ELSEVIER

Animal Feed Science and Technology
98 (2002) 103–119

ANIMAL FEED SCIENCE AND TECHNOLOGY

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# Effect of protein and energy supplements on milk production in organic farming

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Received 10 May 2001; received in revised form 22 May 2001; accepted 29 November 2001

**Abstract**

The effects of protein and energy supplementation on milk production responses were studied in three experiments. In experiment 1, eight cows were used in a duplicated 4 × 4 Latin square design to study the effects of a commercial conventionally produced rapeseed meal and organically cultivated field pea (*Pisum sativum* L.) as protein supplements for organic grass-red clover silage-based diets. Silage was fed ad libitum and concentrates at a rate of 8 kg per day. A mixture (1:1) of oats and barley was used as a control supplement (C). Rapeseed meal (R), a mixture of (1:1 on a CP basis) of rapeseed and field pea (RP) or field pea (P) were incorporated into three other supplements. Compared with C, R increased ($P < 0.05$) silage intake by 0.64 kg per day and improved the daily yields of milk (1.7 kg), energy corrected milk (ECM) (1.6 kg), and milk protein (78 g), but decreased ($P < 0.001$) the efficiency of dietary N utilisation from 0.295 to 0.264. Replacement of R with P linearly decreased ($P < 0.05$) ECM yield from 24.5 to 23.3 kg per day and milk protein yield from 764 to 719 g per day. In experiment 2, the effects of energy (organically grown oats–barley, 1:1) and protein (commercial rapeseed meal) supplements on milk production in cows grazing organic grass swards was studied in a duplicated 4 × 4 Latin square design. Treatments consisted of herbage alone (H), 4 kg per day of energy supplement (E), 1.25 kg per day of protein supplement (P) and 2.75 kg per day of energy and 1.25 kg per day of protein supplements (EP). Both E and P supplementation improved ($P < 0.01$) the daily yields of milk (2.3 and 1.4 kg, respectively) and milk constituents. Yields of milk and milk protein were highest when part of the E supplement was replaced with P. In experiment 3, the effects of supplement (g per day, oats (450), barley (450), rapeseed meal (100)) level (2.5 or 5 kg per day) and herbage allowance (HA, 18 or 24 kg DM per day) on milk production of cows at pasture was studied in a duplicated 4 × 4 Latin square. Higher levels of supplementation increased

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0377-8401/02/$ – see front matter 
PII: S0377-8401(02)00005-6

($P < 0.01$) milk yield and protein output by 1.7 kg and 64 g per day, respectively. In conclusion, the protein value of pea was markedly lower than that of rapeseed meal, while both the use of energy and protein supplements improved milk production at pasture. 

*Keywords:* Milk production; Protein supplement; Field pea; Rapeseed; Energy supplement; Silage; Pasture; Organic farming

## 1. Introduction

Increasing numbers of farmers are adopting organic farming systems where soluble mineral fertilisers are not permitted (2092/91, CEC 1991). Both low fertilizer inputs and organically produced protein rich legume forages are crucial for plant and soil nitrogen transfer. Milk production under organic conditions is based on feeds cultivated according to established standards. At present only a minor proportion of feeds can be conventionally and/or commercially produced for this purpose (EC No. 1804/1999). Diets should also contain at least proportionately 0.60 (on a dry matter (DM) basis) forage. However, during the first 3 months after calving the proportion of concentrates can be increased to a maximum of 0.50. Furthermore, cattle should have daily access to pasture during the grazing season. In Finland, mixtures of grass–swards are used as the basal forage for milk production under both organic and conventional farming practices. Field pea is the most important legume forage for organic farming, while rapeseed meal has been shown to be a good protein supplement for conventional grass silage-based diets (Huhtanen, 1998). However, there is a lack of available information concerning milk production responses to energy and protein supplements in cows fed organically cultivated feeds or feeds produced with low fertilizer inputs.

Grasses and particularly legumes are able to satisfy rumen microbe N requirements, but increasing N supply through this approach is inefficient due to the extensive rumen N degradation resulting in substantial losses of ammonia and N (Beever and Siddons, 1986). Protein supplementation has increased milk production for lucerne silage (Dhiman and Satter, 1993; Broderick et al., 2000), red clover silage (Broderick et al., 2000), and grass silage-based diets (Thomas and Rae, 1988; Huhtanen, 1998; Rinne et al., 1999a) but responses in grazing animals have been variable (Delaby et al., 1996; Delagarde et al., 1997; Delagarde et al., 1999; Jones-Endsley et al., 1997). Replacement of cereals with slowly degraded protein has been reported to improve milk production to a greater extent in cows offered low compared with heavily fertilized grass swards (Delaby et al., 1996).

It is well-known that high forage diets do not provide sufficient energy for milk production during early- and mid-lactation indicating the necessity for energy supplements. For cows offered high quality forage ad libitum, use of concentrate supplements, is based on attaining high milk production during the indoor feeding period. However, for cows at pasture, adopting the same strategy is complicated due to differences in grazing management.

The current study, comprised of three individual experiments, was conducted to evaluate the effects of protein and energy supplements on milk production. The first study, relative milk production responses to field pea, rapeseed or a combination of the two of cows offered organic grass-clover silage-based diets were assessed. The two additional experiments were designed to examine the effects of protein and energy supplements on milk production of cows at pasture. The effects of grazing strategy and herbage (H) allowance on milk production are reported in a companion paper (Kuusela and Khalili, 2002).

## 2. Material and methods

### 2.1. Animals, feeding, experimental design and experimental diets

Experiments were conducted at the Siikasalmi experimental farm of the University of Joensuu (62°30′ N, 29°30′ E) in North Karelia with Finnish Ayrshire dairy cows. Cows were fed individually at 07:30 and 15:30 h and milked at 06:00 and 16:00 h. Oats, barley, field pea (*Pisum sativum* L.) and grass-legume swards were cultivated under organic conditions (2092/91, CEC 1991). Use of soluble mineral fertilizers and most agrochemicals are prohibited for the production of organic crops within the EU. As a result long-term soils fertility is maintained or improved through crop-rotation using fertility enhancing (grass-legume leys, field pea) and other crops (oats, barley), applications of organic matter (farmyard manure), lime rock potassium and phosphate. Rapeseed meal (RSM) was conventionally produced (Raisio Feed Ltd., Raisio Finland).

#### 2.1.1. Experiment 1

Silage prepared from secondary growths of swards containing on the average 60% grass (timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*)) and 40% legume, mainly as red clover (*Trifolium pratense*) was offered ad libitum providing 0.05 refusals. Herbage was ensiled after wilting for 6 h using a formic acid-based additive (6 l $t^{-1}$) and placed in a tower silo. Field pea grain was crushed with a hammer mill to pass through a 4 mm sieve.

The experiment was carried out during winter 1999 with eight (66 $\pm$ 20 days in milk) cows, four of which were in their first lactation, and conducted as two balanced 4 $\times$ 4 Latin squares with 21 days periods that comprised of a 14 days adjustment period and a 7 days recording period. Cows were allocated to squares according to milk yield and diets were randomly assigned to each cow within a square. Four supplements including 250 g per day of a commercial mineral mixture (containing (g $kg^{-1}$) Ca (160), P (64), Mg (80) and Na (90) and 20 g per day of NaCl) were fed at 8 kg per day (on an air-dry basis). Supplements were formulated (g $kg^{-1}$) from oats (500) and barley (500) for C, oats (392), barley (391) and rapeseed meal (217) for R, oats (338), barley (337), rapeseed meal (128) and field pea (197) for RP, and oats (258), barley (258) and field pea (484) for P. Supplements R, RP and P were formulated to be isonitrogenous. The intake of RSM (kg DM per day) corresponded to 1.52 and 0.89 for supplements of R and RP, respectively, while supplements P and RP provided 3.35 and 1.38 kg pea per day, respectively.

Composition of supplements used and chemical composition and calculated feeding values of dietary ingredients are shown in Table 1.

Table 1
Composition of supplements used and chemical composition and calculated feeding values of dietary ingredients (experiment 1)[a]

| | C | R | RP | P | S[b] |
|---|---|---|---|---|---|
| Concentrates ($g\ kg^{-1}$) | | | | | |
| Oats | 500 | 392 | 338 | 258 | |
| Barley | 500 | 391 | 337 | 258 | |
| Rapeseed meal[c] | – | 217 | 128 | – | |
| Field peas | – | – | 197 | 484 | |
| Dry matter ($g\ kg^{-1}$) | 870 | 880 | 872 | 866 | 260 |
| Crude protein ($g\ kg^{-1}$) | 129 | 186 | 181 | 175 | 140 |
| Ether extract ($g\ kg^{-1}$) | 48 | 52 | 46 | 38 | – |
| NDF[d] ($g\ kg^{-1}$) | 280 | 281 | 254 | 214 | 561 |
| AAT[e,f] ($g\ kg^{-1}$) | 98 | 111 | 110 | 110 | 80 |
| Me[f,g] (MJ kg per DM) | 13.0 | 12.6 | 12.9 | 13.3 | 10.3 |

[a] C: mixture of oats and barley; R: C + rapeseed meal; RP: C + rapeseed meal and pea; P: C + pea; S: grass-red clover silage.
[b] Silage: pH 4.00; dry matter 257 ($g\ kg^{-1}$); in dry matter ($g\ kg^{-1}$): water-soluble carbohydrates 18; lactic acid 52; acetic acid 19; in total N ($g\ kg^{-1}$): ammonia N 61; soluble N 528. In vitro OM cellulase digestibility 0.70.
[c] Solvent extracted.
[d] Neutral detergent fibre.
[e] Amino acids absorbed from the small intestine.
[f] Values derived from feed tables (Tuori et al., 1996).
[g] Metabolizable energy.

### *2.1.2. Experiment 2*

Experiment 2 was conducted with cows grazing swards predominating in timothy, meadow fescue and white clover (*Trifolium repens*) during 1997. At the beginning of experiment the average clover content was 9% which remained relatively low during the entire grazing period. Cows were either strip grazed (herbage allowance (HA) above 3 cm was 22.5 kg DM per cow per day) or allocated to paddocks over several days, with an average grazing cycle length of 21 days. Grazing management is described in more detail by Kuusela and Khalili (2002). The experiment was conducted with eight lactating ($152 \pm 51$ days in milk) cows, as two $4 \times 4$ Latin squares with 21 days periods that comprised of a 14 days adjustment period and a 7 days recording period.

Cows were offered pasture alone (H) or supplemented with 4 kg per day of an oat and barley mixture (500/500 $g\ kg^{-1}$ (E)), 1.25 kg per day of RSM (P) or 2.75 kg of the oat and barley mixture and 1.25 kg per day of RSM (EP) (Table 2). Supplements were formulated such that E and P supplements supplied the same amount of crude protein (CP) and supplements E and EP provided equal amounts of energy.

### *2.1.3. Experiment 3*

Experiment 3 was conducted with grazing cows during 1998. Pasture was strip grazed and contained an average clover content of 36% at the beginning of the experiment that marginally increased during the summer. Cows had access to either 18 or 24 kg DM of fresh herbage. Due to severe tramping additional areas of pasture had to be included into

Table 2
Composition of supplements used and chemical composition and calculated feeding values of dietary ingredients (experiments 2 and 3)[a]

| | Herbage[b] | | E | P | EP | HA[b] | | Supplement |
|---|---|---|---|---|---|---|---|---|
| | Strip | Paddock | | | | 18 | 24 | |
| Concentrates ($g\ kg^{-1}$) | | | | | | | | |
| Oats | | | 500 | – | 344 | | | 450 |
| Barley | | | 500 | – | 344 | | | 450 |
| Rapeseed meal[c] | | | – | 1000 | 312 | | | 100 |
| Dry matter ($g\ kg^{-1}$) | 217 | 219 | 860 | 890 | 870 | 166 | 161 | 863 |
| Ash ($g\ kg^{-1}$) | 86 | 83 | 30 | 80 | 46 | 90 | 91 | 35 |
| Crude protein ($g\ kg^{-1}$) | 171 | 176 | 129 | 390 | 210 | 173 | 167 | 155 |
| Ether extract ($g\ kg^{-1}$) | ND[d] | ND[d] | 48 | 66 | 54 | ND[d] | ND[d] | 50 |
| NDF[e] ($g\ kg^{-1}$) | 556 | 551 | 280 | 284 | 281 | 438 | 467 | 280 |
| AAT[f,g] ($g\ kg^{-1}$) | 90 | 90 | 98 | 157 | 116 | 90 | 90 | 104 |
| ME[g,h] (MJ kg per DM) | 11.0 | 11.0 | 13.0 | 11.3 | 12.5 | 11.0 | 11.0 | 12.8 |
| In vitro OM digestibility | 0.72 | 0.73 | ND[d] | ND[d] | ND[d] | 0.76 | 0.74 | ND[d] |

[a] E: mixture of oats and barley; P: rapeseed meal alone; EP: oats and barley and rapeseed meal; supplement: mixture of oats and barley and rapeseed meal.
[b] Herbage Strip: strip grazing, paddock: paddock grazing system; HA: herbage allowance (kg DM).
[c] Solvent extracted.
[d] ND= not determined.
[e] Neutral detergent fibre.
[f] Amino acids absorbed from the small intestine.
[g] The values derived from feed tables (Tuori et al., 1996).
[h] Metabolizable energy.

the grazing rotation. Grazing management is described in more detail elsewhere (Kuusela and Khalili, 2002). The experiment was conducted with eight lactating (148 $\pm$ 45 days in milk) cows, as two 4 $\times$ 4 Latin squares with 21 days periods that consisted of a 14 days adjustment period and 7 days recording period.

Four experimental treatments in a 2 $\times$ 2 factorial arrangement consisted of two herbage allowances (HA, 18 or 24 kg DM per day) and two levels of concentrate supplementation (S, 2.5 or 5.0 kg per day as an air-dry basis). Concentrate was formulated ($g\ kg^{-1}$) from oats (450), barley (450) and RSM (100) (Table 2).

### *2.2. Measurements and analytical procedures*

Milk yield and feed intake (experiment 1) were measured daily in each experiment. Silage dry matter (DM) content was determined by drying at 105 °C for 24 h and corrected for volatile losses according to Huida et al. (1986). Results presented are based on measurements of intake (experiment 1) and milk production (all experiments) collected during the last 6 days of each experimental period. Intake from pasture was not estimated (experiments 2 and 3). Representative samples of feeds were collected during the last 6 days of each experimental period and pooled within period. During the last 5 days of each period faecal grab samples (experiment 1) were taken twice daily from each cow at milking, pooled within cow, stored frozen and analysed for acid insoluble ash (AIA) according to Van Keulen and Young (1977). Diet digestibility was estimated using AIA as an internal marker. Samples of feeds and faeces were analysed for organic matter (OM) by ashing at 600 °C for 12 h, nitrogen (Kjeldahl-N) and neutral detergent fibre (NDF) (Van Soest et al., 1991). Fat was extracted with diethyl ether (AOAC, 1990) after boiling for 1 h in 3 M HCl. Silage samples were also analysed for water soluble carbohydrates (Somogyi, 1945), lactic acid (Haacker et al., 1983), VFA (Huida, 1973) and ammonia N (McCullough, 1967). Silage in vitro OM digestibility was measured using a cellulase-based method (Friedel, 1990). During each collection period milk samples were collected over six consecutive milking from each cow and analysed for fat, protein and lactose using an infrared milk analyser (Milcoscan 605) and urea according to McCullough (1967). During each collection period cows were body condition scored using a scale from 1 (very thin) to 5 (very fat).

### 2.3. Calculations and statistical methods

For experiment 1, intakes of metabolizable energy (ME) and the supply of amino acids absorbed from the small intestine (AAT) were calculated based on published values for dietary ingredients (Tuori et al., 1996). Intakes of ME were estimated based on digestible organic matter (DOM) intake assuming a ME content of 16 MJ kg$^{-1}$ DOM (MAFF, 1984).

Data from all experiments were analysed using the SAS systems for linear models for a Latin square design (Littell et al., 1992) according to the following model:

$$y_{ijlkm} = \mu + S_i + C_J(S_i) + P_k + D_l + PxS_{ki} + DxS_{li} + f_{ijklm},$$

where $\mu$ is the overall mean, $S$, $C$ and $P$ the random effects of square, cow and period, respectively, $D$ the fixed effect of treatments and $f_{ijlkm}$ the random error term. Treatment effects were further separated into single degree of freedom orthogonal contrasts. For experiment 1, contrasts assessed the effect of RSM (C versus R) and linear (L) and quadratic responses (Q) to replacing R with P. In the second experiment, the sum of squares for treatment effects were divided into single degree of freedom comparisons to evaluate the effects of energy (E) supplementation (C + P versus E + EP), protein (P) supplementation (C + E versus P + EP) and their interaction (E × P). There were no interactions between supplement types. The effect of grazing method rotational versus strip grazing on milk production was also analysed, the results of which are documented in a companion paper (Kuusela and Khalili, 2002). For experiment 3, the effect of supplementation level (2.5 versus 5.0) and HA were tested. There were no interactions between supplement level and HA. The effects of HA are reported in a companion paper (Kuusela and Khalili, 2002).

## 3. Results

### 3.1. Experiment 1

Concentrations of CP in R, RP and P supplements were 186, 181 and 175 g kg$^{-1}$, respectively. Due to a higher ME content of P (13.3 MJ kg per DM) compared with R supplement (12.6 MJ kg per DM) the CP content of both supplements was very similar.

The grass-clover silage was of good fermentation quality in terms of a low pH, low concentrations of fermentation acids and a small proportion of ammonia N in total N (Table 1).

Compared with the control, feeding supplement R increased ($P < 0.05$) daily silage and total DM intake and estimated ME and AAT intake (Table 3). Replacing RSM with pea decreased ($P < 0.05$) total DM intake. Due to the higher energy content of pea compared with RSM, estimated ME intake was not affected by protein source. Silage intake tended to decrease ($P = 0.08$) and the supply of AAT was reduced ($P < 0.05$) with increases in the proportion of pea in the protein supplement. Digestibility of DM, OM and NDF was higher ($P < 0.05$) for C than R diets. Increasing the proportion of pea in the supplement linearly improved OM digestibility ($P < 0.05$) but linearly decreased N and ether extract digestibility ($P < 0.01$).

Compared with the control, feeding supplement R increased daily milk yield ($P < 0.05$) and the concentration ($P < 0.05$) and output ($P < 0.001$) of milk protein (Table 4). Increases in ECM, fat and lactose secretion attained with supplement R were significant relative to the control treatment. Milk urea concentration was increased ($P < 0.001$) and apparent N utilisation was decreased by feeding supplement R. Replacement of R with P linearly decreased ($P < 0.05$) ECM, milk fat and protein yields.

Table 3
Mean treatment effects on nutrient intake and digestibility (experiment 1)

| | Supplements[a] | | | | S.E.M. | Significance of effect | |
|---|---|---|---|---|---|---|---|
| | C | R | RP | P | | C vs. R | Protien L |
| Feed intake (kg DM per day) | | | | | | | |
| Silage | 9.96 | 10.60 | 10.45 | 10.14 | 0.180 | * | $P = 0.08$ |
| Supplement | 6.51 | 7.02 | 6.98 | 6.92 | | | |
| Total intake | 16.47 | 17.62 | 17.43 | 17.06 | 0.174 | *** | * |
| ME1[b](MJ per day) | 186.7 | 194.6 | 196.2 | 193.4 | 1.83 | ** | |
| ME2[c](MJ per day)) | 187.2 | 197.6 | 197.6 | 196.5 | 1.83 | *** | |
| N intake (g per day) | 365 | 455 | 444 | 429 | 4.0 | *** | *** |
| AAT[c](g per day) | 1435 | 1627 | 1603 | 1572 | 14.2 | *** | ** |
| Digestibility | | | | | | | |
| Dry matter | 0.738 | 0.723 | 0.730 | 0.733 | 0.0034 | ** | $P = 0.06$ |
| Organic matter | 0.757 | 0.743 | 0.751 | 0.753 | 0.0033 | ** | * |
| N | 0.717 | 0.732 | 0.719 | 0.705 | 0.0059 | | ** |
| Ether extract | 0.717 | 0.726 | 0.702 | 0.666 | 0.0063 | | *** |
| NDF[d] | 0.667 | 0.654 | 0.664 | 0.660 | 0.0041 | * | |

Number of observations per each treatment was eight. S.E.M.: standard error of mean.

[a] C: mixture of oats and barley; R: C + rapeseed meal; RP: C + rapeseed meal and pea; P: C + pea.

[b] Calculated from the intake of digestible organic matter determined in cows using acid insoluble ash as an internal marker.

[c] Calculated using values from feed tables (Tuori et al., 1996).

[d] NDF = neutral detergent fibre.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 4
Mean treatment effects on milk production and efficiency of N utilization for milk production (experiment 1)

| | Supplements[a] | | | | S.E.M. | Significance of effect | |
|---|---|---|---|---|---|---|---|
| | C | R | RP | P | | C vs. R | Protein L |
| Milk yield (kg per day) | 21.8 | 23.5 | 23.1 | 22.4 | 0.47 | * | |
| ECM[b] yield (kg per day) | 22.9 | 24.5 | 24.4 | 23.3 | 0.33 | ** | * |
| Milk composition (g kg$^{-1}$) | | | | | | | |
| Fat | 45.5 | 43.8 | 44.9 | 44.4 | 0.84 | | |
| Protein | 31.7 | 32.7 | 32.3 | 32.5 | 0.28 | * | |
| Lactose | 48.2 | 48.0 | 48.1 | 47.8 | 0.18 | | |
| Urea (mg kg$^{-1}$) | 234 | 306 | 313 | 285 | 8.8 | *** | |
| Yield of milk constituents (g per day) | | | | | | | |
| Fat | 983 | 1026 | 1039 | 983 | 14.1 | * | * |
| Protein | 686 | 764 | 742 | 719 | 13.5 | *** | * |
| Lactose | 1048 | 1128 | 1111 | 1067 | 21.8 | * | $P = 0.06$ |
| Feed efficiency | | | | | | | |
| ECM kg$^{-1}$ DM | 1.40 | 1.39 | 1.40 | 1.37 | 0.012 | | |
| N utilization | | | | | | | |
| N in milk per N intake | 0.295 | 0.264 | 0.263 | 0.264 | 0.0042 | *** | |

Number of observations per each treatment was eight. S.E.M.: standard error of mean.
[a] C = mixture of oats and barley (C); R: C + rapeseed meal; RP: C + rapeseed meal and pea; P: C + pea.
[b] ECM = energy corrected milk (Sjaunja et al., 1990).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

### 3.2. Experiment 2

There were clear differences in the CP content of experimental supplements due to differences in proportionate RSM inclusion (Table 2). Herbage CP and NDF content and in vitro OM digestibility were similar between strip and paddock grazed swards.

Both energy and protein supplementation increased ($P < 0.05$) the yields of milk and milk constituents (Table 5). Supplement E decreased ($P < 0.05$) milk protein concentration, increased ($P < 0.001$) milk lactose content, but had no ($P > 0.05$) effects on milk urea concentrations. Supplement P tended to increase ($P = 0.06$) lactose concentration and increased ($P < 0.001$) milk urea concentration. Both E and P supplements improved daily milk constituent yields ($P < 0.05$). There were no interactions between E and P supplements, such that the effects on milk production were mainly additive.

### 3.3. Experiment 3

RSM included in the oat and barley mixture increased concentrate CP content to 155 g kg$^{-1}$ DM (Table 2). Herbage offered at both allowance had a similar CP content (173 and 167 g kg$^{-1}$), as was the case for concentrations of NDF (438 versus 467 g kg$^{-1}$). In vitro OM digestibility was relatively high for herbage offered at both levels (0.757 and 0.739).

Table 5
Mean treatment effects on milk production (experiment 2)

| | Diets | | | | S.E.M. | Significance of effect | |
|---|---|---|---|---|---|---|---|
| | Herbage[a] | E[b] | P[b] | EP[b] | | E | P |
| Milk yield (kg per day) | 16.8 | 19.1 | 18.2 | 20.4 | 0.42 | *** | ** |
| Milk composition (g kg$^{-1}$) | | | | | | | |
| Fat | 41.7 | 41.1 | 44.2 | 41.6 | 0.73 | $P = 0.06$ | $P = 0.06$ |
| Protein | 31.6 | 30.9 | 32.0 | 31.4 | 0.25 | * | |
| Lactose | 46.1 | 47.2 | 46.6 | 47.8 | 0.25 | *** | $P=0.06$ |
| Urea (mg kg$^{-1}$) | 210 | 232 | 272 | 293 | 11.5 | | *** |
| Yield of milk constituents (g per day) | | | | | | | |
| Fat | 697 | 783 | 803 | 846 | 28.2 | * | ** |
| Protein | 524 | 591 | 574 | 635 | 14.3 | *** | ** |
| Lactose | 772 | 902 | 848 | 975 | 23.8 | *** | ** |

Number of observations per each treatment was eight. S.E.M.: standard error of mean.
[a] Herbage: herbage only including two grazing strategies (strip or paddock).
[b] Supplement type: E: energy supplement, mixture of oats and barley; P: protein supplement, rapeseed meal; EP: mixture of oats and barley and rapeseed meal.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Increasing concentrate supplementation from 2.5 to 5.0 kg per day increased milk yield by 1.7 kg ($P < 0.01$) (Table 6). Milk protein and lactose concentrations were both improved ($P < 0.05$) following increases in concentrate supplementation. Milk constituent yields were also enhanced ($P < 0.001$) when higher levels of concentrate supplements were fed.

Table 6
Mean treatment effects on milk production (experiment 3)

| | Supplement level[a] (kg per day) | | S.E.M. | Significance of effect Supplement level |
|---|---|---|---|---|
| | 2.5 | 5.0 | | |
| Milk yield (kg per day) | 19.1 | 20.8 | 0.35 | ** |
| Milk composition (g kg$^{-1}$) | | | | |
| Fat | 40.7 | 40.4 | 0.59 | |
| Protein | 31.7 | 32.3 | 0.15 | * |
| Lactose | 46.2 | 46.7 | 0.14 | * |
| Urea (mg kg$^{-1}$) | 144 | 149 | 8.0 | |
| Yield of milk constituents (g per day) | | | | |
| Fat | 775 | 826 | 13.5 | * |
| Protein | 597 | 661 | 7.5 | *** |
| Lactose | 880 | 968 | 13.6 | *** |

Number of observations per each treatment was eight. S.E.M.: standard error of mean.
[a] Mixture of oats, barley and rapeseed meal.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

## 4. Discussion

### 4.1. Effect of protein supplementation on milk production

In low input or organic farming systems based on forage where applications of soluble mineral fertilizers are not permitted, legumes such as clover represent important sources of protein for dairy cows. However, this is an ineffective approach due to extensive degradation of herbage protein in the rumen that leads to considerable N losses (Beever and Siddons, 1986). Furthermore, Peyraud et al. (1995) and Delagarde et al. (1997) reported that lowering N fertilizer applications reduced the supply of amino acids in animals fed fresh herbage. For milk production based on organic or low input systems protein supply may often be a limiting factor. Therefore, use of protein supplements could be an efficient and effective means of improving milk production. However, there is only limited data to support this hypothesis. The potential of commercial conventionally produced RSM and organically grown field pea for this purpose were currently assessed.

#### 4.1.1. Effect of rapeseed meal supplement

*4.1.1.1. Milk yield.* Rapeseed feeds are well-established protein supplements for cows fed grass silage-based diets (Tuori, 1992; Huhtanen, 1998; Rinne et al., 1999a,b; Dewhurst et al., 1999). Similarly, RSM supplementation increased milk yield by 1.12 kg $kg^{-1}$ RSM DM when RSM was included into the concentrate supplement (experiment 1). This response was higher than that observed in a previous experiment with cows fed similar diets (Khalili et al., 1999), but is consistent with responses generally attained with grass silage-based diets (Huhtanen, 1998). Currently, RSM increased ECM yield 0.20 kg per incremental MJ ME, a response consistent with a theoretical value of 0.19 (Tuori et al., 1996) indicating that marginal increases in ME intake could explain the improvements in milk yield. Furthermore, the magnitude of response suggest that the efficiency of energy utilisation was neither improved nor impaired with RSM supplementation. In the present study the increase of 0.20 was lower than an average response of 0.35 reported for RSM, but this response is in line with a greater increase in silage intake (0.64 kg DM) for R diet compared with average increases of 0.25 kg reported in the literature (Huhtanen, 1998).

Although RSM impaired N utilisation (N secretion in milk per N intake) compared with the control diet, RSM increased milk yield by 0.89 kg per 100 g increase in AAT intake indicating a sub-optimal supply of amino acids from the basal diet. This value is much lower than an estimate of 1.45 reported for grass silage-based diets (Huhtanen, 1998). The reason for the lower marginal response to additional AAT observed in experiment 1 is not clear, but increases in silage intake or the use of grass-clover swards may explain this difference due to positive associative effects on digestion, since feeding red clover-grass silage improves microbial N flow to the duodenum compared with grass silage (Vanhatalo et al., 1995).

Nocek and Russell (1988) concluded that the effects of protein supplementation on milk yield appear to be largely driven by DM intake. Increases in silage intake and DM digestibility have been reported following increases in the proportion of protein in concentrate supplements (Thomas and Rae, 1988). The effect on silage intake has also been observed when RSM is included in concentrate supplements (Tuori, 1992; Huhtanen, 1998; Rinne et al., 1999a). In experiment 1, the increase in silage intake and consequently ME supply can explain the increase in ECM production.

*4.1.1.2. Milk protein yield*. Increases in milk protein yield to RSM supplementation were manifested both by a increase in milk protein content and milk yield equivalent to 51.3 g $kg^{-1}$ RSM DM. This response was marginally higher than those previously reported (Tuori, 1992; Huhtanen, 1998; Khalili et al., 1999) to RSM. The response of milk protein yield to additional AAT of 0.41 g $g^{-1}$ was similar to the response (0.42) reported for cows fed a similar diet (Khalili et al., 1999), but lower than a mean marginal response of 0.55 reported for grass silage-based diets (Huhtanen, 1998). The lower response to additional AAT was influenced by greater than expected increases in silage intake for diet R compared with average increases of 0.25 reported for grass silage-based diets (Huhtanen, 1998). Recently RSM has been shown to increase the supply of rumen undegradable protein entering the omasal canal in cows fed grass-red clover silage-based diets (Ahvenjärvi et al., 2002). Such an increase and possibly improvements in the balance of absorbed amino acids supply could potentially explain the observed responses with protein, since RSM has been shown to increase histidine supply, currently considered to be the first limiting amino acid for milk production in cows fed grass silage-based diets (Vanhatalo et al., 1999).

*4.1.2. Comparison of rapeseed meal and pea protein supplements*

Earlier studies had suggested that pea could successfully replace other protein supplements such as soybean meal without having negative effects on milk production (Syrjälä-Qvist et al., 1981; Khorasani et al., 1992; Corbett et al., 1995; Petit et al., 1997). The quality of pea protein is less ideal for milk production since it contains more rumen degradable protein and less AAT (g kg per DM) than soybean or RSM. Combining RSM and pea protein sources had no effect on ECM yield compared with RSM alone, but when RSM was completely replaced by pea, yields of ECM and milk protein decreased. In contrast, no differences in ECM or milk protein yields were observed in a recent comparison of RSM and pea protein supplements (Khalili et al., 1999).

Increases in energy supply could explain most of the improvements for ECM yield for P diets in both experiments indicating that the effect of pea supplements was mediated primarily through an increase in ME supply. Values for pea supplemented diets were close to responses of 0.09 kg reported for increases in concentrate feeding (Huhtanen, 1998) supporting the suggestion of a response to energy for P diet. Inclusion of pea supplements had no additional effects on milk production compared with the basal diet as highlighted by lower increases in ECM per increase in ME intake (0.06 versus an average value of 0.18) for P compared with RSM supplement.

Silage and AAT intake increased with RSM supplement. It is also likely that RSM improved the balance of absorbed amino acids, since RSM is relatively rich in histidine. With diet P milk yield increased by 0.44 kg per 100 g additional AAT intake, a marginal response that is only half of that attained with RSM. On the other hand, previous study has demonstrated a corresponding response of 0.97 kg for pea supplements (Khalili et al., 1999). The relationship between supply of AAT and milk protein yield intake (mp = 152 + 0.37 g AAT) indicated that the mean marginal response across all diets was higher than that of 0.24 attained with pea supplements. A greater marginal response of 0.44 has been observed when diets comparable to those in the current study have been fed. The reason for differences in the magnitude of response tentatively suggests that either protein was a more limiting factor on animal performance or that the efficiency of N utilization was higher when ME intake was higher for P than RSM supplemented diets.

#### 4.1.3. Effect of rapeseed meal supplement in grazing cows

*4.1.3.1. Milk and milk protein yield.* Responses to protein supplementation reported in grazing cows have been variable. Milk production has been shown to increase with supplements of soybean meal (Peyraud et al., 1994; Delagarde et al., 1997; Delagarde et al., 1999). In experiment 2 milk yield increased by 1.20 kg kg RSM per DM with diet P and ECM yield increased by 0.17 kg per additional MJ ME from RSM compared with grazed herbage. This response was close to a theoretical marginal response of 0.19 ECM MJ per ME (Tuori et al., 1996) indicating that RSM supplementation had neither a negative nor a positive associative effect on energy utilisation. Milk yield increased 0.79 kg additional 100 g $AAT^{-1}$ when RSM supplements were fed to grazing cows. This suggests that the supply of metabolisable amino acids derived from pasture was sub-optimal, and therefore improvements in animal performance can be attributed to increased amino acid supply. When RSM was used as a supplement for grazing cows incremental increases in milk protein yield g per g additional AAT was 0.28, a value lower than corresponding responses in cows fed grass silage-based diets. When part of diet E was replaced by RSM (diet EP) milk and milk protein yield responses were similar to those reported for grass silage-based diets (Huhtanen, 1998).

Milk and milk protein yields have been increased with formaldehyde treated casein in cows fed high quality pasture including *Trifolium repens* (Rogers et al., 1980). Such responses were attributed to increases in amino acid supply rather than as a direct consequence on herbage intake response. Delagarde et al. (1999) noted that soybean meal increased herbage intake compared with grazing alone, suggesting that the current responses to protein supplementation may, also in part, be due to increases in herbage DM intake.

### 4.2. Effect of energy supplement in grazing cows

#### 4.2.1. Milk yield

Milk yield increased by 0.66 and 0.77 kg per kg DM additional concentrate in experiments 2 and 3, respectively. These responses are high compared with studies reviewed by Leaver (1985), but are within the range of more recent reports in the literature (Rook et al., 1994; Berzaghi et al., 1996; O'Brien et al., 1996; Carruthers et al., 1997; O'Brien et al., 1999; Sayers et al., 2000). Responses have been shown to decline with increased levels of concentrate supplementation (Kibon and Holmes, 1987; Hoden et al., 1991; Meijs and Hoekstra, 1984; Grainger and Mathews, 1989; Syrjälä-Qvist et al., 1996). It has also been shown that responses to concentrates have been greater when herbage intake is restricted (Patterson et al., 1998; Dillon et al., 1997). Sward clover content also influences milk production responses to supplementation, since response are lower in animals fed clover rich swards (Wilkins et al., 1994). In spite of an almost four-fold higher clover content in pasture offered in experiment 3 compared with experiment 2, milk production responses were almost similar.

Marginal ECM yield responses of 0.05 (experiment 2) and 0.06 kg (experiment 3) per additional MJ ME were much lower than a mean marginal response of 0.09 kg of milk per MJ additional ME reported for grass silage-based diets supplemented with similar levels of concentrates (Huhtanen, 1998). Furthermore, these responses are also much lower than a theoretical response of 0.19 kg ECM per MJ ME (Tuori et al., 1996). Herbage intake was not measured but the low marginal ECM responses suggests that herbage intake was depressed by concentrate supplementation in both experiments despite the relatively low levels fed. In general substitution rate increases with concentrate intake and forage digestibility. Recently, O'Connell et al. (2000) reported a response of 0.63 kg milk $kg^{-1}$ concentrate DM that was associated with a reduction of herbage DM intake of 0.50 in grazing cows. In recent studies at this Institute a mean substitution rate of 0.53 has been attained with grass silage-based diets. Pasture intake is affected by substitution rate and the effect of supplementary concentrates on milk production in grazing cows is known to depend on herbage intake (Leaver, 1985). It is possible that substitution rate exceeded 0.5 in the present study.

#### *4.2.2. Milk protein yield*

N utilisation for milk production in grazing cows fed pasture as the sole feed has been shown to be low (Van Vuuren, 1993). One reason for this is an imbalance between the supply of energy and amino acids. Increased energy feeding has been shown to increase milk protein yield in grazing cows ((experiments 2 and 3), Rook et al., 1994; Berzaghi et al., 1996; O'Brien et al., 1996; Carruthers et al., 1997; O'Brien et al., 1999; Sayers et al., 2000)) and milk protein content ((experiment 3) Spörndly, 1986)).

Energy supplementation also increases the amount of amino acids absorbed in the small intestine through increased microbial protein supply (Khalili and Huhtanen, 1991; Jaakkola and Huhtanen, 1993), DM intake and as a consequence of a higher AAT content of energy supplements compared with herbage. Such increases will improve the balance between energy and amino acids, allowing improvements in animal performance. In line with earlier studies (Hoden et al., 1991; Rook et al., 1994; O'Brien et al., 1996) feeding concentrate to grazing cows elicited milk protein responses of 18.6 (experiment 2) and 28.4 (experiment 3) g $kg^{-1}$ additional concentrate DM. Marginal milk protein yield responses to additional AAT intake from additional concentrates of 0.19 and 0.27 g $g^{-1}$ were observed in experiments 2 and 3, respectively. These responses are lower than an average of 0.42 derived from studies examining the influence of concentrate feeding in grass silage fed animals (Huhtanen, 1998). The low marginal responses observed suggest that herbage intake was decreased in both experiments.

### *4.3. Comparison and interactions between rapeseed meal and energy supplements in grazing cows*

EP diet increased milk yield by 21% compared with feeding pasture alone. RSM elicited similar responses when grazing cows were offered RSM alone or when RSM was included into the concentrate supplement. Milk yield was greater with EP diet compared with diets

E and P. The additive nature of this response indicated that there were no interactions between energy and protein supplements on milk yield. Delagarde et al. (1999) reported that protected soybean meal improved milk production more than wheat concentrate due to increased herbage intake when pasture received low fertilizer N applications. When grass-red clover silage was supplemented with RSM, barley or both, RSM increased milk yield to a greater extent than barley (Ahvenjärvi et al., 2002). The effect of barley was due to higher DOM intake and microbial flow entering the omasal canal, but the effect with RSM was primarily due to increases in the supply of rumen undegradable protein. (Sutton et al., 1994) also reported greater milk and milk protein responses to additional protein than energy in cows fed grass silage-based diets.

## 5. Conclusions

Inclusion of rapeseed meal in concentrate supplements for cows fed grass-clover-based diets improved milk production. Replacement of RSM with pea protein impaired milk production, but a combination of pea and RSM resulted in similar performance compared with RSM alone. The relative value of pea protein was lower than that of RSM and had no additional benefits on animal performance compared with the basal barley and oat concentrate. Both protein and energy supplements elicited positive production responses in grazing cows, effects that were additive. Therefore, a concentrate supplement including additional protein appears to be necessary for milk production from cows grazing grass-clover swards grown under organic or low input systems.

## Acknowledgements

The authors are grateful to the Finnish Ministry of Agriculture and Forestry for financial support. We would also like to thank staff of the Siikasalmi farm at the University of Joensuu and Animal Production Research at Agrifood Research Finland.

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