11.6 Fatty acid analysis of feed

Reagents

- Ethanol (absolute)
- 5 N sodium hydroxide (NaOH) (200 g of NaOH pellets / 1 litre of distilled water).
- 5 N hydrochloric acid (HCL) (500 ml of concentrated acid added to 500 ml of distilled water)
- Petroleum ether (PE) (Boiling range 40-60ºC)
- 1% sulphuric acid in methanol.
- 5% Salt solution (NaCl)

Procedures

Saponify

- Mix thoroughly and take a representative sample.
- Grind sample to a fine consistency.
- Add 2-5 ml of ethanol
- Add 2-5 ml of 5 N sodium hydroxide (NaOH)
- Shake well and cover with foil
- Place in oven @ 80ºC for 1.5-2 h and then allow to cool.

Acidify

- Add 5 N hydrochloric acid approx 3 ml and invert test tube with care.
- pH must be checked for each sample using pH paper till acid (must be pink)
- When sample has cooled sufficiently
- Extract fatty acids with 4 ml of petroleum ether, shake well, pipette the supernatant into a labelled 15 ml test tube.
- Repeat above step – pooling the extracts
- Evaporate pooled PE extracts to dryness in a warm water bath under a stream of nitrogen.

Methylate

- To the dried sample add 3 ml of 1% sulphuric acid in methanol
- Reflux on a heating block at 50-60ºC for 1.5 h
- Add 3 ml of 5% NaCl (salt solution)
- Add 3 ml of PE
- Cool, shake well and centrifuge and decant supernatant into GLC vial

Reference: Christie, W.C. 1993, Gas chromatography and lipids. The Oily Press Ltd., Dundie, Scotland, UK.; Gulati S.K. (1976). Protected triacylglycerol and sterol supplements for ruminants. MSc thesis.