Introduction

Selenium (Se) is a non-metallic element with a chemical nature similar to sulphur and is an essential mineral for animals. Its concentration in the earth’s crust is 0.1 ppm, with wide variation depending on the area (Saruwatari and Ikeda, 1993). Selenium was formerly considered a poisonous substance for animals, but recently its physiological and nutritional importance has been pointed out and it is now recognized as an essential element. In animal nutrition, the requirement range of Se is very narrow, e.g., 50–300 ng · g⁻¹ DM in feeds for ruminants. Since ruminant feeds have an Se content range from 100 to 5000 mg · kg⁻¹ DM, the occurrence of both toxicity and deficiency is very likely (NRC, 1983; McDowell, 1985).

In most tropical and subtropical areas of the world, the Se content of the soil is generally low due to heavy rainfall and soil erosion, thereby exposing grazing animals to Se deficiency (Harrtey, 1963; Trinder et al., 1969; Fujihara et al., 1992).
It is, therefore, imperative that some form of feeding intervention be practiced to prevent clinical or sub-clinical Se deficiency symptoms.

Inorganic (selenite and selenate) and organic (selenocysteine and selenomethionine) selenium are known to be nutritionally useful. Se exists as selenocystine, selenocysteine, selenomethionine, or Se-methyl selenomethionine in most cereals or forages (NRC, 1983; Serra et al., 1996). In practice, selenate and selenite are used as supplements in Se-deficient diets (Nicholson et al., 1991; Saruwatari and Ikeda, 1993). Conversely, Se in the animal body is mostly in an organic form that is bound with amino acids. Increased biosynthesis of Se-proteins provides protection against oxidative stress in muscles and other vital organs of mammals (Schomburg et al., 2004).

There are several factors that influence Se bioavailability in ruminants, including antagonism to sulphur (S) and reciprocity with copper (Cu), where a positive response to Se supplementation was observed in Cu-deficient sheep (NRC, 1985). Some reports pointed out the interrelationships of Se with mercury, arsenic (As) and cadmium (Cd) (Hidiroglou et al., 1968; Whanger et al., 1978; McDowell, 1985; Grosicki and Kowalski, 2002). Moreover, Se availability could be influenced by animal species and the protein and conjugated linoleic acid contents of the diet (Schomburg et al., 2004; Czauderna et al., 2011).

Serra et al. (1994) demonstrated that the bioavailability of selenite and selenate is similar in providing supplemental Se to sheep. Conversely, Se absorption among ruminants is low after its incorporation into microbial tissue under a highly reduced rumen environment (Cousins and Cairney, 1961; Peterson and Spedding, 1963; Hidiroglou et al., 1968; Paulson et al., 1968; Harrison and Conrad, 1984; Hudman and Glenn, 1984; Lyons et al., 2006). In vitro experiments revealed that about 40% of rumen microbial selenite is converted to an insoluble form, while the incorporation of Se into microbial cell walls is more efficient and faster. Selenium was incorporated into bacterial protein within an hour post feeding, reaching a peak level in 2 hours (Hidiroglou et al., 1968). Recent findings showed that the addition of conjugated linoleic fatty acids (CLA) to diets enriched with selenized yeast (0.5 µg Se · g⁻¹) stimulated the accumulation of Se in the liver and muscle of rats (Czauderna et al., 2011). These authors attributed the increased Se biosynthesis to elevated oxygen consumption associated with higher oxidation of fat. Furthermore, they pointed out that dietary inorganic Se (selenate) or organic Se (as selenized Se) exhibited a similar influence on the metabolic capacity of CLA isomers in rats (Czauderna et al., 2004, 2011).

In view of the recent developments on Se nutrition and limited information on the utilization of bacterial Se and protozoal Se (Duran and Kawashima, 1980; McDowell, 1985), the current study was conducted to determine the utilization of rumen microbial Se using rats as the experimental animal to simulate the lower digestive tract of ruminants. Likewise, Czauderna et al. (2011) indicated that Se in tissues of mammals reflects the chemical form of Se and its level in the consumed diet. Therefore, the Se concentration in the muscles and other organs such as the liver, kidney, lungs, brain and heart were analysed to estimate the Se bioavailability of microbial Se. Part of the results of this work were published by Fujihara et al. (2004).

Material and methods

Preparation of microbial Se

Two rumen-fistulated sheep (castrated male and female; average body weight 39.3 ± 1.7 kg) were used as sources of microbial tissue Se. They were fed a mixed diet consisting of timothy hay and concentrate (wheat bran and soyabean meal, 3:1/dry matter (DM), 3:2/DM) to meet the daily energy requirement of 0.4 MJ per kgW⁰.⁷⁵. Selenite (Wako Pure-chemical Industrial Co.) was incorporated at the rate of 0.4 mg · kg⁻¹ DM (Fujihara and Ichinohe, 2012).

A sufficient amount of rumen liquor was collected from the host animals 2 h post Se-feeding and filtered to separate solids from the liquid. The filtrate was mixed with 0.1 M sucrose at a 3:1 ratio (v/v) in a separation flask and incubated at 39°C for 2 h, which allowed segregation of feed particles, bacteria and protozoa. Protozoa were separated by low-speed centrifugation, washed, and dried for 48 h at 60°C, while the bacterial layer was further centrifuged at 20 000 g for one h and the sediment dried. Dried bacteria and protozoa matter was ground and stored at 4°C prior to incorporation in the experimental diets.

Experimental animals and diets

A total of 43-female Wistar rats (8 weeks old) were used in the study. They were kept in individual cages in a temperature controlled room at 23°C and randomly assigned to the one of the 4 dietary treatments as follows: control – without Se supplement (12 animals); kg⁻¹ DM: ISe – inorganic Se (0.1 mg selenite Se) (11 animals); PSe – 0.1 mg protozoal Se (10 animals); BSe – 0.1 mg bacterial Se (10 animals).
The diet composition is given in Table 1.

### Experimental procedure

**Feeding management.** After a 7-day preliminary period, a 7-day metabolism trial was conducted where daily feed intake and excreta were measured. During the preliminary period, the animals were fed *ad libitum* with commercial feed for rats (MF Shimizu Experimental Materials Co.; 0.05 ppm Se detected as a contaminant). Fresh water (distilled) was made available at all times. Body weight (BW) was determined before and after the metabolism trial. Feed intake and water consumption were checked at 10 a.m. every day during the trial. The animals were cared for throughout the experimental period according to the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee).

**Sample collection.** Total faecal and urinary excretions were collected. Faecal samples were oven-dried at 60°C for 48 h, ground and stored prior to analysis. Similarly, urine was collected using filter papers that were placed under the cages. Absorbed urine was dried and stored prior to analysis.

### Bioavailability of microbial Se in rats

On the final day of the trial, all rats were anaesthetized using diethyl ether (Wako Pure-Chemical Industrial Co.) and blood samples were collected through the ventral aorta using a heparinized syringe. One millilitre of whole blood was taken from the syringe and stored at –20°C prior to Se analysis, while the remaining portion was centrifuged at 1 600 g for 20 min to separate plasma from blood corpuscles. Vital organs such as the brain, lung, heart, liver, spleen, kidney and the thigh muscles were removed, washed with Ringer’s solution with vitamin B1 (Mitaka Pharmaceutical Co.) and stored at –20°C.

### Analytical methods

**Dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) contents of the diets and faeces were measured according to the AOAC procedure (1960).**

After wet digestion with nitric and perchloric acids (3:1 v/v), the Se contents of the diets, faeces, urine, whole blood and vital organs were determined by the fluorometric method of Watkinson (1966). All of the data were subjected to ANOVA (Yoshida, 1975) using a commercially available computer programme (SAS, 1999).

### Results

**Nutrient content of dietary treatments**

Table 1 presents the nutrient content of the experimental diets. Except for vitamin E, variations in the OM and CP contents of the experimental diets are quite low. This would indicate that the diets are iso-nitrogenous, negating the possible advantage of incorporating microbial matter in the Se-supplemented groups. Both bacteria and protozoa are rich sources of organic nutrients.

**Selenium concentration in rumen microbes**

The 0.4 mg Se · kg⁻¹ DM incorporated into the diet made up of timothy hay and concentrate fed to the host animal produced 0.42 mg protozoal Se · kg⁻¹ DM, while the harvested bacterial Se was only 0.26 mg · kg⁻¹ DM (Table 2). With this concentration, the total volume of collected rumen fluid was 580 ml for protozoa and 1630 ml for bacteria to arrive at a similar concentration of 0.67 mg Se · kg⁻¹ DM. It is interesting to note that protozoa had a better ability to synthesize cellular Se than bacteria utilizing a similar rumen substrate. This result could be explained by the nature of protozoa to engulf bacteria in the rumen (Hungate, 1978).
Feed and water consumption, body weight

Table 3 presents the average daily feed and water intake, and body weight of rats fed with different Se-sources. Feed intake did not vary significantly, but water consumption was found to be higher in the microbial Se-supplemented group. Animals fed BSe had the highest \((P < 0.05)\) water intake, followed by those in the PSe, control and ISe groups.

Table 3. Feed and water intake of rats as influenced by different forms of Se supplements

<table>
<thead>
<tr>
<th>Intake</th>
<th>Control</th>
<th>ISe diet</th>
<th>PSe diet</th>
<th>BSe diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed, g · day(^{-1})</td>
<td>13.91 ± 0.32(^{a})</td>
<td>14.80 ± 0.18(^{a})</td>
<td>14.76 ± 0.13(^{a})</td>
<td>14.46 ± 0.76(^{a})</td>
</tr>
<tr>
<td>Water, ml · day(^{-1})</td>
<td>180.94 ± 10.71(^{a})</td>
<td>169.04 ± 15.47(^{a})</td>
<td>257.14 ± 14.28(^{a})</td>
<td>357.14 ± 13.69(^{a})</td>
</tr>
</tbody>
</table>

Despite a similar initial BW, rats fed BSe had the lowest \((P < 0.05)\) liveweight after 7 days of the balance trial (Table 4). The 4 g gain in weight of animals in the BSe group is more than 3-fold lower than the 12.9 g total gain in weight in the ISe group. The 4 g gain in weight of animals across the control, PSe and ISe groups.

Table 4. Body weight changes and apparent digestibility of nutrients in rats fed diets with different forms of Se supplements

<table>
<thead>
<tr>
<th>Indices</th>
<th>Control</th>
<th>ISe diet</th>
<th>PSe diet</th>
<th>BSe diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/head</td>
<td>179.4 ± 2.5</td>
<td>178.6 ± 1.5</td>
<td>181.0 ± 2.5</td>
<td>177.2 ± 2.1</td>
</tr>
<tr>
<td>final</td>
<td>190.7 ± 2.3(^{a})</td>
<td>191.5 ± 1.4(^{a})</td>
<td>190.9 ± 1.5(^{a})</td>
<td>181.2 ± 2.6(^{a})</td>
</tr>
<tr>
<td>gain</td>
<td>11.3 ± 1.6(^{a})</td>
<td>12.9 ± 0.9(^{a})</td>
<td>10.0 ± 1.8(^{a})</td>
<td>4.0 ± 1.8(^{a})</td>
</tr>
<tr>
<td>Dry matter, %</td>
<td>73.6 ± 2.5(^{a})</td>
<td>76.1 ± 2.3(^{a})</td>
<td>71.3 ± 2.8(^{a})</td>
<td>66.6 ± 2.7(^{a})</td>
</tr>
<tr>
<td>Organic matter, %</td>
<td>76.2 ± 2.3(^{a})</td>
<td>78.0 ± 2.1(^{a})</td>
<td>73.1 ± 2.6(^{a})</td>
<td>67.8 ± 2.8(^{a})</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>74.3 ± 2.1(^{a})</td>
<td>77.4 ± 2.4(^{a})</td>
<td>70.6 ± 2.9(^{a})</td>
<td>58.6 ± 3.0(^{a})</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>74.7 ± 4.0(^{a})</td>
<td>82.6 ± 1.7(^{a})</td>
<td>81.5 ± 1.3(^{a})</td>
<td>68.3 ± 2.2(^{a})</td>
</tr>
</tbody>
</table>

**Selenium balance**

As shown in Table 5, Se intake was about 3 times higher \((P < 0.05)\) in Se-supplemented diets than in the control (Table 1). The consumed Se in the control was contributed by both the various feed ingredients that composed the diet. Although total feed consumption varied significantly \((P < 0.05)\) among the Se-supplemented diets, Se intake was not affected.

Table 5. Selenium balance in rats as influenced by different forms of Se supplements

<table>
<thead>
<tr>
<th>Indices</th>
<th>Control</th>
<th>ISe diet</th>
<th>PSe diet</th>
<th>BSe diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng · kg(^{-1}) · BW · day(^{-1})</td>
<td>2523 ± 188(^{a})</td>
<td>7938 ± 563(^{a})</td>
<td>7229 ± 222(^{a})</td>
<td>7049 ± 1130(^{a})</td>
</tr>
<tr>
<td>Fe intake</td>
<td>1393 ± 186(^{a})</td>
<td>3513 ± 826(^{a})</td>
<td>3171 ± 1503(^{a})</td>
<td>2513 ± 1631(^{a})</td>
</tr>
<tr>
<td>Urinary excretion</td>
<td>34.7 ± 5.9</td>
<td>134.9 ± 63.7</td>
<td>61.8 ± 36.1</td>
<td>78.3 ± 40.5</td>
</tr>
<tr>
<td>Absorbed Se(^{a})</td>
<td>1129 ± 240(^{a})</td>
<td>4424 ± 945(^{a})</td>
<td>4112 ± 1439(^{a})</td>
<td>4535 ± 2418(^{a})</td>
</tr>
<tr>
<td>Retained Se(^{a})</td>
<td>1094 ± 237(^{a})</td>
<td>4289 ± 931(^{a})</td>
<td>4050 ± 1503(^{a})</td>
<td>4457 ± 2448(^{a})</td>
</tr>
<tr>
<td>Se utilization(^1)</td>
<td>96.2 ± 1.4</td>
<td>96.3 ± 1.9</td>
<td>99.0 ± 1.0</td>
<td>98.6 ± 1.9</td>
</tr>
</tbody>
</table>

Absorbed and retained Se was significantly higher \((P < 0.05)\) in Se-supplemented groups than in the control. On the other hand, faecal Se excretion was high in ISe, and urinary Se excretion tended to be high in Se-supplemented groups as compared with the control. The high retention of absorbed Se in PSe and BSe, which reached 98.6% and 99.0%, respectively, was due to the small amount of Se excreted through the urine.

**Se content of blood, muscle and organs**

The Se concentrations in whole blood, muscles and vital organs are shown in Table 6. Except for BSe, the positive effect of Se supplementation was very evident as blood Se concentrations of rats fed ISe and PSe were higher \((P < 0.05)\) than those in controls. No significant effect on blood Se concentration was observed between the two microbial Se sources, thus both PSe and BSe were similar in terms of absorption and incorporation into erythrocytes. Except for the liver and kidney \((P < 0.05)\), other organs showed similar Se concentrations across treatments.
The addition of selenite to the diet of sheep produced 0.40 g microbial matter Se per kg rumen matter, which is comparable to the 0.45 mg microbial Se · kg⁻¹ DM from our previous study (Fujihara and Ichinohe, 2012). This value is twice the 0.2 mg Se · kg⁻¹ DM reported by Serra et al. (1997), despite the similar feeding rate of selenite to the host animal.

In addition to the elevated Se content in groups PSe and BSe, their vitamin E content was also higher than the control. This could be attributed mainly to the inclusion of rumen bacteria and protozoa influencing the influx of ingested vitamin E through rumen microbial activity (Chikunya et al., 2004). Likewise, the greater amount of bacterial matter in BSe resulted in a higher dietary vitamin E content than PSe. With a lower Se concentration per unit of bacterial tissue, its inclusion rate was adjusted to maintain an iso-Se level among the Se-supplemented diets. Therefore, protozoa could provide a greater amount of Se than bacteria per unit of microbial matter. As shown in Table 3, water consumption of rats fed diets with rumen microbial Se tended to be higher than those fed other diets. Some microbial matter in PSe and BSe, could have stimulated thirst, although the substances that triggered higher water intake in the present experiment have not been identified yet.

The low consumption in the BSe group could be attributed to the low digestibility of its nutrient contents. With similar ingredients used in formulating the various experimental diets, the 32% inclusion rate of bacterial matter greatly influenced nutrient digestibility in the BSe group. One of the known factors that tend to adversely affect digestibility of bacterial matter is its high content of cell wall constituents, which are mostly indigestible substances.

### Se balance and utilization
Selenium in the body is eliminated via three routes, namely, urinary excretion through the kidney, faecal output through the lower digestive tract, and respiratory excretion through the mouth. The amount and ratio of Se excretion through these routes are influenced mainly by the form and amount of dietary Se (Reilly, 1992). Selenium is absorbed mainly through the jejunum and caecum. Likewise, the rate of Se utilization depends largely on whether it is from organic or inorganic sources. In the lower gut, selenomethionine is absorbed up to 90% compared with only 60% for selenate when used as supplemental Se (Reilly, 1992). Selenium utilization can be measured as the difference between intake and output, with faecal Se appearing as insoluble inorganic Se or protein-binding Se in almost similar proportions (Butler and Peterson, 1961). Despite similar Se consumption in the supplemented diets, Se excretion was highest in rats fed ISe. With similar Se output in PSe and BSe, these results indicate that rumen protozoa and bacteria are more available due to their higher digestibility and utilization. On the other hand, inorganic Se was poorly digested. Faecal Se even exceeded Se intake, indicating that selenite was unavailable.

The higher amount of Se in the liver, kidney, and muscle of rats fed PSe and BSe further proved that microbial Se is more bioavailable than selenite. This result is similar to the findings of Serra et al. (1997), who reported higher Se concentrations in some vital organs of rats fed bacterial Se than in those supplemented with selenite or selenate. Likewise, Ornman and Pehrson (1999) observed the same positive effects of bacterial-Se supplementation in elevating the blood Se concentration of cattle, but not with selenite or selenate. The mechanism by which microbial Se is more available at the tissue level than inorganic Se sources was explained by Hudman and Glenn (1984). They concluded that microorganisms under the highly ionized rumen environment transform ⁷⁵Se-selenite to a sulphur-amino acid-selenium complex that is rendered unavailable to the host animal because of its strong ligand. Furthermore,

### Table 6. Se concentration in whole blood, muscle and other organs of rats as influenced by different forms of Se supplements

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>ISe diet¹</th>
<th>PSe diet²</th>
<th>BSe diet³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haematocrit, %</td>
<td>41.5 ± 0.8</td>
<td>42.3 ± 0.5</td>
<td>42.5 ± 0.8</td>
</tr>
<tr>
<td>Whole blood, ng Se · ml⁻¹</td>
<td>174.5 ± 27.9</td>
<td>284.0 ± 33.0</td>
<td>257.6 ± 20.1</td>
<td>208.9 ± 10.1</td>
</tr>
<tr>
<td>ng Se · g⁻¹ wet tissues</td>
<td>824.5 ± 26.8</td>
<td>908.2 ± 70.0</td>
<td>865.2 ± 14.5</td>
<td>690.9 ± 42.0</td>
</tr>
<tr>
<td>Liver, g</td>
<td>392.6 ± 55.6</td>
<td>533.8 ± 54.4</td>
<td>209.8 ± 43.6</td>
<td>367.7 ± 71.4</td>
</tr>
<tr>
<td>Kidney, g</td>
<td>824.5 ± 26.8</td>
<td>908.2 ± 70.0</td>
<td>865.2 ± 14.5</td>
<td>690.9 ± 42.0</td>
</tr>
<tr>
<td>Spleen, g</td>
<td>234.8 ± 11.8</td>
<td>227.1 ± 26.1</td>
<td>247.7 ± 14.5</td>
<td>245.3 ± 19.9</td>
</tr>
<tr>
<td>Brain, g</td>
<td>111.6 ± 6.9</td>
<td>120.8 ± 6.0</td>
<td>93.3 ± 18.7</td>
<td>95.5 ± 13.0</td>
</tr>
<tr>
<td>Heart, g</td>
<td>222.2 ± 9.5</td>
<td>204.7 ± 9.2</td>
<td>219.8 ± 17.0</td>
<td>227.6 ± 6.4</td>
</tr>
<tr>
<td>Lung, g</td>
<td>189.2 ± 11.4</td>
<td>203.1 ± 10.4</td>
<td>228.0 ± 18.6</td>
<td>209.5 ± 17.8</td>
</tr>
<tr>
<td>Muscle, g</td>
<td>119.5 ± 7.4</td>
<td>126.4 ± 3.9</td>
<td>126.0 ± 4.4</td>
<td>123.6 ± 5.5</td>
</tr>
</tbody>
</table>

²³⁴ see Table 3; a muscle of the thigh; a,b means with different superscripts within a row are significantly different at P ≤ 0.05

### Discussion

#### Diets, feed and water intake, and nutrient digestibility
The addition of selenite to the diet of sheep produced 0.40 g microbial matter Se per kg rumen matter, which is comparable to the 0.45 mg microbial Se · kg⁻¹ DM from our previous study (Fujihara and Ichinohe, 2012). This value is twice the 0.2 mg Se · kg⁻¹ DM reported by Serra et al. (1997), despite the similar feeding rate of selenite to the host animal.

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Conclusions

Rumen microbes can synthesize Se compounds in their cells that resemble selenite in terms of digestibility and bioavailability. Protozoa had a greater ability than bacteria to incorporate Se, as indicated by the variation in microbial Se concentration collected from host animals (sheep) receiving a higher level of Se supplementation. Nonetheless, the supplementation of Se from microbial matter increased the concentration of plasma Se and retention in the muscles and some vital organs, like the liver and kidneys, with a similar efficiency as inorganic Se sources. Higher accumulation of Se in the liver and muscles provides better protection from the degrading effects of peroxides that could result in Se deficiency symptoms such as liver necrosis and white muscle disease.

Acknowledgements

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References


