Conventional and homeopathic treatments in late pregnant goats: effects on metabolic status and immune response

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ABSTRACT - The study was aimed at assessing the effects of conventional and homeopathic treatments on metabolic status and immune response in late pregnant goats. Administration of an anti-ketogenic preparation and of *Echinacea purpurea* in homeopathic dilution did not exert unequivocal effects on metabolic status, but improved some immunological parameters of periparturient goats.

Key words: Anti-ketogenic preparation, Echinacea, Metabolic status, Immune response.

Introduction - Previous studies demonstrated that peri-parturient dairy cows, sheep and goats are likely to experience metabolic disturbances and impairment of immunoresponsiveness (Kehrli et al., 1999; Lacetera et al., 2004; Lacetera et al., 2006). Furthermore, it has been also reported that the immune suppression, which may take place in transition dairy ruminants, may be at least partially dependent on the energy deficit due to the high demands of pregnancy (small ruminants) or lactation (dairy cows) (Lacetera et al., 2001; Lacetera et al., 2005). Finally, previous studies also documented that dietary interventions in peri-parturient dairy goats may affect both metabolic status and immunoresponsiveness (Agazzi et al., 2004; Celi et al., 2008). The present study was aimed at assessing the effects of conventional and homeopathic treatments on metabolic status and immune response in late pregnant goats.

Material and methods - The study was carried out in a commercial unit by using 38 healthy peri-parturient Camosciata delle Alpi goats. Three weeks before the expected parturition, assessed by echography, the 38 goats were randomly assigned to four experimental groups and fed the same basal diet (hay and concentrate) throughout the study. Control group (C) was administered subcutaneously 1 ml of physiological solution daily; the anti-ketogenic preparation group (A) was orally administered 30 ml of the commercial anti-ketogenic preparation from three weeks before up to kidding; the *Echinacea purpurea* group (E) was treated daily with 1 ml of *E. purpurea* in homeopathic dilution (30 centesimal) subcutaneously (treatment was suspended only during the last week of pregnancy); the group A+E received the combined treatments of group A and E. The anti-ketogenic preparation contained monopropylenic glycole (500 g/kg), sodium propionate (100 g/kg), sorbitol (52.5 g/kg), niacin (20 g/kg), and vitamin B12 (0.02 g/kg). Measurements and samplings were carried out from 3 weeks before up to 3 weeks after kidding. Blood samples were collected weekly from goats via jugular venipuncture, using two evacuated glass tubes coated with sodium heparin. One tube was used for plasma separation. Plasma was obtained by centrifuging blood at 3,500 xg for 15 minutes and was stored at -20°C until analyzed to measure glucose, nonesterified fatty acids (NEFA), and β-hydroxybutyrate (BHBA) concentrations, as described elsewhere (Lacetera et al., 2001). Plasma samples were also used to measure total immunoglobulin (Ig) G (see below). The other tube was utilized for separation of peripheral blood mononuclear cells (PBMC). PBMC were isolated by density gradient centrifugation, as already described (Lacetera et al., 2001). After isolation, PBMC were re-suspen-
ded at a concentration of 1x10^6 cells/ml in RPMI-1640 enriched culture medium. Triplicate cultures were assayed, by using 96-well tissue-culture plates. Each well contained 1x10^6 mononuclear cells in 100 µl of ECM. Control wells contained 100 µl of PBMC suspension without mitogens. Additional control wells were used that contained 100 µl of ECM without cells and 100 µl of PBMC suspension without the pyrimidine analogue 5-bromo-2'-deoxyuridine (BrdU). An optimal concentration of concanavalin A (ConA, 1.25 µg/ml) or pokeweed mitogen (PWM, 0.02 µg/ml) was added to plates. Plates were thus incubated in an atmosphere of 95% air and 5% CO2 for 48h at 39°C. Afterwards, 100 µM BrdU were added to each well, and plates were incubated for an additional 18h. The DNA synthesis was quantified by an ELISA assay. The assay was performed with a commercial kit (Amersham Biosciences, Milan, Italy) that is based on measurement of BrdU incorporated during DNA synthesis in proliferating cells. Values for DNA synthesis were expressed as the optical density (OD) for test wells minus the OD for control wells that did not contain BrdU. Finally, a blood sample was taken from kids at 48-72 hours after birth via jugular venipuncture, using evacuated glass tubes without anticoagulant. Serum was obtained by centrifuging blood at 3,500 x g for 15 minutes, and was stored at -20°C until analyzed to measure concentration of total immunoglobulin (Ig) G. This analysis was carried out by radial immunodiffusion using a commercial kit (Bethyl Laboratories, Inc., Montgomery, USA). Data were analysed by a one way ANOVA declaring the differences significant at P<0.05.

Results and conclusions – Treatments did not exert unequivocal effects on metabolic parameters of periparturient goats. Results of the ANOVA are reported in table 1.

| Metabolic parameters | Sum of squares | Mean square | F value | P value |
|----------------------|----------------|-------------|---------|---------|
| Glucose              | 0.193          | 0.064       | 0.215   | ns      |
| NEFA                 | 0.906          | 0.302       | 5.311   | <0.01   |
| BHBA                 | 1.714          | 0.571       | 1.955   | ns      |

ns=not significant; NEFA= non esterified fatty acids; BHBA= β-hydroxybutyrate.

Treatments did not affect plasma glucose, and in all cases, the lowest values were recorded at 11 and 4 days before parturition (not shown). However, plasma glucose was always above the lower critical value (30 mg/dl) for the onset of pregnancy toxaemia (Morgante and Stelletta, 2005). Conversely, treatments significantly influenced plasma NEFA. In details, compared to control group, groups A and A+E showed higher (P<0.01) concentrations of plasma NEFA at -11 and +4 days from kidding (not shown).

These results conflict with previous findings indicating decrease of plasma NEFA in sheep given propylene glycole, and are not easy to explain (Chiofalo et al., 2005). We can only hypothesize that this result may be at least partially dependent on the higher incidence of multiple pregnancy observed in A and A+E goats (mean ±SD: 2.00±0.47 and 2.00±0.00 kids per goat vs. 1.84±0.65 and 1.73±0.80 for the C and E groups, respectively). Treatments did not affect plasma BHBA, and in all groups the highest values were observed at 11 and 4 days before kidding. In the same sheep study cited above, Chiofalo et al. (2005) reported lower concentrations of ketones following dietary administration of propylene glycole. However, altogether our results confirm that late pregnancy has to be considered as the phase of the periparturient period with the higher risk of energy deficit for dairy goats (Barakat et al., 2007). Treatments did not affect plasma IgG both in goats and in their kids (not shown), and levels of serum immunoglobulins in post-coital kids testified that the transfer of colostral immunoglobulins throughout colostrums was adequate in all groups. Conversely, all treatments affected to a certain extent the response of PBMC to mitogens (Figures 1a and 1b). In general terms, such effect was positive, in that the ability of PBMC to proliferate in response to mitogens did not change in the treated groups during the trial, whereas in the control group it was subjected to an impairment, which was par-
particularly evident at 4 days before parturition. Results of the control group are in line with those from previous studies (Lacetera et al., 2006). Finally, in PBMC isolated from goats treated with E. purpurea, a high proliferation was also observed (P<0.05) before kidding for PBMC not stimulated with mitogens (data not shown). In conclusion, the experiment pointed out that treatments utilized may be of some help to improve the immune status of periparturient goats, and that their effects may not be explained by their effects on energy metabolism as evaluated by measuring plasma parameters.

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