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A comparison of three vaccine programs on the health, growth performance, and carcass characteristics of high-risk feedlot heifers procured from auction-markets

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Abstract

A total of 2,575 beef heifers (BW = 568 ± 28.1 lb; 258 ± 12.7 kg) at high-risk of developing bovine respiratory disease (**BRD**) were enrolled in a randomized complete-block design study at a commercial feedlot to evaluate the effect of 3 vaccine programs on health, growth performance, and carcass characteristics. Dates of arrival to the feedyard served as the blocking factor and 10, 3-pen blocks were enrolled in the study (n = 10 replications per vaccine program). Chute-order randomization was used during arrival processing to assign heifers to 1 of 3 vaccine programs that differed by vaccine products or timing of the pentavalent modified-live viral vaccination: 1) Pyramid® 5 and Presponse® SQ during arrival processing (**PRE**), 2) Titanium® 5 and Nuplura® PH during arrival processing (**TNA**), and 3) Nuplura® PH during arrival processing with Titanium® 5 delayed until 28 days-on-feed (**TND**). No booster vaccinations were administered. Overall mortality, BRD morbidity, and BRD treatment success risks did not differ among the vaccine programs ($P \geq 0.13$). There were numerically fewer mortalities attributable to acute interstitial pneumonia in TNA heifers than the TND and PRE heifers (probability of difference = 0.99 and 1.00, respectively). Mortality attributable to BRD did not differ between vaccine programs (probability of difference ≤ 0.48). Endotoxin concentrations were measured in the *Mannheimia haemolytica* vaccines, and were lower in Nuplura® PH than Presponse® SQ. An arrival vaccine program implementing Titanium® 5 and Nuplura® PH had similar efficacy on BRD-related health outcomes as vaccinating with Pyramid® 5 and Presponse® SQ. Delaying a pentavalent viral vaccine until 28 days-on-feed did not affect health or growth-performance outcomes in this study.

Key words: bovine respiratory disease, BRD, endotoxin, feedlot, vaccination

Résumé

Un total de 2 575 génisses de boucherie (PC = 568 ± 28.1 lb; 258 ± 12.7 kg) à haut risque de développer le complexe respiratoire bovin (CRB) ont été utilisées dans une étude avec plan en blocs aléatoires complets dans un parc d'engraissement commercial afin d'évaluer l'effet de trois programmes de vaccin sur la santé, la performance de croissance et les caractéristiques de la carcasse. Les dates d'arrivée au parc d'engraissement ont servi pour former les blocs. Il y avait 10 blocs avec chacun trois enclos dans l'étude (n = 10 réplicas par programme de vaccintion). L'allocation aléatoire des génisses à l'un des trois programmes de vaccination a pris place au moment du traitement des animaux à l'arrivée. Ces trois programmes de vaccination différaient selon les produits de vaccination ou selon le moment d'administration du vaccin pentavalent à virus vivants modifiés : 1) vaccination avec Pyramid* 5 et Presponse* SQ au moment du traitement à l'arrivée (PRE). 2) vaccination avec Titanium* 5 et Nuplura* PH au moment du traitement à l'arrivée (TNA), et 3) vaccination avec Nuplura* PH au moment du traitement à l'arrivée et avec Titanium* 5 28 jours après le début de l'engraissement (TND). Il n'y a pas eu de rappel de vaccination. Il n'y a pas eu de différence entre les trois programmes de vaccination ($P \geq 0.13$) pour la mortalité en général, la morbidité associée au CRB et les chances de succès du traitement contre le CRB. La mortalité attribuable à la pneumonie interstitielle aiguë était numériquement moindre chez les génisses TNA que chez les génisses TND ou PRE (probabilité de différence = 0.99 et 1.00, respectivement). La mortalité attribuable au CRB n'a pas varié selon le programme de vaccination (probabilité de différence ≤ 0.48). La concentration d'endotoxine dans les vaccins avec *Mannheimia hemolytica* était moins élevée avec Nuplura* PH qu'avec Presponse* SQ. Un programme de vaccination mis en place à l'arrivée avec Titanium* 5 et Nuplura* PH a produit les mêmes résultats de santé associés

au CRB qu'un programme de vaccination avec Pyramid* 5 et Presponse* SQ. L'administration du vaccin pentavalent après un délai de 28 jours en engraissement n'a pas eu d'impact sur les résultats reliés à la santé ou à la performance de croissance dans cette étude.

Introduction

An array of infectious agents have been implicated in the multifactorial etiology of bovine respiratory disease (**BRD**). Because of this, viral and bacterial vaccines are commonly utilized during arrival processing at the feedlot in an attempt to reduce the incidence of BRD.[17,32] *Mannheimia haemolytica* (***Mh***) is the primary bacterial species involved with BRD,[13] and consequently the most common bacterial pathogen with an antigen incorporated into vaccine programs for high-risk cattle.[17,32] There are differences between how vaccines which confer immunity against this important pathogen are manufactured. Generally speaking, the bacterin and leukotoxoid antigens of most commercially available *Mh* vaccines are harvested through propagation of whole-cell *Mh* cultures. Nuplura® PH[a] contains both toxoid and cellular-associated antigens, but differs as the leukotoxoid is manufactured using recombinant technology, and the outer membrane proteins are extracted from the bacterial cell walls of a wild-type isolate using filtration processes. Prior to this study, the relative efficacy of vaccine programs incorporating Nuplura® PH versus a program incorporating a product whose leukotoxoid is derived from whole-cell *Mh* culture had not been evaluated in a large-pen feedlot setting. Calves administered a vaccine program incorporating Titanium® 5 at branding and weaning subsequently had reduced feedyard mortality in a small study,[16] but no large-pen feedlot studies evaluating Titanium® are reported in the scientific literature. The objective of this study was to compare 3 vaccine programs designed to protect against *Mh* and respiratory viruses on the health, growth performance, and carcass characteristics of feedlot heifers.

Materials and Methods

Summary of experimental design

A total of 2,575 beef heifers (BW = 568 ± 28.1 lb; 258 ±12.7 kg) at high-risk of developing respiratory disease were procured from auction-markets in the southern United States during May and June of 2017, and transported to a commercial feedyard in southwest Kansas to be enrolled in a randomized complete-block design study to evaluate the effect of 3 feedlot vaccine programs on health, growth performance, and carcass characteristics. Dates of arrival to the feedyard served as the blocking factor and 10, 3-pen blocks were enrolled in the study (n = 10 replications per vaccine program). The 3 vaccine programs differed by either vaccine products or timing of the pentavalent vaccine containing modified-live virus (**MLV**): 1) **PRE** (a *Mh* culture supernatant leukotoxoid vaccine[a] administered concurrently during arrival processing with a pentavalent MLV vaccine[b] containing infectious bovine rhinotracheitis, bovine viral diarrhea [types 1 and 2], parainfluenza 3, and bovine respiratory syncytial viruses; both vaccines given during arrival processing), 2) **TNA** (a recombinant *Mh* leukotoxoid vaccine[c] administered concurrently during arrival processing with a pentavalent MLV vaccine[d] containing strains of the same 5 families of viruses), and 3) **TND** (the same recombinant *Mh* leukotoxoid vaccine[c] as TNA administered during arrival processing, and the same pentavalent MLV vaccine[d] as TNA but delayed until the 28th day-on-feed (**DOF**)). No booster vaccinations were administered. Daily health observations were performed by pen riders blinded to vaccine program, and the heifers were harvested based upon visual estimate of fatness. The average DOF was 219 (range 189 to 238 days). The 3 pens within a block were harvested on the same day at the same abattoir. Endotoxin concentrations were measured in the 2 *Mh* vaccines represented in the vaccine programs.

Endotoxin measurements

Three 100 mL bottles of each *Mh* vaccine were submitted to a good manufacturing practice compliant and Food and Drug Administration approved laboratory[e] for the evaluation of endotoxin concentrations. Over the 10 blocks, the recombinant leukotoxoid vaccine administered to heifers in the TNA and TND treatments represented a single manufacturing lot, whereas the leukotoxoid supernatant vaccine administered to the PRE heifers represented 3 different manufacturing lots. These lots were represented in the vaccine submitted for measurement of endotoxin concentrations. An additional 2 manufacturing lots of the recombinant leukotoxoid vaccine were submitted to compare variation in endotoxin concentration within 3 manufacturing lots of each vaccine. Endotoxin-specific gel-clot assays were performed using a limulus amebocyte lysate (**LAL**) reagent[f] reconstituted with a buffer[g] formulated to render the reagent insensitive to (1→3)-ß-D-glucan interference. The potency of the Control Standard Endotoxin used to prepare the standard curve and positive product controls was determined in comparison to the FDA Reference Standard Endotoxin (10,000 endotoxin units (**EU**)/vial) using the lot of LAL reagent to be used for the vaccine assays. Next, 100 µL of the vaccine was reacted in an endotoxin-free reaction tube with 100 µL of LAL/buffer combination. The tubes were then vigorously shaken for 30 seconds before being incubated for 60 minutes at 98.6°F (37°C). Endotoxin concentrations were determined by visual evaluation of a series of vaccine dilutions and a positive test was indicated by gel formation that didn't collapse when the tube was inverted. An aliquot from each vaccine lot was tested in duplicate. The amount of endotoxin in the vaccine was calculated by multiplying the sensitivity of the limulus amebocyte lysate reagent[f] (0.03 EU/mL) by

the dilution factor at the endpoint and expressed as EU/mL of the vaccine.

Arrival processing

Heifers were housed in receiving pens upon arrival to the feedyard and provided *ad libitum* access to hay and water until processing. If the number of heifers required for a block was unable to be satisfied from a single auction market, then additional heifers were acquired from another auction market by no later than 3 days after the arrival of the first load to the feedyard. In such cases, groups of heifers remained separated by source through arrival processing to equally distribute source across treatments and eliminate the potential of treatments being confounded by source. Arrival processing occurred the day after the last truckload of heifers arrived at the feedyard to ensure all heifers were provided a minimum of 24 hours rest prior to processing. The heifers were evaluated by a veterinarian (MET) immediately before processing, and those severely affected by respiratory disease or another abnormal health condition that could potentially impact growth were excluded from the study.

Heifers were allocated to vaccine program during arrival processing using a chute-order randomization scheme that consisted of sequential, independent permutations of the 3 vaccine programs. Order of vaccine program treatments for all permutations within a block was determined by drawing the treatments out of a hat. The first vaccine program drawn was assigned to the first heifer in the chute, the second vaccine program drawn was assigned to the second heifer in the chute, and the third vaccine program drawn was assigned to the third heifer in the chute. The treatment order was used for the entire block, and a new order was drawn at the beginning of each subsequent block.

The viral and bacterial fractions were administered subcutaneously (**SQ**) in the right neck as separate injections for each vaccine program (2 mL per product; Nuplura® PH only during arrival processing for TND cattle). Because the viral vaccines were modified-live products, vaccine not administered within 120 minutes of rehydration of the desiccated virus was discarded and a new bottle was rehydrated. In addition to vaccine treatments, the following products were administered during arrival processing:

- Duplicate, serially numbered ear tags color-coded for each pen (tag colors were randomly assigned to each individual block to maintain blinding)
- Tilmicosin[h] (2 mL/100 lb [13 mg/kg]; nearest cwt to mean body weight for the block) administered SQ in the left neck
- Moxidectin[i] (1.0 mL/110 lb [0.2 mg/kg]; nearest cwt to mean body weight for the block) administered SQ in the left neck
- Oxfendazole[j] (1.0 mL/110 lb [4.5 mg/kg]; nearest cwt to mean body weight for the block) administered *per os*
- Dinoprost trimethamine[k] (25 mg/heifer) administered intramuscularly in the right neck
- Trenbolone acetate/estradiol growth promoting implant[l] administered SQ in the upper middle third of the left ear.

Group weights were captured for each individual vaccine program using certified scales after arrival processing was completed for all heifers enrolled within a block. The 3 groups comprising the block were then randomly assigned to 1 of 3 adjacently located pens oriented the same cardinal direction. Pen square footage and bunk space were equivalent within a block. An average of 86 heifers (range 77 to 91) were enrolled per pen with an average enrollment weight of 568 lb (258 kg; [range 524 to 618 lb or 238 to 280 kg]) across all blocks.

Feed and water

Heifers were fed diets formulated to meet or exceed requirements for growing beef cattle[20] (Table 1) using a slick bunk feeding program. Feed bunks were assessed daily by a trained observer who estimated orts and determined the amount to be delivered in order to provide near *ad libitum* access to feed. Daily feedings were provided over 2 deliveries with the exception of dietary transition periods during which feed was provided over 3 deliveries. The 3 pens within a block were transitioned to diet 2 on the 32nd day on feed to avoid transitioning diets during delayed viral vaccination. The day on feed which the transition to finishing diet (diet 3) occurred was dependent on feed intake of the pen and was not restricted to the same day for each pen within a block. With the exception of tylosin[m] for the reduction of incidence of liver abscesses, no additional concomitant feed-grade antibiotics were permitted during the trial. Ractopamine hydrochloride[n] (290 mg/head/day target) was included in the diet when the heifers were estimated to be 30 days from harvest and was started on the same day for each pen within a block. Water was provided *ad libitum* through an automatic float-activated system.

Table 1. Ingredient composition (percent as fed) for the 3 diets fed throughout the duration of the study.

	Diet		
Ingredient	**1**	**2**	**3**
Steam flaked corn	20.5	42.2	67.7
Wet distillers grain	38.4	28.3	16.7
Ground alfalfa hay	34.8	21.2	3.9
Chopped corn stalks	2.0	2.0	3.9
Fat	0.0	1.0	2.0
Liquid supplement	3.3	4.3	5.1
Micro ingredients*	1.0	1.0	0.7

* Formulated to provide the following: 26 g/ton monensin[t] (90% dry matter basis), 8 g/ton tylosin[m] (90% DM basis), 0.4 mg/heifer/day melengestrol acetate,[u] and 290 mg/heifer/day ractopamine hydrochloride[n] (last 28 to 32 days-on-feed only)

Delayed vaccination

The viral vaccine was administered to the TND heifers on the 28th day after arrival processing. This delayed vaccination was performed by the processing crew during the morning before the delivery of first feeding to ensure animal health observers and feed delivery personnel remained blinded to treatments throughout the duration of the study. Heifers receiving the TNA and PRE vaccine programs remained in their home pens and did not receive a booster vaccination. Administration of the delayed viral vaccine to the TND heifers was consistent with the standards applied during arrival processing when the TNA and PRE heifers received their viral vaccine.

Reimplant

Heifers were removed from their home pens and reimplanted with a second trenbolone acetate/estradiol growth promoting implant[l] at an average of 111 DOF (range 110 to 113). An interim body weight was collected for each pen by weighing the heifers in drafts before being implanted. All pens within a block were reimplanted on the same day and no additional products were administered during reimplant.

Health observations, BRD case definition, and therapeutic regimens

Following administration of tilmicosin during arrival processing, a 3 day post-metaphylactic interval was observed during which heifers were not eligible for antibiotic treatment for BRD. Health was evaluated daily by trained pen riders in accordance with the study site's standard operating procedures. The pen riders remained blinded to vaccine program treatments throughout the duration of the study, and each pen within a block was evaluated by the same pen rider within a single day.

Heifers with abnormal health conditions not related to BRD were handled and treated in accordance with standard operating procedures set forth by the study site's consulting veterinarian. Potential BRD morbidities were identified in the home pen and moved to a nearby hospital using low-stress handling methods to be examined further. In order to be considered a BRD case and receive antibiotic treatment, heifers must have had a rectal temperature ≥ 104.0°F (40°C) and at least 1 of the following clinical signs indicative of BRD: depression/lethargy, incoordination, dyspnea/abnormal respiration (rate, character, etc.), sunken eyes/dehydration, nasal and/or ocular discharge, lowered head carriage, and/or depressed ruminal fossa. Rectal temperature was measured using a digital thermometer.[o] Non-febrile (< 104°F) heifers determined to be severely affected with BRD based upon clinical presentation could be administered antibiotic therapy at the discretion of hospital personnel. Heifers requiring treatment for BRD were administered a single subcutaneous injection of enrofloxacin[p] (5 mg/lb [11 mg/kg] of body weight) in the neck with a 3-day post-treatment moratorium. Heifers still displaying clinical signs of BRD after the treatment moratorium or any time later in the feeding period were considered treatment failures and eligible for additional treatments. A single injection of florfenicol[q] (18.1 mg/lb [40 mg/kg] of body weight) or oxytetracycline[r] (9 mg/lb [19.8 mg/kg] of body weight) were administered subcutaneously to heifers requiring second and third BRD treatments, respectively, with a 3-day post-treatment moratorium observed for both antibiotics. Danofloxacin[s] (3.6 mg/lb [8 mg/kg] of body weight) was used for treatment of late-day BRD morbidities when the withdrawal period of the antibiotic called for by the study treatment regimen would have exceeded the projected days until harvest. Heifers receiving treatment with danofloxacin were included in the analyses of overall morbidity and appropriate levels of BRD treatment, but subsequent treatments were omitted from the dataset. Treatment success was defined as heifers which did not require subsequent BRD treatment for the remainder of the feeding period nor was their cause of death attributable to BRD.

Heifers receiving antibiotic therapy were subjectively evaluated by feedlot personnel and returned to their home pen the same day of treatment if deemed well enough to thrive. Hospital pens were available to house non-thriving calves and were subjectively evaluated daily by feedlot personnel who monitored response to treatment and eligibility of individual heifers to be returned to their home pen. Feed consumed by heifers in the hospital pens was prorated back to the appropriate home pen by dividing the feed delivery by the number of heifers in the hospital pen that day. Heifers non-responsive to the third BRD treatment were considered chronically-ill and not eligible to be returned to the home pen. Chronically-ill heifers were weighed the same morning their pen cohorts were shipped to packing plant so that their weight could be included in the deads-in analyses. Mortalities were necropsied by an attending veterinarian (MET) or other trained feedlot personnel who determined the probable cause of death based upon gross examination. No additional diagnostics were performed to confirm or differentiate causes of mortality.

Harvest

Heifers were harvested based upon visual estimate of adequate fatness. Average DOF was 219 (range 189 to 238 days), and all pens within a block were harvested on the same day at the same abattoir. Heifers were weighed by pen in drafts on a livestock ground scale during the morning before harvest, and a 4% shrink was applied to final weights. Pen-level carcass data was provided by the abattoir, and the abattoir at which the heifers were harvested varied by block. Because all heifers within a block were harvested on the same day at the same abattoir, the potential variability across packing plants was considered to have a minimal impact on the study. One block was omitted from the statistical analyses of carcass characteristics due to logistical issues at the abattoir and heifers being harvested over multiple days.

Economics

The heifers were marketed on a live-basis, and profitability was calculated for each pen at closeout with the following equation: NP = SP x SW – BIC – FC – MC – PC – YD – DC where: NP = net profit, SP = sale price ($/cwt), SW = total saleable weight (including railers), BIC = beef improvement checkoff fees, FC = feed costs, MC = medicine costs, PC = processing costs, YD = yardage, and DC = delivered cost (purchase price plus transport costs). Profitability was calculated on a deads-in basis and is expressed as both per heifer enrolled and per heifer harvested. Sale price and delivered cost were the same within a block as each pen was delivered and sold on the same day(s). Shrink-adjusted body weights were used to determine the total saleable weight.

Statistical analyses

Data were imported into a commercial software package.[v] Endotoxin concentrations were log-transformed to meet normality assumptions and a linear model was used to analyze log transformed endotoxin concentrations by vaccine. Differences in endotoxin concentration between the *Mh* vaccines was considered statistically significant when $P \leq 0.05$. The model adjusted means and their 95% confidence intervals were back transformed to the original scale for reporting.

Continuous variables were analyzed using a linear mixed model that included the fixed effect of vaccine program and the random effect of block. Categorical variables were analyzed with a generalized linear mixed model that included the same designation of fixed and random effects as the model for continuous variables. For the categorical variables, the data were modeled with a binomial distribution of outcomes in an $\frac{events}{trials}$ analysis where the count of reactors (pulls, etc.) represented the events and the exposed population (count of heifers enrolled or harvested, depending on the outcome) represented the trials. Least squares means and standard errors were converted to percentages by multiplying by 100. Pen served as the experimental unit for all outcomes. Performance outcomes were evaluated on a deads-out (railers also excluded) and deads-in (railers included) basis. A statistical effect of vaccine program treatment was declared and pairwise comparisons were performed amongst all vaccine programs for outcomes when $P \leq 0.05$ for the overall F-test. For pairwise comparisons, vaccine programs were considered to be statistically different when $P \leq 0.05$. Tendencies were declared when $0.06 \leq P \leq 0.10$. Descriptive cumulative BRD first treatment and overall mortality curves were evaluated by vaccine program and DOF.

Bayesian latent-class models were used to evaluate causes of mortality (BRD, digestive, acute interstitial pneumonia (**AIP**), or other) because generalized linear mixed models failed to converge due to 0 AIP mortalities in 1 of the vaccine programs. Bayesian models included binomial outcomes with block included as a random effect. Mean and 95% probability intervals were calculated for posterior distributions. Two-chain models were utilized and convergence evaluated by visual assessment of density, autocorrelation, trace plots, and Brooks-Gelman-Rubin diagnostics for each parameter. Bayesian analyses outcomes provide probability of difference between vaccine programs based upon posterior distributions of the data, whereas traditional frequentist P-values indicate the probability the magnitude of difference observed would be as great or greater if there was truly no difference between vaccine programs. Numerically greater probabilities from Bayesian analyses suggest a higher likelihood the difference observed between vaccine programs is the true outcome given the model assumptions and study design.

Results and Discussion

Endotoxin measurements

Mannheimia haemolytica is a gram-negative bacterial species and contains endotoxin as a constituent of the cell wall. As a result, an inherent amount of endotoxin is present in vaccines that are produced using *Mh* cell cultures. The recombinant *Mh* leukotoxoid vaccine had less variability and significantly lower endotoxin concentrations compared to the vaccine whose leukotoxoid is a supernatant derived from whole-cell *Mh* culture (Table 2; $P<0.01$).

Endotoxins are highly reactive substances that can contribute to the pathophysiological effects observed in gram-negative infections.[25] However, outer membrane proteins and a ruminant-specific leukotoxin are believed to be the antigens primarily responsible for providing immunity against *Mh* in cattle.[8,13,31] Thus, endotoxin present in *Mh* vaccines is undesirable as it could have potentially negative implications such as interfering with the ability of the vaccine to confer immunity against these antigens, and contribut-

Table 2. Model-adjusted least square mean (95% confidence interval) and standard deviation of endotoxin concentrations in the *Mannheimia haemolytica* vaccines.*

Vaccine	Endotoxin concentration (EU/mL)	95% confidence interval	Standard deviation	*P*-value†
Nuplura® PH	1,588	(679.3, 3,710.5)	1,069.9	< 0.01
Presponse® SQ	56,120	(24,012.4, 131,160.9)	11,951.2	

* Model included log-transformation of endotoxin concentrations. Model adjusted means and 95% confidence intervals were back transformed to the original scale for reporting.

† Differences between the vaccines were considered statistically significant if $P \leq 0.05$.

ing to the phenomena known as the "vaccine sweats". In naturally occurring infections, endotoxin is released during periods of rapid bacterial proliferation or when the bacterium becomes lysed by host inflammatory cells.[26] Multiple studies have intravenously administered endotoxin to simulate naturally occurring infectious disease, and describe increases in acute phase proteins and pro-inflammatory cytokines in cattle.[5,6] A study evaluating the innate immune response of calves weaned at 80 versus 250 days-of-age reported a more profound acute-phase response in the calves weaned later in life,[6] suggesting some populations may be more sensitive to endotoxin than others. It is important to note that the endotoxin used in that study was derived from *Escherichia coli*. Because the weight and potency of endotoxin can vary across different bacterial species,[25] endotoxin concentrations are expressed using EU to allow for comparisons of biological activity. Considering the potency (5 EU/ng) of the endotoxin and the dosage administered (1 µg/kg; BW = 233 kg [513 lb]) in the study evaluating calves weaned at different ages,[6] the calculated EU/kg of body weight provided intravenously is over 11 times greater than the amount of endotoxin provided to calves that were administered the supernatant *Mh* vaccine SQ in the current study. Another study evaluated the pharmacodynamics of intravenously administered endotoxin in Jersey cows and reported clinically healthy cows cleared the endotoxin within 30 minutes, whereas healthy cows administered non-steroidal anti-inflammatory drugs immediately before infusion had delayed clearance of the endotoxin.[1] Future research should aim to better understand the biological significance of endotoxin present in *Mh* vaccines within populations of cattle with various immunocompetencies, in addition to populations receiving concurrent gram-negative vaccines containing additional endotoxin.

Health outcomes

Health outcomes during the portion of the feeding period leading up to reimplant (average 111 DOF) and for the entire feeding period (average 219 DOF) are presented in Tables 3 and 4, respectively. Descriptive cumulative BRD morbidity (first treatment only) and overall mortality curves by vaccine program and DOF are also presented (Figure 1). Overall mortality, BRD mortality, and BRD morbidity did not differ among vaccine programs at reimplant (Table 3; $P \geq 0.22$). Overall mortality (Figure 1B), removals, and BRD morbidity (Figure 1A), treatment success rates and case fatality risk did not differ at closeout (Table 4; $P \geq 0.13$). Other studies report delaying a viral vaccine 7 to 14 days had no effect on mortality or BRD morbidity in newly received beef cattle in confined and stocker settings.[10,22,26,27,28] A separate study reported delaying the viral vaccine used in the PRE vaccine program for 30 days reduced the re-treatment risk in feedlot heifers that had already received an initial treatment for BRD during the feeding period.[30] However, overall BRD morbidity and mortality were not affected by delaying the vaccine 30 days, similar to the current study. It is important to note the heifers administered the viral vaccine during arrival processing in the current study remained in the home pen on day 28 and were not revaccinated, whereas the study that delayed the vaccine 30 days[30] also revaccinated the cattle that received the vaccine during arrival processing. The degree to which the additional vaccination or handling of the cattle in the arrival vaccination treatments might have attributed to

Table 3. Model-adjusted least square means for enrollment weight and health and performance of feedlot heifers at reimplant (average 111 days-on-feed) by vaccine program.

Variable	PRE*	TNA†	TND‡	SEM§	*P*-value‖
No. calves (pens)	860 (10)	855 (10)	860 (10)	-	-
Enrollment weight, lb	565.7	568.6	570.3	9.19	0.09
BRD first treatment, %	13.42	12.39	14.57	2.05	0.40
BRD second treatment, %	6.01	5.73	6.45	1.18	0.80
BRD third treatment, %	2.69	2.39	3.10	0.77	0.62
BRD mortality, %	2.33	2.74	1.94	0.77	0.48
Overall mortality, %	2.53	3.15	1.94	0.85	0.22
Interim body weight, lb	951.0	954.9	946.9	12.21	0.18
ADG, lb¶	3.21	3.15	3.20	0.10	0.77
ADG, lb#	3.47[a]	3.48[a]	3.39[b]	0.05	0.05

* PRE = Pyramid® 5 + Presponse® SQ (Boehringer Ingelheim, St. Joseph, MO) during arrival processing

† TNA = Titanium® 5 + Nuplura® PH (Elanco Animal Health, Greenfield, IN) during arrival processing

‡ TND = Nuplura® PH during arrival processing and Titanium® 5 (Elanco Animal Health, Greenfield, IN) administered 28 days-on-feed

§ Largest SEM in the analysis

‖ *P*-value for the overall treatment effect *F*-test. A statistical effect of vaccine program treatment was declared and pairwise comparisons were performed amongst all treatments for outcomes when $P \leq 0.05$ for the overall *F*-test

¶ Dead animals included in analysis

Dead animals excluded in analysis

[a,b] Means without common superscripts differ ($P \leq 0.05$)

Table 4. Model-adjusted least square means for health outcomes of feedlot heifers at closeout (average 219 days-on-feed) by vaccine program.

Variable	PRE*	TNA†	TND‡	SEM§	*P*-value‖
BRD first treatment, %	14.27	13.24	15.77	2.11	0.31
BRD second treatment, %	6.64	6.24	7.53	1.26	0.54
BRD third treatment, %	2.87	3.10	3.62	0.80	0.64
BRD first treatment success,¶ %	47.22	47.19	45.99	4.75	0.97
BRD second treatment success,¶ %	49.46	39.22	45.69	6.95	0.54
BRD third treatment success,¶ %	43.79	42.03	66.10	10.50	0.13
BRD case fatality risk, %	18.67	21.91	13.61	4.62	0.18
Overall mortality, %	4.00	3.82	3.06	0.94	0.50
Overall removals, %	0.20	0.31	0.20	0.22	0.86

* PRE = Pyramid® 5 + Presponse® SQ (Boehringer Ingelheim, St. Joseph, MO) during arrival processing

† TNA = Titanium® 5 + Nuplura® PH (Elanco Animal Health, Greenfield, IN) during arrival processing

‡ TND = Nuplura® PH during arrival processing and Titanium® 5 (Elanco Animal Health, Greenfield, IN) administered 28 days-on-feed

§ Largest SEM in the analysis

‖ *P*-value for the overall treatment effect *F*-test

¶ Treatment success defined as not requiring additional antibiotic treatment for BRD or cause of death was non-related to BRD

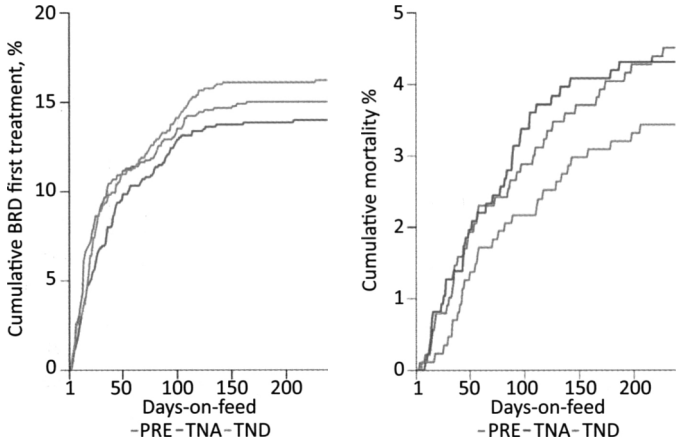

Figure 1. Descriptive cumulative BRD morbidity (first treatment only; A) and overall mortality (B) curves by vaccine program* and days-on-feed.
* **PRE**: Pyramid 5® + Presponse® SQ (Boehringer Ingelheim, St. Joseph, MO) during arrival processing; **TNA**: Titanium 5® + Nuplura® PH (Elanco Animal Health, Greenfield, IN) during arrival processing; **TND**: Nuplura® PH during arrival processing and Titanium 5® (Elanco Animal Health, Greenfield, IN) on the 28th day-on-feed

the discrepancy of the effect of delayed vaccination on BRD treatment success rates between these 2 studies is unclear.

Traditional frequentist statistical models failed to converge appropriately because there were no mortalities attributable to AIP for heifers that received the TNA vaccine program. Because of this, Bayesian analyses were used to evaluate cause of mortality through closeout. Bayesian analyses are commonly used for diagnostic test evaluation, disease population estimates, and are able to provide posterior estimates for rare events.[12,15] The posterior estimates allowed for comparison of mortality risk by causation category (AIP, BRD, digestive, or other) between vaccine programs. To the authors' knowledge, this is the first study using Bayesian analyses to evaluate risk of mortality by causation in feedlot cattle. However, there are examples in the literature where Bayesian analyses have been used to model average daily gain variance,[7] diagnostic test accuracy,[11,19,35,36] meta-analyses,[33] and disease prevalence.[21]

Vaccine program had no effect on mortality attributable to BRD, digestive disorders, or causes classified as "other" (probability of difference ≤ 0.70). To the authors' knowledge, this is the first large-pen feedlot study comparing the vaccines used in the TNA and TND vaccine programs to other commercially available vaccines. A 2015 study compared a combination vaccine containing the same *Mh* vaccine and viral desiccate used in the current PRE treatment to another commercially available combination vaccine containing a *Mh* bacterin-toxoid derived from whole-cell culture and reported no differences in health outcomes in a population similar to the one evaluated in the current study.[29] Another study conducted in Colorado compared 2 viral vaccines containing the same MLV antigens contained in the different combination vaccines compared in the 2015 study and reported reduced BRD morbidity and BRD relapses in the cattle receiving the viral vaccine used in the current PRE treatment, but no difference between the viral vaccines for overall or BRD mortality.[3] Whether the discrepancy in treatment effect on BRD morbidity between these 2 earlier studies is attributable to the presence of the *Mh* fraction (i.e. combination vs MLV only), gender of the population, or unknown factors cannot be delineated.

Both *Mh* vaccines used in this study have been previously shown to reduce BRD morbidity and mortality compared to controls that did not receive a *Mh* vaccine,[4,14] although the study evaluating the recombinant *Mh* leukotoxoid vaccine was an earlier version. Another study evaluated lung lesions and reported the addition of cellular-associated antigen (recombinant *Mh* outer membrane protein Plpe) improved protection of the *Mh* supernatant vaccine used in the PRE vaccine

program to a transthoracic *Mh* experimental challenge.[8] The recombinant *Mh* leukotoxoid vaccine in the current study also contains outer membrane proteins to provide cellular-associated antigen, although they are not recombinant. It is difficult to discern why the possession of outer membrane protein antigen did not translate to reduced BRD morbidity and mortality for the TNA and TND heifers in the current study, but adding recombinant Plpe improved protection of the supernatant vaccine to *Mh* challenge.[8]

The authors emphasize that diagnosis of AIP was based solely upon gross examination. With this in mind, the TNA heifers had numerically fewer mortalities attributable to AIP than the heifers that received the TND and PRE vaccine programs (Table 5; probability = 0.99 and 1.00, respectively). It is important to note the small sample size as there were only 9 mortalities attributed to AIP in heifers receiving the PRE vaccine program, 2 in the heifers receiving the TND vaccine program, and none for the heifers receiving the TNA vaccine program. The probability of difference between the TND and PRE vaccine programs for AIP mortality was lower (probability = 0.79). The 9 AIP mortalities in the PRE heifers occurred over 5 blocks (DOF range: 126 to 227), and were not clustered within a single block. The proportion of mortality in the feedyard attributable to AIP is small compared to BRD,[38] and the difference in AIP mortalities between the vaccine programs was surprising. Generally speaking, AIP mortalities occur relatively late in the feeding period and are believed to have a multifactorial etiology with a variety of risk factors such as gender (heifer predilection), and heat stress.[9,18] A greater degree of fatness has been speculated to be a predisposing factor for AIP, but this is inconsistent when looking solely at the arrival vaccine programs in our study. The TNA heifers tended (P=0.08) to have a greater percentage of yield grade 4 carcasses than the PRE heifers, but the TNA heifers also had fewer AIP mortalities (0 vs 9). Prior or concurrent bacterial bronchopneumonia has also been associated with an increased risk of developing AIP in feedlot cattle,[34,37] suggesting better protection against BRD early in the feeding period could subsequently reduce the incidence of AIP. However, the presence of concurrent bacterial pneumonia was not recorded for the AIPs in the current study, and BRD morbidity did not differ between treatments. Dust has also been implicated as a risk factor associated with increased incidence of AIP in feedlots,[2,38] and feedyard dust has been shown to carry endotoxin.[24] Sheep exposed to aerosolized feedyard dust containing endotoxin developed an interstitial pneumonia in a challenge setting.[23] The potential for endotoxin load of the *Mh* vaccines administered during arrival processing to contribute AIP mortality that occurs later in the feeding period is unclear, although development of a delayed type hypersensitivity might be considered. Another limitation when trying to consider potential reasoning behind the differences in number of AIP mortalities between treatments is that the study evaluated vaccine programs, resulting in the *Mh* vaccine being confounded by the viral vaccine. This is the first study suggesting differences in AIP mortality between vaccine treatments, and the repeatability of this finding should be verified.

Growth performance

Growth performance leading up to reimplant and for the entire feeding period are presented in Tables 3 and 6, respectively. Deads-out ADG was lower for TND heifers compared to the heifers receiving either the PRE (P=0.04) or TNA vaccine program (P=0.02) at reimplant. The decrease in deads-out ADG observed in TND heifers was not reflected in interim body weight (P=0.18), and ADG was not different between vaccine programs (P=0.77) when calculated on a deads-in basis. There was no effect of vaccine program on ADG or feed conversion (deads-in or -out), dry matter intake, cost of gain, or final body weight over the course of the entire feeding period (P≥0.34). In the study mentioned earlier that evaluated the effect of delaying the pentavalent viral vaccine used in the PRE treatment until 30 DOF versus giving it during arrival processing, the heifers vaccinated during arrival processing tended to have a greater final body weight and carcass weight following an average of a 209 day feeding period, although average daily gain did not differ.[30] The study reported in 2015 that compared combination vaccines also reported no effect on growth performance,[29]

Table 5. Disease risk and 95% probability interval for cause of mortality of feedlot heifers by vaccine program.

	PRE*		TNA†		TND‡		PRE vs TNA§	TNA vs TND§	PRE vs TND§
Cause	Mean	95% PI	Mean	95% PI	Mean	95% PI			
BRD, %	2.60	(1.37, 4.94)	3.30	(1.75, 6.11)	2.23	(1.16, 4.24)	0.30	0.48	0.19
Digestive, %	0.11	(0.03, 0.57)	0.15	(0.03, 0.58)	0.28	(0.06, 0.69)	0.67	0.66	0.60
AIP,‖ %	1.01	(0.37, 1.81)	0.00	(0.00, 0.01)	0.05	(0.01, 0.44)	1.00	0.99	0.79
Other, %	0.05	(0.00, 0.33)	0.34	(0.08, 1.12)	0.07	(0.00, 0.60)	0.70	0.55	0.23

* PRE = Pyramid® 5 + Presponse® SQ (Boehringer Ingelheim, St. Joseph, MO) during arrival processing

† TNA = Titanium® 5 + Nuplura® PH (Elanco Animal Health, Greenfield, IN) during arrival processing

‡ TND = Nuplura® PH during arrival processing and Titanium® 5 (Elanco Animal Health, Greenfield, IN) administered 28 days-on-feed

§ Probability of difference between vaccine programs based upon Bayesian posterior distributions.

‖ Acute interstitial pneumonia

whereas the 2008 study conducted in Colorado reported the viral vaccine used in the PRE treatment in the current study had superior feed-conversion when adjusted on a carcass weight basis compared to the other pentavalent MLV viral vaccine.[3]

Carcass characteristics

The proportion of carcasses that were yield grade 4 was lower (Table 7; P=0.01) for heifers receiving the PRE vaccine program than for the TND heifers, and tended (P=0.08) to be lower than for the TNA heifers. The proportion of carcasses that were yield grade 4 did not differ (P=0.29) between TNA and TND programs. No other carcass trait differed among vaccine programs (P≥0.14). The confounding of the *Mh* vaccine by the viral vaccine in the TNA and PRE vaccine programs in the current study limits our ability to interpret potential reasoning behind the differences observed in the proportion of carcasses that were yield grade 4. In addition to vaccine products, the TND and PRE treatments are confounded by timing of viral vaccine administration; although the similarity between the TNA and TND heifers suggests timing has no effect on yield grade. A lower proportion of yield grade 4 carcasses was also reported in the 2015 study where no differences were observed in health outcomes for feedlot heifers receiving the combination vaccine that incorporates the vaccines used in the PRE treatment compared to another MLV/*Mh* bacterin-toxoid combination vaccine.[29] The authors speculated the differences in yield may have been reflective of subtle differences in subclinical disease, although they saw no differences in health outcomes. With regards to the current study, the authors speculate the nature of both groups that received the recombinant *Mh* leukotoxoid having a greater proportion of yield grade 4 carcasses than the group that received the supernatant vaccine could potentially indicate it is less likely to be a type I experimental error. The previous authors' hypothesis would support the increased proportion of yield grade 4 heifers in PRE compared to both the TNA and TND treatments if there was a vaccine effect, yet the current study also reported no differences in morbidity or mortality, nor is the increase in yield grade 4 heifers reflected by a greater dressing percentage or quality grade distribution. Furthermore, no difference was observed in the proportion of yield grade 4 carcasses from steers in the 2008 study that compared the same pentavalent vaccines as the 2015 study but did not include a *Mh* fraction.[3] Similar to BRD morbidity, it is difficult to discern if the discrepancy of vaccine treatment on yield grade between these 2 previously published studies is attributable to the presence of the *Mh* fraction.

Economic analysis

Profitability of the vaccine programs is reported on a per heifer enrolled and per heifer sold basis in Table 6. While profitability did not differ by vaccine program (P≥0.35), the TNA and TND programs yielded $8.21 and $20.30 numerical advantages per heifer sold, respectively, compared to heifers administered the PRE vaccine program. Because these analyses were deads-in, the numerical differences observed in profitability are mostly attributable to the numerically greater level of mortality that was observed in the PRE heifers relative to the TNA and TND heifers. The 2015 study comparing 2 commercially available combination vaccines containing respiratory viruses and *Mh* bacterin/toxoid in a single injection also reported no differences in economic outcomes between vaccines in feedlot cattle.[29]

Table 6. Model-adjusted least square means for live performance and economic outcomes of feedlot heifers at closeout (average 219 days-on-feed) by vaccine program.

Variable	PRE*	TNA†	TND‡	SEM§	*P*-value‖
Final body weight, lb¶	1248.7	1248.8	1248.3	8.75	0.99
ADG, lb#	2.87	2.86	2.91	0.08	0.73
ADG, lb**	3.13	3.12	3.11	0.04	0.79
F:G#	6.41	6.36	6.24	0.11	0.34
F:G**	6.15	6.11	6.06	0.06	0.43
Dry matter intake, lb	18.31	18.17	18.14	0.28	0.65
Cost of gain,# $/100 lb	80.39	79.78	78.64	1.38	0.46
Cost of gain,** $/100 lb	77.15	76.72	76.29	0.78	0.57
Profit, $/heifer enrolled	76.62	84.30	96.44	13.47	0.39
Profit, $/heifer sold	79.30	87.51	99.60	13.69	0.35

* PRE = Pyramid® 5 + Presponse® SQ (Boehringer Ingelheim, St. Joseph, MO) during arrival processing

† TNA = Titanium® 5 + Nuplura® PH (Elanco Animal Health, Greenfield, IN) during arrival processing

‡ TND = Nuplura® PH during arrival processing and Titanium® 5 (Elanco Animal Health, Greenfield, IN) administered at 28 days-on-feed

§ Largest SEM in the analysis

‖ *P*-value for the overall treatment effect *F*-test.

¶ Adjusted for 4% shrink.

Dead animals included in analysis

**Dead animals excluded in analysis

Table 7. Model-adjusted least square means for carcass characteristics of feedlot heifers by vaccine program.

Variable	PRE*	TNA†	TND‡	SEM§	*P*-value‖
Hot carcass weight, lb	796.33	793.92	795.70	4.29	0.81
Dressing percent,¶ %	63.44	63.46	63.49	0.34	0.98
Carcass adjusted ADG,# lb	3.14	3.11	3.12	0.04	0.60
Prime, %	2.03	1.40	1.63	0.58	0.61
Choice, %	66.93	70.16	65.45	2.97	0.15
Select, %	29.65	27.23	30.89	3.30	0.30
Standard/No roll, %	0.74	0.73	1.34	0.47	0.35
Yield grade 1, %	6.67	6.22	6.80	1.45	0.88
Yield grade 2, %	37.96	37.06	34.46	3.66	0.36
Yield grade 3, %	46.15	44.87	44.26	3.76	0.76
Yield grade 4, %	6.71[a]	9.09[a,b]	10.68[b]	1.79	0.02
Yield grade 5, %	0.38	0.64	1.27	0.46	0.14

* PRE = Pyramid® 5 + Presponse® SQ (Boehringer Ingelheim, St. Joseph, MO) during arrival processing
† TNA = Titanium® 5 + Nuplura® PH (Elanco Animal Health, Greenfield, IN) during arrival processing
‡ TND = Nuplura® PH during arrival processing and Titanium® 5 (Elanco Animal Health, Greenfield, IN) administered at 28 days-on-feed
§ Largest SEM in the analysis
‖ *P*-value for the overall treatment effect *F*-test
¶ Adjusted for 4% shrink
# ADG calculations based upon common dressing percent of 63.46% in the 9 blocks with carcass outcomes captured
[a,b] Means without common superscripts differ ($P \leq 0.05$)

Conclusions

Our findings confirm reduced endotoxin concentrations in the *Mh* vaccine produced using recombinant leukotoxoid technology and purification of outer membrane proteins compared to a vaccine that is a supernatant of whole-cell *Mh* culture. There was no effect of vaccine programs incorporating these different *Mh* vaccines on BRD-related outcomes or growth performance. Delaying the viral vaccine 28 days had no effect on BRD-related health outcomes or growth performance over the entire feeding period. Additional research is warranted to verify the repeatability of the differences in AIP mortalities observed between the vaccine programs.

Endnotes

[a] Presponse® SQ, Boehringer Ingelheim Vetmedica, St. Joseph, MO
[b] Pyramid® 5, Boehringer Ingelheim Vetmedica, St. Joseph, MO
[c] Nuplura® PH, Elanco Animal Health, Greenfield, IN
[d] Titanium® 5, Elanco Animal Health, Greenfield, IN
[e] Associates of Cape Cod Incorporated, East Falmouth, MA
[f] Pyrotell®, Associates of Cape Cod Incorporated, East Falmouth, MA
[g] Glucashield®, Associates of Cape Cod Incorporated, East Falmouth, MA
[h] Micotil®, Elanco Animal Health, Greenfield, IN
[i] Cydectin®, Bayer Animal Health, Shawnee Mission, KS
[j] Synanthic®, Boehringer Ingelheim Vetmedica, St. Joseph, MO
[k] Lutalyse®, Zoetis Animal Health, Parsippany, NJ
[l] Component® TE-200 with Tylan, Elanco Animal Health, Greenfield, IN
[m] Tylan™, Elanco Animal Health, Greenfield, IN
[n] Optaflexx®, Elanco Animal Health, Greenfield, IN
[o] GLA M700 Thermometer, GLA, San Luis Obispo, CA
[p] Baytril®, Bayer Animal Health, Shawnee Mission, KS
[q] Nuflor®, Merck Animal Health, Desoto, KS
[r] Biomycin® 200, Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO
[s] Advocin®, Zoetis Animal Health, Parsippany, NJ
[t] Rumensin®, Elanco Animal Health, Greenfield, IN
[u] MGA®, Zoetis Animal Health, Parsippany, NJ
[v] R Studio Team® 2016, Boston, MA

Acknowledgements

The authors would like to acknowledge the staff at the Hy-Plains Educational Center (Hy-Plains Feedyard, Montezuma, KS) for their assistance conducting this study, and Arcadia Commodity Opportunity, LLC for ownership of the heifers. This study was funded by Elanco Animal Health, although no Elanco employees were directly involved with conduct or statistical analyses related to the study.

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