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# EFFECT OF FEED PROCESSING ON VOLATILE FATTY ACID PRODUCTION RATES MEASURED WITH $^{13}$C-ACETATE IN GRAZING LACTATING DAIRY COWS

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## SUMMARY

***The effect of processed cereal grain supplementation on volatile fatty acid (VFA) production rates of grazing, lactating Holstein-Friesian cows were measured in a 5x5 Latin square experiment. The experimental treatments were as follows: control (only grazing, no supplement addition, NS), pelleted barley (PB), pelleted maize (PM), toasted and subsequently pelleted barley (TPB), and toasted and subsequently pelleted maize (TPM) as supplements. An isotope dilution technique using stable isotope of carbon ($^{13}$C) as an internal marker was employed for the estimation of VFA production. At the beginning of a 3-hour long allowed grazing time, 100 mg of 99% enriched $^{13}C_2$ Na-acetate were introduced in the rumen and repeated after grazing with 50 mg isotope, after which the cows were starved for 6 hours until evening milking. During grazing disappearance rate ($k_{dis}$) and production rate ($k_{prod}$) of acetate, propionate and butyrate were significantly higher ($P \leq 0.05$) in supplemented than in NS cows. Moreover the effect of barley grain and pelleting treatment was higher than the effect of maize grain and toasting. During starvation significantly higher ($P \leq 0.05$) $k_{dis}$ and $k_{prod}$ of VFAs were observed in PM and TPM treatments. Total VFA production for the experimental period (grazing + starvation) were 49.5, 78.7, 69.9, 88.5, 80.8 mol/day for NS, PB, TPB, PM and TPM, respectively. The higher VFA productions measured in supplemented animals emphasis the extensive digestion that occurs in the rumen after feeding processed grains. In methodological terms, $^{13}C_2$ Na-acetate labelling appears to be a useful means for examining the VFA acetate production in ruminants.***

***Key-words: dairy, grazing, supplementation, VFA production, stable isotope***

## INTRODUCTION

Rate of change in ruminal VFA is the difference between rate of production from fermentation and rates of absorption and passage. The majority of the VFA produced in the rumen are absorbed from the rumen through the rumen wall by simple diffusion of the undissociated acids (Allen, 1997). Ruminal pH and osmolality, type and concentration of VFA significantly affect absorption rates of VFA from the rumen. The absorbed VFA are important because they provide two-thirds of the energy supply of the ruminant (Sutton, 1985). Because of the importance of VFA in ruminant metabolism several methods have been developed to measure VFA production in the rumen. Indirect methods make use of the stoichiometric relationships which exist between the production of VFA and the production of methane or between the production of VFA and the amount and composition of the mixture of substrates fermented (Murphy et al., 1982). The agreement between _ VFA molar proportions in rumen fluid and VFA proportions produced have been questioned, because the proportions of VFA in the rumen may not precisely reflect their relative production rates, especially when the diet contains a substantial proportion of concentrates (Sutton, 1985). The $^{13}$C labelling technique was initially used in agronomy (Thompson, 1996), but became gradually also applied in animal nutrition after labelling forages (Pellikaan, 2004). Appropriate aspects to be investigated are the digesta passage, the diet digestibility and carbon metabolism in the body (Boutton, 1991a; Svejcar et al., 1993). With $^{13}$C enriched VFA the ruminal VFA production can also be measured (Kristensen, 2001). Supplementation of lactating grazing dairy cows with processed cereal grains affects the

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ruminal fermentation and VFA production. Therefore the objective of this study was to quantify the influence of fresh grass and processed cereal grains on the production of VFA in the rumen of grazing, lactating dairy cows using $^{13}C_2$ Na-acetate as an internal marker.

## MATERIAL AND METHODS

**Animals and management.** Five multi parity Holstein-Friesian dairy cows (173±28 DIM; mean±SD) fitted with a rumen fistula (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) were used. The animals were milked twice daily at 0630 and 1700 h. **Experimental design.** The experiment was based on a 5x5 Latin Square design with five cows, five treatments and five periods. Each experimental period lasted for 14 days. Days 1$^{st}$ to 9$^{th}$ were used for adaptation and days 10$^{th}$ to 14$^{th}$ for sample collection. **Treatments and feed processing.** The five treatments were as follows: control (no supplement addition, NS) pelleted barley (PB), pelleted maize (PM), toasted and subsequently pelleted barley (TPB), and toasted and subsequently pelleted maize (TPM). A laboratory scale pressurised toaster was used for pressure toasting the grains for 1.5 minute at 135 $^{o}$C. After toasting, the grains were dried in a forced air oven for 16 h at 35 $^{o}$C followed by pelleting. Pelleting (80 $^{o}$C, 10 s) was carried out with a 5 x 65 mm (bore x hole) die, using a V2-30 pelleting press (Robinson milling system B.V., Boxtel, The Netherlands). **Sample collection.** Next to a control treatment of grazing only, the four forms of processed grains were fed as a supplement in the milking parlour (3 kg at morning and 3 kg at evening milking). Rumen evacuations (Børsting and Weisbjerg, 1989) were conducted after morning milking and 100 mg of 99% enriched $^{13}C_2$ Na-acetate (Euriso-top, Bât, France) was introduced in the rumen for estimation of VFA production. The cows were allowed to graze individually. After 3 hours of grazing each cow was brought to the barn. Rumen evacuations and enrichment procedure was repeated with 50 mg of 99% enriched $^{13}C_2$ Na-acetate. Then the animals were kept inside the barn and starved until evening milking. Rumen liquid samples were taken each half an hour starting at 08.00 h until 11.00 h then every hour until 17.00 h. **Chemical analyses.** Rumen liquid samples were kept frozen for isolating VFA by distillation with the Kjeldahl distillation equipment. After isolating VFA by distillation samples were analysed using isotope ratio mass spectrometry (IRMS) as described by Boutton (1991b) to determine the $^{13}C$ to $^{12}C$ ratios. **Calculations for for $^{13}C$ enrichment.** For the estimation of acetate production rates, the procedure based on the stable isotope $^{13}C$ labelling technique developed by Chen et al. (1997) was used. Acetate production is assumed to occur at a fixed fractional rate ($k_{prod}$) and is estimated from the decline in the $^{13}C$ to $^{12}C$ ratio. The percentage enrichment with $^{13}C$ was calculated for each collection time in each cow in each respective period. From IRMS we had the total carbon concentration in each sample of ruminal fluid and the percentage of $^{13}C$ present into these samples. The first evacuation time was taken as the basal level of enrichment. Regression equation was applied to these corrected values to find the fractional rate of enrichment disappearance. Rumen pH influence rates of VFA absorption, therefore we assumed that the disappearance of propionate and of butyrate was 1.5 times that of acetate. **Calculations for hexose fermentation.** To validate or refute our estimates of VFA production measured with the $^{13}C$ labelling technique we calculated fermentable organic matter (FOM) from our *in vivo* and *in situ* measurements using several approaches. In the first approach ($FOM_{VFA}$) total hexose (kg) fermented in the rumen was calculated from VFA production. In the second approach ($FOM_A$) the available organic matter (kg) was calculated from the *in vivo* measurements (Tóthi et al., 2003). In the third approach ($FOM_B$) we assumed that the water-soluble fraction of the grass was instantly and totally degraded in the rumen. Therefore we modified the $FOM_A$ calculation formula with *in sacco* data measured in our former experiments (Tóthi, 2003). In the fourth approach we assumed that rumen degradation of the degradable fractions of each OM component (rumen content, fresh grass, and supplements) occur according to the first order kinetic function ($FOM_C$). **Statistical Analysis.** All data were subjected to least squares ANOVA for a 5 x 5 Latin square design using the GLM procedure (SAS, 2001). When significant differences due to the treatment were detected, the multiple comparison procedures were used.

## RESULTS AND DISCUSSION

**Disappearance and production rates of individual acids.** During grazing $k_{dis}$ of VFAs were lower than the $k_{prod}$ for each individual VFA because the VFA pool size increased during grazing time when

cows were eating and new substrate entered and fermented in the rumen (Table 1). In comparison to NS animals supplementation significantly increased $k_{dis}$ for PB, PM and TPM. The $k_{prod}$ of supplemented cows were also significantly higher than NS cows because of availability of more fermentable starch at grazing time. In starvation period $k_{dis}$ of VFAs were higher in each treatment than $k_{prod}$ because the VFA pool size decreased during the starvation period. The differences in $k_{dis}$ existed in the grazing period between NS and supplemented cows with barley grain were eliminated, while $k_{dis}$ of maize grain was still significantly higher than NS animals. This phenomenon was also observed in $k_{prod}$ of VFAs. Higher starch concentration and slower ruminal starch degradation rate of maize, make more fermentable substrate available for the microbes during the starvation period. During grazing pelleted cereal grains supplemented animals had higher $k_{dis}$ and $k_{prod}$ than toasted and subsequently pelleted cereal grains supplemented cows. However, this was inverse during the starvation period and toasted and subsequently pelleted cereal grains supplemented cows had higher $k_{dis}$ and $k_{prod}$ than pelleted cereal grains supplemented animals. This indicates that toasting might have a protective effect of the starch in maize and barley resulting in delaying its fermentation for awhile.

**Table 1. Disappearance ($k_{dis}$) and production ($k_{prod}$) rates (/h) of acetate (HAc), propionate (HPr) and butyrate (HBu) in the rumen of dairy cows grazing grass pasture[1], and supplemented with processed cereal grains[2]**

| | NS | Barley grain | | Maize grain | | SEM | P3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PB | TPB | PM | TPM | | G | H | GxH |
| **Grazing period** | | | | | | | | | |
| HAc $k_{dis}$ | $0.69^{S}$ | 1.34 | 0.94 | 1.19 | 1.08 | 0.14 | 0.9 | 0.1 | 0.3 |
| HPr $k_{dis}$ | $1.04^{S}$ | 2.00 | 1.14 | 1.79 | 1.63 | 0.21 | 0.9 | 0.1 | 0.3 |
| HBu $k_{dis}$ | $1.04^{S}$ | 2.00 | 1.14 | 1.79 | 1.63 | 0.18 | 0.9 | 0.1 | 0.4 |
| HAc $k_{prod}$ | $0.80^{S}$ | 1.35 | 1.04 | 1.30 | 1.14 | 0.12 | 0.7 | 0.1 | 0.5 |
| HPr $k_{prod}$ | $1.18^{S}$ | 2.03 | 1.55 | 1.93 | 1.72 | 0.17 | 0.8 | 0.1 | 0.5 |
| HBu $k_{prod}$ | $1.14^{S}$ | 2.01 | 1.52 | 1.91 | 1.69 | 0.17 | 0.8 | 0.1 | 0.5 |
| **Starvation** | | | | | | | | | |
| HAc $k_{dis}$ | $0.62^{T}$ | $0.62^{a}$ | $0.65^{a}$ | $0.83^{a}$ | $1.04^{b}$ | 0.08 | 0.008 | 0.2 | 0.3 |
| HPr $k_{dis}$ | $0.92^{T}$ | $0.94^{a}$ | $0.97^{a}$ | $1.25^{ab}$ | $1.56^{b}$ | 0.13 | 0.008 | 0.2 | 0.3 |
| HBu $k_{dis}$ | $0.92^{T}$ | $0.94^{a}$ | $0.97^{a}$ | $1.25^{ab}$ | $1.56^{b}$ | 0.12 | 0.007 | 0.2 | 0.2 |
| HAc $k_{prod}$ | $0.46^{T}$ | $0.54^{a}$ | $0.57^{a}$ | $0.74^{ab}$ | $0.97^{b}$ | 0.09 | 0.01 | 0.2 | 0.3 |
| HPr $k_{prod}$ | $0.73^{T}$ | $0.84^{a}$ | $0.88^{a}$ | $1.14^{ab}$ | $1.48^{b}$ | 0.12 | 0.009 | 0.2 | 0.3 |
| HBu $k_{prod}$ | $0.75^{T}$ | $0.84^{a}$ | $0.89^{a}$ | $1.15^{ab}$ | $1.49^{b}$ | 0.12 | 0.01 | 0.2 | 0.3 |

[1] NS: no supplement addition. [2] PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize. [3] G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction. $^{S}$ figure with superscript is significantly ($P<0.05$) different from PB, PM and TPM. $^{T}$ figure with superscript is significantly ($P<0.05$) different from PM and TPM. $^{a,b}$ figures with different superscript in the same row differ significantly ($P<0.05$).

**Volatile fatty acid production.** In grazing as well as in starvation total VFA (TVFA) production and the individual VFA production were higher in supplemented animals than in NS animals (Table 2). Supplementation with PB resulted in significantly higher HAc production in grazing because PB contains the highest amount of water soluble starch which is a fast energy source for the ruminal microbes. The opposite occurred during starvation than in grazing period where the differences between NS and PB were eliminated and due to feeding maize, significantly higher HAc, HPr, Hbu and TVFA productions were observed.

**Table 2. Acetate, propionate, butyrate and total VFA production (mol/period) in the rumen of dairy cows grazing grass pasture[1], and supplemented with processed cereal grains[2]**

| | NS | Barley grain | | Maize grain | | SEM | P3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PB | TPB | PM | TPM | | G | H | GxH |
| **Acetate** | | | | | | | | | |
| Grazing | 14.96$^{S}$ | 25.10 | 18.06 | 22.71 | 16.82 | 3.4 | 0.6 | 0.1 | 0.9 |
| Starvation | 14.15$^{T}$ | 14.82$^{a}$ | 18.66$^{ab}$ | 23.58$^{ab}$ | 25.22$^{b}$ | 2.8 | 0.02 | 0.3 | 0.7 |
| **Propionate** | | | | | | | | | |
| Grazing | 6.44$^{S}$ | 16.74 | 10.27 | 13.21 | 10.38 | 2.5 | 0.5 | 0.1 | 0.5 |
| Starvation | 6.21$^{T}$ | 9.22$^{a}$ | 10.93$^{ab}$ | 14.98$^{b}$ | 14.73$^{ab}$ | 1.7 | 0.02 | 0.7 | 0.6 |
| **Butyrate** | | | | | | | | | |
| Grazing | 3.86$^{S}$ | 8.01 | 5.84 | 6.60 | 5.23 | 1.1 | 0.4 | 0.1 | 0.7 |
| Starvation | 3.86$^{T}$ | 4.81$^{a}$ | 6.10$^{ab}$ | 7.41$^{ab}$ | 8.42$^{b}$ | 0.9 | 0.04 | 0.3 | 0.9 |
| **TVFA**[4] | | | | | | | | | |
| Grazing | 25.27$^{S}$ | 49.86 | 34.18 | 42.52 | 32.44 | 6.7 | 0.5 | 0.1 | 0.7 |
| Starvation | 24.24$^{T}$ | 28.87$^{a}$ | 35.69$^{ab}$ | 45.98$^{b}$ | 48.38$^{b}$ | 5.4 | 0.02 | 0.4 | 0.7 |

[1] NS: no supplement addition. [2] PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize. [3]G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction. [4] TVFA (total VFA) = HAc+HPr+HBu; [S]figure with superscript is significantly (P<0.05) different from PB; [T]figure with superscript is significantly (P<0.05) different from PM and TPM; [a,b]figures with different superscript in the same row differ significantly (P<0.05)

Supplementing pelleted cereal grains resulted in higher VFA productions during the grazing period compared to toasted grains and this was inverse in starvation. This mean that toasting has a protective effect on cereal grains starch and this is consistent with the fact that even processed maize starch is much slower degraded in the rumen than barley starch. TVFA productions for the whole experimental period were 49.5, 69.9, 78.7, 80.8 and 88.5 mol for NS, PB, TPB, PM and TPM, respectively. The molar proportions of HAc:HPr:HBu were 60:25:15 (58:26:16) for NS and 50:34:16 (51:32:17), 53:30:17 (52:31:17), 53:31:16 (51:33:16) and 52:32:16 (52:30:17) for PB, TPB, PM and TPM, respectively. These results indicate that supplementation of pasture grass with processed cereal grains favours the development of HPr producing bacterial species and are associated with an increase in the proportion of HPr at the expense of HAc, although acetate is always the most abundant of the acids. The higher VFA productions measured in supplemented animals emphasise the extensive digestion that occurs in the rumen after feeding processed cereal grains.

**Hexose fermentation.** The results of $FOM_{VFA}$ calculated based on our VFA production data are higher than $FOM_A$, $FOM_B$, and $FOM_C$ values in each experimental period (Table 3). Production rates of VFA as measured by isotope dilution techniques showed a wide variability and errors as been discussed previously by Sutton (1985) and Dijkstra (1994). In some other studies carbon exchange between HAc and HBu was considered a source of error because acetate label was detected in butyrate. Label from HAc has also previously been detected in HPr and other VFA (Kristensen, 2001). Substantial amount of endogenous acetate might also increase the acetate pool in the rumen (Van Soest, 1994). Salivary mucin a carbohydrate source for the ruminal microbes, that results in VFA production (Bansil et al., 1995). Also the $^{13}C$ to $^{12}C$ ratio and the $k_{prod}$ calculations might be affected by the naturally occurring differences in the ratio of the stable carbon isotopes $^{12}C$ and $^{13}C$ between plant species belonging either to the $C_3$ group (cool-season, barley) or to the $C_4$ group (warm season, maize). The $^{13}C$ content of $C_4$ plants is higher than that of $C_3$ plants (O'Leary, 1981), being an explanation for the high values calculated for maize. More research is needed to elucidate the uncertainties and possible variation in the assumptions needed to make this validation.

**Table 3. Estimated amount of fermented carbohydrate (kg/period) in the rumen of dairy cows grazing grass pasture[1], and supplemented with processed cereal grains[2]**

| | NS | Barley grain | | Maize grain | |
|---|---|---|---|---|---|
| | | PB | TPB | PM | TPM |
| **Grazing period** | | | | | |
| $FOM_{VFA}$ | 2.36 | 4.69 | 3.24 | 3.98 | 3.05 |
| $FOM_A$ | 1.59 | 2.35 | 1.91 | 1.32 | 1.96 |
| $FOM_B$ | 2.22 | 2.87 | 2.50 | 1.69 | 2.43 |
| $FOM_C$ | 1.89 | 3.14 | 3.12 | 2.46 | 2.60 |
| **Starvation** | | | | | |
| $FOM_{VFA}$ | 2.27 | 2.73 | 3.38 | 4.32 | 4.60 |
| $FOM_A$ | 1.59 | 2.57 | 2.99 | 2.39 | 2.16 |

[1]NS: no supplement addition; [2] PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize

## CONCLUSION

Supplementation of pasture grass with different processed cereal grains significantly increased VFA production in dairy cows compared to the non supplemented animals. Toasting and subsequently pelleting of cereal grains might have a protective effect on barley and maize starch, which delay its ruminal fermentation or shift a certain amount of starch to the small intestine. From a methodological point of view $^{13}C_2$ Na-acetate labelling appears to be an easy and useful way for examining the VFA production of ruminants but VFA production estimates should be treated carefully. Notably the uncertainty of the assumptions needed to be made for its validation needs further attention. Higher enrichments than those used here (100 mg +50 mg) and the use of other labelled VFA than $^{13}C_2$ Na-acetate ($^{13}C$ Na-propionate and $^{13}C$ Na-butyrate) may improve the reliability of this labelling technique.

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