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THE USE OF COBALT ETHYLENEDIAMINE TETRAACETIC ACID AS A SOLUBLE MARKER IN THE DETERMINATION OF RUMEN OUTFLOW RATE IN SHEEP

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SUMMARY

Rumen outflow rate in sheep was studied using cobalt ethylenediamine tetraacetic acid as soluble rumen marker in three rumen fistulated sheep of an average body weight of 9.6±0.07kg. Single dose time sequence spot sampling technique was adopted at various time intervals of 2, 4, 6, 8, & 24 h. The ruminal concentrations of cobalt ethylenediamine tetraacetic acid after each post-dosing spot sampling time was determined by a graphical comparison of spectrophotometric absorbance of known concentrations of cobalt ethylenediamine tetraacetic acid and the absorbance of equal volume of ruminal fluid collected from each sheep at given time intervals post-dosing with marker. Depletion of marker with time from the rumen reflected the liquid turnover in the rumen. The ruminal volume of marker distribution was estimated as 1.5499±0.0198L. Using the exponential equation for determining the fractional turnover rate, the average fractional turnover rate was 0.09624±0.0017/h or 9.624%. The rumen half- life of the marker was 7.204±0.12h. The average outflow rate was 0.149±0.002L/h. The data obtained will help in regulating feeding time of intensively reared sheep towards achieving high voluntary intake of dry matter and effective rumen degradability of ruminant feed fractions, which are influenced by rumen outflow rate.

KEY WORDS: Cobalt ethylenediamine tetraacetic acid, rumen, outflow rate, sheep.

INTRODUCTION

The term rate of passage or outflow rate has come to mean the time taken by undigested residues from a given meal to reach the faeces or any point in the gut, and the length and pattern of the subsequent appearance of residues from the meal at the same point (Dougherty, 1965).

The rate of passage of digesta is one of the important factors in determining the efficiency with which the animal utilizes a given amount of food (Uden *et al.,* 1980).

In experiments on digestive functions in ruminants, markers can be used to estimate the rate of movements of digesta , the volumes of a viscous and the rate of absorption of solutes from the gut (Downes and McDonald 1964).

Hyden (1961) has given a full mathematical treatment. of the principle involved in the use of soluble markers to measure the rate of flow and volume of rumen fluid, and has indicated the inherent limitation of the method. Radioactivity was selected as a means for sensitive and complete specific analysis. The complex

of chromium ethylenediamine tetraacetic acid (51CrEDTA) was tested, and was shown to be a highly satisfactory soluble marker in sheep. However, Jacques *et al.*,(1987) have used cobalt ethylenediamine tetraacetic acid to measure ruminal outflow rate and ruminal volume.

Rate of passage or outflow rate is closely related to retention time, voluntary intake (Forbes,1998). High voluntary intakes of dry matter have been associated with low retention time or high outflow rate and high thyroxine levels; (Miller *et al.*, 1974; Ross *et al.*,1985). Increase in rate of passage resulted in a decrease in efficiency of microbial synthesis and an increase in the quantity of food nitrogen which escape degradation in the rumen. Ground hay makes metabolizable energy available to the animal, decreases eating time, and increases consumption but increases digesta flow rate (Weston and Hogan, 1962).

Tropical breeds of cattle have been shown to have a faster rate passage for any given food (Mann *et al.*, 1987). This suggests that they are useful where only poor quality foods are available, a situation typical of our environment. It becomes, therefore, necessary that for an efficient ruminant production to be carried out, estimation of outflow rate is highly desirable. When this is achieved, it becomes relatively easier to develop a diet formulating system that will make for increase feed intake and utilization for our ruminants. The aim of the present experiment was to use the ruminal disappearance of CoEDTA to estimate the rumen outflow rate in West African Dwarf sheep, by spectrophotometric analysis of the colorimetric changes of ruminal fluid mixed *in vivo* with the soluble marker.

MATERIALS AND METHODS

Single dose sampling technique was adopted as described by Downes and McDonald (1964), Faichney (1975), Jacques *et al.* (1975), Orskov and McDonald (1979), and Kristensen *et al.* (1981). In carrying out this experiment, assumption was made that condition in the rumen approached a steady state.

The sheep were fed once daily using freshly cut grasses and legumes. Feed was daily removed at 16.00h (Downes and McDonald, 1964). The rumen fistulas were implanted using the surgical technique as described by Dougherty (1955). Prior to feeding, ruminal samples were collected from each sheep. These were the zero hour ruminal fluid samples. The samples provided the background matrix for cobalt analysis and baseline values for pH. The wavelength for maximum absorbance of the marker was determined (610 nm) using an ordinary spectrophotometer. Again, at the same standard wavelength (610 nm) (Downes and McDonald, 1964), the maximum absorbance of the rumen fluid alone was obtained. This served as the ''blank'' (Downes and McDonald, 1964).

After feeding, a pulse dose of 1.8 g of cobalt in 300 ml (Jacques *et al.*, 1987) distilled water prepared according to Uden *et* al. (1980) and warmed to 37°C was introduced into the rumen of each sheep through the fistula aperture using a repeating syringe. Various sites of the rumen were chosen to aid in mixing (Jacques. *et al*, (1987). Ruminal fluid samples were collected at 2, 4, 6, 8, and 24h post-dosing using suction syringe. 1ml of rumen fluid was diluted in 4ml of distilled water. Into this set-up, 5ml of 20% trichloroacetic acid (TCA) was added to reduce the turbidity and precipitate the

proteins in the ruminal fluid (Hyden, 1955). The set-up was well shaken and allowed to stand for 5 min. It was centrifuged at 15,000rpm for 20 min. and filtered (Jacques et al., 1987). The spectrophotometric absorbance of 1ml of filtrate was determined at 610 nm

Estimation of cobalt concentrations in rumen at different post-dosing time intervals

Rumen capacity of 5 L was assumed for each sheep (McDonald *et al.,* 1994). Since 1.8g of cobalt in 300mls was introduced into each sheep, the total fluid volume in the rumen was 5.3L. Therefore, 1ml of rumen fluid contained 0.33mg of cobalt at zero time. A graph of spectrophotometric absorbance against time was plotted for 1ml of known concentration of cobalt (maximum conc., 0.33mg; minimum conc., 0.06 mg in 4 ml of distilled water). By matching the spectrophotometric absorbance of 1ml of the filtrate with that of known concentrations of cobalt, the concentrations of cobalt in ruminal fluid at different time intervals (post-dosing) were estimated. The half times of the markers were calculated from the slope of the curve by plotting log concentrations of marker against time. Calculation of ruminal fluid was as described by Warner and Stacy, (1968).

$$F = \frac{0.6931 \times V}{t_{1/2}}$$

Parameters obtained where fitted into the exponential equation; $Ct=Coe-kt$

Where Co=marker concentration at time zero.
t= time since marker injection.
Ct=marker concentration at time t
k= constant of marker elimination or liquid turnover rate, obtained by regression analysis (Faichney, 1975).

Volume of distribution
= Quantity of marker (wt) injected into the rumen
Extrapolated marker conc. At time zero conc./vol)

Outflow rate (vol/h)[F] = VK

Functional turnover rate = outflow rate / Volume of distribution

Rumen pH and pH of marker were measured using a pH meter. The pH of marker was adjusted to that of the rumen environment (pH 7.5) before introduction of marker into the rumen. This was achieved using few drops of sodium hydroxide and concentrated hydrochloric acid.

RESULTS

TABLE I: **Spectrophotometric absorbance of graded concentrations of cobalt ethylene tetraacetic acid complex (at 610 nm)**

Co++EDTA conc. (mg/4ml H2O)	Spectrophotomeric absorbance	Wt (g) of cobalt
0.32	0.521	1.29
0.30	0.50	1.21
0.28	0.483	1.13
0.26	0.481	1.05
0.24	0.471	0.97
0.22	0.427	0.89
0.20	0.401	0.81
0.18	0.392	0.73
0.16	0.373	0.65
0.14	0354	0.57
0.12	0.330	0.48
0.10	0.301	0.40
0.08	0.286	0.32
0.06	0.274	0.24

TABLE II: **Spectrophotometric absorbance of cobalt ethylenediamine teyraacetic acid complex and ruminal fluid samples obtained at various time intervals post-dosing (610 nm)**

Animal	Time (hrs)/absorbance				
	2	4	6	8	24
Sheep I	0.80	0.78	0.71	0.69	0 55
Sheep II	0.81	0.76	0.70	0.68	0.53
Sheep III	0.81	0.79	0.71	0.68	0.55

The spectrophotometric absorbance of rumen fluid alone ("blank ") at 610 nm was 0.293 (approx. 0.3)

TABLE III: ***Spectrophotometric absorbance of cobalt remaining in rumen fluid at various time intervals post-dosing**

Animal	Time (hrs)/absorbance				
	2	4	6	8	24
Sheep I	0.5	0.48	0.41	0.39	0.25
Sheep II	0.51	0.46	0.40	0.38	0.23.
Sheep III	0.51	0.49	0.41	0.38	0.25

*= Obtained as the difference between the spectrophotometric absorbance of Co ++ EDTA and ruminal fluid (Table II) and spectrophotometric absorbance of rumen fluid alone or the "blank" (0.3) at 610 nm.

TABLE IV: Ruminal concentration of cobalt at different time intervals post-dosing

Animal	Time (hrs)	Conc. (mg/L)	Log conc.
Sheep I	2	305	2.4843
Sheep I	4	295	2.4698
Sheep I	6	205	2.3118
Sheep I	8	185	2.2672
Sheep I	24	40	1.6021
Sheep II	2	315	2.4983
Sheep II	4	260	2.4150
Sheep II	6	200	2.3010
Sheep II	8	175	2.2430
Sheep II	24	35	1.5441
Sheep III	2	305	2.4843
Sheep III	4	295	2.4698
Sheep III	6	205	.2.3118
Sheep III	8	175	2.2430
Sheep III	24	40	1.6021

TABLE V: Average rumen outflow rate parameters in sheep

"b	"a"	T1/2	K	V	r
0.09624 ±.0017	1161.728±15.075	7.204±0.12	0.09624±.0017	1.5499±.0198	0149±0.002

a = Log conc of marker at zero time
b = Regression coefficient of fractional turnover r = k
k = Liquid turnover rate
note b = k
v = Volume of marker distribution in the rumen
t½ = Time for half of the marker to disapear from the rumen.
r = rate of passage.
Rate of passage or flow rate (F) (Vol 1hr) = V.k.

DISCUSSION

The rumen outflow rate observed in this experiment, given as 0.096 or 9.6% did not vary much from the value of 0.08 or 8% proposed by AFRC (1980) for temperate breed of sheep. The relatively high fractional turnover rate observed in the present study could be justified by three reasons;

(a) According to AOAC (1980), poor performing animals have higher fractional turnover rate.

This increase outflow rate is beneficial to the poor performing animals like those used in the present experiment. This is because in a steady state an increase in outflow rate increases the dilution rate. When dilution rate is equal to the

(b) Temperature increases rumen outflow rate. Temperate animals have slower rate of passage than tropical animals (Mann *et al.*, 1987).

(c) Species difference in rumen fractional turnover rate has been reported (Weston and Hogan, 1962).

It has been reported that sheep are very sensitive to particle size (ARC, 1980). It is therefore very possible that an interplay between these factors may have accounted for the relative increase in fractional turnover rate observed in the present experiment.

multiplication rate of rumen bacteria, maximum microbial yield would occur (; AFRC, 1992, Orskov, 1992). It is supposed that the efficiency of microbial protein synthesis can be increased by 20%, if rumen outflow rate is increased (Verbic *et al.* 1999).

Rumen outflow rate exerts much influence on voluntary dry matter intake (Hartnel and Satter, 1979). Faster passage causes increased voluntary intake. It could be assumed that the efficiency of microbial protein synthesis in the rumen can be increased by an increase in dry matter intake. Since such increase in rumen outflow rate increases rumen microbial protein synthesis. The increase in outflow rate is reflected in the depletion rate of cobalt (conc./L), from the rumen within the time intervals as show in Table IV. From the Table, it could be seen that as the spot sampling time increased, there was gradual decrease in the ruminal concentration of cobalt. This was also shown as a gradual decrease in the spectrophotometric absorbance of rumen fluid (post-maker dosing) as spot sampling time increased (Table III).

CONCLUSSION

For poor performing ruminants like our local breed of sheep, a faster rumen outflow rate is necessary. This is because it improves the voluntary intake and ruminal protein synthesis. Since these animals receive poor quality foods, the ruminal microbial protein being of high biological value is very essential.

Again, the influence of outflow rate on effective degradability of feeds further explains the important role it plays in ruminant nutrition. It is therefore desirable that before the quality and utilization of common ruminant feeds (especially grasses and legumes) can be clearly estimated an estimation of the outflow rate is recommended.

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