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Original Article

Influence of Anti-Plasmodial Metal Complex on Rumen Microbial Populations and Serum Parameters in Sheep

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ABSTRACT: A locally synthesized transition metal complex, cobalt-lumefantrine was assessed through laboratory and feeding trials to ascertain its usefulness as an agent for manipulation of the rumen in sheep grazing tropical pasture. The antimicrobial properties of the metal complex were examined on fresh rumen fluids at different concentrations (0, 0.010, 0.025 and 0.050 mg metal complex/50 ml) to estimate the optimum dosage for the sheep. Thereafter, twelve adult male sheep (11.27 ± 0.62 kg, initial body weight) were divided into three similar treatment groups. Sheep were drenched individually with 2.5 mg metal complex/head dissolved in 10 ml distilled water, either once or at two-week interval over 28 days. The control group received water without the metal complex. Total protozoa population was lowest (P < 0.05) when 50 ml rumen sample was incubated with 0.025 mg cobalt-lumefantrine metal complex. Serum alanine amino transferase, ALT (23.73 and 18.16 vs. 16.92 μg/L) and cholesterol (0.94 and 1.18 vs. 1.40 mmol/L) levels were significantly (P < 0.05) influenced by the antiplasmodial metal complex treatments (single and repeated treatments vs. the control). It was concluded that 2.5 mg cobalt-lumefantrine complex treatment had no adverse effects on rumen pH, favourably modified rumen microbial populations and improved serum cholesterol level in the sheep.

KEYWORDS: Antiplasmodial, metal-complex, rumen, sheep.

INTRODUCTION

The use of transition metal complexes both as feed additives and medicaments for livestock production is fast becoming popular in the recent time. The development is sequel to favourable applications of the unique characteristics of the metal complexes for pharmaceutical drugs production (Garcina *et al*. 2010). Metal ions can create either labile or inert bonds with coordination numbers ranging from one to twelve, and with numerous geometries including linear, trigonal planar, tetrahedral, or octahedral (Messori *et al.* 2000; Zaric, 2010). This makes it possible to construct therapeutic molecules with shapes and structures otherwise impossible or extremely difficult to achieve with carbon-based compounds (Singh and Chaudhary, 2004).Transitional elements form coordinate covalent bonds, with a unique ability to form stable coordination complexes or chelates. The primary chelated minerals used in livestock production include iron, manganese, zinc, copper, cobalt and nickel (Adeloye and Yousuf, 2001; Bao *et al*. 2007). Chelated minerals have been reported (Walraven *et. al*., 2009) to reduce manure-nutrient excretion and improve animal performance in pigs. A major challenge to the nutrition of ruminants grazing tropical pasture is how to create favorable rumen ecology for efficient microbial digestion (Nagaraja *et al.* 1997).

Table 1: Effects of Cobalt Complex on Rumen pH and Microbial Populations

Parameter/Treatment (mg per 50 ml rumen fluid)	Treatments				
	Control	**0.010**	**0.025**	**0.050**	**± SEM**
Bacteria	40.70[a]	16.57[c]	28.70[b]	4.87[d]	0.01
Protozoa	1.53[a]	1.03[a]	0.87[b]	0.63[b]	0.01
Fungi	7.37[a]	0.80[c]	1.97[b]	1.13[b]	0.01
pH	6.09	5.75	6.42[a]	5.97	1.34

a,b, c – values within a row with similar superscript are similar ($p < 0.05$)

SEM - Standard error of treatment means

Several chemical compounds including detergents, ionophores and antibiotics have been used (Frumholtz, 1999) as antimicrobial agents to reduce rumen protozoal population and enhance the cellulose-digesting bacteria. The aim of the present study is to test the hypothesis that, a transition metal complex with antiplasmodial activities, produced locally from the Chemistry laboratory, University of Ilorin, Nigeria, could influence rumen microbial populations for improved performance in sheep, grazing tropical pasture. Chemical substances in chelates have slow rate of release into animal body system resulting in low toxicity. The use of locally produced chemical agent for improved livestock production would have an added advantage of ease of availability at low cost over commercial proprietary products.

MATERIALS AND METHODS

Source and Collection of Rumen Sample

Fresh rumen samples were obtained at the time of slaughter from five sheep maintained on native pasture in the preceding two weeks. Rumen contents were strained through four layers of cheese clothes into thermal flask, gassed with carbon dioxide and immediately taken to the laboratory in ice-packed bucket for microbial evaluation. The rumen fluid pH was determined immediately after collection, with a digital pH meter.

Microbial Evaluation

Bacterial, protozoa and fungi concentrations of the rumen fluid were estimated in 50 ml samples containing 0, 0.010, 0.025 and 0.050 mg metal complex. Each treatment was carried out in triplicates and incubated at 39 ^{o}C as described by Perumbakkam and Craig (2012), over 24-hour period.

About 1 ml rumen fluid was collected after the incubation period and kept in a plastic bottle to which 9 ml of 10 ml/l formalin solution (1:9 v/v, rumen fluid: 10 ml/L formalin) was added and stored at 4 °C for measuring protozoal population by the total direct counts methods of Galyean (1989) on a haemacytometer. A serial dilution technique (Fawole and Oso, 1998) was used to estimate bacteria concentration on nutrient agar medium. Fungi counts were made following the roll tube method (Frumholtz, 1999).

Animals and Treatments

Fifteen physically healthy growing lambs were divided into three treatment groups similar in body weights (11.27± 0.62 kg) and sex ratio (4 males, 1 female) following a two-week period of adaptation to grazing conditions.

Sheep were drenched individually with 2.5 mg Dichlorobis(lumefantrine)cobalt(II) dihydrate $\{CoCl_2(LUM)_2\}.2H_2O$ at zero (control), once (on the first day) or twice (on the first and fourteenth day) treatment level during a 28-day grazing period. The effective dosage was estimated by relating the optimum concentration (0.025 mg per 50 ml rumen fluid) obtained from the incubation trial with the average volume of rumen fluid (5 liter) in the slaughtered sheep. Serum metabolites (glucose, total protein, urea, creatinine and cholesterol) and enzymes (alanine amino transferace, ALT and aspartate amino tarnsferase, AST) concentrations were measured in triplicates on the sheep, initially and at the end of the 28-day grazing period.

Table 2: Effects of Metal Complex on Serum Metabolites and Enzymes Concentrations

Parameter/dose	Control	Single Dose	Repeated Dose	± SEM
Metabolites				
Glucose, mg/dL	2.75	2.74	2.65	0.83
Protein, g/dL	59.17	51.12	60.20	2.74
Urea-N, mg/dL	19.71	18.63	17.55	4.27
Creatinine, mg/dL	105.86	101.08	102.22	3.66
Cholesterol, mg/dL	1.40[a]	0.94[b]	1.18[b]	0.24
Enzymes				
ALT, u/L	16.92[b]	23.73[a]	18.16[b]	2.59
AST, u/L	40.63	35.84	39.04	7.46

a,b, c – values within a row with similar superscript are similar ($p < 0.05$)

SEM - Standard error of treatment means

Chemical Analyses

About 10 ml blood was withdrawn from each animal through jugular vein puncture into vacuum glass tubes containing no anticoagulant. Blood samples were centrifuged at 3,000 rpm for 10 minutes and the serum obtained stored at –25 °C for chemical analyses. Serum metabolites (glucose; total protein; urea; creatinine and cholesterol) and enzymes (alanine aminotransferase, ALT; aspartate aminotransferase, AST) concentrations were estimated on a semi–automated clinical chemistry analyzer Microlab 300 Vilat Scientific, Dieren, The Netherlands)

Statistical Analysis

Data were analyzed by General Linear Model of SAS using the Duncan's multiple range test to separate differences among treatment means (SAS, 1988).

RESULTS AND DISCUSSION

Table 1 shows data on changes in rumen pH and microbial population as influenced by the different amounts (010, 0.025 and 0.050 mg) of the metal complex incubated in 50 ml rumen fluid samples. The antiplasmodial metal complex had no effect ($p > 0.05$) on rumen pH measurements after a 24-hour incubation period.

The pH of 6.09 recorded for the fresh rumen sample was within the pH range of 5.75 and 6.42 obtained for the three incubated samples. Bishehsari (2010) had reported no significant difference in rumen pH of Mehraba sheep fed inorganic cobalt chloride supplements. The rumen pH is probably the most important factor affecting microbial population and activities (Lana *et al*, 1998). Rumen fluid incubated with 0.025 mg cobalt-lumefantrine had pH value (6.42) within the range of 6.0 to 6.9 considered (Kamra and Pathak 1996) optimum for the growth of rumen bacteria.

A 24-hour incubation of fresh rumen fluid with cobalt-lumefantrine confirmed the antimicrobial property of the metal complex (Table 1). Bacteria, protozoa and fungi populations in the rumen fluid were reduced ($p < 0.05$) at the end of the incubation period. The 0.025 mg cobalt-lumefantrine in 50 ml rumen fluid caused the greatest ($P < 0.05$) reduction in protozoa population with minimal effects on bacteria or fungi population. Defaunation of the rumen could cause a reduction in methanogenesis (Santra *et. al.* 1994)) and an improvement in feed conversion efficiency especially when the animal is on high roughage diet (Pal *et.al.* 1992). Equilibrium among the rumen microbes would be required for optimum fermentation. The cobalt-lumefantrine treatment did not eliminate the protozoa in the incubated rumen fluid but caused a significant reduction ($P < 0.050$ in protozoa population at the 0.025 mg treatment level.

Table 2 shows data on serum metabolites and enzymes concentrations as influenced by periodic drenching of sheep with 2.5 mg cobalt-lumefantrine complex while grazing on native pasture. Drenching sheep with antiplasmodial metal complex had no effect ($p > 0.05$) on serum glucose, total protein, urea-nitrogen or creatinine level. Serum cholesterol concentration (mg/dL) was reduced from 1.40 in the control group to 0.94 and 1.18 in sheep that received single and repeated dosages of 2.5 mg cobalt lumefantrine complex respectively. Alanine transaminase level in the serum was higher ($P < 0.05$) for the sheep that had the single dosage of the metal complex.

A reduction in the level of serum cholesterol level in the serum of sheep drenched with cobalt-lumefantrine complex could be attributed to the effect of cobalt from the metal complex on cholesterol metabolism. Supplemental copper reduced serum cholesterol in steers (Engle and Spears, 2000). Cobalt and copper share similarities in their physical and chemical properties; both are divalent transition metals required for metabolism by the rumen microorganisms and the host ruminant. Serum enzymes and metabolites concentration are affected by age of the animal, type and amount of fat in the diet and nature of supplements.

In conclusion, cobalt-lumefantrine complex produced locally in Chemistry Laboratory, as an antiplasmodial compound, could safely be used to modify rumen microbial profile in sheep under grazing environment. The trace mineral complex caused reduction in the population of protozoa in the rumen fluid and lowered cholesterol level in the blood serum of sheep. All serum metabolites and enzymes concentrations however, were within serum reference-value ranges. The serum enzymes and metabolites were measured to detect any indication of toxicity resulting from treatments and provide evidence on the health status of the animals.

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