Nylon bag - Procedure.

Animals
Rumen fistulated (RF)

- 2 – 3 years old Brahman cross bred steers
- Average liveweight 350kgs
- Fitted with large diameter (10cm i-d) rumen cannulae (Bar Diamond #2C)
- Area around cannulae clipped and clean
- Housed in individual pens

Preparation of feed types

a) Grains or concentrates as fed or as received
b) Hay ground through a 3 mm screen and air dried
c) Control hay ground through a 3 mm screen

Preparation of nylon bags

a) **Bag** monofilament polyester
b) **Dimension** 24 x 10 cm in dimension with a pore size of 45 μm. The seams of the bag are French stitched (Allied Filter Fabrics).
c) **Nylon Bag Preparation**
    - Need **two** bags / feed / time / animal except for "0" hours when we only need four **4** bags / feed / time / animal.
    - Usually have a poor quality hay feedstuff to act as a control.
    - The clean clearly numbered empty bags were checked for holes and placed in a forced-draught drying oven at 5^{50}C for 24 hrs prior to use. They were removed in small batches and cooled to room temperature in a desiccator prior to weighing.
    - Empty bag weight recorded. Approximately 5 grams of grain / concentrate or 4 grams of plant material placed in the weighed bag. The bag number, bag weight and combined bag and feed weight is then recorded. The bag is then allocated to a particular time for an individual animal.
    - The top of the filled weighed bag is folded length ways three times over itself, then with a twisting motion folded down, thus sealing the top of the bag. The tail should only be 3 cms, if any larger you are taking up too much of the bag volume.
    - A 4-inch cable tie is placed along the fold of the bag with the knob of the tie facing inwards and a size 16 rubber band is tightly wrapped around the tie and top of the bag. The flexible end of the tie is not removed. (Auto Electrical Importers 79 Randolph St Rocklea 32743077)
    - Bags are filled and tied until the required number of replications is completed.
    - The bags of the different feedstuff for each time / animal are placed in small sealable plastic bag, which are marked with time and animal number. These are then placed in a larger plastic bag marked for each animal.

- Two sub samples of the feed material are collected at the start, middle and end of the bag filling process. These samples are weighed and recorded before putting into a drying oven at 105^0C for 48 hrs. The samples are removed from the oven and placed for cooling in a desiccator. The cooled samples are then weighed and average dry matter % calculated for each feedstuff. This result is the used in calculating rate of degradability.
- A sample of each feedstuff is taken and stored for later chemical analysis.
- Samples of the ration are also taken for later chemical analysis.

d) **Incubation Times**

Concentrates and grains	0, 3, 6, 9, 13, 16, 24, 48 & 72 hrs
Hay	0, 3, 6, 9, 13, 16, 24, 48, 72 & 96 hrs

e) Record Keeping

Prepare Excel file listing animal number, feedstuff, incubation time, bag #, dry bag weight, dried bag and undried sample weight, dried bag and dried residue weight, time bag insertion and time of bag removal. Master lists sorted by animal number, time and bag number and sorted by animal number bag number and times are also prepared.

Incubation of bags

Equipment required -

Chain Weight 1.87 kg Length 55 cms
Links Number 15 Length 60 mm
String length 35 cms with ring attached
Shoulder length plastic gloves
Disposable latex gloves
Screwdriver
Multigrips
Bucket for each animal
Animal headstall or halter

Procedure

Calculate bag insertion and removal times so that the animal has to be disturbed as little as possible. Try to combine times for insertion or removal.
See Appendix (1)

a) Insertion

- The first insertion of nylon bags (96 hrs) is usually completed on a Monday at 3:00 PM and times for subsequent insertion or removal are calculated from this time.
- Arrange plastic bags containing filled nylon bags in order. Starting with 96 hrs. Check and tick off bag number against master list.
- Prior to insertion the 96 hr bags are loosely attached by ties to end of chain. No more then 4 bags / link. If more then 4 bags / time to be attached subsequent bags are to be attached on next link of chain.

- In bucket marked with animal number soak chain and bags for that animal in clean water 3 – 5 minutes before insertion into animal
- Halter tie animal securely in such away that you can safely get access to the rumen cannular. May have to put animals in crush.
- Put on shoulder length gloves and secure with rubber band or clip onto overalls. Put latex disposable glove over shoulder glove, as this will stop large gloves tearing.
- Clean around cannular area. Gently remove plug by either inserting screwdriver under outer rim of cannular and with multigrips carefully pull plug out or by pushing plug into animal and pulling plug out sideways.
- Insert chain and 96 hr bags into animal making sure that the bags are pushed down below the feed raft in the rumen.
- Either tie the end of string to the plug or hang it outside of the animal with end attached to a ring.
- Clean surface of cannular and after wetting the plug gently insert the plug into the cannular. The plug doesn't have to be inserted all the way in, but it needs to be inserted far enough that it will not fall out or allow rumen content to spill.
- Note time of insertion of bags. This is the most critical time as subsequent times for insertion and removal are based on this time.
- Repeat procedure with other animals making note of time of insertion.
- Clean equipment.
- Subsequent insertions are carried out at the required times in a similar manner. The pre soaked bags are attached loosely to the chain, missing a link between each time grouping. This makes it easier to distinguish between groups of bags when they have to be removed. Make sure that all bags are below the rumen feed raft.
- The 15 hrs bags are inserted with the 24 hr bags on separate links of the chain; these bags are then removed the next morning when the 9 hr bags are inserted. See Appendix (1).

b) **Animal feeding**

- During the sampling period 96 hrs to 24 hrs the animals are fed their ration in two equal portions at 7:30 AM and after the bags have been inserted at 3:00 PM. On the final day because of problems with gut fill and bag removal the feeding routine is slightly modified. At 6:00 AM on Friday after the bags are inserted or removed the animals are fed their normal ½ concentrate diet but only 1/3 of their total hay ration. This allows for easier bag insertion or removal. The final ½ of the concentrate diet and 1/3 of the hay diet is fed after the 12:00 AM bag insertion. The final hay is fed after the final bags are removed.

c) Removal Procedure 15 hr bags

Equipment required -

Blunt nose scissors
Screwdriver
Multigrips
Shoulder length plastic gloves

Disposable latex gloves
Bucket of clean cold water for each animal

- Consult master list for bag numbers to be removed.
- Secure animal.
- Remove cannular plug and gently lift chain and bags towards outside of animal.
- Find group of bags to be removed, they should be on same link. Check again bag numbers against master list.
- Bring bags towards opening and carefully slip blunt nose scissors through plastic tie loop. While holding bag cut loop. Bring bag outside and place into bucket of cold water. Remove remaining 15 hr bags. Keeping buckets separate do this for each animal.
- Remove rumen content from the outside of each bag by gently washing them under cold water. Separately wrap a rubber band around the top of the 15 hr bags for each animal and place them in a freezer, making sure that they do not stick to anything in the freezer as you will want to remove them at the end of the experiment.
- Clean equipment.
- Feed animal ration.

d) Final Removal of bags

Equipment required -

Blunt nose scissors
Screwdriver
Multigrips
Shoulder length plastic gloves
Disposable latex gloves
Bucket of clean cold water for each animal
Buckets for rubbish

- Secure animal
- Remove plug and gently remove chain and bags from animal. Flick chain to remove any solid rumen content from outside of bags. Lower chain and bags into bucket of cold water.
- Record time of removal.
- Clean around bung area and reinsert bung.
- Repeat for each animal.
- Remove halters.
- Feed remaining ration.

e) Washing Procedure

Equipment required –

Blunt nose scissors
Buckets for rubbish
Underwear bags
Washing machine

- Remove 15 hr bags from freezer and thaw in cold water.

- Using blunt nose scissors cut ties and remove bags from chain. Pull ties from bags being careful to leave rubber bands wrapped around top of bags.
- Wash bags under running cold water giving them 10 – 25 squeezers in the process.
- Place bags into underwear bag.
- "0" hr bags are similarly washed after they are soaked for 3 – 5 minutes in clean cold water.
- Repeat process for animals putting their bags into a separate underwear bag.
- Weigh down underwear bag with weight to ensure all bags are submersed in water.
- Place underwear bags into washing machine.
- Wash in cold water for two 6-min cycles, then spin dry.
- After washing place in force-draught oven at 55^0C for 48 hrs, keeping bags from different animals separate.

f) Weighing and bulking of Feedstuff.

Equipment required -

Master sheet - sorted on bag#, feedstuff and animal#
Desiccator
Rubbish bins
Trays

- Remove small number of bags from oven and allow them to cool in a desiccator.
- Once bags are cool, take a bag out remove rubber band from top and remove any dried rumen content from the outside of the bag.
- Quickly weigh the bag and record weight.
- After checking bag# against Master list, place bag in tray being careful that no feedstuff falls out.
- Bags are placed according to feedstuff and time.
- Repeat for all animals putting bags together according to feedstuff and time.
- Check groups of bags against Master list.
- The bags were then emptied into a labelled 250 ml screw top jar for later analysis.
- The empty bags were washed in Lux soap flakes and given two deep rinses.
- **Caution very caustic solution** If bags are still dirty after washing soak in Chromic acid solution for 12 – 24 hrs and then rewashed in clean water. See Appendix 2 for Chromic acid solution. **Caution very caustic solution**

g) Analysis of samples.

- Bulk residue feedstuff ground to 1 mm for OM, NDF etc

h) Record keeping.

- All times and weights are added to the Excel file.

- Degradability is calculated using the formula

  A = dry bag weight
  B = dry bag weight + undried feed sample weight
  C = dry bag weight + dried residue sample weight
  D = average dried sub-sample feed Dry Matter %
  E = dry weight of feed sample
  = ((B - A)*D/100)
  F = dry weight of residue sample
  = C – A

  Degradability = 100 – ((F / E)* 100)

i) Animal Health.

- Check animal's feet and legs for signs of swelling from being on concrete.
- Clip and clean around cannular.
- Check around cannulae for proud flesh, put zinc cream on to edge of wound.
- At end of experiment mix copper sulphate and zinc cream and put onto any proud flesh.
- Spray or put in insecticidal ear tags to counteract buffalo or house fly

Diets and feeding procedures

The RF steers were fed on a standardised diet designed to provide adequate CP for efficient microbial activity (*c*. 16 % CP). The diet consisted of *c*. 1/3 lucerne hay, 2/3 green panic hay and 1kg of cottonseed meal, and was fed at 90 % of *ad lib* intake to facilitate nylon bag removal. The steers underwent a preliminary feeding period of 6 days minimum prior to bag insertion. During the incubation period, the diet was fed in two equal portions at *c*. 0730 h and 1530 h.

The dried feed sample under study was ground through a 3 mm screen prior to weighing (*c*. 5 g DM) into nylon bags. The required number of filled nylon bags was attached to a length of chain (16 x 6 cm links) and then soaked in water for *c*. 5 - 10 minutes prior to immersing in the rumen of RF steers. Bag replications and times of removal are described in individual chapters.

Upon removal of the bags from the rumen, the excess material was washed off the bags and they were immediately frozen if more times of removal followed. Once all bags had been removed from the rumen, the frozen bags were defrosted by placing in cool water. The bags were then each given a constant number of squeezes by hand under running tap water (10 to 25 squeezes, depending on experiment) before being rinsed on a set cycle in an automatic washing machine. Upon removal from the

washing machine the bags were oven dried (55^0C for 48 h), cooled in a desiccator, and then weighed to determine the proportional DM loss. Dried bag residue was ground through a 1mm screen prior to chemical analyses.

Appendix 1

Nylon Bags					
		Time In		**Time Out**	
Mon 3:00 PM		**96**			
Tues 3:00 PM		**72**			
Wed 3:00 PM		**48**			
Thur 3:00 PM		**24, 15**			
Friday					
6:00 AM		**9**		**15**	
9:00 AM		**6**			
12:00 AM		**3**			
3:00 PM				**72, 48, 24, 9, 6, 3**	

Appendix 2

Chromic acid solution for washing nylon bags.

**** Caution VERY CAUSTIC ****

Wear rubber gloves, eye protection and protective clothing

$Na_2Cr_2O_7$ or $K_2Cr_2O_7$

Concentrated H_2SO_4

To make 8L of 4% (w/w) solution.

- Weigh 12.8 g of chromate into a 2L measuring cylinder.
- Add 500 mls of distilled water.
- Swill until chromate is dissolved.
- Very slowly add 320 mls of conc H_2SO_4 to mixture. Cool side of cylinder under running cold water.
- Make up to 2L with distilled water.
- Carefully pour into large heavy-duty plastic container and then make up to 8L with distilled water.
- Carefully place bags into solution making sure that you do not get any of the solution on your hands or your clothes.
- Leave bags in for 12 – 24 hrs at 55^0C.
- Carefully remove clean bags and rinse 3 – 4 times under clean tap water.
- Wash in Lux and give them a good rinse.
- Dry bags at 55^0C for 12 hrs.