

11.0 ANALYSIS OF BYPASS PROTEIN AND FAT SUPPLEMENTS

11.1 *In vitro* determination of degree of protein protection in bypass protein supplement

Equipment

- Stoppered ground glass 30 ml test tubes – Quick fit catalogue number MF 24/2/6 socket size 19/26 plus stoppers – catalogue number SB19.
- Test tube racks to fit both sizes of test tubes
- Suba seals
- Wide rubber bands
- Vortex mixer
- Acid dispensers and precision pipettes
- Tecator Kjeltac Auto 1030 nitrogen analyzer with digestion system 20 and scrubbing unit together with the necessary digestion tubes and racks.



Fig. 11.1 Shaking incubator

Notes:

- Weigh all samples the day before and cover with foil.
- Turn on incubator the night before and set temperature to 39°C.
- Keep strained rumen fluid at 39°C flushed with N₂ and commence ruminal incubation within an hour of collection.
- Keep test tubes with samples warm
- Keep rumen fluid warm and flush continuously with N₂ whilst dispensing

Procedure

1. Weigh 100 mg of protected protein, unprotected protein, known standards, pure casein and blanks into ground glass stoppered test tubes in triplicate.
2. Pipette 10 ml of strained rumen fluid into test tube.
3. Flush the sample triplicate set with nitrogen using a pasteur pipette attached to a gas cylinder via rubber tubing.
4. Cap with suba seals and tightly seal with rubber bands to maintain anaerobic conditions.
5. Incubate samples in a shaking incubator (Fig. 11.1) at 39°C for 20-24 hours.
6. To stop incubation add 2 ml of 1 N sulphuric acid.
7. Filter the content through cotton plug in other test tubes.
8. Take 2 ml of filtrate into Tecator tubes.
9. Proceed to titrate using a Tecator distillation set up.

Calculation for the degradation of protein

$$\begin{aligned} \text{\% Protein} &= \frac{(\text{mls titrant} - \text{blank titrant}) * 0.1(N) * 14.01 * 6.25 * 6.25 * 100}{1000 * \text{sample wt.} * \text{protein} / 100 * \text{dry matter} / 100} \\ \text{Degradation (RDP)} &= \text{---} \\ \text{\% Protection (RUP)} &= 100 * (1 - \text{protein degradation} / \text{casein protein degradation}) \end{aligned}$$

References: Gulati S.K. (1976). Protected triacylglycerol and sterol supplements for ruminants. MSc thesis, Macquarie University, North Ryde, NSW, Australia.; Ashes *et al.* (1979) J. Amer. Oil Chem Soc. 56:522; Gulati *et al.* (1999). 90th AOCS, Florida, USA. S41-S42.