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FULL CONTROL OF RUMINANT NUTRITION BY INTRAGASTRIC INFUSION OF LIQUID DIETS

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Sıvı besin maddelerinin mide içi infüzyonu ile ruminant beslenmesinin tam kontrolü

Özet: *Ruminant hayvanların beslenmesinin tam kontrolü sıvı besin maddelerinin mideye infüzyonu ile yapılabilir. Bu çalışmada, operasyon prosedürü ve hayvan bakımı tarif edilmiştir. Bu denemede rumen kanülü ve abomasum infüzyon katateri takılmış dört koyun kullanıldı. Hayvanların rumenlerine uçucu yağ asitleri, tampon solusyon ve mineraller, abomasuma da kazein infüze edildi. Bütün infüzyon peristaltik pompa ile ve* 24 *saat boyunca yapıldı. Toplam enerji, yaşama payı ihtiyacı* 450 *kJ / kg* $^{0.75}$ */ gün, toplam protein* 350 *mg N / kg* 0.75 */ gün esas alınarak hesaplandı. Dört aylık infüzyon boyunca deney hayvanlarının sağlıkları bozulmadı.*

Summary: *Full control of ruminant nutrition can be achieved by infusion of liquid diets into the stomach. In this work, surgical procedures and animal management are described. Four sheep with a rumen cannula and abomasal infusion catheter were used in this experiment. Animals were maintained by intragastric infusion in which solutions of volatile fatty acids (VFA) buffer and major minerals were infused into the rumen and casein infused into the abomasum. All infusions were achieved by means of peristaltic pumps. Volume of infusates were delivered continuously over* 24 *h. The calculation of total energy to be supplied was based on the assumption that the maintenance requirement for energy was* 450 *kJ / kg* $^{0.75}$ *per day and nitrogen requirement for maintenance were taken to be* 350 *mg N / kg* $^{0.75}$ *per day. The health of the experimental animals remained good throughout the four months of infusion.*

Introduction

Diets for ruminants must satisfy both the requirements of the rumen microbes and those of the host animal. Nutrients, especially those influencing energy and protein metabolizm, are greatly modified by

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microbial activity before they can be absorbed from the intestines so the needs of the host are obscured.

The nutrient requirements of the host can be studied by infusing liquid diets into the stomach so that microbial action is eliminated. Sheep have been infused with either free volatile fatty acids (VFA) (2) or parlty neutralized VFA (11) into the rumen, but at levels well below estimated energy requirement. We have been able to sustain by infusing VFA, protein, minerals and vitamins for several months, at VFA levels sometimes over twice the estimated energy requirement.

An attempts has been made in the present experiment to sustain animals at maintenance level of energy requirement by continuously over 24 h infusion of nutrients into the rumen and abomasum without the complications which arise from normal rumen activity.

Materials and Methods

Animals and Management: Four female of Suffolk × Scottish Blackface breeding were used in these experiments. The lambs were 7 months of age at the start of the experiment and had an average liveweight of 37 kg. Each animal was fitted with a rumen cannula, an abomasal infusion catheter and an ileal infusion catheter as described by Orskov et al. (8).

Lambs were housed indoors in metabolism crates under continuous lighting. The animal room was well ventilated and was maintained at a temparature of about 20 C° by the use of infra-red heating lamps suspended above each crate. All animals were sheared at the start of the experiment.

After surgery recovery all animals were transferred from solid food (pelleted barley diet) to total intragastric nutrition during the introductory stage of the experiment. The procedure was to increase the amount of infusate in steps of multiples (0.25) of the maintenance requirement for energy and maintain the new level for one or two days. At the same time the amount of food given by mouth was reduced over 6 days.

Surgical Procedures: The rumen cannulas were made at the Rowett Research Institute and had an internal diameter of approximately 14 mm. (7). Anaesthesia was induced by using Halothane. The cannulas were exteriorized between the last rib and the lumbar transverse processes. For the abomasal infusion a transparent polyvinyl tube vas

used (Portex Ltd., Hythe, Kent). The tube was approximately 0.4 m. long with 4 mm. internal and 7 mm. external diameter. A circular flange, 20 mm. in diameter and 2 mm. thick was made from polyethylene sheeting and glued (Portex Vinyl Cement; Portex Ltd., Hythe, Kent) to the end of the catheter. An incision was made within a "purse-string" suture midway between the greater and lesser curvature on the right lateral wall of the abomasum, the flanged end of the catheter was inserted and the "purse-string" tightened. Subseqiently a second flange was pushed down the catheter so that the abomasal wall was contained between the two flanges. A collar of 7 mm. internal diameter and with 11 mm. external diameter was brought hard up against the second flange and glued in position. This provided a very secure and leak-proof abomasal entry. The free and of the catheter was exteriorized by passing the catheter through the eye of a large needle and puncturing the body wall from within, just below the transverse processes of the 5 th lumbar vertebra. To ensure that the tube was not pulled directly from outside it was securely anchored to the wool of the sheep.

During the surgery, 2 nylon pan scrubbers were inserted into the rumen; these were used in an attempt to ensure that rumen motility and muscle tonus were maintained.

A post-operative recovery period of 4 weeks was allowed before experiments were undertaken. During this time the wounds were washed with antiseptic solution and infusion lines were flushed with water every few days. The changeover from solis food to total infusion took place over the final three weeks of this period.

Intragastric Infusion Procedures: The methods used to maintain animals by intragastric infusion were essentially those described by Orskov et al. (8), McLeod et al. (6) and Hovell et al. (5) in which solutions of volatile fatty acids (VFA), buffer and major minerals were infused into the abomasum.

The concentrated solutions from which the daily infusions were prepared were made up as follows: VFA, mineral buffer and casein stock solutions were prepared as described by MacLeod et al. (6). The mixture of VFA consisted of (mmol / mol) 650 acetic, 250 propionic and 100 n-butyric acid and was approximately 10 M concentration. Calcium carbonate (18 g / kg) was dissolved directly in the VFA solutions when they were prepared. The calcium phosphate (15 g / kg) and magnesium chloride (7.5 g / kg) were dissolved separetly in warm

water and then combined. The buffer solution contained sodium and potassium bicarbonates and sodium chloride.

The casein (143 g N / kg) was prepared as a 100 g / kg solution by adding 53 g sodium carbonate / kg air dry lactic casein and homogenizing for 20 min. in warm water and kept refrigerated at 4° C. During homogenization a solution of water soluble vitamins was added in amounts calculated to meet the animal's requirement at maintenance. Vitamin A, D and E were given by intramuscular injection of Vetrivite (C-Vet Ltd., Bury St. Edmunds, England) at 3 weekly intervals.

Trace minerals at 1 ml /kg bodyweight $^{0.75}$ (kg $^{07.5}$) were injected daily via the abomasal catheter. To prevent blockage by precipitated casein, the abomasal catheter was rinsed with water after injection.

Details of all mixtures are given in Tables 1a and 1b.

Table 1a Composition (g / kg) of concentrate preparations (6)

Solutions		*Comments*
Casein Solution		
Casein	100.0	Casein: 143 g / N / kg
Na_2CO_2	5.3	Gross energy: 20.239 kJ / g
Vitamin sol.	25.7	
Water	869.0	
Volatile Fatty Acids Solution		
Acetic (C_2)	388.2	Gross energy: 11.66 kJ / g.
Propionic (C_3)	183.9	Mixture (mmol / mol): 650 C_2, 250 C_3, 100 C_4
Butyric (C_4)	87.8	
$CaCO_3$	18.0	
Water	332.1	
Buffer Solution		
$NaHCO_3$	73.0	Daily amounts of the buffer infusates were
$KHCO_3$	38.0	related to the amonut of volatile fatty acids
NaCl	7.0	infused at about 1.8 times the amount of con-
Water	882.0	about 1.8 times the amount of concentrate volatile fatty acids at the maintenance level.
Mineral Solution		
$Ca(H_2PO_4)_2.H_2O$	15.0	Dissolved in the VFA solution at the rate of
$MgCl_2.6H_2O$	7.5	40 g / kg 0.75 at the maintenance level.
Water	977.5	

Table 1b Composition of vitamin and trace mineral solutions (6)

Solutions		
Vitamin Preparation		
Thiamine hydrochloride	0.76	The vitamin-linoleic acid mixture was homo-
Riboflavine	3.04	genized at a rate of 1 kg in 7.59 l ethanol-=
Nicotinic acid	3.04	water mixture (30:70, v/v) and the homo-
Choline chloride	113 89	genate incorporated in the casein concent-
Pyridoxin hydrochloride	0.30	rated solution at 25.7 g/kg. A further intra-
P-amino-benzoic acid	0.08	muscular injection of vitamins A, D and E
Calcium DL-pentothenate	2.28	was administered at 3 week intervals.
Folic acid	0.01	
Cyanocobalamin	0.01	
Myo-inositol	113.89	
D-biotin	0.05	
2-Methyl-1,4-Napthaquinone	0.38	
DL-c-tocopherol acetate	3.04	
Linoleic acid	759.23	
Trace Minerals		
$FeSO_4.7H_2O$	822.79	Trace minerals were dissolved at a concentrati-
$ZnSO_4.7H_2O$	48.6	on of 253 g in 10 a water and solution then used
KI	43.91	at the rate of 1 ml/kg 0.75/d at the main-
$MnSO_4.4H_2O$	22.94	tenance level. Copper was not used in this
$CoSO_4.7H_2O$	8.70	solution because of the high level of Cu con-
NaF	31.25	centration of tap water which was used for diluting the concentrates.

The above concentrated solutions were diluted as required to give daily infusate solutions. Four reservoirs were used per animal. These contained, respectively, a solution of casein, one of VFA plus major minerals, one of buffer solution, and one of strach plus celluose mixture. The mineral solution (40 g/kg $^{0.75}$ per day) was added to the VFA solution. This solution was diluted approximately 4–5 times for infusion into the rumen.

The buffer solution was diluted approximately 6–7 times for infusion. The quantities infused were adjusted to maintain the rumen pH between 6.0 and 6.5 and the amounts required were calculated in relation to level of VFA infused.

The daily volume of VFA plus mineral mixture and buffer solutions into the rumen was approximately 550 g/kg $^{0.75}$ per day.

The casein solution was diluted approximately 6 fold for infusion and daily volume of this solution into the abomasum was about 180 g/kg $^{0.75}$ per day.

All infusions were achieved by means of peristaltic pumps (Watson Marlow Ltd., Falmouth, Cornwall). Volume of infusates were delivered continuously over 24 h. Reservoirs were changed at 10.00 am daily and weights of resildues were recorded. The daily volumes infused, residues recorded, and the energy content of VFA and the energy and N content of casein solutions were later entered in a computer programme which calculated daily intakes of nutrients.

Rumen pH and osmotic pressure were measured routinly twice daily to assess rumen conditions. Rumen pH was normally maintained between 6.0 and 6.5 and at about 250 mosmol /kg.

The calculation of total energy to be supplied was based on the assumption that the maintenance requirement for energy was 450 kJ /kg $^{0.75}$ per day (5) and the quantities of VFA and casein infused were adjusted to achieve this total energy intake.

Nitrogen requirement for maintenance were taken to be 350 mg N /kg $^{0.75}$ per day (1) and the casein infused was taken to have an availability of 0.80 (5). Thus the casein N allowance was equivalent to 350 /0.80 or 438 mg casein N /kg $^{0.75}$ per day. This quantity of casein provides 62.2 kj /kg $^{0.75}$ per day and this was deducted from the total energy requirement to arrive at the daily allowance of energy to be supplied as VFA. The VFA concentrate solution contained 11.66 kj /g, hence the daily allowance of VFA concentrate was (450-62.2) /11.66 = 33.25 g /d.

The intakes of both energy and nitrogen were adjusted each period according to the animal liveweights. N intake was maintained throughout at a level of 1 x maintenance.

Analytical Methods: Rumen samples (10 ml) were taken routinely twice a day. Samples were taken from each animal by a 60 ml syringe attached to the rumen sampling line. The syringe was flushed 3–4 times (30–40 ml each time) to mix the rumen contents throughly before a sample was withdrawn. Rumen fluid urease activity was measured by production of NH_3 from urea at 37 C° (3). Rumen ammonia was analysed by the method described by Fawcett and Scott (4). The C_2-C_6 VFA in rumen fluid were assayed by gas-liquid chromatography essentially are described by Ottenstein and Bartley (10). pH was determined electrometrically and osmotic pressure by freezing point depression.

Results and Discussion

The health of the experimental animals remained good throughout the experiment. However, one sheep showed symptoms of severe rumen distention shortly after its first introduction to abomasal infusion. Radiological examination indicated ileal blockage and this was treated by discontinuing infusion and allowing access to chopped dired grass for about 1 week. Once normal faecal material was passed intragastric infusions were re-introduced and the animal was reinstated on the previous experimental schedule after a suitable preliminary period.

Table 2. Some mean valus of 4 infused sheep

Mean liveweight (kg 0.57)	14.93
Energy intake (kJ / kg 0.75 / d)	444
Nitrogen intake (g / kg 0.75 / d)	0.453
Rumen ammonia concentration (mg / 100 mk)	10.76
Rumen pH	6.51
Rumen osmotic pressure (mosmol / kg)	224.3
Rumen urease activity (umol / ml / mn)	0.56

The abomasal catheters were, in all cases, trouble free. The rumen cannula of one sheep developed a slight leakage round the rim of the cannula during the third period.

Adjustment of volumes infused (VFA and buffer solution) made it possible to keep rumen pH approximately 6.5 and osmotic pressure approximately 230. Mean energy and nitrogen intakes of animals are shown in table 2. Intakes of anergy and nitrogen remained constant throughout the infusion (approximately 450 kj /kg 0.75 /d and 0.450 g N /kg 0.75 /d) and there was no difference between animals. Mean cocentration of ammonia, pH, osmotic pressure and urease activity in rumen fluid of 4 animals are shown in Table 3. Rumen VFA molar proportions (%) were measured and showed mean values of 66.7 acetic, 24.2 propionic and 8.8 butyric. These values were similar to the composition of the VFA mixture infused and did not differ between animals.

Post-mortem examinations of animals were carried out some 3 week after the end of the experiment. During this time all had been maintained on intragastric nutrition. The rumen epithelia was found similar to that of normally fed animals, and the rumen papillae are large, flat and well-seperated. The small intestine was thinwalled (9).

The technique whereby ruminants can be totally maintained by the infusion of VFA and bicarbonate salts into the rumen and of

protein solution into the abomasum removes most of the normal flora of the rumen fluid because usual nutrients for microbial growth are not available (12). The remaining microbial population is mostly attached to, and actively digest the sloughed epithelial cells from rumen wall. The rumen fluid of infused lambs has therefore been used as a model system for the rumen wall-bound or 'epimural' population. The results suggest that the rumen fluid from infused lambs are ureolytic. The total infusion system of ruminant nutrition provides a tool whereby aspects of energy and protein metabolisms can be examined without the complications which arise from normal rumen activity.

References

1. **Agricultural Research Council** (1984): *The nutrient requirement of ruminant livestock*, Suppl. No. 1. Commonwealth Agricultural Bureaux, Slough.
2. **Armstrong, D.G., Blaxter, K.L. and Graham, N. McC.** (1957): *The heat increments of mixtures of steam volatile fatty acids in fasting sheep.* Br. J. Nutr. 11, 392—408.
3. **Cook, R.R.** (1976): *Urease activity in the rumen of the sheep and the isolation of ureolytic activity.* J. Gen. Microbiol. 92, 32—48.
4. **Fawcett, J.K. and Scott, J.E.** (1960): *A rapid and precise method for the determination of urea* J. Clin. Path. 13, 156—159.
5. **Hovell, F.D. DeB., Orskov, E.R., Grubb, O.A. and MacLeod, N.A.** (g983): *Basal urinary nitrogen excretion and growth response to supplemental protein by lambs close to energy equilibrium.* Br. J. Nutr. 50, 173—187.
6. **MacLeod, N.A., Corrigal, W., Stirton, R.A. and Orskov, E.R.** (1982): *Intragastric infusion of nutrients in cattle.* Br. J. Nutr. 47, 547—554.
7. **McKenzie, J.D. and Kay, R.N.B.** (1968): *Rumen cannulus made from vulcathene.* J. Sci. Technol. 14, 15—16.
8. **Orskov, E.R., Grubb, D.A., Wenham, G. and Corrigall, W.** (1979): *The sustance of growing and fattening ruminants by intragastric infusion of volatile fatty acids and protein*, Br. J. Nutr. 41, 553—558.
9. **Orskov, E.R., MacLeod, N.A., Kay, R.N.B. and Gregory, P.C.** (1984): *Method and validation of intragastric nutrition.* Can. J. Anim. Sci. 54, 138—139.
10. **Ottenstein,D.M. and Bartley, D.A.** (1971): *Separation of free acids* C_2—C_5 *in dilute aqueius solution column technology.* J. Chromat. Sci. 9, 673 —681.
11. **Tao, R.C. and Asplund, J.M.** (1975): *Effect of energy sources on plasma insulin and N metabolism in sheep totally nourished by infusion.* J. Anim. Sci. 41, 1653—1659.
12. **Wallace. R.J.** (1984): *A comparison of the ureolytic and proteolytic activities of rumen bacteria from lambs fed conventionally and by intragastric infusion.* Can. J. Anim. Sci. 64, 140—141.