

***The concentration of free glycerol in goat milk increases during feed restrictions.***

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SUPPLEMENTARY FILE

Milk samples are initially diluted in water (1+2 H<sub>2</sub>O) and heat-treated, 75 °C 5 min.

Samples and reagents (30 or 60 + 100 µl) are pipetted in black 96-well plates. Incubation with reagents (both processes) 40 min at 37 °C before reading.

Reaction conditions step 1-3 (30 µl diluted sample): [sample fraction 0.077; Tris-buffer 55 mM (pH 7.8); ATP 1.50 mM; Mg<sup>++</sup> 1.37 mM; K<sup>+</sup> 2.75 mM; ADHP 589 mM; Glycerol kinase 1.82 U/ml; Glycerophosphate oxidase 1.82 U/ml; Peroxidase 1.41 U/ml]

Reaction conditions step 2-3 (60 µl diluted sample): [sample fraction 0.125; Pipes-buffer 48 mM (pH 6.5); ADHP 589 µM; Glycerophosphate oxidase 2.27 U/ml; Peroxidase 1.41 U/ml]

Standards for both processes are based on glycerol in milk pre-liberated from intrinsic glycerol. Glycerol free milk: 25 ml milk is heat-treated at 75 °C for 10 min. After cooling 40 U glycerol kinase and 40 U glycerol oxidase solubilized in 3.0 ml Tris 150 mM, pH 8.5 plus 15 mg ATP (25 µmoles) are added, and the mixture is incubated for 20 minutes at 37 °C. 20 µmoles of thiosulphate is added and the mixture further incubated 50 minutes. Finally, the mixture is heated to 90 °C for 10 minutes.