**Supplementary Material S1**

**Added dietary cobalt or vitamin B12, or injecting vitamin B12 does not improve performance or indicators of ketosis in pre- and post-partum Holstein-Friesian dairy cows**

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Plasma vitamin B12 analysis was conducted using the ADIVA Centaur CP VB12 assay at the AHVLA laboratory, Shrewsbury, Shropshire, UK. The limit of detection was 33 pmol/l, and coefficients of variation (CVs) for low, medium and high quality controls were 12.2, 12.4 and 12.4% respectively. Plasma homocysteine (Hcy) was determined using an Imola Auto analyser (RX imola; Randox Laboratories Ltd., Antrim, U.K.) using a kit supplied by Randox Laboratories (catalogue no. HY4036). The limit of detection was 1.74 µM and CV’s for low, medium and high quality controls were 1.03, 0.83 and 0.95% respectively.

Plasma methylmalonic acid (MMA) and succinic acid (SA) concentrations were determined by GC with mass spectroscopic detection (GC-MS) following derivatisation and extraction, modified from the method of Kanakkaparambil *et al.* (2009). Briefly, 50 µl of plasma and 5 ul of internal standard (4-chlorobutyric acid (CBA) 250 µM) were added to 250 µl 12% BF3-methanol in a 2.5 ml screw capped glass vial, vortexed for 30 s and heated at 95°C for 15 minutes on a heating block. After cooling, 250 µl distilled water and 250 µl dichloromethane (CH2Cl2) was added to the vial and vortexed for 30 s. The mixture was then centrifuged for 8 minutes at 2500 g at 4°C to separate the layers. The lower dichloromethane layer was transferred to a screw capped auto-sampler vial with insert for GC-MS analysis. The method used a DB-WAX (crosslinked polyethylene glycol; J&W Scientific Agilent technology) 30 m long column of 0.25mm i.d and 0.15 µm film thickness. The carrier gas was He and flow rate was 1.0 ml/min. Injection mode was splitless and volume was 1 µl for both SCAN mode, for qualification, and SIM mode, for quantification. Injection port temperature was 260°C. The MS selective detector interference temperature was 280°C. The chromatograph was programmed for an initial temperature of 50°C for 2 minutes, increased to 150°C at 8°C per minute, then increased to 220°C at 100°C per minute and held for 5 minutes at the final temperature. The MS was operated in electron impact (EI) ionization mode with the ionization energy of 70eV. SCAN mode measured at m/z: 20-500 and SIM (selected ion-monitoring) ions were set at 105 (for CBA), 115 (for MMA and SA). The same method was used for standards of MMA and SA at concentrations 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10 and 20 µmol/l. Calibration was carried out by comparison of peak areas of CBA with MMA and SA in the standards and the final results expressed in µmol/l plasma. The limit of detection and limit of quantification was 0.156 µmol/l. The CV’s for low, medium and high quality controls were 0.44, 1.03, 0.83% for MMA and 1.2, 1.1 and 2.3% for SA respectively.

For hepatic triacylglycerol (TAG) analyses, samples were reduced to powder under liquid N, and known amounts (around 90-120 mg) weighed and homogenised in 1.6 ml of 0.47 M sodium sulphate, followed by the addition of 2 ml of 0.47 M sodium sulphate and 5.4 ml hexane:isopropanol (3:2, v/v). The mixtures were vortexed for 30 s and centrifuged at 2000 g for 5 minutes at room temperature, then 2.5 ml of the top layer transferred to a glass tube and dried under nitrogen. The dried lipid was reconstituted in 1 ml of hexane and 10 or 60 µl transferred to a second tube and dried under nitrogen. Dried samples were then re-suspended in 120 µl of isopropanol and 50 µl was mixed with 250 µl of colour reagent from Wako LabAssay triglyceride kit (Catalogue No. 290-63701; Alpha Laboratories, Hampshire, UK) for triacylglycerol measurement. Different concentrations of triolein standard (Catalogue No. T7140, Sigma-Aldrich, Gillingham, UK) were processed in parallel with liver samples to generate a standard curve. Different concentrations of glycerol and non-extracted triolein were included in each plate to monitor enzyme activity and completeness of lipolysis by lipoprotein lipase. In addition, a single liver analysed in each plate served as a quality control. The inter-assay CV was 8.4%.

**References**

Kanakkaparambil R, Singh R, Li D, Webb R and Sinclair KD 2009. B-vitamin and homocysteine status determines ovarian response to gonadotrophin treatment in sheep. Biology of Reproduction 80, 743-752.



**Supplementary Figure 1** Plasma glucose (a), ß-hydroxybutyrate (3-OHB) (b), non esterified fatty acids (NEFA) (c) and urea (d) in pre- and post-partum of dairy cows offered diets that contained no added cobalt (Co) (C: ◼), added dietary Co (DC: ●), added dietary B12 (DB: ○) or injected B12 (IB: Δ).