Increasing Selenium Concentration in Milk: Effects of Amount of Selenium from Yeast and Cereal Grain Supplements

J. W. Heard,* C. R. Stockdale,*¹ G. P. Walker,* C. M. Leddin,* F. R. Dunshea,† G. H. McIntosh,‡ P. M. Shields,§ A. McKenna,|| G. P. Young,‡ and P. T. Doyle*

*Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Kyabram Centre, 120 Cooma Road, Kyabram, Victoria 3620, Australia

†National Centre of Excellence in Functional Foods, Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Werribee Centre, 600 Sneydes Road, Werribee, Victoria 3030, Australia

‡Department of Medicine, Flinders University of South Australia, Bedford Park, South Australia 5042, Australia

§Alltech Biotechnology Pty. Ltd., 68–70 Nissan Drive, Dandenong South, Victoria 3175, Australia

||Tatura Milk Industries Ltd., PO Box 213, Tatura, Victoria 3616, Australia

ABSTRACT

Two experiments were conducted to establish responses in milk Se concentrations in grazing dairy cows to different amounts of dietary Se yeast, and to determine the effects of the Se concentration of the basal diet. The hypothesis tested was that the response in milk, blood, and tissue Se concentrations to supplemental Se would not be affected by whether the Se was from the basal diet or from Se yeast. In addition, by conducting a similar experiment in either early (spring; experiment 1) or late (autumn; experiment 2) lactation, we hypothesized that different Se input-output relationships would result. Both 6-wk experiments involved 60 multiparous Holstein-Friesian cows, all of which had calved in spring. They were allocated to 1 of 10 dietary Se treatments that included 2 types of crushed triticale grain (low Se, approximately 165 µg of Se/kg of DM; or high Se, approximately 580 µg/kg of DM) fed at 4 kg of DM/d, and 1 kg of DM/d of pellets formulated to carry 5 quantities of Se yeast (0, 4, 8, 12, or 16 mg of Se). Daily total Se intakes ranged from <2 to >18 mg/cow in both experiments. Milk Se concentrations plateaued after 15 and 7 d of supplementation in experiments 1 and 2, respectively, and then remained at plateau concentrations. Average milk Se concentrations for the plateau period increased as the amount of Se yeast increased, and low- and high-Se grain treatments were different at all quantities of Se yeast, although there was a tendency for this difference to diminish at the greatest concentrations of yeast. There were significant positive, linear relationships between Se intake and the concentrations of Se in milk, which were not affected by the source of Se, and the relationships were similar

for both experiments. Therefore, the output of Se in milk in experiment 1 was greater than that in experiment 2 because the milk yield of the cows in early lactation was greater. The estimated proportions of Se partitioned to destinations other than milk and feces increased with the amount of Se in the diet and were greater in experiment 2 than in experiment 1, a result that was supported by Se concentrations in whole blood and plasma and in semitendinosus muscle tissue. If high-Se products are to be produced for human nutrition, it is important to be able to develop feeding systems that produce milk with consistent and predictable Se concentrations so that products can consistently meet specifications. The results indicate that this objective is achievable.

Key words: selenium, selenium yeast, dairy cow, pasture-fed

INTRODUCTION

Recommended dietary allowances of Se for humans are approximately 60 µg/d, and tolerable upper intakes are approximately 400 µg/d for adults (Institute of Medicine, 2000). Even at maximum tolerable amounts, much of this could be supplied by dairy products from milk with elevated Se concentrations. Selenium concentrations in cow's milk vary within and between dairy regions in Australia (Tinggi et al., 2001). Average Se concentrations in milk from cows grazing pastures and supplemented with cereal grain-based concentrates are approximately 10 µg/kg in northern Victoria (Heard et al., 2004b; Walker et al., 2004) and 6 to 11 µg/kg in northern Queensland (R. G. Walker, Department of Primary Industries and Fisheries, Kairi, Queensland; personal communication). Binnerts (1979) reported milk Se concentrations of 3 to 5 µg of Se/kg of milk from cattle in low-Se areas in the Netherlands and 6 to 11 µg/kg for those from high-Se areas. Variations in Se

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¹Corresponding author: richard.stockdale@dpi.vic.gov.au

concentrations in pastures (10 to >50 μg of Se/kg of DM; Caple et al., 1980) may lead to variations in concentrations in milk, but the effects are likely to be relatively small unless significant amounts of Se are added in fertilizer (Aro et al., 1998). The Se concentrations in concentrate supplements, such as wheat (1 to 117 μg /kg of DM) and lupins (10 to 488 μg /kg of DM), vary to a greater degree than those in pasture (White et al., 1981) and are therefore more likely to affect concentrations in milk.

Many dairy companies are increasing their interest in developing specialty products. Specifically in relation to Se, the challenge is to develop dairy feeding systems that produce milk with consistent and predictable Se concentrations so that Se-enriched products can consistently meet predetermined specifications. The purpose may be to meet recommended daily intakes for humans (MacRae, 2004), or to provide products for use in preventive health (Heard et al., 2004b). Considerable interest exists in supplementing the diets of cows with organic Se as a means of enhancing concentrations in milk and dairy products. Better responses (increase in Se in milk per unit of Se consumed) have been obtained from feeding Se yeast compared with feeding equivalent quantities of inorganic Se sources such as sodium selenate (Ortman and Pehrson, 1999) or sodium selenite (Ortman and Pehrson, 1997, 1999; Givens et al., 2004). As well as the source of Se, other factors that may influence the output of Se in milk include milk protein concentration or production, stage of lactation, and diet. Most Se in milk is associated with milk proteins. Van Dael et al. (1991, as cited by Givens et al., 2004) reported 55 to 75% of milk Se in CN, 17 to 38% in whey, and only 7% in fat, and milk protein concentration and composition can be affected by both stage of lactation and diet.

Two experiments were conducted to establish responses in milk Se concentration to different amounts of Se yeast, and to determine the effects of the Se concentration in the basal diet. The hypothesis tested was that the responses in milk, blood, and tissue Se concentrations to additional dietary Se would not be affected by whether the Se was supplied by the basal diet or from Se yeast. In addition, by conducting a similar experiment with grazing dairy cows in either early (spring) or late (autumn) lactation, we hypothesized that different Se input-output relationships would result.

MATERIALS AND METHODS

Experimental Design

All procedures were conducted with protocols approved by the Northern Victorian Animal Ethics Com-

mittee. Two experiments were conducted at the Kyabram Center of the Victorian Department of Primary Industries (36°20'S, 145°04'E) with spring-calved, multiparous Holstein-Friesian cows. Both experiments involved 60 cows that were allocated to 10 groups of 6 based on milk production [experiment 1, 29.9 ± 3.13 (SD) kg/d; experiment 2, 15.8 ± 1.76 kg/d], milk protein concentration ($3.25 \pm 0.259\%$; $3.45 \pm 0.214\%$), BCS (4.4 ± 0.55 units; 4.3 ± 0.45 units), and BW (522 ± 57.8 kg; 578 ± 54.3 kg). The cows were 43 ± 11.5 (spring) and 238 ± 11.7 (autumn) DIM at the start of experiments 1 and 2, respectively. Cows on all dietary treatments grazed irrigated perennial pasture, composed principally of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), as a single herd at daily allowances of 35 to 40 kg of DM/cow.

Dietary treatments included 2 types of concentrate supplement, low- or high-Se rolled triticale, fed at 4 kg of DM/d, and 5 quantities of Se yeast produced from *Saccharomyces cerevisiae* CNCM I-3060 (Sel-Plex, Alltech Biotechnology, Nicholasville, KY; 0, 4, 8, 12, and 16 mg of Se/d) carried in 1 kg of DM/d of triticale grain pellets. The pellets were made from the low-Se triticale.

Cow Management and Measurements

Before treatments commenced in experiment 1, the cows grazed as one group for 7 d and received 4 kg of DM/d of low-Se triticale. Body weight and BCS were measured 3 d before treatments commenced and milk production was measured daily. Milk composition was determined on one occasion. Treatments commenced at the a.m. feeding on October 5 (d 1) and finished after the p.m. feeding on November 15 (d 42). Before treatments commenced in experiment 2, the cows grazed as one group for 14 d and received 6 kg of DM/d of low-Se triticale. Body weight and BCS were measured 3 d before treatments commenced and milk production was measured daily. Milk composition was again determined on one occasion. Treatments commenced at the a.m. feeding on April 19 (d 1) and finished after the p.m. feeding on May 27 (d 39).

Pasture height was measured on 2 to 3 d each week using a rising plate meter (Earle and McGowan, 1979), and pregrazing pasture mass was then estimated using published equations relating plate meter height to pasture mass (kg of DM/ha; Heard et al., 2004a). The area offered to the cows was calculated to provide a daily pasture allowance of approximately 40 (experiment 1) or 35 (experiment 2) kg of DM/cow. Paddocks were measured and electric fencing erected for the following 2 to 3 d of grazing. The herd was offered almost half of their daily pasture allowance after the a.m. milking and the remaining pasture after the p.m. milking. The cows

were given the opportunity to back-graze over the a.m. allocation in the p.m.

Nutritive characteristics of the pasture consumed were estimated by taking representative samples of pasture cut to a simulated grazing height of approximately 4 cm. At least 15 quadrats along a defined transect across the grazing area were cut and bulked for each week. Subsamples of approximately 100 g of fresh weight were oven-dried at 60°C for 72 h, ground to pass through a 0.5-mm screen, and stored for subsequent analysis.

Supplements were fed individually, with half the daily allocation given immediately following the a.m. milking, and the remainder fed immediately before (experiment 1) or after (experiment 2) the p.m. milking. Representative samples of each supplement were collected each day and bulked to provide a sample for each feed each week. Subsamples of approximately 100 g of fresh weight were oven-dried at 60°C for 72 h, ground to pass through a 0.5-mm screen, and stored for subsequent analysis. Representative samples of each supplement were also collected once per week and dried at 100°C to ensure an accurate measure of DMI of supplement. Where residues occurred, they were collected, separated into grain and pellets, and weighed. Subsamples of residues were dried at 100°C to determine DM contents.

Milk production of individual cows was measured at each milking before an experiment commenced, throughout the treatment period, and for 21 d after treatments finished in both experiments. In experiment 1, milk samples from the p.m. and a.m. milkings were taken from all cows on d 0, 1, 3, 4, 6, 7, 8, 15, 22, 29, 42, 48, 56, and 63 to measure milk composition (protein, fat, lactose concentration) and SCC. A subsample of milk from cows within each treatment was bulked on each of these days to measure milk Se concentration. Five cows that did not consume the Se yeast pellets consistently throughout experiment 1 were excluded from the bulk milk samples for the relevant treatments. Samples from individual cows in the 0, 8, and 16 mg/d of Se yeast treatments from d 22 and 42 were also analyzed for milk Se concentration. In experiment 2, milk samples were taken from all cows on d 0, 3, 6, 9, 13, 15, 21, 22, 28, 29, 35, 36, 45, 51, and 58 to measure bulk milk Se concentration on a treatment basis. A subsample of milk from individual cows in the 0, 8, and 16 mg/d of Se yeast treatments from d 21 and 35 were also analyzed for milk Se concentration. In addition, individual cow samples from d 0, 9, 15, 21, 28, 35, 45, 51, and 58 were analyzed for milk composition and SCC. Finally, BW and BCS (according to the 8-point scale of Earle, 1976) were recorded each week after the a.m. milking.

Blood samples were taken from the tail vein of each cow in the morning during wk 3, 6, and 9 in both experiments for analysis of plasma (experiment 1) and whole blood (experiment 2) Se concentrations. Fecal samples were collected from the rectum of all cows on d 18 and 39 in both experiments. For each treatment, a composite sample was created for each of these days by bulking material from cows on an equal DM basis. Muscle tissue biopsies were taken from the semitendinosus muscle of all cows on d 17, 38, and 59 with a biopsy drill fitted with a 14-gauge $\times$ 38-mm needle, removing approximately 150 mg of tissue. Before the biopsy was taken, the site was cleaned with alcohol, and after tissue removal, the site was sprayed with antibiotic. The area from which the biopsy was taken was desensitized with an injection of local anesthetic [2.5 mL of Ilium Lignocaine 20, active ingredient lignocaine hydrochloride (20 mg/mL); Troy Laboratories Pty. Ltd., Smithfield, New South Wales, Australia]. All biopsy samples were analyzed for Se concentration.

Analytical Methods

Samples of grain and pellets were analyzed for DM, nitrogen (by combustion with an FP-428 instrument, Leco Australia Pty. Ltd., Baulkham Hills, New South Wales, Australia), NDF (Van Soest et al., 1991), in vitro DM digestibility (IVDMD; Tilley and Terry, 1963), and Se concentration (see below). Metabolizable energy (MJ/kg of DM) was calculated by using $0.17 \times \text{IVDMD} (\%) - 2.0$ (Standing Committee on Agriculture, 1990), and CP was calculated as nitrogen $\times$ 6.25. Samples of pasture were analyzed as for the supplements, except that IVDMD was determined by a pepsin-cellulase technique (Clarke et al., 1982). Milk samples were analyzed for protein, fat, and lactose concentrations with an infrared milk analyzer, as was SCC (Foss 4000/5000 Combi, Foss Electric, Hillerød, Denmark).

Total Se in milk, feed, fecal, plasma and whole blood, and muscle samples was determined after digesting subsamples in a mixture of nitric and perchloric acids by using test tubes fitted with reflux funnels in an electrically heated digestion block. In the end stage of the digestion process, hydrochloric acid was added to the tubes to reduce selenium(V) to selenium(III), with the final matrix being 30% hydrochloric acid. The digestates were diluted (to 20 mL final volume) and analyzed by inductively coupled plasma mass spectrometry/vapor generation. Duplicate samples were included at 3 to 4 per 100 samples, and reproducibility was at least 90%. Both standard reference material and laboratory control samples were carried through all analyses. The standard reference materials were National Institute of Standards and Technology standard 1549

(nonfat milk powder) and standard 1577b (bovine liver). “In-house”-prepared laboratory control samples were ovine kidney, chicken meat, and nonfat milk powder. In each run, 2 randomly selected samples were spiked, with acceptable recoveries being between 80 and 120%. Three blanks (empty tubes) were also included in the digestion and analysis process in each run. For muscle, additional tuning was performed on the inductively coupled plasma mass spectrometry/vapor generation system to increase sensitivity.

Statistical Analysis and Calculations

Treatment responses for animal variables were compared by repeated measures analysis of covariance (Genstat 8, Lawes Agricultural Trust, 2005) with a 2×5 factorial with triticale (low- and high-Se concentration) and amount of Se yeast (nominally 0, 4, 8, 12, and 16 mg of Se/d) as factors. Data for individual cows were used, and significant differences between factors were established at $P = 0.05$. The same analysis was undertaken in which individual cow data were available for milk, blood, and muscle tissue Se concentrations, but without the use of covariates. For the 5 cows that did not consume their supplements, the Genstat 8 (Lawes Agricultural Trust, 2005) program estimated missing values based on data from other animals in a group.

Relationships between milk Se concentration (Y ; $\mu\text{g/kg}$), or milk Se output (Y ; mg/d), and total Se intake (X ; mg/d) were examined by using group data in stepwise regression analyses in Genstat 8 (Lawes Agricultural Trust, 2005) according to the following model:

$$Y = a + b \cdot X + c \cdot X^2 + d \cdot \text{experiment} + e \cdot \text{grain} + f \cdot \text{interactions},$$

where “experiment” is a pseudovisible to distinguish between experiments 1 (+1) and 2 (+2); “grain” is a pseudovisible to distinguish between low-Se (−1) and high-Se (+1) triticale grain; and interactions refers to the first-order interactions between each of the variables. A variable was included in a regression only if it was significant at $P < 0.05$. Average plateau data during the treatment period were used, which were defined as being from d 15 in experiment 1 and from d 7 in experiment 2. The same model was used, in which muscle Se concentration was the dependent variable, but separate regressions were generated for wk 3, 6, and 9.

Fecal output was calculated by using estimated total DMI and the IVDMD of dietary components. The DMI of pasture was estimated by using Diet Check (Heard et al., 2004b), and total DMI was calculated by using

this value and measured supplement intakes. Fecal Se excretion was calculated from estimated fecal DM output and the Se concentration of the composite feces grab samples.

RESULTS

Feeds and Intake

In experiment 1, the pasture mass ($\pm\text{SD}$) was 5.2 (± 0.57) tonne of DM/ha and daily pasture allowance was 39 (± 2.6) kg of DM/cow. In experiment 2, the pasture mass was 4.0 (± 0.26) tonne of DM/ha and daily pasture allowance was 37 (± 2.1) kg of DM/cow. The ME of pasture consumed was similar in both experiments, but CP, NDF, and Se concentrations were slightly greater in experiment 2 (Table 1). The ME and NDF concentrations of the 2 triticale grains and the 5 Se yeast pellets were similar, but the CP concentrations of the grain were less than those of the Se yeast pellets. The low- and high-Se grains were consistent in their difference in average Se concentration between experiments (Table 1), but the coefficients of variation (CV) within a grain were between 11 and 23%, indicating the existence of considerable variation. The targeted Se concentrations in the Se yeast pellets were generally achieved (Table 1), but again there was variation within a pellet, with CV ranging from 3 to 13%; however, this variation was not related to treatment.

Average intakes of DM and Se from grain and pellets were similar in both experiments (Table 2); however, the pattern of intake of the grain and pellets varied between experiments (Figure 1). In experiment 1, cows did not consume all of their grain or pellets until approximately d 15 of the experiment, whereas in experiment 2, this occurred almost immediately. The estimated DMI of pasture averaged approximately 13.0 and 12.5 kg/cow in experiments 1 and 2, respectively, and constituted approximately 70% of total DMI.

Animal Production

Repeated measures analyses indicated that time was not a significant factor in either experiment; hence, only means for the whole experimental period are given in Table 3. There were no effects of grain type or Se yeast treatment on any aspect of animal production in either experiment. The exception was a small increase in milk lactose concentration in experiment 2 as the amount of Se yeast increased, but there was no effect on lactose production.

Selenium Concentrations and Output

Experiment 1. Selenium concentrations in milk plateaued after 15 d of supplementation, and then re-

Table 1. Nutritive characteristics of the pasture and supplements consumed by the cows in both experiments (mean $\pm$ SD)

Item	Pasture	Triticale grain ¹		Selenium yeast pellets ²				
		Low	High	0	4	8	12	16
Experiment 1								
ME (MJ/kg of DM)	12.0 ± 0.39	13.0 ± 0.14	12.9 ± 0.17	13.3 ± 0.11	13.4 ± 0.10	13.4 ± 0.09	13.4 ± 0.07	13.4 ± 0.09
CP (% of DM)	15.5 ± 2.03	11.6 ± 0.08	10.7 ± 0.83	16.7 ± 0.31	16.6 ± 0.25	16.5 ± 0.27	16.6 ± 0.16	17.7 ± 0.26
NDF (% of DM)	45.2 ± 4.14	17.0 ± 1.21	17.0 ± 0.64	16.4 ± 1.22	16.1 ± 0.97	16.5 ± 0.63	15.1 ± 0.94	16.3 ± 0.56
Selenium (μg/kg of DM)	65 ± 21.5	157 ± 35.6	578 ± 70.5	147 ± 16.3	3,500 ± 178.9	6,683 ± 299.4	11,333 ± 816.5	16,167 ± 2137.0
Experiment 2								
ME (MJ/kg of DM)	11.9 ± 0.82	13.6 ± 0.09	13.3 ± 0.07	13.5 ± 0.10	13.5 ± 0.16	13.3 ± 0.32	13.0 ± 0.39	13.4 ± 0.16
CP (% of DM)	17.0 ± 2.13	12.4 ± 0.28	10.3 ± 0.60	17.7 ± 0.18	18.0 ± 0.50	17.4 ± 0.17	17.6 ± 0.11	17.8 ± 0.14
NDF (% of DM)	51.2 ± 4.85	17.1 ± 1.37	17.4 ± 1.43	16.1 ± 0.27	16.2 ± 0.86	14.7 ± 0.71	15.1 ± 0.60	15.0 ± 0.64
Selenium (μg/kg of DM)	99 ± 19.0	168 ± 18.3	580 ± 76.9	140 ± 17.9	4,267 ± 445.7	8,183 ± 397.1	12,167 ± 752.8	14,833 ± 408.2

¹Low- and high-selenium grain supplements.

²Pellets with nominal concentrations of 0, 4, 8, 12, and 16 mg of selenium yeast/kg of DM.

mained at plateau concentrations until treatments ceased (Figure 2A). After supplementation with grain and Se yeast had ceased, concentrations declined rapidly to approach pretreatment values. When individual cow data for wk 3 and 6 were analyzed, Se yeast increased milk Se concentration across the range, but the Se in triticale had no effect at the greatest concentration of Se yeast (Table 4). Between-cow variation in milk Se concentration within treatments was similar across treatments, with CV averaging 10.1, 12.1, and 12.0% for the 0, 8, and 16 mg/d of Se yeast treatments, respectively. Plasma Se concentrations changed more slowly than did milk Se concentrations. They increased from wk 3 to 6 (89 vs. 110 μ g/kg, on average; SE of the difference = 1.5) and only returned to wk 3 concentrations 3 wk after treatments had ceased (87 μ g/kg). Plasma Se concentrations were consistently different because of the triticale treatment, and Se yeast increased plasma Se concentrations up to the second highest concentration of Se yeast (Table 4). Muscle Se concentrations increased with the amount of Se in the diet regardless of its origin, but were slower to change than those of plasma, with muscle Se concentrations 3 wk after the cessation of treatment being no different from those recorded after 6 wk of Se supplementation (Table 4). Mean fecal Se concentrations were 0.2, 0.6, 0.8, 1.2, and 1.5 mg/kg of DM when low-Se triticale was fed, and were 0.5, 0.7, 1.1, 1.2, and 1.5 mg/kg of DM when high-Se triticale was fed to cows supplemented with 0, 4, 8, 12, and 16 mg of Se yeast/d, respectively.

Experiment 2. Milk Se concentrations reached plateau values within a week of commencing supplementation, and these were maintained for the rest of the treatment period (Figure 2B). When milk samples from individual cows during wk 3 and 6 were examined, Se concentrations increased with both Se yeast and Se in triticale (Table 4). In contrast to experiment 1, between-cow variation in milk Se concentration decreased as the concentration of Se yeast increased. For the 0, 8, and 16 mg/d of Se yeast treatments, CV averaged 19.8, 14.1, and 9.7%, respectively, principally because of greater increases in means relative to SD. Whole blood Se concentrations rose between wk 3 and 6 (176 vs. 212 μ g/kg, on average; SE of the difference = 2.4), but had not returned to wk 3 concentrations 3 wk after treatments had ceased (192 μ g/kg). Whole blood Se concentrations generally increased consistently with both triticale and Se yeast treatments (up to the second highest concentration), and muscle Se concentrations followed similar trends (Table 4). Finally, mean fecal Se concentrations were 0.3, 0.8, 1.1, 1.3, and 1.7 mg/kg of DM when low-Se triticale was fed, and were 0.5, 0.9, 1.3, 1.5, and 1.8 mg/kg of DM when high-Se triticale was fed to cows

Table 2. Estimated daily DMI and selenium intake for all treatments averaged across whole experimental periods

Item	Selenium yeast pellets ¹									
	Low-selenium triticale grain ²					High-selenium triticale grain ²				
	0	4	8	12	16	0	4	8	12	16
Experiment 1										
DMI (kg/cow)										
Grain	3.91	3.73	3.80	3.78	3.93	3.92	3.94	3.82	3.92	3.89
Pellets	1.00	0.94	0.94	0.99	0.98	1.01	1.01	0.99	1.02	0.98
Pasture	12.6	12.6	13.4	13.0	12.7	13.3	13.4	13.4	13.2	12.7
Total	17.5	17.3	18.1	17.8	17.6	18.2	18.4	18.2	18.1	17.6
Selenium intake (mg/cow)										
Grain	0.61	0.59	0.60	0.59	0.62	2.27	2.28	2.21	2.27	2.25
Pellets	0.15	3.29	6.28	11.22	15.84	0.15	3.54	6.62	11.56	15.84
Pasture	0.82	0.82	0.87	0.85	0.83	0.87	0.87	0.87	0.86	0.83
Total	1.58	4.70	7.75	12.66	17.29	3.28	6.68	9.70	14.68	18.92
Experiment 2										
DMI (kg/cow)										
Grain	3.96	3.96	3.95	3.94	3.94	3.92	3.90	3.93	3.93	3.90
Pellets	1.06	1.06	1.03	1.02	1.01	1.06	1.06	1.03	1.03	1.03
Pasture	12.8	12.1	12.6	12.8	12.1	12.6	12.2	12.7	12.5	12.7
Total	17.8	17.1	17.6	17.8	17.1	17.6	17.2	17.7	17.5	17.6
Selenium intake (mg/cow)										
Grain	0.67	0.67	0.66	0.66	0.66	2.27	2.26	2.28	2.28	2.26
Pellets	0.15	4.52	8.43	12.41	14.98	0.15	4.52	8.43	12.53	15.28
Pasture	1.27	1.20	1.25	1.27	1.20	1.25	1.21	1.26	1.24	1.26
Total	2.08	6.39	10.34	14.34	16.84	3.67	7.99	11.96	16.05	18.80

¹Pellets with nominal concentrations of 0, 4, 8, 12, and 16 mg of selenium yeast/kg of DM.

²Low- and high-selenium grain supplements.

supplemented with 0, 4, 8, 12, and 16 mg of Se yeast/d, respectively.

Relationships Between Se Input and Output.

There were significant positive, linear relationships between total Se intake and the concentration of Se in milk, and these were similar in both experiments (Table

5 and Figure 3). Hence, the Se output in milk in experiment 1 was greater than that in experiment 2 (Figure 4) because of the difference in milk yield between experiments (Table 3). The regression equation relating total Se intake (mg/d) and milk Se output (mg/d) in the experiments (experiment; experiment 1 = +1, experiment 2 = +2) was

$$\begin{aligned} \text{milk Se output} &= 0.12 (\pm 0.066) \\ &+ 0.32 (\pm 0.010) \text{ total Se intake} - 0.11 (\pm 0.005) \\ &\text{experiment} \times \text{total Se intake} \\ 100R^2 &= 98.6 (P < 0.001); \\ \text{RSD} &= 0.14; \text{CV} = 8.2\%; n = 20, \end{aligned}$$

where RSD is the residual standard deviation.

The proportional output of Se in feces was similar at both stages of lactation, and it tended to decline with Se intake (Figure 4). Therefore, the estimated proportions of Se partitioned to destinations other than milk and feces increased with the amount of Se in the diet and were greater in experiment 2 than in experiment 1 (Figure 4). The relationships between total Se intake and muscle Se concentration were positive and linear (Table 5). In all relationships considered, the source of the additional Se had no influence on a relationship,

Figure 1. Change in intake of pellets containing selenium yeast (bottom half of the graph) and triticale grain (top half of the graph), averaged across treatments, during experiment 1 (■) and experiment 2 (◇).

Table 3. The effect of selenium supplementation on aspects of daily milk production, and BW and BCS averaged over the experimental periods in experiments 1 and 2

Item	Selenium yeast pellets ¹						Triticale grain ²		
	0	4	8	12	16	SED ³	Low	High	SED ³
Experiment 1									
Milk yield (kg/cow)	26.2	25.6	27.0	26.8	26.3	1.15	26.5	26.3	0.73
Milk fat (%)	3.52	3.68	3.27	3.44	3.52	0.154	3.40	3.57	0.098
Milk protein (%)	3.03	3.04	2.93	2.92	3.05	0.093	2.99	2.99	0.059
Milk lactose (%)	5.01	4.95	4.94	4.97	5.06	0.088	5.02	4.95	0.056
Fat yield (kg/cow)	0.93	0.95	0.89	0.91	0.94	0.049	0.90	0.95	0.031
Protein yield (kg/cow)	0.80	0.78	0.79	0.77	0.81	0.033	0.79	0.79	0.021
Lactose yield (kg/cow)	1.32	1.28	1.35	1.31	1.35	0.063	1.33	1.32	0.040
SCC (× 1,000)	72	105	178	59	39	60.7	66	114	38.4
BW (kg)	534	539	539	534	526	9.1	532	538	5.8
BCS ⁴ (units)	4.3	4.4	4.4	4.2	4.3	0.09	4.3	4.4	0.06
Experiment 2									
Milk yield (kg/cow)	14.4	14.2	16.0	13.7	14.7	1.26	14.7	14.5	0.79
Milk fat (%)	4.37	4.20	4.22	4.32	4.40	0.233	4.30	4.31	0.146
Milk protein (%)	3.79	3.79	3.77	3.90	3.84	0.073	3.81	3.82	0.046
Milk lactose (%)	4.85 ^a	4.78 ^a	4.95 ^{ab}	5.01 ^b	5.00 ^b	0.070	4.90	4.93	0.044
Fat yield (kg/cow)	0.64	0.61	0.67	0.60	0.65	0.048	0.64	0.63	0.030
Protein yield (kg/cow)	0.56	0.57	0.59	0.54	0.58	0.036	0.58	0.56	0.023
Lactose yield (kg/cow)	0.71	0.69	0.80	0.71	0.74	0.068	0.73	0.73	0.042
SCC (× 1,000)	237	215	198	267	177	54.6	200	237	34.5
BW (kg)	591	587	586	592	588	4.7	589	589	3.0
BCS ⁴ (units)	4.4	4.4	4.4	4.4	4.4	0.05	4.4	4.4	0.03

^{a,b}Means in a row for main effects with different superscripts differ ($P < 0.05$).

¹Pellets with nominal concentrations of 0, 4, 8, 12, and 16 mg of selenium yeast/kg of DM.

²Low- and high-selenium grain supplements.

³SED = standard error of difference.

⁴On the 1- to 8-point scale of Earle (1976).

including those for plasma and whole blood (relationships not shown). In addition, Se concentration relationships were not affected by interactions between any of the independent variables.

DISCUSSION

The hypothesis that the response in milk, blood, and tissue Se concentrations to additional dietary Se would not be affected by whether the Se was supplied by the basal diet or from Se yeast was confirmed in both experiments for total Se intakes up to 19 mg/d. Both sources of additional Se appeared to be equally effective in elevating Se concentrations. This occurred in spite of there being a tendency toward a lack of effect of grain Se concentration on milk Se concentration at the greatest amounts of Se yeast. At 4 mg of Se yeast/d, the basal diet provided approximately 30 and 45% of total dietary Se when the diet included low- and high-Se triticale, respectively, but these values were only 10 and 18% at 16 mg of Se yeast/d. The apparently smaller effect of Se in triticale with greater Se yeast supplementation is most likely a result of this dilution rather than because the Se from the basal diet became less biologically available at the higher inclusion rates of Se yeast. This notion is supported by the variation in Se concentration

seen in Table 1, much of which is probably caused by the inherent variability associated with the analysis of Se.

The relationships between total Se intake and Se concentrations in milk presented here are different from those reported by Givens et al. (2004), Heard et al. (2004b), and Juniper et al. (2006; Figure 3), even though all studies used Se yeast from the same source. Differences in analytical techniques when determining Se concentrations may be an issue here, but cow and diet differences may also be implicated, although all cows were of Holstein origin and milk yields were similar. Givens et al. (2004) and Juniper et al. (2006) fed TMR in intensive feeding systems with cows at various stages of lactation, and Heard et al. (2004b) conducted their research with grazing dairy cows in early lactation. We acknowledge that seasonal effects on the pasture available for grazing and stage of lactation are confounded in the present study, with the experiments not being specifically designed to test for these factors. The similarities in nutritive characteristics and DMI of the various feeds in both of our experiments, however, lead us to suggest that it was principally stage of lactation, and the associated differences in milk and milk protein yields, that differentiated our 2 studies. There was little difference in the relationships between milk

Figure 2. Effect of Se from Se yeast ($\square, \blacksquare$: 0 mg of Se yeast treatment; $\triangle, \blacktriangle$: 4 mg of Se yeast treatment; $\circ, \bullet$: 8 mg of Se yeast treatment; $\nabla, \blacktriangledown$: 12 mg of Se yeast treatment; $\diamond, \blacklozenge$: 16 mg of Se yeast treatment) and triticale grain (low Se concentration: open symbols; high Se concentration: full symbols) on milk Se concentrations in A) experiment 1 and B) experiment 2.

Se concentration and Se intake between the 2 experiments (as described in Table 5 and demonstrated in Figure 3), but the output of Se in milk differed greatly because of the greater milk production in early lactation. Although we suggest that stage of lactation is the principal difference between our experiments, we believe there is still a need for further clarification of the roles of diet and stage of lactation on milk Se concentration.

To be able to provide high-Se milk for the commercial production of health ingredients or products, it is important to be able to predict the time taken to reach plateau concentrations after the commencement of supplementation with high-Se feeds. In experiment 2, and

in previous reports with cows that had adequate Se intake (McIntosh and Royle, 2002; Heard et al., 2004b), milk Se concentrations peaked at approximately 7 d after the introduction of Se yeast. The results of experiment 1 illustrate the importance of cows consuming the desired amount of supplement. With experiments 1 and 2 coinciding with early and late lactation, respectively, we suggest that, on commercial farms where seasonal calving is practiced, it would be best to implement strategies to produce high-Se milk in mid to late lactation to avoid issues with freshly calved cows that tend to have variable DMI.

Effects of Se supplementation on animal production in our experiments were not anticipated. The estimated ME intakes were not different between treatments, and several variables clearly indicate the adequacy of the Se status of the cows we used. It is principally when Se supplements are fed to Se-deficient cows that production responses can be expected. Witchel et al. (1994) suggested cows grazing pasture with less than 30 μg of Se/kg of DM would have inadequate Se intakes, whereas Caple et al. (1980) considered that pasture with 50 μg of Se/kg of DM would provide adequate Se. The Se concentrations in our pastures and supplements were always greater than 60 μg of Se/kg of DM, indicating sufficiency of Se in the basal diet. Furthermore, blood Se concentrations of greater than 21 μg of Se/kg are generally considered to be indicative of adequate Se in lactating dairy cows (Witchel et al., 1994; Thompson et al., 1998; Grace et al., 2001). A blood Se concentration of 21 μg of Se/kg is associated with a milk Se concentration of 4 μg of Se/kg according to a regression developed by Grace et al. (2001). The minimum average milk Se concentration before or during either experiment was approximately 12 to 13 μg /kg, and concentrations of Se in whole blood measured in experiment 2 were in excess of 100 μg of Se/kg.

In experiment 1, plasma Se concentrations increased with dietary Se supplementation up to approximately 12 mg of Se yeast/d, beyond which there was no further increase regardless of the source of Se. The results were similar for whole blood Se in experiment 2, although blood Se was higher than in plasma. Kim and Mahan (2001) suggest that, at least for pigs, red blood cell Se is a better indicator of Se status than plasma Se, particularly for organic forms of Se, for which no plateau in the dose-response curve was apparent when up to 40 mg/kg of feed was consumed daily. Apparently, Se can be incorporated into red blood cells only during erythropoiesis, and because this process occurs slowly, there will be a lag in blood Se response following changes in Se supplementation (Thompson et al., 1980). After treatments ceased on d 42 or 39 in the present experiments, milk Se concentration from cows receiving

Table 4. Summary statistics of the effect of 6 wk of selenium treatment on the concentrations of selenium ($\mu\text{g/kg}$) in milk and blood or plasma, and in muscle biopsies, in experiments 1 and 2

Item	Selenium yeast pellets ¹						Triticale grain ²		
	0	4	8	12	16	SED ³	Low	High	SED ³
Experiment 1									
Average of individual cow milk data collected during wk 3 and 6									
Low selenium grain	13 ^a		68 ^c		131 ^e	5.6			
High selenium grain	27 ^b		88 ^d		129 ^e				
Plasma samples collected from individual cows									
Week 3	44 ^a	85 ^b	88 ^b	123 ^d	107 ^c	4.1	83 ^a	95 ^b	3.2
Week 6	58 ^a	100 ^b	113 ^c	141 ^d	138 ^d		104 ^a	116 ^b	
Week 9	59 ^a	78 ^b	90 ^c	101 ^d	107 ^d		84 ^a	90 ^b	
Muscle biopsy samples collected from individual cows									
Week 3	97 ^a	114 ^{ab}	130 ^{bc}	147 ^{cd}	158 ^d	9.0	120 ^a	139 ^b	5.7
Week 6	103 ^a	133 ^b	166 ^c	179 ^c	208 ^d		151 ^a	164 ^b	
Week 9	104 ^a	143 ^b	162 ^c	181 ^d	208 ^e		151 ^a	168 ^b	
Experiment 2									
Average of individual cow milk data collected during wk 3 and 6	27 ^a		91 ^b		138 ^c	4.0	79 ^a	92 ^b	3.3
Whole blood samples collected from individual cows									
Week 3	145 ^a	158 ^a	182 ^b	192 ^{bc}	201 ^c	8.6	174	177	5.4
Week 6	157 ^a	189 ^b	216 ^c	246 ^d	252 ^d		205 ^a	219 ^b	
Week 9	144 ^a	165 ^b	195 ^c	226 ^d	230 ^d		184 ^a	200 ^b	
Muscle biopsy samples collected from individual cows									
Week 3	140 ^a	158 ^{ab}	173 ^b	175 ^b	173 ^b	11.9	159	169	7.5
Week 6	142 ^a	171 ^b	208 ^c	243 ^d	233 ^d		191	207	
Week 9	126 ^a	177 ^b	176 ^b	214 ^c	225 ^c		177	190	

^{a-e}Means in a row for main effects, or for interactions, with different superscripts differ ($P < 0.05$).

¹Pellets with nominal concentrations of 0, 4, 8, 12, and 16 mg of selenium yeast/kg of DM.

²Low- and high-selenium grain supplements.

³SED = standard error of difference.

Se yeast declined to near pre-experimental concentrations within a week, although small effects were still apparent 3 wk after the cessation of supplementation. The plasma and blood Se concentrations declined much more slowly, indicating a continuing influx into the circulatory system of Se from body tissues. Furthermore, although milk is synthesized and removed daily, the turnover of plasma Se-protein and red blood cells is a much slower process.

The NRC (2005) recommends that the maximum tolerable amount of Se for ruminants is 5 mg of Se/kg of DM. Based on this figure, a high-producing dairy cow should be able to consume up to 100 mg of Se/d for extended periods without developing symptoms of toxicity. In developing feeding systems that include significant amounts of organic Se supplements to produce high-Se products, it is important to consider changes in indicators of animal health and accumulation in tissues

Table 5. Regression relationships between total selenium intake (mg/d) and selenium concentration in milk or the selenium concentration in muscle biopsies

Dependent variable	Significant regression equation ¹	100R ²	RSD ²	CV
Milk Se concentration ($\mu\text{g/kg}$)				
Plateau averages ³	18.9 (± 4.66) + 7.5 (± 0.23) total selenium intake – 9.2 (± 2.63) experiment	98.4 ($P < 0.001$)	5.9	7.1
Muscle Se concentration ($\mu\text{g/kg}$)				
At wk 3	65.7 (± 9.34) + 3.2 (± 0.47) total selenium intake + 31.8 (± 5.28) experiment	84.1 ($P < 0.001$)	11.8	8.1
At wk 6	57.0 (± 10.40) + 6.6 (± 0.52) total selenium intake + 35.6 (± 5.89) experiment	92.4 ($P < 0.001$)	13.1	7.3
At wk 9	80.4 (± 10.70) + 6.2 (± 0.54) total selenium intake + 17.9 (± 6.07) experiment	89.7 ($P < 0.001$)	13.5	7.9

¹Only significant ($P < 0.05$) terms have been included. Total selenium intake (mg/kg of DM) includes selenium from pasture, grain, and selenium yeast. The term “experiment” is a pseudovariate to distinguish between experiment 1 (+1) and experiment 2 (+2). Treatment means were used ($n = 20$).

²RSD = residual standard deviation of regression equation.

³Weeks 3 to 6 in experiment 1 and wk 2 to 6 in experiment 2.

Figure 3. Relationships between total Se intake and the concentration of Se in milk in experiment 1 (■: average of d 15 to 42) and experiment 2 (◆: average of d 9 to 36). Results from other experiments (△: Givens et al., 2004; ○: Heard et al., 2004a; □: Juniper et al., 2006).

other than milk, and to establish the quantities excreted in urine and feces. The efficiency of incorporation of Se into milk, as described by the proportions of intake partitioned to various destinations (Figure 4), was greater in experiment 1 than in experiment 2, presumably because of the greater output of milk and milk protein by the cows in experiment 1. Supplementation with organic Se does lead to accumulation of Se in muscle and other organs, as well as in milk. Only limited data are available on accumulation of Se from Se yeast in tissues of lactating dairy cows, and this is only when small amounts have been fed (Ortman and Pehrson, 1997). The research reported here clearly indicates that, at least in skeletal muscle, concentrations increase in response to increases in Se from the basal diet and from Se yeast. The results also clearly indicate that the elimination of the accumulated Se from muscle takes longer than 3 wk after supplementation has ceased. The implications of this for both continued on-farm production of high-Se milk and its management at the factory level need to be investigated.

CONCLUSIONS

Supplementation of grazing dairy cows with increasing amounts of Se led to similar linear increases in milk Se concentrations in both experiments, regardless of whether the increase in Se intake was from Se in the triticale supplement or from the Se yeast. With the nutritive characteristics and intakes of the various

Figure 4. Effect of Se from Se yeast (0 mg of Se yeast treatment; 8 mg of Se yeast treatment; 16 mg of Se yeast treatment) and triticale grain (low- and high-Se concentrations) on the proportions of Se in milk (black), feces (light gray), and unknown (dark gray) destinations in dairy cows in A) experiment 1 and B) experiment 2.

feeds being similar in the experiments reported here, we suggest it was principally the greatly different DIM, and the associated differences in milk and milk protein yields, that differentiated the 2 experiments. Therefore, it appears that stage of lactation is unlikely to affect the relationship between milk Se concentration and Se intake. If high-Se products are to be produced for human nutrition, it is important to be able to develop feeding systems that produce milk with consistent and predictable Se concentrations so that products can consistently meet specifications. The results indicate that this objective is achievable.

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