Rapeseed Products from Double-Low Cultivars as Feed for Dairy Cows: Effects of Long-Term Feeding on Thyroid Function, Fertility and Animal Health

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> Ahlin, K.-Å., M. Emanuelson and H. Wiktorsson: Rapeseed products from double-low cultivars as feed for dairy cows: Effects of long-term feeding on thyroid function, fertility and animal health. Acta vet. scand. 1994, 35, 37-53. - Eighty-five dairy cows of the Swedish Red and White Breed (SRB) were included in a long-term experiment during 3 consecutive lactations. The cows were divided into 3 different dietary groups that received no rapeseed (NR), up to 1.2 kg dry matter (DM) 00-rapeseed meal plus 0.2 kg DM full-fat 00-rapeseed (MR), and up to 2.5 kg DM 00-rapeseed meal plus 0.9 kg DM full-fat 00-rapeseed (HR) per day. No significant differences in culling rates or disease rates were found between the feeding groups at any time during the experiment. The interval from calving to conception among the primiparous cows was longer for the HR-group (125 days) than for the NR-group (100 days). The response to a thyrotroph releasing hormone around 90 days postpartum during the first lactation was significantly higher for the HR-group (86.7 μ /L/h) than for the NR-group (55.2 μ g/L/h). This indicates that at the highest level of rapeseed feeding, glucosinolates had a very mild, suppressive influence on thyroid hormone release, apparently compensated for by an increased activity along the hypothalamic-pituitary-thyroid axis. No significant differences in fertility or thyroid function were found among the pluriparous cows. During 2nd lactation the concentration of serum urea was higher in the NR-group (7.31 mmol/L) than in the HR-group (6.83 mol/L). The effects of independent environmental factors influenced fertility and thyroid function to a much greater extent than the rapeseed feeding. It was concluded that the feeding of rapeseed products from certified double low varieties of B. napus to adult dairy cows in amounts up to 3 kg rapeseed meal per cow and day would not have any negative effects on animal health or fertility

glucosinolates; TRH; TSH; reproduction; metabolic blood profile.

Introduction

Rapeseed products are a valuable source of protein and energy in rations to dairy cattle. Several reports are in favour of increasing the amounts of rapeseed products in the concentrates (*Clandinin & Robblee* 1978, *Thomke* 1981, *Bell* 1984). The double low cultivars of *Brassica napus oleifera* and *B. campestris olei*- *fera* contain very low concentrations of antinutritional substances like erucic acid and glucosinolates (00-varieties or LG-varieties or canola).

In spite of the economic advantages by cultivating improved varieties of Brassica oil crops, the usefulness of rapeseed as a feedstuff has been limited. The main reason for

this are undesirable and toxic effects associated with the level of glucosinolates (Hill 1979, Bell 1984). Glucosinolates from Brassicaceae plants (progoitrin, gluconapin, glucobrassicanapin, napoleiferin, glucobrassicin and neoglucobrassicin) are hydrolysed by the enzyme myrosinase (thioglucoside glucohydrolase; EC 3.2.3.1) at neutral pH. This results in the formation of glucose, KHSO₄, goitrogenic compounds, such as 5-vinyl-2-oxazolidinethione (goitrin, OZT, VTO), various isothiocyanates and thiocyanate (SCN-). At a low pH, nitriles are also formed, which depress feed intake and growth in addition to having goitrogenic properties (Hill 1979). Low-glucosinolate rapeseed meal (00-RSM) is more palatable than high-glucosinolate rapeseed meal (HG-RSM). However, other toxic substances, such as sinapines and tannins, can cause consumption problems.

Standards for double low cultivars differs between countries, which complicate comparisons between trials. According to Swedish specifications for 00-RSM, erucic acid should not account for more than 2% of the total fatty acids and 1 gram dry matter (DM) of fatfree meal should not contain more than 40 µmol total glucosinolates. The Canadian (canola-variety) standards are similar for erucic acid but the glucosinolate limit (30 µmol/gram fatfree DM), does not include indolyl-glucosinolates. The limit for countries within the European Community is 20 µmol glucosinolates per gram air-dried rapeseed, indolyl-glucosinolates included.

Hypothyroidism (overt or subclinical), caused by iodine deficiency, sometimes in combination with goitrogenic agents, has been reported to influence fertility in ruminants. The condition is expressed as increased prevalence of congenital goitre and stillborn calves. In addition, retained placentas and ketonaemia tend to be more common and resistance to infections (endometritis) can be reduced (Wilson 1975).

Most reports on the use of rapeseed products are based on few animals in changeover trials or trials with a short experimental period – often less than 4 months. Consequently, possible long-term negative effects of feeding 00-RSM on metabolism or health have not been shown. The aim of this study was to explore the possibilities of increasing the levels of 00rapeseed products in dairy cow rations by evaluating the effects on thyroid function, reproduction and animal health.

Materials and methods

Experimental design

A total of 95 heifers of the Swedish Red and White Breed (SRB) were introduced during 2 consecutive years. Batch 1 consisted of 47 SRB-heifers calving during 1982-1983, and batch 2 of 48 SRB-heifers calving during 1983-1984. Each animal was used for a maximum of 3 lactations or until it was culled. Cows were culled if they were accidentally injured, produced less than 4,000 kg of 4% fat corrected milk (FCM) per lactation or less than 17 kg FCM per day, did not conceive within 200 days postpartum or required more than 7 artificial inseminations (AI) to get pregnant. Culled animals were excluded from all calculations concerning fertility. After initial cullings, 85 first calvers were available for more extensive comparisons of different variables (Table1).

Within each batch, the animals were divided into 2 sire groups according to their sire's relative breeding value for milk yield. Within each sire group, the animals were blocked 3 by 3 (blocks 1-16) according to their predicted calving time and were randomly allotted to 3 different dietary groups as applied in the method of stratified and restricted randomisation (*Altman* 1991).

			E	Experiment	al year			
		1982/83	1983/8	34	1984/8	35	1985/86	
Lacta	ation number	1	1	2	2	3	3	
Batch	Dietary group ¹							Final number ²
	NR	14		12		10		8
1	MR	14		13		12		9
	HR	13		13		11		7
	NR		15		13		10	8
2	MR		15		12		11	10
	HR		14		11		9	8

Table 1. Number of cows in each of the dietary groups at the beginning of each lactation and experimental year

¹ No rapeseed (NR), medium level (MR) and high level (HR) of low-glucosinolate rapeseed products.

² Number of cows left in each dietary group at the end of the third lactation.

Feeding

All cows were fed 6.5-7.5 kg DM of grass-red clover silage and 1.2-2.0 kg DM of grass hay (timothy and meadow fescue). Cows in the NR (no rapeseed) group were fed a concentrate mixture in which the protein supplements were mainly based on soybean meal and with tallow as the main fat supply. In group MR (medium rapeseed level) the cows received a maximum of 1.2 kg DM extracted 00-RSM and 0.2 kg DM heat-treated, crushed full-fat rapeseed (00-TFR). In group HR (high rapeseed level) the cows were fed up to 2.5 kg DM of 00-RSM plus 0.9 kg DM 00-TFR. All diets were isonitrogenous and isocaloric and contained the same levels of fat and minerals. A detailed description of the feeding regimes was presented elsewhere (Emanuelson et al. 1993).

The rapeseed products originated from spring-sown, double-low cultivars of *B. napus oleifera* (Karat or Topas or Hanna). The total glucosinolate content ranged from 17.6 to 31.0 μ mol/g DM in 00-RSM, with a mean of 24.4 μ mol/g DM (N = 6), and from 4.0 to 15.5 μ mol/g DM in 00-TFR, with a mean of 7.2

 μ mol/g DM (N = 7). By the end of the second year, there had been a decrease in the total glucosinolates in 00-RSM to a level about half that of the first year, and in 00-TFR the original level was halved by the start of the second year.

Fertility

Data from clinical examinations, analysis of progesterone in whole milk, and heat observations were used. Every animal was examined clinically by rectal palpations starting about 1 week postpartum and once a week thereafter until the first AI. Rectal palpations were performed 6 weeks after the last AI to determine pregnancies.

Samples for radioimmunological analysis (RIA) of progesterone in whole milk were taken twice weekly until a functional corpus luteum (CL) was confirmed. Thereafter, the samples were taken once a week until the first AI. Additional milk samples were taken on the day of AI, 10 and 21 days after AI or if indicated by signs of reproductive problems. The samples were preserved using sodium azide or Bronopol and stored at 4°C until as-

sayed with a commercial progesterone kit (Farmos Diagnostica, Turku, Finland), usually within 1 week after sampling.

The detection limit, calculated as the concentration at 10% displacement of the labelled ligand, has been reported to 1.6 nmol/L (Kassa et al. 1986). The standard curve ranged from 5 nmol/L over 10, 20, 30, and 50 to 100 nmol/L. The intra- and inter-assay coefficients of variation were estimated from the duplicates of the low- and high-control pools in 20 assays. The intra-assay coefficients of variation were 8.4% for the low pool ($8.8 \pm 1.0 \text{ nmol/L}$) and 3.8% for the high pool (56.1 \pm 6.7 nmol/L). The inter-assay coefficients of variation were 8.4% for the low pool and 11.4% for the high one.

Every animal was observed 3 times daily, and heat symptoms or external signs of disturbed fertility were recorded. Records from the clinical examinations, progesterone assays, and heat observations were assembled for every animal to create an individual progesterone and fertility profile covering 1 postpartum period. With an established methodology (Larsson et al. 1984), these fertility profiles determined reproductive events and fertility measures during the postpartum period. The following definitions were used:

Uterine involution (UI) was considered complete when the uterine horns were approximately symmetrical and well contained within the pelvic cavity, the uterine wall was soft and pliable, when neither uterine contents nor caruncles could be palpated, and the cranial part of the cervix had a diameter less than or equal to 5 cm.

Ovulation day (OV) was defined as either the day before a post-oestrus bleeding, the day after a fertile AI, 5-10 days before a progesterone level above or equal to 10 nmol/l whole milk, at least 5 days before the palpation of an active CL (15 mm in diameter or larger), or the day after a normal or strong heat (heat code 3 or 4).

Oestrus. An animal was regarded to be in oestrus (H) if the total score for all the heat symptoms (including behavioural changes) amounted to heat code 2 or higher. The scale consisted of 5 levels, where 0 = no oestrus, 1 =possible oestrus, 2 = weak oestrus, 3 = normal oestrus and 4 = strong oestrus.

Ovulatory oestrus. When external signs of heat (at least code 2) were recorded in connection with ovulation this was defined as ovulatory oestrus (OVH).

Normalised reproductive functions (NRF) were considered re-established if OVH was recorded for the first time after calving on the same day or the day after UI.

Other fertility measures used in the statistical analysis were the interval from calving to first AI (CFI), the interval from calving to conception (CC), and the number of AI per pregnancy (AIP). Any AI repeated within 6 days was excluded from the calculation of AIP. The CC-interval was also calculated with a correction (CCC) when pregnant animals had been accidentally inseminated and later aborted. Such animals were considered to have become pregnant at the time of the first fertile AI. In the same way, a correction was also applied to AIP, denoted AIPC.

Thyroid function

The thyroid function was evaluated using the thyrotropin releasing hormone (TRH)-test (Laarveld et al. 1981b), performed on around 90 and 300 days after calving. Blood samples were collected from the jugular vein into heparinised Vacutainer tubes 10 min and immediately before the intravenous administration of 300 µg of TRH. Additional blood samples were collected 10, 20, 30, 40, 50, 60, and 120 min after the injection. Within 30 min after collection, the samples were centrifuged at

40

2,100 G for 20 min. The plasma was transferred to plastic tubes, and frozen until assay. All samples were analysed for thyroid stimulating hormone (TSH) using a double antibody RIA-method. The TSH-assay used an antiserum against ovine TSH (NIAMDDanti-oTSH-1) at a final dilution of 1:90,000 and bovine TSH (NIADDK-bTSH-I-1) as a standard (A.F. Parlow, Harbor-UCLA Medical Center, Torrance, CA, USA).

A highly purified bovine TSH (NIADDKbTSH-11) for iodination was used as a tracer. The iodination (carrier-free ¹²⁵I, Amersham International plc, Buckinghamshire, England) was performed by the chloramine-T method (Greenwood et al. 1963) using 16 g of chloramine-T per 2 g of protein and an exposure time of 50 s. This is a highly specific RIAsystem for bovine TSH with reported crossreactions of less than 0.2% with luteinizing hormone (LH), of less than 0.02% with follicular stimulating hormone (FSH), of less than 0.001% with prolactin, and of less than 0.07% with growth hormone (GH) (A.F. Parlow, Harbor-UCLA Medical Center, Torrance, CA, USA). The antiserum generally bound 30% of the iodinated bTSH, and the standard curve ranged from 0.5 ng to 16 ng of bTSH. The nonspecific binding was less than 4%.

After adding 100 μ L of antiserum to the samples, the tubes were incubated for 4 h at room temperature. 100 μ L of tracer (¹²⁵I-bTSH-11) was added, and the tubes incubated overnight at room temperature. The antibody-bound hormone was separated from the free hormone by 2 mL of a second antibody against rabbit gamma globulin coupled to a solid phase (DASP, Organon, Oss, The Netherlands) and rotated overnight at room temperature. The tubes were rotated at 2,100 G for 3-5 min, and the precipitate was washed 4 times (first wash with 1 mL, then with 2 mL) with a 0.5% solution of TWEEN 20 (Sigma) in 0.9% saline before the radioactivity was quantified in a gamma counter (G.D. Searle, Uithoor, The Netherlands). The intra-assay coefficients of variation, calculated from 4 assays, were less than 10% between 0.5-9.4 µg/L. The inter-assay coefficients of variation in 4 assays were 5.8% for the plasma-pool of 0.3 ± 0.02 µg/L, 13.7% for 0.9 ± 0.1 µg/L, and 11.1% for 2.2 ± 0.2 µg/L respectively. The minimum average detectable concentration was 0.09 µg/L with the inter-assay coefficients of variation, amounting to 9.3% in the 4 assays.

To evaluate the TSH-response with a single measurement, the area under the curve (AUC) was calculated for a period of 60 min. Basal levels for each individual were calculated as the arithmetical mean of the 2 '0-samples' before the TRH-injection, which was then subtracted from each value before the AUC was calculated. The AUC represents the quantity (μ g) of TSH released during 1 h (μ g/L/h).

Animal health

The animals were observed by the herdsmen throughout the experimental period, and any signs of disease were recorded. Animals with acute disease were diagnosed and treated by veterinarians. Blood samples were taken from the jugular vein approximately 90 and 300 days after calving (in connection with the TRH-tests).

Serum-TP was determined according to *Peters Jr. et al.* (1982), serum-urea according to *Oltner & Berglund* (1982) and plasma glucose as described by *Trinder* (1969) with a modification described by *Oltner & Berglund* (1982). Serum-P₁ was determined according to *Itaya & Ui* (1966) and modified by *Oltner & Berglund* (1982). Serum-Na and serum-K were determined in a flame photometer (*Hewett* 1974). Serum bile acids were assayed according to *Mashige et al.* (1981) and serum-

gamma-glutamyltransferase (EC 2.3.2.2) in a kinetic test (Szasz 1969). After analyses metabolic blood profiles were constructed using a computer.

Statistical analyses

The effects of the independent factors batch, sire group, block, and dietary group on the reproductive measures, the components of the metabolic blood profile and thyroid function were analysed by a least-squares analysis as applied in the General Linear Model (GLM)procedure (SAS Institute Inc. 1987). The slightly imbalanced design makes the GLMprocedure the method of choice. Because repeated measurements were done on a limited statistical material, each lactation was analysed separately, using the following model:

$$y_{ijkl} = \mu + a_i + s_{ij} + b_{ijk} + d_l + e_{ijkl}$$

where

- μ = overall mean
- $a_i = ith batch (i = 1,2)$
- $s_{ij} = jth$ size group within ith batch (j = 1,2)
- $\dot{b_{ijk}}$ = random effect of kth block within ith batch and jth sire group assumed to be normally distributed with mean = 0 and variance = σ_b^2 (k = 1,...8)
- $d_l = lth dietary group (l = 1,2,3)$
- e_{ijkl} = random residual assumed to be normally distributed with mean = 0 and variance = σ_e^2

As a consequence of the fluctuation in the non-return-rate (NR) over the year, with a nadir during the darkest winter-months, the model was adjusted taking into account the time of calving. For first calvers the effect of block was already included in the analysis; therefore no other adjustments were made. For older cows the blocks were no longer complete and were therefore replaced by calving season. When analysing the fertility measures and thyroid function, calving seasons were divided up into 3 periods (1 = July-September, 2 = October-December, 3 = January-June). In the model for estimating factor effects on components of the blood profile, the calving seasons were divided up into only 2 periods (1 = June-November, 2 = December-May) to enable the quality of the silage during 1984/85 to be monitored more closely.

Since age at first calving influences the CCinterval (*Strandberg* 1986), calving age, broken down into 3 periods (1 = 24-26 months, 2 = 27-29 months, 3 = 30-33 months), was included in the model for the primiparous cows. To eliminate the negative influence of outliers and skewed distributions on the assumption of normality, the raw fertility data and the AUC-values of TSH were transformed using natural logarithms and the geometrical least-squares means and the upper and lower borders of a 95% confidence interval (CI) were transformed back to the original scale.

The distributions of different diseases and reproductive disorders were analysed using the FREQ procedure in SAS with the options Fisher's Exact Test and Mantel-Haenszel Chisquare for trends across the dietary groups. In the analysis the groups were compared separately (NR, MR, HR) and categorically (NR, MR+HR). The frequencies of at least 1 occurrence per individual cow of a particular disease or reproductive disorder, both for separate lactations and for the whole experimental period, were calculated for the dietary groups.

Results

Glucosinolate content

The average intake of glucosinolates was 42 mmol per cow and day among the first calvers in the rapeseed-fed groups in batch 1 (Table

				Die	etary grou	p ¹		
Batch ⁴		$\frac{MR + HR^2}{1}$		$\frac{MR + HR^2}{2}$		MR ³ 1 + 2		HR ³ 1 + 2
Lactation	40	(41 44)		(10, 10)	24	(22, 24)	50	(50 55)
1		(41 - 44)	44	(42 - 46)	34	(32 - 36)	52	(50 - 55)
2	55	(52 - 57)	41	(38 - 43)	36	(33 - 38)	60	(57 - 63)
3	32	(29 - 35)	31	(28 - 34)	23	(21 - 26)	40	(37 - 43)

Table 2. Least-squares means (with 95% confidence interval) for the average daily total amount of glucosinolates consumed during lactation weeks 1-13 (mmol per cow and day).

1 Medium level (MR), and high level (HR) of low-glucosinolate rapeseed products.

2 Average of dietary groups MR + HR.

3 Average of batch 1 and 2.

4 Batch 1 was introduced in 1982/83 and batch 2 in 1983/84.

2). This did not differ significantly from the mean consumption in the second batch of first calvers (44 mmol/cow/day). During 1983/84 second calvers in batch 1 consumed the largest average amounts of glucosinolates (55 mmol/cow/day) throughout the experimental period. Due to the reduction in glucosinolate content of the rapeseed over the years, third calvers in both batches consumed significantly less (33 mmol/cow/day) glucosinolates, in spite of the larger intake of rapeseed products among the multiparous cows.

Culling and animal health

Initial cullings were made during the first lactation, before any relevant reproductive data could be recorded, (3 cows in group NR, 5 cows in group MR, and 2 cows in group HR). The reasons for these exclusions were not related to rapeseed feeding. The most common causes for culling during the experimental period per se were low milk yield (NR = 6 cows, MR = 2 cows, HR=1 cow) and non-pregnancy (NR = 4 cows, MR = 4 cows, HR = 8 cows). Culling rates did not differ significantly between the dietary groups (Table 1).

There were no significant differences in disease rates or in the occurrence of any reproductive disorder between the dietary groups (Table 3), but during the first lactation there were tendencies towards a higher frequency of mastitis in the NR-group and an increased number of acyclic cows in the HR-group.

Fertility and thyroid function

For first calvers there was no significant main effect of dietary group on any of the fertility traits. Comparisons of least-squares means (LSM) across different levels of the independent variable dietary group revealed, that the UI-interval was longer for the MR-group than for the HR-group (Table 4). The main effect of dietary group was almost significant for the CCC-interval (p = 0.057) and for the TSH-response to the TRH-test (p = 0.051), but did not have any effect on the CC-interval (p = 0.11). Both the CC- and CCC-intervals were longer for the HR-group than for the NRgroup, and there was also a tendency for the number of AI per pregnancy to increase with the amount of rapeseed fed (Table 4). In the TRH-test performed at 90 days postpartum the TSH-response was larger for the HRgroup than for the NR-group (Table 4 and Fig. 1). No such differences were seen in the TRHtests made around 300 days after calving.

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Table 3. Numerical distribution of cows with at le-
ast 1 instance of a specific disease or reproductive di-
sorder during all 3 lactations.

	D	netary group	p ¹
	NR	MR	HR
Diseases.			
Inappetence	17	20	20
Mastitis	18	9	14
Diarrhoea	12	16	11
Laminitis	8	5	7
Ketosis	8	3	5
Bloat	2	3	6
Udder injury	1	-	6
Foot rot	2	1	3
Parturient paresis	1	1	1
Reproductive disorders:			
Acyclic ovaries	7	5	13
Cystic ovaries	9	6	9
Endometritis	4	5	5
Abortion	4	2	2
Retained placenta	2	3	-

1 No rapeseed (NR), medium level (MR), and high level (HR) of low-glucosinolate rapeseed products.

For first calvers calving age had a statistically significant main effect on the dependent factors CFI-interval (p = 0.009) and CC-interval (p = 0.038). Heifers calving at an age of 24-26 months had shorter intervals (p<0.01) to CFI (70 days; 64-75) and CC (87 days; 77-99) than heifers calving at an age of 27-29 months (CFI = 99 days; 81-121 and CC = 142 days; 103-195). The CFI (69 days; 58-82) of the oldest heifers (30-33 months) was similar to that of the youngest heifers, and the oldest heifers did not differ significantly from the other 2 age groups regarding the CC-interval (107 days; 82-141).

For the pluriparous cows dietary group had no main effect on the fertility measures (Table 5) or the results of the TRH-stimulations (Fig. 1) at any time during the lactation. The only difference in LSM occurred during lactation 2, where cows in the MR-group showed a larger TSH-response than cows in the NRgroup. In second calvers, calving season had

Table 4. Geometrical least-squares means (with 95% confidence interval) for the reproductive measures (in days) and the TSH-response (in $\mu g/L/h$) to the TRH-test during 1st lactation.

			Dieta	ry group ¹		
	NR		MR		HR	
Reproductive measures:						
Uterine involution (UI)	37 ^{ab}	(34-42)	41ª	(37-46)	36 ^b	(32-40)
First ovulation (OV1)	34	(22-50)	33	(22-52)	37	(24-59)
First ovulatory oestrus (OVH1)	60	(46-77)	56	(42-73)	60	(46-78)
Normal repr. functions (NRF)	76	(63-92)	72	(59-88)	81	(67-99)
Calving to first AI ² (CFI)	74	(66-82)	82	(73-92)	79	(70-89)
Calving to conception (CC)	100ª	(84-118)	106 ^{ab}	(88-129)	125 ^b	(103-152)
Corrected CC ³ (CCC)	94ª	(79-111)	107 ^{ab}	(89-129)	123 ^b	(102-149)
AI per pregnancy (AIP)	1.68	(1.27-2.20)	1.81	(1.34-2.44)		(1.65-3.04
Corrected AIP ³ (AIPC)	1.54	(1.18-2.01)	1.84	(1.37-2.46)	2.16	(1.61-2.91)
Thyroid function						
TSH-response	55.2ª	(40.9-74.5)	61.4 ^{ab}	(43.1-87.3)	86.7 ^b	(62.1-121.1)

ab Least-square means (LSM) within the same row with different superscripts differ significantly (p<0.05).

1 No rapeseed (NR), medium level (MR), and high level (HR) of low-glucosinolate rapeseed products.

2 Artificial insemination.

3 Refers to correction when a cow was accidentally inseminated during pregnancy and aborted due to this. Fertility was calculated using only the first fertile insemination.

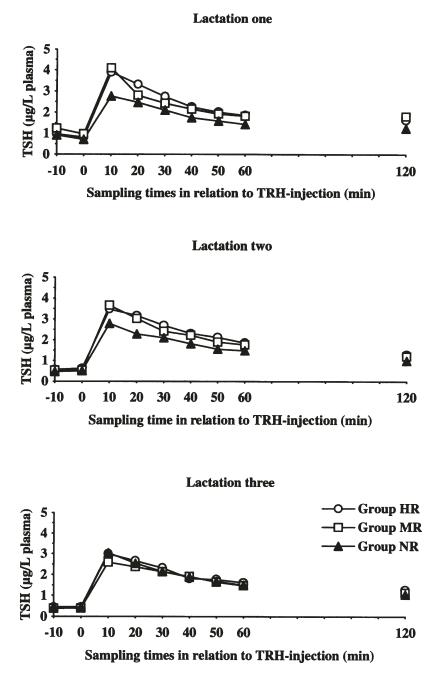


Figure 1. Arithmetical means of the TSH-response after TRH-test around day 90 after calving for the different feeding groups during lactation 1-3.

I KH-test during lactations 2-5.											
		Diet	Lactation 2 Dietary group ¹	2 Ip ¹				Lac Dieta	Lactation 3 Dietary group		
	NR		MR		HR		NR		MR	HR	
Reproductive measures.											
Uterine involution (UI)	39 (3:	35-44)	39	(34-44)	40	(35-46)	38 (3:	33-44)	34 (29-40)	36	(30-42)
First ovulation (OV1)	23 (1'	17-30)	25	(19-34)	30	(22-46)	29 (1)	19-34)	29 (21-39)	23	[17-33]
First ovulatory oestrus (OVH1)	60 (4)	49-73)	09	(49-74)	67	(54-84)	68 (5)	(50-82)	64 (50-82)	68	52-89)
Normal repr. functions (NRF)	71 63	(3-81)	73	(64-83)	81	(70-93)	68 (5'	(57-82)	75 (61-91)	89	55-85)
Calving to first AI ² (CFI)	74 (6	68-80)	75	(69-81)	78	(71-86)	81 (7:	73-89)	78 (70-87)	77 ((26-87)
Calving to conception (CC)	100 (8)	85-118)	98	(82-118)	102	(84-123)	94 (7	78-115)	104 (85-128)	93	(74-118)
Corrected CC ³ (CCC)	94 (8	81-109)	93	(79-110)	101	(85-119)	92 (7	76-111)	109 (89-132)	95 ((76-120)
AI per pregnancy (AIP)	1.81 (1	1.42-2.31)	1.60	(1.22-2.10)	1.55	(1.17-2.05)	1.40 (1.	1.04 - 1.90	1.65 (1.20-2.27)	1.47 ((1.02-2.11)
Corrected AIP ³ (AIPC)	1.62 (1	1.29-2.04)	1.44	(1.11-1.87)	1.55	(1.19-2.02)	1.33 (0	(0.99-1.77)	1.75 (1.29-2.37)	1.52 ((1.07-2.16)
Thyroid function:											
TSH-response	78.4 ^a (6	78.4 ^a (64.6-95.2)	102.5 ^b	(83.4-126.0)	92.3 ^{at}	^o (75.0-116.1)	81.0 (6	5.0-101.0)	102.5^{b} (83.4-126.0) 92.3 ^{ab} (75.0-116.1) 81.0 (65.0-101.0) 79.0 (62.5-99.9)	76.2 (76.2 (59.3-97.9)
ab Least-squares means (LSM) within the same row with different superscripts differ significantly (p<0.05) 1 No rapeseed (NR), medium level (MR), and high level (HR) of low-glucosinolate rapeseed products.	vithin the vel (MR)	same row , and high	with dif level (I	ferent super HR) of low-g	scripts	differ signıfıca ıolate rapesee	untly (p< d produc	0.05). ts.			
2 Artificial insemination.		11 - 7						iland cali			المن المن المن الم
3 Refers to correction when a cow was accidentally inseminated during pregnancy and aborted due to this. Fertuinty was calculated using only the furst fertile insemination.	ow was acc	adentally 1	nsemin	ated during]	pregna	ncy and aborte	a que lo	LIIIS. FETUII	Ly was calculated	io guisn	ny me mst

Table 5. Geometrical least-squares means (with 95 % confidence interval) for the reproductive measures (days) and the TSH-response (g/L/h) to

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significant main effects on the UI-interval (p = 0.026) and the NRF-interval (p = 0.012). Cows calving in July-September completed their UI earlier (34 days; 28-41) than cows calving in October-December (45 days; 41-49), while cows calving in January-June did not differ significantly from cows calving in other seasons (UI = 40 days; 35-46). During July-September and October-December the cows in second lactation resumed NRF earlier (67 days; 55-82 and 70 days; 64-77 respectively) than cows calving during January-June (90 days; 78-103). For the second calvers there was also a significant (p<0.001) main effect of batch on the TSH-response. Cows calving in 1983/84 showed a much stronger TSH-response (121.7 µg/L/h; 102.5-144.5) compared with cows calving during 1984/85 (67.8 µg/L/h; 56.8-81.0). For the third calvers batch had a significant main effect on the UI-interval (p = 0.012). Cows calving during 1984/85 completed their UI later (41 days; 35-47) than cows calving in 1985/86 (32 days; 28-36).

Metabolic blood profile

In the first lactation dietary group did not have a significant influence on any of the components of the metabolic blood profile around 90 days postpartum. During the second lactation there was no significant main effect of dietary group on urea, but the NR-group showed higher LSM-levels of urea than the HR-group. During the third lactation the main effect of dietary group on urea level was nearly significant (p = 0.07): Cows in the NRgroup had higher LSM-values of urea than cows in the MR-group. During the whole experiment the urea values in the NR-group tended to be higher than in the rapeseed fed groups.

During lactation 2 batch influenced both urea (p = 0.037) and glucose (p = 0.013) significantly. Cows calving in 1983/84 had lower lev-

els of urea (6.81 mmol/L; 6.53-7.09) and higher plasma glucose levels (3.04 mmol/L; 2.90-3.18) than those in 1984/85 (urea = 7.26 mmol/L; 6.98-7.54 and plasma glucose = 2.80 mmol/L; 2.66-2.94).

No significant main effects of dietary group were found for any blood constituents sampled around 300 days after calving during lactations 1-2. A significant effect (p = 0.016) of dietary group on urea was only found during lactation 3: Cows in the NR-group had higher levels of urea than cows in the MR-group.

Discussion

During lactations 2-3, levels of serum urea were elevated around 90 days after calving among cows in the NR-group, which was in accordance with the milk urea levels presented elsewhere (*Emanuelson* 1993). Milk urea is positively correlated with the protein/energy ratio in the diets (*Gustafsson* 1993), but since there were no differences in the ratio between dietary groups (*Emanuelson et al.* 1993) this could not explain the different serum urea concentrations in the present study.

Several reports have been published relating either milk urea or plasma urea levels to fertility. Some investigators reported a negative relationship (Ropstad & Refsdal 1987), while others have found no correlations or a curvilinear relationship (Gustafsson 1993). The tendencies towards impaired fertility for the cows in group HR during lactation 1 could not be explained by the urea level, since there were no differencies between diets. The serum urea values and all the other blood parameters were within the reference ranges. Because of homeostatic mechanisms nutrition-induced alterations in blood parameters may not be detected unless they are pronounced and persistent.

Neither the culling rate nor the disease rate were influenced by the feeding of rapeseed. Only a few reports have documented significant negative effects on the health from consumption of rapeseed. Ahlström & Thomke (1978a) found that weight gain was depressed and nostril discharges increased in heifers fed 50% rapeseed hulls in the concentrates from a high glucosinolate (HG) cultivar. Others reported significantly increased thyroid weights in growing calves fed either HG-RSM or 00-RSM (Ekman & Iwarsson 1974, Ahlström & Thomke 1978b, Papas et al. 1979). Vincent et al. (1988) fed heifers 2 different levels of HG-RSM for 2 5-month periods over 2 consecutive years and found histological evidence of goitrogenicity (but no weight change), significantly elevated plasma levels of SCN-, and depressed levels of plasma thyroxin (T4).

In all of the cited experiments young, growing animals were used, and the rapeseed products were mainly from HG varieties. In an experiment where growing bull calves were fed with 10% and 20% of 00-RSM in the concentrates (Andersen & Sørensen 1985), no negative effects on the T4 levels or weights of thyroids or livers were found. The total glucosinolate level in their 00-RSM was less than 12 μ mol/g DM. The much higher levels (31 μ mol/g DM of the 00-RSM) given to the first batch of primiparous cows in our study probably explains why fertility and thyroid function were depressed.

The relationship between hypothyroidism in cattle and reproductive disorders has been described in several reports (*Wilson* 1975). *Djurdjević et al.* (1982) reported that neonatal calf mortality was 16% owing to asphyxiation from severe congenital goitres of a parenchymatous, but compensated, euthyroid type. This condition was eliminated by iodine supplementation. Cows which had given birth to calves with goitres had lowered T3- and T4-ti-

tres and were regarded as subclinically hypothyroidic. *Reddi & Rajan* (1984) studied goats with normal reproductive functions, delayed puberty, postpartum anoestrum and repeat breeding. They found that levels of proteinbound iodine (PBI) in goats with reproductive disorders were significantly lower than in normal goats, with the lowest levels of PBI in the group with postpartal anoestrum.

During the first lactation in our experiment there was a tendency for fertility to be impaired and thyroid function mildly disturbed. This was most likely due to the greater sensitivity to glucosinolates in still growing first calvers as compared with older cows. The 2 batches of first calvers consumed almost the same daily amounts of glucosinolates (Table 2), and no relationship between the daily intake of glucosinolates and reproductive capacity could be found. Although cows in batch 1 of lactation 2 were exposed to the highest levels of glucosinolates they showed no signs of infertility. A parallel can be drawn to a study on induced hypothyroidism in small ruminants (Reddi & Rajan 1985): Thiourea at oral doses of 50 and 100 mg was administered daily to adult female goats and female kids. The ovaries were small, pale, and inactive. The uteri, which were small and infantile, only contained a few mucosal glands which all were inactive. The changes were dose dependent, more severe in young kids than in adults and could be reversed by withdrawing the goitrogenic treatment.

In earlier reports (Lindell 1976, Lindell & Knutsson 1976), where cows were given up to 1.39 kg HG-RSM, only tendencies toward reduced fertility were observed, such as increased numbers of AI per pregnancy and slightly prolonged CC- and calving-intervals. Ahlstrom (1978) reported a significant (p<0.05) increase in the number of AI per pregnancy in a group of primiparous dairy

cows given expeller-crushed fines based on a HG-variety of winter rape. These findings contradict those of most studies on the use of HG-RSM or 00-RSM in concentrates for dairy cows, in which no negative effects on fertility were found (*Sharma et al.* 1977, *Papas et al.* 1978, *Laarveld et al.* 1981a, 1981b, *Sanchéz & Claypool* 1983). However, the disturbed fertility of the first calvers in our study further indicate that the younger, and still growing, first calvers might have been more sensitive to glucosinolates.

Among the reports from trials using HG-RSM as feed for dairy cows are significant depressions of iodide concentration ([I-]) and elthiocyanate concentrations evations of ([SCN]) in milk (Iwarsson 1973, Lindell 1976, Laarveld et al. 1981a), increased [SCN-] (Ahlström 1978), increased [SCN-] and decreased levels of T4 (Vincent et al. 1988) and depressed levels of T4 in serum (Papas et al. 1979). By contrast, when 00-RSM was used [I⁻] decreased while [SCN⁻] increased (Papas et al. 1978, Laarveld et al. 1981b), and when 25% 00-RSM was given (Sharma et al. 1977), T4 levels were depressed. The crucial question here is whether these findings represent genuinely hypothyroid conditions or merely indicate a subclinical hypothyroid state, which might even be compensated.

A sufficiently sensitive and reliable test of thyroid function is necessary, especially if there are no significant morphological or histological changes in the thyroid gland. The TSH-response recorded 20 and 60 min after intravenous administration of 200 μ g of TRH is regarded as a simple, safe, sensitive, and reliable test of thyroid function in humans in the absence of pituitary or hypothalamic disease (*Ormston et al.* 1971). In hyperthyroidism or abnormalities in the pituitary or hypothala-mus the TRH-test could be accompanied by assays of T3 and T4. The test has also success-

fully been applied to dairy cattle to evaluate rapeseed feeding (Laarveld et al. 1981b) and to determine non-toxic levels of iodine supplementation (Convey et al. 1978). Patients with primary, overt hypothyroidism show an exaggerated and prolonged response to the TRH-test. Even the significantly elevated basal TSH-levels are sufficient evidence of an impaired thyroid function in cases of symptomatic hypothyroidism. The TSH-response alone is an extremely sensitive indicator of thyroid function, even in subjects with subclinical hypothyroidism down to the compensated euthyroid state, in which the concentrations of T3 and T4 could fall well within the normal range (Evered 1976).

In the present experiment basal levels of TSH did not differ between the 3 dietary groups. The main effect of dietary group on the TSHresponses was nearly statistically significant in lactation 1, and the largest differences between treatments were found for the 20- and 30-min samplings. No prolonged response, characteristic of a more serious hypothyroid condition, was observed. Therefore, it seems most likely that cows in the HR-group suffered from a very mild, subclinical, and probably compensated form of hypothyroidism induced by the depressed [I-] and elevated [SCN-], which also were found in this experiment (Emanuelson et al. 1993). These findings are in line with the results of Laarveld et al. (1981a, 1981b), who found that dietary HG-RSM (76.1 µmol glucosinolates per gram DM) contents led to a significant depression of T4 levels and an elevated TSH-response (interpreted as a mild impairment of the thyroid activity). However, no such effects were found when using similar contents of 00-RSM (11.5 µmol/g DM) resulting in a daily intake of 14-47 mmol glucosinolates.

Although the consumption of glucosinolates was significantly higher among the first batch

of second calvers in 1983/84 compared with all other cows (Table 2), the fertility of this batch was not significantly reduced. On the other hand, their TSH-response was significantly higher than that of the second batch in 1984/85. In addition, the enhanced TSH-response of the MR-group during the second lactation and the lack of any main effect of dietary group were not associated with any significant reduction in fertility of the rapeseed-fed groups (Table 5). Since the proportion of cows with milk acetone concentrations over 1.00 mmol/L tended to be larger in the MR-group during the second lactation (Emanuelson 1989), the picture could also be complicated by the reported relationship between ketosis and decreased thyroid activity (Tvet et al. 1980, Durdevic et al. 1980).

The independent environmental factors, i.e batch, calving age and calving season, had a much greater influence on fertility measures and thyroid function than did dietary group alone. There was no evidence of any significant main effects of dietary group in this study; thus the observed differences in CCinterval and TSH-response to the TRH-test between the NR- and the HR-groups must be biologically less important.

The importance of environmental factors other than the rapeseed dietary component is further reflected in the significant effect of calving season on NRF-intervals, which were prolonged during January-June. For the second batch of second calvers this coincided with a period during which they were intermittently fed silage of low hygienic quality (NH₃-N 18.5% of total N; butyric acid 1.1% of DM), which reduced the consumption (*Emanuelson* 1989). In addition, during this period the first batch of third calvers completed their UI significantly later compared with the second batch of third calvers. The significantly higher concentrations of serum urea and

r suboptimal nutritional state. ly A relationship between hypothyroidism and subfertility or infertility should appear when

lower titres of plasma glucose in their meta-

bolic blood profiles are also indicative of a

the hypothyroid condition is severe enough to produce a clinically observable change in the thyroid function. However, the reproductive consequences of subclinical, mild forms of hypothyroidism, even with a compensated euthyroid state, and hypothyroidism in adult animals are of dubious importance. In such a situation other environmental and managemental factors in a dairy herd would probably influence fertility to a much greater extent than would goitrogenic compounds at the levels used in this study. Among the potentially important factors are calving age and calving season, the accuracy of heat observations and the timing of AI in relation to ovulation and calving. Furthermore, the use of carefully adjusted feeding routines to re-establish a positive energy balance as soon as possible after calving, even for the most high yielding dairy cows in the barn, should have a great fertilityenhancing effect.

Conclusions

Calving age, calving season, and experimental year exerted a much greater influence on both fertility and thyroid function than dietary group. The results indicate that it should be possible to feed rapeseed products from certified double-low varieties of B. napus in the concentrates to adult dairy cows in amounts up to 3 kg rapeseed meal per cow and day without negatively affecting either animal health or fertility. However, it would be wise to avoid feeding rapeseed products in excessive quantities to calves and primiparous cows.

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Sammanfattning

Rapsprodukter från dubbellåga sorter till mjolkkor: Effekter av långtidsutfodring på skoldkörtelfunktion, fruktsamhet och djurhalsa.

Under 3 på varandra foljande laktationer studerades 85 mjolkkor av SRB-ras for att utrona eventuella effekter på skoldkortelfunktion, fruktsamhet och djurhalsa av utfodring med varierande mangder rapsprodukter från dubbellåga (00) sorter av Brassica napus. En grupp (NR) utfodrades med ett icke-rapshaltigt koncentrat. En annan grupp (MR) fick maximalt 1.2 kg torrsubstans (ts) 00-rapsmjol samt upp till 0.2 kg ts fettrikt 00-rapsfro, medan en tredje grupp (HR) erholl upp till 2,5 kg ts 00-rapsmjol och upp till 0.9 kg ts fettrikt 00-rapsfro. Det fanns inga statistiskt sakerställda skillnader mellan utfodringsgrupperna med avseende på utgallrings- eller sjukdomsfrekvens. Intervallet från kalvning till draktighetsgivande insemination (CC) var langre for HR-gruppen (125 dagar) jamfort med NR-gruppen (100 dagar) under forsta laktationen. Svaret vid en TRH-test av skoldkortel-funktionen ca 90 dagar efter kalvning var signifikant hogre for HR-gruppen (86,7 µg/l/tim) an for NR-gruppen (55,2 µg/l/tim) under forsta laktationen. Detta indikerar en mycket svag och sannolikt kompenserad negativ inverkan på skoldkortelfunktionen av den hogsta rapsgıvan. Inga skillnader 1 fruktsamhet forelåg hos de aldre korna. Under andra laktationen observerades dock ett storre TSH-svar 1 MR-gruppen (102,5 $\mu g/L/tim$) amfort med NR-gruppen (78,4 $\mu g/L/tim$) samt en hogre koncentration av urea i plasma i NRgruppen (7,31 mmol/l) an i HR-gruppen (6,83 mmol/l). Effekterna av oberoende miljofaktorer påverkade fruktsamhet och skoldkörtelfunktion i mycket hogre grad an rapsutfodringen. Raps från verifierat dubbellåga sorter av Brassica napus i kraftfodret till vuxna mjolkkor i mangder upp till 3 kg per ko per dag kan inte anses medfora någon okad risk for negativa effekter på djurhalsa eller reproduktionsformåga. Eftersom vaxande djur sannolikt ar mera kansliga for hoga intag av glukosinolater an vuxna bor dock maximala givor till forstakalvare undvikas.

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