Selenium levels in cows fed pasture and concentrates or a total mixed ration and supplemented with selenized yeast to produce milk with supra-nutritional selenium concentrations

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ABSTRACT

Seventy multiparous Holstein-Friesian cows were fed different amounts of pasture and concentrates, or a total mixed ration (TMR), for 42 d in mid-lactation to test the hypothesis that the concentration of Se in milk would depend on the amount of Se consumed, when the Se is primarily organic in nature, regardless of the diet of the cows. Of the 70 cows, 60 grazed irrigated perennial pasture at daily allowances of either 20 or 40 kg of dry matter (DM)/cow. These cows received 1 of 3 amounts of concentrates, either 1, 3, or 6 kg of DM/cow per day of pellets, and at each level of concentrate feeding, the pellets were formulated to provide 1 of 2 quantities of Se from Se yeast, either about 16 or 32 mg of Se/d. The other 10 cows were included in 2 additional treatments where a TMR diet was supplemented with 1 kg of DM/d of pellets formulated to include 1 of the 2 quantities of supplemental Se. Total Se intakes ranged from 14.5 to 35.9 mg/d, and of this, the Se-enriched pellets provided 93, 91, and 72% of the Se for cows allocated 20 and 40 kg of pasture DM/d or the TMR, respectively. No effects of the amount of Se consumed on any milk production variable, or on somatic cell count, body weight, and body condition score, for either the pasture-fed or TMR-fed cows were found. Milk Se concentrations responded quickly to the commencement of Se supplementation, reaching 89% of steady state levels at d 5. When milk Se concentrations were at steady state (d 12 to 40), each 1 mg of Se eaten increased the Se concentration of milk by 5.0 µg/kg ($R^2 = 0.97$), and this response did not seem to be affected by the diet of the cows or their milk production. The concentration of Se in whole blood was more variable

than that in milk, and took much longer to respond to change in Se status, but it was not affected by diet at any time either. For the on-farm production of Se-enriched milk, it is important to be able to predict milk Se concentration from Se input. It seems that type of diet does not affect this relationship.

Key words: dietary selenium, milk selenium, dairy cow, type of diet

INTRODUCTION

Considerable research has recently been undertaken where dairy cows have been fed diets with between 2 and 134 mg of Se/d (e.g., Palacios et al., 2005; Hoac et al., 2008; Juniper et al., 2008a; Ceballos et al., 2009). This has resulted in milk Se concentrations ranging from 7 to 551 µg of Se/L or kg. Much of this research has been concerned with producing milk or milk products for human consumption to help alleviate Se deficiencies, whereas some has been aimed at providing supranutritional Se levels for human health. Selenium enters the food chain when it is taken up from the soil and incorporated into plant protein, which is then used directly by humans or consumed by animals and incorporated into animal products. On reviewing the literature, Weiss (2003) and Ceballos et al. (2009) found that supplementing the diet of cows with an organic source of Se, such as Se yeast, was more effective at increasing the concentration of Se in milk than similar amounts of Se given in an inorganic form (e.g., sodium selenite or sodium selenate). Selenium in this form is absorbed with a high efficiency and is more effectively used and retained by the ruminant compared with absorption from an inorganic source of Se (e.g., Knowles et al., 1999), and it is safer to use. The bioavailability of an organic source of Se that has been incorporated into milk has also been shown to be greater than Se from other sources in pigs (McIntosh et al., 2008; Uglietta et al., 2008), and its efficacy in colorectal cancer prevention in mice is better (Hu et al., 2008). There

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is increasing interest from local Australian dairy companies in marketing high-Se products, particularly in the Asia region. These markets require products that are consistently high in Se, and the Se needs to be in a bioactive form. Currently, there are limitations to the amount of Se that is permitted to be fed to dairy cows in some regions of the world, but as human populations age, with an increasing number of people afflicted by cancers and other immune system issues, these restrictions may change.

It is already technically feasible to increase Se concentrations in milk to supply a significant amount of Se (e.g., Heard et al., 2007; Juniper et al., 2008a; Walker et al., 2010). To do this on a commercial scale, however, feeding systems must be developed that can provide specified concentrations of Se in milk. Although relationships between milk Se concentrations and Se intake are good within experiments, they often differ between experiments (e.g., Givens et al., 2004; Wang et al., 2009; Calamari et al., 2010). Heard et al. (2007) demonstrated that stage of lactation of Holstein-Friesian cows grazing pasture and fed concentrates did not affect this relationship, but some of the variation between experiments may be due to feed differences because diets have ranged from grazed pasture plus concentrates to TMR in intensive feeding systems. The role of the basal diet needs clarification because Koenig et al. (1997) found that Se absorption and retention were greater in sheep fed a concentrate-based diet than in those fed forage.

To determine whether diet might influence the relationship between Se concentration in milk and Se intake, with the Se principally being contributed by Se yeast, this study was undertaken in which cows were fed different amounts of pasture and concentrates or a TMR. It was hypothesized that the concentration of Se in milk would depend on the amount of Se consumed, regardless of the diet of the cows.

MATERIALS AND METHODS

All procedures were conducted using protocols approved by the Northern Victorian Animal Ethics Committee. The experiment was undertaken at the Victorian Department of Primary Industries' Kyabram Center (36°20'S, 145°04'E) for 6 wk in November/December 2006 using 70 multiparous Holstein-Friesian cows that were in mid-lactation. Cows were 78 d (SD $\pm$ 15.4) into lactation at the start of the experiment, and were producing 33.5 ($\pm$ 4.20) kg of milk/d with milk fat, protein, and lactose concentrations of 3.7 ($\pm$ 0.52), 3.1 ($\pm$ 0.19), and 5.0 ($\pm$ 0.12)%, respectively. Their mean BW and BCS were 561 ($\pm$ 50.1) kg and 4.3 ($\pm$ 0.42) units on the 8-point scale of Earle (1976), respectively, and they had an average SCC of 151,000/mL ($\pm$ 368,400). Data

were collected during the 2 wk before the experiment began for selection of cows and allocation to treatment, and for correction of data for between-cow variation. During this time, all cows grazed pasture, and were fed about 4 kg of DM of pelleted concentrates in the dairy at milking times.

Experimental Design and Management

Of the 70 cows, 60 grazed irrigated perennial pasture consisting of white clover (*Trifolium repens* L.), perennial ryegrass (*Lolium perenne* L.), and paspalum (*Paspalum dilatatum* Poir.) at allowances of either 20 or 40 kg of DM/cow per d. These cows received 1 of 3 concentrate supplement treatments, either 1, 3, or 6 kg of DM/cow per d of pellets, and at each level of concentrate feeding, the pellets were formulated to provide 1 of 2 quantities of Se from Se yeast produced from *Saccharomyces cerevisiae* CNCM I-3060 (Sel-Plex; Alltech Biotechnology, Nicholasville, KY), either about 16 or 32 mg of Se/d. There were 2 additional treatments where a TMR was fed to the remaining 10 cows. The TMR included corn silage, cereal-grain based pellets, canola meal, alfalfa hay, and a mineral mix (Table 1). The cows fed the TMR were also supplemented with 1 kg of DM/d of pellets formulated to provide either of the 2 quantities of supplemental Se (these pellets were the same as those offered to the grazing cows that were fed 1 kg of pellets). Additionally, 0.25 kg of cereal-grain based pellets was fed to all cows at each milking to facilitate movement of cows into the milking shed. There were 5 cows per treatment.

Cows were allocated to treatments by stratified randomization based on milk yield, milk protein concentration, milk fat concentration, age, BCS, and BW before the experiment began using Baird's method (Baird, 1994) according to the ALLOCATE procedure in Genstat (Genstat 10, 2007; Lawes Agricultural Trust, Rothamsted, UK). All cows allocated to the low pasture allowance grazed as 1 group on irrigated perennial pasture, and the cows allocated to the high pasture allowance grazed as a separate group. Each paddock used during the experiment was subdivided by an electric fence to allow the 2 herds to graze side-by-side in that paddock, but at their allocated pasture allowances. The cows fed the TMR were fed as 1 group on a shaded concrete feeding strip adjacent to a rice hull loafing area. All cows were individually offered half of their Se-enriched pellets immediately following the a.m. milking, and the other half immediately following the p.m. milking. After being allowed sufficient time to consume their supplement, the pasture-fed cows returned to their paddock to graze and the TMR-fed cows returned to the feeding facility.

Table 1. Composition and nutrient analysis of the TMR diet and selenium-enriched pelleted concentrates, and nutritive characteristics of the pasture

Item	TMR	Pasture	Selenium-enriched pelleted concentrates
DM (%)	60.6	—	—
Feed ingredients (% of DM)			
Corn silage	42.1	—	—
Alfalfa hay	14.6	—	—
Pelleted concentrates	23.2	—	—
Cereal grain fines	—	—	69.5
Canola meal	19.3	—	11.8
Palm kernel meal	—	—	10.0
Millrun	—	—	2.4
Molasses	—	—	1.0
Rice hulls	—	—	0.9
Mineral mix	0.8	—	4.4
Nutrient content (DM basis)			
ME ¹ (MJ/kg)	11.3	10.7	12.7
CP (%)	17	16	14
NDF (%)	33	50	22
Calcium (%)	0.74	0.49	1.01
Phosphorus (%)	0.39	0.29	0.51
Potassium (%)	1.14	2.31	0.56
Sodium (%)	0.15	0.42	0.55
Magnesium (%)	0.30	0.28	0.28
Chlorine (%)	0.48	1.48	0.46
Sulfur (%)	0.24	0.25	0.16
Manganese (mg/kg)	73	110	146
Copper (mg/kg)	11	8	53
Zinc (mg/kg)	49	28	200
Iron (mg/kg)	244	430	165

¹ME was estimated using $0.17 \times \text{in vitro DM digestibility} - 2.0$ (Standing Committee on Agriculture, 1990).

After 6 wk of feeding Se-enriched pellets, treatments ceased and the cows returned to the research center herd where their milk and blood Se concentrations were monitored for a further 9 wk. During this time, the herd was fed a partial mixed ration of triticale hay, canola hay, corn silage, and pelleted concentrates, in addition to a minimal amount of dry pasture.

Measurements

Pasture height was measured on Monday (for the following 3 d), and Thursday (for the following 4 d) for the 6 wk of the experiment by taking up to 240 height readings using a rising plate meter (Earle and McGowan, 1979). Pre-grazing pasture mass was estimated using standard pasture height/pasture mass equations in a locally-generated dairy nutrition model, Diet Check (Heard et al., 2004b). Mean meter reading and pasture mass ($\pm$ SD) were 16.5 ($\pm$ 2.51) cm and 5.9 ($\pm$ 0.59) t of DM/ha, respectively. The area offered to each group of cows was calculated to provide their allotted pasture allowances. Cows were offered half of their daily pasture allowance after the a.m. milking and the remaining pasture after the p.m. milking. Pasture intakes of individual cows were estimated using the Diet Check nutrition model (Heard et al., 2004b).

Each week, a bulked pasture sample (collected on Monday and Thursday) representative of pasture consumed by cows was collected by cutting a minimum of 15 small quadrats at predetermined positions along a transect across the grazing area. Representative subsamples of approximately 100-g fresh weight were oven-dried at 60°C for 72 h, ground through a 0.5-mm screen, and stored for subsequent analysis. Total N was determined by a LECO FP-428 autoanalyzer (LECO Corporation, St Joseph, MI), and CP content was calculated as $N \times 6.25$. In vitro DM digestibility (IVDMD) was determined using the pepsin-cellulase methodology described by Clarke et al. (1982), and ME (MJ/kg of DM) was estimated using the equation $0.17 \times \text{IVDMD} - 2.0$ (Standing Committee on Agriculture, 1990). Neutral detergent fiber was determined using a modification of the method described by Van Soest et al. (1991), with the amylase step omitted because the samples filtered freely. Mineral analyses were carried out using inductively coupled plasma emission spectrometry.

The pellets offered to, and refused by, each cow were weighed daily, and samples were dried at 100°C for 24 h to determine DM content. Samples of pellets were collected each week throughout the experimental period, dried at 60°C, ground, and analyzed for IVDMD

using a rumen fluid-pepsin method (Tilley and Terry, 1963). Nitrogen, NDF, and mineral concentrations were determined as described above, except that the amylase step was retained when analyzing for NDF.

Daily DMI of the group of cows consuming the TMR was measured by weighing the feed offered and that refused each day, and taking samples to determine DM% by drying at 100°C for at least 48 h. Each week, bulked samples of the TMR were dried at 60°C, ground, and analyzed for IVDMD, N, NDF, and mineral concentrations as described for pellets above.

Cows were milked at 0530 and 1545 h each day and individual milk yields were recorded at each milking for 2 wk before the experiment began and throughout the treatment period. Aliquot samples of the milk from each cow were taken at a p.m. and a.m. milking before the experiment, each week during the experiment, and at 3, 6, and 9 wk after the feeding of Se-enriched pellets had ceased. These samples were analyzed for fat, protein, and lactose concentrations, and SCC, by a Foss 4000/5000 Combi (Foss Electric, Hillerød, Denmark) and for total milk Se concentration after the p.m. and a.m. aliquots had been combined. Samples for Se analysis were stored at -20°C before being analyzed. Cows were weighed, and their BCS assessed (Earle, 1976), before treatments commenced and each week thereafter for the duration of the treatment period.

Whole blood samples (about 10 mL) were taken from the tail vein of each cow for analysis of blood Se concentration before the start of the experiment, during the experimental period (wk 3 and 6), and at 3, 6, and 9 wk after the withdrawal of feeding treatments. Samples were collected in the morning, after the cows had been fed their supplements, into an evacuated heparinized blood tube and immediately put on ice before being stored at -80°C before analysis for Se concentration.

The concentration of total Se in all samples (feeds, milk, whole blood) was determined by inductively coupled plasma mass spectrometry/vapor generation following digestion of samples in a mixture of nitric and perchloric acids, as described by Heard et al. (2007).

Statistical Analysis

Analysis of variance was used to determine treatment responses, with the 12 treatments associated with grazed pasture being analyzed together, and the TMR treatments analyzed separately. Treatment responses of pasture-fed cows were analyzed by the repeated measures ANOVA procedure in Genstat 10 (2007; Lawes Agricultural Trust) in a 2 × 2 × 3 factorial design, with daily pasture allowance (nominally 20 or 40 kg of DM/cow), amount of Se in pellets (nominally 16 or 32 mg of Se/d), and weight of pellets (1, 3, or 6 kg of DM/cow

per d) as the factors. For each variable analyzed, the data were covariately adjusted using the related information collected before the imposition of treatments. Repeated measures of the data from the TMR animals were analyzed separately for each time by simple analysis of covariance (amount of Se in pellets; nominally 16 or 32 mg of Se/d) because the small number of animals limited the use of the repeated measures facility in Genstat. A cow was the experimental unit.

Relationships between milk or blood Se concentration (Y ; µg/kg) and total estimated Se intake (X ; mg/d) were examined by using group data in stepwise regression analyses in Genstat 10 according to the following model:

$$Y = a + b \times X + c \times X^2 + d \times \text{diet} \\ + e \times \text{interaction},$$

where a , b , c , d , and e are all coefficients, diet = a pseudovariable to distinguish between the pasture-fed (+1) and TMR-fed (-1) cows, and interaction = the first-order interaction between X and diet. A variable was only included in a regression if it was significant at $P < 0.05$. For milk Se, average steady-state data during the treatment period, defined as being for the last 5 wk of the experimental period, were used, whereas the 2 blood sampling times during treatment were analyzed separately.

RESULTS

Feeds and Intake

Nutritive characteristics of the feeds offered to the cows in the study are given in Table 1. The estimated DMI of pasture by cows averaged 11.4 and 15.3 kg/cow in the low and high allowance groups, with variation around these averages (Table 2) being due to the different amounts of supplement consumed. The cows in the TMR treatments had an average daily DMI of 24.8 (day-to-day SD ± 1.27) kg/cow, exclusive of their intake of Se yeast pellets. The DM content of the TMR was 61%, and refusals averaged 7.5% of that offered each day. Average measured DMI of pellets are given in Table 2, with intakes stabilizing by about d 7 of the study in all treatments.

Concentrations of Se in the various feeds consumed by cows in all treatments are given in Table 2. Where the nominal provision of Se yeast in pellets was 16 mg/d, the measured amount of Se offered in the 1, 3, and 6 kg of DM of pellets was 15.3, 18.3, and 13.6 mg, respectively, and in the 32-mg pellets, it was 24.7, 32.1, and 36.7 mg of Se, respectively. Total Se intakes

Table 2. Estimated daily DM and selenium intakes for all treatments averaged across the whole experimental period

Item	Pasture treatment ¹												TMR treatment ²	
	20 kg of DM/cow per d						40 kg of DM/cow per d							
	1 + 16	1 + 32	3 + 16	3 + 32	6 + 16	6 + 32	1 + 16	1 + 32	3 + 16	3 + 32	6 + 16	6 + 32	1 + 16	1 + 32
DM intake (kg/cow)														
Pasture/TMR	12.5	12.4	11.5	11.7	10.1	10.0	16.1	16.8	15.6	15.5	13.4	14.2	24.6	24.2
Se pellets	1.01	1.00	3.00	2.98	5.76	5.90	0.99	0.96	2.94	2.98	5.75	5.56	0.77	0.85
Dairy pellets	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Total DMI	13.9	13.9	14.9	15.1	16.3	16.3	17.5	18.2	18.9	18.9	19.6	20.1	25.8	25.5
Se concentration ³ (mg/kg of DM)														
Pasture/TMR	0.12 (0.042)	0.12	0.12	0.12	0.12	0.12	0.12 (0.042)	0.12	0.12	0.12	0.12	0.12	0.23 (0.019)	0.23
Se pellets	15.32 (0.726)	24.74 (1.579)	6.11 (0.741)	10.69 (0.942)	2.27 (0.403)	6.12 (0.361)	15.32	24.74	6.11	10.69	2.27	6.12	15.32	24.74
Dairy pellets	0.47 (0.076)	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Se intake (mg/cow)														
Pasture/TMR	1.50	1.49	1.38	1.40	1.21	1.21	1.93	2.02	1.87	1.86	1.61	1.70	5.66	5.57
Se pellets	15.47	24.74	18.33	31.86	13.08	36.11	15.17	23.75	17.96	31.86	13.05	34.03	11.80	20.78
Dairy pellets	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Total Se intake	17.16	26.42	19.90	33.45	14.48	37.51	17.29	25.96	20.02	33.91	14.85	35.92	17.65	26.54
% from Se pellets	90	94	92	95	90	96	88	91	90	94	88	95	66	78

¹Base feed = pasture allowance of 20 or 40 kg of DM/cow per day; concentrates for all treatments are shown in the form x + y, where x = kg of DM/cow per d and y = mg of Se from Sel-Plex (Alltech Biotechnology, Nicholasville, KY).

²TMR treatments, with 1 kg of DM/cow per d of concentrates nominally carrying either 16 or 32 mg of Se from Se yeast (Sel-Plex).

³Standard deviations are given in parentheses.

ranged from 14.5 to 35.9 mg/d, and of this, the Se-enriched pellets provided 93, 91, and 72% for the cows allocated 20 and 40 kg of pasture DM/d or the TMR, respectively (Table 2).

Animal Production

No interactions were found between time and any aspect of diet for milk production during the experimental period after the first week, when cows were adjusting to diets; therefore, only summary statistics for the last 5 wk are presented for animal production variables (Table 3). Average daily milk yield ranged from 22 to 40 kg/cow, with ranges in milk fat, protein, and lactose concentrations of 2.4 to 4.1%, 2.8 to 3.2%, and 4.8 to 5.2%, respectively. The TMR-fed cows produced more milk than any of the pasture-fed cows, and this milk was lowest in milk fat concentration, toward the top of the range in protein concentration, and highest in lactose concentration (Table 3). No effects of amount of Se yeast on any milk production variable or on SCC, BW, and BCS, for either the pasture-fed or TMR-fed cows were noted.

Selenium Concentrations in Milk and Blood

Pre-experimental concentrations of Se in milk averaged 15 µg/kg (Table 4). With the introduction of treatments, milk Se concentrations responded quickly, reaching 89% of steady-state levels, on average, at d 5. Milk Se concentrations had declined to about double pretreatment levels by 3 wk after treatments had ceased, but there was little further change in the ensuing 6 wk. For the pasture-fed cows, pasture allowance, amount of concentrates, and amount of Se yeast all significantly affected milk Se concentration during the treatment period, in line with intakes of DM and Se. Similar trends with amount of Se yeast were evident in the TMR-fed cows (Table 4).

The regression equation relating total Se intake (mg/d) and milk Se concentration (µg/kg) using steady-state data (d 12 to 40) was:

$$\text{milk Se} = 11.0 (\pm 6.21) + 4.99 (\pm 0.242) \text{ total Se intake,}$$

$$R^2 = 0.97 (P < 0.001); \text{RSD} = 7.1; \\ \text{CV} = 5.4\%; \text{ and } n = 14.$$

Each 1 mg of Se eaten increased the Se concentration of milk by 5 µg/kg, and this response was not affected by the diet of the cows (Figure 1a).

Table 3. The effect of diet and selenium supplementation on aspects of covariate-corrected milk production, and SCC, BW, and BCS, averaged over the last 5 wk of the 6-wk experimental period

Item	Pasture treatment ¹										TMR treatment ²									
	Pasture allowance		Selenium pellet intake			Se from Se yeast			P-value for contrast ⁴				1 + 16			1 + 32			SED	P-value
	20	40	1	3	6	16	32	SED ³	Se-Plex	Pasture	Pellets	1 + 16	1 + 32	SED	P-value					
Milk yield (kg/d)	24.5	29.3	24.7	26.7	29.2	26.8	27.0	1.11	0.74	<0.01	<0.01	39.8	39.9	3.41	0.97					
Milk fat (%)	3.83	3.78	3.86	3.83	3.74	3.83	3.79	0.268	0.70	0.73	0.62	2.81	2.36	0.310	0.20					
Milk protein (%)	2.95	3.16	2.98	3.05	3.14	3.06	3.06	0.054	0.93	<0.01	<0.01	3.12	3.20	0.077	0.35					
Milk lactose (%)	4.84	5.00	4.87	4.91	4.99	4.91	4.93	0.059	0.42	<0.01	<0.01	5.16	5.13	0.068	0.73					
Fat + protein yield (kg/d)	1.65	2.02	1.68	1.82	2.00	1.84	1.83	0.110	0.79	<0.01	<0.01	2.30	2.24	0.190	0.80					
SCC ⁵ (×1,000/mL)	44	48	43	55	41	43	49	21.1	0.45	0.68	0.35	77	40	34.4	0.33					
BW (kg)	574	593	580	582	588	584	583	7.0	0.72	<0.01	0.04	605	607	15.0	0.92					
BCS ⁶ (units)	4.1	4.3	4.3	4.3	4.1	4.2	4.3	0.22	0.22	0.06	0.09	4.3	4.0	0.25	0.28					

¹Treatments were 20 or 40 kg of DM/cow per d pasture allowances; 1, 3, or 6 kg of DM/cow per d of concentrates nominally carrying either 16 or 32 mg of Se from Se yeast (Sel-Plex; Alltech Biotechnology, Nicholasville, KY).

²TMR treatments, with 1 kg of DM/cow per d of concentrates nominally carrying either 16 or 32 mg of Se from Se yeast (Sel-Plex).

³Standard error of difference.

⁴P-values for contrasts for grazing cows; Sel-Plex: 16 vs. 32 mg of Se from Sel-Plex; Pasture: 20 vs. 40 kg of DM/cow per d pasture allowances; Pellets: 1 vs. 3 vs. 6 kg of DM/cow per d of pellets.

⁵Cows with mastitis were excluded (6 cows on 16 mg; 2 cows on 32 mg).

⁶On the 8-point scale of Earle (1976).

Table 4. Selenium concentrations in milk and blood before the experiment, during the experimental period (to 42 d), and for 9 wk after the feeding of selenium-enriched pellets had ceased

Se concentration (µg/kg)	Pasture treatment ¹											TMR treatment ²			
	Pasture allowance		Selenium pellet intake			Se from Se yeast		SED ³	<i>P</i> -value for contrast ⁴						
	20	40	1	3	6	16	32		Sel-Plex	Pasture	Pellets	1 + 16	1 + 32	SED	<i>P</i> -value
In milk															
Pre-experimental	15	15	15	15	15	15	15	—	—	—	—	13	14	—	—
d 5	131	116	110	134	128	90	158	8.5	<0.01	0.01	<0.01	75	121	19.6	0.04
d 12–40	145	131	126	146	143	100	176					91	137	22.5	0.08
d 62	29	27	27	28	28	26	30					24	27	2.3	0.26
d 82	25	23	23	24	25	23	25					22	23	1.4	0.51
d 104	26	24	24	25	27	24	26					23	24	1.4	0.51
In whole blood															
Pre-experimental	116	122	123	117	117	119	119	—	—	—	—	104	108	—	—
d 21	202	204	196	209	203	179	226	12.8	<0.01	0.03	<0.01	187	214	20.3	0.26
d 42	250	258	237	263	262	218	290					238	276	31.7	0.26
d 63	208	222	198	220	227	192	238					213	250	24.5	0.17
d 83	203	219	196	215	222	193	229					212	246	24.2	0.20
d 105	201	211	193	206	218	189	222					209	233	22.6	0.33

¹Treatments were 20 or 40 kg of DM/cow per d pasture allowances; 1, 3, or 6 kg of DM/cow per d of concentrates nominally carrying either 16 or 32 mg of Se from Se yeast (Sel-Plex; Alltech Biotechnology, Nicholasville, KY).

²TMR treatments, with 1 kg of DM/cow per d of concentrates nominally carrying either 16 or 32 mg of Se from Se yeast (Sel-Plex).

³Standard error of difference.

⁴*P*-values for contrasts for grazing cows; Sel-Plex: 16 vs. 32 mg of Se from Sel-Plex; Pasture: 20 vs. 40 kg of DM/cow per d pasture allowances; Pellets: 1 vs. 3 vs. 6 kg of DM/cow per d of pellets. These cover all times because of repeated measures analysis, and no significant interactions with time were found.

Figure 1. Relationships between total Se intake and a) milk (average of d 12 to 40) and b) blood (full line = after 3 wk of supplementation; broken line = after 6 wk of supplementation) Se concentrations in cows grazing pasture and fed concentrates (◆,▲) or a TMR (◇, △).

Pre-experimental concentrations of Se in whole blood averaged 117 µg/kg, and responded more slowly than milk Se concentration to change in dietary Se intake (Table 4). After the feeding treatments ceased, Se concentrations were lower than at the end of treatment, but they were still almost 100% higher than those before Se supplementation commenced. For the pasture-fed cows, pasture allowance, weight of pellets, and amount of Se yeast were all significantly associated with blood Se concentration, whereas similar, but nonsignificant, trends with the inclusion of Se yeast were evident in the TMR-fed cows (Table 4). As found for milk Se concentration, blood Se concentration (µg/kg) was primarily influenced by total Se intake (mg/d), as described by the following regression equations generated from the data presented in Figure 1b:

$$\text{blood Se at wk 3} = 130 (\pm 7.7) + 2.90 (\pm 0.302) \text{ total Se intake,}$$

$$R^2 = 0.89 (P < 0.001); \text{RSD} = 8.9; \text{CV} = 4.4\%; \text{and } n = 14, \text{ and}$$

$$\text{blood Se at wk 6} = 137 (\pm 10.4) + 4.78 (\pm 0.407) \text{ total Se intake,}$$

$$R^2 = 0.92 (P < 0.001); \text{RSD} = 12.0; \text{CV} = 4.7\%; \text{and } n = 14.$$

These responses were not affected by diet, but at 6 wk, there was a trend ($P = 0.063$) for blood Se concentrations to be greater with the TMR.

DISCUSSION

The most important result from this study was that Se intake, principally from Se yeast, was a very good predictor of Se concentration in milk ($R^2 = 0.97$), thereby supporting our hypothesis that the concentration of Se in milk would depend on the amount of Se consumed regardless of the type of diet or the resulting milk yield of the cows, and in spite of there being limited data for the TMR diet. The diets used here covered the range of feeding systems likely to be encountered on farms in Australia, and it is really only the quantity of Se that would differ because farmers would only offer enough Se to provide for the dietary requirements of their animals. Milk yields varied greatly between treatments, but the response from pasture-fed cows and TMR-fed cows were similar even though Se yeast supplied more than 90% of dietary Se in the pasture-concentrate diets and only about 72% with TMR.

The response in milk Se concentration found here, of 5 µg/kg for each 1 mg of Se consumed, was quite different from that found by Heard et al. (2007) and Walker et al. (2010), who together reported a response of 7 µg of Se/kg of milk for each mg of Se consumed, up to a total of 42 mg of Se/d. Each set of data was collected under similar conditions at Kyabram, and the Se analyses were conducted at the same laboratory using the same technique. A key difference between experiments, however, was that the Se-enhanced pellets used in the current experiment were manufactured at a commercial pelleting plant, whereas the pellets used by Heard et al. (2007) were produced under less severe conditions in a research facility and Walker et al. (2010) introduced Se yeast directly into the rumen through rumen fistulae. It was postulated that the heat and pressure associated with the commercial pelleting process may have affected

the bioavailability of Se from the Se yeast in our case, but a subsequent study by Stockdale and Gill (2010) suggested that this was unlikely; thus, an explanation for the different milk Se responses is not clear.

The other important result from this study was that blood Se concentration was slow to react to both the inclusion and exclusion of Se-enriched pellets in the diet of the cows. This response confirmed results from the study of Heard et al. (2007) in which blood Se was monitored for 3 wk after 6 wk of Se supplementation. Selenium is incorporated into red blood cells only during erythropoiesis, and because this process occurs slowly, there is a lag in blood Se responses following an increase in Se supplementation (Thompson et al., 1980). Juniper et al. (2008b) estimated that asymptotic values of blood Se in beef cattle were not likely to occur until about 150 d after the start of exposure to Se supplementation, reflecting the average lifespan of erythrocytes. Conversely, after the withdrawal of supplementation, the decline in blood Se concentrations will depend on erythrocyte turnover and the rate of release of Se from tissue reserves. Together, these processes account for the continued maintenance of milk Se concentrations above pre-experimental levels after Se supplementation had ceased. Body tissues store considerable quantities of organic Se, and Lee et al. (2006, 2007) recently reported high correlations between blood Se concentration and the concentration of Se in leg muscle and liver in steers offered supplements containing Se from Se-enriched organic sources. Heard et al. (2007) have also shown a strong association between whole blood and muscle Se concentrations in lactating dairy cows. In both instances, however, daily Se intakes tended to be lower than those found here, up to about 8 mg/d in the research of Lee et al. (2006, 2007), and up to about 18 mg/d in the case of Heard et al. (2007). It is suggested that the stores of Se in body tissue could continue to re-enter the blood stream for a considerable time after supplementation with large amounts of Se has ceased, but the length of time for which elevated blood, and therefore, milk, Se concentrations are likely to remain is currently unknown.

As in most research conducted recently where Se yeast has been fed for short periods (e.g., Heard et al., 2004a, 2007; Juniper et al., 2006, 2008a), dairy cow milk production was not affected by the amount of Se consumed. Although Harrison et al. (2006) reported an increase in milk protein when using Se yeast, it is principally when Se supplements are fed to Se-deficient cows that milk production responses can be expected. A blood Se concentration greater than 21 µg/kg, which is associated with a milk Se concentration of 4 µg/kg (Grace et al., 2001), is generally considered to be indicative of adequate Se in lactating dairy cows (Wich-

tel et al., 1994; Thompson et al., 1998; Grace et al., 2001). Before the commencement of the present study, the mean milk and blood Se concentrations were 15 and 117 µg/kg, respectively.

Differences existed in milk production associated with pasture allowance, amount of concentrates fed, and type of basal diet. The responses to pasture allowance and concentrate feeding seen in this study were typical of those recorded previously under irrigation in the northern Victorian region (Wales et al., 1999, 2006). The difference in production between the cows well fed on pasture plus concentrates and the cows offered TMR is also similar to that reported by Kolver and Muller (1998). Thus, the production responses of the cows in this study were not different from expectations.

Supranutritional quantities of Se for the prevention of human health conditions associated with reduced immunity may be 3 to 4 times higher than daily recommended intakes (150–200 µg of Se/d; McIntosh et al., 2008). To consume such quantities of Se, foods or ingredients must have high Se concentrations. Fish and brazil nuts are recognized as naturally high-Se foods, and McIntosh et al. (2008) have recently reported that a milk protein concentrate product, Tatura-Bio Se (Tatura Milk Industries, Tatura, Victoria, Australia), is a dairy source with high bioavailability and bioefficacy for disease prevention (e.g., for colorectal cancer; Hu et al., 2008). To provide the milk to manufacture the milk protein product, large amounts of Se must be fed to cows, and research has shown that the transfer of Se into milk is greater from organic sources of Se than from inorganic sources (Weiss, 2003; Ceballos et al., 2009). Selenium yeast is one such organic source, and Hu et al. (2008) have demonstrated that the efficacy of the milk protein concentrate for the prevention of colorectal cancer is much greater than that of the parent Se yeast. The quantities of supplemental Se needed to be fed to cows to provide the concentrations of milk Se required for such dairy products (e.g., in excess of 50 mg/d) are not universally permitted (e.g., in the US, the FDA regulations limit Se supplementation of dairy cows to 0.3 mg/kg of DM), but some regions have daily recommendations rather than legislation against specific amounts. Therefore, these countries have the opportunity to take advantage of potential increases in consumer demand for high-Se milk protein products for the enhancement of human health, and the results presented here would help in developing the on-farm strategies to significantly increase milk Se concentrations to meet product requirements.

CONCLUSIONS

Selenium intake was a very good predictor of Se concentration in milk in this instance, and the diet of the

cows, or their level of milk production, did not seem to influence the Se intake/Se concentration relationship. Although milk Se concentration changed rapidly in response to changes in the amount of Se consumed, blood Se concentrations were much slower to react to both the inclusion and the exclusion of Se-enriched pellets in the diet of the cows, and after cessation of Se supplementation, this helped to maintain milk Se at higher concentrations than might otherwise be encountered. The apparent lack of effect of diet on milk Se concentration suggests that feeding systems can be developed in which milk is produced with predictably consistent Se concentrations to enable processing into Se-rich products if the demand emerges for human health products.

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