The effect of vitamin C supplementation on plasma concentration and urinary excretion of vitamin C in cattle

L. Padilla,* T. Matsui,*¹ S. Ikeda,† M. Kitagawa,† and H. Yano*

*Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan; and †Livestock Farm, Graduate School of Agriculture, Kyoto University, 144 Tomitakomono, Kyotanba-cho, 622-0203, Japan

ABSTRACT: We investigated the plasma concentration and urinary excretion of vitamin C in cows supplemented with vitamin C. Five cows (mean BW = 597 kg) were allocated to a 5 × 5 Latin square design and supplemented with a vitamin C preparation coated with hydrogenated soybean oil at 0, 10, 20, 40, or 60 mg of vitamin C per kg of BW per day for 9 d. Plasma and urine samples were collected for measuring vitamin C concentration. Urinary excretion of vitamin C was expressed as the ratio of vitamin C to creatinine. Plasma vitamin C concentration and urinary vitamin C excretion increased quadratically as dietary vitamin C increased (P < 0.001); that is, the lowest dose affected neither plasma vitamin C concentration nor urinary vitamin C excretion but the plasma vitamin C concentration and urinary vitamin C excretion increased (P < 0.05) with increasing supplementation of vitamin C at greater doses. This suggests that plasma vitamin C concentration affects urinary excretion of vitamin C in cattle and that plasma vitamin C concentration exceeded the renal threshold for vitamin C in the cows receiving vitamin C at 20 mg/kg of BW per day. Furthermore, increased urinary excretion of vitamin C appears to limit plasma vitamin C concentration in response to vitamin C intake. The daily excretion of vitamin C was estimated by the reported value of daily creatinine excretion, indicating that the daily amount of vitamin C excreted into urine was more than half of supplied vitamin C. Therefore, a large part of supplied vitamin C probably escapes ruminal degradation and is absorbed but excreted into urine.

INTRODUCTION

Vitamin C (VC) is the most abundant and probably most important water-soluble antioxidant in mammals (Sauberlich, 1994). Although cattle can synthesize VC in the liver and it is not considered to be an essential nutrient for healthy cattle (McDowell, 1989), a large reduction in plasma VC concentration was reported in calves stressed by housing conditions (Cummins and Brunner, 1991), in lactating cows with artificially induced mastitis (Weiss et al., 2004), and in heat-stressed cows (Padilla et al., 2006). Moreover, supplementation with VC reduced the incidence of diarrhea in calves (Cummins and Brunner, 1989) and stimulated recovery from acute mammary inflammation in dairy cows (Chaiyotwittayakun et al., 2002).

Dietary VC is extensively degraded in the rumen (Cappa, 1958). Thus, special preparations for ruminants have been developed and some experiments indicated that the supplementation with these preparations increased plasma VC concentration in cattle (Hidiroglou, 1999; Tyler and Cummins, 2003). Urinary excretion of VC generally increases when the plasma concentration exceeds the renal threshold (McDowell, 1989). Additionally, urinary VC excretion is partially responsible for closely controlling plasma VC concentration in humans (Graumlich et al., 1997). To our knowledge, there has been just one report demonstrating urinary VC excretion in adult ruminants, in which the plasma VC concentration increased linearly but the urinary VC concentration changed quadratically in cows as dietary VC increased; that is, the urinary concentration of VC was numerically greatest in cows without supplementation (Weiss, 2001). Therefore, the relationship between VC supplementation and urinary excretion of VC may be different between cattle and other animals. This experiment examined the effect of increasing dietary...
VC on plasma VC concentration and urinary VC excretion in cattle.

MATERIALS AND METHODS

Animals and Experimental Design

Animals were cared for according to the Guide for the Care and Use of Laboratory Animals (Animal Care Committee, Kyoto University). Five Japanese Black × Holstein cows with a mean BW of 597 ± 30 kg and a mean age of 45 ± 4 mo were used. The cows were given a concentrate mixture (Table 1) twice daily (0600 and 1700) at 7 kg/d and rice straw at 1 kg/d on a DM basis. After a 14-d adaptation period, the animals were randomly allocated to a 5 × 5 Latin square design. In each 16-d period, a VC preparation coated with hydrogelatinized soybean oil (HSO; Technocoat V-70, Central Techno, Tokyo, Japan) was supplied at 0, 10, 20, 40, or 60 mg of VC/kg of BW per day for 9 d. The cows were not supplemented with VC in the following 7 d. The VC preparation was mixed with a small amount of concentrate mixture and given before the morning feeding. After the VC preparation was completely ingested, the remaining feeds were given. Blood samples were collected from the jugular vein into heparinized tubes (VP-H050K, Terumo Medical Products, Tokyo, Japan) on d 7 of supplementation in each period, and urine was spot-sampled from d 7 to d 9 of supplementation. Blood and urine samples were collected before giving the VC preparation or before the morning feeding on each sampling day.

Analytical Methods

A heparinized plasma sample was treated with a reducing reagent, dithioerythritol, and frozen at −80°C for the VC analysis. A fresh urine sample was divided into 2 subsamples. One of the subsamples was immediately treated with dithiothreitol and frozen at −80°C for the VC analysis. The other subsample was frozen at −20°C for the creatinine analysis. Urine samples collected in each period were pooled before analyses. Plasma and urinary VC concentrations were determined as ascorbic acid by HPLC (Haiying et al., 2003; Kashiba et al., 2002) within 4 d of sampling.

Urinary creatinine concentration was determined by a commercial kit (Creatinine Test, Wako, Osaka, Japan). The urinary excretion of VC was expressed as the ratio to urinary creatinine. The excretion of urinary creatinine ranged from 15 to 20 mg/kg of BW per day in cattle (Kaneko, 1989). The amount of excreted VC was estimated according to the excretion of urinary creatinine as 17.5 mg/kg of BW per day.

Statistical Methods

The effect of VC supplementation on plasma VC concentration and urinary VC excretion was analyzed using a model that included VC dose, period, and animal. The effect of dose was partitioned into linear and quadratic orthogonal contrasts. Unequal spacing of treatment was accounted for when treatment contrasts were tested. Dunnett’s 1-tailed t-test was used to identify the doses that significantly increased plasma VC concentration and urinary VC excretion above control values. A P-value of 0.05 was used to accept or reject the null hypothesis. All statistical analyses were carried out using the GLM procedure of SAS (SAS Institute Inc., Cary, NC).

RESULTS

The average concentration of plasma VC was 15.2 μmol/L in the cows not given the VC preparation (Table 2). The concentration of plasma VC increased as dietary VC increased (linear effect, P = 0.002; quadratic effect, P < 0.001). The lowest dose did not affect plasma VC concentration, but the plasma VC concentration was greater (P < 0.05) in the cows given VC at 20 mg/kg of BW per day or greater compared with the control cows.

The average excretion of urinary VC was 0.258 mol/mol of creatinine in the cows not given VC (Table 2). Urinary VC excretion increased with increasing dietary VC (linear effect, P = 0.021; quadratic effect, P < 0.001). The lowest dose did not affect urinary VC excretion, but the urinary excretion was greater (P < 0.05) in the cows given VC at 20 mg/kg of BW per day or greater compared with the control cows.

The amount of daily VC excretion was estimated by daily creatinine excretion as 17.5 mg/kg of BW per day (Figure 1). The cows without supplementation excreted VC into urine at 6 mg/kg of BW per day. The VC excretion rate ranged from 16 mg/kg of BW per day in the cows supplemented with VC at 20 mg/kg of BW per day to 32 mg/kg of BW per day in the cows supplemented with VC at 60 mg/kg of BW per day. Thus, the daily amount of excreted VC into urine was more than half of supplied VC.

Table 1. Composition of the concentrate mixture

<table>
<thead>
<tr>
<th>Ingredient, % of DM</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rolled barley</td>
<td>38.2</td>
</tr>
<tr>
<td>Flaked corn</td>
<td>31.5</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>14.1</td>
</tr>
<tr>
<td>Rice bran</td>
<td>8.9</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>5.0</td>
</tr>
<tr>
<td>Premix1</td>
<td>2.3</td>
</tr>
<tr>
<td>Calculated value2</td>
<td></td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>13.4</td>
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<tr>
<td>DE, Mcal/kg of DM</td>
<td>3.6</td>
</tr>
</tbody>
</table>

1Premix supplied (per kilogram of feed): NaCl, 9.75 g; CaCO3, 9.75 g; retinyl palmitate, 5,000 IU; cholecalciferol, 1,000 IU; and DL-α-tocopheryl acetate, 5 mg.

2Based on composition values from Agriculture, Forestry and Fisheries Research Council Secretariat (2001).
Table 2. Effect of vitamin C (VC) supplementation on plasma concentration and urinary excretion of VC in cows

<table>
<thead>
<tr>
<th>Item</th>
<th>Dose of vitamin C, mg/kg of BW per day</th>
<th>P-value</th>
<th>SEM</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Plasma VC, μmol/L</td>
<td>15.2</td>
<td>16.8</td>
<td>18.1*</td>
<td>18.9*</td>
<td>23.2*</td>
</tr>
<tr>
<td>Urinary VC, mol/mol of creatinine</td>
<td>0.26</td>
<td>0.27</td>
<td>0.69*</td>
<td>0.88*</td>
<td>1.36*</td>
</tr>
</tbody>
</table>

*Means differ from the cows not receiving VC preparation (P < 0.05).

**DISCUSSION**

The plasma VC concentration increased as dietary VC increased, which indicated that at least some of the HSO-coated VC preparation was not degraded in the rumen and was subsequently absorbed across the small intestine. The bioavailability of VC preparations in ruminants has been studied using plasma VC concentrations. Plasma VC concentrations increased in wethers given a VC preparation coated with silicone at 80 to 88 mg/kg of BW per day (Hidiroglou et al., 1997) and in heifers given ascorbyl-2-phosphate at 38 mg/kg of BW per day (Tyler and Cummins, 2003). Weiss (2001) indicated that the plasma VC concentration increased linearly with the supplementation of ascorbyl-2-phosphate ranging from 3 to 30 g/animal per day in lactating cows. The experiment reported here showed a quadratic increase in plasma VC concentration with increasing supplementation of HSO-coated VC in cattle, and the minimally effective dose was between 10 and 20 mg/kg of BW per day for increasing plasma VC concentration in cows. Increased dietary VC was reported to suppress VC production in mouse liver (Tsao and Young, 1990). If VC supplementation similarly inhibits endogenous VC production in cattle, it could explain the lack of an effect on plasma VC concentration in the cows given the lowest VC dose.

The plasma concentration and urinary excretion of VC increased as dietary VC increased. Although Weiss (2001) reported that urinary VC concentration did not increase with increasing dietary VC, the present experiment indicates that urinary VC excretion depends on dietary VC in cattle. Toutain et al. (1997) suggested that most of the VC was eliminated through the kidneys when the plasma VC concentration was below a renal threshold in suckling calves at 6 d of age, but that VC was excreted into urine at a low level when plasma VC concentration was above the renal threshold. A study in humans showed that urinary excretion of VC was stably low when plasma VC concentration was lower than the renal threshold for VC, but that urinary VC excretion increased as plasma VC concentration increased above the renal threshold (Graumlich et al., 1997). The excretion of VC in urine depends on glomerular filtration and tubular reabsorption of VC in the kidney (Kallner et al., 1979). The filtered amount of VC is determined by the glomerular filtration rate and by the plasma VC concentration, whereas tubular reabsorption is a saturable process. The simultaneous increase in plasma concentration and urinary excretion of VC suggests that the plasma VC concentration exceeded the renal threshold for VC in cows given VC at 20 mg/kg of BW per day and thus, urinary VC excretion is affected by plasma VC concentration in cattle. Urinary VC excretion is proposed to be responsible for control of plasma VC concentration in humans based on increasing plasma VC concentration (Graumlich et al., 1997). We consider that increased urinary excretion of VC limits plasma VC concentration in response to VC intake in cattle.

In this study, the daily amount of VC excreted into urine was more than half of that supplied in the HSO-coated form. Considering that VC supplementation suppresses endogenous VC production in mouse liver (Tsao and Young, 1990) and that VC is partially metabolized (McDowell, 1989), we consider that at least half of the HSO-coated VC escaped ruminal degradation and was absorbed. Furthermore, it is likely that much of it was not utilized and was excreted in urine. Therefore, excretion in urine is probably the major route of elimination of VC in cattle receiving supplemental VC.
In conclusion, plasma concentration and urinary excretion of VC increased with increasing dietary VC. Urinary excretion of VC was shown to be affected by plasma VC concentration. Plasma VC concentration can exceed the renal threshold for VC in cattle receiving supplemental VC, and urinary VC excretion is probably the major route of its elimination, which helps to regulate plasma VC concentration. In addition, it is likely that cattle absorb more than half of ingested HSO-coated VC but a large amount of absorbed VC is not utilized and is excreted in urine.

LITERATURE CITED


