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Correlations between phenolic fractions in tropical forages and ruminal C18 fatty acid metabolites *in vitro*

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Introduction Increasing the contents of α-linolenic acid (C18:3 *n*-3) and conjugated linoleic acids (CLA) in ruminant products is desirable due to their beneficial effects on human health. Quite a large proportion of dietary linoleic acid and α-linolenic acid undergo biohydrogenation processes in the rumen by the action of ruminal microorganisms which results in various intermediate fatty acids (FA) including CLA (mainly *c*9,*t*11-C18:2; Chilliard *et al.* 2007). Several feeding strategies have been proposed in order to increase α-linolenic acid such as feeding forage-based diets, adding various PUFA sources, using protected lipids and supplementing essential oils. In this respect, the role of plant phenolic compounds is also of increasing interest (Khiaosa-ard *et al.*, 2009, Cabiddu *et al.*, 2010). The present study aimed at establishing relationships between phenolic compounds in tropical forages, where these frequently occur in particular high concentrations (Jayanegara *et al.*, 2011), and proportions of different C18 fatty acids after *in vitro* incubation with rumen fluid.

Materials and methods Samples of 27 tropical plant species were obtained from two sampling sites near Bogor, Indonesia, consisting of one grass, four herbs, nine shrubs and 13 tree species. For each species, approximately 3 kg fresh weight of the leafy part was collected from several individuals each. The samples were oven-dried at 50°C and ground to pass a 1-mm sieve. Determination of chemical composition of the plants included proximate analysis (crude protein, ether extract), detergent analysis (neutral detergent fibre, acid detergent fibre, acid detergent lignin), phenolic fractions (total phenols, non-tannin phenols, total tannins, condensed tannins, hydrolysable tannins) and FA profiles. About 200 mg dry matter (DM) of plant samples were incubated for 24 h at 39°C with 30 ml of buffered-rumen fluid (in four replicates) using the Hohenheim gas test method (Menke and Steingass, 1988). The incubation was done by adding 50 mg linseed oil per g plant DM, emulsified in 1:99 v/v aqueous solution of Tween® 80. After incubation, the fermentation fluid was subjected to FA analysis through extraction, transesterification into fatty acid methyl esters (FAME) and separation using a gas chromatograph (Khiaosa-ard *et al.* 2009). The individual FA data were treated as mg/g of total FAME. Correlation analysis was performed between chemical composition and FA metabolites in fermentation fluid using SPSS Software version 17.0.

Results Concentrations of total phenols in the plants ranged from 14 to 235 g/kg DM. There was a clear positive relationship between total phenols in the plants and concentrations in fermentation fluid of C18:3 *n*-3 (correlation coefficient r=0.67), C18:2 *n*-6 (r=0.69) and C18:1 *n*-9 (r=0.75 (all P<0.001) after 24 h of incubation. The relationship was of a type that increasing concentrations of total phenols decreased the degree of biohydrogenation of these fatty acids. Total phenols in plants were also positively correlated with the occurrence of *c*9,*t*11-C18:2 after incubation (r=0.54; P<0.01), but negatively correlated with C18:0 (r=–0.46; P<0.05). Similar correlations as with total phenols were found with total tannins. However, no significant relationships were detected between non-tannin phenols and any of the C18 FA. Condensed tannins revealed a different pattern compared to hydrolysable tannins; the former were positively correlated with C18:1 *n*-9 (r=0.75; P<0.001) and *c*9,*t*11-C18:2 (r=0.59; P<0.01) and negatively correlated with C18:0 (r=–0.49; P<0.01), while the latter were positively correlated with C18:3 *n*-3 (r=0.61; P<0.01) and C18:2 *n*-6 (r=0.55; P<0.01). Therefore, hydrolysable tannins turned out protecting these fatty acids from the first step of biohydrogenation.

Conclusions The significant correlations found between total phenols and several ruminal C18 FA metabolites after ruminal incubation suggest the potential of phenols to modify FA biohydrogenation and to reduce FA biohydrogenation right from the first step, at least in case of tropical forages with basically high phenol concentrations. Within the phenolic fractions, total tannins exhibited a much stronger influence on lowering biohydrogenation than the non-tannin phenols. The relationship between condensed tannins and *c*9,*t*11-C18:2 suggests that these phenolic compounds could have a role in increasing this CLA isomer in ruminant-source foods. The relatively simple determination of total phenols appears to be useful for a preliminary screening of plants with respect to their inhibitory potential in terms of ruminal ALA biohydrogenation before testing these plants in more depth.

References

Cabiddu, A., Salis, L., Tweed, J.K.S. *et al.* 2010. Journal of the Science of Food and Agriculture. 90, 829–835.
Chilliard, Y., Glasser, F., Ferlay, A. *et al.* 2007. European Journal of Lipid Science and Technology. 109, 828–855.
Jayanegara, A., Wina, E., Soliva, C.S. *et al.* 2011. Animal Feed Science and Technology. 163, 231-243.
Khiaosa-ard, R., Bryner, S.F., Scheeder, M.R.L. *et al.* 2009. Journal of Dairy Science. 92, 177–188.
Menke, K.H., Steingass, H. 1988. Animal Research and Development. 28, 7–55.