11.5 Total fat extractions using acidic chloroform: methanol (2:1 v/v) method

This procedure is to be used for samples containing protected lipid supplement either pure or as part of a ration.

Equipment

- Soxhlet extraction apparatus with individually controlled serial heating mantle.
- Extraction thimbles to fit soxhlet apparatus
- Rotary film evaporator
- Drying oven and boiling chips

Reagents

- Chloroform / Methanol 2:1 v/v
- 1 N HCL

Procedure

1. A 5-10 g sample is weighed into a 30 x 80 mm soxhlet thimble.
2. The sample is extracted by refluxing for 5-6 h in a soxhlet apparatus using approximately 150 ml of chloroform – methanol (2:1, v/v) plus 1 ml of 1 N HCl.
3. The solvent extract is allowed to cool and filtered and the volume measured in a 250 ml stoppered measuring cylinder.
4. The filtrate is washed with 1/5 volume of water.
5. The mixture is allowed to separate into two clear phases; the upper aqueous phase is removed by aspiration and discarded.
6. The lower organic phase is evaporated to dryness in a tarred 250 ml round bottom flask using a rotary film evaporator and the remaining lipid is estimate gravimetrically after drying at 100ºC for 1h.

Calculation

$$\% \text{ Fat} = \frac{(\text{wt flask} + \text{fat}) - \text{wt flask} * 100}{\text{wt of sample}}$$

References: Gulati S.K (1976). Protected triacylglycerol and sterol supplements for ruminants. MSc thesis, Macquarie University, North Ryde, NSW, Australia.