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# THE USE OF A "TT" SHAPED CANNULA FOR COLLECTION OF DUODENAL DIGESTA IN SHEEP

By

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BORHAMI, B. E. A., K. HOVE and F. SUNDSTØL: *The use of a "TT" shaped cannula for collection of duodenal digesta in sheep.* Acta vet. scand. 1980, *21*, 546—558. — Six sheep were equipped with both a rumen fistula and a duodenal cannula. The duodenal cannula was made of two ordinary T shape cannulas which were vulcanized into a double T cannula. Both the rumen and the duodenal cannulas were made of soft rubber.

In two experiments six different diets were fed. In the first experiment the diets consisted mainly of ammonia-treated straw ($NH_3$-straw) plus barley or sodium hydroxide-treated straw (NaOH-straw) plus concentrate containing different N-supplements. In the second experiment, the treated straw (either $NH_3$-straw or NaOH-straw) was fed alone. The animals in the two experiments were fed at maintenance level.

Duodenal digesta were collected for periods of 12 h by inflating a balloon in the distal flange of the cannula, using pieces of foam rubber. The volume of fluid leaving the rumen was measured using the marker Cr-EDTA. The effect of digesta removal on blood constituents was studied. Three to four observations on each ration were made.

Digesta flow and dry matter entering the duodenum were higher with $NH_3$-straw than NaOH-straw fed either alone or with concentrate. However, there was a considerable variation of which a large part was of individual nature.

The volume of fluid reaching the duodenum was always lower than the volume leaving the rumen, indicating a net absorption of water in the omasum and abomasum. This was estimated to be from 10.1 to 11.6 l/24 h in Experiment 1 and from 0.3 to 1.4 l/24 h in Experiment 2 (51—59 and 5—31 % of the ruminal outflow, respectively).

A significant increase in plasma K and Mg and decrease in $P_i$ and α-amino N concentration were observed due to 12 h collection of duodenal digesta. However, plasma Na, Ca, glucose and urea nitrogen concentrations remained at their pre-collection levels.

Post-mortem examination of duodenum in sheep slaughtered after six and 10 months revealed a normal gut with no sign of dilatation in the immediate vicinity of the cannula.

double cannula; duodenum; sheep.

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Considerable efforts have been devoted to develop techniques suitable for cannulation of the various parts of the alimentary tract in ruminants. Such techniques have been a prerequisite for the study of the processes taking place in the various parts of the digestive system. Different operative procedures and types and shapes of the various cannulas have been reported (*Phillipson* 1948, 1952, *Hogan & Phillipson* 1960, *Singleton* 1961 and *Ash* 1962). *MacRae* (1975) and *Wenham* (1979) discussed some of the differences between the T shape cannulas and the re-entrant cannulas of the Ash type. In short, T shape cannulas cause only small abnormalities in the propulsion of the gut contents, while serious dilatations and disturbances of the flow may occur after some time with re-entrant cannulas. The re-entrant cannula is, however, superior, when quantitative collections of intestinal contents are to be performed. The use of T shape cannulas for collection requires blocking of the gut lumen by an inflatable balloon inserted distal to the cannula. Such blocking may impede the peristaltic movements of digesta due to the continuous pressure exerted on the gut wall, and make mechanical obstructions to the outflow from the cannula. In the present work we report the use of a double T shaped (TT) cannula in the duodenum. During periods of collection of intestinal contents the gut lumen is blocked by a balloon inserted through the distal T. The balloon is inflated with small pieces of foam rubber to maintain the balloon expanded, thus avoiding the use of positive pressure. It is thought that this flexible balloon gives lower resistance to the gut contractions and less interference with the gut movements. The effect of continuous collection of duodenal digesta for 12 h on rumen outflow, digesta flow to the duodenum and blood components is reported.

## MATERIALS AND METHODS

### *Cannula*

Cannulas were made of soft rubber. Two ordinary T shape cannulas were vulcanized into a double T cannula (Fig. 1). The same material has earlier proved useful for prolonged implantations of rumen cannulas in both cows and sheep.

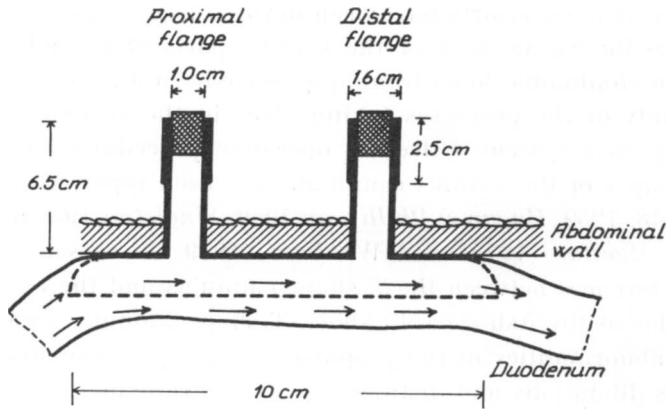


Figure 1. Schematic drawing of the cannula positioned in the duodenum. Arrows indicate direction of the digesta flow in the gut.

*Surgery*

Six mature, castrated sheep were starved for 24 h before start of the operation. Ten mg atropine was given subcutaneously 30 min before the induction of anaesthesia with sodium thiobarbital. The anaesthesia was maintained by inhalation of an oxygen/flouthane mixture in a closed circuit apparatus. A 10—15 cm incision was made through the abdominal wall on the right side, and the duodenum was located. The cannula was introduced through a 1.5 cm incision in the duodenum placed 3—5 cm distal to the pyloric orifice leaving the proximal flange outside the gut. The distal flange was taken out through a second incision in the duodenum approx. 5 cm distal to the first. The gut wall was tightened around the neck of the flanges by double purse string sutures. The two flanges were taken out through separate stab wounds a few cm cranial to the abdominal incision. Subsequently the animal was turned and a wide neck (4 cm) cannula was placed in the rumen.

*Feeding experiments*

Two experiments involving collections of duodenal digesta were carried out. In the first experiment four experimental diets containing straw, barley and various nitrogen supplements were fed (Table 1). The diets were calculated to be isonitrogenous and isocaloric on a gross energy basis. The intake was calculated to be about 1.2 $\times$ maintenance of metabolizable energy (M.E.).

Table 1. Composition of the diets fed (g DM/d).

| | Expt. 1 | | | | Expt. 2 | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 |
| Ammonia-treated straw | 623 | | | | | 822 |
| NaOH-treated straw | | 573 | 572 | 572 | 820 | |
| Barley meal | 322 | 320 | 206 | 250 | | |
| Molasses | 16 | 16 | 15 | 16 | | |
| Protein supplements | | 16 | 114 | 74 | 6 | |
| Mineral „ | | | | | 16 | 16 |
| Total DM intake | 977 | 941 | 923 | 929 | 842 | 838 |
| Total N intake | 16.3 | 16.7 | 16.7 | 16.7 | 13.3 | 11.7 |

In the second experiment, two different diets were fed to each of three sheep (Table 1). The diets were isonitrogenous and provided the maintenance requirement of M.E.

Each diet was given for a five week period. During the last two weeks, measurements of rumen fluid volume and collections of duodenal digesta were performed. In this period the daily ration was fed in two equal portions at 08 a.m. and 08 p.m. The results of measurement of rumen function and nitrogen metabolism will be discussed in separate papers.

*Collection of digesta*

During periods of collection of digesta, the duodenum was blocked at the level of the distal cannular flange by a small balloon. Inflation of the balloon was done by introduction of small pieces of foam rubber to give a volume of about 20 $cm^3$. Digesta leaving the abomasum was allowed to flow freely into a plastic bag tightened around the tip of the proximal flange. The digesta reaching the duodenum between two meals were collected at six 2-h intervals without any return of digesta to the animal during the period of collection.

Digesta flow in sheep fed sodium hydroxide-treated straw supplemented with three different N supplements showed no significant differences, accordingly only the mean results for these three diets are presented (Table 2).

*Rumen volume and rate of outflow*

The volume and outflow of rumen liquid were calculated from the dilution of chromiumethylenediamine-tetra-acetate (Cr-

EDTA) as described by *el-Shazly et al.* (1976). Three measurements were carried out in each animal at the following times; before feeding, 3 h and 6 h after feeding. Averages of the three measurements of rumen volume and outflow were used when comparing the rates of rumen outflow with the amounts of digesta collected at the duodenum.

*Blood samples*

With the present technique of collection no digesta will enter the gut for 12 h. The pattern of metabolism in ruminants is directed towards a continuous absorption of nutrients from the intestine and reabsorption of the various electrolytes added during the passage through the stomachs. The complete collection of the abomasal outflow may disturb the normal metabolism. To get an indication of the extent to which such disturbances occur, blood samples were withdrawn after 12 h of collection. The concentrations of glucose, α-amino nitrogen, urea N, Na, K, Ca, Mg and $P_i$ were compared to values observed one or two days before the collection.

*Analytical procedures*

Plasma urea was determined as $NH_3$ after enzymatic hydrolysis. Plasma free amino nitrogen determination was carried out after *Palmer & Peter* (1969). Glucose was determined as reducing sugar by the ferricyanide method (*Hawk et al.* 1947). Na, K, Ca, Mg and Cr were estimated using atomic absorption or flame emission spectrophotometer. Inorganic phosphorus was estimated using molybdate and Fiske and Subbarow reagent (*Hawk et al.*).

## RESULTS

*Post-operative examinations*

The sheep resumed normal feeding activity within two—three days after the operation. One sheep developed a necrosis of the jejunum six months after the operation. Post-mortem examination of the duodenum revealed a normal gut with no signs of dilatation in the area cranial to or in the immediate vicinity of the cannula. In sheep slaughtered six and 10 months post-operatively the intestinal anatomy was normal.

*Digesta flow*

The amounts of digesta collected at six 2-h intervals after feeding are presented in Fig. 2. It is noticed that the digesta flow was higher with ammonia-treated straw than sodium hydroxide-

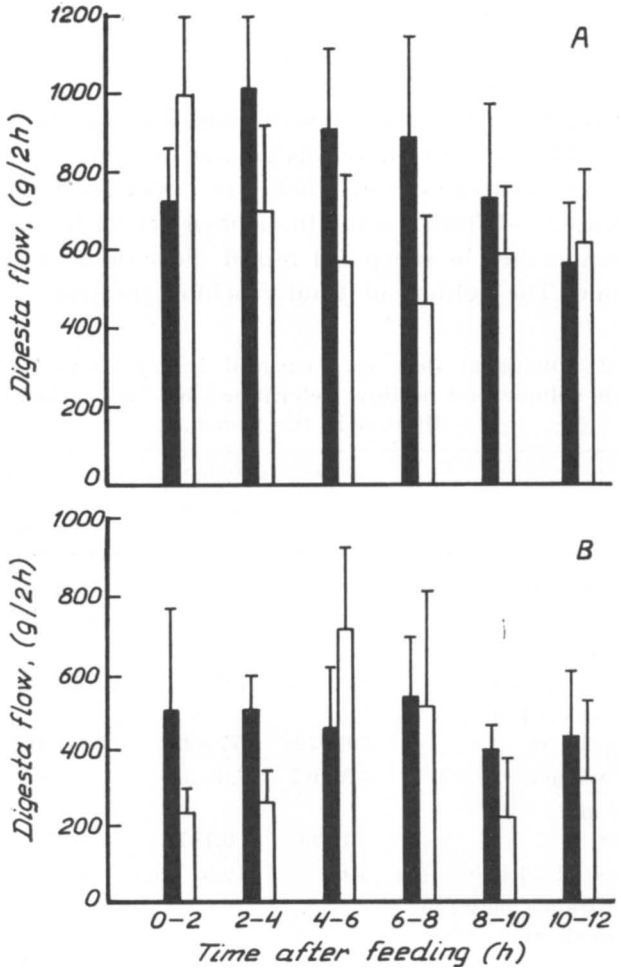


Figure 2. Amounts of duodenal digesta collected at 2-h intervals between two consecutive feedings.

A: ■ Ammonia-treated straw + barley alone (Diet 1)
□ Sodium hydroxide-treated straw + various N supplements

B: ■ Ammonia-treated straw alone
□ Sodium hydroxide-treated straw + urea

treated straw, fed either alone or with concentrate. Also less variation in duodenal digesta flow between the 2-h intervals were observed when the ammonia-treated straw was fed alone. A large part of the variation was of individual nature.

The amount of dry matter entering the duodenum was higher for ammonia-treated straw diets than the sodium hydroxide-treated straw diets.

*Rumen volume and fluid outflow*

The rumen volumes and rates of fluid outflow are presented in Table 2. The rumen volume was greater in animals consuming straw plus concentrates than when straw was fed alone.

The volume of fluid leaving the rumen per 24 h was approx. three times higher in sheep fed mixed diets than in sheep fed straw alone. The volume of fluid reaching the duodenum was

Table 2. Duodenal flow as measured by continuous collection and rumen volume and outflow determined by the marker Cr-EDTA dilution in the rumen.

| Diets : | Straw + concentrate | | Straw alone | |
|---|---|---|---|---|
| Straw treatment: | ammonia | sodium hydroxide[a] | ammonia | sodium hydroxide |
| Number of observations | 4 | 12 | 3 | 3 |
| Total flow of digesta at the duodenum: (kg/24 h, mean ± s.e.m.) | 9.6±2.3 | 7.9±1.1 | 5.6±1.6 | 4.5±2.8 |
| Dry matter collected at duodenum (g/24 h) | 336±79 | 277±32 | 336±106 | 163±103 |
| Rumen fluid volume (l) | 8.3±0.7 | 7.6±0.5 | 3.2±0.3 | 3.2±0.4 |
| Hourly fractional turn-over rate | 0.099 | 0.107 | 0.077 | 0.077 |
| Rumen outflow (l/24 h)[b] | 19.7 | 19.5 | 5.9 | 5.9 |
| Fluid disappearance between rumen and duodenum[c] | | | | |
| l/24 h | 10.1 | 11.6 | 0.3 | 1.4 |
| % of rumen outflow | 51.3 | 59.5 | 5.1 | 31.1 |

[a] Average data for the three diets containing sodium hydroxide plus nitrogen supplements.

[b] Calculated as = Rumen volume × fractional turn-over rate × 100 × 24.

[c] The difference between fluids leaving the rumen and fluids collected at duodenum.

always lower than the volume leaving the rumen, indicating a net absorption in the omasum and abomasum. The volume of fluid which disappeared between the rumen and duodenum was estimated to be from 10.1 to 11.6 l/24 h in Experiment 1 and from 0.3 to 1.4 l in Experiment 2 (51—59 and 5—31 % of the ruminal outflow, respectively).

*Plasma parameters*

Changes in the plasma concentrations of sodium, potassium, calcium, inorg. phosphorus, magnesium, glucose, urea nitrogen and α-amino nitrogen due to 12-h collection of duodenal digesta are presented in Table 3. A significant increase in plasma K and Mg and a decrease in $P_i$ and α-amino N were observed, while the other parameters remained approximately at their pre-collection levels.

T a b l e 3. Concentrations of Na, K, Ca, Mg, inorg. P, glucose, α-amino nitrogen and urea nitrogen in blood plasma* (mmol/l; mean ± s.e.m.)

| Components | Normal | After digesta collection |
|---|---|---|
| Sodium | 139 ± 2.88 | 142 ± 3.38 |
| Potassium | $4.74^a$ ± 0.38 | $6.87^b$ ± 0.67 |
| Calcium | 2.35 ± 0.04 | 2.43 ± 0.05 |
| Magnesium | $0.86^a$ ± 0.04 | $0.95^b$ ± 0.05 |
| Inorg. phosphorus | $1.59^a$ ± 0.11 | $1.30^b$ ± 0.12 |
| Glucose | 3.33 ± 0.06 | 3.23 ± 0.07 |
| Urea N | 0.52 ± 0.06 | 0.46 ± 0.05 |
| α-amino nitrogen | $4.32^a$ ± 0.13 | $3.55^b$ ± 0.18 |

* Means with different superscripts differ significantly ($P < 0.05$).

## DISCUSSION

When comparing the different types of cannulas hitherto used for duodenal collection or sampling, it seems clear that the double T shape cannula used in the present study retains the good properties of a simple T cannula. This refers both to the propulsion of digesta and the dilatation of the gut which have been reported with re-entrance cannulas (*MacRae* 1975, *Wenham* 1979). In addition, the cannula requires little maintenance when first placed in the gut. When comparing single and double T shaped cannulas it was found that the double T shaped can-

nula may prolong the life time of a cannulated sheep due to the fact that the cannula is almost impossible to pull out by the animal (*Borhami*, unpublished data).

*Phillipson* (1952) observed a reduced outflow from the abomasum when the lumen of the gut was blocked by an air-filled balloon. In re-entrant cannulated animals, *Hogan & Phillipson* (1960) reported an increase in digesta flow to the duodenum when no digesta was returned to the animal during periods of collection.

The rates of outflow observed during the 12-h collections in our sheep did, however, not show fluctuations indicating irregularities in the emptying of the abomasum. Although a recycling of gut contents to the distal T during the collection would obviously have been more satisfactory, it seems that the rates of outflow observed presently are in good agreement with results obtained in re-entrant cannulated sheep fed comparable diets. This refers both to the use of continuous collection/recycling techniques as reviewed by *MacRae* (1974) and *Faichney* (1975) and when the flow was evaluated with spot-sampling techniques in animals infused with markers (*Laughren & Young* 1979).

A large part of the variation in the present study was due to great variation between animals. *Leibholz & Hartmann* (1972) observed marked variation in flow of digesta in sheep (up to 10-fold variation at 2-h intervals). *Nicholson & Sutton* (1969) reported a high variability both within and between sheep in the flow of digesta. They found no clear relationship between rates of outflow on one hand and eating, ruminating or resting on the other. This variability seems to be a true biological phenomenon rather than a result of any serious inadequacy of the collection procedure (*Ulyatt & MacRae* 1974).

The results obtained with the present method of cannulation are generally in good agreement with results observed by others with different techniques.

When comparing the volume of fluid leaving the rumen with the volume collected at the duodenum, we found that 55 % of the fluid volume disappeared between the rumen and duodenum of sheep fed mixed diets and 17 % when straw was fed alone. These results are in good agreement with the previous observations on water absorption in the omasum of steers (56 %) and sheep (13 %) fed comparable diets (*Engelhardt & Hauffe* 1975,

*Edrise & Smith* 1979). In the present study the volume of digesta collected at the duodenum in sheep fed straw and concentrate mixture was higher than when straw was fed alone. Similar results have been reported by *Nicholson & Sutton*. The higher dry matter concentrations in the duodenal collection when feeding ammonia-treated straw than when feeding NaOH-treated straw reflect the lower digestibility of ammonia-treated straw. This is in agreement with results of digestibility trials with the same diets (*Borhami*, unpublished data).

The 12-h collection of digesta led to significant changes in the concentrations of several plasma constituents. When evaluating the effects of the collection on the concentrations of plasma constituents, a distinction must be made between substances absorbed in the fore-stomaches and in the intestines. The observed reduction in the plasma levels of inorg. phosphorus and α-amino nitrogen can most probably be explained by a reduced input from gut to blood, since no digesta were allowed to reach the absorptive sites for these substances during the collection. Calcium is also absorbed predominantly in the small intestine. The lack of change in plasma Ca during the collection may, however, be attributed to the close regulation of plasma Ca by several endocrine systems (*Ramberg et al.* 1975). It seems likely to assume that the reduction in Ca influx from the gut which must have occurred during the collection period was compensated for by the homeostatic mechanisms maintaining a normal blood Ca level.

The plasma concentration of the components absorbed from the fore-stomachs, or derived from precursors absorbed there, would seem to be less influenced by the collection procedure. This was in fact the case for both sodium, urea and glucose. Significant increases in the plasma concentrations of Mg and K were, however, observed. Since both minerals are absorbed actively from the reticulo-rumen (*Scott* 1975, *Martens et al.* 1976, *Edrise & Smith*), these changes in plasma concentration might reflect an increased absorption. The collection of digesta led to withdrawal of considerable volumes of isotonic fluid which was compensated for by increased intake of water. We can therefore not exclude the possibility that the increase in Mg and K in plasma might result from a cellular leakage of these minerals. Plasma osmolarity was, however, not measured. The plasma concentrations of K after 12 h of collection were in the upper

normal range, but lower than the concentrations reported to be toxic (*Neathery et al.* 1980).

In conclusion the present results indicate that the TT shaped cannula may give satisfactory measurements of digesta flow to the duodenum. Further work is needed to compare this technique with the marker technique. The effect of reinfusion of digesta into the duodenum during the collection remains to be studied.

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## SAMMENDRAG

*Bruk av „TT"-formet kanyle for oppsamling av chymus fra tolvfingertarmen hos sau.*

Seks sauer ble utstyrt med både vomfistel og en kanyle i tolvfingertarmen. Tolvfingertarm-kanylen besto av to vanlige T-formede kanyler som var vulkanisert sammen til en dobbel T-kanyle. Både vomfistlene og tolvfingertarm-kanylene var laget av mjuk gummi.

Forsøksdyrene ble gitt seks forskjellige fórrasjoner i to forsøk. Rasjonene i det første forsøket besto hovedsaklig av ammoniakkbehandla halm ($NH_3$-halm) plus byggrøpp eller natriumhydroksydbehandla halm (NaOH-halm) plus kraftfór med forskjellig N-tilskudd.

I det andre forsøket ble behandlet halm (enten $NH_3$-halm eller NaOH-halm) gitt som eneste fór. Forsøksdyrene ble fóret til vedlikeholdsnivå.

Chymus fra tolvfingertarmen ble samlet opp i 12 timer ved å føre en ballong gjennom den distale åpningen av kanylen, fylle denne med skumgummi og derved blokkere passasjen videre bakover gjennom tarmen. Mengden av væske som forlot vom/nettmage ble målt ved å bruke Cr-EDTA som markør. Endringen i en del blodkomponenter ved fjerning av chymus ble også undersøkt og det ble utført tre til fire målinger for hver fórrasjon.

Mengden av total chymus og tørrstoff som strømmet inn i tolvfingertarmen var større for $NH_3$-halm enn for NaOH-halm både med og uten kraftfórtilskudd. Det var imidlertid en betydelig individuell variasjon i materialet.

Mengden av væske som nådde tolvfingertarmen var alltid lågere enn mengden som passerte ut av vom/nettmage. Dette indikerer en netto absorpsjon av vann i bladmage og løpe, og den ble beregnet til 10.1—11.6 l/24 t i forsøk 1 og 0.3—1.4 l/24 t i forsøk 2 (henholdsvis 51—59 og 5—31 % av mengden som forlot vom/nettmage).

Det ble funnet en signifikant økning i konsentrasjonen av plasma K og Mg, og nedgang i $P_i$ og α-amino N ved fjerning av chymus fra tolvfingertarmen i 12 t. Konsentrasjonen av plasma Na, Ca, glukose og urea avvek ikke fra nivået før fjerningen av chymus.

Post-mortem undersøkelser av tolvfingertarmene hos sauer slaktet 6 og 10 måneder etter fistulering viste at tarmen var normal uten tegn til dilatasjon rundt kanylen eller i nærmeste tarmavsnitt.

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