Carbohydrate fermentation and nitrogen metabolism of a finishing beef diet by ruminal microbes in continuous cultures as affected by ethoxyquin and(or) supplementation of monensin and tylosin

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ABSTRACT: Long-term feedlot studies have shown positive effects (i.e., improved ADG and reduced morbidity and mortality) of dietary supplementation with ethoxyquin (AGRADO). This may be due to improving the antioxidant capacity at the ruminal, postruminal, or postabsorption levels. This study was designed to investigate the role of ethoxyquin at the rumen level. A finishing diet (12.5% CP; DM basis) was formulated to contain (on a DM basis) 77.5% flaked corn, 10% corn cobs, 10% protein/vitamin/mineral supplement, and 2.5% tallow. In a randomized complete block design experiment, the treatments were arranged as a 2 × 2 factorial. The main factors were two ethoxyquin treatments (without or with 150 ppm) and two monensin/tylosin treatments (without or with monensin and tylosin at 0.0028 and 0.0014% of dietary DM, respectively). Eight dual-flow, continuous culture fermenters were used in two experimental periods (blocks; 8 d each with 5 d for adjustment and 3 d for sample collection) to allow for four replications for each treatment. No inter-actions (P > 0.05) were detected for any of the measurements evaluated. Therefore, results of the main factors were summarized. Ethoxyquin supplementation improved (P < 0.05) true digestibility of OM (from 38.8 to 45.0%) but it did not alter (P > 0.05) concentrations of total VFA (averaging 131 mM) or acetate (averaging 58.8 mM). Ethoxyquin decreased (P < 0.05) propionate concentration from 51.1 to 42.4 mM and increased (P < 0.05) butyrate concentration from 18.4 to 22.9 mM. Digestion of total nonstructural carbohydrates was not altered (P > 0.05) by the treatments and averaged 86%. With the exception of increased (P < 0.05) concentration of propionate (from 42.0 to 51.5 mM) and decreased (P < 0.05) concentration of butyrate (from 25.9 to 16.3 mM), no effects (P > 0.05) were detected for monensin/tylosin. Ruminal N metabolism, including efficiency of bacterial protein synthesis (averaging 21.2 g N/kg OM truly digested), was not affected (P > 0.05) by the treatments. Results suggest positive effects of ethoxyquin on ruminal digestion of OM and unique changes in VFA production.

Key Words: Ethoxyquin, Nitrogen Metabolism

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Introduction

The synthetic antioxidant ethoxyquin (6-ethoxyl-1, 2-dihydro-2, 2, 4-trimethylquinoline) has been used in feedstuffs since the 1950s (Coelho, 1995). Recently, ethoxyquin supplementation of feedlot diets increased ADG (Krumsieck and Owens, 1998a; McBride, 2000), decreased morbidity, mortality, and medication cost (Stovall et al., 1999; Kegley et al., 2000), improved beef shelf life (Krumsieck and Owens, 1998b), and reduced beef rancidity (Walenciak et al., 1999). Beef cattle response to ethoxyquin, however, has not been always consistent. For example, feedlot performance was not affected when ethoxyquin was added to the starting diets of beef steers (Kegley et al., 2000) or heifers (Stovall et al., 1999).

The positive feedlot responses to ethoxyquin with beef cattle could be explained by its potential antioxidant role at the gastrointestinal tract level and(or) at the postabsorption level. At the gastrointestinal tract, ethoxyquin may improve fermentation patterns (e.g., production or composition of VFA) in the rumen and the colon and(or) enhance the antioxidant capacity and health of the intestinal mucosa. This study was designed to assess the effects of ethoxyquin at the ruminal...
level. Because a large number of finishing diets contain monensin and tylosin, it was important to examine the possible interactions between ethoxyquin and these feed additives, especially monensin. Monensin improves ruminal fermentation through its action on bacterial transmembrane ion fluxes and dissipation of cation and proton gradients (Bergen and Bates, 1984). Because ethoxyquin can protect the integrity of bacterial cell membrane against oxidation, it was hypothesized that its effect on ruminal fermentation may be additive to that of monensin. Therefore, the objective was to determine the effects of ethoxyquin alone or in combination with monensin and tylosin on ruminal fermentation characteristics of a finishing beef diet.

**Materials and Methods**

*Animals and Collection of Rumen Fluid.* Two ruminally cannulated, mature Angus steers were used as donors of rumen fluid to be used as the inoculum for the dual-flow, continuous culture fermenter system. The steers were gradually (1 mo) adapted to a high-concentration finishing diet (containing 90% corn and 10% alfalfa hay on a DM basis) and then had ad libitum access to this diet for 2 wk before and throughout the study. This diet was formulated to meet or exceed the nutrient requirements of the steers (NRC, 1996). The rumen fluid was collected (by using a vacuum pump) from each steer approximately 2 h after feeding (0800) and strained immediately after removal from the rumen through four layers of cheesecloth into a prewarmed, insulated container.

*Continuous Culture System and Operation.* The dual-flow, continuous culture fermenter system was developed (Hoover et al., 1976) and modified (Hannah et al., 1986) to simulate differential solid-liquid removal rates occurring in the rumen environment. Evaluation and validation of the efficacy of this system in simulating ruminal fermentation in cattle (Hannah and Stern, 1985; Hannah et al., 1986) or sheep (Hussein et al., 1991a,b) were documented. Such evaluation revealed fermentation characteristics similar to those obtained in vivo when the same diets were tested.

Fermenters (1,020 mL working volume each) were equipped with an automated feeding system and were continuously infused with a mineral buffer solution (Weller and Pilgrim, 1974) containing urea (0.5 g/L) at a rate of 1.5 mL/min to obtain a liquid dilution rate of 0.082/h. Solid (overflow) dilution rate was maintained at 0.041/h by removing liquid through a filter at 0.75 mL/min. The liquid (Poore et al., 1990; Streeter et al., 1995) and solid (Streeter et al., 1995) dilution rates were based on in vivo studies with diets containing forage:concentrate ratios similar to those evaluated in this study. A pH of 6.0 ± 0.05 was maintained by automated infusion of 3 N HCl or 5 N NaOH regulated by a pH controller (Cole-Parmer, Vernon Hills, IL). The pH of 6.0 was chosen as the average daily value of ruminal pH of steers fed diets containing similar forage:concentrate ratios (Streeter et al., 1995; Calderon-Cortes and Zinn, 1996; Zinn and Shen, 1996). Anaerobic conditions were achieved by continuous infusion of N₂ at a rate of 40 mL/min. Maintaining the fermenters’ temperature at 39°C and mixing of their contents were achieved by using VirTis Omni-Culture fermenter base units (The VirTis Company, Gardiner, NY).

Upon arrival at the laboratory, the rumen fluids from both steers were combined (on an equal volume basis) and used to inoculate the eight fermenters. Each fermenter was supplied daily with 75 g DM of a ground (2-mm screen) diet by an automated feeding mechanism adjusted to deliver the diet in 12 equal portions over a 24-h period to establish steady-state conditions.

*The Finishing Diet and Treatments.* The finishing diet was formulated to contain 12.5% CP on a DM basis. This diet was previously evaluated (McBride, 2000) without (control) or with ethoxyquin (150 ppm). The only exception was replacing cottonseed hulls with corn cobs (similar in their nutritional value) to avoid the delivery problems of cottonseed hulls in the automated feeding mechanism used in our continuous culture system. Table 1 shows the ingredient composition of the four experimental diets evaluated in this study. Treatments (Table 1) were arranged as a 2 × 2 factorial with the main factors being two ethoxyquin treatments (without or with 150 ppm [DM basis] from AGRADO; Solutia Inc., St. Louis, MO) and two monensin/tylosin treatments (without or with monensin and tylosin at 0.0028 and 0.0014% of dietary DM, respectively). The levels of monensin and tylosin used in this study were those commonly fed to finishing cattle. The maximum concentration of ethoxyquin in feeds permitted by the FDA is 150 ppm (except for dog food, in which the maximum suggested concentration is 75 ppm). Each dietary ingredient was ground through a 2-mm screen before mixing each diet. Because the chemical analysis of the four diets was similar, the average values are presented in Table 2.

*Experimental Design.* The experimental design was a randomized complete block design (Steel et al., 1997) and consisted of two experimental periods (blocks) of 8 d each. The first 5 d of each period were used for stabilization (e.g., adaptation to the diet) and the last 3 d were used for sample collection. The four diets (Table 1) were allocated randomly to fermenters, giving two replications for each diet per period.

*Sample Collection and Preparation.* Solid and liquid fractions of the effluent were collected in two vessels submerged in a refrigerated water bath (2°C) to retard microbial metabolism. On each sampling day, both overflow and the filtered fraction of the effluent for each fermenter were combined and homogenized (T25 Basic Homogenizer; IKA Labortechnik, Wilmington, NC) for 5 min. A 600-mL sample was removed via vacuum aspiration during homogenization. At the end of each period, the samples of each fermenter from each of the 3 d were composited, and two 500-mL portions (to allow replication for DM determination) were lyophilized to
Table 1. Ingredient composition of finishing diets supplied to rumen bacteria in continuous culture fermenters

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE/MT</td>
<td>NE/NMT</td>
</tr>
<tr>
<td>Flaked corn</td>
<td>77.5</td>
<td>77.5</td>
</tr>
<tr>
<td>Corn cob</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Supplement A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.0</td>
<td>—</td>
</tr>
<tr>
<td>Supplement B&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>10.0</td>
</tr>
<tr>
<td>Tallow</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>AGRADO, dry</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup>The diets were with (E) or without (NE) ethoxyquin (150 ppm of dietary DM) provided by AGRADO (Solutia, Inc., St. Louis, MO) and/or were with (MT) or without (NMT) monensin and tylosin.

<sup>b</sup>A supplement containing (per kg of DM) cottonseed meal (743.6 g), calcium carbonate (110.0 g), urea (55.2 g), rice bran (50.0 g), salt (23.0 g), ammonium sulfate (9.2 g), dicalcium phosphate (3.1 g), ferrous sulfate (0.9 g), zinc sulfate (0.8 g), sodium selenite (0.8 g), manganese sulfate (0.6 g), vitamin E (0.5 g), copper sulfate (0.4 g), vitamins A and D3 (0.1 g), ethylenediamine dihydroiodide (0.04 g), cobalt sulfate (0.004 g), and rumensin 80 and tylan (to provide 0.0028% monensin and 0.0014% tylosin of dietary DM).

<sup>c</sup>A supplement similar in composition to supplement A with the exception of excluding rumensin 80 and tylan.

Table 2. Chemical composition of diets<sup>a</sup> supplied to rumen bacteria in continuous culture fermenters

<table>
<thead>
<tr>
<th>Item</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>96.8</td>
</tr>
<tr>
<td>CP</td>
<td>12.5</td>
</tr>
<tr>
<td>NDF</td>
<td>21.8</td>
</tr>
<tr>
<td>Ether extract</td>
<td>4.9</td>
</tr>
<tr>
<td>Total nonstructural carbohydrates</td>
<td>65.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Because the four diets evaluated were similar in their ingredient (Table 1) and chemical (data not shown) compositions, the average values are presented.

A constant weight. Dried samples were ground through a 1-mm screen and used for all subsequent analyses. During homogenization of the effluent, subsamples were taken with a wide-pore pipette for total N, NH<sub>3</sub>N, and VFA analyses.

Bacterial samples (fluid-associated bacteria) were collected from fermenter contents on the last sampling day of each experimental period (2 h post feeding) by straining the total content of each fermenter through eight layers of cheesecloth. This strained fluid was centrifuged at 500 x g for 20 min to remove feed particles and protozoal cells. Bacteria were separated from the supernate by centrifuging at 26,000 x g for 20 min. Bacterial samples then were lyophilized and ground using a mortar and pestle.

Sample Analyses. Absolute DM determination was conducted on freeze-dried digesta, bacterial, and diet samples by drying at 105°C for 24 h, followed by ashing at 500°C for 16 h in a muffle furnace to determine OM. The NDF (Jeraci et al., 1988) and ether extract (AOAC, 2000) concentrations of the diets were determined. Total nonstructural carbohydrates (TNC) in the diet and digesta samples were determined as described by Smith (1969). Effluent samples were prepared for VFA analysis by the procedure of Erwin et al. (1961). Concentrations of VFA were determined using a gas chromatograph (model 3800, Varian, Walnut Creek, CA) equipped with a glass column (180 cm x 4 mm i.d.) packed with GP 10% SP-1200/1% H<sub>3</sub>PO<sub>4</sub> on 80/100 Chromosorb W AW (Supelco, Bellefonte, PA). Helium was used as the carrier gas with a flow rate of 85 mL/min. The oven, injection port, and detector (FID) port temperatures were 125, 175, and 180°C, respectively.

Total N content of the diet, effluent, and bacterial samples were determined by the macro-Kjeldahl procedure (AOAC, 2000). Purine concentrations in effluent and bacterial samples were determined using the method of Zinn and Owens (1986). Concentrations of NH<sub>3</sub>N in the effluent were measured colorimetrically according to the procedure of Chaney and Marbach (1962). Digestibilities were calculated as described by Hannah and Stern (1985).

Statistical Analysis. The data (ruminal fermentation characteristics and flow of N fractions) were analyzed as a randomized complete block design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Because treatments were arranged as a 2 x 2 factorial, treatment sums of squares were separated into the main factors (ethoxyquin and monensin/tylosin) and their interactions. Because no interactions (P > 0.05) were detected for most of the measurements evaluated, means of the main factors were separated by ANOVA.

Results and Discussion

The diet examined in this study was previously evaluated in the feedlot (McBride, 2000). During a 182-d finishing period, beef steers receiving ethoxyquin had 10.9% greater (P < 0.05) ADG and 18% more (P < 0.05) DMI than those fed the control diet. During the last 14 d of the trial, steers receiving ethoxyquin gained more (P < 0.05) weight than those fed the control diet (1.53 vs 1.06 kg/d). Because of the significant and consistent
Digestibility of OM and TNC. Digestibility of OM and TNC are summarized in Table 3. No interactions ($P > 0.05$) between ethoxyquin and monensin/tylosin supplementations were detected and, therefore, results of the main factors are presented. Apparent OM digestibility tended to increase ($P = 0.05$) with feeding ethoxyquin. Feeding ethoxyquin improved ($P < 0.05$) true OM digestibility (corrected for bacterial OM in the effluent) by 16.0%. No studies on site and extent of digestion of diets containing ethoxyquin were found. However, performance studies with feedlot cattle have shown improvement in ADG and DMI (McBride, 2000) with feeding ethoxyquin. The diets were without or with 0.0028 and 0.0014% of dietary DM, respectively.

Concentrations of VFA and NH$_3$ N in the Effluent. Concentrations of VFA and NH$_3$ N in the effluent are presented in Table 4. With the exception of butyrate and isobutyrate, no interactions ($P > 0.05$) between ethoxyquin and monensin/tylosin supplementations were detected for concentrations of total or individual VFA. Concentrations of butyrate and isobutyrate were highest ($P < 0.05$) for the diet containing ethoxyquin without monensin/tylosin (30.6 and 0.33 mM, respectively) and were lowest ($P < 0.05$) for the diet containing monensin/tylosin without ethoxyquin (15.5 and 0.12 mM, respectively). Table 4 summarizes the effects of the main factors on concentrations of VFA and NH$_3$ N in the effluent. Concentrations of total VFA and acetate were not affected ($P > 0.05$) by ethoxyquin or monensin/tylosin supplementation and averaged 131 and 59 mM, respectively. Feeding ethoxyquin decreased ($P < 0.05$) propionate concentration by 17% and increased ($P < 0.05$) butyrate concentration by 25%. Monensin/tylosin supplementation increased ($P < 0.05$) propionate concentration by 23% and decreased ($P < 0.05$) butyrate concentration by 37%.

No effects were detected ($P > 0.05$) for ethoxyquin or monensin/tylosin supplementations on concentrations of isovalerate or valerate (Table 4). The average values were 0.47 and 3.77 mM, respectively. Table 4 also shows that isobutyrate concentration was increased ($P < 0.05$) by 85% with feeding ethoxyquin, and it was decreased ($P < 0.05$) by 39% with monensin/tylosin supplementation. Because isobutyrate is produced by the deamination of the branched-chain amino acid valine (Harwood and Canale-Parola, 1981), ethoxyquin appeared to enhance the ruminal degradation of dietary valine without affecting the rate of degradation of other branched-chain amino acids. It is also possible that ethoxyquin...
Table 4. Effects of ethoxyquin and monensin/tylosin supplementations on concentrations of VFA and NH$_3$ N when finishing diets were supplied to rumen bacteria in continuous culture fermenters

<table>
<thead>
<tr>
<th>Item</th>
<th>Ethoxyquin$^a$</th>
<th>Monensin/tylosin$^b$</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With</td>
<td>Without</td>
<td>With</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>128</td>
<td>135</td>
<td>131</td>
</tr>
<tr>
<td>Individual VFA, mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>57.5</td>
<td>60.5</td>
<td>58.2</td>
</tr>
<tr>
<td>Propionate$^{c,d}$</td>
<td>42.4</td>
<td>51.1</td>
<td>51.5</td>
</tr>
<tr>
<td>Butyrate$^{c,d}$</td>
<td>22.9</td>
<td>18.4</td>
<td>16.3</td>
</tr>
<tr>
<td>Isobutyrate$^{c,d}$</td>
<td>0.24</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>0.48</td>
<td>0.46</td>
<td>0.36</td>
</tr>
<tr>
<td>Valerate</td>
<td>3.31</td>
<td>4.23</td>
<td>3.96</td>
</tr>
<tr>
<td>NH$_3$ N, mg/100 mL$^d$</td>
<td>1.93</td>
<td>1.99</td>
<td>1.79</td>
</tr>
</tbody>
</table>

$^a$The diets were without or with 150 ppm of ethoxyquin (DM basis).
$^b$The diets were without or with monensin and tylosin at 0.0028 and 0.0014% of dietary DM, respectively.
$^c$Ethoxyquin effect ($P < 0.05$).
$^d$Monensin/tylosin effect ($P < 0.05$).

reduced isobutyrate utilization. Based on the limited information available on the mode of ethoxyquin action at the rumen level, no explanation for this effect can be provided. It should be noted, however, that the reduced ($P < 0.05$) degradation of valine to isobutyrate when monensin/tylosin were supplemented is consistent with the role of monensin in decreasing protein degradation in the rumen environment (Whetstone et al., 1981).

Only one study (McBride, 2000) on the effect of ethoxyquin on ruminal concentrations of VFA was found. In his study, steers were fed a finishing diet (similar in composition to ours) without or with ethoxyquin at the same level (150 ppm) used in our study. Concentrations of total VFA and molar proportions of individual VFA on d 28 of feeding ethoxyquin were reported. Similar to our findings (Table 4), feeding ethoxyquin did not alter ($P > 0.05$) total VFA concentrations. McBride (2000) also reported that ethoxyquin did not affect ($P > 0.05$) molar proportions of VFA. Our results on the effects of ethoxyquin on molar proportions of VFA (data not shown) also showed that ethoxyquin did not alter ($P > 0.05$) molar proportions of acetate, isovalerate, or valerate. Our results (data not shown), however, showed that ethoxyquin decreased ($P < 0.05$) the proportion of propionate from 38.1 to 32.5 mol/100 mol and increased ($P < 0.05$) the proportions of butyrate from 13.6 to 19.6 mol/100 mol and isobutyrate from 0.10 to 0.19 mol/100 mol. In our study, VFA concentrations were based on effluent samples collected over the 24 h of the day. In the study of McBride (2000), however, VFA concentrations were based on one-time sampling (on d 28 of feeding the experimental diets) from the ventral blind sac of the rumen by rumenocentesis (using a stainless steel needle and a syringe).

The effects of monensin/tylosin on molar proportions of VFA in our study (data not shown) were consistent with those concentrations in Table 4. No interactions ($P > 0.05$) between ethoxyquin and monensin/tylosin supplementations were detected for the acetate:propionate ratio in the effluent (data not shown). Although ethoxyquin did not affect ($P > 0.05$) acetate:propionate ratio (the average value was 1.36), monensin/tylosin supplementation decreased ($P < 0.05$) it from 1.57 to 1.14. The increase in propionate concentration and the decrease in the acetate:propionate ratio observed in our study when monensin/tylosin were supplemented are consistent with the well-established effects of monensin on shifting ruminal fermentation by selecting for bacterial species that produce propionate (Chen and Wolin, 1979).

No interactions ($P > 0.05$) between ethoxyquin and monensin/tylosin supplementations were detected for NH$_3$ N concentrations in the effluent. Therefore, the effects of the main factors are presented in Table 4. Ethoxyquin feeding did not affect ($P > 0.05$) NH$_3$ N concentrations, which averaged 1.96 mg/100 mL. Monensin/tylosin supplementation, however, reduced ($P < 0.05$) NH$_3$ N concentration in the effluent by 16%.

No interactions ($P > 0.05$) between ethoxyquin and monensin/tylosin supplementations were detected for any of the N metabolism measurements evaluated. Therefore, the effects of the main factors are presented in Table 5. Results showed that neither ethoxyquin nor monensin/tylosin affected ($P > 0.05$) any of the measurements in Table 5. The NH$_3$ N fraction of total N in the effluent was very small and ranged from 0.04 to 0.05 g/d. This reflects the very low CP degradation of dietary protein (averaging 23.6%). This may be explained by the high level of dietary protein (54%) derived from corn, and corn protein is known for its low ruminal CP degradation (35%; NRC, 1985). As a result, a very high flow of non-NH$_3$ N from dietary origin was observed (1.30 g/d; 67.7% of total non-NH$_3$ N). Efficiency of bacterial protein synthesis was not altered ($P > 0.05$) by ethoxyquin or monensin/tylosin supplementation and averaged 21.2 g N/kg OM truly digested. Readily fermentable energy (from starch) was not limited when...
our diets were fed (65.8% TNC on DM basis). Therefore, it appears that bacterial protein synthesis was limited because of the very low concentrations of NH₃ N (averaging 1.96 mg/100 mL) in the effluent. Early studies (Satter and Slyter, 1974) indicated that bacterial protein synthesis was reduced when ruminal concentrations of NH₃ N were lower than 5 mg/100 mL.

In recent years, the practice of ethoxyquin supplementation (at the approved 150 ppm level) of beef cattle diets has shown several benefits. For example, Krumsiek and Owens (1998a) and McBride (2000) showed 5% and 11% increases in ADG when ethoxyquin was fed. These feedlot benefits may have been due to increased true OM digestibility when ethoxyquin was fed (Table 3).

**Implications**

Results of this study illustrate the positive effects of dietary supplementation of the antioxidant ethoxyquin (150 ppm) on ruminal digestion of organic matter with no benefits on ruminal protein metabolism. These results may explain why feedlot cattle in several investigations gained faster or consumed more feed when ethoxyquin was fed. The modes of such actions, however, remain to be elucidated.

**Literature Cited**


