The effect of extended milking intervals (24, 48 and 72 h) on milk yield, milk composition, mammary physiology and welfare traits in dairy ewes

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SUPPLEMENTARY FILE

Supplementary Materials and Methods

Determination of plasmin (PL), plasminogen (PG), and plasminogen activator (PA) activities

Activities of PL and PG in milk were determined as described by Politis et al. (1989a, b). In detail, a milk sample of 3 ml was mixed with 1 ml of 0.4 M sodium citrate and centrifuged at 27,000 × g for 20 min. The supernatant was recovered and assayed for PL and PG. Plasminogen-derived activity is defined as the PL activity generated after addition of urokinase. The sum of PL+PG was calculated by adding the activity of PL plus the activity of PG and indicates the quantity of PG, PL, or both, moving from the blood into milk. Both assays were performed in 250µl of 0.1MTris-HCl buffer (pH 7.4) containing 0.6 mM Val-Leu-Lys-p-nitroanilide (V7127; Sigma Chemical Co., St Louis, MO), 30 Plough units (2.5 µl) of urokinase (U0633; Sigma), and 30 µl of the milk supernatant. All assays were duplicated. The reaction mixture was incubated at 37°C and absorbance at 405 nm was recorded at hourly intervals. A sample without supernatant served as a control for the detection of spontaneous breakdown of the substrate. In all cases, spontaneous hydrolysis was negligible. Plasmin activity was measured in the same reaction mixture without added urokinase. Plasmin and PG activities were determined from the linear part of the absorbance vs. time curve. One unit of PL was defined as the amount of enzyme that produced a change in absorbance of 0.1 at 405 nm in 60 min. A colorimetric assay was used to measure PA activity in the casein nitrogen (CN) fraction. The principle of this methodology is that PA in the CN fraction converts exogenously supplied PG to PL. The PL hydrolyzes the chromogenic substrate, Val-Leu-Lys-p-nitroanilide, liberating the chromophore, p-nitroaniline. Changes in color were directly related to PL level and therefore indirectly to PA activity. Details of this method are outlined by Gilmore et al. (1995).

Determination of lactose concentration in blood plasma

Lactose concentration in blood plasma was determined as described by Stelwagen *et al.* 1994a). Briefly, 500 µl of plasma were mixed with 1 ml of 1 M perchloric acid. After samples stood for 5 min and were centrifuged for 30 min at 2000 x g, 4°C, 900µl of supernatant were mixed with 300 µ1 of neutralizing buffer (containing equal volumes of 4 M KOH and 1 M K3PO4, pH 7.0). Following standing and centrifugation as before, the supernatant was either frozen or used immediately for lactose analyses. All solutions were kept at 4°C. Lactose standards for the assay were subjected to the same deproteinization procedure as the plasma samples. The enzymatic assay is based on two reactions, one measuring galactose and the other measuring lactose and galactose; the difference between the two provided a measurement of lactose concentration. Deproteinized samples and standards were loaded (100 µl) onto 96-well polystyrene microtiter plates and mixed with an equal volume of enzyme reagent A (to measure galactose) or B (to measure lactose and galactose). Reagent A consists of 1 ml of MOPS [3-(N-morphilino)-propanesulfonic acid] buffer (3 M MOPS and 1 M NaOH to pH 7), 0.5 mg of thio-NAD, 10 µl of MgSO4 in H20, and 4.5 µ1 of galactose dehydrogenase-S (50 U/ml of 3.2 M ammonium sulfate, pH 6). Reagent B consists of 1 ml of reagent A and 15 µl of β-galactosidase (1500 U/ml of 3.2 M ammonium sulfate, pH 6). Mixtures were incubated at 20°C for 60 min after which absorbance was measured at 405 nm, using 490 nm as a reference wavelength.

Supplementary References

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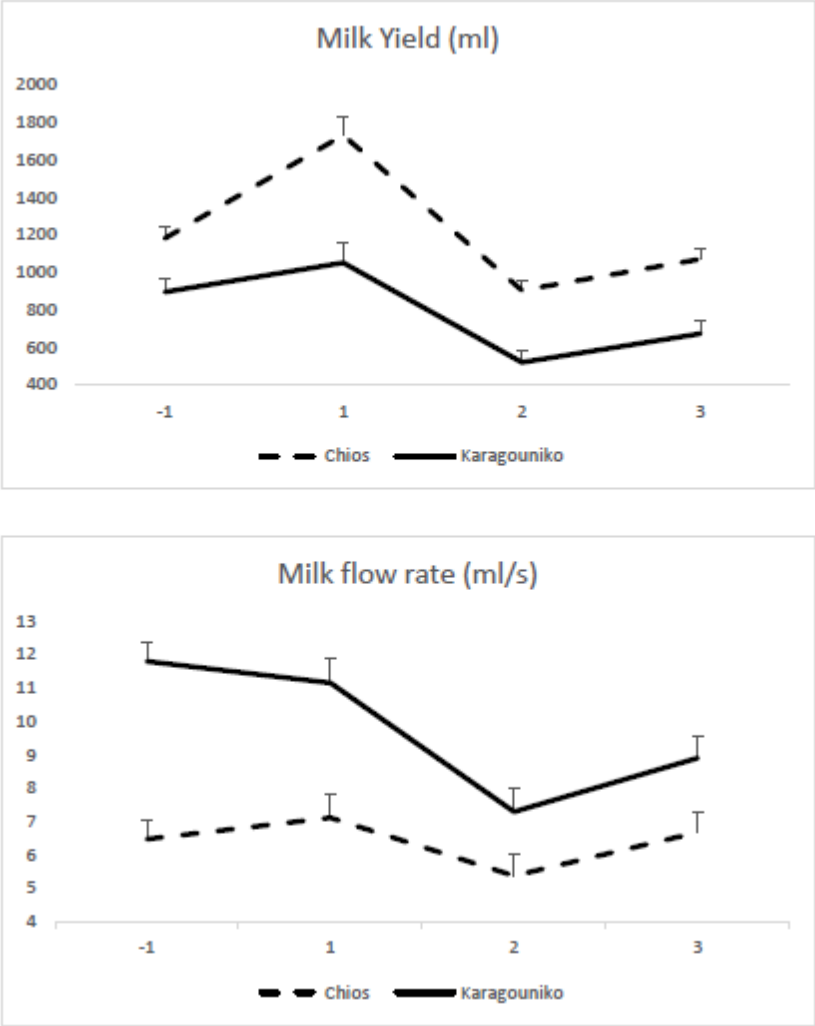


Fig. S1. Effect of breed (Chios and Karagouniko) on milk yield (ml) and milk flow rate (ml/s) (-1: 1 day before cessation, 1, 2 and 3 : 1, 2 and 3 days after re-milking)

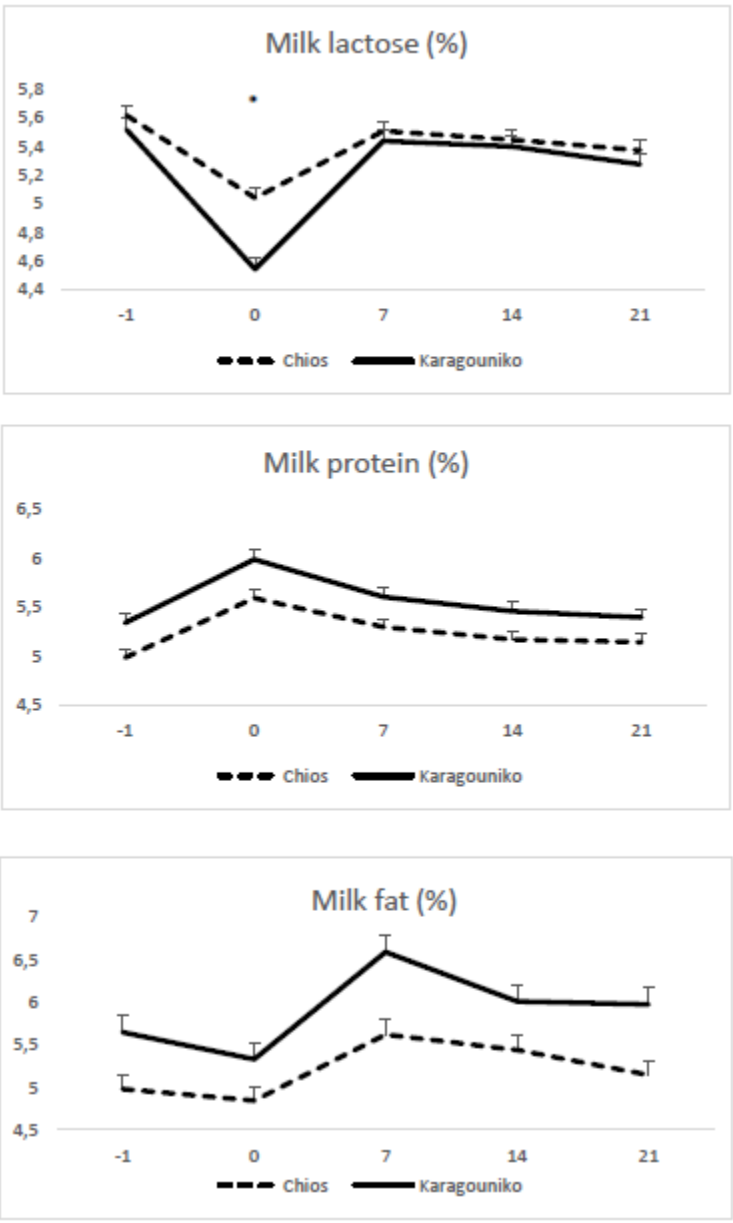
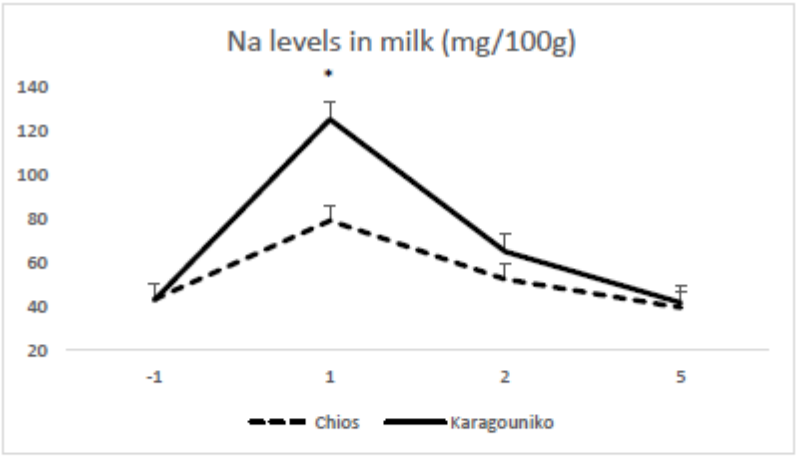
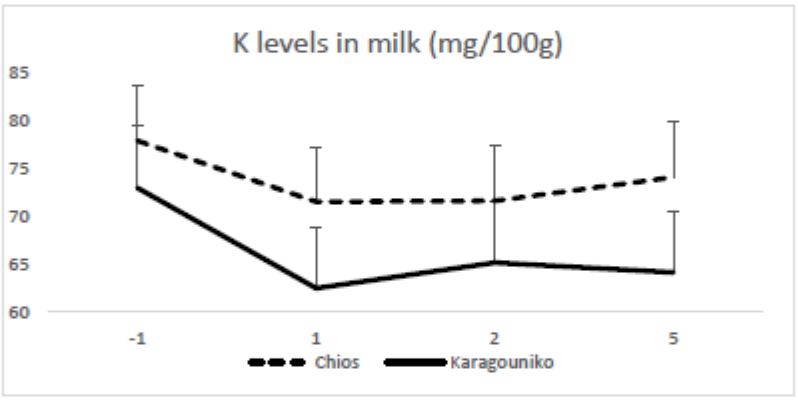


Fig. S2. Effect of breed (Chios and Karagouniko) on the levels (%) of milk protein, lactose and fat (-1: 1 day before cessation, 0: re-milking and 7, 14, 21 and 28: 7, 14, 21 and 28 days after re-milking)





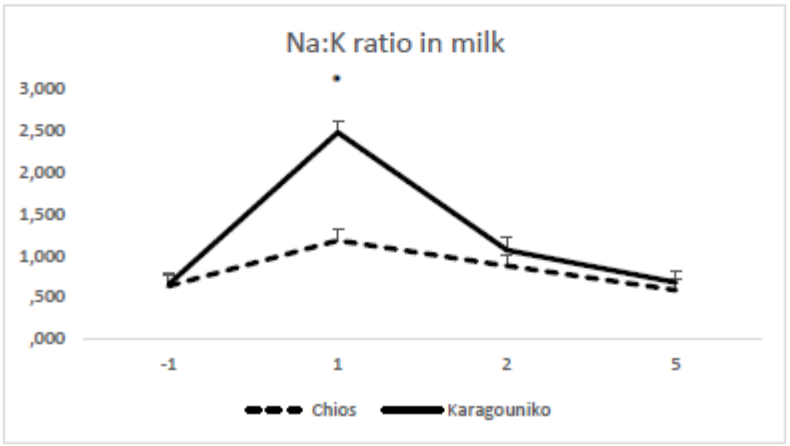


Fig. S3. Effect of breed (Chios and Karagouniko) on sodium (Na+) and potassium (K+) levels (mg/100g) and the Na+/K+ ratio in milk (-1: 1 day before cessation, 1, 2 and 5: 1, 2 and 5 days after re-milking) (*P<0.05)

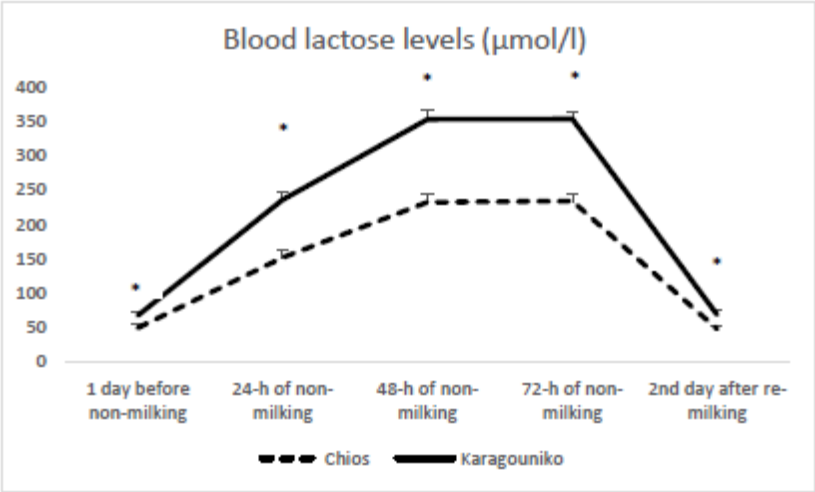


Fig. S4. Effect of 24-, 48- and 72-h period of non-milking on blood lactose levels in Chios and Karagouniko ewes (*P<0.0