

# Selenium biofortified alfalfa hay fed in low quantities improves selenium status and glutathione peroxidase activity in transition dairy cows and their calves

## Research Article

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### Abstract

The hypothesis of the study was that feeding a relatively low amount of Se biofortified alfalfa hay during the dry period and early lactation would improve selenium status and glutathione peroxidase activity in dairy cows and their calves. Ten Jersey and 8 Holstein primiparous dairy cows were supplemented with Se biofortified (TRT;  $n = 9$ ) or non-biofortified (CTR;  $n = 9$ ) alfalfa hay at a rate of 1 kg/100 kg of BW mixed with the TMR from 40 d prior parturition to 2 weeks post-partum. Se concentration in whole blood, liver, milk, and colostrum, the transfer of Se to calves, and the glutathione peroxidase (GPx) activity were assessed. TRT had 2-fold larger ( $P < 0.05$ ) Se in blood *v.* CTR that resulted in larger Se in liver and colostrum but not milk and larger GPx activity in plasma and erythrocytes but not in milk. Compared to CTR, calves from TRT had larger Se in blood but only a numerical ( $P = 0.09$ ) larger GPx activity in plasma. A positive correlation was detected between Se in the blood and GPx activity in erythrocytes and plasma in cows. Our results demonstrated that feeding pregnant primiparous dairy cows with a relatively low amount of Se-biofortified alfalfa hay is an effective way to increase Se in the blood and liver, leading to greater antioxidant activity *via* GPx. The same treatment was effective in improving Se concentration in calves but had a modest effect on their GPx activity. Feeding Se biofortified hay increased Se concentration in colostrum but not in milk.

High producing dairy cows typically experience heightened oxidative stress during the early post-partum period (Abuelo *et al.*, 2015) as a consequence of larger production of free radicals, generally as consequence of aerobic metabolism (Mehdi *et al.*, 2013). Free radicals are also produced from immune cells, such as macrophages and neutrophils, to kill phagocytized bacteria (Sordillo, 2013). Oxidative stress is associated with increased incidences of diseases (Sordillo and Mavangira, 2014), therefore, it is imperative to improve the anti-oxidative status of dairy cows.

Selenium (Se) is an integral component of selenoenzymes, such as glutathione peroxidase (GPx) which play a significant role in the removal and detoxification of free radicals (Zigo *et al.*, 2014). Thus, supplementation of Se during the peripartum can be an effective means to decrease oxidative stress (Gong and Xiao, 2018). As an added benefit, Se supplementation also improves the immune system of the cows (Sordillo and Mavangira, 2014) and the amount of immunoglobulin in the colostrum, benefitting the calves (Hall *et al.*, 2014a).

Due to its toxic properties when supplemented in excess, Se is the only micronutrient regulated as a feed additive in the USA by the FDA, setting the limit of Se supplementation in cattle to 0.3 mg/kg DM (NRC, 2001). Se can be supplemented as an organic source, such as selenized yeast, or inorganic sources, such as selenite or selenate (Weiss, 2005). Organic Se has a larger bioavailability (>50%) compared with inorganic Se (45%) when fed to dairy cows (Séboussi *et al.*, 2016). Animals supplemented with organic Se, such as hydroxy-selenomethionine and selenized yeast, have a larger concentration of Se in whole blood and milk, and larger antioxidant capabilities, including larger blood GPx activity compared to cows supplemented with inorganic Se (Séboussi *et al.*, 2016; Sun *et al.*, 2018).

Biofortification of forages with Se (through fertilization of the soil with inorganic Se that is then absorbed and accumulated into the plant) is an effective approach to improve Se in the diet of cattle (Hall *et al.*, 2011; 2013; Novoselec *et al.*, 2018). Feeding Se biofortified forages successfully improves the Se status of pregnant beef cows and improves the concentration of immunoglobulins in colostrum and the Se status and performance of their calves (Hall *et al.*, 2013; Ranches *et al.*, 2017; Wallace *et al.*, 2017). These studies were performed in beef animals and, except for one study (Ranches *et al.*, 2017), the researchers fed a relatively large amount of Se biofortified forages (up to 2.5% of BW). Unlike beef cattle, non-grazing

high producing dairy cows require a more specialized ration with, in general,  $\leq 60\%$  forages. Furthermore, because of the need to balance the requirements of these animals, several types of forages are usually used in the formulation of the ration. Therefore, it is essential to demonstrate the effectiveness of using a relatively low amount of agronomically Se biofortified forage in the ration of high-producing dairy cows. In a prior study, a ration containing 25% DM of Se biofortified alfalfa/timothy silage improved the Se status of mid-lactation dairy cows (Séboussi *et al.*, 2016). However, an adequate amount of Se is even more crucial for peripartum cows, considering the high oxidative stress and the need to boost the immune status. Furthermore, it is also important to boost the immune status of the calves.

The objectives of the present study were to evaluate the effects of feeding 1% BW of Se biofortified alfalfa to dairy cows during the peripartum period on (1) Se concentration in whole blood, liver, milk, and colostrum; (2) transfer of Se to calves; (3) and the antioxidant status of cows and calves *via* measurement of GPx activity. We hypothesized that supplementing dairy cows with a relatively low amount of Se biofortified hay during the dry period and early lactation would improve the Se and antioxidant status in cows and their calves.

## Materials and methods

### Animals, ration, and experimental design

Experimental procedures used in this study were approved by the Institutional Animal Care and Use Committees (IACUC) of Oregon State University (protocol# 4894).

Details of the animals, ration, and experimental design are available in the online Supplementary File. Briefly, ten Jersey and eight Holstein pregnant heifers from the Oregon State University Dairy Center were enrolled in the study. Around 45 d before expected parturition, the animals were moved into a pen equipped with Calan gates (American Calan, Northwood, NH). Cows were randomly assigned to two groups blocked by breed and expected time of calving. Starting from 40 d ( $38 \pm 5$ ) before expected calving, the cows received either (TRT; 5 Jerseys and 4 Holsteins) 1 kg DM/100 kg BW of Se biofortified alfalfa hay (3.25 mg Se/kg DM) or (CTR; 5 Jerseys and 4 Holsteins) 1 kg of control hay (0.43 mg Se/kg DM) mixed into the TMR. Chemical analysis of the hays and TMR is reported in online Supplementary Tables S1 and S2. All the cows received the supplemental hay until 14 d in milk (DIM). Animals were milked twice a day at 0500 and 1800 h and milk yield was recorded by the Afilab system (Afimilk, Israel). Dry matter intake was monitored daily and BW weekly.

All calves born in calving pen from the cows enrolled in the experiment received 3 l of colostrum from their own mothers within 6 h after birth and 2 l of colostrum for their second feeding 12 h after birth. From day 2 to weaning all calves were fed raw cow milk at 7 AM collected from the bulk tank plus ad libitum access to a starter concentrate and water. Calves were kept in individual Calf-Tel Pen System Calf hutches (122 cm W  $\times$  182 cm L  $\times$  114 cm H) bedded with wheat straw for the entire experimental period.

### Blood, liver and milk samples and measurement of GPx and microminerals

Blood samples were collected from cows at  $-40$  ( $39 \pm 5$ ),  $-20 \pm 3$ ,  $-10 \pm 3$ , 14 DIM and from calves at 2 and 24 d after birth to

measure GPx activity and Se and other trace minerals. Liver biopsies were performed at 40 ( $39 \pm 5$ ) and 10 ( $\pm 3$ ) days before expected parturition and at 10 DIM. Colostrum and milk samples were collected at 1, 3, 7, and 14 DIM. Analysis of trace minerals was performed at the Keck Collaboratory for Plasma Spectrometry at Oregon State University. GPx was measured using a commercial kit. See online Supplementary File for details.

### Selenium balance and % transfer into calves and Se excretion into milk

The balance of Se was estimated using the calculated amount of Se fed *via* TMR and hay corrected by the residuals and two estimated requirements: (1) NRC recommendation (i.e., 0.3 mg of Se/kg DM) or (2) losses (as % of Se intake) from a prior experiment (Séboussi *et al.*, 2016) plus the calculated Se excretion in milk by using the Se concentration measured in milk  $\times$  daily milk yield, and the accumulation of Se in the fetus as suggested by NRC (0.055 mg/d) (NRC, 2001). The % of Se transferred to calves was calculated as (calf ng Se  $\times$  blood volume)/(cow ng Se  $\times$  blood volume) as performed for immunoglobulin transfer in a prior work (Hall *et al.*, 2014a).

### Statistical analysis

Outlier data were checked using PROC REG of SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) prior to statistical analysis. Data with a studentized  $t > 3.0$  were removed. Data were analyzed by GLIMMIX of SAS with the fixed effect of treatment, breed, time and their interactions with the cow (or calf) as random effect using the Spatial Power covariate model with homogeneous variance. PROC CORR was used for correlation analysis among the variables. Significance was declared with a  $P$ -value  $\leq 0.05$ .

## Results

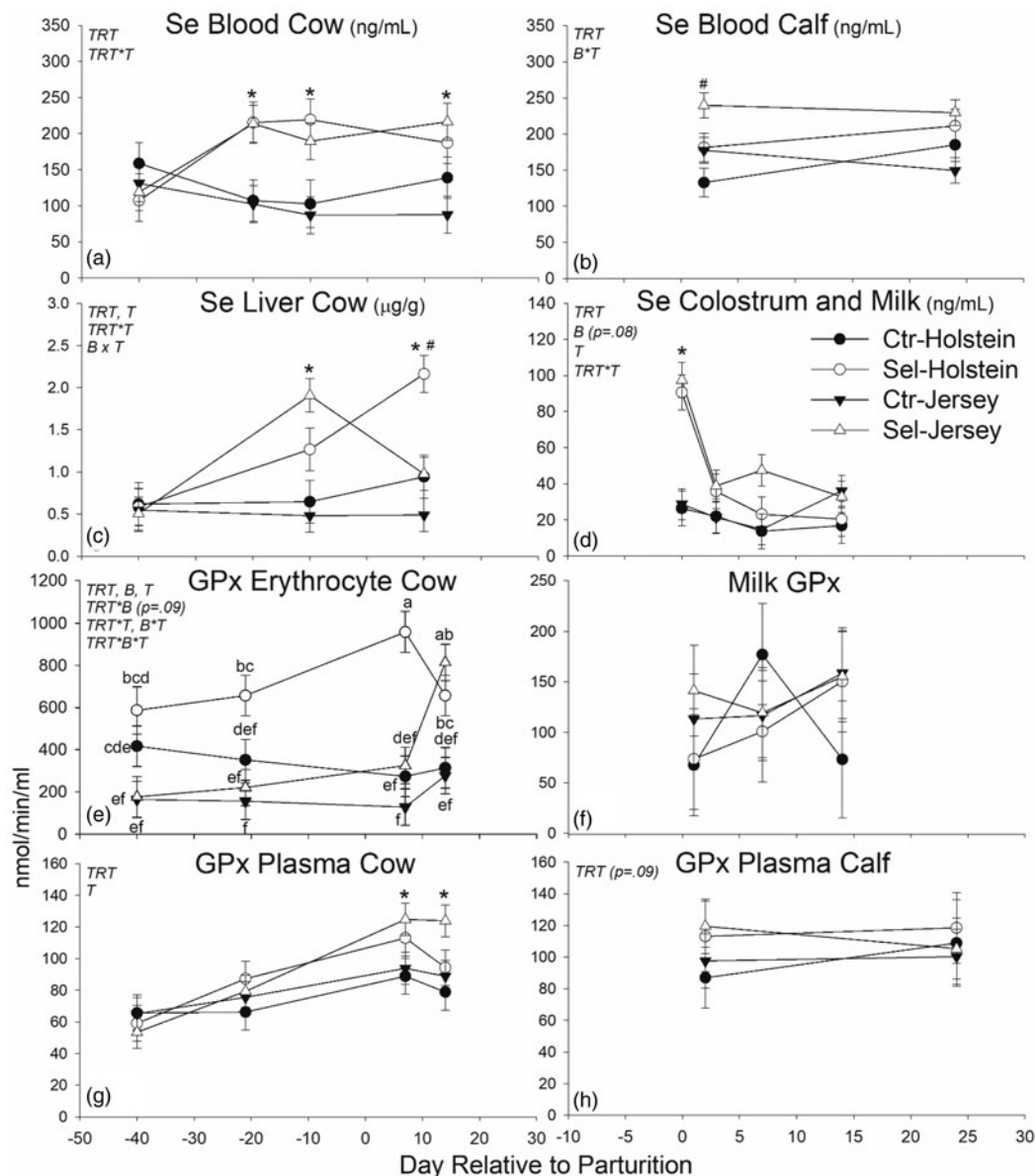
### Selenium balance

Results are presented in online Supplementary Table S3. Cows in our study received between 33 and 37% of Se-biofortified or control alfalfa hay in their diet (as DM). The combination of TMR plus alfalfa hay provided between 3.3 and 3.7 mg Se/d in CTR and 14 and 17 mg Se/d in TRT during the dry period and between 8.6 and 10.4 mg Se/d in CTR and 19 and 25 mg Se/d in TRT. Based on the calculated requirements, following the NRC recommendation (NRC, 2001) or losses estimated using a previous work (Séboussi *et al.*, 2016), the Se balance was positive in all cases. A larger positive Se balance was detected in TRT *v.* CTR. The Se balance was more positive when using NRC losses *v.* the ones from Séboussi *et al.* (2016) due to larger estimated losses in the latter (online Supplementary Table S4).

### Concentration of Se in blood, liver, and milk

The treated group had a significant increase in Se concentration in blood *v.* CTR reaching a plateau after 20 d of supplementation with not differences between breeds (Fig. 1a). In CTR the concentration of Se in blood tended to decrease during the first 20 d of the experiment (from 144.9 at  $-40$  DIM to 95.0 ng/ml at  $-10$  DIM;  $P = 0.08$ ).

Calves born from TRT had an increase in the Se concentration in plasma, reaching 215.5 ng/ml at 2-d post-birth. The



**Fig. 1.** The concentration of Se in whole blood, liver, and milk and glutathione peroxidase (GPx) activity in erythrocytes, blood plasma, and milk in Jersey (Jer;  $n=5/\text{group}$ ) and Holstein (Hol;  $n=4/\text{group}$ ) cows supplemented with 1% BW of Se biofortified alfalfa hay (Sel) or no biofortified alfalfa hay (Ctr) from 40 d prior expected parturition to 14 d after calving. Reported are also the concentration of Se in whole blood and GPx activity in plasma of their calves during the first 24 d after birth. Significant ( $P < 0.05$ ) effects of Selenium (TRT), breed (B), and time (T) and their interactions are indicated in the graph. \*Denotes difference between treatments ( $P \leq 0.05$ ) when TRT  $\times$  Time  $P < 0.05$ ; #Denotes difference between breeds ( $P \leq 0.05$ ) when Breed  $\times$  Time  $P < 0.05$ .

concentration of Se in plasma remained larger when compared to calves born from CTR (161.2 ng/ml) up to 24 d post-birth (Fig. 1b). Overall, Jersey calves had greater concentration of Se in blood during early life in comparison to Holstein calves. However, the difference disappeared at 24 d of age due to an increase of Se in Holstein calves (Fig. 1b).

The concentration of Se in the liver of cows increased in TRT from  $-40$  to  $-10$  DIM reaching  $>2$ -fold larger concentration in TRT v. CTR (1.56 v. 0.56  $\mu\text{g/g}$ ; Fig. 1c and Supplementary Table 5). The increase of Se in liver was faster in Jersey compared to Holstein cows reaching the plateau at  $-10$  DIM, with a decline afterward. In comparison, the increase was continuous until the end of the trial for Holstein cows (Fig. 1c).

Se in colostrum was  $>3$ -fold larger in TRT than in CTR but differences disappeared quickly during the first week of lactation,

and no differences were observed in milk at 14 DIM (Fig. 1d and online Supplementary Table S5). There was also a numerically larger Se in milk (39.7 v. 31.2 ng/ml;  $P = 0.06$ ) in Jersey compared to Holstein cows. The efficiency in transferring Se into the calf was lower in TRT v. CTR (6.1 v. 8.5%; online Supplementary Table S5).

#### Other microminerals

Data are available in the online Supplementary Table S5. The concentration of Co, Cu, and Zn in whole blood of the cows, calves, and milk (both as colostrum and mature milk) was not affected by the supplementation of Se biofortified hay. There was a larger Zn concentration in the blood of Holsteins relative to Jerseys in both cows and calves. Holsteins cows had greater Co in liver while

Holstein calves had greater Co in blood compared to Jerseys. In the liver, the concentration of Co was lower in TRT *v.* CTR only in Holstein cows (online Supplementary Table S5).

### Glutathione peroxidase activity in erythrocyte, plasma, colostrum and milk

The GPx activity in erythrocytes was >2-fold larger in Holstein than Jersey cows (Fig. 1e). TRT had larger GPx activity in erythrocytes *v.* CTR (549.2 *v.* 260.0 nM/min/ml) with a more rapid response in Holsteins *v.* Jerseys reaching a peak at 7 DIM but with a decrease afterward. Jersey cows had a rapid increase in GPx activity in erythrocytes from 7 to 14 DIM (Fig. 1e). GPx activity in milk was not affected by feeding Se biofortified alfalfa or the breed of the cows (Fig. 1f). Compared to CTR, TRT had larger GPx activity in plasma (77.9 *v.* 92.8 nM/min/ml), which was more apparent during lactation, especially due to larger values in Jersey *v.* Holstein cows (Fig. 1g). The activity of GPx in the plasma of the calves from TRT cows was numerically larger compared with calves from CTR cows (114.1 *v.* 98.4 nM/min/ml;  $P = 0.09$ ; Fig. 1h).

### Correlations

A positive correlation was detected between Se concentration in blood and GPx activity in the erythrocytes of the cows (Table 1). Se in blood was also positively associated with the concentration of Se in the liver but there was no statistical association with milk or colostrum ( $P = 0.06$ ). Se concentration in colostrum and mature milk was positively associated with the concentration of Se and GPx activity of the calves' plasma but was not associated with the activity of GPx in colostrum and mature milk. There were no associations between the Se in the blood of cows and the Se concentration in calves, however, a positive association was detected between the concentration of Se in the liver of cows and the concentration of Se in the blood of calves.

### Discussion

#### Feeding the cows with one % BW of Se biofortified alfalfa hay improves the Se status

The cows enrolled in our experiment had an adequate concentration of Se just before starting the trial ( $128.6 \pm 46.9$  ng/ml; mean  $\pm$  SD), with only two cows (one Holstein and one Jersey) with a concentration of Se <100 ng/ml, considered to be marginal (Dargatz and Ross, 1996). During the trial, the control animals did not receive any Se supplementation. This can explain the marginal decrease in Se in blood during the first 40 d of the trial, where cows in the CTR had a concentration of Se of  $93.0 \pm 19.4$  ng/ml, with 7 out of 9 cows with concentrations <100 ng/ml. Thus, control animals in the close-up group can be considered marginally deficient for Se despite having a calculated positive Se balance. This observation indicates that classical means to calculate Se requirements are inadequate. In our case, we did not measure real Se losses, but we used previously published data (Séboussi *et al.*, 2016) to provide a more realistic estimate of the Se requirement. Even with that approach, we failed to capture the real requirement of Se.

The supplementation with 1% BW of Se biofortified alfalfa hay was sufficient to double the concentration of Se in whole blood with a relatively short period of supplementation (20 d),

reaching a plateau in whole blood considered high adequate ( $214.3 \pm 48.0$  ng/ml) (Dargatz and Ross, 1996). We estimated that dry and lactating cows in TRT group received between 14 and 17 mg and between 19 and 25 mg of Se/d, respectively.

Pregnant beef cows receiving 28 mg of Se/d *via* Se biofortified hay had a linear increase in the amount of Se in whole blood reaching approximately 250 ng/ml after four weeks and >300 ng/ml after 10 weeks of feeding (Wallace *et al.*, 2017). In our case, we did not observe a linear increase, rather, the cows reached a plateau of Se in blood in 3 weeks with values slightly lower than those detected in beef cows after 4 weeks of treatment. Similar to findings in beef cows, Jersey cows receiving 15 mg/d of Se-yeast for 8 weeks prior to parturition had 1.6-fold increase of Se in whole blood compared to control cows on the day of calving, reaching >380 ng/ml (Hall *et al.*, 2014b). In a study conducted in Holstein cows (Ran *et al.*, 2010), the supplementation of 5 mg/d of Se-yeast allowed to increase the concentration of Se reaching a relatively high concentration after three months of supplementation ( $179.8 \pm 10.9$  in whole blood) with a numerical increase following up to 4 months of supplementation ( $187.8 \pm 8.4$  ng/ml). In another study, lactating multiparous Holstein cows received a ration with 0.15 or 0.4 mg of Se-yeast/kg of DM for five weeks. All cows, including cows not receiving Se supplementation, had >200 ng of Se/ml in whole blood. The authors detected a linear increase of blood Se due to doses of Se in the ration (Juniper *et al.*, 2011). In that experiment, cows receiving 0.4 mg of Se/kg of DM had >250 ng/ml of Se in whole blood. In our experiment, control cows received around 0.8 mg of Se/kg of DM while treated cows received approx. 1.7 mg of Se/kg DM. Despite this, the concentration of Se in all our animals was <200 ng/ml. In a more recent study, Holstein cows in late lactation fed a ration containing up to 25% of alfalfa/timothy silage with 1.97 mg of Se/Kg of DM for 43 d (total of 17.3 mg of Se/d) reached a concentration of Se in blood similar to our study (Séboussi *et al.*, 2016). However, in our study, Holstein cows during early postpartum received an average of 24.7 mg of Se/d, 42.7% more Se compared to cows in that study.

Se concentrations in the liver can range between 1.2 and 2.0  $\mu$ g/g of dry weight for all species regardless of age (Stowe and Herdt, 1992). In our experiment, TRT had a substantial increase in the concentration of Se concentration in the liver, reaching >1.2  $\mu$ g/g after 30 d of Se supplementation compared to CTR (0.6  $\mu$ g/g). Our data are somewhat similar to a prior study where beef cows treated with 0.5 mg/kg DM of Se-yeast had larger Se in the liver (1.6  $\mu$ g/g) compared to non-supplemented cows (0.64  $\mu$ g/g) or cows supplemented with sodium selenite (0.54  $\mu$ g/g) for a period 112 d (Davis *et al.*, 2008).

None of the cows in our study had health issues despite being fed a significant amount of organic Se. This observation supports prior data indicating that supplementing dairy cows with organic Se is effective and may have less potential for toxicity (Séboussi *et al.*, 2016).

A synergism in the absorption of Se and other trace minerals has been known for a long time (O'Dell, 1985) but also some interference in intestinal absorption exists (Van Campen, 2018). Our data did not indicate any synergism or interference in intestinal absorption of measured trace minerals.

Overall, the above data indicated some marginal differences of our data compared to prior data, likely due to differences in age of animals (primiparous *v.* multiparous), baseline Se status, and/or feeding method. However, our data confirmed the efficacy for using Se biofortify hay to improve Se status in cows.

**Table 1.** Correlation coefficient between whole blood, plasma, liver, and milk concentrations of Se and GPx activity of Jersey and Holstein primiparous cows supplemented with 1% BW of Se-biofortified alfalfa hay or control alfalfa hay from 40 d prior expected parturition to 14 d after calving

	Cows					Calves plasma	
	Se		GPx			GPx Plasma	Se Plasma
	Liver	Milk	Erythrocytes	Plasma	Colostrum + Milk		
Se Cow Blood	<b>0.32<sup>a</sup></b>	0.33 <sup>b</sup>	<b>0.40</b>	0.27	0.01	0.27	0.16
P-value	0.03	0.06	<0.01	0.06	0.98	0.31	0.55
Se Cow Liver		0.34	<b>0.43</b>	<b>0.60</b>	-0.08	0.35	<b>0.52</b>
P-value		0.06	0.02	<0.01	0.65	0.20	0.04
Se Colostrum + Milk			-0.03	0.11	-0.08	<b>0.48</b>	<b>0.61</b>
P-value			0.86	0.55	0.57	0.05	0.01
Erythrocytes Cow				<b>0.31</b>	0.06		
P-value				0.01	0.72		
Plasma Cow GPx					-0.12		
P-value					0.51		
Milk Cow GPx						0.14	0.44
P-value						0.61	0.07
Calves Plasma GPx							0.08
P-value							0.66

Reported are also the correlation coefficient in plasma of their offspring during the first 24 d after birth.

<sup>a</sup>Bold and italic value = significant effect.

<sup>b</sup>Italic value = numerical non-statistical difference (0.1 > P > 0.05).

### Selenium is efficiently transferred to colostrum but not milk

Using Se biofortification can be a good strategy to increase dietary Se concentration in livestock products, including milk (Lyons *et al.*, 2003). Se concentrations in milk have been indicated as a means to assess the Se status in cows (Wichtel *et al.*, 2004). According to the reference provided (Wichtel *et al.*, 2004), the concentration of Se in the colostrum in our cows was more than adequate while the Se in milk would be considered marginal, even those in TRT group. Thus, our data indicated a high transfer of Se in colostrum but a poor transfer of Se in milk.

Larger transfer of Se in colostrum relative to milk was also observed in beef (Slavik *et al.*, 2008; Ranches *et al.*, 2017) and dairy cows (Salman *et al.*, 2013). The high Se concentration in colostrum compared to milk is likely due to high amounts of selenoproteins, as observed in humans, where Se concentration decreases as lactation progresses (Dorea, 2002). It is also possible that the higher Se in colostrum compared to milk was due to the paracellular transfer of Se through leaky tight junctions, which is typical of the colostrum phase in mammals, including dairy cows (Kessler *et al.*, 2019). The tight junctions closure happens quickly at lactogenesis and it is essential to obtain mature milk (Stelwagen and Singh, 2014).

The lack of difference in Se content of milk between TRT and CTR in our study differs from prior reports (Juniper *et al.*, 2006; Ran *et al.*, 2010; Séboussi *et al.*, 2016). Those studies were conducted in mid and late lactating cows whereas our study evaluated early lactating cows. Our data are similar to other studies where a lack of Se enrichment in milk was detected in early post-partum dairy cows supplemented with Se (Salman *et al.*, 2013) or mid-

lactation cows with doses equal or below NRC recommendations (Ivancic and Weiss, 2001).

Se supplementation is associated with an increased amount of Se in various tissues, but, as observed previously in human, does not appear to be highly associated with Se in milk (Bianchi *et al.*, 1999). In goats, the Se concentration in milk increases from early to late lactation (Rozenká *et al.*, 2013). The reason for the larger content of Se in milk of late *v.* early lactating goats, the enrichment of Se in milk of mid or late lactating cows fed larger concentration of Se, and the lack of Se enrichment in milk in our study, may be explained by a larger expression of the main Se-methionine transporters (i.e., *SLC3A1*) in mammary glands of late *v.* early lactating animals, as observed in pig (Chen *et al.*, 2018). No data exist of the expression of *SLC3A1* in mammary tissue in bovine through lactation.

### Transfer of Se in calves

In ruminants, the transfer of nutrients across the placenta or the mammary gland into the fetuses and newborn is very important for proper development and growth (Abdelrahman and Kincaid, 1995). The transfer of Se is known to be more efficient *via* the placenta than through the milk (Dargatz and Ross, 1996). In our experiment, the calves received colostrum from their mother, thus, the Se concentration in blood of calves just after birth can be considered an accumulated transfer from placenta and colostrum. However, the lack of association between the concentration of Se in the blood of cows and calves and the high association of Se in calves' blood with the concentration of Se in colostrum indicate a more important role of Se transfer *via* the colostrum compared to the placenta.

### Glutathione peroxidase activity

Glutathione peroxidase activity is known to decrease around parturition with the nadir at calving (Cigliano *et al.*, 2014; Gong and Xiao, 2016). The pattern of GPx activity in CTR in our experiment is consistent with such pattern. However, in TRT the concentration of GPx activity increased avoiding the decrease at calving, consistent with prior findings (Ran *et al.*, 2010; Hall *et al.*, 2014b). Thus, our data support an improvement of the anti-oxidative capacity of cows supplemented with Se biofortified hay.

Our study indicates that improving the Se concentration in cows improves the Se in plasma of their calves early in life but only marginally (if at all) augments the GPx activity measured in calves' plasma. A prior study detected a positive effect of erythrocytes GPx and blood Se in calves, however, the effect disappeared after the colostrum phase (Rowntree *et al.*, 2004). Thus, our data do not allow us to conclude that feeding Se biofortified hay to cows benefits the anti-oxidative status of their calves. However, different from the prior study (Rowntree *et al.*, 2004), we only measured GPx activity in plasma of calves.

Our study presents several limitations. It is well-known that multiparous dairy cows are more subjected to oxidative stress compared to primiparous cows (Bühler *et al.*, 2018). Findings from our study should be confirmed in multiparous dairy cows. However, our study was designed to determine the feasibility of feeding a relatively low amount of Se biofortified hay using a low number of animals. The use of primiparous cows was a safeguard to minimize diseases that could have compromised the study. The use of two breeds, although providing a window of possible differences in response to Se, especially GPx activity, has reduced the statistical power, especially considering the low number of animals used. The lack of whole blood GPx activity in calves also has limited our ability to make clear conclusions about the anti-oxidative status of dairy calves. Measurements of other anti-oxidative parameters on the plasma could help overcome this limitation.

In conclusion, and despite the above limitations, our data demonstrate that supplementing a relatively low amount of Se biofortified alfalfa hay to peripartum dairy cows is an effective method to increase Se concentration in cows and their calves, improving the anti-oxidative status of the cows. Colostrum is an effective means to transfer selenium to calves, but early postpartum cows have a very poor transfer of Se into milk. Our study revealed some differences in the response to Se biofortified hay between Jersey and Holstein cows that deserve further investigations.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S002202992000028X>.

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