Time-dependent changes in plasma concentrations of 3-methylindole and blood concentrations of 3-methyleneindolenine-adduct in feedlot cattle

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Objective—To describe time-dependent changes in plasma concentrations of 3-methylindole (3MI) and blood concentrations of 3-methyleneindolenine (3MEIN)-adduct in feedlot cattle.

Animals—64 yearling steers.

Procedures—Steers were assigned to 2 groups (32 steers/group). During the first 8 weeks, blood samples were collected from group 1 before the morning ration was fed, whereas samples from group 2 were collected 2 to 3 hours after the ration was fed. Blood samples were collected from all steers approximately 4 times/wk for 3 weeks and 3 times/wk for the subsequent 5 weeks. Samples were collected at the same time for all steers for an additional 10 weeks. Plasma samples were analyzed for 3MI concentrations. Blood samples collected from cattle in group 2 during the first 8 weeks were analyzed for 3MEIN-adduct concentrations.

Results—Mean blood concentration of 3MEIN-adduct increased to a maximum value on day 33 (0.80 µg of protein) and then decreased to a minimum on day 54 (0.40 µg of protein). Plasma 3MI concentrations initially decreased and remained low until after day 54. Group-1 cattle had lower plasma 3MI concentrations, compared with concentrations for group-2 cattle. Blood 3MEIN-adduct concentrations and plasma 3MI concentrations were not associated with deleterious effects on weight gains.

Conclusions and Clinical Relevance—Blood 3MEIN-adduct concentrations peaked during the period of greatest risk for development of bovine respiratory disease complex. Conversely, plasma 3MI concentrations decreased during the same period. Animal-to-animal variation in metabolic capacity to convert 3MI to 3MEIN may be of more importance than differences in plasma 3MI concentration.

Bovine respiratory disease complex (BRDC) is the leading cause of morbidity and mortality in feedlot cattle. Most episodes of BRDC occur soon after cattle arrive at feedlots; accordingly, the disease complex is commonly referred to as shipping fever. It is commonly accepted that BRDC results from an interaction of host susceptibility, stressors, and infectious pathogens. Traditionally, viral pathogens are believed to be primary invaders of the respiratory tract, which may give rise to bacterial colonization of the smaller airways in the respiratory tract and, consequently, bronchopneumonia.

Although toxins have not generally been considered important in the pathogenesis of BRDC in feedlot cattle, rumen-generated 3-methylindole (3MI) may be a contributing toxicant. Tryptophan is fermented by microbes in the rumen to produce 3MI. Following its production, 3MI is readily absorbed across the ruminal and intestinal walls; it is then hematogenously disseminated throughout the body. Excessive ruminal production of 3MI is associated with development of severe pulmonary injury known as acute interstitial pneumonia (AIP). It is possible that at concentrations below those required to induce AIP, 3MI (acting through its reactive metabolites) may injure pulmonary tissues and compromise host defenses, thereby potentiating the development of BRDC.

Experimentally induced respiratory tract disease was most severe when calves were challenge-exposed to 3MI and bovine respiratory syncytial virus (BRSV), compared with calves challenge-exposed with 3MI or BRSV alone. The synergy observed between 3MI and BRSV may also be evident for other respiratory pathogens of importance in the pathogenesis of feedlot-associated BRDC. Data from a field study documented that feedlot cattle with greater serum concentrations of 3MI were more likely to be treated for BRDC than cattle with lower serum concentrations of 3MI.

It is unlikely that 3MI itself contributes to the pathogenesis of BRDC, because it must be bioactivated in the lungs to induce pulmonary injury.
enzyme systems can bioactivate 3MI to reactive species that are believed to be involved in the pathogenesis of 3MI-related pulmonary disease. These enzyme systems are cytochrome P450 enzymes and prostaglandin H synthetase (PHS). Inhibition of both systems provides protection to in vivo and in vitro challenge-exposure to 3MI. The functionality of both enzymes may be required for 3MI to effect cellular toxicity seen in naturally occurring disease, and these enzymes are abundant in Clara cells in ruminants.

The proximate metabolite of cytochrome P450 metabolism of 3MI, 3-methyleneindololene (3MEIN), is believed to be responsible for most 3MI-induced pulmonary diseases. This electrophile covalently binds to cellular macromolecules resulting in profound cellular dysfunction. Other 3MI metabolites also have been identified, and these species of radicals may also contribute to cellular injury. Feedlot cattle that died from bronchopneumonia had greater 3MEIN-adduct concentrations in their lungs than cattle without pulmonary disease. If 3MI and 3MEIN are at greatest risk of developing BRDC. However, to our knowledge, variations in 3MI or 3MEIN concentrations in feedlot cattle during times of greatest risk for developing respiratory tract disease have not been reported. Thus, the study reported here was undertaken to determine time-dependent patterns and magnitudes of plasma concentrations of 3MI and blood concentrations of 3MEIN-adduct in feedlot cattle.

Materials and Methods

Animals—Sixty-four crossbred yearling steers (mean weight, 318 kg) were procured from a single source in western Oklahoma for use in the study. The study protocol was reviewed and approved by the Colorado State University Animal Care and Use Committee. Upon arrival at the feedlot, steers were moved to a receiving pen and provided with long-stem grass hay and unlimited access to water. Within 24 hours after arrival at the feedlot, cattle were moved through a cattle handling facility and vaccinated with modified-live virus vaccine containing bovine herpesvirus 1, parainfluenza virus 3, bovine viral diarrhea virus, and BRSV. Cattle also were administered Clostridium perfringens C and D toxoids and doramectin (20 mg/100 kg, SC) and were implanted with a growth promotant (20 mg/100 kg, SC) containing 120 mg of trenbolone acetate and 24 mg of estradiol. All cattle were monitored daily by trained feedlot personnel for manifestations of illness.

The following day (day 0), steers were weighed, provided with unique identification ear tags, and metaphylactically administered long-acting oxytetracycline (2,000 mg/100 kg, SC). Metaphylactic administration of antibiotic was used to reduce the likelihood of cattle developing BRDC. Cattle were assigned to pens (8 steers/pen). Allocation to pens was determined on the basis of body weight such that mean body weight of each pen was similar. Steers were weighed again on days 33 and –1, at approximately 2-week intervals (days 13, 28, 42, 54, 68, 82, and 97), and twice at the completion of the study (days 117 and 118). Mean value for the 2 initial (days –34 and –33) and 2 final (days 117 and 118) weight measurements were used as arrival and shipment weights, respectively.

Steers selected for use in the study had been consuming a diet that consisted primarily of mature pasture for at least 4 months prior to their arrival at the feedlot. After steers were moved from the receiving pen to their allocated pens, they were fed a straw-based diet to mimic the intake of cattle consuming mature pasture. Concentration of crude protein in the straw diet was 6.1% (dry-matter basis). A trace-mineral block designed for range cattle was included in each feedbunk of each pen while cattle consumed the straw-based diet. This diet was fed for 32 days to allow the cattle to thoroughly adapt. On day –1, cattle were fed only in the morning; cattle were not fed again until the afternoon of day 0. This period of feed restriction was used to simulate feed restriction associated with typical transportation of cattle from the ranch of origin or sale barn to a feedlot.

On day 0, cattle were fed the first of 4 diets that contained increasing concentrations of nonstructural carbohydrates (ie, step-up diets; Appendix). Beginning on day 1, steers were fed twice daily and provided subsequent step-up diets during the afternoon feedings on days 5, 8, and 12. The finishing diet was first provided to the cattle during the afternoon feeding on day 15. Weight of each feed delivery was recorded and adjusted to reflect dry-matter content. Delivery of dry-matter content was further adjusted to reflect feed refusal and provide a more accurate estimate of dry matter consumed in each pen.

Cattle were transported to a commercial abattoir on day 118. Carcass characteristics including hot carcass weight, liver abscess score, marbling units, cross-sectional area of the longissimus muscle, fat thickness over the 12th rib, kidney-pelvic-heart fat score, and USDA quality and yield grades were recorded. Adjusted final weights were calculated to reflect transportation shrink by multiplying the final weights by 0.96. Dressing percentage of each steer was calculated by dividing hot carcass weight by adjusted final weight.

Collection of samples—Steers were assigned to 2 equally sized groups. Cattle in each group were moved to the animal handling facility for collection of blood samples. There were 4 pens of cattle in each group. On each day of sample collection, all cattle in a specific pen were brought to the animal handling facility, using a randomized order for the pens within each group. Blood samples were collected approximately 4 times/wk for 3 weeks and then 3 times/wk for the following 5 weeks. Blood samples were collected on days –2, –1, 1, and 2 as well as days 3, 6, 9, 12, 13, 15, and 16 (ie, most days that the diet was changed and each day subsequent to a diet change). Because of severe inclement weather, samples were not collected on day 8, which was the day the third step-up diet was initially delivered to the cattle.

During days –2 through 54 (intensive collection of samples), group-1 steers were moved to the animal handling facility immediately prior to the morning delivery of feed, whereas group-2 cattle were moved from their pens to the animal handling facility approximately 2 to 3 hours after the morning delivery of feed (ie, allowed to eat for 2 to 3 hours prior to collection of samples). Following the period of intensive collection of samples, both groups were moved from their pens to the animal handling facility for collection of blood samples at approximately the same time.

Blood samples were collected by jugular venipuncture into 2 evacuated 10-ml blood collection tubes, of which contained potassium EDTA and the other of which contained sodium heparin. Blood samples were kept on ice and processed immediately after the cattle were returned to their pens. Blood samples that contained potassium EDTA were centrifuged (2,125 X g for 20 minutes at 4 C), using a refrigerated centrifuge. Aliquots of plasma were harvested, snap-frozen in liquid nitrogen, and stored at –20 C. Aliquots of heparinized blood samples were washed and incubated at –20 C.

Analysis of samples—All plasma samples (n = 2,204) were analyzed for 3MI concentration. Blood samples collect-
ed from steers of group 2 during the first 8 weeks of sample collection (n = 864) were analyzed for 3MEIN-adduct concentrations.

Blood samples were analyzed for 3MEIN-adduct absorbance per microgram of protein, using methods described elsewhere. Briefly, prepared samples of known protein content were placed into wells of a standard 96-well polystyrene plate. Primary polyclonal antibodies to thioether adducts of 3MEIN were added to each well. Following incubation, a second antibody (donkey anti-sheep IgG coupled with horseradish peroxidase) was added to the wells. After a second incubation, developer was added, and color was measured spectrophotometrically. Absorbance per microgram of protein is an arbitrary unit directly proportional to binding of primary antibody to tissue proteins.

Plasma 3MI concentrations were determined by use of a microplate method adapted from procedures described elsewhere. Samples were extracted with absolute ethanol and then centrifuged. Supernatant was harvested and mixed with a solution containing 4-dimethylaminobenzaldehyde. A purple product is formed when 3MI is present. Absorbance was determined spectrophotometrically, and the concentration of 3MI was calculated by comparing the results for samples to those of a standard curve.

Statistical analysis—Mean daily weight gains (MDG) were calculated for each steer and each pen for the periods during which cattle were fed straw-based and typical feedlot diets. Mean daily dry-matter intake (DMI) for cattle in a pen was calculated for each 2-week period, using amount of dry matter consumed daily per pen of cattle divided by the number of animal-days for the period. Feed efficiency (FE) ratios were estimated for each weigh period by dividing MDG for a pen by DMI for that same pen.

Statistical analyses were performed, using commercially available software. Period 1 was considered day –33 to day –1, and periods 2 through 9 consisted of 14, 15, 14, 12, 14, 14, 15, and 21 days, respectively. Each steer was considered the experimental unit for the analysis of live weights, MDG, carcass characteristics, plasma concentration of 3MI, and blood concentration of 3MEIN. Pens were considered the experimental unit for analysis of DMI and FE. Time period was considered a classification variable for the analysis of MDG, DMI, and FE. Day of the study was treated as a continuous variable when constructing models for analysis of 3MI and 3MEIN concentrations. First-order autoregressive matrices were estimated for each weigh period by dividing MDG for a period by the number of cattle in a pen.

Carcass characteristics measured on a continuous scale were analyzed as a 1-way ANOVA with group as the factor of interest. Categoric carcass characteristics (ie, USDA quality and yield grades) were analyzed, using a χ² goodness-of-fit test. Arrival weight was included as a covariate when analyzing MDG, DMI, continuous carcass characteristics, 3MI concentrations, and 3MEIN concentrations for values of P < 0.10.

Effect of maximum 3MEIN and 3MI concentrations on feed efficiency (FE) ratios negatively correlated with plasma 3MEIN-adduct concentration. Each increase in plasma 3MI was evaluated. Arrival weight and pen-level DMI were included in the models as covariates for values of P < 0.10.

Results

Manifestations of illness were not detected during the study. None of the cattle became sick or died during the study period.

Plasma concentrations of 3MI ranged from 0.12 to 7.57 μg/ml (median, 1.58 μg/ml). Averaged over time, plasma 3MI concentration differed significantly (P = 0.002) between groups 1 and 2 (mean ± SEM, 1.77 ± 0.04 and 1.60 ± 0.04 μg/ml, respectively). A significant (P = 0.002) interaction was identified between day of sample collection and group in the final model of plasma 3MI concentrations, indicating that differences in 3MI concentrations between groups varied significantly over time. Plasma 3MI concentration on the day following a diet change (1.53 ± 0.08 μg/ml) did not differ significantly (P = 0.60), compared with concentrations for the day of a diet change (1.55 ± 0.08 μg/ml). Plasma 3MI concentrations were significantly (P = 0.01) lower during days –2 to 54 (1.58 ± 0.07 μg/ml), compared with concentrations during the period when samples were collected weekly (2.00 ± 0.07 μg/ml; Fig 1).

Mean 3MEIN-adduct concentration was 0.60 ± 0.01 U/μg of protein. Blood concentrations of 3MEIN-adduct ranged from 0.30 to 1.05 U/μg of protein (median, 0.59 U/μg of protein). Blood 3MEIN-adduct concentrations varied significantly (P < 0.001) in a quadratic manner with day of sample collection. Concentrations increased after day –1, peaked on day 33, and then decreased to day 54 (Fig 2). Mean 3MEIN-adduct concentrations were 0.80 ± 0.02 and 0.40 ± 0.01 U/μg of protein on day 33 and 54, respectively. Blood 3MEIN-adduct concentration on each of the days following a diet change (0.68 ± 0.02 U/μg of protein) was significantly (P < 0.001) greater, compared with the 3MEIN-adduct concentration on the day of the diet change (0.61 ± 0.02 U/μg of protein).

Plasma 3MI concentrations were significantly (P = 0.04) negatively correlated with blood 3MEIN-adduct concentration. Each increase in plasma 3MI was evaluated. Arrival weight and pen-level DMI were included in the models as covariates for values of P < 0.10.
concentration of 1 μg/ml was associated with a decrease in 3MEIN absorbance of 0.24 U/μg of protein (Fig 2).

Mean weights of steers on day of arrival and day –1 were 318 ± 3 and 368 ± 3 kg, respectively (Fig 3). We did not detect a significant difference in weight between groups 1 and 2 on day of arrival (P = 0.35) or day –1 (P = 0.83). However, final weight varied significantly (P = 0.02) between groups 1 (564 ± 8 kg) and 2 (584 ± 8 kg). Mean daily weight gains of animals in groups 1 and 2 did not differ significantly (P = 0.53) while receiving the straw-based diet (period 1), and overall MDG was 1.57 ± 0.05 kg. A significant (P = 0.002) interaction between group and period was identified in the final model of MDG from which period 1 was excluded, suggesting that differences in the rates of weight gain between groups 1 and 2 varied over time. Group-2 steers gained at a significantly faster rate than group-1 steers during periods 2 (P < 0.001) and 9 (P = 0.04). However, group-1 steers gained at a significantly (P = 0.003) faster rate than group-2 steers during period 5. Averaged over the entire study period, MDG differed significantly (P = 0.02) for steers in group 1 (1.68 ± 0.04 kg) and 2 (1.82 ± 0.04 kg).

Maximum blood 3MEIN-adduct concentration on days 1, 2, 5, 6, or 9 was significantly (P = 0.03) associated with an increase in the overall MDG while cattle received feedlot diets. Each increase in 3MEIN-adduct concentration of 0.1 U/μg of protein was accompanied by an increase in MDG of 0.52 kg. Maximum plasma 3MI concentration for the same period was not significantly (P = 0.92) associated with MDG. When analyzed on an individual-animal basis, blood 3MEIN-adduct AUC (P = 0.03) but not plasma 3MI AUC (P = 0.06) was significantly associated with improvements in MDG. Each increase in plasma 3MI and blood 3MEIN-adduct AUC of 1 unit was associated with an increase in MDG of 0.02 and 0.12 kg, respectively. There was a significant (P < 0.001) interaction between period and blood 3MEIN-adduct AUC on a per-pen basis. Averaged over time, 3MEIN-adduct AUC increased during period 6 and was significantly greater during that period than during periods 2 (P = 0.01), 3 (P = 0.01), 4 (P = 0.02), 5 (P = 0.05), 8 (P = 0.01), and 9 (P = 0.01) but not during period 7 (P = 0.09).

Feed efficiency did not differ significantly (P = 0.33) between groups for period 1 (mean value for both groups, 0.24 ± 0.01). Excluding period 1 from the model, we did not detect a significant interaction between group and period, nor did we detect a significant (P = 0.67) effect of group on FE. Overall FE was 0.19 ± 0.01. Feed efficiency varied significantly over...
time (P < 0.001). Excluding period 1 from the model, the combined FE for periods 3, 4, 5, and 8 were significantly (P < 0.001) greater than the combined FE for periods 2, 4, 6, and 7.

Hot carcass weights differed significantly (P = 0.05) between steers of groups 2 (353.0 ± 4.9 kg) and 1 (341.0 ± 4.9 kg). We did not detect significant (P = 0.20) differences between groups for other carcass characteristics.

Discussion

The vast majority of BRDC episodes are diagnosed soon after cattle enter feedlots, and most occur within 8 weeks after arrival.3,4,31,32 During this period, blood 3MEIN-adduct concentrations increased, with greatest mean values on days 16, 23, and 33, and then declined to their lowest concentration on day 54. This pattern of change in blood 3MEIN-adduct concentrations coincides temporally with curves of typical BRDC epidemics. Plasma 3MI concentrations were expected to increase early in the feeding period in a similar manner to 3MEIN-adduct concentrations; however, this was not observed. None of the cattle developed clinical manifestations of respiratory tract disease in this study. Therefore, evaluation of associations between 3MI or its metabolite, 3MEIN, and the occurrence of BRDC was not possible.

Plasma 3MI and blood 3MEIN-adduct concentrations were negatively correlated. A possible explanation for this unexpected finding is that steers with a high capacity to metabolize 3MI may have converted 3MI to 3MEIN more efficiently than steers with a low metabolic capacity. Therefore, those steers with high metabolic capacity for 3MI would have lower 3MI and higher 3MEIN-adduct concentrations than steers with low metabolic capacity.

Blood samples were collected more frequently during the period of greatest risk for development of BRDC. Frequency of sample collection was decreased to weekly intervals after day 54, because this stage of the feeding period is associated with a lower risk for development of BRDC. Frequent handling may have resulted in lower feed consumption during the first 54 days after arrival in the feedlot, compared with consumption in cattle exposed to more typical feedlot management practices. Because ruminal generation of 3MI and, therefore, plasma 3MI concentrations, are largely dependent on tryptophan intake, a decrease in DMI may have resulted in decreased 3MI production during the period of intensive sample collection. It is possible that the frequency of handling resulted in 3MI and 3MEIN-adduct concentrations that were not representative of feedlot cattle under typical management conditions.

Plasma 3MI concentrations initially decreased and remained relatively low until after day 54 (Fig 1). Monensin4 was included in the diets used in the study reported here and may have contributed to the reduction in plasma 3MI concentrations by reducing fermentation of tryptophan within the rumen.33 Plasma 3MI concentrations increased following the period of intensive sample collection during the time cattle are believed to be at low risk of developing infectious respiratory tract disease. However, DMI increased during period 6 and was greater than all other periods (Fig 4). Therefore, reduced DMI may have accounted for lower 3MI concentrations during the period of intensive sample collection.

Because of limited fiscal resources, it was not possible to determine 3MEIN-adduct concentration in all blood samples. Blood samples collected from steers in group 2 during the period of intensive sample collection were assayed for 3MEIN, because the period of intensive sample collection coincided with greatest risk for development of BRDC, as determined in other studies.34,35 Additionally, group-2 steers were less affected by sample collection than group-1 steers and may have provided a better model for blood 3MEIN-adduct concentrations in feedlot cattle under more commonplace production settings.

Concentrations of 3MI measured in this study may represent basal production of 3MI that, on average, do not result in adverse outcomes in feedlot cattle. However, bioactivation of 3MI to 3MEIN by cytochrome P450 enzymes in Clara cells may be of consequence, because 3MEIN, a potent pneumotoxin, peaked during the period of greatest risk for development of BRDC. In another study,36 3MEIN-adduct concentrations in lung tissues were greater in cattle affected with bronchopneumonia, compared with cattle without histologic evidence of respiratory tract disease. Therefore, the metabolic capacity of the cytochrome P450 enzymes that bioactivate 3MI may be of more importance as a predictor of the likelihood of disease in feedlot cattle than is the concentration of 3MI. A mechanism for increased 3MEIN production in tissues of the respiratory tract may be the induction of cytochrome P450 enzymes that catalyze the formation of this putative reactive intermediate in susceptible lung cells. Induction of these enzymes could be associated with drug treatment, exposure to viral pathogens, changes in diet, or other environmental factors.36 Evaluation of this hypothesis will require further characterization of the cytochrome P450 enzymes in respiratory tract tissues of cattle.

In another study,37 an increase in serum 3MI concentration of 1 µg/ml was associated with a significant reduction in MDG of 0.02 kg. However, an association between serum 3MI concentration and MDG was not detected in another study.38 We did not find a significant association between maximum plasma 3MI concentration on days 0 through 9 with MDG. However, maximum blood 3MEIN-adduct concentration from the same time period was associated with significant improvements in MDG. Blood 3MEIN AUC was significantly positively associated with increases in MDG. It is likely that MDG and 3MI (and potentially 3MEIN) are associated with DMI. In our models, we attempted to control for DMI intake; however, DMI was measured on a per-pen basis, and plasma 3MI and blood 3MEIN concentrations were measured on a per-animal basis. Hence, it was not possible to appropriately control for per-animal DMI. This may explain the reason that 3MI and 3MEIN-adduct concentrations were positively correlated with MDG in some models.

The study design resulted in lower DMI and MDG for cattle of group 1, compared with values for
cattle of group 2. This may have been a consequence of cattle handling procedures. During the period of intensive sample collection, steers of group 1 were removed from their pens before they received their morning ration, whereas steers of group 2 were removed from their pens 2 to 3 hours after they received their morning ration. Differences in DMI, MDG, and 3MI concentrations between groups 1 and 2 persisted even after the frequency of sample collection was reduced to once each week (ie, all cattle were removed from their pens prior to the morning feeding) and lasted for the duration of the study. Analysis of these data suggests that procedures that require handling of feedlot cattle, whether for production (such as insertion of a second growth-promotant implant) or research purposes, should be performed in a manner that will allow cattle to be in their pens at the time feed is normally delivered.

BoviShield 4, Pfizer Animal Health, New York, NY.
Fortress CD, Pfizer Animal Health, New York, NY.
Dectomax, Pfizer Animal Health, New York, NY.
Revalor-S, Hoechst-Roussel Vet, Warren, NJ.
Dectomax, Pfizer Animal Health, New York, NY.
BoviShield 4, Pfizer Animal Health, New York, NY.
Fortress CD, Pfizer Animal Health, New York, NY.
BoviShield 4, Pfizer Animal Health, New York, NY.
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BoviShield 4, Pfizer Animal Health, New York, NY.

Appendix

Dietary dry-matter (%) of each commodity included in each of 4 diets that contained increasing concentrations of nonstructural carbohydrates (step-up diets) and a finishing diet fed to steers in a feedlot

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Finishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steam flaked corn</td>
<td>34.715</td>
<td>45.972</td>
<td>52.571</td>
<td>65.359</td>
<td>69.141</td>
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<tr>
<td>Alfalfa hay</td>
<td>31.271</td>
<td>24.030</td>
<td>11.541</td>
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<tr>
<td>Corn silage</td>
<td>27.796</td>
<td>22.678</td>
<td>27.229</td>
<td>17.260</td>
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<td>CCDS</td>
<td>3.979</td>
<td>4.964</td>
<td>3.903</td>
<td>4.124</td>
<td>4.003</td>
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<tr>
<td>Soybean meal</td>
<td>1.798</td>
<td>2.547</td>
<td>2.631</td>
<td>1.924</td>
<td>1.634</td>
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<tr>
<td>Salt (NaCl)</td>
<td>0.173</td>
<td>0.178</td>
<td>0.169</td>
<td>0.179</td>
<td>0.174</td>
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<tr>
<td>Limestone</td>
<td>0.171</td>
<td>0.413</td>
<td>0.697</td>
<td>0.920</td>
<td>1.106</td>
</tr>
<tr>
<td>Soy oil</td>
<td>0.008</td>
<td>0.014</td>
<td>0.026</td>
<td>0.036</td>
<td>0.044</td>
</tr>
<tr>
<td>Fat</td>
<td>NI</td>
<td>NI</td>
<td>0.677</td>
<td>1.430</td>
<td>2.083</td>
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<tr>
<td>Urea</td>
<td>NI</td>
<td>NI</td>
<td>0.320</td>
<td>0.607</td>
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<td>Manganese*</td>
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<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.014</td>
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<tr>
<td>Tylosin*</td>
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<td>0.003</td>
<td>0.004</td>
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<tr>
<td>Trace minerals</td>
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<td>0.015</td>
<td>0.014</td>
<td>0.015</td>
<td>0.014</td>
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<tr>
<td>Vitamin E</td>
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<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Vitamin A</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Dry-matter content*</td>
<td>0.194</td>
<td>0.748</td>
<td>0.770</td>
<td>71.52</td>
<td>69.43</td>
</tr>
</tbody>
</table>

*Dry-matter content (%) on an as-fed basis.
CCDS = Condensed corn distiller’s solubles. NI = Not included.

References
24. Kaster JK, Yost GS. Production and characterization of specific


