Milk Production from Leguminous Forage, Roots and Potatoes

Effects on microbial protein supply and nitrogen efficiency

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Abstract

The aim of the present work was to investigate the effects of replacing grain concentrates with roots and potatoes in dairy cow diets based upon large amounts of grass/alfalfa silage. The emphasis was on the possible improvement of microbial protein synthesis and nitrogen balance. Alfalfa dominated silage has a large excess of ruminally degradable protein that must be balanced with feed carbohydrates to avoid urinary nitrogen losses. The effects on ruminal fermentation pattern, intake and production were also studied.

The thesis is based on two batch culture in vitro experiments and three animal experiments. The in vitro experiments compared fodder beets, barley/oats and raw, boiled or frozen potatoes as supplements to a silage diet incubated with rumen fluid from cows fed different diets. With respect to amounts fermented during 5 h incubation, supplements were ranked ($P < 0.05$) fodder beets = boiled potatoes > barley/oats > raw potatoes = frozen potatoes = unsupplemented silage. Substrates were numerically ranked in the same order with respect to microbial protein production, but due to larger variation they could only be divided into two groups, where fodder beets, boiled potatoes and barley/oats gave microbial yields not different from each other, but higher than for raw potatoes, frozen potatoes or unsupplemented silage. Butyrate proportion was little affected by incubation substrate but fodder beets fed to rumen fluid donor cows increased butyrate molar proportion in vitro from 10.7 to 13.0%.

A change-over design experiment compared barley supplementation with fodder beet and potato supplementation of a silage diet for lactating cows. The fodder beet/potato diet lowered ad libitum silage intake by 0.9 kg DM/d and milk yield decreased correspondingly by 1.7 to 2.3 kg/d. Microbial protein production and nitrogen balance were not increased by the fodder beet supplementation, but a part of N excretion was redirected from urine to feces. Fodder beets tended to decrease the ratio lipogenic/glucogenic VFA, by increasing propionate and butyrate at the expense of acetate.

In an intake experiment, most of the cows consumed the maximum allowance of fodder beets (4.6 kg DM/d) while there was a huge variation in the potato intake. A more synchronous feeding of degradable protein and readily available carbohydrates lowered the urinary nitrogen loss and increased allantoin excretion numerically but not significantly. A close correlation ($R^2 = 0.94$) was found between total urinary N excretion and the ratio urea/creatinine in urine, which implies that spot sampling of urine may be a way to facilitate N balance measurements in lactating cows.

In conclusion, a full replacement of grain by roots and potatoes can be done and the effects will be lowered urinary N losses but also a reduction in silage consumption and hence also milk production.

Keywords: allantoin, ATP, creatinine, hindgut fermentation, in vitro technique, N efficiency, potato starch, rumen, sucrose

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Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Eriksson, T. & Murphy, M. Ruminal digestion of leguminous forage, potatoes and fodder beets in batch culture. 1. Fermentation pattern. Animal Feed Science and Technology. (*Accepted)*

II. Eriksson, T., Ciszuk, P., Murphy, M. & Wilson, A.H. Ruminal digestion of leguminous forage, potatoes and fodder beets in batch culture. 2. Microbial protein production. Animal Feed Science and Technology. (*Accepted)*

III. Eriksson, T., Murphy, M., Ciszuk, P. & Burstedt, E. Nitrogen balance, microbial protein production and milk production in dairy cows fed fodder beets and potatoes, or barley. Journal of Dairy Science. *(Accepted)*

IV. Eriksson, T., Ciszuk, P. & Burstedt, E. The effect of voluntary intake of potatoes and fodder beets in dairy cows on nitrogen metabolism assessed by urinary parameters. *(Manuscript)*

List of abbreviations

AAT	Amino acids absorbed in the digestive tract
ADF	Acid Detergent Fibre
ATP	Adenosine triphosphate
BW	Body weight
DM	Dry matter
ECM	Energy corrected milk
FIA	Flow injection analysisME
MJ	Megajoule
NDF	Neutral Detergent Fibre;
NEL	Net energy for lactation
NSC	Non-structural carbohydrates
OM	Organic matter
PBV	Protein balance in the rumen
VFA	Volatile fatty acids

Introduction

Root crops, such as fodder beets (*Beta vulgaris*), swedes (*Brassica napus*) and turnips (*Brassica campestris*), were widely used in Swedish dairy rations some decades ago. They were then regarded as "succulent" feeds, exchangeable for grass silage or green fodders. The technical development after World War II in growing, harvesting, processing and feeding of grains has increased the relative costs of root fodder crops and the current Swedish land allocation to roots for feed purposes is probably about 100 - 200 ha. This could be compared to the 100 000 ha grown in 1920 (Utvecklingsvägar inom grovfoderområdet, 1977). In Denmark, where fodder root crops have been used to a larger extent than in Sweden, the land grown with fodder beets has decreased from 120 000 ha in 1985 to 10 000 ha 2002 (Dansk kvaeg, 2002). This development has in Denmark been accompanied by a corresponding increase in maize silage production. Unlike root crops, potatoes are generally not grown as a forage crop and their use for feed purposes is restricted to cull potatoes and food industry by-products and probably most frequent in regions with a large potato production for the food industry. Although it has been more common to feed potatoes to pigs, potatoes have also been used for cattle. Currently, it seems that potatoes are used to about the same extent as roots. From compilation of data for 1112 Swedish dairy farms reporting rations to the extension service during 2002, it was found that six herds fed fodder beets, two herds fed carrots and four herds fed potatoes (Swedish Dairy Association, 2003).

There are several reasons for investigating roots and potatoes as dairy cow feeds. The protein efficiency of dairy production is poor, and is in Northern Europe usually within the range of 15 to 25% if calculated as farm gate balances (Swensson, 2002). An increasing concern has been raised regarding the environmental consequences of the poor protein and hence nitrogen utilization (Tamminga, 1992; Kuipers & Mandersloot, 1999; Swedish Government, 2001). A major route of nitrogen loss in ruminants is the urinary excretion of ruminally degraded feed protein not utilized for microbial protein production. This loss could be reduced if there is a balance in the rumen between carbohydrates and nitrogen available for microbial growth. Unlike the maize silage rations predominant in continental Europe, Swedish dairy rations are usually based on silage made from grass, clover or alfalfa. These silages are characterized by a high protein content and also by a high ruminal degradation rate of the protein fraction. The producers of organic milk, where forage has to comprise a large part of the total ration may face certain problems in balancing the ration, especially since the proportion of legumes is relatively high in typical silages on organic farms. Feeds with large carbohydrate excess, like roots and potatoes are required to balance the large amounts of ruminally degradable feed protein. The, compared to the common Swedish feed grains, different properties of the carbohydrate fractions in roots and potatoes could be expected to differ with respect to extent of and site of digestion. This would have influence on microbial protein production, ruminal fermentation pattern, intake and milk production. In addition, the physical form in which roots and potatoes are fed may also contribute to differences when compared to grains. Results in literature from experiments with roots and potatoes fed to dairy cows

were often obtained with basal forages different from what is used in Sweden today. The cows in these experiments were also different with respect to genetic merit for milk yield and milk composition. An evaluation of these feeds as supplements to large amounts of silage harvested and preserved with the current technology, in rations for Swedish cows with high genetic merit, would therefore contribute with information regarding the current potential of roots and potatoes as dairy cow feeds.

Aim of the thesis

The aim of this thesis was to gather information relative to the replacement of grain concentrates with roots and potatoes in dairy cow diets based upon large amounts of legume-rich silage. The emphasis was on the possible improvement of microbial protein synthesis and nitrogen balance. Other questions to be answered were the effects on:

- Milk yield and milk composition.
- Intake capacity.
- Ruminal fermentation pattern with respect to balance between glucogenic and lipogenic volatile fatty acids.

Materials and methods

This thesis is based upon two in vitro experiments and three animal trials conducted at the Kungsängen Research Center in Uppsala 1998 and 1999. The experiments are reported in the four papers included in this thesis as described below.

The Substrate study (in vitro): Papers I and II

Seven silage-based diets were tested with three different silage types and ruminal fluid from cows on three diets, yielding a total of 189 observations after three replicates. Data regarding fermentation pattern and carbohydrate metabolism are reported in Paper **I**, whereas Paper **II** covers microbial protein production and nitrogen metabolism.

The Rumen fluid study (in vitro): Papers I and II

Based upon results from the Substrate study, ruminal fluid from cows fed three different levels of potatoes were incubated simultaneously with a silage and barley diet, to give a total of 54 observations. Fermentation pattern data are reported in Paper **I** and microbial protein production is reported in Paper **II**.

The Change-over experiment: Papers III and IV

Fourteen midlactation cows were used in a change-over experiment with three periods and three treatments, consisting of different carbohydrate supplements to a basal silage ration. Four ruminally cannulated cows were included and quantitative collection of feces and urine was performed on eight cows. The main results of the experiment are reported in Paper **III** and additional data on urinary composition are reported in Paper **IV**.

The Intake experiment: Paper IV

Twenty-three lactating cows were switched from a standard grass silage/concentrate ration to fodder beets and potatoes, both allowed at 30 kg fresh weight/d, as the only carbohydrate supplements. The experiment is reported in Paper **IV**.

The Simultaneous feeding experiment: Paper IV

Six of the cows from the intake experiment were maintained on their rations but with fodder beets and silage fed simultaneously. The experiment is reported in Paper **IV**.

Experimental feeds and rations

Silage was made in large bales from prewilted pure stands of alfalfa and from mixed grass stands dominated by perennial ryegrass. An additive containing hexamine and sodium nitrite (Kofasil Ultra™, Hansson & Möhring, Halmstad, Sweden) was used. In the animal experiments, alfalfa and grass silages were mixed 60:40 on a dry matter (DM) basis prior to feeding. Fodder beets of the variety Kyros from a producer in southern Sweden were stored at the research farm. Portions for three days were dry cleaned and chopped into cube-like pieces with about 5-cm faces prior to feeding. Cull potatoes, mainly oversized, were delivered washed from a local packing plant and prior to feeding chopped to cube-like pieces with about 3-cm face.

Silage was fed ad libitum in the Change-over experiment, but was restricted to 10 kg dry matter/d in the Intake experiment and the Simultaneous feeding experiment. In all three animal experiments, 1 kg/d (as fed) of heat-treated rapeseed cake (EXPRO, Carlshamn Crushing, Karlshamn, Sweden) was fed and, in the Change-over experiment, also 1 kg/d (as fed) of perennial ryegrass hay. The carbohydrate supplement in the Change-over experiment was fed with 5 kg DM/d and consisted of rolled barley (diet BA), rolled barley/raw potatoes 80:20 (diet BAP) or fodder beets/raw potatoes 80:20 (diet BEP).

In vitro diets in the Substrate study consisted of silage (S) or a mixture 60:40 of silage and one of the following carbohydrate supplements: Barley/oats grain 1:1 (BO); raw potatoes (RP); steam-boiled potatoes (BP); frozen potatoes (FP); fodder

beets (FB); and, barley/oats/raw potatoes (BORP) 1:1:2. In the Rumen fluid study, a diet with silage/barley mixed 60:40 on a DM basis was used.

In vitro procedures

In vitro experiments were performed as 5-h incubations in a batch culture system (Czerkawski & Breckenridge, 1969), with eight separate incubation vessels. To each incubation vessel, 200 ml of coarsely strained ruminal fluid (1-mm screen) and 300 ml of buffer (Lindgren, 1979) were added together with 20g DM of feed sample. Ruminal fluid was obtained either from non-lactating cows on a standardized maintenance diet or from lactating cows adapted to different diets. Feed samples were either stored frozen (grain, silage) or prepared for each incubation (fodder beets, potatoes) to facilitate the use of low DM samples, thereby mimicking real feeding situations. Samplings and registrations comprised continuous pH registration, liquid sampling and gas registration on an hourly basis and endpoint measurement of total microbial mass.

Animal trial procedures

The feeding experiments utilized the experimental herd at Kungsängen Research Center, which comprises two selection lines bred for similar production of energy corrected milk (ECM) but with different milk fat concentrations. Milk yield was recorded daily with a FloMaster milk meter (Alfa Laval Agri, Sweden) or for two consecutive days in each sampling period by the Tru-Test technique (Tru-Test Ltd, Auckland, New Zealand).

Fecal samples were either obtained as grab samples or from quantitative collection (the Change-over experiment) during 96 h. Samples were lyophilized prior to analysis, except for the total nitrogen analysis in the Change-over experiment, where samples were analyzed promptly after thawing. Urine samples were from spot samples obtained at every spontaneous urination (the Intake experiment) or from quantitative sampling during 48 or 72 h. Samples were preserved by 1.8 M H_2SO_4 and stored frozen. Ruminal fluid samples were taken from cannulated cows (the Change-over experiment), following a schedule that covered 17 h during four days. The pH was measured immediately and the samples were then stored frozen. Total rumen contents were evacuated, weighed and sampled on three occasions.

Chemical analysis

Standard procedures were followed for most of the analysis of milk, feeds, urine, feces and ruminal samples from cannulated cows and in vitro vessels. The milk was analyzed for fat, protein, and lactose by infrared spectroscopy (DairyLab2 A7S Foss Electric, Denmark), for somatic cell count (Fossomatic 90, A/S Foss Electric, Hillerød, Denmark), and for urea by a flow injection analysis (FIA) method using pH difference (Ramsing, Ruzicka & Hansen, 1980). Energy corrected milk (ECM) was calculated according to Sjaunja et al. (1991). Acetone concentration in milk

was determined by a FIA method (Marstorp, Anfält & Andersson, 1983). Kjeldahl nitrogen in feeds, in vitro vessels, urine and feces were determined according to Nordisk Metodikkommitté (1976). Ash, DM and ether extract were determined as described by Murphy, Åkerlind & Holtenius (2000). Neutral detergent fiber (NDF) was analyzed by the oven method (Chai & Udén, 1998), and acid detergent fiber (ADF) and lignin according to Van Soest, Robertson & Lewis (1991). Crude fiber for the Weende analysis was determined according to Jennische & Larsson (1990). Non-structural carbohydrates (sugar, maltodextrines and starch) were analyzed enzymatically (Larsson & Bengtsson, 1983). Metabolizable energy (ME) was calculated for all feeds except silage and hay from the Weende analysis with standard digestibility coefficients and energy values (Axelson, 1941). The ME of hay and silage was determined from the 96-h in vitro digestible organic matter (OM) (Lindgren, 1979). In situ determination of degradation rates for crude protein and NDF in the feeds was performed by standard methods (Lindgren, 1991) and buffer solubility of crude protein was analyzed according to Lindberg et al. (1982). Amino acids absorbed in the digestive tract (AAT), protein balance in the rumen (PBV), and rumen protein degradability were calculated according to Madsen (1985) and Spörndly (1999).

Microbial protein concentration in ruminal contents and in in vitro incubation vessels was determined by the total purine method (Zinn & Owens, 1986; Aharoni & Tagari, 1991). For the in vitro experiments, the total N:purine ratio of isolated bacteria samples (Legay-Carmier & Bauchart, 1989) from rumen fluid donor cows was used for calculation. Urine was analyzed on a Technicon Auto Analyzer for allantoin (Lindberg & Jansson, 1989), urea (Technicon, 1974a) and creatinine (Technicon, 1974b). Liquid in vitro and ruminal samples were analyzed simultaneously for NH_3-N and total α-amino-N on a Technicon Auto Analyzer (Broderick & Kang, 1980) and for volatile fatty acids (VFA) by gas chromatography (Murphy, Åkerlind & Holtenius, 2000). A minor set of in vitro samples was analyzed for lactate by high-performance liquid chromotography (Andersson & Hedlund, 1983). Lactate concentration for the Substrate study was then estimated from the subset analyzed for lactate by rearranging the equation of Argyle & Baldwin (1988). Fermentation balances described by Wolin (1960) and Van Soest (1994) were used for calculation of substrate degradation and gas formation from production of VFA. Adenosine triphosphate (ATP) formation was calculated from VFA analysis and published ATP values (Stadtman, 1967; Baldwin, 1970; Baldwin, Lucas & Cabrera, 1970; De Vries, van Wyck-Kapteyn & Stouthamer, 1973; Tamminga, 1979; Russell & Wallace, 1988; Van Soest, 1994).

Statistical analysis

All statistical analyses were performed with the SAS software (1996), by GLM, REG STEPWISE and MIXED procedures (Littell et al., 1996).

Results

The Substrate study (in vitro): Papers I and II

Larger amounts of substrates were fermented from fodder beets and boiled potatoes than from barley or raw potatoes during 5 h in vitro incubation. This difference was greatest if inocula from cows fed at maintenance energy level was used. The estimated lactate concentrations after 5 h were with fodder beet supplementation 25 to 72 mM, with inocula from lactating or maintenance fed cows, respectively. The only other substrate that was estimated to give a significant lactate concentration with inocula from lactating cows was the boiled potatoes. Propionate molar proportion was within the range 21 to 25% for all combinations of inocula and substrates and butyrate molar proportion was within the range 8 to 14%. Butyrate proportion was more dependent on inocula than on incubation substrate, with molar proportions of 8.0, 10.7 and 13.0 for inocula from cows on a maintenance ration, a lactation level potato ration and a lactation level fodder beet ration, respectively. Microbial protein production was better correlated to the amounts of fermented substrate than to the ATP supply calculated from VFA production, with highest yield for fodder beets with inocula from maintenance fed cows. Boiled potatoes produce more microbial protein than raw potatoes with inocula from maintenance fed cows. With inocula from lactating cows, microbial yield from fodder beets was not different from the yield with barley/oats or raw or boiled potatoes. Microbial efficiency increased with alfalfa proportion of the basal silage in incubation diets. The inocula also differed in microbial efficiency, with highest efficiency for a lactation level potato ration and lowest efficiency for a maintenance energy level diet. Ammonia concentrations were lower with barley/oats than with fodder beets, except for the similar concentrations recorded with inocula from maintenance fed cows.

The Rumen fluid study (in vitro): Papers I and II

Fermentation was slightly more rapid with inocula from cows with 1 kg DM potatoes in the ration than with inocula from cows fed 0 or 2 kg DM potatoes, as could be monitored by development of pH and ammonia concentration. Microbial yield only increased numerically and not significantly, by 3 and 8% compared to inocula from cows fed 0 or 2 kg DM potatoes, respectively.

The Change-over experiment: Papers III and IV

Fodder beet supplementation lowered ad libitum consumption of silage by 0.9 kg DM, compared to barley supplementation and yield of ECM decreased by 1.7 kg. Milk fat concentration did not differ but milk protein concentration was slightly higher (3.21 vs. 3.15, $P = 0.04$) with barley supplementation than with fodder beet/potato supplementation. Average rumen pH was 6.0 to 6.1 for all diets. Propionate molar proportions were between 18 and 20%, with the highest value ($P = 0.07$) for the fodder beet diet and butyrate molar proportions were all within the

range 13.8 to 14.5 %. Rumen ammonia concentration tended to be lower with fodder beet supplementation. Allantoin excretion and N balance were not increased by fodder beet supplementation, but exchange of 1 kg DM barley for potatoes tended to increase allantoin excretion. The fodder beet diet altered N excretion so that 5% of dietary N was removed from urine and instead fecally excreted. Overall urinary N excretion was well correlated to the protein balance in the rumen (R^2 = 0.81) and to the ratio urea/creatinine in urine, if scaled for body weight (R^2 = 0.94).

The Intake experiment: Paper IV

Most of the cows consumed the maximum allowed amount of fodder beets, while potato intakes were evenly distributed from 0.1 to 5.6 kg DM after a 20 d escalation period. Yield of ECM decreased from 29.4 to 22.3 kg/d during the 30 d of adaptation to the fodder beet/potato diet from the previous grain concentrate supplemented diet. Intakes of ME and AAT were not correlated (P = 0.18) to yield of ECM. Concentrations of fat and protein in milk were not altered, but milk urea was reduced from 6.3 to 4.5 mM. Multiple regression coefficients were similar for potatoes and fodder beets when milk urea concentration and the ratio urinary urea/urinary creatinine were response variables. The coefficient for potatoes tended to be lower than for fodder beets when the ratio urinary allantoin/urinary urea was the response variable.

The Simultaneous feeding experiment: Paper IV

Milk urea concentration declined from 4.3 to 3.9 mM with the synchronous feeding regimen and the ratio urea/creratinine in urine declined by 17%. The ratio allantoin/creatinine in urine was only numerically increased by 5% (P = 0.28).

General discussion

Nitrogen balance

To minimize nitrogen loss in animal husbandry, an appropriate amount of an amino acid mixture matching the animal's demands should be presented to the absorptive organs of the animal. In dairy cows, microbial protein constitutes 34 to 89% of this amino acid mixture, on average 59% (Clark, Klusmeyer & Cameron, 1992). The amino acid composition of microbial protein (Volden, Harstad & Mydland, 1999) generally agrees well with the composition of milk protein. The microbial protein could partly be regarded as upgraded, because rumen microbes utilize non-protein nitrogen compounds in the feed to a relatively large extent. Although rumen microbes require that a certain amount of feed protein be degraded in the rumen if fermentation of feed carbohydrates should not be limited, the degradation is often in excess of the needs. The ultimate nitrogenous ruminal end-product of protein degradation is ammonia, which, if not captured into microbial protein, is transferred to the liver, dimerized to urea and finally excreted in urine. The

exception is if the dietary N intake is so low that almost all the urea is recirculated to the rumen. This loss of ruminally-degraded feed protein via urine is usually the largest pathway for excretion of N wastes in dairy cows (Tamminga, 1992). It is also the form of N excretion that is most likely to cause atmospheric pollution, when the urea is hydrolyzed to ammonia in the barn (Monteny et al., 2002). A logical step for reducing N losses would therefore be to reduce the proportion of ruminally-degradable protein in the ration. Several protein evaluation systems, such as the Scandinavian AAT/PBV system (Madsen, 1985; Madsen et al., 1995) and the Dutch DVE/OEB system (Tamminga et al., 1994) include a parameter that describes the balance between rumen degradable protein and potential microbial growth from digestible carbohydrates or fermentable organic matter in the feed. A balance close to zero should mean that urinary N losses are minimized without reduction of ruminal fermentation. If the ration is based upon large amounts of clover or alfalfa silage, with a high PBV, feeds with a relative carbohydrate excess are necessary for balancing the ration. Fodder beets and potatoes are two of the domestic feed crops with the largest carbohydrate excess, or most negative PBV, according to current feed evaluation (Spörndly, 1999). Judging from the PBV values, these feeds should be able to improve N balance in protein-rich diets, compared to grain supplements as oats, barley and wheat.

Experimentally, an improved N balance should be manifested by decreased concentrations of ruminal ammonia and of urea in plasma, milk and urine. The proportion of intake N excreted as waste should also be reduced and the proportion recovered as milk protein should increase. In the Substrate study (Paper **II**), ammonia concentrations were lower with oats/barley supplementation than with fodder beet supplementation, the only exception being the equal endpoint concentrations when inocula was from maintenance fed cows. The latter was in spite of a higher microbial yield from fodder beets. However, the connection between ammonia concentration and microbial growth is confounded by different rumen microbe's preferences for different N sources. For example, Russell, Sniffen & Van Soest (1983) concluded that 66% of cell protein was derived from casein taken up without passing through the ammonia pool when sufficient amounts of non-structural carbohydrates (NSC) were supplied. Other researchers have reported lower microbial N proportions derived from amino acids (Maeng et al., 1976) or a growth response saturating at very low amino acid concentrations (Argyle & Baldwin, 1989).

If ammonia production proceeds, but the uptake is dependent on the preference of the microbial populations thriving on different carbohydrate sources, ammonia concentration may remain high in spite of an extensive microbial growth. This is more likely to occur with the substrate excess present in the Substrate study (Paper **II**) than in real feeding situations, where removal of peptides and amino acids would reduce the potential for ammonia production. In the Change-over experiment (Paper **III**), rumen ammonia concentration tended to be lower with beet supplementation than with barley supplementation, but the decrease was proportional to the lower N intake with that diet. However, total excretion of urinary N, as well as total milk urea excretion were instead lowered in proportion to the lower PBV intake. Because milk yield decreased, concentration of milk urea was not affected as much as the total milk urea excretion (Table 1). Regression

analysis of total urinary excretion against PBV (Paper **IV**) also showed a slope of 0.17, which is very close to the calculated N content 0.16 of crude protein. This indicates that the PBV value estimated urinary N very accurately in the Change-over experiment. It should be noted that these PBV values in the Change-over experiment were calculated from rumen degradability determined in sacco on the actual feeds. If PBV instead was calculated from the higher, tabulated degradability used for routine feed evaluation (Spörndly, 1999), the slope for the linear regression of urinary N excretion against PBV intake increased to 0.18 and R^2 increased from 0.81 to 0.87.

Table 1. *Relative excretion of urinary N when protein balance in the rumen (PBV) was lowered by replacing 4 kg DM barley with fodder beets and by a lower silage intake (Paper **III**).*

Diet	PBV, g/d	TUN[1], g/d	UUN[2], g/d	Milk urea, mM	Kg milk	Milk urea, mmoles/d[3]
BEP[4]	495	187	142	4.88	21.8	106.4
BAP[5]	647	244	196	5.83	23.2	135.3
Relative[6]	76.5%	76.6%	72.4%	83.7%	-	78.7%

[1]*Total urinary N*
[2]*Urinary urea N*
[3]*Kg milk × concentration*
[4]*Fodder beets and potatoes*
[5]*Barley and potatoes*
[6]*BEP/BAP*

The nitrogen efficiency in terms of feed N recovered in milk was not improved by replacing barley with fodder beets (Paper **III**), because fecal N excretion was not reduced at all in spite of a lower N intake. It is likely that this resulted from increased hindgut fermentation and excretion of undigested microbes. The effects on urine excretion and milk urea would be about the same as if this part of fermentation had occurred in the rumen, because plasma urea would still be removed to serve as an N source for this hindgut fermentation (Van Soest, 1994).

Raw potato supplementation resulted in higher ammonia concentrations than both fodder beets and barley/oats in the Substrate study (Paper **II**). This may be explained by the slow fermentation rate of raw potato starch and by the highly soluble protein in potatoes, which could be decreased by boiling (Paper **II**). Boiling then also led to decreased ammonia concentration. For the cannulated cows in the Change-over experiment (Paper **III**), ruminal ammonia concentration was not at all affected if 1 kg DM barley was replaced by potatoes. Neither were amounts or routes for N excretion affected by this exchange. In the Intake experiment (Paper **IV**), where larger amounts of potatoes were fed, potatoes according to regressions, reduced milk urea concentration and urinary N excretion similar to fodder beets. A tendency for lower urinary allantoin excretion together with numerically larger fecal N excretion indicated a larger hindgut fementation with potatoes than with fodder beets in the Intake experiment.

For all three dairy cow experiments reported in Paper **IV**, N recovery in milk was relatively poorly correlated to different N intake parameters and also to milk urea concentration, with the best fit ($R^2 = 0.27$) against total N intake. However, if recovery of feed N in milk could not be estimated accurately, urinary N excretion could. Besides the previously described correlation with $R^2 = 0.81$ towards PBV intake, it was possible to establish a linear regression with $R^2 = 0.94$ for total urinary N excretion per kg body weight (BW) as a function of the ratio urea/creatinine in urine (Paper **IV**). Creatinine is mainly a function of animal muscle mass (Antoniewicz, Heinemann & Hanks, 1981) and should therefore be a reliable marker for urine volume. The ratio urea/creatinine should then give an accurate estimate of urinary N excretion per kg BW, because of the very close correlation between total N and urea in urine (Ciszuk & Gebregziaher, 1994). Factors that may impair the use of urea/creatinine as a tool are if urinary N constituents other than creatinine (Bristow, Whitehead & Cockburn, 1992) are not constant in proportion to urea or if creatinine excretion is altered. Recent experiments where creatinine has served as a marker in urinary spot samples include unsatisfactory results (Shingfield & Offer, 1998) as well as reports of a good sensitivity for the method (Valadares et al., 1999; Broderick, 2003). If factors responsible for between-experiment variation in excretion of creatinine and other urinary constituents could be identified and corrected for, spot samples would be possible to use for assessing N metabolism in commercial dairy herds.

In conclusion, supplementation with fodder beets or potatoes did not improve the N efficiency of mid-lactation cows, compared to supplementation with barley. However, N excretion in urine was in good agreement with the intake of PBV, while fecal N was moderately increased by fodder beet supplementation and with a greater increase if larger amounts of raw potatoes were fed. The redirection from urinary urea N towards microbial fecal N would prevent a part of ammonia volatization in barns.

Microbial protein production

The production of microbial protein in the Substrate study (Paper **II**) was generally associated with the amounts of substrate fermented during 5 h, which were larger if silage was supplemented with fodder beets or boiled potatoes than with barley/oats. The variation in microbial protein production was still considerable and the fermented amount only explained 39% of this variation. Several feed evaluation systems calculate microbial protein from the intake of either total digestible nutrients (NRC, 2001) or of totally digestible carbohydrates (Madsen, 1985; Madsen et al., 1995). This would be accurate, given that the measurement used reflects ruminally fermentable substrate and that microbial protein production simply is a function of amount of fermented substrate. In the Change-over experiment (Paper **III**), intakes of DM, digestible carbohydrates (calculated according to Madsen, 1985) and NDF explained 55, 61 and 64% of the variation in microbial protein production, respectively. In the Intake experiment (Paper **IV**), allantoin excretion indicated similar microbial protein production per kg DM of fodder beets and silage, but a tendency ($P = 0.17$) for lower microbial protein production per kg DM potatoes. Judging solely from the contents of digestible

carbohydrates, fodder beets and potatoes should facilitate the same microbial protein production, while production should be lower from silage. The response for NDF and for silage is in agreement with the results of Hvelplund & Madsen (1985), in the experiments forming the basis of the Scandinavian protein evaluation system. They found a 60% higher microbial production per kg digestible carbohydrates for diets classified as roughage diets than for concentrate diets. The influence of NDF per se and of roughage may be related to particle-associated bacteria constituting the main part of microbial flow to the small intestine (Legay-Carmier, Bauchart & Doreau, 1988).

When fermentation in the Substrate study (Paper **II**) not was hampered by end product accumulation, fodder beets had the highest microbial efficiency and the numerical differences for other substrates also indicated higher efficiency with higher production. Similarly, the means for different inocula and silage types showed increased efficiency with increased production. In attempts to improve estimation of microbial protein supply, the fermentation rate of feed carbohydrates has been incorporated into feed evaluation systems (Russell et al., 1992). The rationale for this is that fewer carbohydrates would be used for microbial maintenance at a high microbial growth rate and hence the microbial yield from a certain amount of carbohydrates would be greater (Pirt, 1965). However, this would only be fully valid if the ruminal washout rate increases at an equivalent rate in relation to the increase in fermentation rate, so the microbial pool requiring maintenance not is increased. In a batch culture, effects on microbial efficiency directly related to less maintenance from different growth rates would therefore probably be limited to the first hours of incubation. The ranking of different substrates, with respect both to total microbial yield and to microbial efficiency, is in batch cultures strongly dependent on incubation time. Hall & Herejk (2001) showed higher yield and microbial efficiency for sucrose and pectin than for starch during the first hours, while curves converged after 10 h and the starch then gave both higher yield and efficiency than the other substrates.

Although a high carbohydrate fermentation rate may as such promote microbial efficiency, the low pH that may occur if the fermentation acids accumulate in the rumen might be negative for microbial efficiency (Therion, Kistner & Kornelius, 1982; Strobel & Russell, 1986). However, in the Substrate study (Paper **II**), a negative correlation existed between pH and microbial efficiency, although with a poor fit ($R^2 = 0.13$). If the incubation had been allowed to proceed, it is possible that the advantage from high fermentation rate would have been overtaken by the disadvantage from the increased maintenance costs and lower generation of ATP associated with lower pH (Shi & Weimer, 1992; Russell & Strobel, 1993).

Sucrose has, compared with starch, decreased rumen ammonia and increased microbial protein in some in vivo experiments (Chamberlain, Robertson & Choung, 1993; Oh et al., 1999), but has occasionally resulted in decreased microbial protein production (Sannes, Messman & Vagnoni, 2002), in spite of a simultaneous ammonia reduction. In the Change-over experiment (Paper **III**), fodder beet supplementation did not result in higher microbial protein production or efficiency than barley supplementation. The decreased ruminal ammonia concentration with the fodder beet diet could be explained by a lower intake of

rumen degradable protein and did not reflect a compared to barley improved microbial growth.

Another hypothesis is that microbial efficiency could be enhanced not only by increasing fermentation rate, but also by continuously matching ruminal supply of available carbohydrates and protein (Herrera-Saldana & Huber, 1989; Herrera-Saldana et al., 1990; Sinclair et al., 1993; Sinclair et al., 1995; Kim et al., 1999a; Kim et al., 1999b). This could experimentally be achieved by feeding carbohydrate and protein feeds with matched degradation rates in a mixed ration, by feeding different feeds according to different time schedules, or by ruminal infusion of soluble substrates. In a high-producing dairy cow, the two goals of synchrony and high fermentation rate may partially converge, because the animal is dependent on a rather intensive fermentation to cover the high nutritional requirements. Thus, a mixed ration with relatively high degradation rate of both starch and protein resulted in higher milk yield than diets with low degradation rates for either starch or protein or for both fractions (Herrera-Saldana & Huber, 1989). Duodenal flow of microbial protein from these diets was highest with high starch degradation rate and was less affected by protein degradation rate (Herrera-Saldana et al., 1990).

In rations based on large amounts of clover or alfalfa silage, the ruminal protein degradability is usually very high. Synchrony would there mainly mean that carbohydrates with high degradation rate should be added to the ration and preferably mixed with the silage. In the Simultaneous feeding experiment (Paper **IV**), fodder beets were fed on the same occasions as silage. This feeding regimen increased allantoin excretion only numerically by 5% ($P = 0.28$), when compared with the Intake experiment (Paper **IV**), where the same amounts were fed to the same cows, but with a 5 h time lapse between silage and fodder beets. However, ruminants have mechanisms to control short-term imbalances of protein and carbohydrates, which may explain why the synchronization effect is less than expected. The ammonia released from ruminally-degraded protein may be circulated as urea for some time and then enter the rumen again via saliva and by diffusion into the rumen from the bloodstream (Van Soest, 1994). A temporary excess of carbohydrates can be stored as endogenous polysaccharides by both bacteria (Wallace, 1980) and protozoa (Coleman, 1992) and starch grains may simply be engulfed by some protozoa, resulting in a delayed release of carbohydrates for fermentation (Coleman, 1992).

Besides the assumed advantage of matching hourly total release of carbohydrates and protein, combination of different types of carbohydrates may be beneficial also from other points of view. A proportion of slowly degradable starch may help to even out the diurnal intensity of fermentation. This could explain why there was a tendency for increased microbial protein production when one kg of barley DM was exchanged for potatoes in the Change-over Experiment (Paper **III**), even if not coupled to any positive response in yield or composition of milk. According to Cone et al. (1989), more resistant starch led to a prolonged amylolytic activity in rumen fluid. In the Substrate study (Paper **II**), potato inclusion in donor cow diets appeared to increase microbial protein production, while the Rumen fluid study (Paper **II**) resulted in decreased ammonia concentration for inclusion of 1 kg DM potatoes/d, compared to 0 or 2 kg. Microbial protein production only increased

numerically and not significantly with the ammonia reduction. The Intake experiment (Paper **IV**) indicated lower microbial protein production/kg DM for potatoes than for fodder beets. It is possible that small amounts of raw potato starch are beneficial, by maintaining a persisted amylolytic activity from the microbial flora, while some negative effect from larger amounts of raw potato erases this advantage. In the Intake experiment, it is likely that more starch escaped rumen fermentation when more potatoes were fed and this could explain why potatoes tended to give less microbial protein than fodder beets in that experiment.

In conclusion, fodder beet supplementation did not result in higher microbial protein production in vivo than did barley supplementation, even if batch culture production was higher with fodder beets. One kg DM potatoes in the diet tended to increase microbial protein production, but there was no production response. With fodder beet and potato intakes up to 4 and 5 kg DM, respectively, fodder beets tended to give more microbial protein than potatoes.

Fermentation pattern

Swedish dairy cows have ruminal fermentation patterns characterized by relatively low propionate proportions and relatively high butyrate proportions (Van Gylswyk & Murphy, 1993; Burstedt & Murphy, 1999). Considering the the tendency for sugars to increase butyrate proportion (Friggens et al., 1998), a concern with root feeding is hence that butyrate must not increase at the cost of propionate production and impair the supply of glucogenic substrates for the cow. The in vitro fermentations in the Substrate study (Paper **I**) resulted in propionate molar proportions of 22 to 25% with inocula from lactating cows. This is higher than the 18 to 20% measured in the cannulated cows of the Change-over experiment (Paper **III**). The large lactate accumulation occurring in vitro (Paper **I**) suggested an even higher propionate proportion, if the lactate was further fermented according to Jaakkola & Huhtanen (1992) where 52% would be converted to propionate. Also a conversion of 35% of the lactate to propionate (Gill et al., 1986) would mean an increase of propionate proportion. There are probably several reasons that in vivo propionate proportions were lower than what was obtained in vitro. A major difference is that the total intake of non-structural carbohydrates in the Change-over experiment (Paper **III**) was moderate.

Russell (1998a) reported low pH to favor propionate proportion and proposed that inhibition of methane producers was the mechanism for that. This may be true in vivo and for the 48 h incubations Russell performed, but for the 5 h incubations reported in Papers **I** & **II** it would most likely not have occurred. Besides, the propionate proportion in the Substrate study seemed not to be dependent on pH. However, only gas volume was recorded in the Substrate study and the proportions of CO_2, CH_4, and H_2 were not analyzed, so the relationship between propionate production and methanogenesis could not be determined in the present experiment. The in vitro incubations (Paper **I**) resulted in end-point pH about 5.30 with fodder beet supplementation. The fodder beet supplemented diet in the Change-over experiment (Paper **III**) had the same average pH as the barley supplemented diet and a tendency for higher pre-feeding morning pH, probably because less readily

fermented substrate remained with the fodder beet diet. The fermentation rate of sucrose is very rapid (Weisbjerg, Hvelplund & Bibby, 1998), which was supported by the propionate peaks in the Change-over experiment (Paper **III**). Anyhow, the division in four meals meant that only 1 kg DM of fodder beets was fed per meal. The high effect of sliced fodder beets on chewing and rumination activities reported by De Brabander et al. (1999) indicates an extensive buffering from salivation, that may help in maintaining ruminal pH in fodder beet rations.

Another factor that may explain higher propionate proportions in vitro is that there most likely is differences among microbial species in the tolerance for in vitro conditions. This may also explain the lower butyrate proportions in vitro, where a decreased protozoal contribution probably is a common cause. Rumen protozoa are often responsible for a major portion of the butyrate production, in sheep between 70 and 80% (Michalowski 1987). According to our observations, protozoa seems to be hampered to some extent by the continous agitation in the batch culture system. Czerkawski & Breckenridge (1969), when introducing the in vitro system utilized in the current experiments, also reported butyrate proportions lower than what the development in the rumen fluid donor animals suggested. However, butyrate producing bacteria like *Butyrivibrio fibrisolvens* may sometimes be a considerable part of rumen microbial mass under Swedish conditions (Van Gylswyk & Murphy, 1993), resulting in less dependence on protozoa for butyrate production.

The small effect of substrate but large effect of donor cow diet on butyrate proportions in the Substrate study (Paper **I**) is in agreement with the fermentation parameter estimates of Murphy, Baldwin & Kong (1982), where butyrate fermentation from soluble carbohydrates was tripled in concentrate diets compared to roughage diets. However, the fodder beet diet in the Change-over experiment (Paper **III**) only tended to increase butyrate molar proportion, and the decreased acetate proportion resulted in a more glucogenic fermentation pattern with fodder beet supplementation than with barley supplementation.

When fermentation patterns were studied in cows receiving a diet similar to the fodder beet diet in the Change-over experiment, but including 4 kg DM barley in addition to the fodder beets, propionate molar proportion increased to 22% (Eriksson et al., unpublished). The propionate peaks of 22 to 23 molar % that followed all meals in the Change-over experiment, except for the first one in the morning, also indicates that a higher level of non-structural carbohydrates should have increased propionate proportion.

In conclusion, fodder beet supplementation of an alfalfa/grass silage diet increases propionate proportion of total VFA under Swedish conditions mostly due to reductions in acetate production. Butyrate is slightly increased on the expense of acetate. However, the effects of the added sugar in the fodder beets was not as great as expected from the in vitro trials, neither with respect to the increases in butyrate or propionate. Fodder beet inclusion with 4 kg DM/d do not lower ruminal pH compared to a similar amount of rolled barley.

Carbohydrate digestion

The rate and extent of ruminal digestion are the most important characteristics of a carbohydrate supplement. The different amounts fermented during 5-h incubation in the Substrate study (Paper **I**) would mainly reflect different fermentation rates for the different feeds, although the relative substrate excess limited fermentation with the most readily available substrates. The most readily available substrate was sugar, mainly sucrose, in the fodder beets. In vivo determinations with sucrose have shown a very high ruminal degradation rate. Weisbjerg, Hvelplund & Bibby (1998) found a ruminal hydrolysis rate of 1200 to 1400%/h for infused sucrose and a fermentation rate of 400 to 600%/h for the released monosacharides. Sucrose ingested as root crops could be expected to have a slower degradation rate because relatively large pieces may enter the rumen, with delayed release of sucrose. In the Change-over experiment (Paper **III**), ruminal sugar content was the same for all diets, regardless if 2.8 kg or 0.5 kg/d were provided by the diet. This strongly suggests a rapid ruminal degradation rate also for sucrose in roots. In addition, Sabri, Offer & Roberts, (1988) showed an OM disappearance of 77% after 3 h-in sacco incubation with fresh fodder beets, a value that thereafter only increased slightly until the endpoint 16 h. This is close to the 2-h DM disappearance of 76% from in sacco incubation performed on dried samples in the Change-over experiment (Paper **III**). The 76% DM disappearance after 2 h corresponds well to total degradation of the sugar fraction, together with the determined degradation of protein and NDF and partial degradation of an assumed pectin fraction comprising 5% of DM (Kniga & Pahkomov, 1972). In contrast to the indications of rapid degradation also of root sucrose, Zausch & Boldt (1985), though without providing many details, reported sugar to be present in the rumen 10 h after feeding sugar beets, but not after feeding similar amounts of sugar with fodder beets or molassed-beet pulp.

When sucrose is washed out of the rumen, it will not be utilized in the small intestine because ruminants lack sucrase (Van Soest, 1994) and hence hindgut fermentation is the only possible fate. The lower total tract N digestibility with the beet supplemented diet (Paper **III**) indicated increased hindgut fermentation, because fecal N may have been elevated by undigested microbes from such a fermentation (Van Soest, 1994). However, considering the fast sucrose disappearance as monitored by ruminal contents samples, it is most likely that a slight reduction in ruminal fiber degradation (Huhtanen and Khalili, 1992) allowed more fiber to be fermented in the hindgut.

With raw and frozen potatoes, the amounts fermented during 5 h in the Substrate study (Paper **I**) were not different from amounts fermented with the unsupplemented silage diet. The relatively high content of sugar in the silage and the obviously fermentable fraction not accounted for in the chemical analysis, may partly explain why supplementation with raw potatoes did not increase fermentation. The ruminal degradation rate of raw potato starch is slower than for starch from grains like oats, barley or wheat, but similar to or slightly higher than for cornstarch (Cone et al., 1989; Offner, Bach & Sauvant, 2003). The ruminal degradation rate of grain starch seems to be related to the protein matrix of the kernel where the starch granules are embedded. Isolated cornstarch granules are

digested as fast as isolated barley starch granules (McAllister et al., 1993) and are more susceptible to bacterial amylase attack than potato starch granules (Sarikaya et al., 2000). The resistance of raw potato starch is caused by the crystallinity of the granule itself (Gallant et al., 1992). Boiling disrupts the hydrogen bonds in the crystal structure and causes gelatinization of potato starch (Van Soest, 1994). This starch is much more readily degradable than raw potato starch. In the Substrate study (Paper **I**), fermentation intensity with boiled potatoes was close to the fodder beet diet and clearly higher than for the barley/oats supplemented diet. Traditionally, boiling potatoes has not been a common practice for ruminants, but it is obviously an opportunity if readily available carbohydrates are required. The freezing of potatoes in the Substrate study (Paper **I**) was examined as a possible means to increase starch degradability by mechanical damage to granules, thereby promoting enzymatic attack. The behavior of the frozen potato diet, which in all respects was similar to what was recorded for the raw potato diet, does not suggest any difference in starch degradation by only freezing and thawing potatoes. Golachowski (1985) recorded lowered amylose proportion and differences in gelatinization process after freeze-storing, thawing and re-freezing potatoes, but it is unclear whether these changes would have any significant effect on enzymatic degradation.

Besides the effects of potato as in vitro substrate, there also appeared to be an increased fermentation if potatoes were included in the diets for the rumen fluid donor cows in the Substrate study (Paper **I**). The Rumen fluid study (Paper **I**) was then designed to detect linear effects of 0, 1 or 2 kg DM potatoes in donor-cow rations on a silage/barley incubation diet. No such effects were found, although the rumen fluid from cows eating 1 kg DM potatoes showed signs of increased fermentation, with faster decline of pH and ammonia concentration. However, the amount and digestibility of starch in the diet will obviously affect ruminal amylolytic activity. Nozière & Michalet-Doreau (1997) reported a positive correlation between amount of available starch and amylolytic activity for particle associated bacteria. Cone et al. (1989) found interactions between amount and availability of starch fed. High starch levels and a more resistant starch in the ration led to prolonged amylolytic activity, as manifested by the ability of rumen fluid collected at different times after feeding to degrade starch in vitro.

A slow degradation rate means that more of the starch will escape rumen fermentation. In sacco determinations suggests that 45 to 66% of the starch from corn in some cases could pass the rumen undigested (Nocek & Tamminga, 1991; Offner, Bach & Sauvant, 2003). Although the in sacco methodology could be questioned as a tool for determining ruminal starch degradation, more appropriate in vivo techniques also indicate that 40% of ingested corn starch may escape ruminal fermentation at high intake levels (Knowlton, Glenn & Erdman, 1998). The slow ruminal degradation of potato starch suggests that proportions similar to what has been found for cornstarch should escape ruminal fermentation. However, direct comparisons of potatoes and corn for steers by monitoring duodenal passage (Beyer et al. 1993), indicates a higher ruminal digestibility for potato starch than for cornstarch. Südekum & Brandt (1990) found that 86, 5 and 7% of ingested starch was digested in rumen, small intestine and hindgut, respectively, in dairy cows fed raw potatoes providing 3.55 kg/d of starch in a 17 kg DM ration. Because

the proportion digested in the rumen is a function not only of degradation rate, but also of passage rate, the physiological properties, such as the functional specific gravity (Siciliano-Jones & Murphy, 1991) may contribute to differences between feeds.

If starch that escaped rumen was absorbed as glucose by the small intestine, which often has been assumed, it would provide an efficient way to supply the cow with glucose. In addition, it could have a protein sparing effect, because amino acids are used as glucogenic precursors in the gut if other sources not are available (Tamminga, 1992). However, it appears from studies on ileally-cannulated cows (Knowlton, Glenn & Erdman, 1998) that the capacity of the small intestine to digest starch passing out of the rumen is limited, and that post-ruminal starch digestion sometimes reflects hindgut fermentation rather than small intestinal uptake. Starch infused abomasally (Arieli et al., 2001) has occasionally been digested with amounts over 3 kg/d in dairy cows, compared to the negative small intestinal digestion reported by Knowlton, Glenn & Erdman (1998), in spite of 3 kg starch leaving rumen. This suggests a large influence of starch properties on small intestinal digestibility. The starch passing out of the rumen would be the more resistant part of dietary starch, while starch infused abomasally would include also more available starch. Of the rumen-escape potato starch in the experiment of Südekum & Brandt (1990), almost equal proportions were digested in the small intestine and the hindgut, respectively, resulting in 98% total tract digestibility of starch. Total tract starch digestibilities resembling those found by Südekum & Brandt (1990) were recorded in the Change-over experiment (Paper **III**), the Intake experiment (Paper **IV**) and the Simultaneous feeding experiment (Paper **IV**). However, in the Intake experiment (Paper **IV**), starch digestibility was negatively correlated to potato intake ($P < 0.001$), with 94% starch digestibility when 3.6 kg/d of raw potato starch was consumed. The effects on excretion of fecal N and urinary urea and allantoin from potatoes in the Intake experiment (Paper **IV**) indicate that potatoes increased the hindgut fermentation. Potatoes decreased urinary urea but not urinary allantoin to the same extent as fodder beets did. This suggests increased microbial growth in the hindgut, which removed urea N from the blood that after microbial incorporation elevated fecal N by excretion of undigested microbes.

In conclusion, sucrose from fodder beets is readily degraded in the rumen. Raw potato starch degrades at a slower rate than starch from oats, barley and wheat, but somewhat faster than raw cornstarch. The rumen degradability is also higher than for raw cornstarch. Total tract digestibility of raw potato starch depends on amount fed, but could be expected to be > 90% in most feeding situations. Raw potato starch degraded post-ruminal is absorbed in the small intestine and fermented in the hindgut in approximately equal proportions. Boiling of potatoes increases ruminal degradation rate so that it exceeds the rates for oats, barley and wheat. Freezing of potatoes has no detectable effect on ruminal degradation rate.

Intake

The possibilities of roots and potatoes to improve nutrient supply, when substituted for grain, depend largely on how the intake of the other feeds is affected. In the Change-over experiment (Paper **III**), ad libitum silage consumption was reduced from 13.6 to 12.7 kg DM when fodder beets replaced barley. The extent to which 1 kg DM of a feed suppresses ad libitum intake of forage in the ration is known as the substitution rate (Forbes, 1995). The fodder beets in the Change-over experiment then had a substitution rate of about 0.75, assuming the substitution rate for barley to be 0.50 (Huhtanen et al., 2002). Values in the literature for fodder beets, as reviewed by Gruber (1994), ranged from 0.33 to 1.10 and recently Ferris et al. (2003) reported substitution rates from –0.12 to 0.34, when fodder beets were added to a ration of silage at five different concentrate levels. A part of this variation may be explained by different properties of the fodder beets used, where low DM beets have a higher substitution rate than high DM beets (Gruber & Steinwender, 1986; Dulphy, Rouel & Bony, 1990). However, most of the wide ranges of reported substitution rates could be explained by the fact that the substitution rate of a feed is not a constant but rather depends on the feed's relative properties in the actual feeding situation (Leaver, 1973; Huhtanen et al., 2002). If fodder beets are fed in conjunction with a basal ration of low quality in terms of digestibility and intake potential, the "fill value" of fodder beets is relatively low and the substitution rate will be moderate. If on the other hand the basal ration has a high intake potential, ad libitum intake of it will probably be high and the introduction of fodder beets will suppress basal ration intake considerably. Typically, experiments showing low substitution rates for fodder beets (Krohn & Andersen, 1979; Ferris et al., 2003) have used low DM silage, whereas experiments showing high substitution rates (Meijer et al., 1994, Birkenmaier et al., 1996; Paper **III**) have used silage with higher DM concentration. Silage fermentation products depressive to intake, such as acids and ethanol, are generally associated with a low DM (Huhtanen et al., 2002). The silage in the Change-over experiment had a low content of these products. It seems from this that total replacement of grain by roots in a ration based upon silage with high intake potential clearly would impair total feed intake, while addition of roots without removing grains may increase intake to a limited extent in such a ration.

The 0.9 kg DM/d of potato that replaced the same amount of barley in the Change-over experiment was too small to detect any significant change in silage intake, although silage intake was numerically higher and not lower when potatoes were included. De Brabander et al. (1982) found substitution rates for potatoes of 0.53, 0.44 and 0.15 towards grass silage, high-quality grass hay and average-quality grass hay, respectively, where quality was judged as digestibility. Together with numerically lower chewing index (time required for chewing and ruminating of a feed) as well as a less physiological indication of fiber effect for raw potatoes than for fodder beets (De Brabander et al., 1999), this suggests that potatoes have a somewhat lower substitution rate than fodder beets. However, in direct comparison of fodder beets and potatoes (Jans, 1989), intake of the basal diet did not differ between these supplements.

Not only the substitution rate towards other feeds, but also the acceptance of a feed among animals and the intake potential of the feed itself are important. Most of the cows in the Intake experiment (Paper **IV**) consumed the maximum allowed 4.6 kg DM of fodder beets but only a few cows ate the allowed 5.3 kg DM of potatoes. Considering the average free-choice fodder beet intake of 10.7 kg DM (range 4.2 to 16.1 kg DM) found by Krohn & Konggard (1987), many cows in the Intake experiment would clearly have eaten much more fodder beets if allowed to. Sweet taste has also been shown to be preferred among the primary tastes by most early-lactation cows (Nombekela et al., 1994). The poor acceptance for potatoes among some of the animals in the Intake experiment is in agreement with observations on dairy goats (Kessler, 1983) and early-lactation cows (Jans 1989). In the experiments of Jans (1989), the lower acceptance for potatoes among some individuals only occurred during the first month of lactation. According to Faverdin, Baumont & Ingvartsen (1995), negative effects of a feed's taste and smell should be overridden by the motivation to satisfy energy requirements in high-producing animals. This is not supported by the inadequate energy supply, caused by aversion against potatoes among some of the cows in the Intake experiment. The occasional individual aversion against potatoes should be recognized when they are fed to high-yielding cows, where nutrient supply could be jeopardized if the feed is rejected.

Production

In the Intake experiment (Paper **IV**), milk yield and milk fat concentration were only related to the respective pre-experimental values and not to the intake of any certain feed or to total DM intake. However, both fodder beets and potatoes increased milk protein concentration. In the Change-over experiment (Paper **III**) the yield of ECM was 1.7 kg lower when fodder beets replaced 4 kg DM of barley. Concentrations of milk fat and milk protein were not altered by this exchange, whereas exchange of 1 kg DM barley for potatoes reduced milk protein concentration from 3.21 to 3.16%. The decline in ECM yield appeared to be proportional to the intake reduction, because balance of metabolizable energy (ME) was 114 to 116% for all diets, according to Swedish feeding standards (Spörndly, 1999). Meijer et al. (1994) arrived at similar results, when partly replacing concentrates by fodder beets for cows in early lactation, yielding 36 to 40 kg ECM. However, in many experiments fodder beet diets have resulted in lower milk yields than what could be expected from the energy intake. In a review of 15 experiments performed between 1961 to 1990 (Gruber, 1994), fodder beets increased milk production numerically but not significantly by 0.5 kg/d, although energy intake was significantly higher for beet fed cows. If the daily intake of net energy for lactation (NEL) was used as a covariate, beet-fed cows produced 1.8 kg/d less than control groups in these experiments. More recent experiments have also shown poor energy utilization with fodder beets. In respiration experiments with dairy cows fed 7.5 kg DM fodder beets/d (Müller et al., 1994) or similar amounts of pure sucrose (Kirchgessner et al., 1994), milk yield compared to the control declined by 4.7 and 2.4 kg/d for fodder beets and pure sucrose, respectively. The utilization of ME from the sucrose fraction was in these

experiments calculated to be 18% lower than from other feed fractions. Ferris et al. (2003) mixed fodder beets with grass silage prior to the feeding of cows also supplemented with five fixed concentrate levels of 3 to 12 kg DM/d. In spite of an average increase in ME intake of 36 MJ across all concentrate levels, milk yield was not increased by fodder beet inclusion and the energy efficiency hence was reduced.

The calculated energetic efficiency of a feed depends on the method that was used to determine this energy value, in terms of ME or NEL. The respiration chamber experiments (Kirchgessner et al., 1994; Müller et al., 1994) should be able to measure the true ME for the total ration, although the ME for the sucrose fraction must be estimated by difference. The variation among experiments in energy utilization for milk production from fodder beets, not explained simply by the method for estimating ME, may be related to differences in fermentation pattern. Sucrose usually increases butyrate proportion (Strobel & Russell, 1986; Chamberlain, Robertson & Choung, 1993; Friggens et al., 1998). The small increase in butyrate molar proportion, observed when barley replaced fodder beets in the Change-over experiment (Paper **III**), was balanced by a decrease in acetate, so propionate proportion tended to increase. It is possible that the typical Swedish fermentation patterns with relatively low propionate proportions more easily will respond to sucrose with an increased propionate proportion. If the control ration, on the other hand, has a high propionate proportion, a sucrose-rich ration would probably increase butyrate proportion more than propionate proportion, hereby creating a less glucogenic VFA pattern. Another phenomenon that could create lower energy utilization is that a ruminal carbohydrate excess may cause bacteria to spill energy (Russell, 1998b) if there is a shortage of nitrogen compounds for cell growth. The ruminal fermentation heat would then increase. This heat would in a respiration experiment not be distinguished from heat losses occurring by inefficient energy utilization on tissue level.

The constant milk fat concentrations in the Intake experiment (Paper **IV**) and the Change-over experiment (Paper **III**) may be because the ruminal fermentation patterns were not extensively altered, as evidenced by the ruminal samplings (Paper **III**). The positive correlation between milk protein concentration, and the intake of both fodder beets and potatoes (Paper **IV**), is constant with ME intake explaining more than other factors of variation in milk protein concentration (Spörndly, 1989). Increased milk protein concentrations with constant milk volume, reported from experiments where fodder beets have been offered in addition to a control ration (Roberts, 1987; Fisher, Sabri & Roberts, 1994; Ferris et al., 2003), were probably caused by improved energy supply. When also milk fat concentration increased (Roberts, 1987; Fisher, Sabri & Roberts, 1994), it may be due to altered VFA proportions from a constant amount of propionate but an increased amount of butyrate being produced daily. When fodder beets replaced grain and were not just added to the control ration, this has resulted in a more concentrated milk by less volume (Müller et al. 1994), most likely reflecting decreased propionate proportion at constant total VFA production.

The only effect from potatoes on yield or composition of milk noted in the Change-over experiment (Paper **III**) was the small decrease in milk protein content

when potatoes replaced 1 kg DM barley. This was in spite of the, judging from allantoin excretion, higher microbial protein production for the potato-supplemented diet. Data from the literature on the effects of potato feeding on milk yield and composition suggest a response similar to what has been obtained by fodder beets (De Brabander et al., 1982; Jans, 1989). Skjevdal (1974) reported higher milk yield for root (swedes) supplementation than for potato supplementation in a summary of Norwegian feeding trials performed 1948 to 1959. However, this was possible to explain by the larger amount of concentrate fed with the roots to compensate for their assumed lower energy density.

In conclusion, replacement of grain by potatoes and fodder beets only created changes of small magnitude in the concentrations of milk fat and milk protein in the present work. According to the literature, fodder beets and potatoes often have had similar effects on yield and composition of milk, in spite of the large differences in their carbohydrate fraction. These effects include a lesser quantity but more concentrated milk when grain is replaced, but a constant milk yield and higher concentrations of fat and protein if roots or potatoes are fed in addition to the control diet. The latter is under the condition that total intake is increased by addition of roots or potatoes.

Main conclusions

- Roots and potatoes did not improve nitrogen balance compared to similar amounts of barley but N excretion was partly shifted from urinary excretion to fecal excretion.
- The PBV value for fodder beets and potatoes described urinary N excretion correctly.
- There was a correlation with $R^2 = 0.94$ between total urinary N excretion and the ratio urea/creatinine in urine.
- Fodder beets indicated a high potential for microbial protein production in vitro but this was not confirmed by allantoin excretion and production responses in vivo.
- Boiling of potatoes resulted in an in vitro fermentation more rapid than for barley/oats, while freezing of potatoes had no effect.
- The apparent digestibility of raw potato starch was within the range 91 to 100% for all observations with a negative correlation towards potato intake. Urinary allantoin and urea as well as fecal N excretion suggested that a part of potato starch was fermented in the hindgut.
- The production responses for fodder beets and potatoes relative to barley were in agreement with the feeding values for ME and AAT, determined with the current standard coefficients.

- Both propionate and butyrate proportion tended to increase with fodder beet supplementation, whereas acetate proportion decreased. The ratio lipogenic/glucogenic VFA therefore tended to be reduced by fodder beet inclusion.

- Fodder beet supplementation compared to barley supplementation reduced ad libitum intake of alfalfa/grass silage with high intake potential. The milk yield was reduced in proportion to the intake reduction.
- Fodder beets were readily consumed by most cows while there was a much larger individual variation in acceptance for potatoes.

Populärvetenskaplig sammanfattning

Rotfrukter som fodersockerbetor, foderbetor, kålrötter och rovor var fram till mitten av 1900-talet mycket vanliga som foder till mjölkkor. De betraktades då främst som ett ”saftfoder” som var utbytbart mot vallensilage. Den tekniska utvecklingen i odling, skörd och hantering av spannmål gjorde att rotfrukterna relativt blev dyrare efter andra världskriget och odlingen minskade. Idag är den svenska odlingen av foderrotfrukter sannolikt inte större än 100 till 200 ha. Det finns dock skäl att undersöka rotfrukternas värde som foder till mjölkkor på nytt. Stora ansträngningar har de senaste åren satts in för att minska kväveläckage och ammoniakavgång från djurhållning. Man arbetar då bland annat med att balansera foderstaterna bättre. Hos mjölkkor kan en stor del av foderproteinet utsöndras som ureakväve i urinen. Det beror på att mycket av foderproteinet bryts ned snabbt i våmmen av mikroorganismer och bildar ammoniak. Den går sedan med blodet och omvandlas i levern till urea som utsöndras i urinen. Den här snabba proteinnedbrytningen gäller för ensilage och i särskilt stor utsträckning om ensilaget kommer från baljväxter som klöver och lusern. Proteinet som brutits ned i våmmen kan dock tas till vara om det finns kolhydrater tillgängliga så att nya mikroorganismer kan växa till. När de mikroorganismerna dör och flyter ut ur våmmen tas proteinet i dem till vara av kon. Det proteinet kan ofta vara bättre anpassat till kons behov än det ursprungliga foderproteinet. Det är alltså en stor miljömässig vinst om man kan reducera den här förlusten av protein som ureakväve i urinen. Det är också positivt för djurhälsan om kvävemängden i urinen hålls på en måttlig nivå.

För att balansera foderstaten och ta till vara proteinet behövs alltså kolhydrater och det är här rotfrukterna kommer in. De innehåller en stor del socker, som ju är en mycket lättillgänglig kolhydrat och borde kunna förse våmmens mikroorganismer med mycket energi för att ta upp nedbrutet foderprotein. Rotfrukter skulle också kunna vara speciellt intressanta för producenter av ekomjölk. De utfodrar ofta med ensilage från klöver och lusern, eftersom det är grödor som går bra att odla ekologiskt. De vill helst också odla så mycket som möjligt av fodret hemma på gården, vilket gör att en alternativ fodergröda kan vara extra fördelaktig.

En gröda med helt andra kolhydrater än rotfrukterna är potatis. Den innehåller stärkelse som inte alls är lika lättillgänglig som sockret i rotfrukter och även mindre lättillgänglig än stärkelsen i våra vanliga spannmålsslag. Vi får ju själva koka potatisen för att kunna smälta stärkelsen. Det skulle dock vara intressant att undersöka om en del potatis i foderstaten kan förbättra kornas proteinutnyttjande, eftersom det tillsammans med rotfrukter skulle ge både ”korta” och ”långa” kolhydrater. En sådan kombination borde kunna vara gynnsam för den totala våmmiljön. Det finns också alltid en del potatis som inte går att sälja eftersom den har fel storlek eller olika skönhetsfel.

Den här idén att kombinera snabba och långsamma kolhydrater har nu undersökts i ett forskningsprojekt med mjölkkor. Korna fick äta så mycket de ville av gräs-/lusernensilage och fick hackade fodersockerbetor och potatis som

komplement. Resultatet jämfördes med spannmål som komplement. Det visade sig då att korna inte kunde äta lika mycket ensilage när betor och potatis var komplement som när spannmål var komplement. Det beror sannolikt på att det ensilage som går att bereda med modern teknik är så smakligt att korna äter mycket mer av det än vad de kunde göra med det blötare och syrligare ensilage som användes för några decennier sedan. Det från början höga ensilageintaget gör då att intaget lättare minskar när man sätter in ett foder som på ett eller annat sätt tar ”mer plats” i våmmen än vad spannmål gör. Sänkningen i ensilageintaget gjorde att korna mjölkade ungefär 2 kg mindre än med spannmål. Halterna av fett och protein påverkades mycket litet av förändringen. Kornas totala proteinhushållning blev inte bättre med betor och potatis, men det skedde en förskjutning så att utsöndringen via urinen minskade och en större del istället gick ut med träcken. Detta är i regel positivt ur miljösynpunkt, eftersom kvävet i träcken inte ger ammoniakavgång lika lätt som urinkväve.

I ett annat försök fick korna själva välja hur mycket betor och potatis de ville äta, upp till en övre gräns på 30 kg av vardera om dagen. De flesta korna valde då att äta så mycket betor de fick, medan valet av potatisintag var mycket mer utspritt över skalan från strax över 0 till 30 kg. Halterna av olika kväveföreningar i träck och urin tydde då på att betor stimulerade bildningen av mikrobprotein mer än vad potatis gjorde. Både betor och potatis verkade minska den totala kväveutsöndringen med urinen lika mycket, medan kvävemängden i träcken tenderade att bli högre av potatis.

Nackdelen med rotfrukter verkar alltså vara att korna inte kan äta lika stora mängder ensilage. I övrigt fungerade rotfrukter och potatis som utmärkta foder, utom för de kor som hade svårt att vänja sig vid potatis. I foderstater som inte bygger på mycket höga ensilagegivor behöver begränsningen av intaget kanske inte vara ett problem, men det minskar ändå användbarheten av rotfrukter som foder. Kornas kvävehushållning verkade inte bli bättre med rotfrukter och potatis än med spannmål, men utsöndringen försköts från urin till träck, vilket gör att det kan vara lättare att minska ammoniakavgången.

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