RECOMMENDATION FOR QUALITY CONTROL OF *IN SACCO* NYLON BAG TECHNIQUE

Introduction

The incubation of nylon bags filled with feedstuff in the rumen for different incubation times yields a measure of the kinetics of feedstuff degradation in the rumen. Although the *in sacco* nylon bag technique is widely used and forms the basis of many feed evaluation systems, this technique requires precise control of procedures and equipment for achieving desirable reproducibility and reliability of the data generated. A number of factors are known to influence the results. Some of these factors are dimensions and pore size of nylon bags, sample weight, washing conditions etc. These factors should be controlled as far as possible.

Procedure and Suggestions

The nylon bags supplied to all counterparts are of dimensions 9 x 16 cm (40-60 micron).
It is suggested that:

- The sample should be passed through 2 mm screen.
- Three to 5 g samples of feeds should be weighed in the bags. In the rumen of sheep, a total of 6-12 bags (depending on size of the sheep) should be incubated (3 bags attached to each plastic tube; for diagram see: www.rri.sari.ac.uk/ifru). The number of the bags can be increased to 24 to 60 for cattle.
- At least three animals should be used for incubation of the bags.
- The diet of the animals should be as similar to the feeds under investigation. For studies on tanniniferous tree leaves, the animals should be on roughage-based diets (basal diet plus low level of concentrate).
- The incubation time for browses should be 4, 8, 16, 24, 36, 48, 72 and 96 h. Also determine initial water solubility for the samples by immersing a set of bags in triplicate in water (39 ^{o}C) for 1 h.
- The bags should be inserted into the rumen at different time intervals so that all bags are taken out at the same time and subjected together to the following washing condition.
- After incubation, the bags should be immediately placed in cold water to stop fermentation, and to remove the feed particles adhering to the bags. Transfer them to domestic washing machine and wash them for 20 min in water at 22-25 ^{o}C. (The domestic washing machines vary widely around the world. It is suggested that the revolutions per minute (rpm) of the washing machine at which it is presently being used for washing the bags should please be noted, and this information and the other rpm at which the machine can be used must be exchanged with all counterparts, so that an rpm which is as close as possible to one another can be decided.). Dry the bags to a constant weight at 65° C (takes approx. 30 h). Place them in a desiccator kept at room temperature. Weigh the bags after these have come to the room temperature. Calculate percent digestion of feedstuff, and calculate the digestion kinetic parameters using the exponential equation.
- The bags can be re-used after thorough washing. Remove the feed in the bag by turning the bags inside out and dust away loose particles. Soak the bags in hot soapy water overnight, and then wash them in a domestic washing machine. Dry the bags, and re-use them. A thin coating which generally builds up on the mesh after several uses can be

removed by dipping the bags in chromic acid solution. The bags should be thoroughly washed to remove chromic acid.

Quality assurance of the analysis

In order to ensure that each laboratory has good control of the accuracy and reproducibility of the results, use of proper standards is a must. It is suggested that each counterpart should select a feed available in a large quantity (6-8 kg) and similar in nature to the one which will be evaluated in the Co-ordinated Research Projects (CRP). This should be ground to pass through a 2 mm sieve and the ground material should be stored in a dry, cool and dark place. This sample should be included every time for incubation along with the samples to be evaluated under the CRP.

It is suggested that this standard sample should be evaluated 15-20 times by incubating in the rumen for 4, 8, 12, 24, 36, 48, 72 and 96 h. Percent dry matter (DM) digestibility should be calculated at each incubation period. Average of these 15-20 observations of percent DM digestibility at each incubation period should be calculated. Any observed DM digestibility value at an incubation time which deviates by > 10 % from the mean at that particular time should be removed from the set of values, and again average of the rest observed values should be taken. This may be considered as the 'historical value' to check the accuracy of the future assays.

An example: The standard sample was analysed, say 15 times.
At 8 h of incubation, % DM digestibility values are: 20, 21, 22, 20.5, 21, 24, 23, 19, 20, 21, 20.5, 25, 22, 21, 21.5. Please note that each sample is incubated in three animals. Each value is the average of these three values obtained from three animals.
Average of 15 values = 21.4 %
From the above set, the values 24 % and 25 % deviate by > 10 % from 21.4 value (24-21.4 = 2.6 x 100/21.4 = 12 %). Discard these two value and then take average of the rest 13 value to obtain the historic value of DM digestibility at 8 h, and this average is = 20.9 %.

Now in future studies if the observed value of this standard (incubated along with the samples to be evaluated under the CRP) deviates by > 10 % from this mean (20.9 %), the results should be critically assessed for their acceptance. If the deviation is < 10 %, this value may be included in the previous accepted set and then again mean should be calculated to have a new historic value. In this manner, the historic value will become better and better. Similar approach should be applied at other incubation periods.

In addition, calculate the digestion kinetic parameters using individual values (not average) at each incubation period selected using the procedure mentioned above (discarding those values which deviate by > 10 % from the mean) using the exponential equation. Calculate the mean and standard deviation (SD) of these kinetic parameters to obtain the historic values of the digestion kinetic parameters.

If the observed digestion kinetic values of the standard at a later stage when the samples are being analysed for the CRP deviate by greater than 2 times SD of the historical values, repeat the analysis. And if the digestion kinetic parameters are accepted, the new values should also be included into the previous set of the accepted values, and again mean and SD of the new set should be calculated to have the new historic values of mean and SD. In this manner the

set will grow and better historic values of digestion kinetic parameters will be obtained, leading to better quality control of the assay.

Note: The DM digestibility depends on many factors. In the overall environment of the experiments there could be periodic (seasonal, some change in the diet fed to the fistulated animals) changes in digestibility of the standard. This means that the values for the standards will change but the curves will, in general, have similar shape. If this is the case then the values for the standard can be used for a normalising (correction) factor.

The concept or the approach outlined above has not been used earlier by any group. We shall discuss the pros and cons of this approach, difficulties encountered and their possible solutions during the next Research Co-ordinated Meeting (RCM). All counterparts are requested to keep a complete record of the data generated and bring along the raw data. This will help us in modifying, if need be, the approach for quality control of the *in sacco* nylon bag technique.

If possible, counterparts should exchange standard samples with at least one counterpart and compare the values obtained. This set of data should also be presented in the next RCM.

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(agreed by all Agreement Holders)