Development and application of a selenium speciation method in cattle feed and beef samples using HPLC-ICP-MS: evaluating the selenium metabolic process in cattle

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Selenium (Se) is an essential element for mammals with diet being the major source of intake. For this work cattle feed was enriched with combinations of selenium enriched yeast, canola oil, and/or vitamin E in order to evaluate the accumulation and metabolism of selenium in beef cattle. A method to identify and/or quantify the selenium species: selenocystine (SeCys₂), selenomethionine (SeMet), selenomethionine-Se-oxide (SeOMet), and inorganic selenium species, selenate (Se(VI)) and selenite (Se(IV)), was developed and applied to cattle feed and beef samples.

Introduction

Selenium is a trace element in mammalian nutrition and its beneficial properties have been well discussed and reviewed in the scientific literature for over 50 years. For cattle, in particular, selenium has been shown to increase glutathione peroxidase activity and α-tocopherol colostrum levels which may be beneficial to calf health. Evidence supports the fact that selenium deficiencies can lead to several health risks including, among others, decreased immune function. On the other hand high levels of selenium in livestock can lead to selenium toxicity (selenosis) which includes symptoms such as hair loss, decreased appetite, respiratory complications and, in extreme cases, death. Selenium typically enters the food chain as it is first absorbed by plants from the soil and then is incorporated by mammals ingesting said plants and so on; eventually leading to its beneficial effects. One prevalent issue regarding selenium is its non-homogeneous distribution throughout the Earth’s crust leading to very high or low concentrations of selenium according to the geography, thus supplementation has been used to compensate the deficiency in some countries. The narrow range between the essentiality and toxicity of selenium increases the need for appropriate intake. The recommended dietary intake for humans per day is approximately 55 µg and the tolerable upper intake level is ca. 200 µg depending on gender and age.

Canola oil is an edible oil, from different varieties of rape-seed plants, that contains low levels of saturated fatty acids and does not harm humans and livestock. Adding canola oil into cattle feed is expected to increase the unsaturated fat and decrease the saturated fatty acid concentration in beef, thus increasing the beef quality in overall; however, to our knowledge its relationship and interaction with selenium have not been studied. Vitamin E is an antioxidant that prevents lipid oxidation. The combination of selenium and vitamin E in animal feed is associated with the enhanced animal nutrition quality, increased “shelf-life” of beef, and eventually increased antioxidant concentrations in the final consumer’s (human’s) diet.

Determination of total selenium is frequently performed using inductively coupled plasma mass spectrometry (ICP-MS), and a small number of reports have applied this technique to animal feed and beef. The analysis of individual selenium species (speciation) is more complex and well-reviewed by B’Hymer and co-workers. Size exclusion chromatography (SEC) with ICP-MS has been used to analyse distributions of selenium related proteins in foodstuffs and yeast, but to a lesser extent in animal feed and beef. Reports of selenium speciation in animal feed and cattle beef are relatively limited and
noted difficulties in extraction and subsequent identification of selenium species. A popular approach to analyse selenium related proteins is to cleave the proteins by various mechanisms while forming related “seleno-amino acids”. This is commonly achieved using enzyme mixtures or “cocktails”. To separate the resulting seleno-amino acids and other selenium compounds, several chromatographic methods have been coupled to ICP-MS. The most widely utilized methods are ion pairing and ion exchange. Ion pairing (negatively charged ion pairs are most commonly reported in the literature) using a reversed phase column performs especially well for the organic forms of selenium, but often suffers from low retention and resolution of inorganic selenium and selenocysteine from each other and the void volume of the column. Anion exchange is commonly used to address resolution issues of inorganic selenium compounds; however, difficulty arises when resolving the organic selenium compounds using an isocratic separation.

For this work, forty eight Nellore steers were submitted to four different diets (twelve animals per group) for 82 days: control (unfortified animal feed); canola oil (unfortified animal feed with 3% of canola oil in dry matter (DM)); antioxidants (animal feed fortified with 2.5 mg of the as-selenized yeast (Se-yeast) per kg of DM and 1000 IU of vitamin E per day); canola oil and antioxidants (animal feed with 3% of canola in DM fortified with 2.5 mg of Se-yeast per kg of DM and 1000 IU of vitamin E per day).

The primary goal of this project was to develop a method to extract selenium species from the matrices of interest while optimizing a sensitive and selective selenium speciation method to analyse the relevant selenium compounds. The secondary goal was to utilize the developed method to evaluate the selenium metabolites of the cattle as affected by the canola oil, Se-yeast, and vitamin E from the various diets.

Experimental

Materials

All the solutions and dilutions were made with distilled deionized water (DDIW) (Q-Pod, Millipore). For total selenium analysis, samples were digested using trace metal grade nitric acid and 30% (v/v) hydrogen peroxide (both from Fisher Scientific). All the calibration curves were prepared daily with 1. A Se-yeast reference material was used to evaluate the accuracy of the digestion and extraction methods (SELM-1, National Research Council of Canada [NRC]). For size exclusion chromatography (SEC) analysis, tris(hydroxymethyl)aminomethane 99+% (Acros Organics, USA), hydrochloric acid (Fisher Scientific, Canada), sodium dodecyl sulfate (MP Biomedical LLC), and phenylmethylsulfonyl fluoride (Fluka) were used to prepare the extracting solution. The mobile phase was prepared with ammonium acetate (Fisher Scientific), and a saturated sodium hydroxide solution (Fisher Scientific) was used to adjust the solution pH. The column performance was evaluated by using a gel filtration standard set (Bio-Rad) consisting of Thyroglobulin 670 kDa, O-globulin 158 kDa, ovalbumin 44 kDa, myoglobin 17 kDa and vitamin B12 1.35 kDa. The extracts (of SEC and speciation analysis) were centrifuged in 2 mL polypropylene tubes containing 0.45 μm cellulose acetate non-sterile filters (Spin-X Centrifuge Tube Filter, Costar, USA). For selenium extraction for speciation analysis, an extracting solution was prepared with calcium chloride dihydrate, sodium phosphate monobasic dihydrate, and sodium citrate dihydrate; the pH was adjusted with a saturated sodium hydroxide solution (all reagents from Fisher Scientific). The enzymes: proteinase K (from _Trichococci album_), protease XIV (from _Streptomyces griseus_) and pancreatin (from porcine pancreas) were obtained from Sigma Aldrich. For speciation analysis, the mobile phase was prepared using ammonium acetate (Fisher Scientific), ammonium phosphate (Fisher Scientific), acetonitrile (ACN) (Fisher Scientific) and tetrabutylammonium hydroxide (1 M, TBAH) (Fluka). All RP-ICP-MS calibration curves and spikes were prepared using selenium standards: selenomethionine (SeMet, Acros Organics), selenocystine (SeCys2), selenite (Se(vi)), and selenate (Se(vi)) (Sigma Aldrich). High purity argon gas (99.999%) was used as plasma gas (Wright Brothers, USA) and ultra-high purity hydrogen gas (99.9999%) was used in the ORS cell (Matheson Tri-gas, USA).

Samples

Samples were provided for this experiment by Dr Marcus A. Zanetti from the University of São Paulo (Pirassununga, SP, Brazil). Beef samples were obtained from _longissimus dorsi_, an edible muscle of the cattle. A composite of equal portions of beef samples (n = 12) from all animals of each group (n = 4) was prepared. Feed and beef samples were previously freeze dried and cryogenically milled.

Instrumentation

All the samples were weighed in an analytical balance (Mettler Toledo, model XS204). Acid digestion was performed in a microwave equipped with a 40-position carousel in 100 mL PFA vessels (MarsXpress, CEM). The pH was adjusted with a pH meter (AB15, Fisher Scientific) previously calibrated with standard buffer solutions (Fisher Scientific). An ultra-sonic sonication probe was used in order to break cell structures at 60% maximum extrusion of 200 watts (Q-Sonica, Q125). The extracts were filtered to 0.45 μm via inactivated cellulose spin filters at 8000g for 5 minutes using a microcentrifuge (Fisher Scientific, model accuSpin™ Micro 17). For total selenium analysis an Agilent 8800 ICP-triple quadrupole MS (QQQ) with an autosampler was used (Agilent Technologies). A SEC column, TSK gel G4000SWx1 (Tosoh Bioscience, LLC), previously evaluated using a gel filtration standard (BioRad, USA) was used in a 1100 Series HPLC (Agilent Technologies) coupled to a UV-vis detector (Diode Array Detector, Agilent Technologies). For speciation analysis a Zorbax SB-C18 4.6 × 250 mm, 5 μm column (Agilent
Table 1  HPLC and ICP-MS conditions

| SEC analysis | 
| Column | TSK gel G4000SWxl 7.5 x 300 mm, 8 µm |
| Flow rate | 0.55 mL min⁻¹ |
| Eluent | 50 mM ammonium acetate pH 7.5 |
| Injection volume | 100 µL |
| Elution program | Isocratic |

| HPLC for speciation analysis (RP-ICP-MS) | 
| Column | Zorbax SB-C18 4.6 x 250 mm, 5 µm |
| Flow rate | 1.2 mL min⁻¹ |
| Eluent | 50 mM ammonium phosphate, 20 mM ammonium acetate, 1% (v/v) ACN, 1 mM TBAH at pH 6.5 |
| Injection volume | 20 µL |
| Elution program | Isocratic |

| ICP-MS | 
| RF power | 1600 W |
| Sampling depth | 8.3 mm |
| Carrier gas flow rate | 0.95 L min⁻¹ |
| Spray chamber temperature | 2 °C |
| Spray chamber type | Double-pass |
| Nebulizer type | Micromist |
| Nebulizer pump | 0.30 rps |
| Makeup/dilution gas | 0.11 L min⁻¹ |
| Cell gas | H₂ at 3.7 mL min⁻¹ |

Technologies) was used with an HPLC system (1100 Series, Agilent Technologies); an Agilent 8800 ICP-QQQ in MS/MS mode using H₂ as reaction gas was utilized for all analyses. The operating conditions are presented in Table 1. The software MassHunter was used to monitor the selenium signal at m/z = 78 and ChemStation software was used to monitor the UV signal at 280 nm.

Total analysis

Approximately 250 mg of each material was directly weighed into digestion vessels. Five mL of concentrated HNO₃ and 3 mL of DDIW were added to each flask. An additional 1 mL of H₂O₂ was added post sample cooling after the first microwave run. Replicates were done according to the following heating program: the first step was at 1600 W, 20 min ramp to 120 °C and 15 min hold; in the second step 1600 W was used with a 10 min ramp to 190 °C and 15 min hold. The digestion flasks were allowed to cool to room temperature and each sample replicate digest was diluted to fit in a linear curve ranging from 0.5 to 50 µg L⁻¹ with 2% HNO₃ (v/v). Germanium was added to each diluted sample at a final concentration of 5 µg L⁻¹ and evaluated as the internal standard. The analysis was carried out using an ICP-QQQ using H₂ as reaction gas.

Size-exclusion chromatography (SEC)

In an agate mortar and pestle, ~100 mg of sample and 2 mL of a 30 mM tris-hydroxymethylaminomethane-hydrochloric acid (Tris–HCl) (pH 7.5) solution containing 0.08% (w/v) sodium dodecyl sulphate (SDS) and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) were added and the mixture was agitated and macerated for 5 minutes. The extract was collected and filtered in a 0.45 µm spin filter at 4000 g for 5 min; the filtrates (referred to as “protein extracts” herein) were subjected to analysis using SEC-UV-ICP-MS. Duplicates of each sample were analysed to evaluate reproducibility. Possible proteins and protein standards were monitored using UV detection at a wavelength of 280 nm. Additionally enzymatic digests (described subsequently) were also analysed using SEC-UV-ICP-MS.

Speciation analysis

All reversed phase ion pairing (RP-IP) ICP-MS calibration standards and sample fortifications were prepared using selenium species standard solutions prepared daily with a mobile phase and ~1000 mg Se kg⁻¹ stock solutions previously prepared and frozen (~20 °C freezer). The standard selenomethionine-Se-oxide (SeOMet) was synthesized according to Krause et al. An extraction “buffer” solution providing optimal conditions (optimization discussion to follow) for the enzymatic digestion was prepared mixing 5 mM calcium chloride, 20 mM sodium phosphate and 50 mM sodium citrate at pH 7.5. In polypropylene tubes, 3 mL of buffer solution was added to ~100 mg of feed, ~50 mg of supplement and CRM, or ~200 mg of beef samples. Samples were mixed using an ultra-sonic probe for 1 min (1 s on/1 s off) at 80% amplitude. A solution containing ~3 mg of each enzyme previously dissolved in buffer solution was added to each sample. The solutions were incubated at 37 °C for 24 h at 150 rpm. The extract (referred to as “enzyme extract” herein) was filtered with a 0.45 µm filter for RP-IP-ICP-MS analysis or a 1.2 µm filter for total analysis (ICP-MS), the latter in order to evaluate the extraction efficiency. All samples were diluted 2 to 10 times with the mobile phase (if necessary) to maintain the pH 6.5 and remain within the calibration curve range (2 to 50 µg L⁻¹).

Table 2  Total selenium dry weight concentration (mg kg⁻¹) of feed and beef samples analysed by ICP-MS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average conc. (mg kg⁻¹) ± SD (n = 3)(RSD (%))</th>
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<tbody>
<tr>
<td>Feed (control)</td>
<td>0.25 ± 0.01 (4.0)</td>
</tr>
<tr>
<td>Feed + canola oil</td>
<td>0.28 ± 0.02 (7.1)</td>
</tr>
<tr>
<td>Feed + antioxidants⁴</td>
<td>5.31 ± 0.19 (3.6)</td>
</tr>
<tr>
<td>Feed + canola oil + antioxidants⁴</td>
<td>4.43 ± 0.20 (4.5)</td>
</tr>
<tr>
<td>Selenium supplement</td>
<td>963 ± 58 (5.9)</td>
</tr>
<tr>
<td>CRM SELM-1</td>
<td>1990 ± 41 (2.0)</td>
</tr>
<tr>
<td>Beef (control)</td>
<td>0.30 ± 0.01 (3.3)</td>
</tr>
<tr>
<td>Beef + canola oil</td>
<td>0.60 ± 0.01 (1.7)</td>
</tr>
<tr>
<td>Beef + antioxidants⁴</td>
<td>1.84 ± 0.12 (6.5)</td>
</tr>
<tr>
<td>Beef + canola oil + antioxidants⁴</td>
<td>2.62 ± 0.16 (6.1)</td>
</tr>
</tbody>
</table>

⁴ Antioxidants = Se-yeast + vitamin E.
Results and discussion

Total selenium determination

The results for total selenium content are summarized in Table 2. The accuracy was estimated by using the CRM of Se-yeast SELM-1, and was found to be 97%.

For reference, in the United States, the AAFCO (2011) guidelines recommend a 0.3 ppm (mg kg\(^{-1}\)) Se requirement in selenium supplemented feeds on a total diet basis.\(^2\) The results indicate that the feed and feed + canola oil contained ~0.25 mg Se kg\(^{-1}\) and the supplemented feeds contained ~5 mg Se kg\(^{-1}\). The results indicate that the selenium contents are similar to that recommended by AAFCO regulations, and the supplementation (addition of the “antioxidants” including Se-yeast) increased the selenium content of the feed by a factor of ~20.

SEC characterization

Analysis with SEC-UV-ICP-MS was performed on the protein extracts in order to characterize the samples. SEC analysis can infer how selenium is bonded within samples by approximating the molecular weight (hydrodynamic volume) range of the selenium complex. In Fig. 1, SEC-UV-ICP-MS chromatograms for \(m/z = 78\) and \(\lambda = 280\) nm of protein extracts of (a) feed and (b) beef sample (protein and enzymatic extractions, each at 280 nm) are shown.

Daun and co-workers performed SEC-ICP-MS analysis for different animal tissues, including cattle muscle and suggested that peaks between 2 and 200 kDa may represent selenoproteins (i.e. specifically encoded selenium) and or selenium containing proteins (non-specifically incorporated).\(^3\) Observing the chromatograms presented in Fig. 1, selenium is mostly bonded to medium molecular weight (MMW) proteins in these sample types. In order to identify the specific Se-amino acid forms an enzymatic hydrolysis was performed with feed and beef samples. In Fig. 1(b), SEC-UV-ICP-MS chromatograms obtained before and after the enzymatic extraction in order to evaluate the proteolysis efficiency are shown. A decrease of ~80% of the 280 nm absorbance at the MMW and HMW was observed, and was consistent within the studied samples.

Speciation analysis

During the extraction, it is important to assure the integrity of the Se-amino acids as they relate to the previously intact proteins by providing optimal conditions such as pH and temperature for the enzymes. For this work the optimization of the enzymatic extraction was evaluated by using a combination of the change in the molecular weight profile as monitored by the SEC-UV-ICP-MS analysis as well as by comparing the total Se concentration of the extracts of the feed and beef samples. Optimization of various extraction conditions was evaluated, including: pH, solvent concentration, enzyme combination, and time and temperature of incubation. In the literature, many studies have combined techniques in order to improve the selenium extraction efficiency. Capelo and coworkers proposed the use of an ultra-sonic probe to enhance the enzymatic hydrolysis (increase extraction) in yeast for selenium speciation studies;\(^4\) therefore this was added to the extraction method presented in this work as it was shown to further increase extraction efficiency. The best conditions were based on a modification of the method from Phipps and co-workers\(^5\) and were a mix of the enzymes protease type XIV, protease K and pancreatin in pH 7.5 incubated at 37 °C for 24 h in a buffer containing calcium chloride, sodium phosphate and ammonium citrate used in combination with an ultra-sonic probe.

For the procedure adopted, the CRM SELM-1 average extract total Se concentration determined by ICP-MS was 2124 ± 255 mg kg\(^{-1}\) \((n = 2)\) (RSD = 12%), which represents 103% accuracy. As expected, lower recoveries for feed and beef samples were exhibited with average extraction efficiencies of 60% and 66%, respectively.

Steps were then taken to develop and optimize a robust, fast, and selective separation method to separate the compounds surmised to be present in the animal feed and beef samples.

![Fig. 1](image-url) SEC-UV-ICP-MS chromatograms for \(m/z = 78\) and \(\lambda = 280\) nm of (a) feed and (b) beef sample (protein extract and enzyme extract).
Based on limited literature surveys, inorganic selenium (Se(IV) and Se(VI)), SeMet and SeOMet were determined to be the most appropriate. SeCys has been included in many other published methods, is readily available as a high purity commercial standard, and its reporting in the literature has been recently called into question. In order to minimize sensitivity fluctuations commonly associated with a gradient separation utilizing an organic solvent and subsequent carbon based enhancement, a longer run in an isocratic mode was adopted with the purpose to keep the robustness of the method.

As previously mentioned, selenium speciation is commonly carried out using RP-IP (utilizing an anionic pairing agent) or anion exchange, with their unique advantages for resolving organic and inorganic selenium species, respectively. This study attempted to combine these benefits into one separation method. Therefore a cationic ion pair was used with a reversed phase analytical column. Similar work had been performed by Afton and co-workers, but this application area required additional modifications and optimization. The influence of the side chain length of the ion paring agent was studied from methyl to octyl varieties. Enhanced selectivity was achieved with the butyl chain without long chromatographic times associated with the longer chains. Ionic strength was adjusted with ammonium acetate to modulate the elution power of the mobile phase. Additionally, an organic modifier was added at a low level so as to not affect the separation, but to provide enhanced Se sensitivity due to the “carbon effect”. Chromatograms showing the optimization steps of the various chromatographic conditions are shown in Fig. 2. A more in-depth discussion of individual effects of each parameter can be found in the work by Afton and co-workers.

Standards and samples were diluted with a mobile phase to ensure a sample pH near 6.5 and fit in the linear calibration curve (2 to 50 μg L⁻¹). Samples with pH values significantly different compared to the mobile phase exhibited retention time (RT) shifts. External calibration was used with selenium standard solutions; integrated peak areas were plotted versus the expected solution concentration to generate calibration curves within the calibration range with R² values of >0.995.

The separation of the species was evaluated using selenium species standards and their corresponding RTs. In Fig. 3, the chromatograms of the individual standards and a mix solution with all standards are presented.

Peak identities of the following experiments were confirmed by retention time matching and/or standard addition of standards to the extracts. Fig. 4 demonstrates that the enzyme extracts of all feed samples, control (with and without canola oil) and enriched (with and without canola oil), contained...
various levels of SeMet. Note that the control feed samples were assumed to have selenium from a source other than Se-yeast. The enzyme extract of the Se-yeast based additive used to supplement the feed sample was confirmed to contain SeMet as the primary component, which is consistent with the reports in the literature (SeMet $\geq 60\%$ of the total selenium in a quality yeast product). Therefore, since the feed + antioxidant and feed + antioxidant + canola oil samples also showed SeMet as the primary selenium compound, it can be inferred that no significant selenium interconversion occurs when the other components of the antioxidant mixture and/or canola oil are added to the enriched samples (Fig. 4(c) and (d)). As expected, the SeMet concentration was $\sim$3 times greater in the enriched samples as compared to non-enriched samples. The chromatogram presented in Fig. 4(f) confirms the presence of SeMet in agreement with the CRM SELM-1 certificate of analysis.

RP-ICP-MS ($m/z = 78$) chromatograms presented in Fig. 5(a) and (b) for beef samples from animals without selenium supplementation and with and without canola oil to their diet show SeCys$_2$ and SeMet species. However, the retention time matching and standard addition of SeCys$_2$ may not be enough to confirm its presence. Somewhat recently, reports of SeCys$_2$ have come into question,$^{26}$ therefore the identification of SeCys$_2$ will remain tentative for reporting purposes of this manuscript. Complicating the matter, a large number of possible selenium co-eluters are not all available as standards. A few possibilities exist including a recently reported second oxidation production of SeMet (as SeOMet has been ruled out due to retention time differences);$^{31}$ although unlikely, the possibility that selenocysteine (SeCys) is the peak eluting in the place of SeCys$_2$ cannot be completely ruled out at this point as the instability and related reliability of a SeCys standard obscures this issue. Therefore, future work must be undertaken to determine/confirm its identity. Regardless of the peak’s true identity, the chromatograms of the enzyme extracts of beef from diets omitting selenium enrichment (Fig. 5(a) and (b)) resulted in a different metabolism of selenium as compared to the selenium enriched diets. Also, it is worth noting that although both non-enriched and enriched feed types contained SeMet based components as the sole primary selenium species (the former at lower Se levels), only the non-enriched diet produced a metabolite that was not SeMet based. This could be a result of the different metabolism by the cattle of yeast based SeMet versus non-yeast based SeMet; studies focusing on this difference were not located, but distinction between the two types of SeMet are regularly made in the dietary supplement labels and in the

![Fig. 3](image_url) Fig. 3 RP-ICP-MS for 20 $\mu$g L$^{-1}$ selenium standard solutions: (a) mobile phase/blank, (b) SeCys$_2$, (c) SeMet, (d) Se(IV), (e) SeOMet, (f) Se(VI), and (g) mobile phase with a mix solution with all standards: (1) SeCys$_2$, (2) SeMet, (3) Se(IV) (4) SeOMet, and (5) Se(VI).

![Fig. 4](image_url) Fig. 4 RP-ICP-MS speciation analysis of feed samples: (a) feed control, (b) feed + canola oil, (c) feed + antioxidants, (d) feed + canola oil + antioxidants, (e) Se-yeast used in the antioxidant mixture, and (f) CRM SELM-1.

![Fig. 5](image_url) Fig. 5 RP-IP-ICP-MS speciation analysis of beef samples: (a) beef control, (b) beef + canola oil, (c) beef + antioxidants, and (d) feed + canola oil + antioxidants.
Scientific literature. Comparing the chromatograms of the enzyme extraction of the beef samples with and without canola oil (Fig. 5 (a) vs. (b) and (c) vs. (d)) yielded no evidence that the addition of canola oil significantly impacts selenium uptake and/or metabolism.

A previous study which analyzed longissimus dorsi beef compared Se-Yeast feedings versus selenium feeding. Under some feeding conditions, SeCys concentrations in the beef were greater than SeMet concentrations. While a similar study examined blood and milk, with the former having relatively higher SeCys levels and the latter having higher SeMet levels.

Conclusions

An extraction procedure that compared quite well to those reported in the literature for these difficult to extract matrices of animal feeds and beef samples was optimized. A novel isocratic speciation method that resolves inorganic selenium compounds (Se(IV) and Se(VI)) while resolving the most relevant organic selenium forms (SeMet, SeCys, and SeOMet) was developed and optimized. The developed method was determined to be applicable to two different matrices, feed and beef samples for selenium species analysis. Canola oil addition did not significantly alter the selenium profile within the feed samples and provided no hindrance to selenium uptake or metabolism, thus showing that its use with selenium supplementation may yield a better overall beef product. Also, selenium seemed to metabolize differently (an additional selenium species was detected) when comparing the enzyme extracts of the beef samples without Se-yeast to that with Se-yeast. This altered metabolism could potentially be an important area of research. Future work will focus on further optimization of the extraction protocol to maximize extraction efficiency to ensure that all relevant selenium species are detected and quantified, and confirmation/identification of the SeCys peak.

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References