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STATISTICAL PREDICTION OF RUMEN VOLUME IN FRIESIAN BULLS USING FOUR DIFFERENT MARKERS AND LIVE BODY WEIGHT

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ABSTRACT

Twenty four Friesian bulls with a mean live body weight of 450±32 Kg, belonging to Sakha experimentral farms were used in the present study under the same dietary regime. The rumen volume and flow rate were estimated from the rate of decline in concenteration of a marker in the rumen fluid following a single ruminal injection. The results of several determination methods of rumen volume using different markers (lithium sulphate, polyethelienglycol, Cr-EDTA and Cr_2O_3) gave similar values with differences ranging from 6 to 18%. Rumen volumes as determined by different methods are significantly ($p<0.01$) correlated with live body wieght. The correlation coefficient values ranged from 0.60 to 0.79. The direct determination of rumen volum were done by substituting rumen content by water (physical volume) gave rumen volume values ranged from 30 to 36% higher than that estimated by using marker methodes. The flow rate of rumen contentes was affected significantly ($p<0.05$) according markers.The values of flow rate ranged from 3.5% to 4.9% from the total volume per houre. This values equipoise disharge rate ranged from .84 to 1.25 times per day. Using Polyethyleneglycole gave the nearst estemated value to the true rumen volume (Physical volume) $r = 0.92$ comparing with the rest used markers.

Keywords : Rumen volume, Lithium sulphate, PEG, Cr-EDTA, Cr_2O_3

INTRODUCTION

A substantial amount of information is now available on rumen volume and on factors affecting it, since the development and use of markers (such as PEG, Cr-EDTA, Cr_2O_3, and lithium sulphate) are now became applicable. Since the rumen contents are not homogeneous, since there is evidence that the fluid and particulate portions of the rumen contents flow at different rates, the physical state of the marker to be chosen will depend on the requirements. For instance, if one is interested in the movement of solid digesta, chromium derivatives and lignin can be used. A relatively large proportion of rumen contents is a fluid suspension of very small particles, and often the measurement of the flow of soluble markers gives a good estimate of the flow of the small particles as well as the flow of dissolved substances. Several soluble merkers are in use, but perhaps the most common ones are polyethylene glycol (PEG), and lithium sulphate (Czerkawski 1986).

Direct measurments of rumen volume and its turnover imply the use of rumen cannulated animals or stomach tube with its diffeculties and can be performed by means of rumen evacuation (Robinson *et al.* 1987) or by measuring the dilution rate of a single dose of a marker . However there are difficulties associated with these methods such as timing of rumen

evacuations, rumen sampling, type of markers, mathematical models of interpolation, ect. (Owen and Hanson, 1992). In addition, rumen cannulation is a highly invasive technique which necessitates the careful choice of the most appropiate type of cannula and its construction material and an excellent animal care is required to preserve the health and viability of the animals (Harmon and Richards, 1997). Also determination of rumen volume by marker have problemes that arise due to unrepresentive sampling of digesta in terme of the selection of liquid or particulate phases (Teeter and Owen., 1983). There are another problems with the migration of markers between particles and with the variation in marker uptake with particle size (Faichney 1986, and Reynolds *et al.* 2004). On the other hand, the estimated rumen volumes differ from physiological rumen volume due to dilution by saliva, unhomogenous distribution of the marker in the rumen and loss of marker due to passage out of the rumen or to a combination of these factors (Cunningham, 1997).Also the physical volume differs from the physiological rumen volume since the rumen is stratified into indistinct zones of rumen ingesta which are, gas zone(cap), solid zone, ejection zone, slurry zone, and liquid zone (Cunningham, 1997, McAllister, 2000 and Reynolds *et al.*, 2004).It is possible to resolve the above difficulties by using the indirect or statistical methodes to determined rumen volume and turneover of its contentes (Reynolds *et al.*, 2004).

We are concerned here mainly to determine the acurate marker for rumen volume determination with referances to physical volume (true volume) , and postulate a correlation and regression equations which help to know the rumen volume statisticaly (indirect methodes) without using marker techniques (direct method).

MATERIALS AND METHODS

Animals and Feeding:

Twenty four Friesian bulls with a mean live body weight of 450±32 Kg and about two years old belonging to Sakha experimental farms, Kaferelsheikh Governorate in the Nile Delta, Animal Production Research Institute (APRI), Ministry of Agriculture were used in the experiment which lasted for three weeks. The bulls were housed in an open-sided shed, and had free access to water at all times. All animals were fed as a group once a day roughage(rise straw) 20% and a CFM(Concentrate feeding mixture) 80% .The percent of CFM ingredients were 25% wheat bran, 35% yellow corn, 10% rice polish, 25% cotton seed cake, 2% venas, 1% salt and 2% calcium carbonate. The nutritive requirements were calculated depending on animals body weight according to NRC (1985).

Experimental Design and Procedures:

The experiment was latin square designed with each period of 5 days, the first two days were assigned for marker injection and sampling collection days, followed by three rested days. At the end of the experiment bulls were slaughtered .

Drinking water was with-hold about 8 hrs. before each determination. Ruminal fluid samples was with-drawn at hourly intervals after interedusing the feed to animals which was consumed within one hour.

In the first day of the experiment, rumen fluid volume was determined by lithium sulphate marker at low concenterations, 1-2 m-eq.Li^+/L. of rumen fluid, using the method of Mangan and Wright (1968). After the animals were finished their feed by 4 hrs, 6.0 g lithium sulphate dissolved in one liter distilled water was introduced into the rumen through the stomach tube. The samples of the rumen digesta (about 200 ml.) were withdrawn from each animal by sucking through the stomch tube which moved into different depths and directions in the rumen. The crude ruminal fluid was strained through four layers of chees cloth. Samples were cooled immediately in an ice bath . Then it were centerifuged for 45 min. at 5000 r.p.m. giving a clear fluid essentially free from microbial cells and plant debrise. Three ml of the supertnatant fluid was diluted 1:5 with 0.1 HCl in 3 vials which, stored at (-18◦ C) till the assay was executed.

Rumen fluid volume (RFV) was calculated from the following equation(Allam *et al.*, 1976). $RFV= (Q - (C.V))/ (C - Co)$

Where : Q = quantity of the marker(Li+) added to the rumen., V= volume of solution added to the rumen., Co = concenteration of marker before addition., and C= estimated concenteration of marker at the time of addition as determined by extrapolation on a logarithmic scale.

On day 6th of experiment, rumen fluide volume and its flow rates were determined by the method of Hyden(1961) as follows: 80 g. of polyethylene glycol 4000 (PEG) were introduced into the rumen immediately before feeding. The PEG was disolved in 250 ml water, and 50ml of this solution was mixed into five 200 ml samples of fresh rumen liquor malt from the same animals. The five PEG/liquor mixture were immediatly re-introduced into the rumen, a plastic tube being used to ensure as wide distribution as possible. Samples were taken at hourly intervales after feeding and analysed for PEG using the metod of Malawar and Powel(1967). The volume at time 0, and fluid flow rate were calculated from the regression of log PEG concentration on time (Hyden 1961).

On the 11 th day of the experiment, 80 gm of Cr_2O_3 was suspended in approximately 300 ml of warm water, and 1hr was allowed for mixing before animals feeding. Sampling methods were performed as discribed by Purser and Moir(1959) . Analysis of Cr_2O_3 was performed according to the method of Kimura and Miller (1957). Samples had been taken immediately prior to feeding (T0), then foure samples were taken at hourly intervals after feeding. The rumen volume was estimated according to the general formula: $V = M/(C_2 - C_1)$. Where V is volume of the rumen (Litter) , M is milligrams of Cr_2O_3 added, and C_1 & C_2 are the initial and final concenteration of Cr_2O_3 (mg/L), respectivley . Since the initial (T_0,or zero time) rumen volume was only estimated, cosequently the formula in this case becomes V= M/C.

On the 16th day of the experiment, the rumen fluid volume was determined using Cr- EDTA complex as a marker according to Binnerts *et al.*,(1968) . Volume of 250 µl Chromium-51 ethylenediamine tetra- actate (Cr-EDTA) was solved in 500 ml of distilled water and administered into the

rumen via stomach tube. One hour after dosing and every two hours for a period of 8 hrs, approximately 20 ml of rumen liquor was collected . Samples were transferred into counting tubes and read with standard solution by Gamma Counter. The log concentration of the marker was plotted againist time. The zero time concenteration (C) was estimated by extrapolation to zero time using linear regression. The rumen liquid volume (R) can be calculated according to the formula R = D/C. Since (D) is the dose of Cr-EDTA added into the rumen.

Rumen samples were collected by the stomach tube before the introduction of the marker of blank determination and for preparing standard curves for each determination. Four concentrations (10, 20, 30 and 40 ml) of Cr-EDTA/100ml rumen liquor, each in duplicate, were used for drawing the standard curve. A suitable volume of the marker solution (2 liters for bull) was warmed to 40 °C and introduced into the rumen through the stomach tube. The samples were strained through silk cloth, then the strained liquor was added in equal volume to a solution of 10% trichloroacetic acid (TCA) in a 50 ml centrifuge tube. After standing for 1h. or more, the tubes were centrifuged for 30 min at 16000 rpm. The supernatent was decanted through Whatman 42 filter paper and its optical density was measured at 550 nm. In the same samples Cr was estimated as chromate by the method of Stevenson and DeLangan (1960). Rumen volume at the time the marker was added was obtained by extrapolating the line to zero time.

Flow rate determination:

Many models have been developed to discribe the flow in biological systems. Attempts to describe the flow of substances in the rumen in terms of single- compartment model were made by Warner and Stacey (1968) who stated that, when the marker injected as a single shot in a small volume of water, and C refers to concenteration of marker in the rumen, its concenteration in the inflowing water is zero and its net rate of formation is zero, the Warner equation is: $V\,(dc/dt) + FC = 0$. since V is volume of rumen content(L), C is the concenteration of marker in the rumen, D is dilution rate or proportion of water removed (per hr), t is the time(hr), and $F = DV$

After determination of rumen volume by the different markers, all experimental bulls were slaughtered at the time which they would normaly have been fed. One day before slaughter, live body weight was recorded.The slaughter prosses was done throughout 12 separated days as two bulls per day. The oesophagus was tied off immediately after slaughtering , and as soon as the abdomen was opened, ties were made to separate the rumen-reticulum from the omasum. The rumen contents were weighed ,and the rumen volume was estimated as follows: The rumen was completely emptied and washed. Water was then introduced into the rumen through the stomach tube, the esophagus and reticulo-omasal orifice was tied off. The total quantity of water requierd to completely fill the rumen under water conditions was measured and it is termed "Physical volume". Finally, the stomach tube was removed and the moist, empty rumen was weighed and it is termed "Empty weight". Some of the chmical analysis (Cr-EDTA & Lithium sulfate) were done in Animal physiology lab, and centeral lab in Faculty of Agriculture,

Cairo Univ., and the others (PEG & Cr_2O_3) were done in the National Research Center.

Statistical analysis:

All values were expressed as means and SD. The data were analysed for calculating the correlations and regressions using SPSS for windows. Release 10.01 (1999). Markers outflow rate from the rumen were analysed by analysis of variance following the methods proposed by Steel and Torrie (1980) and according the model:

$$Y_{ijk} = \mu + A_i + P_j + D_k + AP_{ij} + AD_{ik} + DP_{jk} + APD_{ijk} + e_{ijk}$$

Where : μ is the overall mean; A_i is the effect due to the animales; P_j is the effect due the period; D_k is the effect due to the marker type; AP_{ij} is the effect due the interaction between animales and period; AD_{ik} is the effect due the interaction between animales and marker type; DP_{jk} is the effect due the interaction between marker type and period, APD_{ijk} is the effect due to the interaction between animales, period and marker type; e_{ijk} is the experimental error.

RESULTS AND DISCUSSION

Data presented in Table (1) indicated a normal trend concerning the volume of the rumen estimated by either rumen weight or its volume using different markers. Statistical analysis revealed significant differences ($P<0.05$) between the values of rumen volume due to the method of determination (Table 1). These differences agree with the results of Purser and Moir, (1966), El-Shazly *et al.*(1976) , Priego *et al.* (1977) and Darlis *et al.* (2000) . The significant differences between the rumen volume values when using different markers may be due to the nature of the dispersed phase where it was a liquid phase in case of lithium sulphate, Cr_2O_3, and, PEG. Meanwhile, the solid particle phase was the medium for Cr-EDTA (Gregory 1984 and Kamler *et al.*, 2003). The previous observation explains the higher values obtained by emptying the rumen and filling it with water (physical volume). A similar trend was observed in the results of El-Shazly *et al.* (1976) who reported higher values obtained by emptying the rumen (20 – 68% on the average) and with Al-Rabbat *et al.* (1971) and Alexander *et al.*, (1969) with 25-30% higher in the physical rumen volume than the volume obtained by using polyethylene glycol (PEG).

All means were calculated using 24 animals.

The difference between the physical rumen volume which estimated by water filling after slaughter and the physiological rumen volume, which determined by different markers before slaughter might be attributed to the gas production phase during rumen fermentation process (McAllister,2000 and Kamler *et al.*, 2003).

Table (1) also shows that, the physical rumen volume was 20% relative to the live body weight, while the rumen fluid volume as determined by different markers ranged from 12 to 14% as related to live body weight.

The highest values of ruminal turnover and flow rate of ruminal contentes per/hr or per/d were found with PEG markers. This difference among PEG and the other marker were significant ($p<0.05$). This results were in a good agreement with the data reported by Bernabbucci *et al.* (1999) and Mauro Spanghero *et al* (1999) who reported that PEG had higher rumen outflow rate values irrespective of diet type than the other markers.

Table (1): Rumen parameters as detected by different methods of estimation

Item	Rumen volume	Turnover rate/day	Flow rate l/h	Flow rate constant %/h
Empty weight of rumen (kg).	21.60	-	--	-
Rumen weight with chym (kg).	53.42	-	-	-
Physical volume.(L).	87.1^{a}	-	-	-
Lithium sulphate volume (L).	55.58^{c}	1.0^{b}	2.3^{b}	4.2^{a}
Cr-EDTA volume (L).	53.08^{c}	0.95^{b}	2.1^{b}	3.9^{b}
PEG volume (L).	62.83^{b}	1.25^{a}	3.12^{a}	4.9^{a}
Cr_2O_3 volume (L).	50.84^{c}	0.84^{b}	2.1^{b}	3.5^{b}
Live body weight (Kg.).	429.33	-	-	-

Means with different superscripts in the same coulumn are significantly ($P<0.05$) different.

Table (2) shows that, the accuracy of estimating rumen volume by using the method of PEG marker (solid state marker) was the highest (r^2 = 0.92) comparing to physical rumen volume (true volume), meanwhile, the accuracy (r^2) of other markers to estimate rumen volume were 0.62, 0.45, 0.31 for lithium sulfate, Cr_2O_3 and Cr-EDTA, respectively, in compare to physical rumen volume (true volume).

Table (2): Accuracy values among live body weight(KG), physical rumen volume (L) and different methods of rumen volume determination.

Marker	R^2 between LBW* and method of estimation	R^2 between Phy.Vol.** and method of estimation
Polyethylene glycol	0.79	0.92
Lithium sulphate	0.71	0.62
Cr_2O_3	0.62	0.45
Cr-EDTA	0.60	0.31

*** LBW = Live Body Weight** **** Phy.Vol.= Physical rumen volume**

There are positive correlations between live body weight and rumen volume determined by different markers methods (Table2). These correlations help in postulating some regression equations that may assist the researchers to determine the rumen volume of the animal without direct determination that requires experience, costs and avoid inconvenience to animals.

The suggested equations (statistical prediction) as determined by regression coefficients are as follows:

$Y_1 = -5.867 + 0.141X$; r = 0.60 $Y_2 = -13.27 + 0.161 X$; r = 0.71
$Y_3 = -19.462 + 0.191 X$; r = 0.79 $Y_4 = -25.725 + 0.263X$
$Y_5 = -15.652 + 0.151 X$; r = 0.62

Since: X is the live body weight of the animal,
Y_1 is the rumen volume that determined by Cr-EDTA marker,
Y_2 is the rumen volume that determined by Lithium sulphate marker,
Y_3 is the rumen volume that determined by Polyethylene Glycol marker,
Y_4 is the rumen volume that determined by physical volume, and
Y_5 is the rumen volume, which determined by $Cr2O_3$ marker.

Conclusion

The highly correlation coeffecient between body weight and rumen volume values indicate that we can detrmine the rumen volume for cattle by using the previous suggested equations instate of the direct determination by the classical (direct) methodes which have a lot of diffeculties, and more expensive in mony and time. Also if we well use the direct methods to determiend the rumen volume we suggested that using polyethelenglycole (PEG) as amarker is better becouse it has more accurecy than the other markers(Lithium sulphate, Cr_2O_3 and Cr-EDTA) in compare to physical rumen volume (true volume).

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التنبؤ الاحصائي بحجم الكرش في عجول فريزيان عن طريق استخدام اربعة مرقمات مختلفة ووزن الجسم

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صممت هذه التجربة للتعرف على أدق طرق استخدام المرقمات لتقدير حجم الكرش وذلك بالمقارنة بحجم الكرش الطبيعي المقدر بطريقة فيزيقية ، وكذلك لحساب معامل الارتباط بين طرق التقدير المختلفة ووزن الجسم ، ووضع معادلة تنبؤ ذات درجة دقة جيدة يمكن منها تقدير حجم الكرش بمعلومية وزن الجسم أو حجم الكرش المقدر بمرقم معين بمعلومية حجم الكرش المقدر بمرقم آخر.

أجريت التجربة على٢٥ عجل فريزيان بمتوسط وزن ٤٢٠ ±٣٢ كجم تمت تغذيتها على المقررات الغذائية اللازمة . تم تقدير حجم الكرش والحيوان حي باستخدام المرقمات (كبريتات الليثيم ، بولى ايثيلين جليكول ، أكسيد الكروميوم ، كروميوم اديتا) كما تم تقدير حجم الكرش الفعلي بعد ذبح الحيوان بطريقة فيزيقية .

كان هناك ارتباط معنوي (مستوى ٠١و٠) بين حجم الكرش والوزن الحي في كل طرق التقدير ، وكانت الاختلافات في قيم معامل الارتباط تتراوح بين ٦و٠ عند التقدير باستخدام المرقم أكسيد الكروميوم اديتا إلى ٧٩و٠ باستخدام المرقم بولى ايثيلين جليكول حيث اعتمد التقدير لحجم الكرش على تقدير النقص الحادث في تركيز المرقم بمرور الوقت بعد الحقن بالكرش والذي كان يتم مرة واحدة يوميا قبل التغذية .

وضح قياس حجم الكرش بالطريقة الفيزيقية (طريقة مباشرة بعد الذبح) عن زيادة في الحجم الفيزيقي للكرش عن تلك الذي تم الحصول علية باستخدام المرقمات بنسب تتراوح بين ٣٠ % – إلى ٣٦%.

وكانت أدق الطرق لتقدير حجم الكرش باستخدام المرقمات هي التي تمت باستخدام البولي ايثيلين جليكول .