## In situ ruminal digestibility of red-osier dogwood in finishing beef heifers

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SHORT COMMUNICATION: *In situ* ruminal digestibility of red-osier dogwood in finishing beef heifers

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Gomaa, W.M.S., Wei, L.Y., Mosaad, G.M., Alexander, T.W., Yang, W. Z. 2017. *In situ* ruminal digestibility of red osier dogwood in finishing beef heifers. An in situ study was conducted to determine the effect of feeding red osier dogwood (ROD) on ruminal digestion of barley, ROD and barley silage in beef heifers. Heifers were fed diets that varied by substituting ROD for barley silage at 0, 3, 7 or 10%. Slowly degradable fraction and effective degradability...
(ED) of ROD crude protein (CP) linearly \( (P < 0.02) \) increased with increasing ROD. The ED of CP of barley and barley silage was reduced \( (P < 0.01) \) by feeding ROD. These results indicate that feeding ROD potentially reduce ruminal protein degradability, thereby improve protein efficiency.

**Key words:** beef heifers, in situ rumen digestion, red osier dogwood

**Abbreviations:** \( a \), soluble fraction; \( b \), slowly degradable fraction; \( c \), fractional disappearance rate constant at which \( b \) is degraded; \( CP \), crude protein; \( DM \), dry matter; \( ED \), effective ruminal degradability; \( NDF \), neutral detergent fibre; \( ROD \), red osier dogwood.

Red osier dogwood (ROD; *Cornus sericea*) is native shrub plant across Northern America. It grows on marginal land and is abundant in low wetlands and pasture land (Isaak et al. 2013). It is one of the most valuable browse species used for food by various wild animals, and is palatable at all growth stages (Scales 2015). Currently, there are some farmers in Manitoba cultivating ROD for use as animal feed. Once it has been established, ROD can be harvested annually year round. There is no need for fertilizer, herbicides, fungicides or any other inputs except for harvesting and delivery charges. New machinery is currently being developed with the ability to harvest and process ROD, in order to meet the large amounts required for use as cattle feed.

The ROD can be fed to cattle as forage source with high feeding value. A farm demonstration carried out in Manitoba showed that feeding heifers 0.9 kg day\(^{-1}\) of ROD (dry matter [DM] basis), in place of barley silage, resulted in an increased daily gain of 0.23 kg compared to cattle given no ROD, over a period of 38 d on feed (Scales 2015). The ROD is rich in bioactive compounds with total phenolic concentrations varying from 4 to 22% of DM depending on the growing season (Isaak et al. 2013). The phenolics include anthocyanins, gallic
acid, ellagic acid, quercetin, kaempferol, and cyanin, which have been shown to display both antioxidant and antimicrobial properties (Zeb 2016). Studies demonstrated that feeding ROD may reduce the use of antibiotics in weaned pigs, and reduce the incidence of both diarrhea and mortality in rabbits (Scales 2015). Therefore, feeding ROD can potentially be used in livestock animals, as an alternative to antibiotics.

Information about the digestibility of ROD in the rumen of cattle, and the effect of feeding ROD on ruminal microbial activity is scarce (Isaak et al. 2013). The present study was conducted to evaluate the effect of increasing substitutions of ROD for barley silage in a high-grain diet, on in situ ruminal digestion kinetics in beef heifers.

The animals used in this study were cared for in accordance with the guidelines set by Canadian Council on Animal Care (Ottawa, ON, Canada). Five ruminally cannulated Angus beef heifers (body weight = 660 ± 40.8 kg) were used in a 5 × 5 Latin square design with 21 d periods, that included 14 d of adaptation and 7 d of measurements. The measurements consisted of in situ ruminal digestion (d 1 and 2), ruminal fermentation and nutrient digestibility in the total digestive tract (d 3 to 7) in each period. Data with ruminal fermentation and digestibility were reported elsewhere. The five experimental diets were: control diet containing 15% barley silage, 82% barley grain and 3% vitamin and mineral supplement (DM basis), control diet that substituted 3%, 7% or 10% of ROD for equal proportions of barley silage, respectively, or control diet supplemented with monensin (28 mg kg$^{-1}$ diet DM). Monensin is widely used in beef cattle diets to improve ruminal fermentation in North American feedlots, and was used as positive control. The chemical composition (DM basis) was similar among the diets and were 95.2% for organic matter, 24.9% for neutral detergent fibre (NDF), 54.9% for starch, and 13.3% for CP. Heifers were fed a total mixed ration ad libitum once daily in the morning at 10:00 h.
The ROD used in this study was provided by Red Dog Enterprise Ltd. (Winnipeg, MB, Canada). It was immature, grown for less than one year, and consisted approximately of 75% leaves, 10% bark, and 15% stem. Samples of barley grain and barley silage for in situ evaluation were collected from the same feeds offered to the experimental animals. Barley grain and ROD were ground through a 4-mm sieve, and fresh silage was processed by grinding twice for 20s with a Knifetec 1095 sample mill (Foss Tecator, Höganäs, Sweden). Rumen digestion kinetics were determined using the in situ method. Briefly, 7 g of ground barley, 5 g of ROD or 5 g of barley silage (DM basis) were separately weighted into bags (10 × 20 cm) made of monofilament PeCAP polyester screen (pore size of 50 ± 3 µm). Duplicate bags were incubated in the rumen of each heifer for each incubation time of 0, 3, 6, 12, 24, and 48 h in reverse order of incubation times so that all bags were removed simultaneously. Upon removal, the bags were washed with running tap water and were oven-dried at 55°C for 48 h for determining DM disappearance. The residues from the bags belonging to the same feed, and incubated in the same animal, were pooled and ground through a 1-mm sieve for NDF and CP analysis.

Ruminal kinetics were estimated using the nonlinear regression procedure of SAS (SAS Inst. Inc., Cary, NC) using the equation:

\[ y = a + b \left( 1 - e^{-c(t - \text{lag})} \right) \text{ for } t > \text{lag} \]

where \( y \) is the ruminal disappearance at time \( t \) (%); \( a \) = soluble fraction; \( b \) = slowly degradable fraction; \( c \) = fractional disappearance rate constant at which \( b \) is degraded; \( \text{lag} \) = lag time (h), and \( t \) = time of incubation (h). Effective ruminal degradability (ED) of DM and CP was calculated using the equation: \( \text{ED} = \frac{a + b c}{c + k} \) where \( k \) is ruminal flow rate which was assumed to be 6% h\(^{-1}\) for grain and 3% h\(^{-1}\) for ROD and silage.
Data were analyzed using the MIXED procedure of SAS software with the model including fixed effect of diets and random effects of period and heifers. Contrasts were generated to compare the average of three ROD doses and monensin supplementation. The effect of increasing ROD inclusion rates was examined through linear and quadratic orthogonal contrasts using the CONTRAST statement of SAS. The PDIFF option was included in the LSMEANS statement to account for multiple comparisons. Effects of the fixed factor were considered significant at P ≤ 0.05, and trends were discussed at 0.05 < P ≤ 0.10.

The chemical composition (% of DM) of ROD, barley and barley silage were, respectively, 92.1, 97.6 and 91.2 for organic matter; 37.3, 20.3 and 44.7 for NDF; and 8.8, 15.4 and 13.6 for CP. Compared with silage, ROD obviously had less NDF and CP content. The ROD contained 12.3% total phenolics including 0.54% gallic acid, 0.92% methyl gallate, 0.15% catechin, 0.80% epicatechin, 0.83% rutin, 0.45% ellagic acid, and 0.72% quercetin. Gallic acid, ellagic acid and quercetin were previously reported to have antioxidant activity (Zeb 2016). The content of total and individual phenolic compounds of ROD vary substantially with growing season. For example, Isaak et al. (2013) reported that rutin content was low in summer and peaked in October whereas quercetin content was high in spring and decreased in summer. Plant parts (leaves, bark, stems) that differ in nutrients or bioactive components also vary in proportion with growing season, thus, the time of harvesting can impact the bioactivity in ROD substantially.

Ruminal in situ DM digestion kinetic parameters of ROD and NDF disappearance were not different (Table 1). However, fraction “b” of CP of ROD linearly (P < 0.02) increased with increasing dietary ROD, and the rate constant was less (P < 0.03) with ROD diets (3.4% h⁻¹) than the control (4.0% h⁻¹). As a result, ED of CP linearly (P < 0.01) increased with increasing ROD in the diets. The DM digestion kinetic parameters of ROD are in the range reported from studies
using hays. Rodrigues et al. (2007) analyzed six meadow hays to determine the correlation of phenolic contents to in situ ruminal digestion kinetics of DM, and reported that the values ranged from 14 to 19% for “a”, 47 to 60% for “b”, 2.1 to 3.3% h\(^{-1}\) for “c”, and 36 to 40% for ED. Those authors indicated that phenolic acid concentration in feed was negatively correlated to the rate constant. Although Rodrigues et al. (2007) showed greater “b” but lesser “c” and ED of DM values for the six meadow hays compared to those of ROD in our study, the values for ROD were likely due to its high NDF content (range of 66 to 70%). In support of this, Arieli et al. (1999) reported that parameters of “a” (14%) and “c” (5.3% h\(^{-1}\)) of DM for vetch hay were higher than the “a” (6.9%) and “c” values (2.1% h\(^{-1}\)) for wheat hay because of the greater NDF content of the wheat hay (63% versus 43% for vetch and wheat hays, respectively). Combined, this suggests that the fibre content of the feed was the primary factor to influence the ruminal digestibility of ROD, rather than the phenolic concentration of ROD. The digestion kinetics were not different between ROD and monensin treatments.

Increasing dietary ROD tended \((P < 0.06)\) to decrease the “a” fraction of barley DM but linearly \((P < 0.03)\) increased the “b” fraction. As a result, the ED of DM was greater \((P < 0.05)\) with low or high ROD than the control diet (Table 2). Processed barley grain is highly degradable in the rumen. However, in situ DM digestion of barley grain has varied among studies depending on the variety and particle size of processed barley being tested. Khorasani et al. (2000) reported that in situ DM digestion kinetics of a, b, and c ranged, respectively, from 33 to 56%, 34 to 60% and 27 to 62% h\(^{-1}\) for 60 different barley varieties that were all ground through a 2-mm sieve. In contrast, Zhao et al. (2015) found lower fractions of “a” (2.8 to 4.7%) and slower rate constants of “c” (6.4 to 7.8% h\(^{-1}\)), but greater “b” (73 to 77%) when barley grain was coarsely dry-rolled. In the present study, the barley grain was ground through a 4-mm sieve
thus it was expected that the DM digestion kinetics that we reported were intermediate compared to the studies by Khorasani et al. (2000) and Zhao et al. (2015). Because high soluble fraction increases the susceptibility of ruminants to acidosis (Beauchemin et al. 2001), the decreased soluble fraction and the increased slowly degradable fraction of barley grain by feeding ROD could be beneficial to cattle fed high-grain diets. Feeding ROD linearly \((P < 0.01)\) decreased the ED of CP and the NDF disappearance of barley.

With barley silage, the in situ digestion kinetics of DM and NDF disappearance at 24 or 48 h of incubation were not affected by feeding ROD (Table 2). Whereas, increasing dietary ROD linearly \((P < 0.01)\) decreased \(c\) and ED of CP. Adding ROD to the high-grain diet consistently reduced protein degradation of barley grain and silage. The reduction in ruminal protein degradability by feeding ROD may be attributed to the protein-binding capacity of phenolic compounds (Cattani et al. 2012). The decrease in protein degradability, thus increase in ruminal by-pass protein, may be beneficial either to improve protein efficiency or to reduce ammonia absorption through the rumen. Consequently, this could decrease urinary nitrogen excretion which is more volatile than the nitrogen excreted in feces. Overall, the effects of ROD on DM and NDF digestion in the rumen appeared to be mixed, but for some factors had a dose-dependent effect. Our results demonstrated likely that the low dose of ROD numerically improved NDF digestion of barley grain, whereas, there was no difference in NDF digestion between the control and low dose of ROD for barley silage.

Hino et al. (1993) reported that microbial growth and fibre digestibility decreased with a high dose \((30–40 \text{ mg L}^{-1})\) but improved with a low dose \((2.5–5 \text{ mg L}^{-1})\) of two antioxidants (a-tocopherol and b-carotene). Cattani et al. (2012) showed that increasing dosages of a blend of natural phenols extracted from red chicory altered digestion and they suggested that the phenols
could have induced a shift in the partition of energy, with a greater proportion of nutrients channeled towards microbial protein synthesis. Monensin is also known to inhibit ruminal protein degradation and decrease the flow of microbial protein to the intestine through its actions on the rumen microbiota. In our study, there was a similar effect for ROD and monensin on protein degradability, suggesting that ROD also acts to inhibit protein degradation, though it is not known if this was due to altering the microbiota. The differences in NDF digestion between ROD and monensin, however suggests that they have different modes of action for altering NDF digestion.

In conclusion, ROD contains high levels of phenolic compounds and showed comparable ruminal degradability with barley silage. Increasing dietary ROD linearly decreased the soluble fraction and increased the slowly degradable fraction of barley grain without adversely impacting degradability in the rumen. The high soluble fraction in grain diets increases cattle susceptibility to acidosis, thus feeding ROD may mitigate this effect by reducing the soluble fraction. Lastly, the results indicated that feeding ROD has potential to reduce dietary protein degradability in the rumen, and to improve protein efficiency.

Table 1. The in situ digestion kinetics of red osier dogwood (ROD) in beef heifers fed a high-grain diet with varying levels of ROD

<table>
<thead>
<tr>
<th>Item</th>
<th>Diets&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, %</td>
<td>16.3</td>
<td>18.4</td>
<td>18.3</td>
</tr>
<tr>
<td>b, %</td>
<td>41.2</td>
<td>35.0</td>
<td>35.1</td>
</tr>
<tr>
<td>c, % h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>3.91</td>
<td>5.12</td>
<td>5.78</td>
</tr>
<tr>
<td>ED, %</td>
<td>57.1</td>
<td>53.1</td>
<td>54.1</td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, %</td>
<td>8.2</td>
<td>9.7</td>
<td>8.5</td>
</tr>
<tr>
<td>b, %</td>
<td>39.9&lt;sup&gt;ab&lt;/sup&gt; 36.1&lt;sup&gt;b&lt;/sup&gt; 43.5&lt;sup&gt;a&lt;/sup&gt; 43.9&lt;sup&gt;a&lt;/sup&gt; 43.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.57</td>
<td>0.02</td>
</tr>
<tr>
<td>c, % h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;a&lt;/sup&gt; 3.30&lt;sup&gt;b&lt;/sup&gt; 3.53&lt;sup&gt;b&lt;/sup&gt; 3.47&lt;sup&gt;b&lt;/sup&gt; 3.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.149</td>
<td>0.03</td>
</tr>
<tr>
<td>ED, %</td>
<td>47.8&lt;sup&gt;ab&lt;/sup&gt; 45.5&lt;sup&gt;b&lt;/sup&gt; 51.6&lt;sup&gt;a&lt;/sup&gt; 53.7&lt;sup&gt;a&lt;/sup&gt; 52.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52</td>
<td>0.01</td>
</tr>
<tr>
<td>NDF, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>26.2</td>
<td>18.5</td>
<td>22.1</td>
</tr>
<tr>
<td>48 h</td>
<td>34.0</td>
<td>36.3</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Note: Means within a row not sharing a lowercased italic letter differ significantly at the P < 0.05 level.

<sup>a</sup>Diet was substituted with 0, 3, 7 or 10% of ROD for equal proportion of barley silage; Mon was diet (0%) supplemented with 28 mg monensin kg<sup>-1</sup> diet DM.

<sup>b</sup>L, Q = linear or quadratic effect of increasing replacement of silage with ROD (0, 3, 7 and 10%); ROD vs. Mon = contrast between average of ROD and Mon.

<sup>c</sup>Standard error of mean.
### Table 2. The in situ digestion kinetics of barley grain and barley silage in beef heifers fed a high-grain diet with varying levels of red osier dogwood (ROD)

<table>
<thead>
<tr>
<th>Item</th>
<th>Diets(^a)</th>
<th>SEM(^c)</th>
<th>P value(^b)</th>
<th>Diet</th>
<th>L</th>
<th>Q</th>
<th>ROD vs Mon</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>10</td>
<td>Mon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley grain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, %</td>
<td>16.6</td>
<td>14.9</td>
<td>14.6</td>
<td>14.7</td>
<td>16.0</td>
<td>1.53</td>
<td>0.08</td>
</tr>
<tr>
<td>b, %</td>
<td>69.3(b)</td>
<td>73.3(b)</td>
<td>72.1(a)</td>
<td>73.1(a)</td>
<td>71.0(ab)</td>
<td>2.65</td>
<td>0.03</td>
</tr>
<tr>
<td>c, % h(^{-1})</td>
<td>28.0</td>
<td>27.4</td>
<td>24.5</td>
<td>25.9</td>
<td>30.7</td>
<td>3.20</td>
<td>0.23</td>
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<tr>
<td>ED, %</td>
<td>85.7(b)</td>
<td>88.0(a)</td>
<td>86.5(b)</td>
<td>87.6(a)</td>
<td>86.9(ab)</td>
<td>1.18</td>
<td>0.05</td>
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<tr>
<td>CP</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>a, %</td>
<td>14.0</td>
<td>11.6</td>
<td>12.4</td>
<td>11.8</td>
<td>11.9</td>
<td>1.77</td>
<td>0.35</td>
</tr>
<tr>
<td>b, %</td>
<td>63.6</td>
<td>66.4</td>
<td>61.9</td>
<td>64.1</td>
<td>64.4</td>
<td>1.61</td>
<td>0.10</td>
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<tr>
<td>c, % h(^{-1})</td>
<td>12.3</td>
<td>11.5</td>
<td>12.6</td>
<td>11.5</td>
<td>13.6</td>
<td>1.07</td>
<td>0.60</td>
</tr>
<tr>
<td>ED, %</td>
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<td>77.7(a)</td>
<td>74.0(b)</td>
<td>75.5(ab)</td>
<td>76.1(ab)</td>
<td>0.64</td>
<td>0.01</td>
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<tr>
<td>NDF, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>49.7</td>
<td>53.1</td>
<td>49.4</td>
<td>48.8</td>
<td>51.4</td>
<td>4.71</td>
<td>0.35</td>
</tr>
<tr>
<td>48 h</td>
<td>53.7(ab)</td>
<td>56.6(a)</td>
<td>52.6(b)</td>
<td>52.3(b)</td>
<td>56.9(a)</td>
<td>5.13</td>
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<tr>
<td>Barley silage</td>
<td></td>
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<tr>
<td>DM</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, %</td>
<td>29.1</td>
<td>29.5</td>
<td>30.1</td>
<td>28.9</td>
<td>29.7</td>
<td>0.77</td>
<td>0.27</td>
</tr>
<tr>
<td>b, %</td>
<td>24.8</td>
<td>24.8</td>
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<td>19.0</td>
<td>29.3</td>
<td>2.88</td>
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<tr>
<td>c, % h(^{-1})</td>
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<td>6.7</td>
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<td>58.9</td>
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<tr>
<td>a, %</td>
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<td>48.9</td>
<td>49.7</td>
<td>48.4</td>
<td>46.5</td>
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<td>0.10</td>
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<td>b, %</td>
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<td>16.3</td>
<td>16.1</td>
<td>15.1</td>
<td>17.6</td>
<td>0.91</td>
<td>0.22</td>
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<tr>
<td>c, % h(^{-1})</td>
<td>9.5(a)</td>
<td>8.6(ab)</td>
<td>7.5(b)</td>
<td>7.9(b)</td>
<td>5.9(c)</td>
<td>0.37</td>
<td>0.01</td>
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<tr>
<td>ED, %</td>
<td>66.0(a)</td>
<td>65.1(a)</td>
<td>65.7(b)</td>
<td>63.5(b)</td>
<td>64.0(ab)</td>
<td>1.10</td>
<td>0.03</td>
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<tr>
<td>NDF, %</td>
<td></td>
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<tr>
<td>24 h</td>
<td>15.8</td>
<td>17.9</td>
<td>19.7</td>
<td>18.2</td>
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<td>2.26</td>
<td>0.04</td>
</tr>
<tr>
<td>48 h</td>
<td>28.4(ab)</td>
<td>27.2(b)</td>
<td>24.6(b)</td>
<td>21.8(b)</td>
<td>35.8(a)</td>
<td>4.27</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Note:** Means within a row not sharing a lowercased italic letter differ significantly at the \(P < 0.05\) level.

\(^a\)Diet was substituted with 0, 3, 7 or 10% of ROD for equal proportion of barley silage; Mon was diet (0%) supplemented with 28 mg monensin kg\(^{-1}\) diet DM.

\(^b\)L, Q = linear or quadratic effect of increasing replacement of silage with ROD (0, 3, 7 and 10%); ROD vs. Mon = contrast between average of ROD and Mon.

\(^c\)Standard error of mean.