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## Indole and skatole as markers for nitrogen utilisation in dairy cows

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### ABSTRACT

Indole and skatole are products of amino acid degradation in the rumen which could be useful to monitor protein utilisation in grazing ruminants. Two groups ("1970s" strain and "current" strain) of mixed age Holstein Friesian cows were housed indoors and fed cut pasture for 21 days. Concentrations of indole and skatole in milk fat and of milk urea nitrogen were determined. No significant differences were observed between strains of cow for indole and skatole concentration in milk fat, but they were different ( $P < 0.01$ ) between sampling dates. Both indole and skatole had moderate positive ( $r \sim 0.50$ ) correlation with the rumen degradable protein (RDP) balance, whilst skatole had a weaker ( $r \sim 0.30$ ) correlation with crude protein intake, metabolisable protein supply and RDP supply. Indole and skatole concentrations in milk fat were more closely correlated to the estimated RDP balance in the rumen than milk urea. A large proportion of individual animal variation in milk metabolites remains unaccounted for by protein intake, and may relate to individual differences in detoxification and elimination of indole and skatole.

**Key words:** protein utilisation; indole; skatole; pasture.

### INTRODUCTION

An understanding of how efficiently dairy cows convert consumed feed into milk is becoming increasingly important as the dairy industry strives to meet productivity targets while maintaining a competitive edge. Current work is investigating methods to identify dairy cows with high or low feed conversion efficiency in New Zealand's herds. Pasture-fed dairy cows often consume diets containing crude protein (CP), calculated as total nitrogen (N) multiplied by 6.25, in excess of their requirements for lactation (Brookes & Nicol, 2007). Therefore the ability to measure the utilisation of dietary protein is crucial to achieving industry targets.

Indole and skatole are two compounds which have the potential to be considered as indicators of protein metabolism. Indole and skatole have been identified as products of ruminal degradation of the amino acid tryptophan (Kremmer *et al.*, 1997, Deslandes *et al.*, 2001). When diets with high degradable protein concentration are fed, such as fresh temperate forages, amino acids from the excess protein are used as an energy source by microbes in the rumen (Bach *et al.*, 2005). Higher concentrations of indole and skatole, and several other products of amino acid degradation have been found in milk from pasture-fed cows compared with milk from cows fed a grain-based total mixed ration (Lane *et al.*, 2002). These authors concluded from an analysis of the pattern of seasonal variation that the concentration of indole and skatole in the milkfat could not be explained by the CP concentration of the pasture alone. Cosgrove *et al.* (2006) demonstrated that diets containing a higher proportion of clover resulted in much higher indole and skatole concentrations in milk compared to

cows fed only ryegrass. The authors proposed that the differences observed in indole and skatole concentrations were most likely the result of differences in the intake and degradability characteristics of the dietary protein, factors which in turn define the amount of rumen degradable protein (RDP) eaten by cows.

However, these studies provided no direct experimental evidence on the strength of the relationship between indicator metabolites in milk and the supply, digestion and utilisation of protein from cows fed known amounts of forage.

The objective of this experiment was to examine the relationship of dietary protein supply and indole and skatole concentrations in the milk fat. The concentration of these two metabolites in milk was also compared to milk urea N (MUN), an accepted indicator of efficiency of protein utilisation in lactating cows (Jonker *et al.*, 1998; Burgos *et al.*, 2007). We hypothesised that indole and skatole concentrations are direct indicators of protein utilisation in lactating cows, thus differences in the concentration of these metabolites in milk could be markers of rumen metabolism of dietary protein surpluses. The amount of RDP supplied in excess of requirements is an important dietary attribute affecting the overall efficiency of N utilisation by lactating cows. This work is part of a larger project aimed at determining the factors underlying individual variation in the efficiency of feed conversion of New Zealand dairy cows.

### MATERIALS AND METHODS

The experiment was carried out with the approval of the Ruakura Animal Ethics Committee. Thirty mixed-age, Holstein-Friesian cows (age 4 - 7

year-old,  $88 \pm 16$  days in milk; mean  $\pm$  standard deviation) consisting of two genotypes (1970s strain  $n = 9$  and current strain  $n = 21$ ) were housed indoors and fed cut pasture for 21 days.

The cows were milked twice daily at approximately 07:00 hours (AM) and 14:30 hours (PM). Milk yields were measured using in-line meters at each milking. Composite samples from the PM and following AM milking were collected on three occasions during the final week of the experimental period for milk composition analysis. On each sampling date, a milk subsample was analysed for fat, protein, casein, lactose and total solids using an infrared spectroscopic milk analyser (FT120, Foss Electric, Hillerød, Denmark). A second sub-sample was centrifuged at 10,800 g for 30 minutes in a refrigerated centrifuge at 4°C to separate fat and skim milk. Indole and skatole concentrations in milk fat were determined by high performance liquid chromatography with fluorescent detection as described by Lane *et al.* (2002). Urea concentration in the skim milk samples were measured using the urease ultraviolet assay (Alpha Scientific, Hamilton, New Zealand). MUN concentration was calculated on a molar basis by multiplying the molar concentrations of milk urea by 2.

Cut pasture was fed *ad libitum* plus 10%, three times a day at 08:30 hours, 15:30 hours and 21:00 hours. Daily intake was determined by weighing

feed offered and feed refused. Composition of feed offered and refused was determined by near infrared reflectance spectroscopy (FeedTECH, Palmerston North, New Zealand). Each cow's live weight was recorded at the start and end of the 21 day experimental period.

The National Research Council Dairy Cow model (National Research Council, 2001) was used to estimate variables to assess the efficiency of nitrogen utilisation in ruminants, namely crude protein (CP), rumen degradable protein (RDP) and metabolisable protein (MP) intakes. Briefly, dry matter (DM) intakes and chemical composition of the forage were used to estimate the RDP balance in the rumen. This was estimated as RDP intake minus requirements for microbial protein synthesis. For these calculations, the soluble protein, the potentially degradable protein and the rate of the protein degradation were assumed to be 50% of the CP, 40% of the CP and 0.16 L/h, respectively (Burke, 2004). Estimated MP intake was compared against the observed yield of milk protein to calculate the MP balance. This was estimated as MP intake minus the requirement for maintenance and milk production. RDP and MP balances provide an assessment of the adequacy of dietary N supply relative to requirements of rumen microbes and the ruminant, respectively. Efficiencies of CP and MP utilisation for milk production were calculated as the ratio of protein secreted in milk divided by the CP and MP supplied to the cows.

Pearson's correlation coefficients between variables related to dietary N supply (intakes of CP, RDP and MP, and the RDP and MP balance) and metabolite concentrations in milk postulated as indicator variables of N utilisation (indole, skatole and MUN), were calculated using the CORR procedure in SAS (SAS Institute Inc., 2002). The

**TABLE 1:** Mean and standard deviation of forage composition variables, estimation of dietary nitrogen supply and milk production characteristics during the experiment.

Variable	Mean	Standard deviation
Forage chemical composition:		
Crude protein (g/100 g DM)	18.4	1.4
Lipid (g/100 g DM)	3.4	0.1
Ash (g/100 g DM)	9.2	0.2
Acid detergent fibre (g/100 g DM)	24.2	0.8
Neutral detergent fibre (g/100 g DM)	42.5	2.4
Soluble sugars and starch (g/100 g DM)	19.3	1.2
Metabolisable energy (MJ/kg DM)	12.4	0.2
Dietary nitrogen supply:		
Dry matter intake (kg/d)	20.7	2.3
Crude protein intake (kg/d)	3.8	0.4
Rumen degradable protein intake (kg/d)	2.1	0.2
Metabolisable protein intake (kg/d)	2.5	0.3
Milk production and composition:		
Milk yield (kg/d)	23.0	3.9
Milk fat (%)	4.5	0.5
Milk protein (%)	3.6	0.2
Milk fat indole (ng/g fat)	79.1	64.4
Milk fat skatole (ng/g fat)	125.5	76.2
Milk urea nitrogen (mmol/L skim milk)	11.8	1.5

**TABLE 2:** Pearson's correlation coefficients ( $r$ ) between the concentration of postulated indicator metabolites in milk and variables of dietary nitrogen supply. Probabilities for the null hypothesis that  $r = 0$  are given in parentheses. CP = crude protein; RDP = rumen degradable protein; MP = metabolisable protein.

Variable	Indole	Skatole	Milk urea nitrogen
CP intake	0.17 (0.11)	0.30 (<0.01)	0.26 (0.01)
RDP intake	0.17 (0.11)	0.30 (<0.01)	0.27 (0.01)
MP intake	0.16 (0.14)	0.29 (0.01)	0.26 (0.02)
RDP balance	0.51 (<0.001)	0.51 (<0.001)	0.45 (<0.001)
MP balance	0.18 (0.08)	0.17 (0.10)	0.22 (0.04)
CP transfer to milk protein	-0.03 (0.79)	0.05 (0.67)	-0.05 (0.63)
MP transfer to milk protein	-0.11 (0.29)	-0.03 (0.73)	-0.12 (0.24)

effects of strain and sampling on milk metabolite concentrations and protein supply variables was assessed using the MIXED procedure in (SAS Institute Inc., 2002) with sampling date and strain as fixed effects, cow within strain as random effect and a simple variance - covariance structure.

## RESULTS

The composition of the forage offered to the cows during the experimental period is presented in Table 1. Mean crude protein intake was 3.81 kg/cow/d with a range of 2.85 to 4.71 kg/cow/d. Cows from the 1970s strain had a lower DM intake than the current strain (19.3 vs 21.3 kg DM/d,  $P < 0.01$ ), resulting in a lower mean intake of CP (3.6 vs 3.9 kg/d), RDP (2.0 vs 2.2 kg/d) and MP (2.3 vs 2.6 kg/d).

Indole and skatole concentrations in milk fat were different ( $P < 0.01$ ) between sampling dates. No differences were observed between the 1970s and the current strains of cows for milk concentrations of indole (71 vs 74 ng/g milk fat,  $P = 0.75$ ), skatole (106 vs 129 ng/g milk fat,  $P = 0.17$ ) and MUN (11.5 vs 12.0 mmol/L skim milk,  $P = 0.23$ ). Since concentrations of indole, skatole and MUN were not different between cow strains, the correlation analysis reported below refers to aggregated data for both strains.

The concentrations of indole and skatole in milk fat were strongly correlated ( $r = 0.74$ ,  $P < 0.01$ ). Both indole and skatole in milk fat were each moderately correlated ( $r \sim 0.44$ ;  $P < 0.01$ ) with MUN in skim milk.

Indole, skatole and MUN were correlated with variables related to the dietary supply of N (Table 2). The strongest correlation was with the RDP balance, which is the protein excess relative to the rumen microbial requirements. However, when a regression analysis was performed, the estimated RDP balance could explain only 27%, 27% and 21% of the variation in indole, skatole and MUN concentrations, respectively. No significant correlations were found between the milk metabolite concentrations studied and the efficiencies of utilisation of CP or MP for milk protein production. ( $P > 0.24$ ).

A scatterplot of the individual observations for estimated RDP balance and skatole concentrations in milk (Figure 1) shows that whilst high skatole concentrations are associated with higher estimated RDP balance, there is large variation in individual responses.

## DISCUSSION

The concentrations of indole and skatole were similar to those published by Lane *et al.* (2002) for cows eating ryegrass based pasture at a similar time of the year. However, the mean CP of the forage in this experiment was only 18% (Table 1), whilst Lane *et al.* (2002) reported CP concentration of

24 to 27% of the DM. This supports the observations that CP of the forage is not the main determinant of milk metabolites resulting from rumen digestion of dietary protein, with variables such as intake and protein degradability having greater influence on milk indole, skatole and MUN (Lane *et al.*, 2002; Cosgrove *et al.*, 2006).

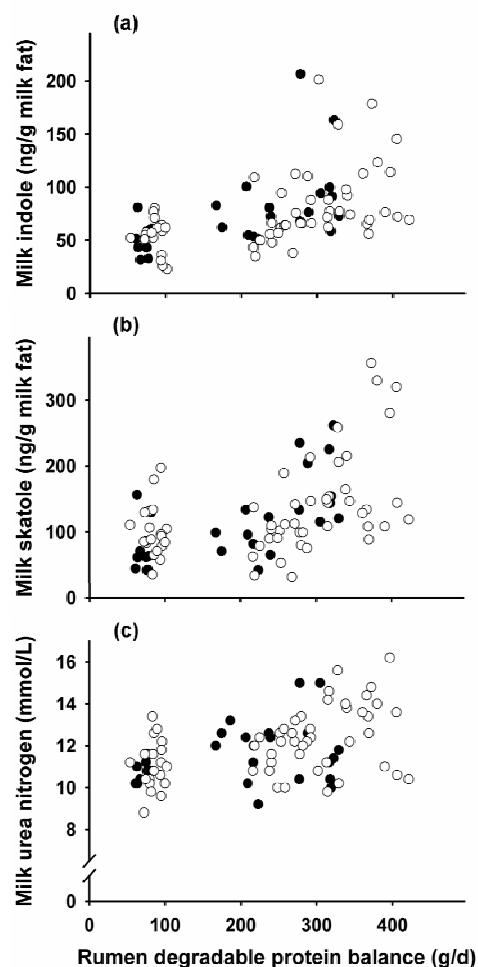
This study established that out of the several variables describing dietary supply of protein, the RDP balance was more closely associated to the observed concentrations of indole and skatole in the milk fat. Whilst this association has been previously hypothesised (Lane *et al.*, 2002; Cosgrove *et al.*, 2006) this study provided a direct measure of the association and information on the magnitude of the change in the milk concentrations of these metabolites in response to changes in the supply of dietary protein.

In this short term experiment, it was apparent (Figure 1) that high concentrations of some nitrogenous milk metabolites were associated with higher surpluses of RDP. However, it was also clear that wide variation in the concentrations of indole, skatole and urea in milk occur between individual animals consuming diets with a similar supply of protein. Individual variation that cannot be explained by the surplus of RDP may be accounted for by individual differences in the ability to metabolise and eliminate skatole as reported by Deighton *et al.* (2006) for sheep receiving controlled ruminal infusions.

Day-to-day variation was found to be significant for most of the dietary variables and milk metabolites measurements. Due to the limited sampling regime, this day-to-day variation made it difficult to determine how consistent the individual responses in the milk metabolite concentrations were relative to changes in protein supply. Furthermore, indole, skatole and MUN concentrations are affected by the timing of the main feeding bouts relative to the milking time (Lane *et al.*, 2008). In the present study, the estimation of RDP balance was performed using a static, feed composition-driven model of rumen metabolism. The use of dynamic models of rumen metabolism could offer opportunities to explore whether individual differences in indole, skatole and MUN occur as a result of temporal differences in feeding bouts between animals eating the same diet.

The concentration of MUN in milk has been used routinely to monitor adequacy of dietary N supply in herds with cows fed total mixed rations (Jonker *et al.*, 1999). Results from this experiment show that indole and skatole concentrations appear to show a two to four fold change to increased RDP surplus compared with a one and a half fold change in the concentration of MUN. One explanation for this finding could be that rapid detoxification and elimination processes are required for metabolites

**FIGURE 1:** Scatterplot of the individual observations for estimated rumen degradable protein balance and (a) indole; (b) skatole and (c) milk urea nitrogen concentrations in milk from cows a “current” strain (open symbols) and a “1970s” strain (closed symbols).



such as indole and skatole (Deighton *et al.*, 2007), whilst recycling mechanisms operate for urea (Lapierre *et al.*, 2005) and may diminish the variation in MUN.

Our results suggested that the concentrations in milk of indole and skatole provide a measure of the adequacy of the amount and degradability of the dietary protein supply to lactating cows. However, as these milk metabolites show variation between individuals unaccounted for by variations in RDP intake, more research is required to determine whether this reflects variation in individual detoxification and elimination of these compounds, or variations in the efficiency of nitrogen utilisation among individuals in the New Zealand herd.

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