg with different sex were used in this study. These animals were belonging to clinical hospital of department of internal medicine and infectious diseases, Faculty of veterinary medicine, Cairo university and housed individually. These animals were fed twice daily with diet based on dehydrated alfalfa and corn grain in 3:1 DM

ratio,vitamins, mineral and ad libtm water were offered. All animals were maintained according to the principles governing the care of laboratory animals.

Samples

Rumen juice sample was collected after 30 day of receiving the diet from each animal by means of silicon stomach tube connected to suction pump in the morning 2 hours after feeding. It was strained through four layers of gauze within 30min of collection.

Methods

Magnesium, copper and zinc chloride salts were used in concentration 5mmol/L for study its effects on rumen fermentation parameters (total volatile fatty acids (TVFAs), ammonia nitrogen level and total protozoal count) and selected rumen fluid enzymes activity.

Strained rumen fluid was divided into four portions. The first portion for determination of TVFAs (with and without each metal salt) according to to Warner (1964),The second portion for determination of ammonia nitrogen level(with and without each metal salt) according to Conway (1957), The third portion for estimating total protozol count(with and without each metal salt) according to Dehorety (1986) and the forth portion for determination of selected rumen fluid enzymes activity(with and without each metal salt) after shaken and incubation for 30min at 37°C. The commercial kits were used in determination the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and glutmate dehydrogenase (GD) (Spinreact company, Spain) with using specific spectrophotometer (Apple 302, USA). All kits were performed according to the manufacturer's recommendations.

Statistical Analysis

The obtained results were expressed as mean and mean of standard error (M±SE) and analyzed statistically by using SPSS 16.0 software package (t-Test) (SPSS Inc., Chicago, IL, USA). Significant differences in the values between groups were indicated by $P^{*}\leq 0.05$, $P^{**}\leq 0.01$and $P^{***}\leq 0.001$.

3. Results:

From our obtained results in vitro addition of metal ions salts have a great effect on rumen fermentation parameters and selected ruminal enzymes activity as the following:

Table (1): Influence of in vitro addition of metal ions salts in concentration (5mmol/L) on rumen fermentation parameters in sheep.

Parameters	TVFAs (mmol/L)	Ammonia concentration (mmol/L)	Total protozoa count ($\times 10^4$/ml)
Control(without addition)	30.39 ± 0.62	331.07 ± 14.82	21.38 ± 3.74
$MgCl_2$	30.79 ± 0.57	373.25 ± 6.20***	11.02 ± 2.88**
$CuCl_2$	30.01 ± 0.45	386.26 ± 3.31***	8.32 ± 2.74***
$ZnCl_2$	22.07 ± 1.30***	320.15 ± 34.88	6.52 ± 1.43***

Denote means values significant at P≤ 0.05, P**≤ 0.01, P***≤ 0.001.

Comparison between rumen fermentation parameters without and with magnesium chloride showed that magnesium significantly increase (P≤ 0.001) of ammonia concentration (331.07 ± 14.82 to 373.25 ± 6.20) and significantly decrease (P≤ 0. 01) of total protozoa count (21.38 ± 3.74 to 11.02 ± 2.88).Addition of copper showed significantly increase (P≤ 0.001) of ammonia concentration (331.07 ± 14.82 to 386.26 ± 3.31) and significantly decrease (P≤ 0.001) of total protozoa count (21.38 ± 3.74 to 8.32 ± 2.74), While addition of zinc significantly decrease (P≤ 0.0 01) of TVFAs (30.39 ± 0.62 to 22.07 ± 1.30) and total protozoa count (21.38 ± 3.74 to 6.52 ± 1.43).

Table (2): Influence of in vitro addition of metal ions salts in concentration (5mmol/L) on activity of selected ruminal enzymes in sheep.

Parameters	ALT (µKat/L)	AST (µKat/L)	GGT (µKat/L)	GDH (µKat/L)
Control(without addition)	0.367 ± 0.006	0.836 ± 0.007	1.15 ± 0.028	0.141 ± 0.002
$MgCl_2$	0.357 ± 0.008	0.827 ± 0.006	1.39 ± 0.025***	0.212 ± 0.003***
$CuCl_2$	0.359 ± 0.009	0.824 ± 0.006	1.14 ± 0.031	0.052 ± 0.001***
$ZnCl_2$	1.026 ± 0.047***	1.730 ± 0.098***	0.88 ± 0.025***	0.224 ±0.002***

Denote means values significant at P≤ 0.05, P**≤ 0.01, P***≤ 0.001

Aspartate Aminotransferase(AST), alanine aminotransferase (ALT), gamma-glutamyltranferase (GGT), glutamate dehydrogenase (GDH).

Comparison of the activity of selected rumen enzymes without and with magnesium showed that magnesium significantly stimulated (P≤ 0.001) both GGT (1.15 ± 0.028 to 1.39 ± 0.025 µKat/L) and GDH (0.141 ± 0.002 to 0.212 ± 0.003 µKat/L) with no significant difference for ALT(0.367 ± 0.006 to 0.357 ± 0.008 µKat/L) and AST (0.836 ± 0.007 to 0.827 ± 0.006 µKat/L).

Copper significantly inhibited (P≤ 0.001) GDH activity (0.141 ± 0. 002 to 0.052 ± 0.001 µKat/L)but did not affect activity of ALT (0.367 ± 0.006 to 0.359 ± 0.009 µKat/L), AST (0.836 ± 0.007 to 0.824 ± 0.006 µKat/L) and GGT (1.15 ± 0.028 to 1.14 ± 0.031 µKat/L).

The effect of zinc was significantly achieved in ALT, AST, GGT and GDH. It was found that zinc stimulated (P≤ 0.001) ALT (0.367 ± 0.006 to 1.026 ± 0.047 µKat/L), AST (0.836 ± 0.007 to 1.730 ± 0.098 µKat/L) and GDH (0.141 ± 0.002 to 0.224 ±0.002 µKat/L) while on the other hand it inhibited (P≤ 0.001) GGT (1.15 ± 0.028 to 0.88 ± 0.025 µKat/L).

Table (3): Influence of in vitro addition of metal ions salts in concentration (5mmol/L) on rumen fermentation parameters in goat.

Parameters	TVFAs (mmol/L)	Ammonia concentration (mmol/L)	Total protozoa count ($\times 10^4$/ml)
Control(without addition)	30.35 ± 0.47	350.74 ± 7.85	20.30 ± 3.45
$MgCl_2$	26.12 ± 1.13**	376.34 ± 8.15*	8.62 ± 1.44***
$CuCl_2$	31.85 ± 4.04	290.27 ± 18.77**	8.62 ± 1.18***
$ZnCl_2$	30.25 ± 0.35	371.86 ± 4.53***	8.33 ± 1.20***

Denote means values significant at P≤ 0.05, P**≤ 0.01, P***≤ 0.001.

Comparison between rumen fermentation parameters without and with magnesium chloride showed that magnesium significantly decrease (P≤ 0.01) of TVFAs (30.35 ± 0.47 to 26.12 ± 1.13) and (P≤ 0.001) of total protozoa count (20.30 ± 3.45 to 8.62 ± 1.44) with significant increase(P≤ 0.05) of ammonia concentration(350.74 ± 7.85 to 376.34 ± 8.15). Addition of copper showed significantly decrease (P≤ 0.01) of ammonia concentration (350.74 ± 7.85 to 290.27 ± 18.77) and total protozoa count (20.30 ± 3.45 to 8.62 ± 1.18), While addition of zinc significantly increase (P≤ 0.001) of ammonia concentration (350.74 ± 7.85 to 371.86 ± 4.53) with significantly decrease (P≤ 0.001) of total protozoa count (20.30 ± 3.45 to 8.33 ± 1.20).

Table (4): Influence of in vitro addition of metal ions salts in concentration (5mmol/L) on activity of selected ruminal enzymes in goat.

Parameters	ALT (µKat/L)	AST (µKat/L)	GGT (µKat/L)	GDH (µKat/L)
Control(without addition)	0.375 ± 0.004	0.836 ± 0.007	1.14 ± 0.032	0.143 ± 0.001
$MgCl_2$	0.370 ± 0.005	0.823 ± 0.007	1.39 ± 0.015***	0.215 ± 0.002***
$CuCl_2$	0.365 ± 0.004	0.828 ± 0.006	1.13 ± 0.029	0.053 ± 0.001***
$ZnCl_2$	1.009 ± 0.042***	1.830 ± 0.107***	0.89 ± 0.008***	0.224 ±0.001***

Denote means values significant at P≤ 0.05, P**≤ 0.01, P***≤ 0.001

Aspartate Aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltranferase (GGT), glutamate dehydrogenase (GDH).

Comparison of the activity of selected rumen enzymes without and with magnesium showed that magnesium stimulated significantly (P≤ 0.001) both GGT (1.14 ± 0.032 to 1.39 ± 0.015 µKat/L) and GDH (0.143 ± 0.001 to 0.215 ± 0.002 µKat/L) with no significant difference for ALT(0.375 ± 0.004 to 0.370 ± 0.005 µKat/L) and AST (0.836 ± 0.007 to 0.823 ± 0.007 µKat/L).

Copper significantly (P≤ 0.001) inhibited activity of GDH (0.143 ± 0. 001 to 0.053 ± 0.001 µKat/L) but did not affect activity of ALT (0.375 ± 0.004 to 0.365 ± 0.004 µKat/L), AST (0.836 ± 0.007 to 0.823 ± 0.006 µKat/L) and GGT (1.14 ± 0.032 to 1.13 ± 0.029µKat/L).

The effect of zinc was significantly achieved in ALT, AST,GGT and GDH. It was found that zinc stimulated (P≤ 0.001) ALT (0.375 ± 0.004 to 1.009 ± 0.042 µKat/L), AST (0.836 ± 0.007 to 1.830 ± 0.107 µKat/L) and GDH (0.143 ± 0.001to 0.224 ±0.001

μKat/L) while on the other hand it inhibited (P≤ 0.001) GGT (1.14 ± 0.032 to 0.89 ± 0.008 μKat/L).

4. Discussion:

The results showed that metal ions could be affect rumen fermentation parameters and activity of various rumen enzymes. Magnesium is an active ingredient of various of enzymes as creatine kinase and activates pyruvic acid carboxylase, pyruvic acid oxidase and the condensing enzymes in the citric acid cycle (Soetan et al, 2010) also it exhibits some functions as an anti stress, antioxidant, antipyretic and anti inflammatory agent (Garica-Gomez et al, 2000) presence of magnesium fly ash in area surrounding factories of metal have a great hazards on animal health and productivity such as depression of fertility, reduced numbers of offspring (Bires et al, 1994).

Data from my study showed that in vitro addition of magnesium salts in concentration (5mmol/L) induced significantly increase (p≤0.001) in ammonia concentration with significantly decrease (p≤0.01) in total protozoa count in sheep while in goat showed significantly decrease (p≤0.01) in TVFAs and (p≤0.001) in total protozoa count with significantly increase (p≤0.05) in ammonia concentration, These results were disagreement with (Hubbert et al, 1958) who reported that in vitro addition of magnesium salts to rumen fluid did not results in increasing cellulose digestion. Magnesium have a great effect on ruminal enzymes activity it significantly stimulated (p≤0.001) both GGT and GDH with no significantly effect on both ALT and AST enzymes in sheep and goats as the results were recorded by (Faixova et al, 2002; 2004 and 2006).

Copper is an essential micro mineral for hematologic and neurologic systems (Tan et al, 2006). It enters in formation of several enzymes as cytochrome oxidase, peroxidase and ceruloplasmin (Soetan et al, 2010) also essential for formation of myelin sheaths in the nervous system and helps incorporation of iron in haemoglobin (Murray et al, 2000). Sheep are more susceptible than cattle to the toxic effects of copper (Merck, 1986).

Data from my study showed that in vitro addition of copper salts in concentration (5mmol/L) induced significantly increase(p≤0.001) in ammonia concentration and significantly decrease (p≤0.001) in total protozoa count with non significant decrease in TVFAs in sheep similar results were recorded by (Moreno, 2012) in yearling steers received supplementary copper in diet while in goat showed significantly decrease (p≤0.01) in ammonia concentration and (p≤0.001) in total protozoa count with non significant increase in TVFAs as the results were reported by (Zhang et al, 2007) in cashmere wether goats received supplementary copper in diet. Copper significantly inhibited (p≤0.001) GDH activity but did not affect activity of ALT, AST and GGT in sheep and goats as the results were reported by (Faixova et al, 2002, 2004 and 2006). Copper and zinc are well known to interact with sulphydryl group in the protein or enzymes or they displace the metal ions from the enzyme, thereby interfering with enzymatic activity (Faixova et al, 2006)

Later (Wallace and Mc Kain, 1996) reported that copper inhibited *Prevotella ruminicola dipeptidase* activity to 15% of control activity in in vitro experiment the same result was observed by (Fahmy et al, 1998) who described the effectiveness of copper as inhibitor of the camel rumen urease at the concentration 5×10^{-3}mmol/L was 94% inhibition in comparison with control. Results of further studies showed that copper have stimulatory effects on some enzymes activates (Zaki et al, 2002; Faixova et al, 2004), whereas Engle and Spears (2000) recorded that the effect of copper on ruminal enzyme activity in vitro is negligible.

Zinc is one of essential micronutrient in animal nutrition which is widely distributed in plant and animal tissues it acts as cofactor and component of many enzymes as glutamic dehydrogenase, DNA and RNA polymerase (Soetan et al, 2010).Zinc is consider as an antioxidant element for central nervous system (Hassan et al,2011) and has a great role in cell replication, gene expression, amino acid metabolism, fertility, normal testicular development, tissue repair, wound healing and digestion (Murray et al, 2000).

Data from my results indicated that in vitro addition of zinc salts in concentration 5mmol/L in sheep showed significantly decrease (p≤0.001) in TVFAs and total protozoa count as the results were recorded by (Spears et al, 2004) in steers received zinc methionine in diet with non significant decrease in ammonia concentration similar result was reported by (Froestchel et al, 1990; Arelovich et al, 2000; 2008 and Moreno, 2012) these results confirm a previous suggestion that zinc at concentration of 130 to 1.300ppm decreases ammonia accumulation from urea during in vitro incubation of ruminal fluid from sheep (Spears and Hatfield., 1978) and also zinc supplementation to the diet in sheep received low quality roughage with urea addition decrease ammonia concentration (Rodriguez et al, 1995) in my opinion zinc decrease ammonia concentration through decreasing rate of NH_3 release from urea. Result of my study indicated that zinc had significant stimulating (p≤0.001) effect on ALT, AST and GDH activities with significant inhibiting (p≤0.001) effect on GGT activity in both sheep and goats the same results were recorded by (Faixova et al, 2002, 2004

and 2006). This in agreement with findings of (Wallace and Mc Kain,1996)who reported that zinc stimulated *Prevotella ruminicola dipeptidase* activity by 26% of control activity in in vitro experiment, While on the other hand (Fahmy et al, 1998) who described that zinc in concentration 5×10^{-3}mmol/L had inhibiting effect on the camel urease activity by 90%, These results were also in agreement with observation of (Martinez et al, 2002) who reported that salts of Zn^{2+} produce 44% inhibition of amylase activity when added in concentration 5mmol/L to the rumen fluid.

Conclusions:

Finally, from obtained results the conclusions were that in vitro addition of heavy metal salts have a serious effect on both rumen fermentation properties and rumen enzymes activity which sequencelly will be reflect on animal health and productivity, These results confirmed that animals reared in industrial areas and exposed chronically to contaminated air, water and plant with heavy metal as copper, zinc …., etc affected in its health and productivity.

Corresponding author
Mousa, S.A
Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University. Giza, 12211, Egypt.
sdiamond_201011@yahoo.com

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