The Effect of Concentrate Supplementation on Nutrient Flow to the Omasum in Dairy Cows Receiving Freshly Cut Grass

A. Sairanen,¹ H. Khalili,² J. I. Nousiainen,² S. Ahvenjärvi,² and P. Huhtanen²

¹MTT Agrifood Research Finland, North Savo Research Station, FIN-71750 Maaninka, Finland

²MTT Agrifood Research Finland, Animal Production Research, FIN-31600 Jokioinen, Finland

ABSTRACT

An experiment was carried out to determine the effect of increasing the amount of grain-based concentrate (0, 3, or 6 kg/d) on nutrient flow to the omasum, rumen fermentation pattern, milk yield, and nutrient use of dairy cows. Harvested timothy-meadow fescue grass was fed individually 3 times daily to 6 rumen-cannulated Holstein-Friesian cows in a duplicated 3 × 3 Latin square experiment. Grass was offered as 6 equal meals daily, and concentrates were fed as 2 equal meals daily. Nitrogen, microbial N, and neutral detergent fiber (NDF) flow from the rumen were measured using an omasal sampling technique in combination with a triple marker method [CoEDTA, Yb, and indigestible NDF (INDF) as markers]. Concentrate supplementation linearly decreased ruminal pH, N degradability, ammonia N concentration, and molar proportion of acetate and increased the molar proportion of butyrate. Supplementation of grass with concentrates linearly increased dry matter intake (DMI), microbial N synthesis, N, and NDF flow to the omasum, and ruminal and total tract NDF digestibility decreased linearly. Decreases in NDF digestibility in response to concentrates was primarily related to a decrease in the rate of digestion. Increased DMI overcame the negative effects of concentrate on NDF digestion, resulting in a linear increase in total metabolizable energy intake and milk production. Physical constraints were found not to limit grass DMI. Concentrate supplementation increased the apparent use of dietary N for milk production because of a reduction in N intake, rather than thorough improvements in N capture in the rumen.

(**Key words:** dairy cow, grass, concentrate, nutrient flow)

Abbreviation key: C0 = no concentrate supplementation, C3 = 3 kg of concentrate, C6 = 6 kg of concentrate, DNDF = potentially digestible NDF, DOM = digestible

OM, ECM = energy-corrected milk, INDF = indigestible NDF, LP = large particle phase, ME = metabolizable energy, MPS = microbial protein synthesis, RDN = rumen-degradable N, SP = small particle phase, SR = substitution rate.

INTRODUCTION

Grass, either cut or grazed, is an important source of nutrients for dairy cows in temperate regions (Manneetje, 2000). Well-managed grass is highly digestible (Virkajärvi et al., 2003), thus representing an important source of energy for dairy cows. Grass N content is usually high and is extensively degraded in the rumen (Kolver and Muller, 1998), resulting in poor capture of total grass N to microbial N. High intakes of dietary CP are related to increased urinary N excretion. Urinary N is prone to both leaching and volatilization as ammonia (Whitehead, 2000). Supplements of low protein concentrates reduce dietary N content and thereby decrease N losses at the animal level (Jonker et al., 2002). However, to ensure high milk yields, concentrates containing RUP may be needed to complement grass-based diets (Bargo et al., 2003), which could lead to substantial N losses.

In the literature, there is a vast quantity of information reporting on supplementary feeding of dairy cows at pasture or fed on cut-grass feed (Bargo et al., 2003). However, there is a need to collect more data about the responses to concentrate feeding with respect to the supply of nutrients in grazing dairy cows. Ingested feeds are extensively degraded in the rumen to fermentation end products, which are partially recovered as microbial biomass. Thus, it is necessary to examine ruminal digestion kinetics and nutrient flow from the rumen both for supplemented and nonsupplemented diets to understand the reasons for the low DMI for grass diets as compared with the genetic potential of the modern dairy cow. Physical distension of the rumen has been proposed as the limiting factor of high fiber diet intake, whereas metabolic regulation controls intake of high-energy diet (Mertens, 1994). A highly digestible, solely grass diet has both a high fiber content and a high energy value so that physical and

Received September 6, 2004.

Accepted December 7, 2004.

Corresponding author: H. Khalili; e-mail: hannele.khalili@mtt.fi.

metabolic regulation work simultaneously, affecting the DMI of the cow. For optimal diet formulation, information is needed on what is the right balance among environmental needs, RUP supply, and energy content in the diet. This study is focused on determining the factors limiting production in dairy cows on a grass diet.

Feeding cows freshly cut grass enables an accurate measurement of intake, which is fundamental to assessing nutrient flows from the rumen. The results obtained from tie-stall feeding studies and grazing studies may not be totally comparable because of selective grazing behavior, which may increase the digestibility of grazed sward (Dalley et al., 1999). These errors are unlikely to be significant if high quality, homogeneous cut grass is fed. The current study was conducted to determine the effects of concentrate supplementation on intake, nutrient flow to the omasum, milk production, and nutrient use in cows fed fresh-cut grass.

MATERIALS AND METHODS

Animals, Diets, and Procedures

The study was conducted using a replicated ($n = 2$) 3×3 Latin square design with 6 rumen-cannulated Holstein-Friesian lactating cows in their second lactation (average DIM, 57 ± 12 ; BW, 546 ± 38 kg at the start of experiment) at MTT Agrifood Research Finland. Each of the 3 periods lasted 23 d including the first 15 d of free grazing and 8 d of indoor feeding when fresh-cut grass was offered ad libitum to cows housed in a tie-stall barn. Only the last 8 d (d 16 to 23) were used for the calculations. Treatments (**C0**, **C3**, and **C6**) consisted of 3 levels of concentrate supplementation (0, 3, or 6 kg/d, respectively) fed as 2 equal meals at 0600 and 1800 h. After each period, cows were changed over to the next diet over a period of 4 d. The concentrate consisted of barley (40.0%), oats (10.0%), molassed sugar beet pulp (25.0%), wheat bran (5.0%), rapeseed expeller (10.4%), molasses (5.5%), rapeseed oil (1.2%), and minerals (2.9%) determined on a weight basis. Cows had free access to water and a salt block, and a mineral mixture (83 g/kg DM of Ca, 44 g/kg DM of P, 62 g/kg DM of Mg, 57 g/kg DM of Na) was offered at 300 g/d. Table 1 shows the chemical composition of the feeds.

The cows grazed timothy (*Phleum pratense*)-meadow fescue (*Festuca pratensis*) pasture in an intensive rotation with an estimated herbage allowance >25 kg of DM/d from the beginning of the summer. Timothy was the predominant species in the first period, whereas meadow fescue dominated the sward during the last period. Grass for indoor feeding was harvested with a

Table 1. Composition of the experimental feeds.

	Concentrate	Grass		
		Period 1	Period 2	Period 3
DM, g/kg	878	164	178	178
OM, g/kg DM	921	914	910	897
NSC, ¹ g/kg DM	453			
NDF, g/kg DM	246	513	532	482
INDF, ² g/kg DM	36	36	68	43
N, g/kg DM	26	38	38	38
ME, ³ MJ/kg DM	12.6	11.8	11.1	11.6
IVOMD, ⁴ g/kg		805	756	807

¹NSC = Nonstructural carbohydrates.

²INDF = Indigestible NDF.

³ME = Metabolizable energy.

⁴IVOMD = In vitro OM digestibility.

Haldrup 1500 plot harvester from a similar field to the grazed area 3 times daily at 0600, 1400, and 2200 h (stubble height 10 cm). The area harvested remained the same during the experiment. The grass was stored in the barn before being fed to the cows. Grass was offered to cows beginning on d 16 at 1500, 1900, 2300, 0300, 0700, and 1100 h. Orts were weighed before new grass was fed.

Grass samples for chemical and DM analysis were collected after each harvest and pooled to provide 2 subsamples within each period. Samples were stored frozen at -20°C until dried at 60°C for chemical analysis. Concentrate samples were collected periodically and pooled within periods. The cows were milked and the amount of milk was recorded at 0700 and 1700 h. Milk samples were collected over 6 consecutive milkings from each cow on d 18 to 19.

The total digesta flow into the omasum was assessed by the triple-marker method (France and Siddons, 1986) using LiCoEDTA (Udén et al., 1980), Yb-acetate, and indigestible NDF (**INDF**) as markers for liquid (fluid) phase, small particle phase (**SP**), and large particle phase (**LP**), respectively. Ytterbium-acetate (5.0 g/d) and LiCoEDTA (12 g/d) were dissolved daily in 7 L of water and infused into the rumen continuously using a peristaltic pump (Watson Marlow, Falmouth, UK). Infusion of Co and Yb started on d 16 with a 7.5- and 18-g primer dose of Yb and Co, respectively, at 87 h before the first omasal sampling on d 19. The primer dose was used to reach steady-state ruminal marker concentrations more rapidly. Samples were collected from the omasum using the method described by Huh-tanen et al. (1997), incorporating the modification of Ahvenjärvi et al. (2000). Briefly, a plastic tube was inserted into the omasum, and samples were collected using a compressor/vacuum pump. The sampling tube (1.2 m in length) was connected to a 200-mL sand-filled bottle in the abomasum to keep the sampling

device in the right place. Spot samples of digesta from the omasum (400 mL) were collected 4 times daily at 3-h intervals over 3 consecutive days, yielding 12 samples per cow within each period. On d 19, samples were collected at 0900, 1200, 1500, and 1800 h. On d 20, samples were taken 1 h earlier and, on d 21, 2 h earlier compared with the first sampling day. The sampling schedule used was considered representative of the 24-h feeding cycle because of the regular feeding system. The samples were pooled during each period for each animal and stored at -20°C . After thawing at room temperature, samples were separated into 3 different phases. Whole digesta was first squeezed through one layer of cheesecloth. Solids retained on the cheesecloth were defined as the LP. The filtrate was separated into SP and fluid phase by centrifugation at $10,000 \times g$ for 15 min, and the supernatant fraction was drawn off by aspiration. All phases were subsequently frozen and freeze-dried before chemical analysis.

Rumen fluid (200 mL) was collected on d 20 using a vacuum pump. The first sample was taken immediately before the morning concentrate feeding at 0600 h and at 2-h intervals thereafter, yielding 6 samples per cow in total. Rumen pH was measured immediately after sampling (Orion 410A; Orion Research Inc, Boston, MA) at each sampling interval. Samples were strained through a cheesecloth and saturated with HgCl_2 (0.5 mL); 1 M NaOH (2 mL) was added to 5 mL of strained samples of rumen fluid for VFA analysis. For ammonia determination, 0.3 mL of 50% H_2SO_4 (vol/vol) was added to 15 mL of rumen fluid. The samples were stored at -20°C until combined across sampling times for each cow and period and submitted for chemical analysis. Microbial samples for determination of purine N:total N were obtained from the reticulum using a plastic bottle over 3 consecutive days starting on d 20 at 0800 h. The sampling time was then advanced 4 h for each subsequent day, yielding 3 samples per cow. The closed bottle was positioned in the reticulum by hand and was opened until 400 mL of sample had been collected. Samples were treated with formaldehyde and stored at -20°C until the microbial fraction was separated for analysis by differential centrifugation and filtration of the sediment bacteria. The bacterial pellet was suspended in distilled water and freeze dried. Details for the preparation of microbial samples have been described by Ahvenjärvi et al. (2000). At the end of each sampling period, rumen evacuation was performed on 2 occasions. The first evacuation started before concentrate feeding on d 21 at 1800 h after removing the omasal sampling device. The second evacuation was conducted 36 h later on d 23 after concentrate feeding. Rumen contents were

collected into a plastic container, weighed, and mixed thoroughly; an approximate 5-kg representative sample of rumen contents was taken for DM and chemical analysis. After sampling, the remaining rumen contents was returned to the rumen. In total, the evacuation lasted 1 h (20 min per cow). Three 200-g subsamples were dried at 105°C for DM determination, and the rest of the sample was kept at -20°C until analyzed.

Fecal excretion was determined using acid-insoluble ash as a marker. Fecal samples were collected at 6-h intervals on d 19, 20, and 21 at 0300, 0900, 1500, and 2100 h. Total urine collection was also performed at the same time to quantify the urinary purine derivatives and N excretion. Urine was collected using a light harness attached to the vulva of each cow and a flexible tube leading to a container behind the cows under floor level. Sulfuric acid (5 M H_2SO_4 ; 1400 g/d) was added to the container to maintain a pH <3.0 .

Chemical Analysis and Calculations

Milk samples were analyzed for fat, protein, lactose, and urea content using an infrared analyzer (according to IDF 141C:2000, MilkoScan FT6000; www.foss-nir-systems.com). Feed, ruminal, omasal, and fecal samples were analyzed for DM, OM, N, NDF, and INDF. Grass samples were also analyzed for in vitro OM digestibility using the method described by Friedel and Poppe (1990), and omasal samples were analyzed for ADF (Van Soest et al., 1991). Acid-insoluble ash was determined for feed and fecal samples using hydrochloric acid solution (Anon, 1971). Omasal and fecal Co and Yb concentrations were determined according to Williams et al. (1962). Concentrations of ruminal VFA samples were measured by gas chromatography (Hewlett Packard model 5890; Avondale, PA), and ruminal ammonia was determined using the method according to McCullough (1967). Dry matter content was determined by oven-drying at 105°C for 24 h, and OM was calculated after ashing at 600°C for 18 h. A Dumas-type N analyzer (Leco Fp-428; Leco Corporation, St. Joseph, MI) was used to determine the N content of dry samples. As the direct determination of NDF content (Robertson and Van Soest, 1981) of omasal small particles is overestimated because of the substantial amount of fiber-associated N (Ahvenjärvi et al., 2002), the NDF content in the SP was estimated based on ADF measurements, assuming the same ratio of NDF:ADF as determined for LP (Ahvenjärvi, 2002). Determination of INDF (Huhtanen et al., 1994) was based on duplicate 12-d rumen incubations using nylon bags of 6- μm pore size. The INDF samples were rinsed after incubation, boiled for 60 min in NDF solu-

tion, rinsed, and weighed before and after ashing. The potentially digestible NDF (**DNDF**) was calculated as the difference between NDF and INDF. Metabolizable energy (**ME**) intake was calculated on the basis of digestible OM (**DOM**) intake and assuming an energy content of 16 MJ/kg of DOM (Ministry of Agriculture, Fisheries and Food, 1984). Dietary rumen-degradable N (**RDN**) was calculated on the basis of flows entering the omasal canal: $1 - [(N \text{ flow to the omasum} - \text{microbial N})/N \text{ intake}]$. Endogenous flow to the omasum is negligible (Ørskov et al., 1986); therefore, this source was ignored in further calculations. The rumen N losses were estimated as $(N \text{ intake} - N \text{ flow to the omasum})$. The digestion kinetics were calculated as follows:

$$\text{Rate of intake } (k_i) = 1/24 \times (\text{intake, kg/d})/(\text{rumen pool size, kg})$$

$$\text{Rate of passage } (k_p) = 1/24 \times (\text{omasal flow, kg/d})/(\text{rumen pool size, kg})$$

$$\text{Rate of digestion } (k_d) = k_i - k_p.$$

Microbial flow to the omasum was estimated on the basis of bacterial purine content (Zinn and Owens, 1986). The microbial purine N:N ratio was determined from the reticular samples, and microbial N flow to the omasum was calculated using purine base as a marker. The microbial purine:N ratio was assumed to be the same in the reticulum and omasum. Microbial N flow from the rumen was also predicted from daily urinary purine metabolite excretion (allantoin and uric acid) using the method according to Verbic et al. (1990).

Statistical Analyses

Data were analyzed using ANOVA for a Latin square design according to the MIXED procedure of SAS with the following model:

$$Y_{ijk} = \mu + C_i + P_j + D_k + e_{ijk}$$

where μ is the overall mean, C_i is the random cow effect, P_j is the period effect, D_k is the diet effect, and e is the residual effect. Sums of squares were further separated into linear and quadratic contrasts to test the effect of supplementation. Least squares means and significance ($P < 0.1$) are presented in the tables.

Rumen pH was analyzed by ANOVA for the repeated measurements method using compound symmetry covariance structure with the model:

$$Y_{ijkl} = \mu + C_i + P_j + D_k + T_l + D_k \times T_l + e_{ijkl}$$

where μ , C_i , P_j , and D_k are as previously described; T_l is the sampling time; and $D_k \times T_l$ is the interaction between diet and time.

RESULTS

The quality of the harvested grass was relatively stable during each period except for some variation between periods (Table 1). In the second period, the growth stage of the grass was more advanced as indicated by the higher NDF and INDF contents and lower in vitro OM digestibility; N content was comparable across all experimental periods. The average value for water-soluble carbohydrates in grass was 130 g/kg DM, based on feed table values.

All diets exhibited high ME and N concentrations (Table 2). The nonsupplemented grass diet had the highest NDF content and N:ME ratio. Supplementation decreased the dietary NDF content because of the lower NDF content of concentrates. Grass DMI decreased linearly ($P < 0.001$), but total DM ($P < 0.001$) and ME ($P < 0.001$) intakes increased linearly when concentrates were fed. Substitution of grass DM for concentrate DM was 0.27 kg/kg between C0 and C3, which increased to 0.73 between C3 and C6.

Concentrate supplementation linearly decreased mean rumen pH ($P < 0.05$) from 6.41 to 6.17 (Table 3). Rumen pH was lowest in the morning, and pH increased during the day. Interaction between diet and sampling time had no effect on rumen pH. Rumen ammonia concentration varied in a curvilinear fashion ($P_{\text{quad}} < 0.01$), the highest being with diet C3. Concentrate supplementation had a tendency ($P = 0.12$) to increase total VFA concentration in the rumen, with no significant effect on the molar proportions of propionate, but it decreased linearly ($P < 0.01$) the proportions of acetate and increased ($P < 0.05$) that of butyrate. Changes in the molar proportions of butyrate and acetate proportions counterbalanced each other so that the concentrate had no effect on the ratio of lipogenic:glucogenic in rumen VFA.

Rumen total content decreased linearly ($P < 0.06$) with concentrate supplementation, but there were no differences in the rumen DM pool (Table 4). Microbial protein synthesis was numerically, but not significantly, highest for diet C6 based on direct measurements at the omasal canal. However, use of purine metabolite excretion indicated that concentrate supplementation linearly increased ($P < 0.05$) microbial protein available for absorption. The data based on the omasal sampling method were used in further calculations. Supplementation linearly decreased ($P <$

Table 2. Effect of concentrate supplementation on total diet composition and feed intake.

	Treatment ¹			SEM	P	
	C0	C3	C6		Linear	Quadratic
Diet composition, g/kg of DM						
OM	907	909	911	0.1	—	—
N	38	36	35	0.1	—	—
NDF	509	473	439	0.2	—	—
DNDF ²	460	426	394	0.2	—	—
ME, ³ MJ/kg of DM	11.5	11.7	11.8	0.02	—	—
Feed intake, kg of DM/d						
Grass	17.18	16.47	14.56	0.534	<0.001	NS
Concentrate	0.00	2.63	5.27		<0.001	NS
Total ⁴	17.18	19.10	19.83	0.534	<0.001	NS
ME intake, MJ/d	200	218	227	5.2	<0.001	NS
AAT ⁵ intake, g/d	1607	1673	1919	87.4	<0.001	NS

¹C0 = 0 kg/d Concentrate, C3 = 3 kg/d concentrate, and C6 = 6 kg/d concentrate.

²DNDF = Potentially digestible NDF.

³ME = Metabolizable energy.

⁴Total intake does not include mineral mixture.

⁵AAT = AA Absorbed from the small intestine, calculated according to Madsen et al. (1995).

0.05) ruminal protein degradability without having a significant effect on the efficiency of microbial N synthesis. Supplementation linearly increased ($P < 0.001$) total N flow to the omasum and N excretion in feces ($P < 0.01$); urinary N excretion varied in a curvilinear manner ($P_{\text{quad}} < 0.03$), being highest (331 g/d) with C3 and lowest with C6 (304 g/d).

Concentrate supplementation had no effect on NDF or DNDF intake, but the measured rumen DNDF pool was lowest with C3 and highest with C6 ($P_{\text{quad}} < 0.05$), increased NDF ($P < 0.001$) and DNDF flows to the omasum ($P < 0.001$), and, as a result, linearly decreased ($P < 0.001$) ruminal NDF and DNDF digestibility. Increases in the DNDF pool size caused by concentrate supplementation was related to lowered digestion rate (Table 5) of DNDF ($P < 0.05$). Supplementation also linearly increased passage rate ($P < 0.05$) and flow of INDF ($P < 0.05$) from the rumen. The apparent total tract digestibility of OM ($P = 0.11$) and DM tended to linearly decrease ($P < 0.1$) and that

of N, NDF, and DNDF linearly decreased ($P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively) in response to increased concentrate supplementation.

Supplementation linearly increased the yields of milk ($P < 0.001$) and energy-corrected milk (ECM; $P < 0.01$) (Table 6) by 0.82 and 0.73 kg of milk/kg of concentrate DM, respectively. The marginal increase in yield of ECM between 0 and 3 kg was higher than that between 3 and 6 kg, but the quadratic component of this response was not significant ($P_{\text{quad}} = 0.16$). Supplementation had no significant effect ($P > 0.05$) on milk fat content, but linearly increased milk protein content ($P < 0.01$) and linearly reduced ($P < 0.001$) milk urea content. Milk lactose content tended ($P < 0.1$) to increase linearly with supplementation, with the highest concentration being observed for diets supplemented with 3 kg of concentrate ($P_{\text{quad}} < 0.01$). Concentrate in the diet led to linear increases in yields of milk fat ($P < 0.05$), protein ($P < 0.001$), and lactose ($P < 0.001$).

Table 3. Effect of concentrate supplementation on rumen fermentation characteristics.

	Treatment ¹			SEM	P	
	C0	C3	C6		Linear	Quadratic
pH	6.41	6.30	6.17	0.091	0.04	NS
NH ₃ , mM/L	19.9	20.2	15.8	0.65	<0.001	0.003
Total VFA, mM/L	125	132	131	4.8	NS	NS
	(mM/mol)					
Acetate (A)	681	675	668	4.5	0.008	NS
Propionate (P)	178	176	185	3.9	NS	NS
Butyrate (B)	99	109	109	3.0	0.03	NS
(A + B)/P	4.40	4.48	4.23	0.116	NS	NS

¹C0 = 0 kg/d Concentrate, C3 = 3 kg/d concentrate, and C6 = 6 kg/d concentrate.

Table 4. Effect of concentrate supplementation on rumen pool size, microbial protein synthesis, nutrient flow to the omasum, and apparent nutrient digestibility.

	Treatment ¹			SEM	<i>P</i>	
	C0	C3	C6		Linear	Quadratic
Rumen content, kg						
Total	95.1	91.7	88.8	2.53	0.06	NS
DM	11.1	10.8	11.1	0.55	NS	NS
Microbial N synthesis, g/d						
Omasal sampling method	359	361	384	20.4	NS	NS
Purine derivative method	307	310	345	15.9	0.01	NS
DOMI, ² g/kg	28.7	26.5	27.1	1.30	NS	NS
N, kg/d						
Intake	0.651	0.694	0.691	0.0203	0.03	NS
Rumen pool	0.427	0.427	0.433	0.0176	NS	NS
Omasal flow	0.460	0.476	0.540	0.0243	<0.001	NS
RDN, ³ %	87.5	86.6	80.6	0.205	0.04	NS
N loss from the rumen	0.191	0.218	0.151	0.0142	0.05	0.02
In feces	0.137	0.157	0.164	0.0075	0.004	NS
In urine	0.321	0.330	0.304	0.0074	0.06	0.02
NDF, kg/d						
Intake	8.76	9.07	8.73	0.273	NS	NS
Rumen pool	5.30	5.14	5.54	0.356	NS	NS
Omasal flow	1.97	2.25	2.35	0.142	<0.001	NS
Ruminal digestibility, %	77.6	75.6	73.3	0.010	<0.001	NS
DNDF, ⁴ kg/d						
Intake	7.90	8.14	7.81	0.249	NS	NS
Rumen pool	3.17	3.11	3.57	0.265	0.005	0.02
Omasal flow	1.11	1.33	1.43	0.120	<0.001	NS
Ruminal digestibility, %	85.9	83.9	81.7	1.14	<0.001	NS
INDF, ⁵ kg/d						
Rumen pool	2.14	2.03	1.98	0.117	NS	NS
Omasal flow	0.85	0.92	0.92	0.027	0.02	0.09
Apparent total tract digestibility, %						
DM	78.1	76.5	76.5	0.71	0.09	NS
OM	79.6	78.1	78.1	0.71	NS	NS
N	79.1	77.6	76.3	0.60	0.007	NS
NDF	83.6	79.4	78.3	0.89	<0.001	NS
DNDF	89.1	86.0	84.5	0.69	<0.001	NS

¹C0 = 0 kg/d Concentrate, C3 = 3 kg/d concentrate, and C6 = 6 kg/d concentrate.

²DOMI = Digestible OM intake.

³RDN = Ruminal N degradability.

⁴DNDF = Digestible NDF.

⁵INDF = Indigestible NDF.

The apparent efficiency of N use for milk production (N milk/N intake) increased linearly ($P < 0.01$) with concentrate supplementation, and N losses from the rumen (N intake – N flow to the omasum) varied non-linearly ($P_{\text{quad}} < 0.05$), being highest with C3 and lowest for C6. Ruminal N losses per kilogram of grass DMI also varied in a curvilinear fashion ($P_{\text{quad}} < 0.05$) and demonstrated highest losses with diet C3 (11.1, 13.3, and 10.4 g/kg grass DMI for C0, C3, and C6, respectively). Supplementation had no significant effect on the efficiency of energy use for milk production.

DISCUSSION

Feed Intake

Harvesting 3 times daily prevented increasing temperature of the offered grass during the day, and heat-

ing spoilage did not limit grass DMI. Substitution of grass DMI for concentrate DMI (substitution rate; **SR**) was smaller (0.27 kg/kg) between C0 and C3 than between C3 and C6 (0.73 kg/kg). The SR between C0 and C3 was comparable with that reported for cows offered limited amounts of grass (Bargo et al., 2003), despite grass being offered ad libitum in the current study. Low SR has also been reported with low to medium quality grass by Dixon and Stockdale (1999). Those researchers suggested that SR is minimized when energy intake is low compared with the energy requirements of the dairy cow. Thus, it can be assumed on the basis of the low SR that the nonsupplemented cows were in negative energy balance in the present study such that energy intake could have been limiting for milk production on the C0 diet.

Table 5. Effect of concentrate supplementation on fiber digestion kinetics and liquid outflow rate.

	Treatment ¹			SEM	P	
	C0	C3	C6		Linear	Quadratic
DNDF, ² 1/h						
k _i ³	0.107	0.115	0.095	0.0074	NS	0.06
k _p ⁴	0.015	0.018	0.017	0.0011	NS	0.09
k _d ⁵	0.092	0.096	0.078	0.0067	0.06	0.05
INDF, 1/h						
k _p	0.017	0.019	0.019	0.0009	0.04	NS
Liquid flow, L/d	330	303	303	22.3	NS	NS
Liquid k _p , L/h	0.163	0.157	0.163	0.0111	NS	NS

¹C0 = 0 kg/d Concentrate, C3 = 3 kg/d concentrate, and C6 = 6 kg/d concentrate.

²DNDF = digestible NDF.

³k_i = Rate of intake.

⁴k_p = Rate of passage.

⁵k_d = Rate of digestion.

Rumen Fermentation

Concentrate supplementation lowered rumen pH, which can be explained as an increase in the amount of rapidly fermentable carbohydrates in the diet (Dixon and Stockdale, 1999). However, the effects of supplementation on rumen pH are often inconsistent in grazing cows, and it has also been reported that rumen pH can remain unchanged in response to increasing amounts of concentrate in the diet (Bargo et al., 2003). The mean rumen pH for C0 was higher compared with the average value of 6.18 for nonsupplemented diets based on a review of the literature (Kolver and de Veth, 2002). The relatively high grass NDF content partly explains the high rumen pH values (Kolver and de Veth, 2002).

Supplementation resulted in significant, but numerically small, effects on rumen fermentation patterns. The proportion of butyrate increased, and that of ace-

tate decreased, in response to concentrate supplementation so that the overall effects on the lipogenic:gluconogenic VFA ratio were not significant. The changes in VFA proportions were in line with earlier studies examining the impact of concentrate supplementation in grazing cows (Khalili and Sairanen, 2000; Bargo et al., 2003). It is possible that a moderate amount of concentrates leads to only small changes in the composition of the rumen-fermentable carbohydrates. This is a consequence of high amounts of water-soluble carbohydrate and highly digestible NDF, both in the concentrate and grass.

Flow and Digestion of Nutrients

Nitrogen. The decrease in the RDN:OM ratio in response to concentrate supplementation was associated with a decrease in ruminal N losses. However, the highest ruminal N losses occurred with C3, which

Table 6. Effect of concentrate supplementation on milk production and live weight.

	Treatment ¹			SEM	P	
	C0	C3	C6		Linear	Quadratic
Milk, kg/d	25.1	28.3	29.6	1.42	<0.001	NS
ECM, ² kg/d	23.6	26.8	27.6	1.56	0.002	NS
Milk composition, g/kg						
Fat	36.8	36.6	35.5	1.74	NS	NS
Protein	31.0	31.5	32.7	0.50	0.03	NS
Lactose	47.4	48.3	47.9	0.56	0.06	0.008
Urea, mg/dL	50.4	47.8	38.9	2.41	<0.001	NS
Yield, g/d						
Fat	928	1047	1043	76.1	0.04	NS
Protein	779	889	963	47.1	<0.001	NS
Lactose	1190	1366	1417	69.3	<0.001	NS
Live weight, kg	543	545	552	12.8	NS	NS

¹C0 = 0 kg/d Concentrate, C3 = 3 kg/d concentrate, and C6 = 6 kg/d concentrate.

²ECM = Energy-corrected milk (Sjaunja et al., 1990).

can be explained by both the extensive degradation of grass N and low substitution of grass DM for concentrate DM at this moderate level of supplementation. Supplementation increased total N intake by 40 g/d, while N flow to the omasum was enhanced 80 g/d, showing increased apparent N capture in the rumen. The proportion of the rumen undegradable N flow was greater (55 g/d) compared with the microbial N flow (25 g/d). Ahvenjärvi et al. (2002) reported that the amount of grass silage N truly digested in the rumen was increased when a grass silage diet was supplemented with 5.1 kg of barley/d. Thus, it appears that the increase in RUP was derived solely from the concentrate supplement.

Despite improving the supply of microbial N, concentrate supplementation had no effect on the efficiency of microbial protein synthesis (MPS), defined as microbial mass/OM intake, a finding consistent with earlier studies (Berzaghi et al., 1996; Carruthers et al., 1997). Garcia et al. (2000) concluded that fermentation of highly digestible fresh forage DM could provide sufficient energy for MPS and that supplementary grain does not improve the efficiency of MPS. Thus, energy supplementation only increases MPS if fermentable OM intake is increased by concentrate supplementation. In the current study, supplementation increased both OM intake and RUP supply, leading to the highest supply of AA for milk production for treatment C6.

The high amount of RDN is one possible explanation for limited DMI (Kertz et al., 1982). Choung et al. (1990) reported a 50% decrease in silage DMI when rumen NH_3 concentration was increased from 20 to 29 mM/L by ruminal urea infusions, leading to the conclusion that the intake of high-protein silage might be depressed because of extensive absorption of NH_3 from the rumen. Oba and Allen (2003) argued that high ammonium concentration in the blood would limit intake if the hepatic capacity of the liver to detoxify ammonia is exceeded. In this experiment, the high N content of grass coupled with the high RDN:OM ratio for the treatment C0 may help to explain the low DMI as compared with the genetic intake potential of modern dairy cows.

Fiber. Concentrate supplementation had no effect on NDF intake but did increase NDF flow to the omasum while reducing ruminal and total tract NDF digestibility. This result is in agreement with a review of responses to concentrate supplements in dairy cows fed grass silage-based diets (Huhtanen, 1998) and in cows at pasture (Bargo et al., 2003). The proportion of INDF in total NDF was higher in concentrates compared with grass, so that substitution of grass DM for concentrate DM also decreased the potential NDF digestibility of the diet. This effect could account for

0.75 percentage units of the observed 4.3% unit decrease in ruminal NDF digestibility in response to 6 kg of concentrate/d. It is also possible that reductions in NDF digestibility were related to simultaneous decreases in rumen pH and adaptation of rumen microbes to starch rather than fiber (Mould et al., 1983). In the current experiment, the importance of carbohydrate was less than the effect of rumen pH because marked "carbohydrate effect" occurs only for diets containing high amounts of concentrate (Mould et al., 1983). According to the *in vitro* study of Calsamiglia et al. (2002), rapid (<4 h) drops in ruminal pH from 6.4 to 5.7 had no effect on NDF or ADF digestion. Although the lowest average ruminal pH value in the present study was above a threshold of 6.2 (Huhtanen and Jaakkola, 1993; de Veth and Kolver, 2001), below which fiber digestion is thought to be impaired, rumen pH remained between 5.8 and 6.2 for 7 h/d for treatment C6, indicating that rumen pH had decreased NDF digestion with concentrate supplementation. This is also supported by the findings that microbial tolerance against low pH is lowered when starch is included in the diet (Kolver and de Veth, 2002).

The rate of DNDF digestion in the rumen was lowest for C6, which is indicative of ruminal conditions resulting in impaired fiber digestion and is in line with previous findings (Huhtanen and Jaakkola, 1993; Stensig and Robinson, 1997). The decrease in k_d for DNDF was partly compensated for by an increase in rumen DNDF pool size for C6, as the actual amount of DNDF digested per unit of time is dependent on DNDF pool size. However, the increase in DNDF pool size was insufficient to prevent an increase in omasal DNDF flow. A numerically higher DNDF passage rate ($P_{\text{quad}} < 0.1$) in response to concentrate exacerbated reductions in ruminal DNDF digestibility because particles were exposed to microbial digestion for a shorter time. The increase in rate of passage of INDF also demonstrated that the particle passage rate from the rumen was increased. Because there was no compensatory digestion in the lower digestive tract (data not shown), total DNDF digestibility was also decreased by concentrate supplementation.

Both rumen NDF and DNDF pools were considerably lower in this study compared with that of cows fed grass silage or red clover-grass silage and lower DMI (Khalili and Huhtanen, 2002; Rinne et al., 2002). Khalili and Huhtanen (2002) reported that silage DMI and rumen NDF pool increased when casein was infused into the duodenum, indicating that rumen fill can be increased if specific constraints on milk production are alleviated by supplementation. A low rumen fill and low SR of grass DM between C0 and C3 sup-

ports the hypothesis that physical factors do not limit the intake of cows fed highly digestible grass.

Total dietary OM digestibility can be expected to increase with concentrate supplementation, because of the higher inherent digestibility of OM in concentrate compared with grass. However, potential beneficial effects of concentrate supplementation were negated by the decreased NDF digestibility so that overall apparent total tract OM digestibility tended to be reduced ($P = 0.11$) with supplementation.

Milk Production and Nutrient Use

Milk yield. An intake of 17.2 kg of grass DM/d with the C0 diet was sufficient to support a milk yield of 25.1 kg. Similar levels of grass DMI and milk production have been reported in the study conducted by Kennedy et al. (2003) for medium genetic merit cows. High genetic merit cows (Kennedy et al., 2003) or high-producing cows (Kolver and Muller, 1998) have been reported to produce almost 30 kg of milk with 16.9 to 19.0 kg of grass DMI/d without concentrate. Based on the conclusions of these studies, it can be postulated that average milk production >30 kg/d with a sole grass diet is difficult to achieve with the genetic potential of cows at the present moment. The cows on the C0 diet did not achieve such a high production level. Freshly cut grass feeding or the sampling procedure were not the reasons for this, because milk production was 0.2 kg higher in confinement compared with an average of 5 d before indoor feeding periods, which suggests that DMI was the same at pasture and with freshly cut grass.

A mean response of 0.85 kg of milk/kg of DM concentrate in this study is quite a typical response for high-yielding cows at pasture (Bargo et al., 2003). However, milk yield tended to increase in a curvilinear fashion ($P_{\text{quad}} = 0.20$) in response to increases in concentrate supplementation, which is consistent with the low substitution of grass DM for concentrate DM.

Marginal milk protein content and protein yield responses to concentrate in the present study were consistent with previous studies (Bargo et al., 2003). The estimated supply of AA absorbed from the small intestine (Madsen et al., 1995) with C0, based on RUP and microbial flow, were 10% higher compared with requirements for AA absorbed from the small intestine for maintenance and milk yield. This result suggests that either specific essential AA or ME intake limited milk production for treatment C0. These 2 factors are impossible to distinguish without direct measurement of AA flows. It is possible that increased concentrate origin RUP has provided limiting AA for milk production. However, Kolver and Muller (1998) concluded

that energy is the first-limiting factor for milk production of cows consuming high-quality pasture, and metabolizable protein does not limit milk production.

Nutrient use. The apparent efficiency of N use for milk production (19%) was low in cows fed grass alone because of the excessive amount of N intake compared with rumen-digestible OM. Bargo et al. (2002) reported a lower efficiency (15.7%) for nonsupplemented grazing dairy cows. Concentrate supplementation improved N use from 19 to 22%, but this is still considerably lower than the typical values of 28% reported for cows across a range of dairy farm diets (Jonker et al., 2002).

Concentrate supplementation decreased ammonia losses from the rumen and lowered milk urea content and urinary N excretion; the amount of fecal N excretion increased. Increasing the fecal:urine ratio is advantageous as the urinary N is both highly vulnerable to volatilization and leaching (Whitehead, 2000). However, the highest N losses from the rumen occurred with C3 when expressed as g of N losses/kg of grass DMI. This, together with no changes in MPS efficiency, indicated that concentrate supplementation had only negligible effects on the use of grass N and that the improvements in total dietary N use for milk production were due to reduced dietary CP content. Supplementation with 6 kg concentrate/d increased N usage by 156 g per cow, and N secreted as milk protein increased 29 g per cow. Thus, the overall utilization of N at the farm level decreased with increases in concentrate supplementation. Van Bruchem et al. (1999) concluded that improving the efficiency of dietary N utilization is a less effective strategy for reducing on-farm N losses than lowering fertilizer and concentrate usage.

Increases in DMI between C0 and C6 resulted in an additional 27 MJ of ME when based on DOM intake; a marginal increase in ME intake of 37 MJ should be attained according to feed tables (Tuori et al., 2000). Thus, the observed increase in ME intake was only 73% of that predicted, which can be attributed to negative associative effects of concentrate supplementation on rumen environment. The observed marginal ME intake responses between C0 and C3 of 0.17 kg of ECM/MJ of ME are relatively high compared with predicted responses of 0.19 kg of ECM/MJ of ME (Tuori et al., 2000) or typical values of between 0.08 to 0.10 attributed to increases in concentrate supplementation (Huhtanen, 1998). The high milk production response to marginal increases in ME intake together with the low SR of grass DM suggests that the cows on the C0 diet were in negative energy balance, despite the high in vivo OM digestibility. It appears that ME intake,

in addition to essential AA supply, limited the milk production of cows offered grass as the sole feed.

CONCLUSIONS

Intake of grass was unable to provide sufficient nutrients to maintain milk production when compared with the genetic potential of the modern dairy cow. Physical regulation did not limit the intake of grass DMI. High milk response to increased ME intake and low substitution of grass DM for concentrate DM suggest that energy supply was the limiting factor for the nonsupplemented diet. Concentrate supplementation increased rumen undegradable protein and microbial flow into the omasum, which provided a higher supply of AA for milk production. The digestibility of NDF was decreased by concentrate supplements, but this effect was minimized by the associated increases in total DMI. A decrease in digestion rate was the primary reason for lowered NDF digestion in response to concentrates. Supplementation increased apparent efficiency of N use for milk production in the animal because of reductions in dietary N content, but had no effect on microbial capture of grass N in the rumen.

REFERENCES

- Ahvenjärvi, S. 2002. Determination of ruminal feed digestibility and microbial synthesis based on digesta sampling from the omasal canal. Ph.D. Diss., Univ. Helsinki, Finland.
- Ahvenjärvi, S., A. Vanhatalo, and P. Huhtanen. 2002. Supplementing barley or rapeseed meal to dairy cows fed grass-red clover silage: I. Rumen degradability and microbial flow. *J. Anim. Sci.* 80:2176–2187.
- Ahvenjärvi, S., A. Vanhatalo, P. Huhtanen, and T. Varvikko. 2000. Determination of reticulo-rumen and whole-stomach digestion in lactating cows by omasal canal or duodenal sampling. *Br. J. Nutr.* 83:67–77.
- Anon. 1971. Determination of ash which is insoluble in hydrochloric acid. *Official J. Eur. Community* 2155:30–31.
- Bargo, F., L. D. Muller, J. E. Delahoy, and T. W. Cassidy. 2002. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. *J. Dairy Sci.* 85:1777–1792.
- Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. *J. Dairy Sci.* 86:1–42.
- Berzaghi, P., J. H. Herbein, and C. E. Polan. 1996. Intake, site, and extent of nutrient digestion of lactating cows grazing pasture. *J. Dairy Sci.* 79:1581–1589.
- Calsamiglia, S., A. Ferret, and M. Devant. 2002. Effects of pH and pH fluctuation on microbial fermentation and nutrient flow from a dual-flow continuous culture system. *J. Dairy Sci.* 85:574–579.
- Carruthers, V. R., P. G. Neil, and D. E. Dalley. 1997. Effect of altering the non-structural:structural carbohydrate ratio in a pasture diet on milk production and ruminal metabolites in cows in early and late lactation. *Anim. Sci.* 64:393–402.
- Choung, J.-J., D. G. Chamberlain, P. C. Thomas, and I. Bradbury. 1990. The effects of intraruminal infusions of urea on the voluntary intake and milk production of cows receiving grass silage diets. *J. Dairy Res.* 57:455–464.
- Dalley, D. E., J. R. Roche, C. Grainger, and P. J. Moate. 1999. Dry matter intake, nutrient selection and milk production of dairy cows grazing rainfed perennial pastures at different herbage allowances in spring. *Aust. J. Exp. Agric.* 39:923–931.
- de Veth, M. J., and E. S. Kolver. 2001. Digestion of ryegrass pasture in response to change in pH in continuous culture. *J. Dairy Sci.* 84:1449–1457.
- Dixon, R. M., and C. R. Stockdale. 1999. Associative effects between forages and grains: Consequences for feed utilization. *Aust. J. Agric. Res.* 50:757–773.
- France, J., and R. C. Siddons. 1986. Determination of digesta flow by continuous marker infusion. *J. Theor. Biol.* 121:105–119.
- Friedel, K., and S. Poppe. 1990. Ein modifiziertes Zellulase Verfahren als Methode zur Schätzung der Verdaulichkeit von Grobfutter. G-4-Bericht, Wilhelm-Pieck Universität Rostock, WB Tierernährung.
- Garcia, S. C., C. J. Santini, and J. C. Elizalde. 2000. Sites of digestion and bacterial protein synthesis in dairy heifers fed fresh oats with or without corn or barley grain. *J. Dairy Sci.* 83:746–755.
- Huhtanen, P. 1998. Supply of nutrients and productive responses in dairy cows given diets based on restrictively fermented silage. *Agric. Food Sci. Finl.* 7:219–250.
- Huhtanen, P., P. G. Brotz, and L. D. Satter. 1997. Omasal sampling technique for assessing fermentative digestion in the forestomach of dairy cows. *J. Anim. Sci.* 75:1380–1392.
- Huhtanen, P., and S. Jaakkola. 1993. The effects of forage preservation method and proportion of concentrate on digestion of cell wall carbohydrates and rumen digesta pool size in cattle. *Grass Forage Sci.* 48:155–165.
- Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Anim. Feed Sci. Technol.* 48:211–227.
- Jonker, J. S., R. A. Kohn, and J. High. 2002. Dairy herd management practices that impact nitrogen utilization efficiency. *J. Dairy Sci.* 85:1218–1226.
- Kennedy, J., P. Dillon, L. Delaby, P. Faverdin, G. Stakelum, and M. Rath. 2003. Effect of genetic merit and concentrate supplementation on grass intake and milk production with Holstein Friesian dairy cows. *J. Dairy Sci.* 86:610–621.
- Kertz, A. F., M. K. Koepke, L. E. Davidson, N. L. Betz, J. R. Norris, L. V. Skoch, B. R. Cords, and D. T. Hopkins. 1982. Factors influencing intake of high urea-containing rations by lactating dairy cows. *J. Dairy Sci.* 65:587–604.
- Khalili, H., and P. Huhtanen. 2002. Effect of casein infusion in the rumen, duodenum or both sites on factors affecting forage intake and performance of dairy cows fed red clover-grass silage. *J. Dairy Sci.* 85:909–918.
- Khalili, H., and A. Sairanen. 2000. Effect of concentrate type on rumen fermentation and milk production of cows at pasture. *Anim. Feed Sci. Technol.* 84:199–212.
- Kolver, E. S., and L. D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or total mixed ration. *J. Dairy Sci.* 81:1403–1411.
- Kolver, E. S., and M. J. de Veth. 2002. Prediction of ruminal pH on pasture-based diets. *J. Dairy Sci.* 85:1255–1266.
- Madsen, J., T. Hvelplund, M. Weisbjerg, J. Bertilsson, I. Olsson, R. Spörndly, O. M. Harstad, H. Volden, M. Tuori, T. Varvikko, P. Huhtanen, and B. L. Olafson. 1995. The AAT/PBV protein evaluation systems for ruminants. A revision. *Nor. J. Agric. Sci.* 19(Suppl.):1–37.
- Mannetje, T. L. 2000. The importance of grazing in temperate grasslands. Pages 3–13 in *Grazing Management, Proc. BGS Occasional Symposium No. 34*, Harrogate, UK.
- McCullough, H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. *Clin. Chim. Acta* 17:297–304.
- Mertens, D. R. 1994. Pages 450–493 in *Forage Quality, Evaluation, and Utilization*. G. C. Fahey, ed. ASA, SSSA, CSSA, Madison, WI.
- Ministry of Agriculture, Fisheries, and Food. 1983. Energy allowances and feeding systems for ruminants. Reference book No. 433. Her Majesty's Stationery Office, London, UK.
- Mould, F. L., E. R. Ørskov, and S. O. Mann. 1983. Associative effects of mixed feeds. I. Effects of type and level of supplementation

- and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. *Anim. Feed Sci. Technol.* 10:15–30.
- Oba, M., and M. S. Allen. 2003. Hypophagic effects of ammonium are greater when infused with propionate compared with acetate in lactating dairy cows. *J. Nutr.* 133:1100–1104.
- Ørskov, E. R., N. A. Macleod, and D. J. Kyle. 1986. Flow of nitrogen from the rumen and abomasum in cattle and sheep given protein-free nutrients by intragastric infusion. *Br. J. Nutr.* 56:241–248.
- Rinne, M., P. Huhtanen, and S. Jaakkola. 2002. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. *J. Anim. Sci.* 80:1986–1998.
- Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123–158 in *The Analysis of Dietary Fibre in Food*. W. P. T. James and O. Theander, ed. Marcel Dekker, New York, NY.
- Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1990. A Nordic proposal for an energy-corrected milk (ECM) formula. Pages 156–157 in 27th Session Int. Comm. of Recording and Productivity of Milk Animal, Paris, France.
- Stensig, T., and P. H. Robinson. 1997. Digestion and passage kinetics of forage fibre in dairy cows as affected by fibre-free concentrate in the diet. *J. Dairy Sci.* 80:1339–1352.
- Tuori, M., K. Kaustell, J. Valaja, E. Aimonen, E. Saarisalo, and P. Huhtanen. 2000. *Feed Tables and Feeding Recommendations*. 3rd ed. Yliopistopaino, Helsinki, Finland.
- Udén, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium, and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31:625–632.
- Van Bruchem, J., H. Schiere, and H. Van Keulen. 1999. Dairy farming in The Netherlands in transition towards more efficient use of nutrients. *Livest. Prod. Sci.* 61:145–153.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Verbic, J., X. Chen, N. A. MacLeod, and E. R. Ørskov. 1990. Excretion of purine derivatives by ruminants: Effect of microbial nucleic acid infusion on purine excretion by steers. *J. Agric. Sci. (Camb.)* 114:243–248.
- Virkajärvi, P., A. Sairanen, and J. Nousiainen. 2003. Sward and milk production response to early turn out of dairy cows to pasture in Finland. *Agric. Food Sci. Finl.* 12:21–34.
- Whitehead, D. C. 2000. *Nutrient Elements in Grassland: Soil-Plant-Animal Relationship*. CABI Publishing, New York, NY.
- Williams, C. H., D. David, and O. Riismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrometry. *J. Agric. Sci. (Camb.)* 59:381–385.
- Zinn, R. A., and F. N. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. Anim. Sci.* 66:157–166.