DEVELOPMENT AND TESTING OF A REMOTE CONTROLLED OESOPHAGEAL FISTULA VALVE FOR GOATS

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submitted in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy (Ph.D)

in the Department of Grassland Science

Faculty of Agriculture

University of Natal

Pietermaritzburg

January 1993

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DECLARATION

This thesis is the result of the author's own original work, unless specifically indicated to the contrary in the text.

The author

ACKNOWLEDGEMENTS

"He gives wise men their wisdom, and scholars their intelligence"

Daniel 2: 21.

I wish to express my sincere appreciation to the following individuals and organisations for the valuable assistance and co-operation that was received during the conducting of this study:

* Mr B K Clarke for the mechanical design, drawing and construction of the equipment. The endless number of prototypes and jigs bear testimony to his perseverance;
* Professors N M Tainton and W S W Trollope for their foresight, consistent interest and encouragement throughout this study;
* Dr. S C Cockcroft who was responsible for the surgical procedures;
* Mr. D A K King for his valuable assistance with the fistulated animals and field trials;
* Messrs. D Pepe, L Webber and Miss C Mogorosi for their valuable assistance with data collection and preparation of samples, together with Messrs. J W A Stead, M Bester, J M Giqwa and V Dyan for the effective nursing care of the fistulated goats;

* Messrs. R D Masters, G G Masters, (Mastercraft), N Honiball (Gentyre), M Stent (Mekelek) and staff of Gentech for technical assistance;
* Mr. A C Beckerling for assistance with analyses of data;
* Mr. A B D Joubert for his advice with design and calculations;
* Mr. G Cooper for his professional advice and preparation of some of the diagrams;
* Dohne Research Station personnel for assistance with the fistulation process.
* Mr. H Ward and Professor J P Mildenhall for the mammoth task of correcting the grammar of the report;
* The Research Committee of the University of Fort Hare and the FRD (University Development Programme) for financial assistance;

Acknowledgement is especially due my wife, Susan and two daughters for their encouragement and patience when the project interfered with family activities.

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ABBREVIATIONS

ADF	-	acid detergent fibre
ADL	-	acid detergent lignin
BW	-	body weight
Ca	-	calcium
CF	-	crude fibre
Cl	-	chlorine
CP	-	crude protein
Cu	-	copper
DM	-	dry matter
DMI	-	dry matter intake
ECG	-	electrocardiogram
EE	-	ether extract
IVDMD	-	in vitro dry matter disappearance
IVOMD	-	in vitro organic matter digestibility
K	-	potassium
Mg	-	magnesium
N	-	nitrogen
Na	-	sodium
NDF	-	neutral detergent fibre
NDS	-	neutral detergent solubles
NFE	-	nitrogen-free extract
OM	-	organic matter
OMI	-	organic matter intake
P	-	phosphorus
S	-	sulphur
VFA	-	volatile fatty acids
WSW	-	water soluble carbohydrates
Zn	-	zinc

ABSTRACT

A remote control sampling technique was developed for the collection of oesophageal fistula samples from goats. Number and size of samples can be varied and collected throughout the day without disturbing the animal's normal feeding behaviour. The equipment developed and tested in this study consists of an oesophageal fistula valve which allows the fistula to be opened and closed, a rechargeable battery pack and motor to operate the valve, a portable radio and receiver to activate the valve motor, and a harness to attach the equipment to the body of the animal. In addition, a closing device to effectively seal large oesophageal fistulae (>1 050 mm^2), which in turn is required to accommodate the valve, was developed. During field tests with the fistula valve, 10 % of 1 027 sampling attempts failed due to blockage of the valve, and an average of 1.3 incidences of equipment failure were recorded per animal per sampling day, from an average of 9.9 extrusa collections per day. Observed feeding behaviour (grazing/browsing) as well as grass / bush ratio of fistula valve and standard fistula bag samples of four goats, formed the basis for the evaluation of this technique. In addition, extrusa recovery rates, measured under controlled conditions, were used in the evaluation of this sampling method. Differences in extrusa composition between the fistula valve and fistula bag techniques varied substantially during the browsing period within a camp and also between camps. Furthermore, during high frequencies of observed grazing, there were large differences between the fistula valve and fistula bag methods. During this study, the fistula valve technique provided a more realistic estimate (R^2=.91) of the observed feeding behaviour of goats than the fistula bag technique (R^2=.63). Under controlled conditions, the large oesophageal fistula, with or without the valve, enables high and consistent extrusa recovery rates (87 % recovery; SD 7.5).

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

In South Africa, natural vegetation provides the bulk of nutrients for domestic livestock and all of the nutrients for wild herbivores. For some 20 million years, both grasses and ruminants have evolved together and are therefore co-adapted (Moir, 1968; cited by Van Dyne, Brockington, Szocs, Duek and Ribic, 1980). In an environment characterized by chronic deficiencies in certain nutrients and periodic intervals of harsh climate and food shortages, the survival of grazing animals is, among other factors, dependent on the ability to digest low-quality roughage, store fat, withstand heat and cold, and recycle certain deficient nutrients.

Productivity, though based on survival and many other individual processes, is primarily dependent on the net amount of nutrients available beyond maintenance requirements, which, in turn, is directly related to the quantity and quality of food intake. In range animals, this process commences with the ability of the animal to select large quantities of nutritious forage components. It is therefore logical to study diet selection as the first step in evaluating the productivity of any rangeland for any particular species of animal.

In this respect, oesophageal fistula samples (Torell, 1954) have been accepted as more indicative of the true diet of grazing and browsing animals than forage and/or browse sampled by other methods (Theurer, Lesperance, and Wallace, 1976; Van Dyne et al., 1980). Furthermore, a number of validation studies, based on indirect methods, have been published and reviewed by McManus (1980) to confirm that the existing oesophageal fistula technique provides extrusa samples that closely resemble the total diet of the animal.

On the other hand, however, due to the limitations imposed by the oesophageal fistula technique on the grazing area sampled and the number of samples that can be collected per day, the reliability of this technique to reflect the total daily feeding period has been questioned. Bredon and Short (1971) concluded that the biggest drawback of the oesophageal fistula technique is the short period of sampling, which might introduce considerable error in representing a full day's grazing. Holechek, Vavra and Pieper (1982a) stated that the inability to obtain a representative sample in a large pasture is the primary shortcoming associated with the oesophageal fistula technique. McInnis, Vavra and Krueger (1983) concluded "there is at least one reason for regarding the oesophageal fistula method with some suspicion: plant fragments found in oesophageal extrusa represent the diet of the animal for only that length of time during which the sample is being collected". Based on results from the faecal carbon isotope ratio technique, Coates, Schachenmann and Jones (1987) stated: "the possibility that one or more extrusa samples may not reflect the total daily intake is well recognized", while Jones and Lascano (1992) argued that "diet selection during the collection period (of only 20 to 30 min) may not reflect the 24 hour average value".

Supportive evidence for these viewpoints is available in a number of studies. Diurnal variation in diets of fistulated animals has been observed in nitrogen (N) content (Langlands, 1965; Van Dyne and Heady, 1965; Hodgson, 1969; Obioha, Clanton, Rittenhouse and Streeter, 1970), lignin (Obioha et al., 1970), digestibility (Hodgson, 1969) and botanical composition (Coates et al., 1987). Furthermore, it is known that ruminants can have between 8 and 10 grazing periods per day (Arnold, 1962), and that diet selection changes with increasing satiation and with changes in pasture and weather (McManus, 1980). Vavra, Rice and Hansen (1978) compared oesophageal fistula and faecal samples from range cattle: total number of grasses occurred significantly less frequently in fistula than in faecal samples, while total number of forbs were significantly lower in faecal than in fistula samples; also, little relationship in botanical composition was found between fistula and faecal samples.

The limitations imposed by the oesophageal fistula technique on the grazing area sampled and the number of samples that can be collected per day, are of particular importance where browsing animals are being studied in a large heterogenous plant community. An obvious solution to these limitations would be to collect a sufficient number of small samples throughout the day in order to adequately represent the total daily feeding period in terms of both time of day and species selected.

Based on this thesis, a study was undertaken with the main objective of developing a multiple sampling device for the oesophageal fistula in order to collect representative forage samples as selected by goats throughout the day, without disturbing their normal feeding behaviour.

Because the oesophageal fistula remains the basis of the sampling procedure, it is appropriate to discuss the development and validity of this technique in a more detail than the other techniques mentioned above.

1.1 HISTORICAL OVERVIEW AND DEVELOPMENT OF THE OESOPHAGEAL FISTULA TECHNIQUE.

One of the earliest reports on the oesophageal fistula, by Magendie and Ryer (cited by Van Dyne and Torell, 1964), appeared in 1847. This was followed by several studies cited by the same author, which were of a physiological or psychological nature, before the first attempt was made by Torell (1954) to adapt the oesophageal fistula technique for range nutritional studies.

During the early developmental work on ruminants, high mortality rates were experienced and the life expectancy of oesophageal fistulated animals was, at best, very limited. However, as early as 1964, Van Dyne and Torell cited several studies in which all animals had survived surgery and field use, some for as long as five years. Development of more efficient closure devices was singled out as the main contributing factor towards solving this problem (Van Dyne and Torell, 1964). The success rate in ten reports reviewed by McManus (1980) averaged 68 percent for 126 sheep and 87 percent for 38 cattle. Since the development of the basic oesophageal fistula technique by Torell (1954), a great number of modifications and refinements have been reported.

1.1.1. Surgical technique:

Reviews of the surgical technique for oesophageal fistulation were published by McManus (1960, cited by Van Dyne and Torell, 1964); McManus (1962a); Van Dyne and Torell (1964); Harris, Lofgreen, Kercher, Raleigh and Bohman (1967); and McManus (1980). A summary of the major advances in the surgical technique for different species is presented in Appendix Table 1.

Besides the simplification of the surgical procedure as first described by Torell (1954) and Van Dyne and Torell (1964), improvements were mainly a result of the development of more effective anaesthetics with limited side effects and suitable for use on a wider range of animal species. Also, gentling of animals prior to surgery, sound practices of hygiene and post-operative care to reduce risk of infection, minimized animal stress and hence mortalities. An effective and well tested procedure for the selection and handling of animals prior to surgery, surgical establishment of oesophageal fistulae, and post-operative care of small ruminants is described by Pfister, Hansen and Malechek (1990).

1.1.2. Problems and complications associated with fistulated animals.

Because of anatomical differences between animals and/or differences in size and placement of the fistula, most types of closing devices do not fit and function well in all animals (Karn, 1987), with the result that the useful life of these animals may be greatly reduced. The following problems may be encountered with fistulated animals: impaction of the oesophagus; formation of a "pouch" anterior to the fistula; necrosis and/or irritation of the oesophageal lumen above or below the fistula; scar tissue accumulation; frequent granulomatous tissue formation during the healing period; expansion of the fistula and resultant plug loss, especially after lambing; contraction of the fistula (recently established fistulae are more prone to contraction than well established fistulae), and difficulty in swallowing if the fistula is more than 50 mm in length (McManus, Arnold and Hamilton, 1962; Arnold, McManus, Bush and Ball, 1964; Bredon and Short, 1971; Denney, 1981; Anderson and Mertz, 1982; Ellis, Bailey and Taylor, 1984; Forwood, Ortbals, Zinn and Paterson, 1985; Pfister et al., 1990; Visser, Pietersen, Spreeth and Meissner, 1990).

In order to overcome some of these problems, fistula dilators for stretching contracted fistulae in cattle (Bredon and Short, 1971; Anderson and Mertz, 1982), or a temporary cannula that prevents fistula contraction during sampling (Olson and Malechek, 1987) were developed. The "off centre" sleeve (Van Dyne 1962 cited by Van Dyne and Torell, 1964), flexible latex moulded plug (McManus et al., 1962), or metal parts coated with soft rubber (Forwood et al., 1985) were developed to reduce scar tissue build-up and rubberized belting has been used as a seal in order to reduce leakage from the fistula (Karn, 1987).

1.1.3. **Closing device:**

Reviews of oesophageal fistula closing devices were published by Van Dyne and Torell (1964); Harris et al. (1967); and McManus (1980). Types of closing devices for oesophageal fistulae include permanently located cannulas, semi-permanent cannulas, and removable moulded, flexible, solid or split-stemmed plug stoppers. A summary of the major advances in the development of the oesophageal fistula closing device is presented in Appendix Table 2.

From the original oesophageal fistula technique (Torell, 1954), two different directions of development evolved, namely, the permanently located cannula (Cook, Thorne, Blake and Edlefsen, 1958; Figure 1a), and the removable plug (Torell, 1954; Figure 1b). Of these two devices, the removable plug seems to be preferred by most researchers. In the case of the permanently located cannula, the bolus has to be forced through a rigid circular or oval lining of the fistula during sampling. This may result in either incomplete recovery of extrusa or blockage (McManus, 1980). A further problem associated with the cannula is the possibility of "pouch" forming anterior to the fistula (Van Dyne and Torell, 1964). Compared to the removable cannula, advantages of the permanently located cannula include minimum animal stress, reduced chance of fistula contraction when the plug is removed for extended periods, and increased ease and efficiency in obtaining diet samples (Taylor and Bryant, 1977). Further modifications on both original designs were mainly directed towards minimizing cost, weight, and animal stress, while maximizing efficiency and durability (Taylor and Bryant, 1977). In the absence of sufficient test results, it is very difficult to single out any one improvement or modification that contributed most towards the success of the present method. However, a few developments should be mentioned for their originality and potential to solve some of the problems associated with the oesophageal fistula technique:

(1) simplicity of the removable sleeve and plug (Torell and Bredon, 1961 cited by Van Dyne and Torell, 1964), and the split plug designs (McManus et al., 1962),

(2) rotation of the "off centre" sleeve (Van Dyne 1962 cited by Van Dyne and Torell, 1964) and use of the latex moulded plug (Hamilton, McManus and Larsen, 1960; McManus et al., 1962) in order to reduce the build-up of scar tissue,

(3) oval shaped plug for larger fistulae (Hofmeyr and Voss, 1964),

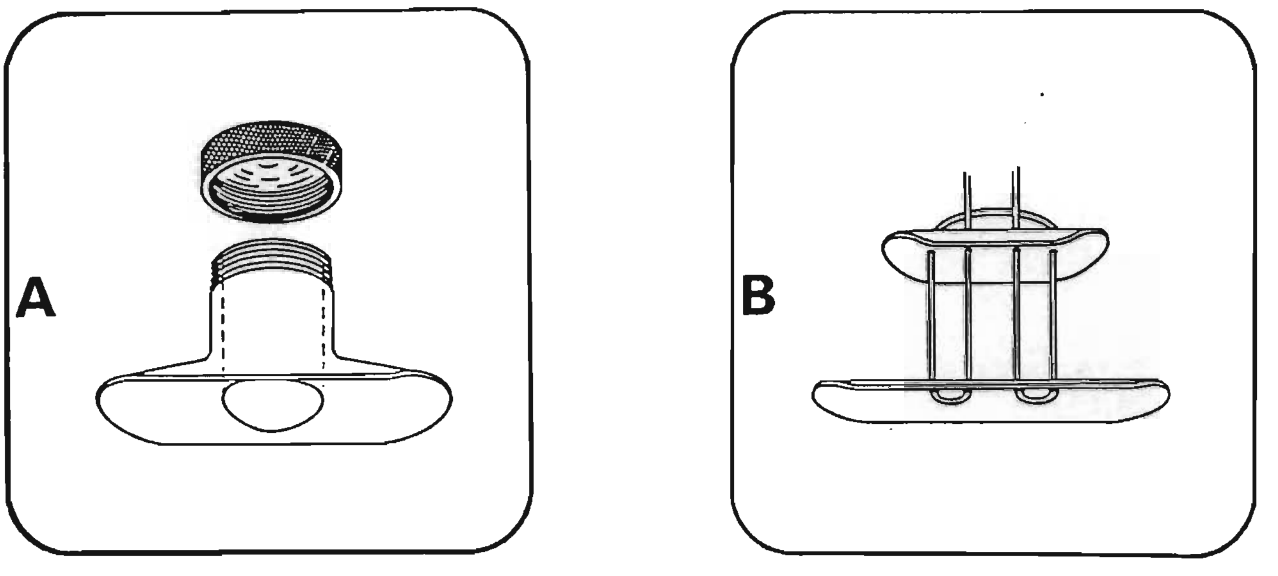


Figure 1 Schematic presentation of (a) permanent or fixed cannula and (b) removable plug

(4) temporary cannula that prevents fistula contraction during sampling (Olson and Malechek, 1987), and

(5) the use of rubberized belting as a seal in order to reduce leakage from the fistula (Karn, 1987).

1.1.4. Fistula size:

Harris et al. (1967) reported that the size of the fistula used in sheep is normally up to 37 mm long and 25 mm wide (800 mm^2) and for cattle 37 to 50 mm long and 25 to 37 mm wide (800 to 1 500 mm^2). McManus et al. (1962) concluded that an optimum size of the fistula in sheep is approximately 600 mm^2, while Arnold et al. (1964) found that fistulae of more than 1 000 mm^2 aperture gave consistently good extrusa recovery rates. Of the 18 fistulae aperture sizes for small ruminants that are summarized in Appendix Table 2, only four are larger than 500 mm^2, of which two exceed 1 000 mm^2. In the same table 50 % of the designs for cattle are larger than 1 000 mm^2.

1.2. VALIDITY OF THE OESOPHAGEAL FISTULA TECHNIQUE.

Oesophageal fistulated animals have been widely used in diet selection studies for many years because the resulting samples have been accepted as being more indicative of the true diet than forage and/or browse sampled by other methods (Theurer et al., 1976). However, due to the sampling procedure and possible effects of fistulation and handling on the physiology and behaviour of fistulated animals, answers to certain questions are of fundamental importance in relation to sampling with such animals, viz:

(1) do oesophageal fistulated animals ingest a diet similar to that of the larger population of non-fistulated animals?

(2) to what extent do mastication, saliva excretion, and possibly ineffective recovery of the bolus affect the total nutrient content of the ingested herbage or the nett intake of nutrients by the animal?

(3) to what extent do fasting, sampling schedule, adaptation period prior to sampling, saliva loss, and general management of fistulated animals affect their physiology and feeding behaviour? and

(4) can one group of oesophageal fistulated animals, used on a roving basis to sample grazing and/or browsing as required, represent animals of different sex, age, livemass, or physiological condition?

1.2.1. Fistulated vs non-fistulated animals.

Because of the problems involved in estimating the true diet of non-fistulated, free ranging herbivores, few studies have critically examined the extent that fistulated animals represent the larger population of their normal counterparts.

Physiology, behaviour and productivity: The diversion of saliva from the rumen during the oesophageal extrusa collection period, as well as saliva and rumen content losses due to fistula plug leakage, could elevate rumen volatile fatty acids (VFA), depress rumen pH, and reduce rumen movement (McManus, 1962b). However, from the same study it was concluded that these conditions would come into effect only after excessive saliva loss (i.e. sampling period in excess of 1 hr.). Based on observations, Arnold et al (1964) concluded that successfully fistulated sheep graze in the same manner and have similar grazing times, herbage intakes and productivity (livemass change and wool growth), as normal sheep. Rumsey, Putnam, Williams and Samuelson (1972) stated that oesophageal fistulation does not affect rumen parameters, nor does it cause permanent changes in heart rate, respiratory rate or electrocardiogram (ECG) patterns. Ellis et al. (1984) concluded that diets of fistulated and non-fistulated animals are qualitatively and quantitatively similar, because no significant differences could be found in either faecal output or chemical analyses of faeces between the two groups. Except for slightly higher faecal N concentrations, Forbes and Beattie (1987) could not find differences in bite rate, grazing time, faecal acid detergent fibre (ADF) or ash concentrations nor in relative proportions of faecal cuticle fragments between oesophageal fistulated and non-fistulated cattle or sheep. On the basis of this evidence they concluded that there is no reason to expect that fistulated and non-fistulated animals of similar history and nutritional background will differ in feeding behaviour or diet selection.

On the other hand, evidence that may indicate physiological and/or behavioural differences between fistulated and non-fistulated animals was reported by Basson (1971) who noted that one of three sheep often refused to eat during the collection period. When its fistula plug was replaced, it immediately resumed with normal eating. Rumsey et al (1972) reported that some oesophageal fistulated steers were observed to regurgitate boluses less frequently than non-fistulated animals. Furthermore, silica (Torell, Bredon and Marshall, 1967) and nitrogen content (McManus, 1960, cited by Forbes and Beattie, 1987) of faeces collected from fistulated and non-fistulated animals were found to be significantly different.

Based on the limited information available, it seems that successfully fistulated animals that are equipped with effective fistula plugs, and are observed to behave normally and are well adapted to the experimental procedure are not physiologically different from non-fistulated animals, though they may differ from non-fistulated animals in feeding behaviour. This may well be a result of differences in handling or management which are discussed in section 1.2.5.

1.2.2. Accuracy of sampling.

Factors that may influence the accuracy by which oesophageal fistula samples represent the ingested food include extrusa recovery rate, mastication, salivation, leaching, contamination with rumen contents, and sample preparation.

Extrusa recovery rate: It is obvious that if all the forage ingested is recovered through the oesophageal fistula, the extrusa samples obtained should accurately represent the forage eaten during the collection period. In practice, however, recovery rates ranging between 10% to 92% of the ingested food have been reported in the literature and are presented in Table 1.

Table 1. **Extrusa recovery rate of ingested food (DM).**

Species	Type of food	% Recovery	
Sheep	Chopped lucerne	90+	(Bath, Weir & Torell, 1956)
Sheep	Lucerne hay	46	(McManus, 1961)
Sheep	Fresh herbage	53 - 73	(Grimes et al., 1965)
Sheep	Pelleted feeds	91	(Basson, 1971)
Sheep	8 Roughages (fresh, 1 hay)	58 - 101	(Armstrong, Common & Davies, 1989)
Cattle	4 Roughages	26 - 81	(Campbell, Eng, Nelson and Pope, 1968)
Cattle	7 Feeds(2 hay)	90	(Kiesling, Nelson and Herbel, 1969)
Cattle	Various feed types	10 - 90	(Little and Takken, 1970)
Cattle	2 Roughages (fresh)	33 - 91	(Barth and Kazzal, 1971)
Cattle	8 Feeds (6 roughages)	33 ± 22	(Little, 1972)
Cattle	8 Roughages (fresh, 1 hay)	77 ± 18	(Cohen, 1979)
Cattle	8 Roughages (fresh, 1 hay)	49 - 103	(Armstrong et al., 1989)

According to Blackstone, Rice and Johnson (1965 cited by Holechek et al., 1982a), the degree of sample recovery from oesophageal fistulated animals is strongly related to the size of the opening. Arnold et al., (1964) concluded that fistulae of more than 1000 mm^2 aperture gave consistently good extrusa recovery rates. In support of this, extrusa recovery of dry matter (DM) and organic matter (OM)) were found to be significantly higher (Hamilton and Hall, 1975; Cangiano and Gómez, 1984) from animals fitted with distal foam plugs which prevent bypass of the fistula than from animals without plugs.

On the other hand, Grimes, Watkin and May (1965), concluded that the percentage recovery does not appear to be related to the size of the fistula, nor to affect the botanical composition of the extrusa. This conclusion was based on results obtained from four sheep(fistula size: 110 mm^2 - 900 mm^2), fed seven different types of freshly cut roughages. Notwithstanding the low and variable recovery rates obtained by Little (1972), it was found that both N and neutral detergent soluble (NDS) contents of extrusa were similar to those of the feeds. Fisher, Burns and Pond (1989) used cellulose sponge plugs in the oesophagus to prevent sorting of the diet to study changes in the masticate of cattle on pasture. They found oesophageal cannula had no effect on particle size distribution, neutral detergent fibre (NDF), or in vitro dry matter disappearance (IVDMD). Furthermore, on the basis of results obtained from oesophageal fistulated sheep fitted with distal foam plugs grazing a wide range of herbage conditions, Hamilton and Denney (1978) found no evidence that stems or any other plant component preferentially bypassed the fistula. Fisher et al (1989) found no significant differences in in vitro organic matter digestibility (IVOMD) or NDF of oesophageal extrusa samples obtained from cattle fitted with or without distal foam plugs.

From the above it is apparent that the size of the fistula has little if any affect on the composition of the extrusa sample. Nevertheless, in the case of a heterogenous diet, it is difficult to believe that the composition of the extrusa would not be affected if only a fraction of the ingested food were collected.

Mastication, salivation, and leaching: The most frequently used method to test the accuracy of sampling is to feed oesophageal fistulated animals a known diet which is then compared to the chemical- and/or species composition of the extrusa obtained from the oesophageal fistula. As far as chemical composition is concerned, a considerable number of studies have been conducted and reviewed by McManus (1980), Holechek et al (1982a) and Olson (1991). A summary of the effects of saliva, mastication and leaching on the chemical composition of oesophageal fistula samples is presented in Appendix Tables 3 and 4.

Although some contradictory results were found for each of these constituents, indications are that N, crude fibre (CF), and calcium (Ca) are least affected, while ash, phosphorus (P), sodium (Na), and potassium (K) are normally overestimated in oesophageal samples. The contradictory results could in part be explained by the interaction that exists between the chemical composition of extrusa samples and drying methods (Scales, Streeter, Denham and Ward, 1974), by type and amount of forage consumed (Harris et al., 1967; Pinchak, Greene and Hunt, 1990), which in turn affects the mineral (Mayland and Lesperance, 1977 cited by Holechek et al., 1982a) and nitrogen content of saliva (Lesperance, Clanton, Nelson and Theurer, 1974; Cohen, 1979), by digestibility or DM content of herbage and by animal species (Armstrong et al., 1989).

Recent studies were reported by Burritt, Malechek and Provenza, (1987); Pinchak, et al (1990); and Olson (1991). In all three studies extrusa samples were freeze dried (see also section 1.2.3 on sample preparation). Burritt et al (1987) concluded that oesophageal extrusa are not suitable for monitoring dietary tannin levels. According to Pinchak et al., (1990), the effects of mastication, salivation, and leaching associated with ingestion of forage precludes quantitative recovery from oesophageal extrusa of dietary ash, Na, P, K, and copper (Cu). Conversely, dietary Ca, N, and probably magnesium (Mg) can be reasonably well estimated from extrusa. Extrusa zinc (Zn) concentrations would appear estimatable for simple forage diets but not from more complex diets because of differential changes related to species and maturity. Olson (1991) found that OM and hemi-cellulose were significantly lower, and acid detergent lignin (ADL) significantly higher, in the oesophageal fistula samples of steers fed native grass hay; N, NDF, ADF, and cellulose were not affected by the oesophageal fistula technique.

In the case where certain plant components react with each other or with the saliva during the process of mastication (Burritt et al., 1987), the relevance of this will largely depend on the purpose for which the oesophageal fistula samples are collected, e.g. to determine the chemical composition of the pasture as against the quality of the food taken in by the animal. These reactions are part of the normal feeding process and should therefore not be regarded as an inaccuracy in determining the quality of the food eaten by the grazing animal, although the opposite would be true if the objective were to evaluate the pasture.

Heady and Torell (1959 cited by McManus, 1980), Grimes et al., (1965), Hall and Hamilton (1975), McInnis et al (1983) have used microscope point techniques to establish botanical composition of extrusa as representative of the diet consumed. Botanical composition of oesophageal fistula samples from stall fed animals is basically the same as that of the material eaten (McManus, 1961).

Contamination with rumen contents: Oesophageal fistula samples contaminated by rumen contents cannot be used for botanical analyses (Holechek et al., 1982a & b). McManus (1961) found that some sheep are more prone than others to contaminate their extrusa samples by regurgitation. According to Price (1963 cited by Van Dyne and Torell, 1964), only five out of 90 oesophageal fistula samples collected from five sheep were contaminated by rumen contents. Results from a study with non-fasted sheep on pelleted diets (Basson, 1971) indicated that contamination of oesophageal fistula samples may take place in as many as 19 % of the samples, notwithstanding the short (15 min.) collection periods employed.

Bath et al (1956) reported that collection periods of longer than 30 minutes increased the chance of samples being contaminated by regurgitated rumen contents. However, Holechek et al (1982b) stated that this problem is primarily related to time since previous eating and can be prevented if animals are starved for a few hours prior to sampling.

1.2.3. Sample preparation:

In order to prevent "non-enzymatic browning" (Van Dyne and Torell, 1964) of extrusa prior to the drying process, samples are often squeezed and/or rinsed.

Squeezing of freshly collected samples: Variable results on the effect of squeezing on certain chemical components have been reported; McManus (1961) found that draining saliva from the extrusa sample did not result in leaching of N from succulent or dry plant material but reduced ash content. Hoehne, Clanton and Streeter (1967) found that squeezed samples have a lower mineral content than non-squeezed samples, but were similar in crude protein (CP) and water soluble carbohydrates (WSC). Barth, Chandler, Fryer and Wang (1970) found no effect of draining grass extrusa samples for 3 to 4 hour on IVOMD or DM, but recorded significant change in legume extrusa. Cohen (1979) found it necessary to apply correction factors for N and sulphur (S) but not for Ca or digestibility.

Rinsing: Cundy and Rice (1968), cited by Holechek et al (1982a), found that rinsing oesophageal fistula samples with tap water significantly decreased DM, N, ash, and IVOMD compared to the forage fed, while cellulose, ADF and lignin were significantly increased.

Quick freezing: Scales et al (1974) stated that little additional accuracy is obtained by freezing lucerne extrusa immediately following collection, compared to samples frozen one hour post collection.

Adaptation period prior to sampling. Because oesophageal fistulated animals are not easy to prepare and require frequent care and attention, it is common to use a group of fistulated animals on a roving basis to sample pastures as required (Coates et al., 1987), often without sufficient previous exposure to the pasture. Langlands (1967) stated that grazing behaviour of sheep adapted to a certain type of grazing is different from that of new arrivals. Animals should have prior experience of the pastures being sampled (Arnold et al., 1964; McManus 1980). Coates et al (1987) concluded that "whenever the application of diet selection work with oesophageal fistulated animals relies on the assumption that the extrusa samples reflect the integrated diet of resident experimental animals, this technique should be treated with great caution unless there is good evidence to support the assumption". Conversely, Hodgson (1969) found no significant changes in either N content or OM digestibility of successive samples of extrusa collected after sheep were introduced to a sward to which they were not accustomed, though ash content of the extrusa was lower when sheep were hungry than when they were not.

1.2.5. Effect of physiological condition, breed, sex, and age.

Fistulated animals seldom represent the larger population of non-fistulated animals in terms of sex, age, livemass and physiological condition. Several important characteristics of an animal's physiological status affect not only the total nutrient requirements, but also the proportions of different nutrients required. Because pregnancy, lactation and growth result in an increased demand for nutrients and thus for food, it is logical to expect that feeding behaviour and forage selection may also be affected. Furthermore, young animals on rangelands are at a disadvantage relative to older animals (other than lactating females) because they not only have higher nutritional requirements per unit body mass, but they also lack foraging experience (Provenza and Malechek, 1986). In addition, young browsers have potentially less browse to select from than mature animals due to the difference in "reach".

Physiological condition: Diets selected by dry and pregnant ewes were found not to be different, but dry matter intake (DMI) (Engels and Malan, 1979) and IVOMD of the diet (Arnold and Dudzinski, 1967) of lactating ewes were significantly higher. Significant differences in organic matter intake (OMI) between single- and twin- rearing ewes were also recorded (Foot and Russel, 1979; Peart, Doney and Smith, 1979; Vulich, O'Riordan and Hanrahan, 1991). However, lactation was not found to change the pattern of diet selection (McManus, Arnold and Ball, 1968), nor did lactation affect digestibility of the diet selected (Cook, Mattox and Harris, 1961). McManus et al (1968) concluded that dry fistulated ewes could be used to obtain extrusa samples for assessing N and WSC contents, but not IVOMD, for ewes in other physiological states.

Age: In a comparative study of lactating cows, weaned calves (age 10 months) and milk-fed calves (age 7 months) strip grazing a ryegrass pasture, no significant differences were found between any group of animals in IVOMD intake, but there was a significant tendency for the cows to be slightly less selective than either group of calves as the sward was grazed down (Le Du and Baker, 1981). Provenza and Malechek (1986) found no difference between goat ewes and kids in the leaf/stem ratios, CP and IVOMD of diets consumed on heavily stocked range. However, in the same study on lightly stocked range, ewes selected a diet different from kids in species composition, lower in IVOMD, but similar in CP and leaf/stem ratios.

Breed and sex: Although differences in grazing time, rate of eating and DM intake were found between breeds (Arnold and Dudzinski, 1967; Engels and Malan, 1979), nitrogen selection was not found to be affected by either breed or sex (Langlands 1967).

From the above it is apparent that dry fistulated females and possibly castrates could be used to collect forage samples representing pregnant females of the same and related breeds. However, in the case of lactating animals, multiple suckled ewes, young growing animals and non-related breeds, fistulated animals of similar physiological status should be used.

From the literature reviewed the need for further research is evident on the following aspects:

(1) methods to improve the oesophageal fistula sampling procedure to more closely reflect (a) the total daily feeding period, which would seem particularly important in mixed (browse/graze) feeders and (b) the diet of resident animals,

(2) procedures which avoid the need to fast animals prior to sample collection, and

(3) interpretation of the chemical analysis data derived from fistula samples.

CHAPTER 2

MATERIALS AND METHODS

The primary objective of this study was to develop a remote control system for the collection of multiple oesophageal fistula samples from goats. In part one of this chapter the design and construction of equipment is described. In part two the methods used to evaluate both the equipment and technique are described. In part three the presentation of the results and statistical analyses of data are discussed.

PART ONE

2.1. DESIGN AND CONSTRUCTION OF EQUIPMENT.

In order to collect multiple ingesta samples without disturbing the animal's normal feeding behaviour, the apparatus must meet certain requirements:

(1) a valve which will allow the oesophageal fistula to be opened and closed without disturbing the animal. At the same time it must ensure maximum extrusa recovery when opened and unrestricted passage of ingesta when closed;

(2) a power source and motor to operate the valve;

(3) a control mechanism, either pre-programmed or remote controlled, to activate the valve motor; and

(4) a comfortable and stable harness to attach the equipment to the body of the animal.

The preliminary phase in the development of the remote controlled oesophageal fistula valve started in 1986, with the objective of establishing a fixed or permanent oesophageal cannula in goats (Figure 2). At the same time a remote controlled "plunger" (Figure 3) was developed to function as a valve during sampling. After about two years and several unsuccessful attempts to maintain goats fitted with permanent cannulae, this idea was rejected in favour of a removable cannula (Figure 4). The reason for this was that necrosis of the tissue surrounding the permanent cannulae occurred irrespective of whether the material used was chrome-plated or teflon-coated bronze.

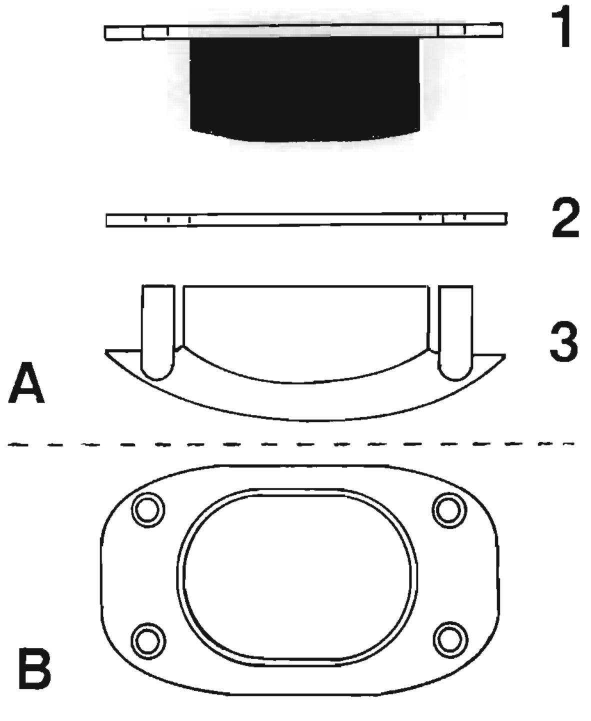


Figure 2 Fixed or permanent oesophageal cannula: (A) exploded side view, (B) plan view; (1) top plate with rubber plug, (2) cover plate, (3) cannula. Construction material: brass, chrome plated or teflon coated.

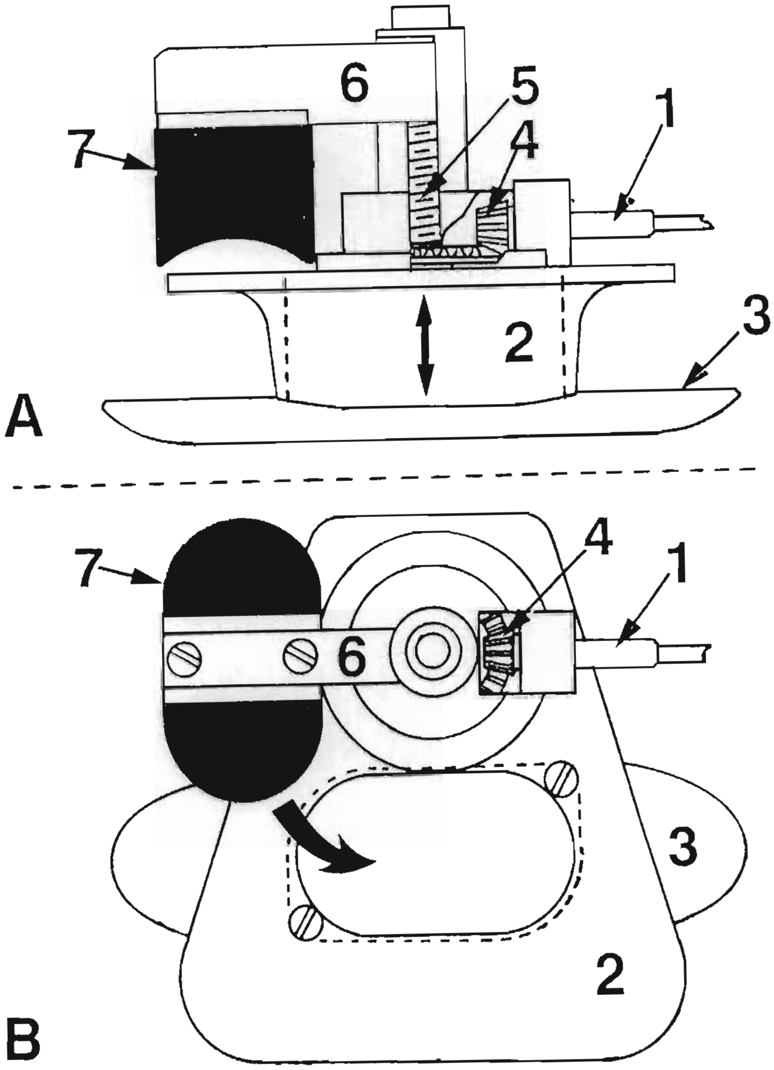


Figure 3 Remote controlled plunger: (A) side view, (B) plan view; (1) drive shaft with flexible extension to reversible motor, (2) cannula and baseplate, (3) sleeve, (4) bevelled reduction gears, (5) feed-screw operating plunger, (6) plunger arm, (7) plunger. Construction material: poly oxymethylene.

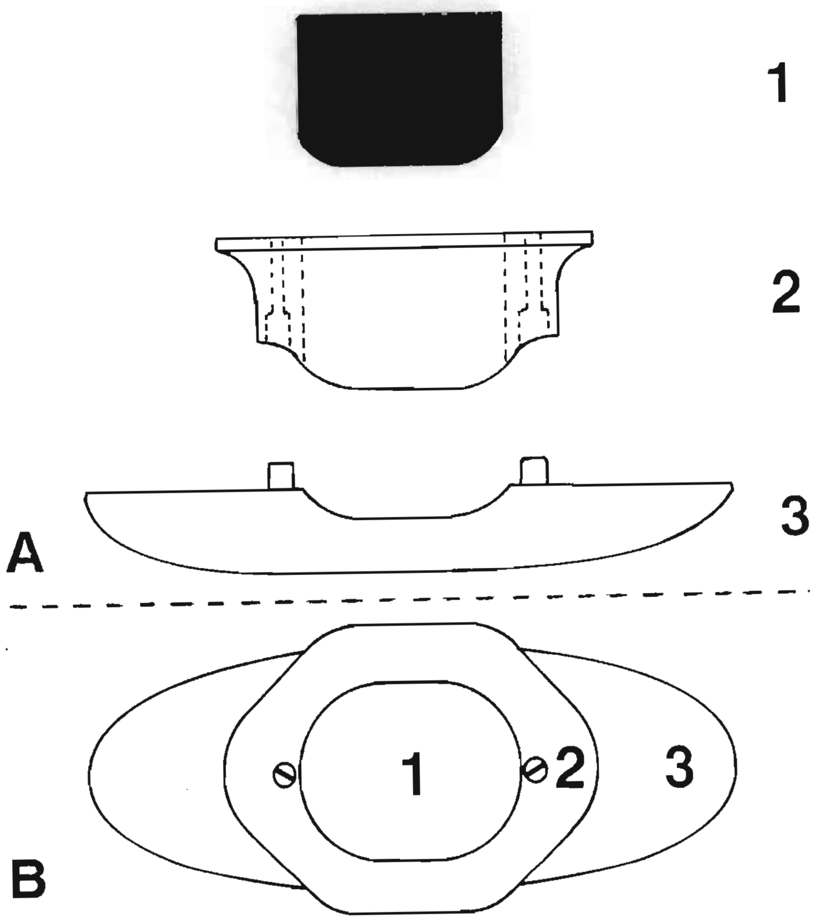


Figure 4 Removable cannula: (A) exploded side view, (B) plan view; (1) rubber plug, (2) cannula, (3) sleeve. Construction material: poly oxymethylene.

With minor modifications the prototype "plunger" that was developed for the fixed cannula (Figure 2) could also be used with the new removable cannula. The test results were, however, disappointing, as the majority of sampling attempts resulted in blockages of the cannula. In an attempt to overcome this problem, the fistula and hence the cannula were progressively enlarged during a number of trials, resulting in an oval shaped fistula with an aperture size of ± 1 050 mm². Although the frequency of blockages was reduced, the enlargement of the fistula brought yet another problem; it was impossible to maintain the test goats for periods in excess of three months after fistulation. Furthermore, the rigid lining of the cannula restricted the flow of ingesta and was demonstrated to be one of the major causes of blockage during sampling.

Alternative designs, including a partially closed fistula cannula for continuous sampling (Figure 5), and a cannula which in the open position restricts the oesophagus caudal to the fistula in order to assist extrusa flow during sampling (Figure 6), were also evaluated and tested, with variable but low success rates.

Finally, an effective fistula closing device as well as a workable prototype remote controlled fistula valve were developed. Both these devices could be considered as major contributors to the successful maintenance of goats with large fistulae and the collection of multiple ingesta samples in this study.

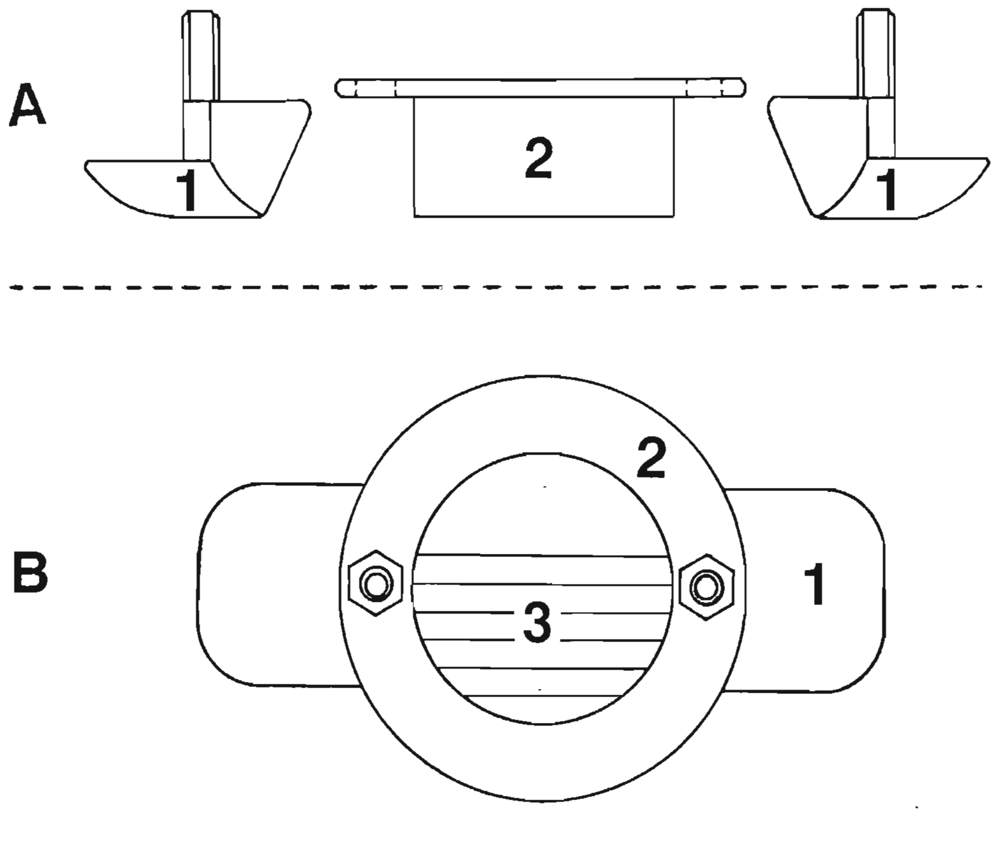


Figure 5 Partially closed fistula cannula: (A) side view, (B) plan view; (1) projecting wings, (2) cannula, (3) partial closure. Construction material: brass, chrome plated.

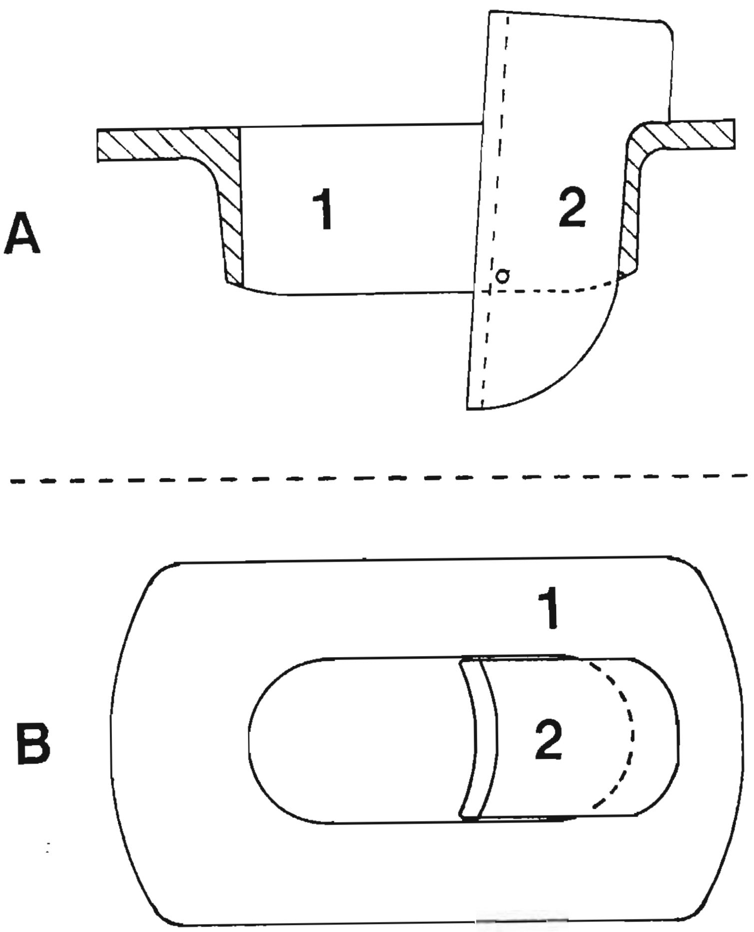


Figure 6 Prototype cannula restricting oesophagus when in open position: (A) side view, (B) plan view; (1) cannula, (2) revolving plug. Construction material: poly oxymethylene.

Following extensive field tests, the design of the prototype fistula valve was further improved in terms of balance, torque, and ease of extrusa flow. This improved fistula valve was, however, tested only during the final stage of this study. Because of this, both the prototype and improved fistula valve designs are described below and referred to as V1 and V2 respectively.

2.1.1. Oesophageal fistula closing device.

The closing device (Figure 7) for a large oesophageal fistula consists of a sleeve and plug. The construction of the sleeve portion was based on the method described for cattle by Karn (1987), with adaptations for use in goats. In the present study a sleeve, 97 mm long and 20 mm wide, was made from a 50 mm PVC pressure pipe with a wall thickness of 2.2 mm. An oval-shaped butyl rubber seal, 105 mm long, 30 to 40 mm wide, 1 to 1.5 mm thick and with a 4 mm central hole, was glued on the convex surface of the sleeve. The seal was cut lengthwise from butyl rubber bicycle inner tube and Cyanoacrylate glue (Superfast IS 495, Loctite) was used as a bonding agent on roughened surfaces. In order to obtain an effective seal, the two convex surfaces of both the PVC sleeve and rubber seal were glued together. This allowed the sides of the seal to curl backwards and press against the inner surface of the oesophagus when fitted.

A hollow plug, 50 mm long, 23 mm wide at the base and with rounded ends of 11.5 mm radius, was machined from poly oxymethylene (Ertacetal C, Erta). The sides, 25 mm high, tapered outward at 10°. Poly urethane casts (15 g) with a shore hardness of 67 D were made commercially in an open casting process.

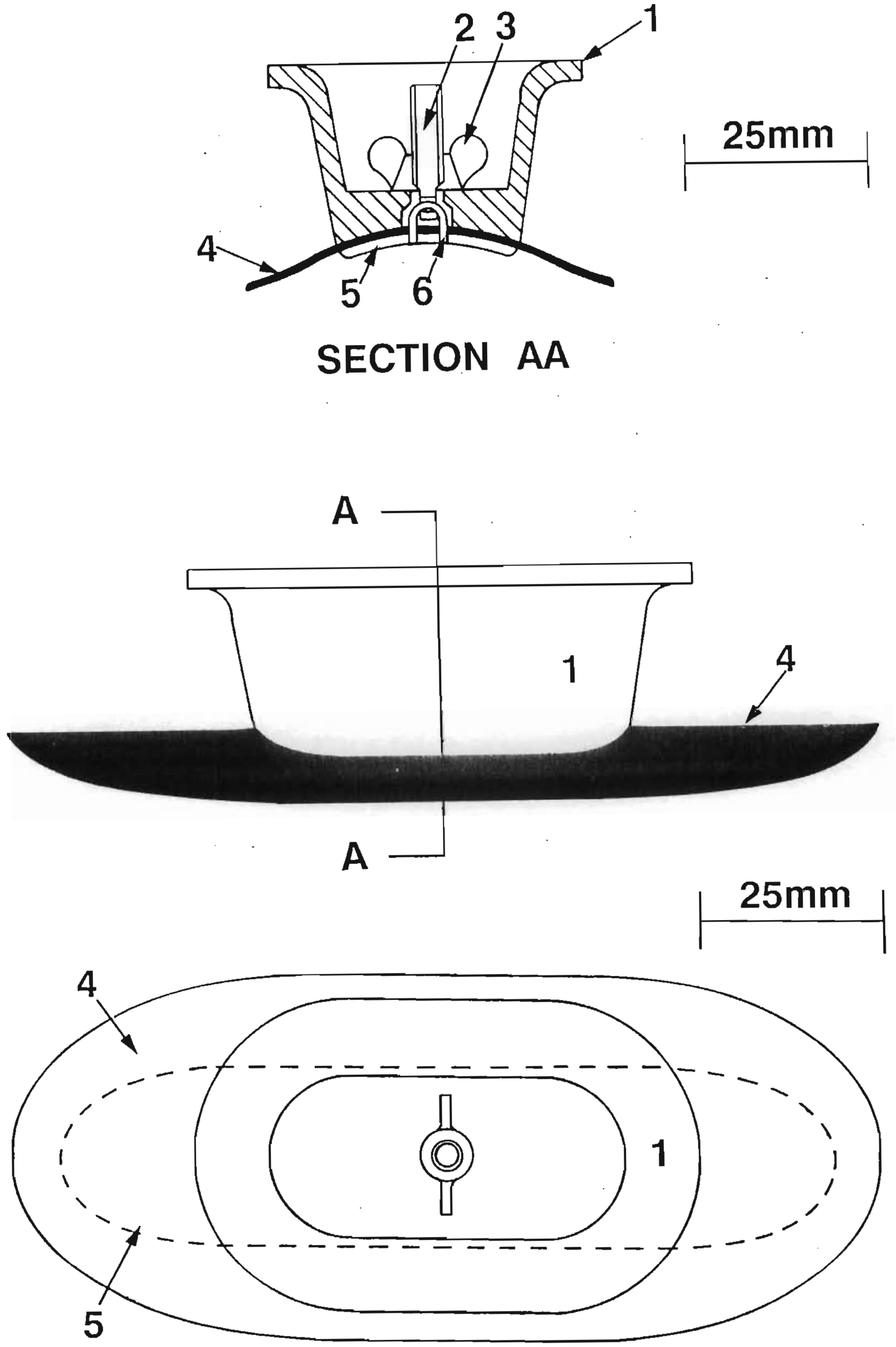


Figure 7 Closing device for a large oesophageal fistula: (1) plug, (2) stainless steel screw, (3) wingnut, (4) oval-shaped rubber seal, (5) sleeve, (6) stainless steel wire hinge.

The flange on the end of the plug facing the animal's head was extended to prevent twisting of the sleeve inside the oesophagus. A flexible PVC strip, 70 mm long, 15 mm wide and 1 to 1.5 mm thick, anchored to the stud and inserted through a slot at one end of the plug, served as an extension to the flange.

2.1.2. Oesophageal fistula valve.

The assembled sampling device consists of a removable cannula, valve, and reversible motor with a combined mass of 84 g (Figure 8). The cannula which fits an elongated oesophageal fistula serves as a support frame for both the valve mechanism and the motor. The cannula consists of a frame which acts as an outer flange above the skin. It is attached by means of two struts to a pair of projecting wings that fit into the oesophagus. The struts situated at either end of the elongated fistula act as a sleeve, preventing fistula contraction during sample collections. The absence of cannula sidewalls and the large frame aperture {1 400 mm^2(V1); 1 900 mm^2(V2)} ensures minimal restriction of extrusa flow. The projecting wings have an oval shaped rubber flange attached to them, acting as a seal between the cannula and the oesophagus.

The whole cannula splits crosswise into two sections which are aligned and held together by pins. The engagement of these pins is all that is required to secure the cannula in position after the two projecting wings have been fitted in the oesophagus. In the prototype design, two removable stainless steel pins (2.4 mm diam), parallel to each other, on either side of the cannula are used, while in the modified fistula valve one of these pins is replaced by a fixed pair of locating pins in order to assemble the two halves.

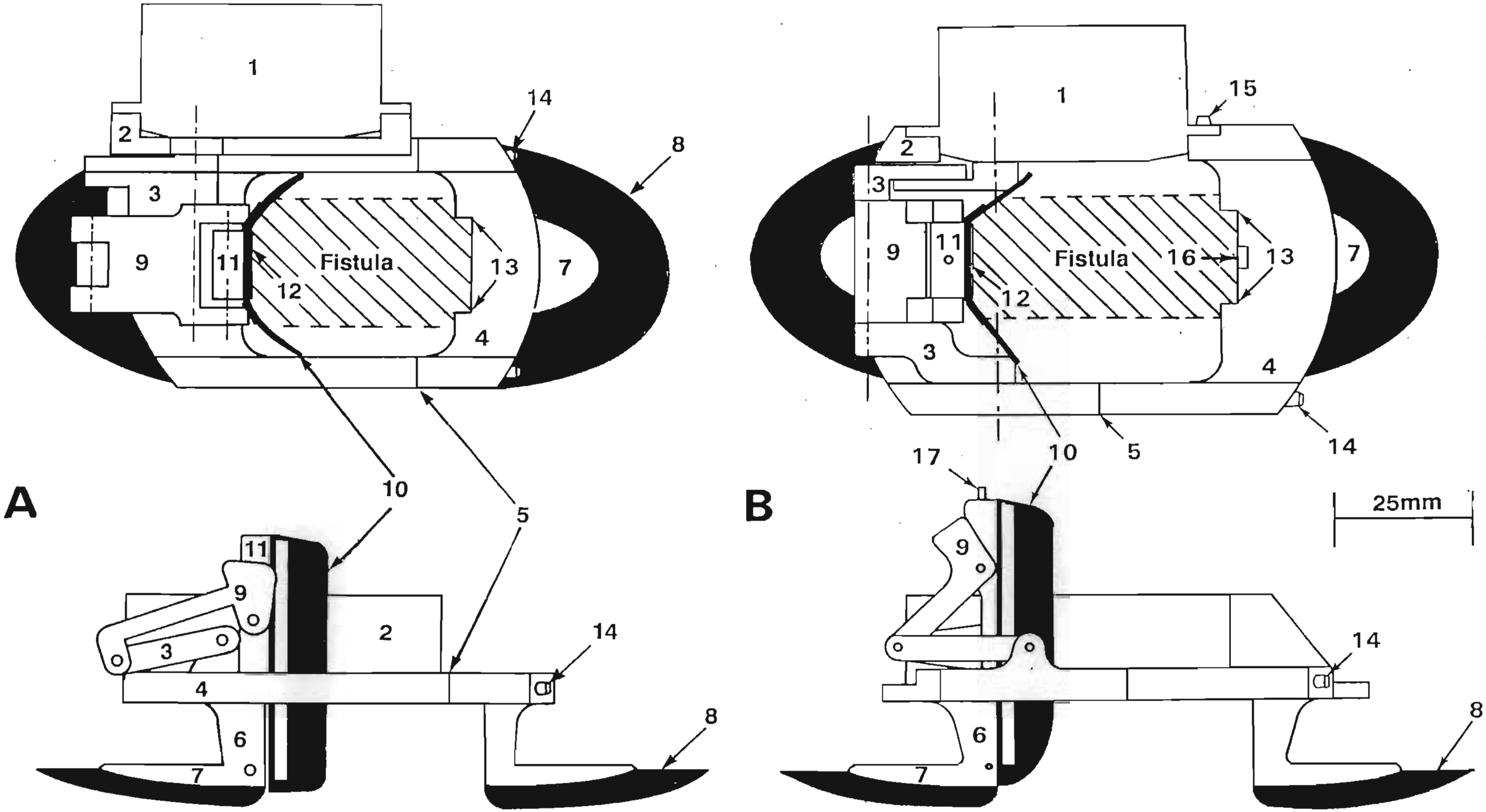


Figure 8 Fistula valve in open position (plan view): (A) prototype [V1] and (B) modified [V2] design, (1) servo motor, (2) motor mounting, (3) drive coupling, (4) outer cannula frame, (5) point of split, (6) strut, (7) projecting wing, (8) oval rubber seal, (9) mechanical linkage with cam, (10) collapsible valve seal with phosphor-bronze strips, (11) valve base, (12) cover plate, (13) groove to fold valve seal, (14) aligning pin, (15) locating pin, (16) stop pin guide, (17) stop pin.

The fistula valve consists of a base {45x14x4 mm(V1); 50x14x4 mm (V2)} on which a collapsible rubber seal is attached. The valve pivots about the inner projection of one of the wings. A cover-plate secures the rubber seal to the valve base. In the closed position, the valve seal lines the inner surface of the oesophagus surrounding the fistula.

In opening the valve, one end of the valve is raised through 90° to its fully open position (Figure 9). During the initial opening of the valve, the sides of the collapsible valve seal are folded in and remain folded until it almost reaches the fully open position, at which stage it unfolds again to allow an unobstructed passage for the extrusa. The initial folding action is facilitated by means of a groove (17 mm wide and 4 mm deep) on the strut opposite the hinge which forces the sides of the rubber seal to collapse as it opens. After about one third of the travelling distance of the valve, the folding action is transferred to a pair of cams, which maintain the collapsed shape until it is released at the fully open position of the valve. Two strips of phosphor-bronze glued onto the rubber on either side of the valve base act as skids for the cam and ensure that the rubber seal folds down its full length.

A retract servo (Futaba FP-S36G) mounted on the outer cannula frame operates the valve through a mechanical linkage. The design of the linkage mechanism is such that a full rotation of the servo motor ($\pm$ 180°) results in a 90° rotation of the valve. In the modified design, the servo has been moved closer to the centre of the cannula (Figure 8). This modification brought about a more balanced design as well as an increase of 35 % (3.91 Newton) in the normal force applied to the valve base at the point of closure. A mechanical linkage calculation was performed to obtain the latter information.

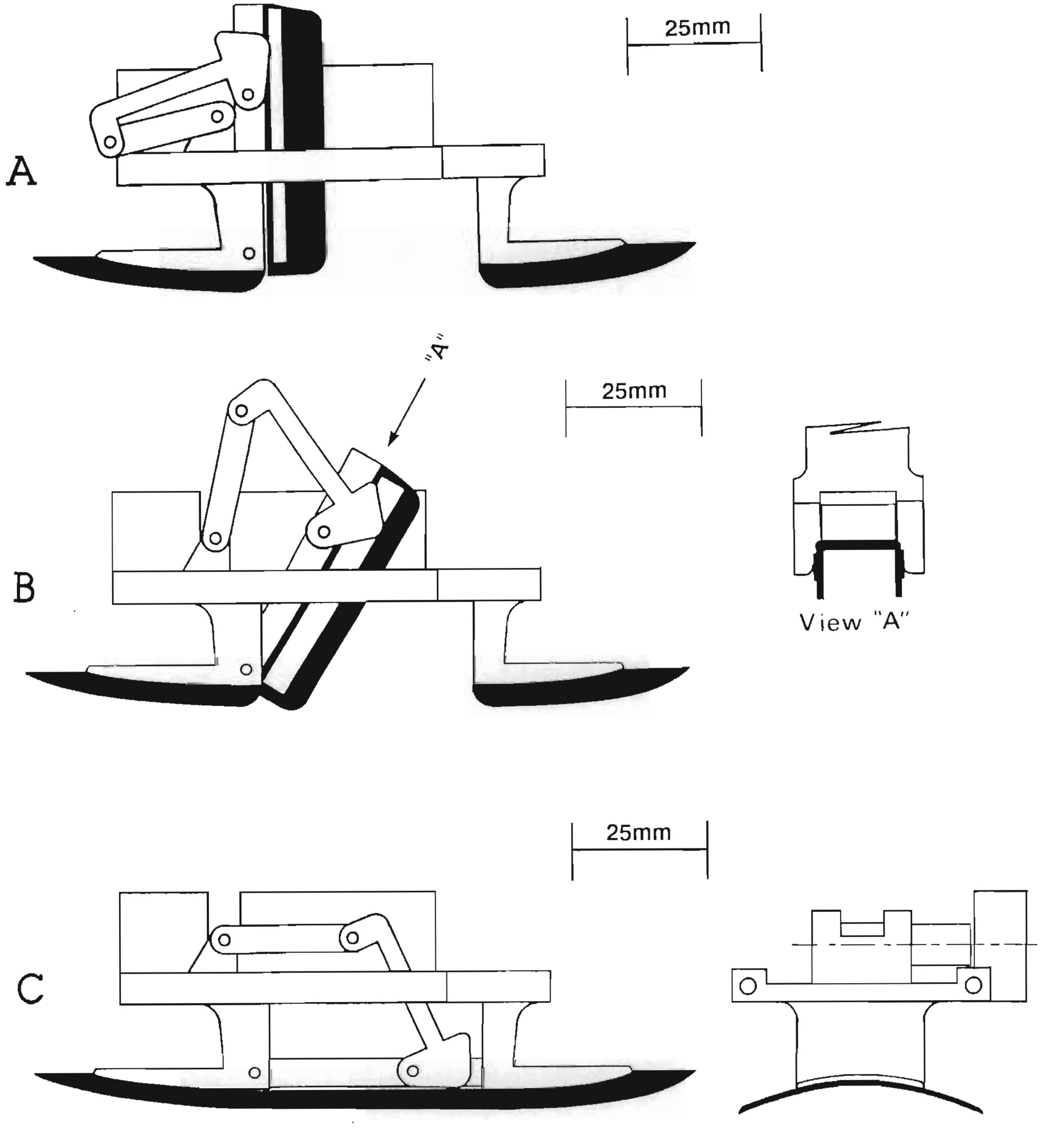


Figure 9 Stepwise illustration of the valve (V1) mechanism: (A) fistula valve in open position, (B) fistula valve partly closed, showing the cam holding the valve seal in folded position, (C) fistula valve in closed position with side view.

The various components of the cannula, valve base and mechanical linkage were machined from poly oxymethylene (Ertacetal C, ERTA). The valve seal was made from natural rubber which provides the high degree of elasticity necessary for the proper functioning of the valve. Butyl rubber (bicycle inner tube) was used for the oval canula seal. Cyanoacrylate (Loctite, Superfast IS 495) was used on roughened surfaces to attach the rubber seals to the various components.

2.1.3. Power source and radio equipment.

The servo is powered via the receiver by a rechargeable battery pack. The receiver and battery pack are mounted separately on a harness strapped to the animal (Figure 10). Initially, a 6 Volt battery pack consisting of five NiCd 1.2 Ah cells was used as the power source for both the receiver and servo. As one of the measures taken to increase the torque of the retract servo, the potential difference of the battery pack was increased to 7.2 Volts by adding one extra 1.2 Ah cell.

A portable model aircraft radio (5 channel multiplex plus) provided direct control of the fistula valve. With slight modifications on the control mechanisms, this system allows for the simultaneous monitoring of five animals (one animal per channel) provided additional receivers are used. Notwithstanding this potential, the maximum number of goats monitored simultaneously during any test period did not exceed three. Following the completion of this study, a six channel portable model aircraft radio (Futaba) was successfully modified and tested for the simultaneous control of six retract servos.

2.1.4. Harness.

A leather harness with an aluminium support frame (Figure 10) was constructed in order to attach the radio receiver and battery pack onto the animal during sampling. The harness consists of a girth, saddle and breast plate. The saddle extends cranially from the girth and over the withers as far as one quarter up the neck. The V-shaped frame of the saddle fits over the dorsal ligament of the neck, preventing the harness from slipping. The saddle is in turn anchored to the breast plate by means of straps running cranial to the shoulder points. Two aluminium boxes used as protection for both the receiver and battery pack are mounted onto straps running diagonally between the saddle and girth on either side of the harness.

The saddle support frame was constructed from three strips of flat aluminium (25 x 3 mm) which were shaped and welded together. The boxes containing the electronic equipment were formed from aluminium sheet 1 mm thick. The girth, breast plate and saddle were made from harness leather (± 4 mm thick), while 3 mm thick leather was used for the straps. Electrical wires, including the receiver antenna, are protected by strips of thin (1 mm) leather glued to the harness.

2.1.5. Surgical procedure.

Following a number of unsuccessful attempts to maintain goats with large oesophageal fistulae, nine female Boer goats (50 to 75 kg BW) were fistulated over a period of 18 months according to the procedure described by Pfister et al (1990), modified as follows.

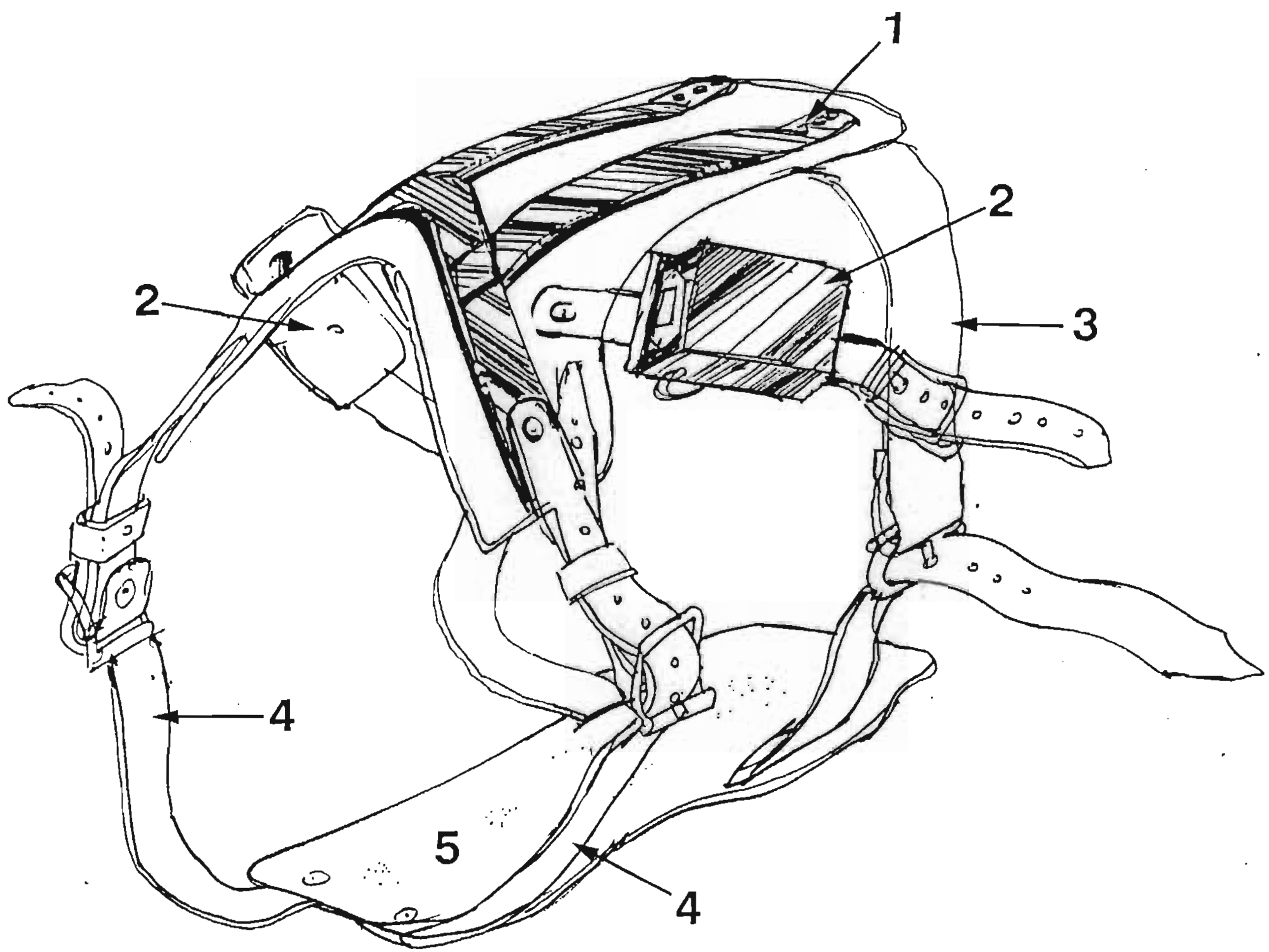


Figure 10 Leather harness: (1) aluminum support frame and saddle, (2) protective boxes for receiver and batteries, (3) girth, (4) anchor straps, (5) breast plate.

Prior to anaesthetic induction, animals were pre-medicated with 0.5 ml propionyl promazine (Combelen, Bayer) plus 1.0 ml atropine sulphate (10 mg/ml Centaur) to sedate the animal and to reduce saliva secretion and retro-peristalsis of the oesophagus. Anaesthesia was induced and maintained at slightly higher dosage of Xylazine HCl (Rompun 2%, Bayer) at 1.0 ml i.m./50 kg BW. Local anaesthetic (Xylocaine, Lignocaine base 100 mg and Cetylpyridinium chloride 0.1 mg/ml Adcock Ingram Lab.) was applied topically to the surgical site, before the incision was made.

To create the fistula, a 70 mm long incision was made on five goats, and a 20 mm long incision on four goats. The oesophageal wall was sutured to the skin using continuous blanket sutures of braided silk followed by insertion of the closing device without any further sutures. The respective fistula aperture sizes were approximately 1 100 mm^2 and 500 mm^2.

Subsequently, the smaller fistulae were passively stretched to an aperture size of approximately 1 100 mm^2 in order to accommodate the remote controlled fistula valve. The fistulae were stretched by a set of two or three intermediate sized plugs, over a period of 2 to 3 weeks. This was possible because the skin is seldom tight round the plug, allowing the insertion of a 5 to 10 mm longer plug every 3 to 7 days without actively stretching the fistula. Removable plugs, as described by Schutte, Wilke and Compaan (1971), were used to maintain the 20 mm fistulated goats, prior to stretching.

PART TWO

2.2. EVALUATION OF EQUIPMENT AND TECHNIQUE.

The major part of this study was undertaken in August 1991, on the University of Fort Hare research farm near Alice, South Africa (32°49'S, 26°51'E) at an altitude of 600 m. The vegetation is representative of Acocks' (1975) "False Thornveld of the Eastern Cape" and ranges from grassland through Acacia karroo savanna to a dense, clumped, medium short, evergreen thicket.

Over a period of eight months, tests were conducted on the reliability of the equipment and also on the ability of the fistula valve technique to reflect on the total daily feeding period. Observed feeding behaviour (grazing/browsing) as well as grass / bush ratio of fistula valve and fistula bag samples formed the basis for the evaluation of this technique. Extrusa recovery rates, measured under controlled conditions, were also used in the evaluation of this sampling method.

2.2.1. Extrusa recovery.

Three randomly selected goats from each of the 70 mm and 20 mm long incised groups were used to determine extrusa recovery rates. Eight weekly extrusa collections were made from these goats. Because certain samples were refused, a total of only 18 and 20 extrusa collections were made from the animals with large and small fistulae, respectively.

Subsequently, three of these animals were also used in a further 18 extrusa recovery tests, with the fistula valve in place. In all of the above tests, the fistulated goats were kraaled overnight and extrusa recovery rates determined the following morning (± 08:00h) without prior feeding. In a further 17 tests with the fistula valve, goats were allowed to browse for about two hours prior to determining the percentage extrusa recovered using the following procedure.

Unmilled lucerne hay samples, with an average mass of 76 g, were offered to the animals and extrusa recovered on canvas, without a fistula bag. Extrusa samples were oven-dried to determine dry matter recovery rates.

2.2.2. Experimental animals.

Of the nine goats fistulated at the beginning of this study, four were used in the evaluation of the fistula valve technique. Two goats, randomly selected from each of the 20 mm and 70 mm incised groups, were used for the equipment evaluation, behavioural studies, and comparisons between the fistula valve- and standard fistula-bag techniques (Table 2). In addition to these four animals, one additional fistulated ewe and two castrates (for faeces collections) were used to make up a flock of seven goats.

Table 2 Grouping of fistulated goats according to studies performed and number and type of samples collected.

Experiment	Fistula size and method of sample collection		Goats (n)	Samples (n)
Extrusa recovery	500 mm^2	without fistula bag	3	20
Extrusa recovery	1050 mm^2	without fistula bag	3	18
Extrusa recovery	1050 mm^2	valve (V1 & V2)	3	35
Evaluation studies	500 mm^2	with fistula bag	2	87
Evaluation studies	1050 mm^2	with fistula bag	2	93
Evaluation studies	1050 mm^2	valve (V1 & V2)	3	1027

2.2.3. Vegetation.

One paddock, classified as False Thornveld of the Eastern Cape (Acocks, 1975), was used as grazing/browsing for the seven experimental goats. Six hundred points and three 50 x 2 m transects were used to determine the botanical composition, as shown in Appendix Table 5. This area was subdivided into eight plots, each approximately one hectare in size. One plot per month was browsed for a period of approximately two weeks, during which time the fistula valve technique was evaluated. The period of occupation per plot varied between 10 and 17 browsing days. Goats were kept in a specific plot until an increase in grazing activity was observed, or until a maximum period of 17 browsing days was reached. A seven strand electric fence was used to confine the experimental goats to a specific plot during each of the two-week browsing periods.

2.2.4. Management of animals and collection of data.

During the two-week evaluation periods, goats had daily access to the experimental plots only, but were kraaled at night and supplied with a commercial lick (block). Similar vegetation in adjacent paddocks was used as browsing for these animals during the interim periods.

On Mondays, Wednesdays, and Fridays, all seven goats were slowly walked to the experimental plots at about 07:00h. On arrival, the four fistulated goats were prepared for standard fistula bag collections, which was followed by a 20 minute sample collection period. Following the removal of the fistula bags, normal fistula plugs were reinserted into two goats, while the other two were equipped with remote controlled fistula valves, after which they were allowed back onto the grazing. Fistula valve samples were collected during the rest of the day (until 15:00h) from both goats at 30 minute intervals, provided that the animals were actively feeding. The samples were allowed to drop on the ground, where they were collected. A sample collection bag was omitted to allow an unobstructed view of the valve.

The feeding behaviour of the four fistulated goats was observed and recorded at two minute intervals for the entire duration of the sampling period. Behaviour was recorded in the following categories: browsing, grazing and non-feeding activities. Browsing activity included bi-pedal stance (recorded separately) and represents the browsing of all woody species, grazing activity represents grazing of both grasses and forbs, while non-feeding activities include separate recordings of walking, standing and lying.

The fistulated goats were trained with great care, to the extent that they would allow the operators to approach and handle them in the open without resistance or signs of stress. This proved invaluable during sample collection if animals had to be restrained in order to remove blockages or to reassemble disconnected electrical wires.

2.2.5. Sample preparation.

All extrusa samples were stored in a deep-freeze (-20°C): fistula bag samples were sub-sampled and deep frozen within one hour after collection, while the fistula valve samples, after being recovered from where they dropped on the ground and parts contaminated with soil or foreign plant material removed by hand, were kept on ice (until ± 16:00h) prior to storage. Each sample was freeze dried and hand separated into bush (woody species) and grass (including forbs) components, after which each component was weighed separately.

PART THREE

2.3. PRESENTATION OF RESULTS AND STATISTICAL ANALYSES OF DATA.

During this study a total of 33 000 observations were recorded on the feeding behaviour of four goats. One thousand and twenty seven fistula valve samples and 180 fistula bag samples were collected. Of these, 537 fistula valve and 72 fistula bag samples were sub-divided into their bush and grass components by hand. A further 73 samples were collected in order to determine extrusa recovery rates. Because of the difficulties involved in direct measurement of the diet of free ranging ruminants, the following indirect parameters were used to evaluate the oesophageal fistula valve technique:

1) **Extrusa recovery rate**: The proportion of ingested plant material (DM) recovered in the extrusa provides a useful measure of the accuracy by which the diet is estimated during the collection period. One-way analyses of variance were used to compare DM recovery rates (%) obtained from the 20 mm and 70 mm long incised fistulae, as well as from the two fistula valve models, V1 and V2. In the analyses, treatments were the different oesophageal fistula sampling techniques; replication was provided by the three goats and day of sampling.

2) **Fistula valve vs. fistula bag:** The difference in either grass or bush content of extrusa samples obtained from the fistula valve and the standard fistula bag techniques provides a meaningful comparison between these sampling methods. In this study, the comparison is based on the difference in the grass content between 35 fistula bag samples and 35 fistula valve samples (daily average), obtained from the same goat on the same day. Because fistula valve samples differ in mass, the grass / bush ratios of each individual fistula valve sample were weighted according to mass of sample after which it was pooled within day for each goat. The reason for comparing only 35 samples from each of the two sampling techniques is mainly due to the spoilage of fistula bag samples with rumen contents. Differences between sampling methods were tested using the T-test. Data obtained from two goats during four of the sampling periods (August, January, February and June) were used in the statistical analyses.

3) **Observed grazing frequency vs. grass content of extrusa samples:** The assumption was made that strong positive relations exist between the observed frequency of grazing and the amount of grass in the extrusa samples from the same goat on the same day. Grass content of fistula valve samples was determined in the same way as described in (2) above. Simple regression procedure was used to quantify these relations in respect of grass content of both the fistula bag and fistula valve samples. Seventy fistula bag samples from four goats and 50 fistula valve samples from two goats, collected during four sampling periods (the same as in 2 above), were used in the regression models.

4) **Sampling success rate of the oesophageal fistula valve:** The reliability of the oesophageal fistula valve is expressed in terms of a) the percentage of sampling attempts that failed due to blockage of the valve and b) equipment failure. Test results from 1 027 fistula valve samples, collected from three goats during eight sampling periods (August, September, October, November, January, February, May and June), are presented.

In view of the very few experimental animals used in this study, data presented on feeding behaviour should be seen only as an indirect measure to validate the oesophageal fistula valve technique. No description of the normal feeding behaviour of goats is implied.

CHAPTER 3

RESULTS AND DISCUSSION

Following the development of the oesophageal fistula valve and the closing device for large fistulae, the secondary objectives were to evaluate this technique in terms of its ability to accurately reflect the total daily food intake and to obtain extrusa samples under a wide range of grazing / browsing activities. The results are presented and discussed in two parts, firstly evaluating the equipment and then the fistula valve technique.

PART ONE

3.1. EVALUATION OF EQUIPMENT

3.1.1. Surgical technique and closing device.

During field tests of the remote controlled fistula valve, extrusa recovery from animals with the stretched fistulae (20 mm incision) was observed to be superior to that of the 70 mm incision fistulae. This could be ascribed to excessive inactivation of oesophageal muscles resulting from the latter surgical procedure. It may be noted that some of the oesophageal fistulated goats fitted with closing devices (as described in section 2.1) have kept in good condition on rangeland for a period in excess of 24 months.

Many closing devices were lost at first, apparently when the goats rubbed against the wire netting of the paddock fence, thereby detaching the closing device from the fistula. This problem was resolved by grinding off both end sections of the plug flange. Repetitive twisting of the sleeve inside the oesophagus, resulting in complete blockages, occurred in one goat. These blockages were prevented by the extension to the plug flange as described in section 2.1.

The rubber seal has been effective in reducing saliva and rumen content losses to a minimum. No erosion of the oesophagus lining has occurred in any of the fistulated animals, thereby solving one serious defect of existing oesophageal fistulae.

3.1.2. Sampling success rate.

A total of 1 027 extrusa samples were collected with the fistula valve from three goats. During sampling, the fistula valve was opened for an average period of three minutes at 30 min. intervals. The commonest problems experienced during the sampling period were:

1. blockage of the valve;
2. disconnection of the electrical wires between the radio receiver and battery pack or servo motor;
3. dislodgement of the valve;
4. uncontrolled movement of the servo motor; and
5. damage to the servo motor.

The total number of samples collected, as well as problems experienced with each of the fistulated goats, are listed in Table 3. On average 9.9 extrusa samples were collected per animal per day. The samples were collected over a period of 54 days, totalling 105 animal sampling days.

One hundred (9.7%) of the 1 027 sampling attempts failed due to blockages which were characterized by bolus compaction during the process of extrusa sampling. The occurrence of blockages varied between animals by approximately five percentage points. In the majority of these cases, the animal had to be restrained and the blockage removed by hand. Blockage induced an increase in salivation which was observed as leakage from the open valve.

Table 3. Sampling success rate of the oesophageal fistula valve (V1 and V2).

Goat		**E1***	**E3***	**E5***	**Total**
Total number of samples		531	437	59	1 027
Sampling days		54	45	6	105
Average number of samples per day		10.0	9.7	9.8	9.9
Blockages during sampling**	(n)	40	56	4	100
	(%)***	7.5	12.8	6.8	9.7
Equipment failure:					
Electrical disconnection	(n)	38	52	4	94
	(%)***	7.2	11.9	6.8	9.2
Valve dislodged	(n)	6	22	0	28
	(%)***	1.1	5.0	0	2.7
Battery/antenna problems	(n)	7	9	0	16
	(%)***	1.3	2.1	0	1.6
Total number of failures	(n)	51	83	4	138
	(%)***	9.6	19.0	6.8	13.4
Mean frequency of equipment failure per sampling day.		0.9	1.8	0.7	1.3
Samples collected manually**	(n)	38	62	0	100
	(%)***	7.2	14.2	0	9.7

* E1, E3, E5 : Goats Ethel 1, Ethel 3 and Ethel 5.
** Samples were salvaged manually for further tests.
*** Percentage of total number of samples.

The frequency of blockages increased with time of day up to 14:00h, as illustrated in Figure 11. A similar tendency and possible explanation for this phenomenon was noted during the extrusa recovery tests, to be discussed in section 3.2.

In addition to the blockage rate of nearly one in ten samplings, the average incidence of equipment failures was 1.3 per animal per sampling day, with highest frequency (1.8/day) recorded for goat E3. Based on observations, the marked difference in frequency of equipment failure between the two goats could in part be explained by difference in behaviour. When entangled in the undergrowth, goat E3 would barge its way through, with the result that either the plug was disconnected or the electric wire broken. Under similar conditions, goat E1 would normally retreat and release itself.

Disconnection of the electrical wire between the radio receiver and battery pack or servo motor occurred 94 times. The majority of these occurrences were due to disconnection of the in-line plugs, the reassembly of which restored full function to the valve. In a limited number of cases, however, either the electrical wire, connector plug, or both was damaged or broken, and/or the fistula valve was dislodged. In such cases, samples (100) were manually collected (these manual samples are in addition to hand collected samples from blockages).

Discharged radio batteries or detachment of the receiver antenna were identified as the main causes of the 16 recorded cases of uncontrolled movement (hunt) of the servo motor.

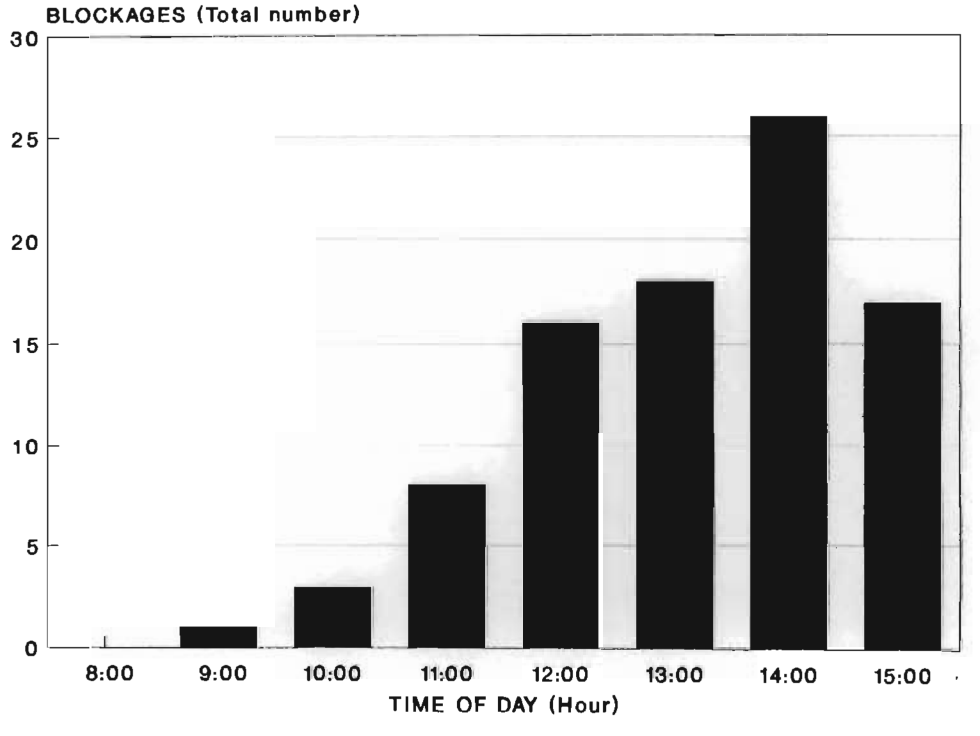


Figure 11 Influence of time of day on frequency of blockage formation.

When this occurred, the valve would oscillate back and forth, on occasion resulting in boluses being trapped by the valve in the oesophagus and consequent dislodgement of the valve. This happened between samplings while the valve was in a closed position. The average transmitting period of a standard radio battery pack was between four and six hours when fully charged.

During the total sampling period, three servo motors were damaged due to incomplete closure of the valve, which arrests the motor during the running phase. This caused overheating, with consequent damage to the electronic components. The reason for the incomplete closure is not clear, but obstruction of the valve opening or weak radio signals could have resulted in this phenomenon. Subsequently, all three servo motors were repaired by a local agent.

Notwithstanding the large number of fistula valve samples collected during this study, the exact reasons why blockages formed are not clear. In this study, the success of sample collection was found to be related to the timing of the opening of the valve and feeding activity i.e. grazing or browsing. Blockages are minimised when opening of the valve is carefully timed to coincide with an empty oesophagus, which normally occurs after the goat has lifted its head and walked a few paces forward. Close observation and operator skills are therefore important factors in success or failure of the technique.

A higher incidence of blockages were recorded during grazing, while equipment failure mainly occurred during periods dominated by browsing activity. The former could be due to the more compact bolus formation during grazing and the latter, to the increased possibility of equipment and/or electrical wires being entangled in bush during peak periods of browse intake. The following conditions were found to induce blockage formation: presence of long grass stems in the extrusa which create large boluses; opening the fistula valve while the animal's head is in a downward position, or during the process of swallowing. Throughout the evaluation, the fistulated goats appeared to behave normally, without any signs of discomfort, whether the fistula valve was open or closed.

Because fistula valve samples were allowed to drop on the ground from where they were recovered, some degree of contamination and possibly loss of samples would be expected. Although this effect was not measured, it did not prove to be a problem in practice. The dense grass cover in the experimental plots and the removal of contaminated parts from the recovered boluses resulted in only a few samples being rejected during this study.

PART TWO

3.2. EVALUATION OF THE FISTULA VALVE TECHNIQUE.

3.2.1. Extrusa recovery rate.

The proportion of DM recovered from the oesophageal fistula as affected by fistula aperture size, presence of the fistula valve, and time of day when samples were collected is shown in Table 3.

At the 08:00h sampling, fistulated goats on average ingested 68 % of the lucerne DM offered. Of this, 49.6 % was recovered in the extrusa from animals with small fistulae compared to 86.9 % from those with large fistulae. The DM recovered from the prototype (V1) and modified (V2) fistula valves was 68.6 % and 86.6 % respectively. In all the above extrusa recovery tests (56), animals were kraaled overnight and extrusa recovery rates determined the following morning (± 08:00h) without prior feeding.

In a further 17 extrusa recovery tests with the fistula valve, goats were allowed to browse for about two hours prior to determining extrusa recovery rate. This procedure resulted in no recovery from the prototype (V1) fistula valve as a result of blockages in all eight extrusa recovery tests. The boluses in these tests were demonstratively dryer than those collected from the same animals following overnight fasting. This observed reduction in saliva content of boluses during the mid-morning recovery tests provides a feasible explanation for the positive relationship between blockage incidence and time of day observed during field sampling (Figure 11). Drier boluses during the mid-morning recovery test notwithstanding, the average DM recovery using the modified fistula valve (V2) was still 62.6 %, with only one blockage recorded.

Table 4. Extrusa recovery (DM %) from different sized fistulae, fistula valves, and time of day.

Time	n	Fistula size and valve	Amount recovered (%)	SD
08:00h	20	Small fistula (500 mm²)	49.6[a] *	24.6
08:00h	18	Large fistula (1050 mm²)	86.9[c]	3.8
08:00h	9	Large fistula + valve (V1)	68.6[b]	21.5
08:00h	9	Large fistula + valve (V2)	86.6[c]	7.5
11:00h	8	Large fistula + valve (V1)	0.0	
11:00h	9	Large fistula + valve (V2)	62.6[ab]	22.2

* Means followed by the same letter do not differ ($P > .05$)

From the standard deviations (SD) presented in Table 4, it is evident that the large fistula, with or without the modified valve (V2), enables consistently high extrusa recovery. The feeding behaviour of the fistulated goats when offered lucerne hay was noticeably different from their behaviour when browsing natural vegetation. The lucerne hay samples were ingested more aggressively than the browse and less time was spend chewing. Nevertheless, the high extrusa recovery obtained from both large fistulae and the fistula valve (V2) ensures that extrusa samples collected under natural conditions could be used to accurately represent the animal's diet during the sampling period.

The mean extrusa recovery obtained in this study is generally higher than the 35 to 73 % reported for small ruminants, as reviewed by McManus (1980) and Holechek et al (1982a) and shown in Table 1. It should be noted, however, that the small samples, as well as the omission of fistula bags, may have enhanced the extrusa recovery rates in the present study.

3.2.2. Comparison of methods.

The average percentage of grass in 35 fistula valve samples (pooled for each goat within each day) was 31.0 % (SD 28.3) as compared to 18.9 % (SD 34.5) for fistula bag samples obtained from the same animals on the same sampling days. The difference of 12 percentage points in grass content between the two techniques is not significant ($P > .05$). This is probably due to the high standard deviations, which in this case are an indication of the large variation in feeding behaviour between days and sampling periods, as illustrated in Figures 12 and 13.

The average daily proportion of grass in the fistula valve and fistula bag samples during four sampling periods (summer and winter) are shown in Figures 12 and 13 respectively. Differences in grass proportion between the sampling methods varied substantially within and between sampling periods. The proportion of grass in fistula valve samples is

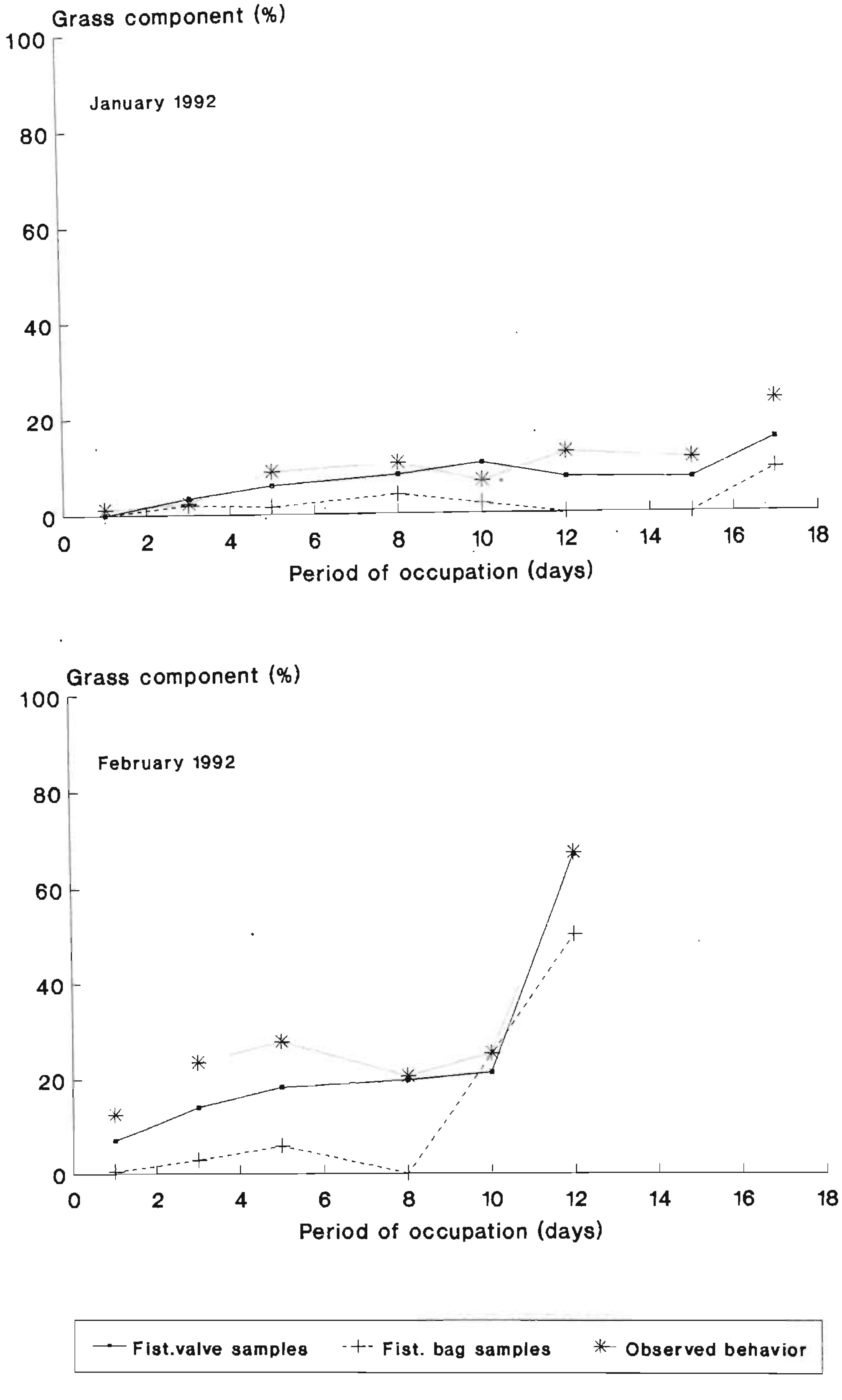
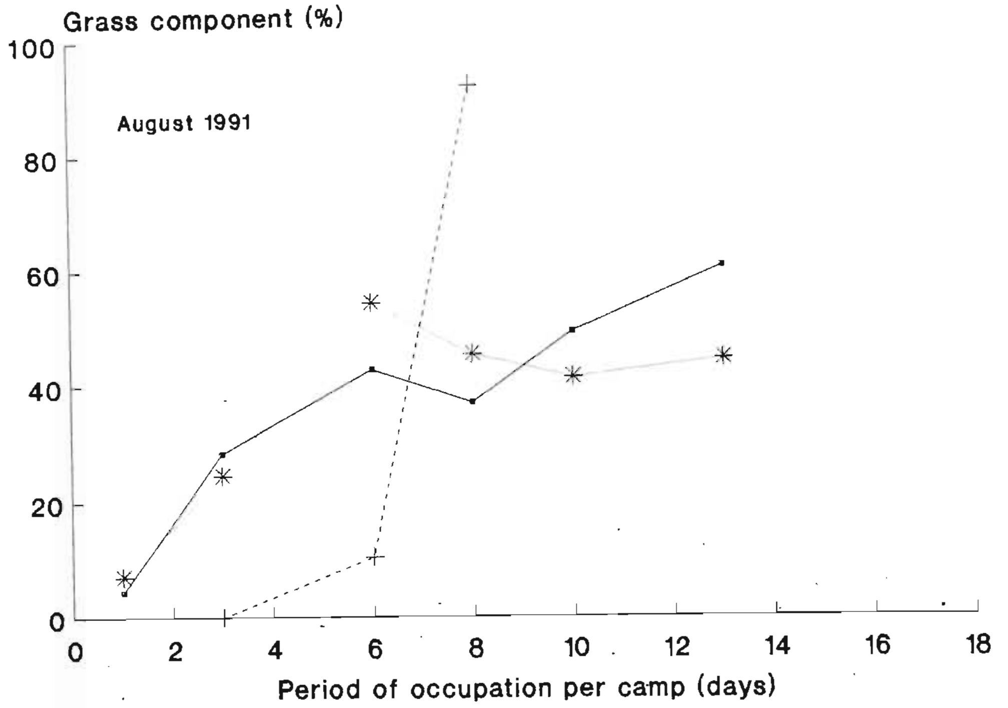


Figure 12 Observed frequency of grazing and average grass content of fistula bag and fistula valve samples during summer.



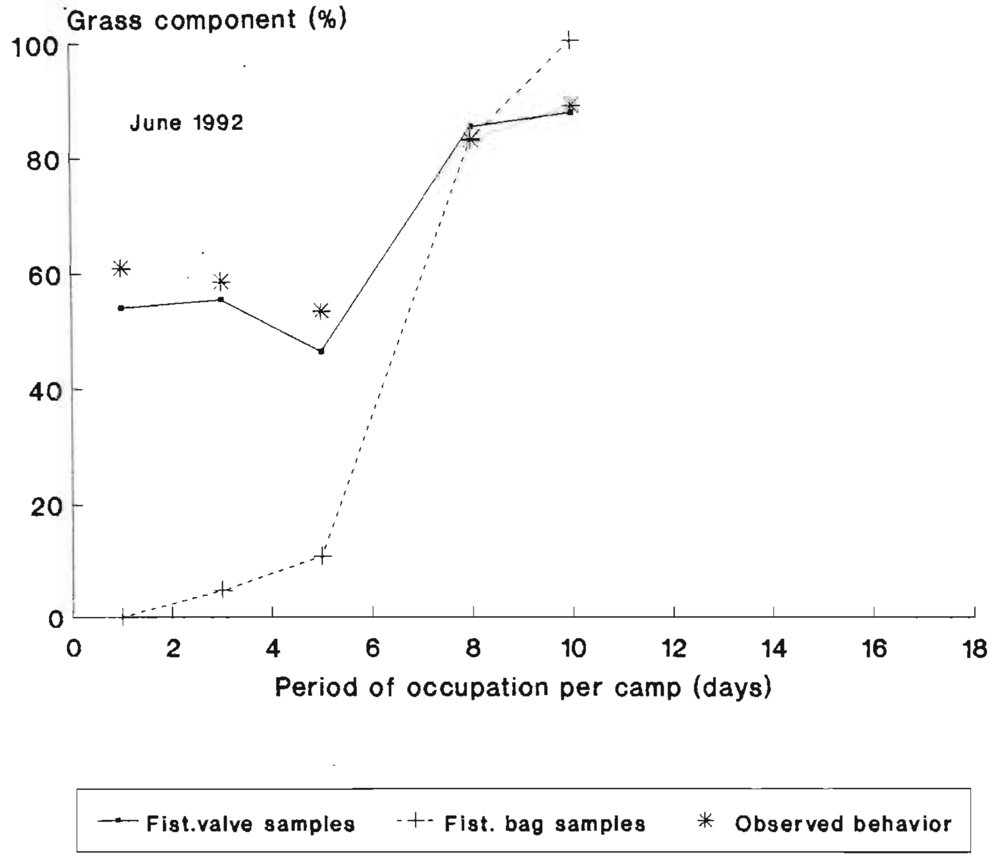


Figure 13 Observed frequency of grazing and average grass content of fistula bag and fistula valve samples during winter.

very similar to the predicted proportions based on observed grazing frequencies. Furthermore, during high frequencies of observed grazing (August and June), large differences between the fistula valve and fistula bag methods occurred. Conversely, during high frequencies of observed browsing (January), the fistula bag technique provides dietary information closely related to the fistula valve samples.

Field observations indicate that the explanation for the tendency for the grass component in the fistula bag samples to be relatively small during the first four to six days of occupation of the plots lies in the observed diurnal variation in feeding behaviour of the goats. During the first few days in a new plot, goats exclusively browsed during the early morning feeding session, when the fistula bag samples were collected. Later in the day, especially during the afternoon, the goats spent more time on grazing. This behavioural pattern is reflected in the fistula valve samples.

The relation between the observed frequency of grazing and the grass content of both the fistula bag and fistula valve samples are shown in Figures 14 and 15 respectively. From these figures it is clear that of the two sampling methods, the fistula valve technique provides a more realistic estimate (R^2=91) of the observed feeding behaviour over a wide range of grazing / browsing ratios. Data in Figure 14 also suggests that observed grazing frequencies of up to 50 % are not likely to be reflected in fistula bag samples. On the other hand, at high frequencies of observed grazing, the data suggests that the fistula bag technique may over-estimate the grazing activity of goats. This observation supports the

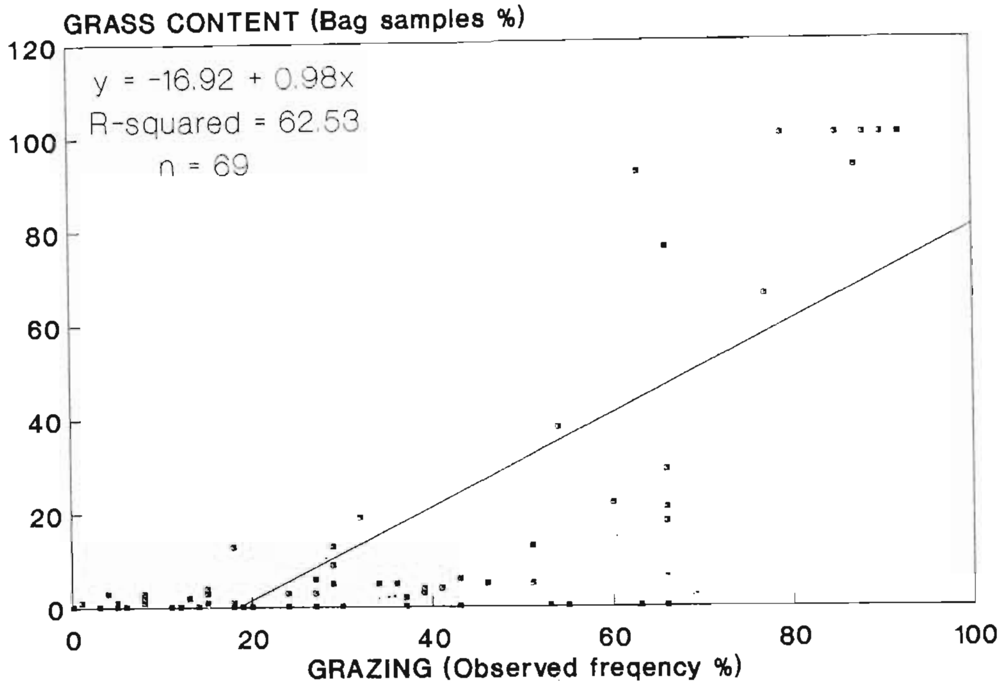


Figure 14 Relation between observed frequency of grazing and grass content of <u>fistula bag</u> samples.

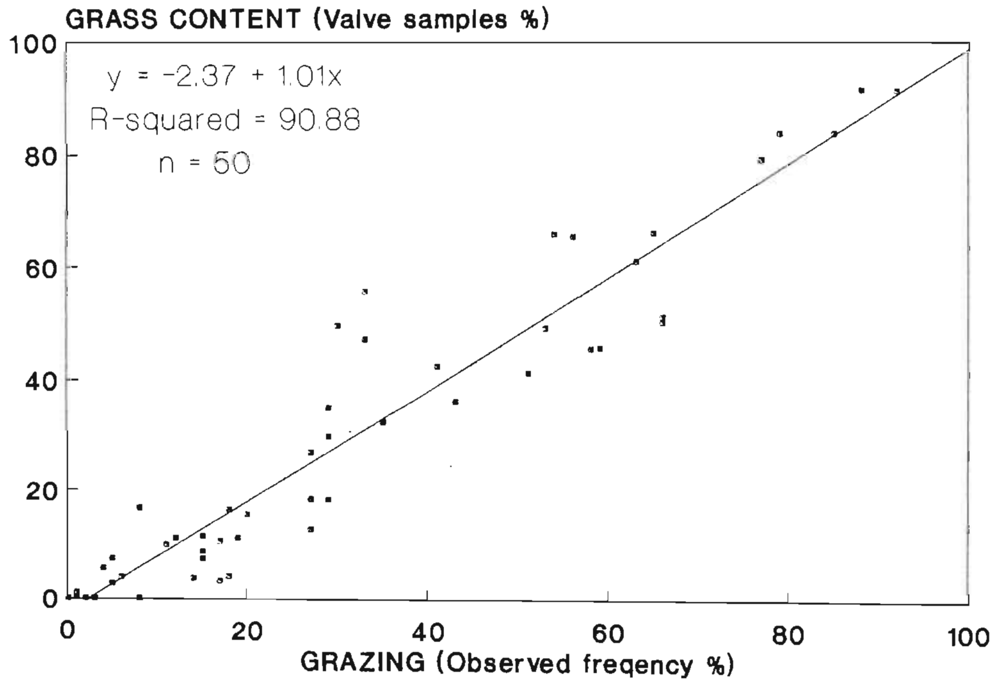


Figure 15 Relation between observed frequency of grazing and grass content of <u>fistula valve</u> samples.

view of Teague (1989), using a pasture discmeter and the oesophageal fistula technique to measure the consumption of bush and grass by Boer goats in an Acacia karroo community. From this study it was concluded that oesophageal fistula samples over-emphasize the amount of grass in the diet.

CHAPTER 4

CONCLUSIONS

The versatility of existing methods for sampling herbage intake by ruminants has been enhanced by the development of the oesophageal fistula valve technique in the following ways:

1) the collection of different numbers and sizes of samples throughout the day is possible without disturbing the animals' normal feeding behaviour;
2) the collection of forage samples as selected by goats in extensive areas having heterogeneous plant populations is possible;
3) sampling from specific plants or plant communities is facilitated;
4) the need to starve animals overnight in order to reduce the possibility of extrusa samples being contaminated with rumen contents is obviated.

The non-surgical enlargement (passive stretching) of the standard oesophageal fistula (500 mm^2) combined with the successful maintenance of these animals over extended periods, enables one to use the fistula valve technique using only a relatively small number of animals. Furthermore, the large fistula on its own may improve the effectiveness of the fistula bag technique as a result of higher extrusa recovery rates.

Although 10 % of the sampling attempts failed due to blockage of the valve, the few animals on which this technique was tested and the failure to identify the actual reasons for blockages made it difficult to predict the reliability and performance of the fistula valve. However, from the consistently high extrusa recovery rates obtained with the modified fistula valve (V2), a reduction in blockages can be expected (the majority of sample collections reported in these trials were collected with the prototype valve (V1)). Moreover, since most of the samples were collected strictly on a fixed time interval (30 min.) during the active feeding periods, careful timing of the opening of the valve may reduce the incidence of blockages even further.

The relatively high incidence of equipment failure (1.3 incidences/animal/sampling day out of an average of 9.9 samples per day) stresses the need to further refine the equipment. Suitable protection of the electrical wires, for example, could reduce the frequency of equipment failure from 1.3 to 0.4 incidences per animal per sampling day. Pre-selection of experimental animals having a placid temperament may also reduce equipment failures. Damage to equipment and blockage of the valve, however, still necessitates the handling of experimental animals in the field. Training of fistulated animals and gentle care are required in order to handle them without affecting their normal behaviour.

As mentioned earlier, one of the major advantages of the fistula valve technique is its suitability for sampling extensive areas of heterogenous plant communities. Because of the small sampling areas (± 1ha plots) used in the present study, this advantage was nullified in the comparison between the fistula valve and fistula bag techniques. Nevertheless, substantial differences were found in the grass / bush ratio of extrusa samples obtained from these methods. The excellent relationship obtained between the observed grazing frequency and the grass content of fistula valve samples confirms the suitability of this technique to obtain forage samples from browsers.

This study has succeeded in providing an alternative method for sampling herbage intake by browsers which may contribute towards an understanding of the complex processes involved in the feeding patterns of free ranging ruminants. However, the fistula valve technique requires further development and research in order to improve the sampling success rate, reduce the total sampling period per day, and adapt the equipment for use with other animal species.

CHAPTER 5

SUMMARY

PART ONE

The primary objective of this study was to develop a remote control system for the collection of multiple oesophageal fistula samples in goats.

The equipment developed and tested in this study consists of an oesophageal fistula valve which allows the fistula to be opened and closed, a rechargeable battery pack and motor to operate the valve, a portable radio and receiver to activate the valve motor, and a harness to attach the equipment to the body of the animal. In addition, a closing device was developed to effectively seal the large oesophageal fistulae (>1 050 mm^2), which in turn is required to accommodate the valve.

Results from this study indicate that the most effective method for creating large fistulae in goats is to use the standard fistulation procedure for creating a 500 mm^2 fistula, which after complete healing is followed by passive stretching to an aperture size of 1 050 mm^2.

Large fistulae are prone to severe leakage. The closing device developed in this study consists of an elongated poly urethane plug, PVC sleeve and butyl rubber seal. The rubber seal, which lines the inner surface of the oesophagus surrounding the fistula, effectively limits saliva and rumen content losses, with no obvious erosion of the oesophagus lining.

The oesophageal fistula valve consists of a removable cannula, valve, and reversible motor with a combined mass of 84 g. The cannula splits into two sections, which are aligned by pins. The engagement of these pins effectively secures the cannula in position after it has been fitted in the fistula. In the closed position, a rubber seal lines the inner surface of the oesophagus surrounding the fistula, which prevents saliva loss during sampling intervals. Most components of the fistula valve were machined from poly oxymethylene.

A retract servo, powered by a rechargeable battery pack, operates the valve through a mechanical linkage. A multi-channelled portable model aircraft radio and receiver provides remote control of the fistula valve. The radio receiver and battery pack are attached to a leather harness, strapped to the animal.

PART TWO

Following the development of the remote control oesophageal fistula sampling technique, the remaining objectives were to evaluate its reliability in terms of sampling success rate and also its ability to accurately reflect the total daily feeding pattern.

Observed feeding behaviour (grazing/browsing) as well as grass / bush ratio of fistula valve and standard fistula bag samples of four goats, formed the basis for the evaluation of this technique. In addition, extrusa recovery rates, measured under controlled conditions, were used in the evaluation of this sampling method.

The remote control sampling technique developed in this study enables the collection of multiple oesophageal fistula samples without disturbing the animal's normal feeding behaviour. Number and size of samples can be varied and collected throughout the day.

During extensive field tests with the prototype fistula valve, 10 % of all sampling attempts failed due to blockage of the valve, and an average of 1.3 incidences of equipment failure were recorded per animal per sampling day, from an average of 9.9 extrusa collections per day. Equipment failures, ranked according to importance, include: disconnection of electrical wires, valve dislodgement, and battery and/or antenna problems.

The exact reasons why blockages formed is not clear. Generally, a higher incidence of blockages were recorded during grazing, while equipment failure mainly occurred during periods dominated by browsing activity.

Differences in extrusa composition between the fistula valve and fistula bag techniques varied substantially during the browsing period within a camp and also between camps. Furthermore, during high frequencies of observed grazing, there were large differences between the fistula valve and fistula bag methods.

During this study, the fistula valve technique provided a more realistic estimate (R^2=.91) of the observed feeding behaviour of goats than the fistula bag technique (R^2=.63).

Under controlled conditions, the large oesophageal fistula, with or without the valve, enables high and consistent extrusa recovery rates (87 % recovery; SD 7.5).

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Appendix

Appendix Table 1. **Advances in the surgical establishment of the oesophageal fistula (OF).**

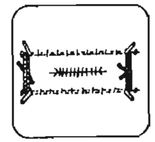
Year	Species, anaesthesia and surgical technique.
1954	**Sheep.** First usable fistula for ruminant nutritional studies. Use of stomach tube to locate oesophagus during surgery. Sutures to fuse oesophagus to skin (Torell, 1954).
1958	**Sheep.** Pentobarbital sodium, blunt dissection (Cook et al., 1958).
1961	**Dairy cattle.** Equithesin (Rusoff and Foote, 1961).
1962	**Calves.** Kemithal (Chapman and Hamilton, 1962)
1962	**Sheep.** Two stage operation; Insursion of cannula between muscular coat and mucous membrane of oesophagus; Deoxycortone acetate as scarifying drug injected into tissue surrounding cannula to reduce leakage (McManus, 1962a).
1964	**Cattle.** Surgery, local anaesthetic (Procaine hydrochloride) only, animal in standing position (Yarns, Whitmore, Norcross and Crandall, 1964).
1964	**Sheep.** Chlorpromazine. Two stage operation: Fixing oesophagus to skin followed by insertion of fistula plug 12 to 15 days later (Hofmeyr and Voss, 1964).
1970	**Cattle.** Skin incision 70 mm. Oesophageal incision 20 mm (Little and Takken, 1970)

Appendix Table 1. Advances: surgery - oesophageal fistula (OF) - continued.

Year	Species, anaesthesia and surgical technique.
1970	**Sheep.** Initial incision, 10 mm long, simultaneously through skin and oesophagus (Bishop and Froseth, 1970)
1971	**Sheep.** Highly polished brass ball on steel rod to locate oesophagus during surgery (Schutte et al., 1971).
1971	**Cattle.** Chloral hydrate (Osbourn and Bredon, 1971).
1972	**Deer.** Pentobarbitone sodium (Veteto, Davis, Hart and Robinson, 1972).
1974	**Goat.** Pentobarbitone sodium, small doses to sedate animal plus local anaesthetic (Aucamp and Nell, 1974).
1977	**Wildebeest/Topi.** Fistulation. Fistula closed successfully, surgery (Usenik, Kreulen and Duncan, 1977 cited by Visser et al., 1990)
1984	**Cattle/sheep/goats.** Xylazine (Ellis et al., 1984).
1985	**Sheep.** Surgical anaesthesia maintained with an oxygen halothane mixture. Gentling of animals prior to surgery; Corrective surgery on enlarged fistulae (Stevens, Thomson and O'Connor, 1985).
1990	**Sheep/goats.** No sutures to fuse oesophagus to skin (Pfister et al., 1990).
1990	**Impala.** Pentobarbitone sodium (Visser et al., 1990).

Appendix Table 2. **Advances in the development of the oesophageal fistula closing device.**

Year	Species, material, design, minimum area of plug/cannula.
1954	**Sheep.** Bandages and stockinette (Torell, 1954).
1954	**Sheep.** Stainless steel pins in embedded polyethylene tubing, one on each side of fistula, and held together by nylon cord (Torell, 1954).[a]
1954	**Sheep.** Two piece plug (two plastic plates drawn together by nylon cord (Torell, 1954).[b]
1958	**Sheep.** Lucite plastic cannula, 500 mm^2 (Cook et al., 1958).[c]
1959	Acrylic plastic cannula (Van Dyne and Van Horn, 1959 cited by Van Dyne and Torell, 1964).[c]
1959	**Cattle.** Two piece plug (two metal plates drawn together by nylon cord) held in position by neck strap (Lesperance et al., 1959 cited by Van Dyne and Torell, 1964).[b]
1960	**Cattle.** Vulcathene cannula, 2 000 mm^2 (Lesperance, Bohman and Marble, 1960).[c]
1961	**Dairy cattle.** Stainless steel cannula, 700 mm^2 (Rusoff and Foote, 1961).
1961	**Cattle.** Removable sleeve and plug (Torell and Bredon, 1961 cited by Van Dyne and Torell, 1964).[d]
1962	Sleeve 'off center' (Van Dyne, 1962 cited by Van Dyne and Torell, 1964).[d]
1962	**Sheep.** Latex, one-piece moulded plug, 200 mm^2 (McManus, 1962a).[e]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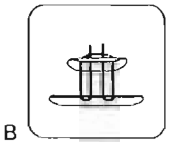
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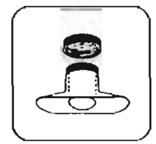
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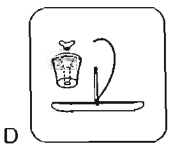
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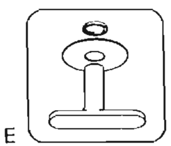
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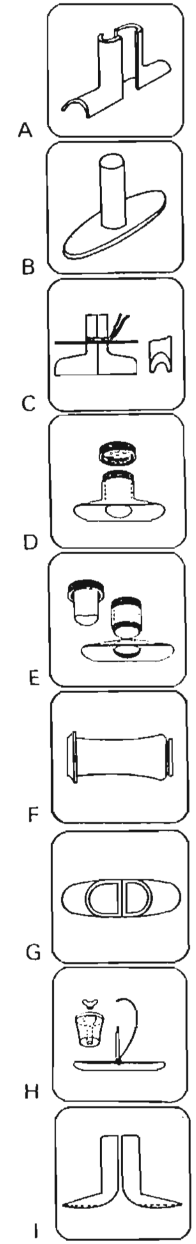
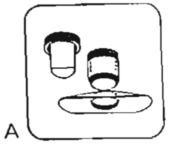
Appendix Table 2. Advances: oesophageal fistula closing device - continued.

Year	Species, material, design, minimum area of plug/cannula.
1962	**Sheep.** Plastic split plug, 300 mm² (McManus et al., 1962).[a;b]
1962	**Calves.** Plastic split plug, 300 mm² (Chapman and Hamilton, 1962).[c]
1963	**Cattle/sheep.** Lucite plastic cannula, cattle: 900 mm², sheep: 500 mm² (Cook, Blake and Call, 1963).[d]
1964	Semi-permanent cannula (Van Dyne and Torell, 1964).[e]
1964	**Cattle.** Soft plastic cannula, 350 mm² (Yarns et al., 1964).[f]
1964	**Sheep.** Perspex split plug with two unequal sized halves which provides three different sized plugs, 760 - 1 050 mm² (Hofmeyr and Voss, 1964).[g]
1970	**Cattle.** Streamlined plastic split plug with curved sleeve (Little and Takken, 1970).[a]
1970	**Sheep.** Rubber and plastic removable plug, 700 - 1 250 mm² (Bishop and Froseth, 1970).[h]
1971	**Sheep.** Wood and plastic removable plug, 350 mm² (Schutte, et al., 1971)[h]
1972	**Deer.** Clear butyrate plastic cannula, 500 mm² (Veteto et al., 1972).[e]
1974	**Goats.** Wood and plastic removable sleeve and plug, 500 mm² (Aucamp and Nell, 1974).[h]
1976	**Cattle.** Polyethylene split plug: stable and light weight, 2 000 mm² (Breen and Hunter, 1976).[i]
1976	**Sheep.** Divisible oesophageal cannula as stopper for collection of saliva (Gunzel and Hoppe, 1976).



Appendix Table 2. Advances: oesophageal fistula closing device - continued.

Year	Species, material, design, minimum area of plug/cannula.
1977	**Sheep/goats.** Plexiglass and PVC, cannula, sheep: 750 mm², goats: 550 mm² (Taylor and Bryant, 1977).[a]
1981	**Sheep/goats.** Polyethylene split plug, 500 mm² (Denny, 1981).[b]
1983	**Sheep.** Stainless steel plate with rubber stopper (McInnis et al., 1983).
1984	**Cattle.** Oesophageal cannula which prevents the passage of food to the rumen during sampling (Cangiano, 1984).
1984	**Cattle/sheep/goats.** Silicone cannula reinforced with stainless steel braces, cattle: 1500 mm², sheep/goats: 500 mm² (Ellis et al., 1984).[c]
1985	**Cattle.** Plastisol molded, flexible plug with outlet at 45°, reinforced with steel wire. Aluminum and stainless steel sleeve coated with pastisol (Forwood et al., 1985).[d]
1986	**Goats.** Polyestyrenic resin moulded split plug, 450 mm² (Grünwaldt and Sosa, 1986).[b]
1987	**Cattle.** Temporary cannula that prevents fistula contraction during sampling, 1 700 mm² (Olson and Malechek, 1987)[e]
1987	**Cattle.** Rubberized belting seal to prevent leakage, 1 400 - 2 300 mm² (Karn, 1987).[f]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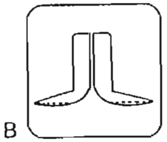
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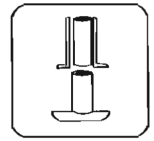
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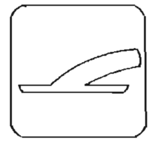
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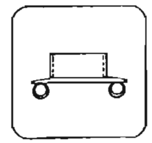
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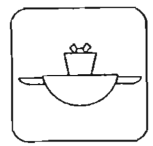
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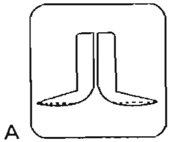
Appendix Table 2. Advances: oesophageal fistula closing device - continued.

Year	Species, material, design, minimum area of plug/cannula.
1988	**Cattle/sheep.** PVC tee-coupler, split plug, cattle: 800 mm², sheep: 300 mm² (Nelson, 1988).[a]
1990	**Impala.** Cannula outlet at 45°, Removable sleeve (PVC) and plug, 300 mm² (Visser et al., 1990).[b; c]
1990	**Sheep/goats.** Polyethylene split plug, 600 mm² (Pfister et al., 1990).[a]



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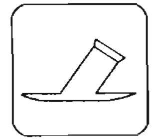

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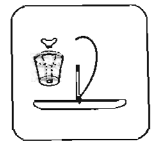

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Appendix Table 3. Effect of salivation and mastication on the chemical composition (organic components) of oesophageal fistula samples. (+ = increased; − = decreased; = = no effect)

Type of feed	Species	Freeze dry	Oven dry	Degree C	Saliva		OM	DM	Nitrogen	CF	NDF	ADF	ADL	Cellulose	Hemi-cel	NFE	NDS	WSW	EE	Fructose	Energy	IVOMD	Tannins	
Luserne hay (milled)	Sheep				+				=	=			=			=			=					(Bath et al., 1956)
Luserne hay	Sheep		=	80	−				=															(McManus, 1961)
Concentrate	Sheep		=	75	+				=	=						=			=					(Lombard & Van Schalkwyk, 1963)
Roughage (fresh & dry)	Sheep		=	75	+				=	=						−			=					(Lombard & Van Schalkwyk, 1963)
Roughage (fresh)	Sheep		=	100	−				=	=														(Arnold et al., 1964)
Roughage (fresh)	Sheep		=	80	+				=					=				=						(Grimes et al., 1965)
Lucerne (fresh & dry)	Sheep		=	70	−				=													−		(Langlands, 1966)
Lucerne hay	Sheep		=	75	+				=	=														(Basson, 1971)
E. curvula hay	Sheep		=	75	+				+	=														(Basson, 1971)
Lucerne + maize	Sheep		=	75	+				+	=														(Basson, 1971)
Roughage (range)	Sheep										−							−				=		(Saul, Flinn & Heard, 1986)
Roughage (fresh + hay)	Sheep	=		0	+																	=		(Armstrong et al., 1989)
Lucerne hay	Cattle				+					+						−					−			(Lesperance et al., 1960)*
Grass hay	Cattle				+					+						−					−			(Lesperance et al., 1960)
Roughage	Cattle		=	50	+				−				=					−		−				(Hoehne et al., 1967)
Roughage (hay)	Cattle								=				=	=					=					(Harris et al., 1967)
Roughage (fresh & dry)	Cattle		=	105	+				+	+														(Marshall, Torell & Bredon, 1967)
Roughage	Cattle				+			−	+	=						−			−					(Campbell et al., 1968)
Concentrate	Cattle				+				=	=						=			=					(Campbell et al., 1968)
Lucerne hay	Cattle		=	70	−				=	+						=			=					(Kiesling et al., 1969)
Tobaso (roughage)	Cattle		=	70	−				=	−						=			=					(Kiesling et al., 1969)
Legumes (5, fresh)	Cattle		=	45	−				=	−		+	+									−		(Barth et al., 1970)
Grass (4, fresh)	Cattle		=	45	−				=	−		+	+									=		(Barth et al., 1970)
Roughage (2, fresh)	Cattle		=	45					=			+	+			−			−					(Barth &Kazzal, 1971)

Appendix Table 3. Effect of salivation and mastication on fistula samples (organic components): – continued
(+ = increased; – = decreased; = = no effect)

Type of feed	Species	Freeze dry	Oven dry	Degree C	Saliva		OM	DM	Nitrogen	CF	NDF	ADF	ADL	Cellulose	Hemi-cel	NFE	NDS	WSW	EE	Fructose	Energy	IVOMD	Tannins	
Roughage (fresh & dry)	Cattle		=	80	+				=								=							(Little, 1972)
Roughage (fresh grass)	Cattle		=	55	–																	+		(Scales et al., 1974)
Roughage (fresh lucerne)	Cattle		=	55	–																	–		(Scales et al., 1974)
Roughage (fresh + hay)	Cattle		=	50	+				=													=		(Cohen, 1979)
Roughage (fresh)									=		=	=	=									=		(Hart, 1983)
Oak	Cattle								=													+	–	(Burritt et al., 1987)
Bitterbush	Cattle								=													=	–	(Burritt et al., 1987)
Maple	Cattle								+													=	–	(Burritt et al., 1987)
Mahogany	Cattle								=													=	–	(Burritt et al., 1987)
Roughage (fresh + hay)	Cattle	=		0	+																	=		(Armstrong et al., 1989)
Roughage dry	Cattle	=		0	–				=															(Pinchak et al., 1990)
Grass dry	Cattle	=		0	–		–		=		=	=	+	=	–									(Olson, 1991)

Appendix Table 4. **Effect of salivation and mastication on the chemical composition (inorganic components) of oesophageal fistula samples. (+ = increased; − = decreased; = = no effect)**

Type of feed	Species	Freeze dry	Oven dry	Degree C	Saliva			Ash	P	Ca	Na	Cl	K	Mg	Cu	Zn	S	
Lucerne hay (milled)	Sheep				+			=										(Bath et al., 1956)
Lucerne hay	Sheep		=	80	−			+										(McManus, 1961)
Concentrate	Sheep		=	75	+			=	=	=								(Lombard & Van Schalkwyk, 1963)
Roughage (fresh & dry)	Sheep		=	75	+			+	+	=								(Lombard & Van Schalkwyk, 1963)
Roughage (fresh)	Sheep		=	100	−													(Arnold et al., 1964)
Roughage (fresh)	Sheep		=	80	+			+										(Grimes et al., 1965)
Lucerne (fresh & dry)	Sheep		=	70	−			+	+	=	+		+					(Langlands, 1966)
Phalaris (fresh)	Sheep		=	70	−			=	+	=	+		+					(Langlands, 1966)
Hay and/or concentrates	Sheep		=	75	+			+										(Basson, 1971)
Luserne hay	Cattle				+			+	+									(Lesperance et al., 1960)*
Roughage	Cattle		=	50	+			+	+	−		+						(Hoehne et al., 1967)
Roughage & concentrates	Cattle				+			+										(Campbell et al., 1968)
Lucerne hay	Cattle		=	70	−			+										(Kiesling et al., 1969)
	Cattle		=	45	−			+										(Barth et al., 1970)
Roughage (fresh & dry)	Cattle		=	80	+			+	+									(Little, 1972)
Roughage (fresh grass)	Cattle		=	55	−			+	+									(Scales et al., 1974)
Roughage (fresh + hay)	Cattle		=	50	+					=							=	(Cohen, 1979)
Roughage (fresh)								+										(Hart, 1983)
Roughage dry	Cattle	=		0	−			+	+	=	+		+	−	+	=		(Pinchak et al., 1990)
Roughage fresh	Cattle	=		0	−			=	+	=	+		+	=	=	=		(Pinchak et al., 1990)

Appendix Table 5. **Veld condition assessment:**

Grass component (600 points). **Veld condition score:**

Species	%
Decreaser:	
Panicum maximum	16.2
Panicum stapfianum	1.3
Setaria neglecta	0.5
Themeda triandra	6.8
Increaser:	
Aristida barbicollis	0.3
Cymbopogon plurinodis	5.5
Cynodon dactylon	5.7
Digitaria eriantha	17.2
Eragrostis capensis	0.3
Eragrostis chloromelas	0.8
Eragrostis curvula	0.3
Eragrostis obtusa	0.5
Eustachys mutica	0.8
Forbs	1.5
Karroochloa curva	0.5
Melica decumbens	0.5
Microchloa caffra	0.6
Sporobolus africanus	1.8
Sporobolus fimbriatus	39.5

Bush component (600 m²). **Tree Equivalents/ha: 1896; Browsing units/ha: 1193.**

Acceptable species:	
Acacia karroo	23.5
Buddleja Saligna	0.7
Coddia rudis	5.9
Ehretia rigida	9.6
Grewia occidentalis	20.6
Lippia javanica	2.9
Lycium ferocissimum	8.1
Maytenus heterophylla	3.7
Rhus lucida	4.4
Scutia myrtina	5.1
Unacceptable species:	
Aloe ferox	2.9
Diospyros lycioides	11.0
Opuntia ficus-indica	0.7
Plumbago Auriculata	0.7

Veld condition assessment was done according to the methods described by Trollope (1980) and Foran, Tainton and Booysen (1978), adapted for the False Thornveld of the Eastern Cape (Danckwerts (1981)