Effects of feeding aspirin and supplemental vitamin E on plasma concentrations of 3-methylindole, 3-methyleneindolenine-adduct concentrations in blood and pulmonary tissues, lung lesions, and growth performance in feedlot cattle

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Objective—To evaluate the effect of feeding aspirin and supplemental vitamin E on growth performance, lung lesions, plasma concentrations of 3-methylindole (3MI), and 3-methyleneindolenine (3MEIN)-adduct concentrations in blood and pulmonary tissues of feedlot cattle.

Animals—256 crossbred steers; 64 cattle were used in experiment 1 and 192 cattle were used in experiment 2.

Procedures—A 2 × 2 factorial design was used for each experiment. Treatment factors were aspirin (0 or 3 g daily) and vitamin E (200 or 1,500 IU daily). Steers were housed in pens (8 steers/pen). Steers were slaughtered on days 59 and 138 for experiments 1 and 2, respectively. Lungs were grossly evaluated. Plasma 3MI concentration was determined, and 3MEIN-adduct concentrations were measured in blood and pulmonary tissues.

Results—Treatment was not associated with improvement or adverse effects on weight gain, dry-matter intake, or feed efficiency in experiment 2. In experiment 1, 36 of 63 (57.1%) steers had lung lesions. Lesions were not associated with treatment or concentrations of 3MI and 3MEIN-adduct. Plasma 3MI concentration and concentrations of 3MEIN-adduct in blood and pulmonary tissues were 3.11 µg/mL, 0.51 U/µg of protein, and 0.49 U/µg of protein, respectively. Aspirin was associated with increased blood concentrations of 3MEIN-adduct for diets that did not contain supplemental vitamin E.

Conclusions and Clinical Relevance—Differences in performance of feedlot steers were not associated with treatment diet. It is possible that concurrent exposure of feedlot cattle to other factors typically associated with development of respiratory tract disease would affect these findings. (*Am J Vet Res* 2002:63:1641–1647)

Abrupt increases in ruminal generation of **3-methylindole (3MI)**, a microbial metabolite of tryptophan, are associated with acute interstitial pneumonia (also known as fog fever or acute bovine pulmonary edema and emphysema) in pastured cattle.[1,2] The 3MI is absorbed predominantly from the small intestine and disseminated hematogenously.[3] Although the mechanism of action of 3MI is not fully understood, it is apparent that bioactivation in pulmonary tissues is a critical step in the pathogenesis of 3MI-induced lung injury.[4,5]

Bioactivation of 3MI by cytochrome P-450 enzymes in Clara cells, type-I alveolar cells, and alveolar macrophages has been studied extensively.[6-9] Clara cells appear to be the primary site for bioactivation of 3MI.[10] It is believed that the predominant metabolite of 3MI is **3-methyleneindolenine (3MEIN)**, and this electrophile is believed to be responsible for the majority of 3MI-induced injury.[7,11] Also, 3MI can be metabolized by **prostaglandin H synthetase (PHS)**.[4,12-14] In 1 study,[15] PHS-dependent free radicals were produced when 3MI was incubated with PHS in vitro and during 3MI challenge exposure in vivo. Free radicals likely potentiate 3MI-induced injury. The pathogenesis of 3MI-induced disease may require co-oxidation by cytochrome P-450 enzymes and PHS.[4]

Although 3MI is a potent pneumotoxic precursor and capable of inducing acute pulmonary injury, it is normally produced at low concentrations in the rumen and does not cause apparent adverse effects.[16,17] However, increases in ruminal generation of 3MI that are not sufficient to induce acute interstitial pneumonia could result in increased concentrations of 3MEIN

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and other electrophiles in pulmonary tissues. Cellular injury and compromise of pulmonary defense mechanisms may be a consequence of these small fluctuations in ruminal generation of 3MI.

Bingham et al[18,19] found that increases in serum concentrations of 3MI at the time of or soon after arrival of cattle at a feedlot were associated with an increased likelihood of treatment because of **bovine respiratory disease complex (BRDC)**. In another study,[17] 3MEIN-adduct concentrations in blood samples were increased during the period typically associated with the greatest risk for BRDC, although plasma 3MI concentrations did not exceed basal concentrations. Therefore, increases in ruminal generation of 3MI or bioactivation in pulmonary tissues may increase the risk for BRDC in feedlot cattle. If this is so, then mitigation of 3MI bioactivation or cellular injury induced by free radicals may reduce the incidence of feedlot-associated BRDC.

Acetylsalicylic acid (ie, aspirin) is an inhibitor of PHS[a] and is associated with a disease-sparing effect during 3MI challenge exposure.[20] This effect is only observed when aspirin is administered prior to 3MI challenge exposure. Authors of that study concluded that the protective effect resulted from the prevention of 3MI metabolism by PHS and not simply from inhibition of prostanoid production. In another challenge-exposure study,[b] 3MI-induced disease was less severe in calves administered aspirin (15.6 g, PO) and α-tocopherol (ie, vitamin E; 1,500 IU, IM) prior to 3MI exposure, compared with disease in control calves or calves administered aspirin or vitamin E prior to 3MI exposure. However, a disease-sparing effect was not detected in a field study[18] in which cattle were administered aspirin (31.2 g, PO) at the time of arrival at a feedlot. The protective effects of aspirin may not have been detected in that study if increases in ruminal generation of 3MI occurred after aspirin-induced inhibition of PHS had waned. A protective effect of aspirin may have been observed if aspirin was administered to maintain inhibition of PHS during periods of increased 3MI bioactivation. In 1 study,[a] investigators documented that low doses of aspirin provided in the diet for 7 days will effectively inhibit PHS.

The study reported here was performed to evaluate the effects of the inclusion of aspirin and supplemental vitamin E in the diet on performance efficiency and carcass characteristics of feedlot cattle. In addition, grossly identifiable lung lesions at slaughter, plasma concentrations of 3MI, and concentrations of 3MEIN-adduct in blood and pulmonary tissues were evaluated.

Materials and Methods

Animals—Two hundred fifty-six crossbred yearling steers (mean body weight, 316 kg) obtained from a single source were used in the study. Sixty-four steers were randomly selected for inclusion in experiment 1, and the remaining 192 steers were included in experiment 2. All steers were monitored daily by trained feedlot personnel to detect manifestations of illness. The study protocol was reviewed and approved by the Colorado State University Animal Care and Use Committee.

Procedure—The first day on which steers were fed the treatment diets was designated as day 0. On the day of arrival at the feedlot (day –2), steers were provided long-stem grass hay and allowed ad libitum access to water. Soon after arrival at the feedlot (< 24 hours), cattle were moved through a cattle handling facility and vaccinated with modified-live virus vaccine[c] that contained bovine herpesvirus 1, parainfluenza 3 virus, bovine viral diarrhea virus, and bovine respiratory syncytial virus. Cattle were also administered *Clostridium perfringens* C and D toxoid[d] and doramectin[e] (10 mg/mL; 2 mL/100 kg of body weight), and implanted with a growth promotant[f] that contained 120 mg of trenbolone acetate and 24 mg of estradiol.

On the following day (day –1), steers were again moved through the cattle handling facility. Steers were weighed, and an individually numbered plastic ear tag was inserted in each steer. On the basis of body weight, steers were assigned to 1 of 6 blocks. Blocks 1 through 4 each comprised 43 steers, whereas blocks 5 and 6 each comprised 42 steers. Sixty-four steers (11 from each of blocks 1 through 4, and 10 from each of blocks 5 and 6) were randomly selected for use in experiment 1; the remaining steers were used in experiment 2. The 64 steers in experiment 1 were allocated into 2 weight blocks, and the 192 steers in experiment 2 were allocated into 6 weight blocks; each block contained 32 steers. Within each weight block, steers were randomly assigned to be fed 1 of 4 treatment diets.

On day 0, the steers were weighed again and then moved to pens (8 steers/pen). Mean value for the body weights recorded on days –1 and 0 was used as the arrival weight.

Treatments and feeding regimen—A 2 × 2 factorial design was used for each experiment; treatment factors were aspirin (0 or 3 g daily) and supplemental vitamin E (200 or 1,500 IU daily). Aspirin and supplemental vitamin E were added to a trace mineral supplement, which was then included in the feed for the steers. The 4 treatment diets were designated as the control, aspirin, vitamin E, and aspirin-vitamin E diets.

Within each treatment diet, steers were fed 4 successive diets (diets 1, 2, and 3 and a finishing diet) that contained increasing concentrations of nonstructural carbohydrates. Expected **dry-matter intake (DMI)** was 5.45, 6.38, 7.36, and 8.11 kg for diets 1, 2, and 3 and the finishing diet, respectively. The amount of aspirin and supplemental vitamin E added to each diet varied depending on the expected DMI for each of the 4 diets **(Appendices 1 and 2)**.

Cattle were provided an untreated, ground hay diet on day –1. Diet 1 was fed beginning on the afternoon of day 0. After that, steers were fed twice daily, and diets 2 and 3 were provided beginning on the afternoon of day 3 and 6, respectively, with the finishing diet provided beginning on the afternoon of day 10. Weight of each feed delivery was recorded and adjusted to reflect dry-matter content. Amount of dry matter delivered was further adjusted to account for feed refusal and to provide a more accurate estimate of DMI for each pen.

Experiment 1—The objective of experiment 1 was to evaluate the effects of aspirin and vitamin E on plasma concentrations of 3MI, 3MEIN concentrations in samples of blood and pulmonary tissues, and grossly identifiable lung lesions at slaughter. The 64 randomly selected steers were weighed on days 26 and 58. They were transported to a commercial abattoir and slaughtered on day 59. Aspirin was removed from the treatment diets on day 51 to allow for a withdrawal period of 8 days. Supplemental vitamin E was not removed from diets.

At the time of slaughter, blood samples were collected from the cranial vena cava into 2 evacuated blood collection tubes[l] (1 contained sodium heparin and the other contained potassium EDTA). Blood samples were stored on ice until fur-

ther processing. Blood samples in tubes containing potassium EDTA were centrifuged at 2,125 × *g* for 20 minutes at 4°C by use of a refrigerated centrifuge.[m] Aliquots of plasma were harvested, frozen in liquid nitrogen, and stored at –20°C. Aliquots of heparinized blood samples were frozen and stored at –20°C.

Lungs were removed at harvest and evaluated grossly to identify pathologic changes. Type of lesion (bronchopneumonia, pleuritis, or interstitial pneumonia) and percentage of lung tissue affected were recorded. A sample of pulmonary tissue (5 × 5 × 2 cm) was obtained from the dorsal aspect of the right caudal lung lobe of each steer, frozen on dry ice, and stored at –20°C.

Plasma 3MI concentrations were determined by use of a microplate method adapted from procedures described elsewhere.[19,21] Heparinized blood samples were analyzed to determine the 3MEIN absorbance per microgram of protein by use of methods described elsewhere.[11]

Experiment 2—The objective of experiment 2 was to evaluate the effect of aspirin and supplemental vitamin E on growth performance and carcass characteristics. Steers were weighed on days 26, 54, 82, 110, 137, and 138. Mean value of the weights recorded on days 137 and 138 was used as the final weight. Aspirin was removed from the treatment diets on day 130, and steers were transported to a commercial abattoir and slaughtered on day 138.

At slaughter, carcass characteristics including hot carcass weight, predicted yield grade, marbling units, cross-sectional area of the longissimus muscle, fat thickness over the 12th rib, percentage of **kidney-pelvic-heart (KPH)** fat, and USDA quality and yield grades were recorded for each steer. Transportation shrinkage was reflected by multiplying the final live weight by 0.96 to yield adjusted final weight. Dressing percentage was calculated by dividing hot carcass weight by adjusted final weight.

Statistical analysis—**Mean daily weight gain of each individual steer (MDG_i)** and **mean daily weight gain of each pen of steers (MDG_p)** were calculated for each 2-week weigh period in both experiments. Mean daily DMI for each steer in a pen was calculated for each 2-week period by using the amount of dry matter consumed daily per pen of cattle divided by the number of animal-days for the period. **Feed efficiency (FE)** was estimated for each weigh period by dividing MDG_p by mean daily DMI.

Statistical analyses were performed by use of commercially available software.[n] Treatments and interaction terms were included in the analysis. Interaction terms were removed from analytical models when the values were $P \geq 0.15$. Each steer was considered the experimental unit for the analysis of live weights, MDG_i, carcass characteristics, 3MEIN-adduct concentrations in blood and pulmonary tissues, and plasma concentrations of 3MI. Pen was considered the experimental unit for analysis of DMI and FE. Time period was considered a classification variable for the analysis of MDG_i, DMI, and FE. First-order autoregressive matrices were used to model the covariance structure within experimental units over time.[22] Lung lesions were classified as detected when lesions associated with bronchopneumonia or pleuritis were identified grossly. Data on lung lesions for 1 steer with interstitial pneumonia were not included in the analyses. Detection of lung lesions was analyzed by use of logistic regression with treatments and their interaction as the factors of interest. The interaction term was removed from the model when values were $P \geq 0.15$. Carcass characteristics measured on a continuous scale were analyzed by use of a 2-way ANOVA. Categoric carcass characteristics (USDA quality and yield grades) were analyzed by use of a χ^2 goodness-of-fit test. Arrival weight was included as a covariate for analysis of MDG_i, DMI, continuous carcass characteristics, and 3MI and 3MEIN concentrations when values were $P < 0.15$.

Results

Animals—Mean arrival weight did not differ significantly ($P = 0.98$) between steers of experiment 1 (316.2 kg) and experiment 2 (316.8 kg). One steer died during experiment 2 as a result of ruminal tympany on day 17. Illness was not observed in any of the remaining steers in either experiment.

Experiment 1—At slaughter, mean weight for the 64 steers in experiment 1 was 405.7 kg. We did not detect an association between final weight and treatment group. Overall MDG_i (± SEM) was 1.55 ± 0.04 kg, and an association between MDG_i and aspirin, vitamin E, or their interaction was not detected.

Feeding vitamin E was associated, although not statistically significant ($P = 0.08$), with an increase in plasma 3MI concentrations. At slaughter, mean plasma 3MI concentrations were 3.51 ± 0.18 and 3.06 ± 0.18 µg/mL for diets with and without supplemental vitamin E, respectively. An association was not detected between feeding aspirin and plasma 3MI concentration, nor did the effect of vitamin E vary significantly ($P = 0.33$) with feeding of aspirin. A significant ($P = 0.01$) treatment interaction was detected for blood 3MEIN-adduct concentration **(Fig 1)**. When diets did not include supplemental vitamin E, aspirin was significantly ($P = 0.01$) associated with an increase in blood 3MEIN concentration; however, this effect was not evident when supplemental vitamin E was included in the diet ($P = 0.37$). We did not detect an association between variation in

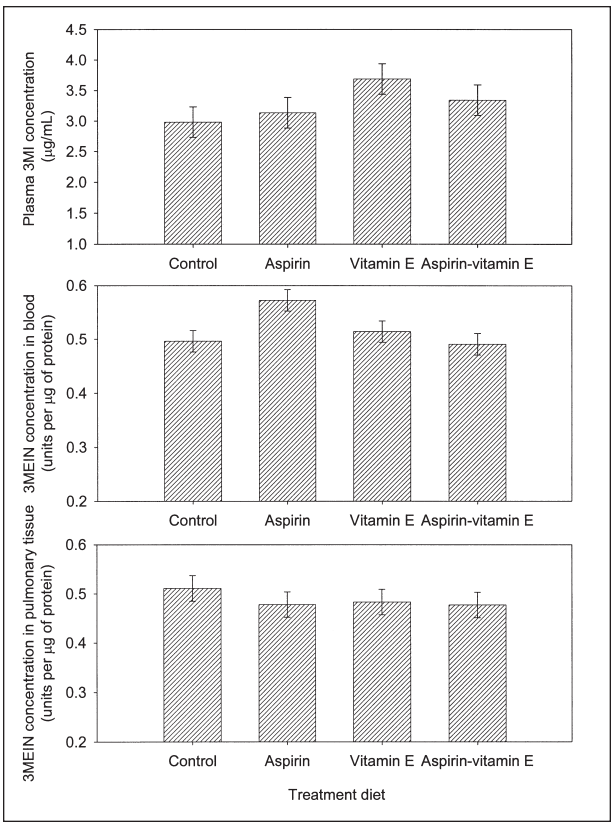

Figure 1—Mean ± SEM plasma 3-methylindole (3MI) concentrations (top) and 3-methyleneindolenin (3MEIN)-adduct concentrations in blood samples (middle) and pulmonary tissues (bottom) for feedlot steers fed 4 treatment diets.

3MEIN-adduct concentration in pulmonary tissues and treatment group. Overall 3MEIN-adduct concentrations in blood and pulmonary tissues were 0.51 and 0.49 U/μg of protein.

We did not detect an effect of plasma concentration of 3MI or 3MEIN-adduct concentrations in blood or pulmonary tissues on MDG_i, final weight, carcass weight, or dressing percentage. Each unit increase in 3MEIN-adduct concentration in blood or pulmonary tissues was associated with an increase of 0.81 (P = 0.06) and 0.69 (P = 0.05) units, respectively, in predicted yield grade. We did not detect an effect of plasma 3MI concentration on predicted yield grade. Each unit increase in 3MEIN-adduct concentration in blood samples was associated (P = 0.11) with an increase of 207 units in marbling score. We did not detect an effect of plasma 3MI concentration or 3MEIN-adduct concentration in pulmonary tissues on marbling score. Cross-sectional area of the longissimus muscle, percentage of carcass as KPH fat, and proportion of carcasses grading USDA choice or higher were not affected by plasma 3MI concentration or 3MEIN-adduct concentrations in blood or pulmonary tissues.

Plasma 3MI concentrations were not predictive of 3MEIN-adduct concentrations in blood or pulmonary tissues. Similarly, we did not detect a correlation between 3MEIN-adduct concentrations in blood samples and pulmonary tissues.

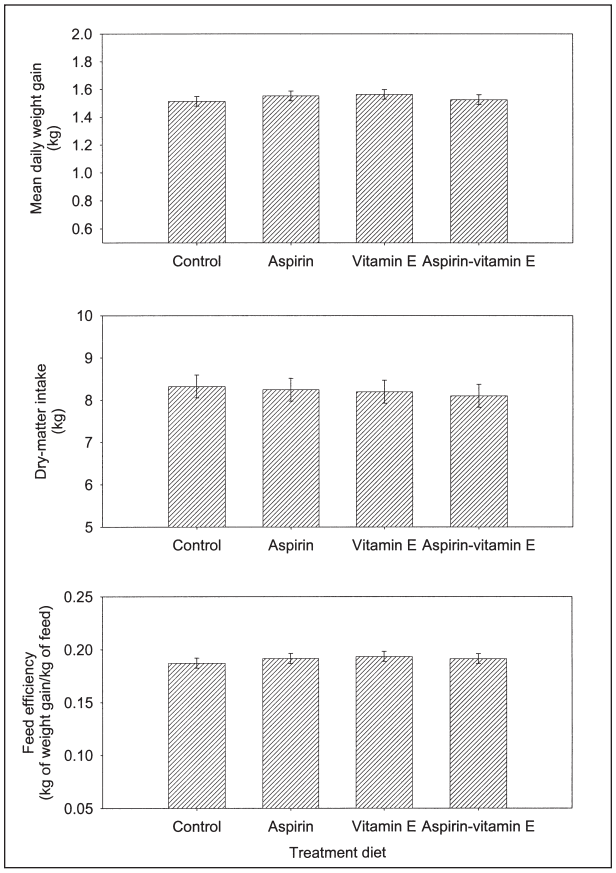

Figure 2—Mean ± SEM values for mean daily weight gain (top), dry-matter intake (middle), and feed efficiency (bottom) for feedlot steers fed 4 treatment diets.

Lungs of 63 steers were evaluated, and 36 (57.1%) had grossly identifiable lung lesions at slaughter. Lesions included interstitial pneumonia (n = 1), bronchopneumonia (21), pleuritis (9), and a combination of bronchopneumonia and pleuritis (5). Only 3 of the lesions were considered active. Data from the steer with interstitial pneumonia were excluded from further analyses, because the steer was not deemed representative of either group. Although not significantly (P = 0.23) different, steers with lung lesions weighed less at arrival (312.8 ± 4.1 kg) than steers without lung lesions (320.2 ± 4.6 kg). We did not detect significant differences in final weight or MDG_i between steers with lung lesions, compared with values for steers without lung lesions.

Although not statistically significant (P = 0.16), steers with lung lesions had a lower dressing percentage (62.4 ± 1.5%), compared with values for steers without lung lesions (63.2 ± 1.5%). Steers with lung lesions had greater, although not significantly different, marbling scores (P = 0.13) and an estimated proportion of their carcass as KPH fat (P = 0.09). However, the proportion of carcasses that graded as USDA select or higher was not associated with lung lesions. Effects of lung lesions on other carcass characteristics were not detected. Odds of a steer having pulmonary lesions at slaughter did not differ with changes in plasma 3MI concentration or 3MEIN-adduct concentrations in blood or pulmonary tissues.

We did not detect an effect of aspirin, vitamin E, or the interaction of vitamin E and aspirin on lung lesions.

Experiment 2—We did not detect an association between differences in body weight and MDG_i with treatment group (**Fig 2**). Overall MDG_i was 1.54 ± 0.04 kg. Body weight and MDG_i varied significantly (P = 0.01) with time (**Fig 3**). Actual amounts of aspirin and vitamin E were calculated (**Table 1**).

We did not detect a significant interaction for aspirin and vitamin E and DMI (P = 0.96) and FE (P = 0.39). Values for DMI and FE were not significantly (P = 0.30) affected by aspirin or vitamin E. Overall values for DMI and FE were 8.16 ± 0.17 kg and 0.19 ± 0.01 kg of feed/kg of weight gain, respectively. Time was significantly (P = 0.01) associated with variation in DMI and FE.

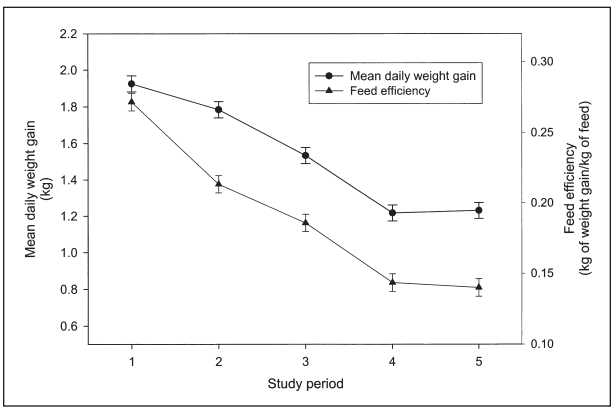

Figure 3—Mean ± SEM values for mean daily weight gain and feed efficiency for feedlot steers during each 2-week interval of the study.

Table 1—Projected and actual dry-matter intake (DMI), and actual intake of aspirin, vitamin E, and monensin by feedlot cattle

Diet	Projected DMI (kg)	Actual DMI (kg)	Actual aspirin intake (g)	Actual vitamin E intake (IU)	Actual monensin intake (mg)
1	5.45	4.98	2.74	1,370.6	82.32
2	6.38	5.65	2.66	1,331.3	93.39
3	7.36	6.34	2.58	1,305.5	104.80
Finishing	8.11	8.57	3.17	1,561.1	283.41

Monensin intake was calculated for all treatment diets, whereas aspirin and vitamin E intakes were calculated only for aspirin- and vitamin E-containing diets, respectively.

We did not detect an effect of aspirin, vitamin E, or the interaction of aspirin and vitamin E on variation in carcass characteristics.

Discussion

Despite evidence in other studies[18,19,23,b] suggesting that treatment with aspirin, vitamin E, or a combination of both may improve production or decrease the risk of developing respiratory tract disease in feedlot cattle, benefits associated with feeding these products were not detected in the study reported here. Although there is limited data available on long-term administration of aspirin in cattle, 3 g/d should have resulted in substantial inhibition of PHS.[a] Ruminant requirements for vitamin E have not been published, but 1,500 IU of vitamin E/d likely exceeds the minimum daily requirement for cattle[24] and provides some degree of protection from 3MI challenge exposure.[b] The study reported here was based on the hypothesis that 3MI production would increase as cattle undergo dietary changes associated with introduction to feedlots. It is possible that the 3MI concentrations in the cattle of this study were insufficient to obtain a beneficial effect from aspirin and vitamin E. It also is possible that other causative factors contributing to 3MI-related diseases were missing. Alternatively, it is possible that aspirin or vitamin E is not effective for preventing deleterious effects of exposure to 3MI or its metabolites.

Several aspects of the study design must be considered when interpreting our study. Prior to initiation of the study, it was decided that steers would be housed in small research pens rather than large pens typically used in most commercial feedlots. This strategy typically improves the ability to measure and objectively compare production variables in feedlot cattle. However, it must also be acknowledged that housing cattle in small pens (8 steers/pen) can substantially disrupt host-agent-environment interactions that produce typical patterns of respiratory tract disease among a greater number of cattle housed in large pens. In addition, steers used in this study were obtained from a single source and were relatively mature when entering the feedlot; both of these factors have been associated with reduced risk of developing BRDC.[o] Thus, the lack of clinical BRDC during our study was not surprising. However, the lack of BRDC may also have limited our ability to detect improvements in performance variables that may have been attributable to treatment with aspirin or vitamin E. Effects of exposure to 3MI are exacerbated when animals are concurrently exposed to a respiratory tract pathogen.[25] As such, improvements in performance variables among cattle treated with aspirin and vitamin E may only be identifiable when there is substantial, concurrent clinical and subclinical respiratory tract disease. Additional field studies to evaluate aspirin and vitamin E prophylaxis would likely require housing of a large number of cattle in large pens in feedlots.

Despite the lack of clinical respiratory tract disease, 36 of 63 (57.1%) steers slaughtered on day 59 in experiment 1 had grossly visible pulmonary lesions. Most of these lesions were mild and considered inactive. Other researchers have reported that a large proportion of feedlot cattle examined at slaughter had visible lung lesions regardless of treatment history.[26,27] Identifiable lung lesions have been associated with decreased MDG_i in other studies.[26,27] Furthermore, Gardner et al[27] found that lung lesions were associated with less desirable carcass characteristics. However, we did not detect an association between gross pulmonary lesions and MDG_i or carcass characteristics in the study reported here. It is possible that a substantial proportion of the lesions observed may have developed prior to arrival at the feedlot, because most of the lesions were resolved and inactive, none of the steers developed detectable illness, and steers with lesions weighed approximately 8 kg less at arrival than steers without lesions.

Steers in experiment 1 were slaughtered prior to reaching optimal carcass condition in an attempt to maximize the potential for identifying differences in active lung lesions that may have been associated with the treatments. If 3MI metabolism was associated with pulmonary injury typically attributed to BRDC, and the experimental treatments were successful in inhibiting this injury, it was hypothesized that differences in pulmonary lesions among treatment groups would be most detectable after the early feeding period, a time when clinical BRDC is typically identified in feedlot cattle. Early slaughter of steers also provided an ideal opportunity to obtain pulmonary tissues for determination of 3MEIN concentrations in addition to allowing us to evaluate plasma concentrations of 3MI and concentrations of 3MEIN-adduct in blood samples. Concentrations of 3MI and 3MEIN-adduct were evaluated to determine whether a similar exposure was evident among treatment groups. It was not surprising that we did not detect differences in tissue concentrations of 3MEIN-adduct associated with treatments, because the treatments were not expected to alter 3MI or 3MEIN production. There is evidence that the cytochrome P-450 enzyme system is integrally involved in metabolism of 3MI to 3MEIN,[8,28,29] but there are no commercially available products approved

for use in cattle intended for slaughter that can inhibit the cytochrome P-450 enzymes involved in 3MI metabolism. It is believed that aspirin exerts its direct effect on 3MI-associated disease by inhibiting PHS metabolism.[20,30] In addition to being commercially available, aspirin modulates 3MI-induced pulmonary disease in goats when given before, but not after, 3MI admnistration.[20,30] Unfortunately, we are not aware of any studies that have directly evaluated the relative contribution of these 2 metabolic pathways. Therefore, the treatments used in our study were not expected to be associated with differences in 3MI or 3MEIN concentrations, but it was considered plausible that aspirin might diminish oxidative injury by directly inhibiting PHS-associated metabolism of 3MI or by less specific inhibition of inflammatory pathways. It was also considered plausible that high amounts of supplemental vitamin E might diminish pulmonary damage associated with oxidative injury without directly affecting 3MI metabolism. Manipulation of the amount of dietary antioxidant consumed has been used to modulate 3MI-induced pneumotoxicity in goats.[20] Vitamin E (D-α-tocopherol) is a potent antioxidant and is readily absorbed from the gastrointestinal tract of cattle.[31-33] Effects of vitamin E on health and immunity of feedlot cattle have been documented.[23]

Restricting collection of biological samples to a single time point limited our ability to identify differences in 3MI and 3MEIN concentrations that may have been associated with differences in production variables or pathologic pulmonary changes. However, the primary objective of the study was to identify production differences associated with the various treatments. It was not considered desirable to impose a more intensive protocol for collection of samples that could affect production efficiency, which was reported in another study[17] that used intensive collection of samples to characterize temporal patterns of 3MI and 3MEIN production. More intensive collection of samples may also have indirectly affected 3MI production by altering feed intake. It was interesting that cattle fed the vitamin E treatment diet had higher plasma concentrations of 3MI on day 59, and cattle fed the aspirin treatment diet had higher 3MEIN-adduct concentrations in blood samples. As mentioned previously, this difference was not expected. It seems likely that a substantial portion of 3MEIN-protein adducts measured in blood samples were derived from pulmonary P-450 enzymes, because these enzymes are believed to be efficient at metabolizing 3MI to this specific reactive intermediate.[5,9,10,28] However, 3MEIN-adduct concentrations in blood samples and pulmonary tissues were not correlated, nor were plasma 3MI concentrations predictive of 3MEIN-adduct concentrations in blood or pulmonary tissues. Plasma concentrations of 3MI and 3MEIN concentrations in blood samples were negatively correlated in another study.[17] The reasons for the unexpected findings reported here were not clear, but it seems likely that differences in these concentrations were coincidental rather than being systematically associated with the treatments.

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[e]Dectomax, Pfizer Animal Health, New York, NY.
[f]Revalor-S, Hoechst-Roussel Vet, Warren, NJ.
[g]Trace mineral mix, Colorado Beef, Lamar, Colo.
[h]Vitamin E, 198 IU/g, BASF Corp, Mount Olive, NJ.
[i]Vitamin A, 500 IU/g, BASF Corp, Mount Olive, NJ.
[j]Rumensin 80, 176 g of monensin/kg, Elanco Animal Health, Indianapolis, Ind.
[k]Tylan 100, 220 g of tylosin/kg, Elanco Animal Health, Indianapolis, Ind.
[l]Vacutainer, Becton Dickinson and Co, Franklin Lakes, NJ.
[m]Centra CL3R, International Equipment Co, Needham Heights, Mass.
[n]SAS System for Windows, release 8.2, SAS Institute Inc, Cary, NC.
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Appendix 1

Composition of 4 successive diets that contained increasing concentrations of nonstructural carbohydrates and were fed to steers after their arrival in a feedlot

	Diet			
Commodity	**1**	**2**	**3**	**Finishing**
Steam flaked corn	49.829	58.093	70.120	77.962
Corn silage	14.926	14.926	11.194	11.194
Alfalfa hay	30.000	20.000	10.000	NI
CCDS	3.000	3.000	3.000	3.000
Soybean meal	1.012	0.980	0.862	0.898
Limestone	0.554	0.902	1.285	1.631
Sodium chloride	0.250	0.250	0.250	0.250
Potassium chloride	NI	NI	NI	0.340
Urea	0.208	0.572	0.943	1.307
Magnesium oxide	0.138	0.179	0.229	0.269
Trace mineral[g]	0.028	0.028	0.027	0.027
Soy oil	0.025	0.040	0.056	0.079
Fat	NI	1.000	2.000	3.000
Vitamin E[h]	0.017	0.017	0.017	0.017
Vitamin A[i]	0.003	0.003	0.003	0.003
Monensin[j]	0.009	0.009	0.009	0.019
Tylosin[k]	NI	0.001	0.003	0.005

Values reported are percentage of each commodity on a dry-matter basis.
CCDS = Condensed corn distillers solubles. NI = Not included.

Appendix 2

Selected characteristics of 4 successive diets that contained increasing concentrations of nonstructural carbohydrates and were fed to steers after their arrival in a feedlot

	Diet			
Characteristic	**1**	**2**	**3**	**Finishing**
Dry matter (%)*	65.91	65.52	67.90	67.54
Crude protein (%)†	13.5	13.5	13.5	13.5
Net energy/gain in body weight (MCal/kg)	1.17	1.30	1.45	1.58
Net energy/maintenance of body weight (MCal/kg)	1.90	2.06	2.24	2.39
Monensin[j] (g/air-dried ton of feed [90% dry matter])	13.5	13.5	13.5	27.0
Monensin[j] (mg/kg of dry matter)	16.53	16.53	16.53	33.07

*Calculated on an as-fed basis.
†Calculated on a dry-matter basis.