13.6 Determination of lactic acid in SRL

Lactic acid, when heated with concentrated H_2SO_4 converts into acetaldehyde, which reacts with p-hydroxydiphenyl to give purple color in the presence of copper ions.

Reagents

1. Copper sulphate 20%: Dissolve 200 g $CuSO_4$ $5H_2O$ in 500 ml distilled water and make the volume to one litre. The solution is stable indefinitely.
2. Copper sulphate 4%: Take 200 ml of reagent 1, and make the volume to one litre.
3. Calcium hydroxide ($Ca(OH)_2$).
4. Concentrated H_2SO_4
5. NaOH 5% - 5 g NaOH, dissolve in 100 ml distilled water.
6. p-hydroxydiphenyl reagent : Take 1.5 g p-dyroxydiphenyl in a 100 ml volumetric flask. Add 100 ml of 5% NaOH and 100 ml distilled water. Warm it with constant stirring to dissolve. Make the volume to 100 ml. Store it in amber color bottle.
7. Stock standard lactic acid: Take 0.1065 g lithium lactate in 100 ml volumetric flask and dissolve in about 50 ml distilled water. Add 0.1 ml concentrated H_2SO_4 and make up the volume to 100 ml with distilled water. The solution contains 1 mg lactic acid per ml and the solution is stable for a long period in refrigerator.
8. Working standard lactic acid solution: Dilute 1 ml of stock lactic acid solution to 100 ml with distilled water. It contains 0.01 mg lactic acid per ml. Prepare fresh working solution at the time of analysis.

Procedure

- Take 1 ml strained rumen liquor in a centrifuge tube. Add 1 ml of 20% $CuSO_4$ and make the volume to 10 ml.
- Add 1 g $Ca(OH)_2$, shake vigorously to make the mixture homogenous.
- Leave the tubes for 90 minutes with periodic shaking.
- Centrifuge at 3000 rpm for 10 min.
- Take 1 ml supernatant in a test tube in duplicate.
- Add 0.05 ml of 4% $CuSO_4$.
- Add 6ml concentrated H SO drop by drop with continuous shaking.

- Keep the tubes in boiling water bath for 5 minutes.
- Cool the tubes at room temperature
- Add 0.1 ml p-hydroxydiphenyl reagent drop by drop. The pipette tip should not touch the wall of the tube. Mix the contents immediately and vigorously.
- Incubate the tubes at 30ºC for 30 min with periodic shaking.
- Keep the tubes in boiling water bath for 90 sec. Remove the tubes and cool to room temperature.
- To plot the standard curve prepare the standard tubes in duplicate as follows:
- Proceed for color development (step 5 to 12).
- Read absorbance (optical density) of all the tubes at 560 nm.
- Find out the concentration of sample on standard curve and multiply by 10 (dilution)

to give µg lactic acid/ml rumen liquor.

Tube No.	1	2	3	4	5	6
Distilled water (ml)	1.0	0.9	0.8	0.6	0.4	0.2
Standard lactic acid solution (ml)	0.0	0.1	0.2	0.4	0.6	0.8
Lactic acid concentration (µg)	0.0	1.0	2.0	4.0	6.0	8.0

Reference: Laboratory manual of animal nutrition. IVRI, Izatnagar, U.P.-243 122.