

Polyclonal antibody preparations from avian origin as a feed additive to beef cattle: ruminal fermentation during the step-up transition diets

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ABSTRACT

This study investigated the effects of feeding an avian-derived polyclonal antibody preparation (PAP; CAMAS, Inc.) against *Streptococcus bovis*, *Fusobacterium necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively) on ruminal fermentation [pH, ammonia-N (NH₃-N), lactate, and volatile fatty acids (VFA)] of beef steers during a 21-d step-up diet adaptation. Eight ruminally cannulated Angus crossbred beef steers (658 ± 79 kg of body weight) were assigned in a crossover design to be transitioned from a diet containing ad libitum bermudagrass hay [*Cynodon dactylon* (L.) Pers.] plus 0.45 kg/d (as fed) of molasses with 0 (CON) or 3 g of PAP (PAP) to a high-grain diet. Transition consisted of three 7-d steps of increased inclusion of cracked corn (35%, 60%, and 82% of the diet DM for STEP1, STEP2, and STEP3, respectively). On each transition day and 7 d after STEP3 (STEP3-7d), ruminal fluid samples were obtained every 3 h for 24 h. Feeding 3 g of PAP daily increased ($P < 0.01$) average ruminal pH during STEP3 compared with CON steers (5.6 vs. 5.4 ± 0.05, respectively). During STEP1, NH₃-N concentration was greater ($P < 0.01$; 9.4 vs. 6.8 ± 0.74 mM, respectively), and time (min/d) and area (time × pH) of ruminal pH below or equal to 5.2 was lesser ($P ≤ 0.03$) for steers consuming PAP compared with steers assigned to CON treatment (33.4 vs. 73.3 ± 21.7 min/d and 187.4 vs. 406.3 ± 119.7 min × pH/d, respectively). Steers consuming PAP had greater acetate:propionate ratio at 0, 3, and 6 h relative to diet change compared with CON (2.42, 2.35, 2.29 vs. 1.66, 1.79, and 1.72 ± 0.17, respectively), whereas butyrate molar proportions increased ($P = 0.02$; 17.1 vs. 11 ± 1.58 mol/100 mol for CON and PAP, respectively) when PAP was not fed at STEP2. Total ruminal lactate concentrations were not affected by PAP feeding ($P > 0.11$). In conclusion, feeding 3 g/d of polyclonal antibody preparation against *S. bovis*, *F. necrophorum*, and lipopolysaccharides was effective in increasing ruminal pH, A:P ratio, and NH₃-N concentrations, possibly attenuating the risks of ruminal acidosis in steers during the step-up transition from forage to high-grain diets.

LAY SUMMARY

Feedlot cattle are fed high-grain diets that require a transition period with gradual increasing amounts of grain. Those diets are associated with changes in microbial populations of the gastrointestinal tract in favor of bacteria that can contribute to cause metabolic disorders by reducing ruminal pH. Feed additives are compounds added to the diet of feedlot cattle to improve animal health and performance by minimizing the effects of microbial changes. An alternative product, polyclonal antibody preparations (PAP), have emerged as a possible tool to ameliorate the effects of high-grain diets on cattle health and performance. Therefore, this research investigated the effects of PAP during diet transition to a high-grain in beef cattle. It was concluded that feeding PAP contributed to increase ruminal pH, which could result in reduced risks of metabolic diseases.

Key words: *Fusobacterium necrophorum*, lipopolysaccharides, step-up process, *Streptococcus bovis*

INTRODUCTION

High-grain diets can temporarily or permanently change the composition and functionality of the reticulo-rumen (Plaizier et al., 2017) in favor of bacteria that produce lactate. Feedlot cattle are fed high-grain diets, which require a transition period with gradually increasing amounts of grain (Coe et al., 1999) that on average lasts 24 d (Samuelson et al., 2016). However, the transition period is still the time when cattle encounter the greatest risk for ruminal acidosis (Fulton et al., 1979).

Ruminal bacteria, such as *Streptococcus bovis* and *Fusobacterium necrophorum*, respond to increased availability

of starch by boosting their growth rates in grain-fed animals (Nagaraja and Titgemeyer, 2010). Additionally, under low rumen pH, growth of *Escherichia coli* also contributes to disbalance in the ruminal environment (Khafipour et al., 2011). In grain fed cattle, ruminal lipopolysaccharides (LPS) concentrations may increase mostly due to intensified lysis or overgrowth of some gram-negative bacteria species (as *E. coli*), presenting a health risk to the host animal. Dai et al. (2020) reported that in vitro dosing of LPS in the presence of glucose stimulated the growth of *S. bovis*, which is associated with development of acidosis, whereas no impact of LPS

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was observed in microbial species that consume lactate or ferment fiber. Acidosis results when cattle consume fermentable carbohydrates in sufficient amounts to cause an accumulation of organic acids in the rumen, with a simultaneous reduction in ruminal pH (Nagaraja and Lechtenberg, 2007). Ruminal pH below 5.6 is considered an indicator of subacute acidosis, while a pH lesser or equal to 5.2 indicates acute ruminal acidosis (Owens et al., 1998; Bevans et al., 2005).

Feed additives, such as ionophores, are extensively used to enhance cattle performance by promoting alterations in ruminal microbial populations and fermentation (DiLorenzo et al., 2006), especially during high-grain feeding. However, other technologies, such as polyclonal antibody preparations (PAP), have been investigated as a possible tool to ameliorate the effects of high-grain diets in cattle health and performance (Silva et al., 2021). Several proposed strategies explain how immunoglobulin Y (IgY), derived from avian antibodies, can protect the host. It is suggested that IgY can agglutinate bacteria, inhibit bacterial adhesion, suppress the bacteria virulence factors, neutralize toxins, and inactivate enzymes (Xu et al., 2011). Previous research demonstrated that a PAP blend against *S. bovis*, *F. necrophorum*, and LPS, presented a positive effect on apparent total tract digestibility of the neutral detergent fiber in beef cattle consuming a nonforage backgrounding diet (Silva et al., 2022). Such effects could be explained by the potential of PAP to increase ruminal pH as observed by DiLorenzo et al. (2006), Blanch et al. (2009), and Marino et al. (2011) when using PAP against *S. bovis* and *F. necrophorum* into high-grain diets of beef steers, beef heifers, and Holstein cows, respectively. However, we recently demonstrated that feeding PAP during the step-up diet transition did not contribute to mitigating host immune responses (Silva et al., 2021). Therefore, investigating the effects of PAP on ruminal fermentation parameters during diet transition is necessary to assess whether the lack of responses on immunity is related to an absence of effects in ruminal parameters or in overall host immune response only. To the best of our knowledge, this is the sole study evaluating the effects of PAP as a tool to mitigate the negative effects of diet change in beef steers during the transition from forage to a high-grain diet on ruminal responses.

We hypothesized that feeding PAP against *S. bovis*, *F. necrophorum*, and LPS, would contribute to maintaining adequate ruminal fermentation parameters during the step-up diet transition, attenuating the risks of ruminal acidosis. Our objective was to evaluate the effects of feeding PAP on ruminal fermentation parameters of beef cattle during a 21-d step-up transition from forage to high-grain diets.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of the University of Florida (protocol #201810277) approved all procedures for the experiments conducted at the North Florida Research and Education Center (NFREC; Marianna, FL).

Polyclonal Antibody Preparations

The PAPs against *S. bovis* (ATCC 9809), *F. necrophorum* (ATCC 27852), and LPS from *E. coli* O157:H7 and bacteria from the genus *Salmonella* (LPS; 40, 35, and 25% of the preparation, respectively) are produced under patented and proprietary procedures (CAMAS Inc., Le Center, MN; DiLorenzo et al., 2006, 2008). The powder preparation used

in the current study comprised the whole egg (egg white and yolk) and contained IgY, immunoglobulin M, and immunoglobulin A. The molasses with PAP provided in the current experiment were analyzed before the start of the study by specific ELISA test plates (Corning Inc., Corning, NY) using the same proportion that was fed to steers (3 g of PAP in 0.450 kg of as fed liquid molasses) to monitor antibody concentrations. Results indicated 0.003 mg/g of IgY in the liquid molasses and PAP mix.

Experimental Design, Animals, and Treatments

The experiment was conducted at the University of Florida, Feed Efficiency Facility (FEF) as described by Silva et al. (2021). Eight ruminally cannulated Angus crossbred steers [658 ± 79 kg of body weight (BW); 4 steers/treatment/period] were used in a cross-over design with 2 periods of 36-d each plus 26 d washout within periods. Steers were randomly allocated to receive 0 (CON) or 3 g/d of PAP (PAP) that was individually fed using 0.45 kg/d (as fed) of liquid molasses as a carrier during the transition from a forage [bermudagrass hay (*Cynodon dactylon* (L.) Pers.)] to a high-grain diet through a 21-d step-up process.

From d -7 to 0, steers were fed only bermudagrass hay [56% total digestible nutrients (TDN) and 13.9% crude protein (CP) on a DM basis] ad libitum. From d 0 to 14, steers received 0.45 kg/d of liquid molasses with or without the addition of PAP and ad libitum bermudagrass hay; feeding PAP 14 d before the diet transition was needed to ensure adequate delivery of PAP in the rumen during the diet change. Chemical composition of the molasses used was (DM basis): 7.8% CP, 1.3% crude fat, 15% ash, 76% TDN, 1.23% Ca, 0.10% P, 0.45% Mg, 4.99% K, 0.127% Na, 1.17% S, 107 mg/kg Fe, 15 mg/kg Zn, 18 mg/kg Cu, 12 mg/kg Mn, and 1.3 mg/kg Mo. The molasses provided had 76% DM on as fed basis.

The diet transition consisted of three steps (STEP1, STEP2, and STEP3) that lasted 7 d each, in which the inclusion of cracked corn increased gradually (35%, 60%, and 82% of the diet DM, respectively) in replacement of cottonseed hulls (Table 1). The diets were offered ad libitum to steers and DM feed intake (DMI) was recorded using GrowSafe feed bunks (GrowSafe System 6.0 version, Ltd., Airdrie, Alberta, Canada).

Ruminal Fluid, pH, and Feed Sampling

Basal samples of ruminal fluid were collected during the day of each diet transition (d 14, 21, and 28) and STEP3-7d (d 35) before the molasses feeding and diet change (0 h), continuing every 3 h for a period of 24 h. All procedures to be described were performed similarly for each of the 2 periods.

Ruminal fluid was collected from 4 rumen sites to obtain a representative sample of digesta and squeezed through 4 layers of cheesecloth. The pH of ruminal fluid was immediately measured using a manual pH meter (Corning Pinnacle M530, Corning, Inc., Corning, NY), additionally a 10-mL sample was taken and 0.1 mL of a 20% (vol/vol) H₂SO₄ solution was added to the fluid to stop fermentation (Schulmeister et al., 2019). Samples were then placed in ice until all the collections were performed, transported to the laboratory, and stored at -20°C for further analysis.

Samples of STEP1, STEP2, and STEP3 diets were collected immediately after delivery on d 14, 21, and 28, respectively. Following collection, samples were dried at 55°C for 48 h in a

Table 1. Ingredients and nutritional composition (DM basis) of experimental diets fed during the step-up transition.

	STEP1	STEP2	STEP3
Ingredients, % DM			
Cottonseed hulls	52	27	5
Corn grain, cracked	35	60	82
Bermudagrass hay	5	5	5
Cottonseed meal	2	2	2
Liquid supplement ¹	5	5	5
Limestone	1	1	1
Nutritional composition, % DM			
DM, %	89	91	89
CP	11.1	12.4	12.3
aNDF ²	58.3	35.5	17.3
ADF ²	38	23.2	9.7
TDN	69	75	81
Starch	21.6	35	60.5

¹Molasses-based supplement containing (DM basis): 76% DM, 7.8% CP, 1.3% crude fat, 15% ash, 76% TDN, 1.23% Ca, 0.10% P, 0.45% Mg, 4.99% K, 0.127% Na, 1.17% S, 107 mg/kg Fe, 15 mg/kg Zn, 18 mg/kg Cu, 12 mg/kg Mn, and 1.3 mg/kg Mo.

²Alpha amylase neutral detergent acid (aNDF) and acid detergent fiber (ADF).

forced-air oven, ground in a Willey mill to pass a 2-mm sieve and sent to a commercial laboratory for chemical analyses (Table 1).

Ruminal pH and Ruminal Fluid Assay Procedures

Ruminal pH was measured every 3 h on d 14, 21, 28, and 35, and data were summarized as average pH, time (min/d), and area (time × pH) that pH was equal or below 5.6 and 5.2 within each 24-h period and during the entire 21-d transition period. Time with rumen pH below 5.6 and 5.2 was calculated and summarized because they may indicate subacute (Owens et al., 1998; Nagaraja and Titgemeyer, 2010) and acute (Owens et al., 1998; Penner et al., 2007) ruminal acidosis, respectively.

Concentration of volatile fatty acids (VFA) in ruminal fluid samples were determined in a liquid-liquid solvent extraction using ethyl acetate (C₄H₈O₂) as described by Ruiz-Moreno et al. (2015). Samples of ruminal fluid were centrifuged for 15 min at 10,000 × g and supernatant was combined with a meta-phosphoric acid:crotonic acid (internal standard) solution at a 5:1 ratio and frozen overnight. Samples were then thawed, homogenized, and recentrifuged for 15 min at 10,000 × g. The supernatant obtained was further transferred into glass tubes and mixed with ethyl acetate in a 2:1 ratio of ethyl acetate to supernatant, with the ethyl acetate fraction (after shaking tubes to allow mixing) transferred to vials for reading (Schulmeister et al., 2019). Samples were analyzed by gas chromatography (Agilent 7820A GC, Agilent Technologies, Palo Alto, CA) using a flame ionization detector and a capillary column (CP-WAX 58 FFAP 25 m 0.53 mm, Varian CP7767, Varian Analytical Instruments, Walnut Creek, CA).

Concentration of ruminal NH₃-N was measured after centrifuging ruminal fluid samples at 10,000 × g for 15 min at 4°C (Avanti J-E, Beckman Coulter, Inc., Palo Alto, CA) following the phenol-hypochlorite technique by Broderick and

Table 2. Dry matter intake (in kg/d) of Angus crossbred cannulated steers (658 ± 79 kg BW) fed free-choice step-up diets that contained increased concentrations of cracked corn¹ plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *Streptococcus bovis*, *Fusobacterium necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively).

Item	Treatment			
	PAP	CON	SEM	P-value
STEP1				
DMI on the day of transition	24.1	26.2	3.3	0.66
Weekly average DMI	18.8	19.0	1.4	0.83
STEP2				
DMI on the day of transition	16.2	18.5	1.8	0.38
Weekly average DMI	16.7	16.6	1.5	0.88
STEP3				
DMI on the day of transition	13.5	15.3	1.6	0.44
Weekly average DMI	11.2	12.1	1.1	0.57
STEP3-7d				
DMI 7-d post-last transition	15.6	13.5	2.4	0.57

¹Diets consisted of gradual inclusion of cracked corn from 0 (Bermudagrass hay only) to 35%, 60%, and 82% for STEP1 (d 14–21), STEP2 (d 21–28), and STEP3 (d 28–35), respectively.

Kang (1980) with modifications as described by Schulmeister et al. (2019).

Total ruminal lactate concentrations were determined using D-lactic acid/L-lactic acid kit from R-Biopharm (R-Biopharm AG, Darmstadt, Germany). The analysis procedure was adapted from the original protocol as described by Dai et al. (2019).

Statistical Analysis

Data were analyzed as a cross-over design using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.4), with steer as the experimental unit. The model for ruminal fermentation parameters (pH, VFA, NH₃-N, and lactate) and DMI throughout the study included the fixed effects of period, order, treatment, time, and treatment × time (hour or day of the study) interaction; random variables for all the analyses included effects of steer within period, order, and treatment. The DMI on the day and week of each transition was tested for the fixed effects of treatment, period, and order. Covariance structures for repeated measures were autoregressive based upon the smallest Akaike Information Criterion values and used steer within order, period, and treatment as the subject. Significance was set at $P \leq 0.05$, and tendencies if $0.05 < P \leq 0.10$.

RESULTS

Dry matter intake was not affected by treatment on the day of each dietary transition ($P \geq 0.38$) or on the week each diet was offered ($P \geq 0.57$; Table 2). However, DMI gradually decreased ($P < 0.01$; Figure 1) from STEP1 to STEP3 (25.1, 16.4, and 11.6 kg/d, respectively).

There was a treatment × STEP effect for average ruminal pH ($P < 0.01$; Figure 2E). Feeding 3 g of PAP daily increased average ruminal pH during STEP3 compared with CON

steers (5.6 vs. 5.4 ± 0.05 , respectively). Time (min/d) and area (time \times pH) with ruminal pH below 5.2 was decreased ($P \leq 0.03$; Table 3) for steers consuming PAP compared to steers assigned to CON treatment during STEP1, whereas no differences were observed during STEP2 and STEP3 ($P \geq 0.12$;

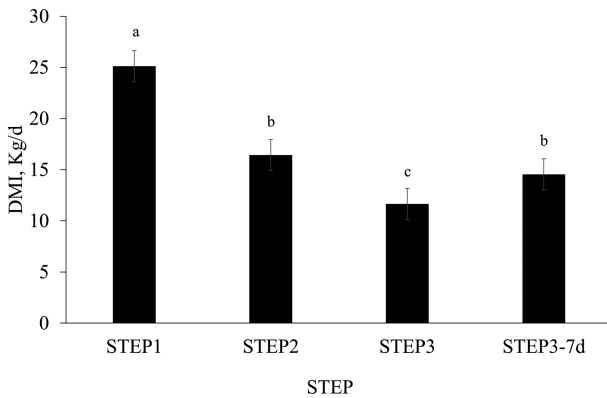


Figure 1. Dry matter intake of Angus crossbred steers fed free-choice step-up diets that contained increased concentrations of cracked corn (35%, 60%, and 82%; STEP1 [days 14–21], STEP2 [days 21–28], and STEP3 [days 28–35], respectively) plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *Streptococcus bovis*, *Fusobacterium necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively). Effect of step-up diet on DMI ($P < 0.01$; SEM = 1.53) but not treatment \times step ($P = 0.68$) or treatment ($P = 0.95$) were detected. Within STEP, means without a common superscript (a–c) differ ($P \leq 0.05$).

Table 3). Increasing the proportion of cracked corn in the diet from STEP1 to STEP3 (from 35% to 82% cracked corn, respectively) reduced ($P = 0.002$; Table 4) average ruminal pH, increased the time ($P = 0.001$) and area ($P = 0.003$) of ruminal pH below 5.6, but not the time and area ruminal pH was below 5.2. Even though the transition from forage to a high-grain diet was gradually performed, the time and area with ruminal pH below 5.2 were greater during STEP3-7d compared to STEP1, STEP2, and STEP3 ($P \leq 0.004$). An effect of hour ($P < 0.01$) was detected for ruminal pH during the hours following each diet transition, but neither a treatment \times hour ($P \geq 0.38$) or treatment ($P \geq 0.50$) effect was detected (Figure 2A–D).

There was a treatment \times STEP effect for ruminal $\text{NH}_3\text{-N}$ concentration ($P = 0.05$), in which steers consuming PAP had greater $\text{NH}_3\text{-N}$ during STEP1 compared with CON (9.4 vs. 6.8 ± 0.74 mM, respectively; Figure 3E). An effect of hour was detected for $\text{NH}_3\text{-N}$ concentration within each 24-h post diet transition ($P < 0.01$; Figure 3A–D). Regardless of STEP, ruminal concentrations of $\text{NH}_3\text{-N}$ were greatest 3 h post-diet transition, whereas $\text{NH}_3\text{-N}$ concentrations were greatest 6 h post PAP feeding during STEP3-7d (Figure 3D).

No effect of treatment \times STEP ($P \geq 0.12$; Table 4) or treatment ($P \geq 0.39$) were observed for VFA concentration during the 21-d diet transition period. However, an effect of STEP ($P \leq 0.01$; Table 5) was detected for concentrations (mM) of acetate, propionate, butyrate, valerate, caproate, total VFA, and A:P ratio. Concentrations of acetate linearly decreased, whereas concentrations of propionate were the least and A:P ratio the greatest in STEP1 ($P < 0.01$). Total VFA

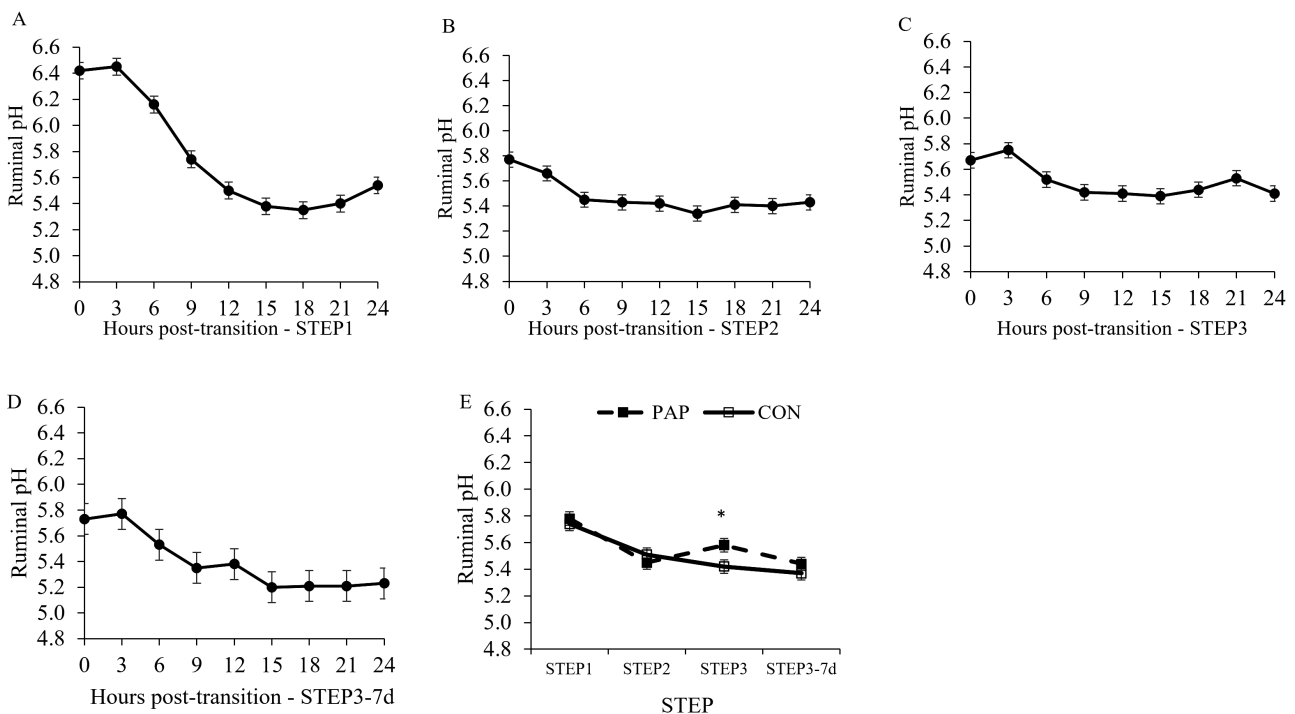


Figure 2. Ruminal pH within the first 24-h post-diet transition of Angus crossbred steers fed free-choice step-up diets that contained increased concentrations of cracked corn (35%, 60%, and 82%; STEP1 [d 14–21], STEP2 [d 21–28], and STEP3 [d 28–35], respectively) plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively). Effect of hour posttransition on ruminal pH [STEP1 (A); $P < 0.01$; SEM = 0.06; STEP2 (B); $P < 0.01$; SEM = 0.06; STEP3 (C); $P < 0.01$; SEM = 0.009; and STEP3-7d (D); $P < 0.01$; SEM = 0.12] but not treatment \times hour ($P \geq 0.38$) or treatment ($P \geq 0.50$) were detected. Effects of treatment \times STEP (E; $P < 0.01$; SEM = 0.05) were detected. Within STEP, means with an asterisk differ ($P \leq 0.05$).

Table 3. Ruminal pH variables of Angus crossbred cannulated steers (658 ± 79 kg BW) fed free-choice step-up diets that contained increased concentrations of cracked corn¹ plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively).

Item	Treatment		SEM	P-value
	PAP	CON		
STEP1				
Average pH	5.78	5.75	0.064	0.60
Time <pH 5.6, min/d	273	286	35.9	0.60
Time <pH 5.2, min/d	33	73	21.7	0.03
Area <pH 5.6, min × pH/d	1566	1621	187.7	0.64
Area <pH 5.2, min × pH/d	187	406	119.7	0.02
STEP2				
Average pH	5.44	5.50	0.064	0.62
Time <pH 5.6, min/d	475	454	26.2	0.61
Time <pH 5.2, min/d	70	25	26.9	0.36
Area <pH 5.6, min × pH/d	2581	2490	1334.2	0.67
Area <pH 5.2, min × pH/d	377	138	144.3	0.36
STEP3				
Average pH	5.58	5.47	0.089	0.24
Time <pH 5.6, min/d	349	441	49.1	0.12
Time <pH 5.2, min/d	82	62	67.3	0.36
Area <pH 5.6, min × pH/d	1917	2397	263.2	0.16
Area <pH 5.2, min × pH/d	440	747	346.1	0.36
STEP3-7d				
Average pH	5.42	5.39	0.123	0.76
Time <pH 5.6, min/d	425	409	51.6	0.80
Time <pH 5.2, min/d	174	309	77.1	0.24
Area <pH 5.6, min × pH/d	2124	2136	271.8	0.97
Area <pH 5.2, min × pH/d	987	1725	487.4	0.30

¹Diets consisted of gradual inclusion of cracked corn from 0 (Bermudagrass hay only) to 35%, 60%, and 82% for STEP1 (d 14 to 21), STEP2 (d 21 to 28), and STEP3 (d 28 to 35), respectively.

Table 4. Ruminal pH variables of Angus crossbred cannulated steers (658 ± 79 kg BW) fed free-choice step-up diets that contained increased concentrations of cracked corn¹ plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively).

Item	Treatment		SEM	STEP				SEM	P-value		
	PAP	CON		STEP1	STEP2	STEP3	STEP3-7d		Treatment	STEP	Treat × STEP
Average pH	5.56	5.51	0.05	5.8 ^a	5.5 ^b	5.5 ^b	5.4 ^b	0.06	0.49	0.002	<0.01
Time <pH 5.6, min/d	308.2	406.4	24	278.6 ^a	462.4 ^b	411.1 ^b	421.1 ^b	30.86	0.45	0.001	0.36
Time <pH 5.2, min/d	93.8	139.2	30.6	43.8 ^a	46.5 ^a	125.2 ^a	250.5 ^b	36.23	0.31	0.004	0.71
Area <pH 5.6, min × pH/d	2053	2190	115	1587 ^a	2521 ^b	2216 ^b	2163 ^b	1550.0	0.40	0.003	0.35
Area <pH 5.2, min × pH/d	535	730.9	165.2	223.2 ^a	263.9 ^a	684 ^a	1339 ^b	193.0	0.39	0.005	0.39

¹Diets consisted of gradual inclusion of cracked corn from 0 (Bermudagrass hay only) to 35%, 60%, and 82% for STEP1 (d 14–21), STEP2 (d 21–28), and STEP3 (d 28–35), respectively.

concentration was lesser during STEP3-7d compared with STEP1 and STEP2, whereas STEP3 was intermediate.

No treatment × hour interaction ($P \geq 0.45$) or treatment ($P \geq 0.23$) effect was observed for VFA molar proportions during STEP1 transition. However, an effect of hour was observed during STEP1 ($P \leq 0.04$; Figure 4) for molar proportions of acetate, propionate, branched chain VFA (BCVFA), butyrate, and concentrations of total VFA and A:P ratio. During STEP2, a treatment × hour interaction was observed for molar

proportions of propionate ($P < 0.01$; Figure 5B) and A:P ($P < 0.01$; Figure 5F); but with no differences within treatments in the molar proportions of propionate when least-square means were separated and compared ($P > 0.11$). Steers consuming PAP had greater A:P ratio during 0, 3, and 6 h relative to diet change compared with CON, whereas butyrate molar proportions were lesser ($P = 0.02$; data not shown) when PAP was not fed (17.1 vs. 11 ± 1.58 mol/100 mol for CON and PAP, respectively).

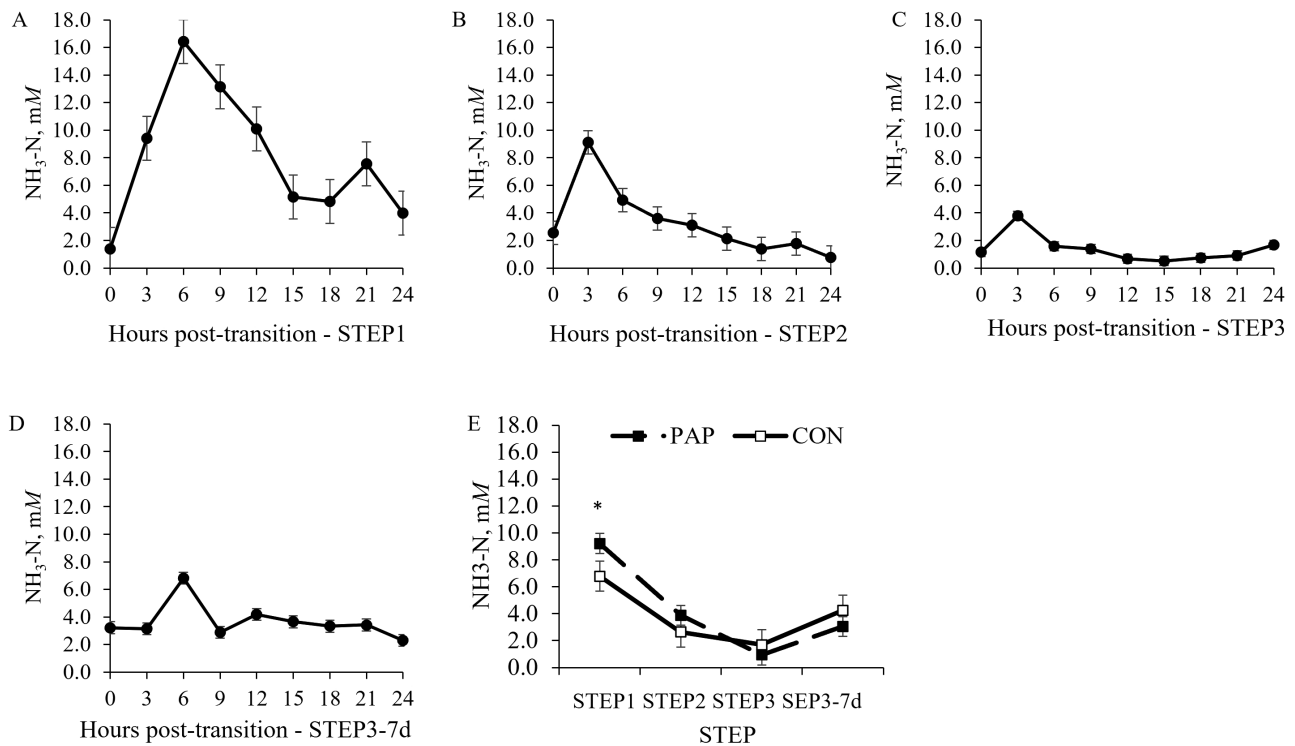


Figure 3. Ruminal $\text{NH}_3\text{-N}$ within the first 24-h post-diet transition of Angus crossbred steers fed free-choice step-up diets that contained increased concentrations of cracked corn (35%, 60%, and 82%; STEP1 [d 14–21], STEP2 [d 21–28], and STEP3 [d 28–35], respectively) plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively). Effect of hour post-transition on ruminal $\text{NH}_3\text{-N}$ [STEP1 (A); $P < 0.01$; SEM = 1.1; STEP2 (B); $P < 0.01$; SEM = 0.8; STEP3 (C); $P < 0.01$; SEM = 0.6; and STEP3-7d (D); $P < 0.001$; SEM = 0.9] but not treatment \times hour ($P \geq 0.90$) or treatment ($P \geq 0.67$) were detected. Effects of treatment \times STEP (E; $P = 0.05$; SEM = 0.74) on overall ruminal $\text{NH}_3\text{-N}$ were detected. Within STEP, means with an asterisk differ ($P \leq 0.05$).

Table 5. Volatile fatty acids (mM) of Angus crossbred cannulated steers (658 ± 79 kg BW) fed free-choice step-up diets that contained increased concentrations of cracked corn¹ plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively).

Item, mM	Treatment		SEM	STEP				SEM	P-value		
	PAP	CON		STEP1	STEP2	STEP3	STEP3-7d		Treat	STEP	Treat \times STEP
Acetate	63.3	60.6	2.5	81.6 ^a	60.9 ^b	54.1 ^c	51.1 ^c	2.58	0.42	<0.01	0.57
Propionate	37.8	36.7	2.5	23.4 ^b	43.4 ^a	40.2 ^a	41.9 ^a	2.58	0.70	<0.01	0.60
Butyrate	15.3	15.9	1.2	16.1 ^a	17.8 ^a	16.1 ^a	12.6 ^b	1.22	0.58	0.02	0.29
BCVFA ²	1.51	0.35	0.35	1.09	0.78	1.86	1.61	0.40	0.77	0.35	0.46
Valerate	2.65	2.36	0.23	0.80 ^c	2.42 ^b	4.12 ^a	2.69 ^b	0.29	0.39	<0.01	0.12
Caproate	0.85	0.86	0.11	0.27 ^b	0.19 ^b	1.44 ^a	1.51 ^a	0.13	0.97	<0.01	0.89
Total VFA	121.5	118.1	4.3	123.3 ^a	126.5 ^a	117.9 ^{ab}	111.4 ^b	4.38	0.59	0.01	0.76
A:P	2.0	1.9	0.1	3.7 ^a	1.5 ^b	1.4 ^b	1.3 ^b	0.12	0.82	<0.01	0.86

¹Diets consisted of gradual inclusion of cracked corn from 0 (Bermudagrass hay only) to 35%, 60%, and 82% for STEP1 (d 14–21), STEP2 (d 21–28), and STEP3 (d 28–35), respectively.

^{a,b}Within a row, means with different superscripts differ, $P \leq 0.05$.

²BCVFA = branched-chain volatile fatty acids: isobutyrate + isovalerate + 2-methylbutyrate.

Effect of hour ($P < 0.01$) postfeeding was observed for molar proportions of acetate, butyrate, valerate (not shown), and caproate (not shown), except for BCVFA ($P = 0.52$; Figure 5D) and total VFA ($P = 0.44$; Figure 5E) at STEP2. During STEP3 and STEP3-7d, an effect of hour was detected for ruminal VFA ($P < 0.01$; Figures 6 and 7), but not for treatment ($P \geq 0.23$). No effects of hour, treatment, or treatment

\times hour interaction was observed for molar proportions of butyrate during STEP3 or STEP3-7d ($P \geq 0.10$; Figures 6C and 7C).

An effect of hour post-transition during STEP2, STEP3, and STEP3d-7 ($P \leq 0.04$; Figure 8) was observed for ruminal lactate concentrations. Ruminal lactate concentrations were greatest at STEP2 ($P = 0.04$; Figure 8E).

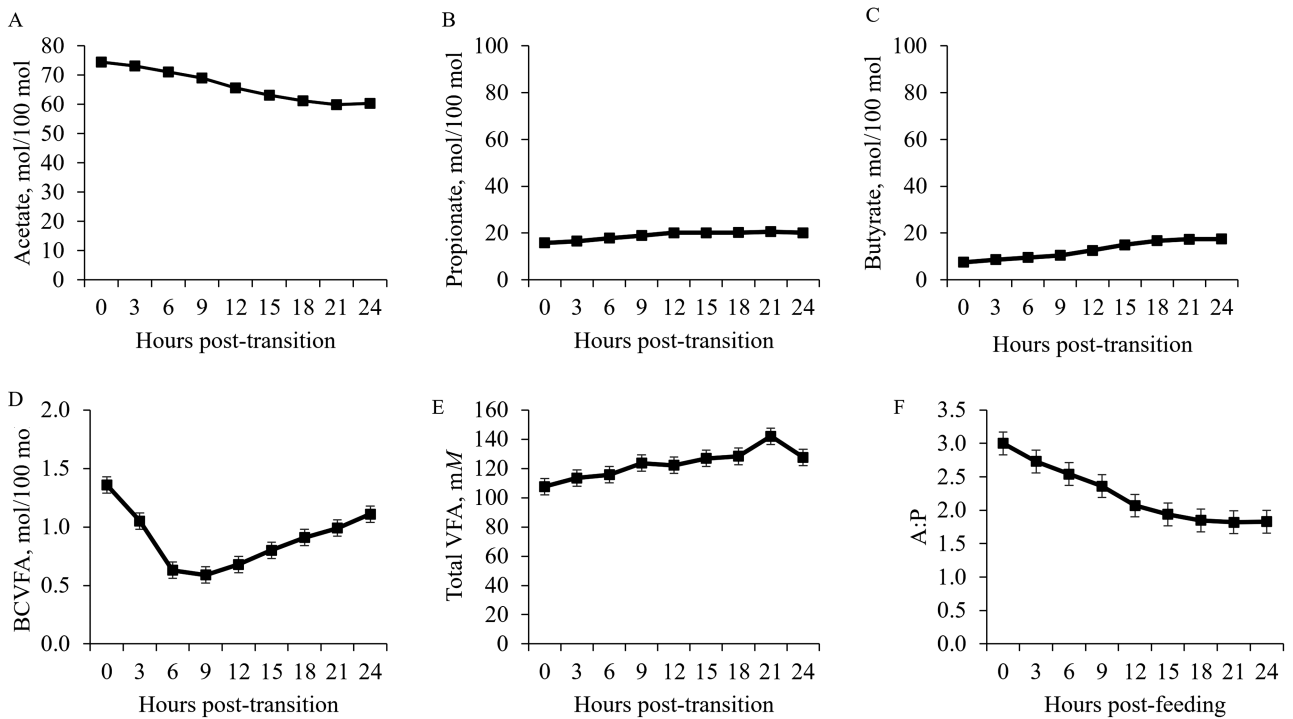


Figure 4. Ruminal volatile fatty acids within the first 24-h post-diet transition of Angus crossbred steers fed free-choice step-up diets that contained increased concentrations of cracked corn (35%, 60%, and 82%; STEP1 [d 14–21], STEP2 [d 21–28], and STEP3 [d 28–35], respectively) plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively) during the STEP1. Effect of hour post-transition on ruminal VFA [Acetate (A); $P < 0.01$; SEM = 0.8; Propionate (B); $P < 0.01$; SEM = 0.7; Butyrate (C); $P < 0.01$; SEM = 0.8; BCVFA (D); $P < 0.001$; SEM = 0.07; Total (E); $P < 0.01$; SEM = 5.6; and A:P (F); $P < 0.001$; SEM = 0.17] but not treatment \times hour ($P \geq 0.45$) or treatment ($P \geq 0.23$) were detected.

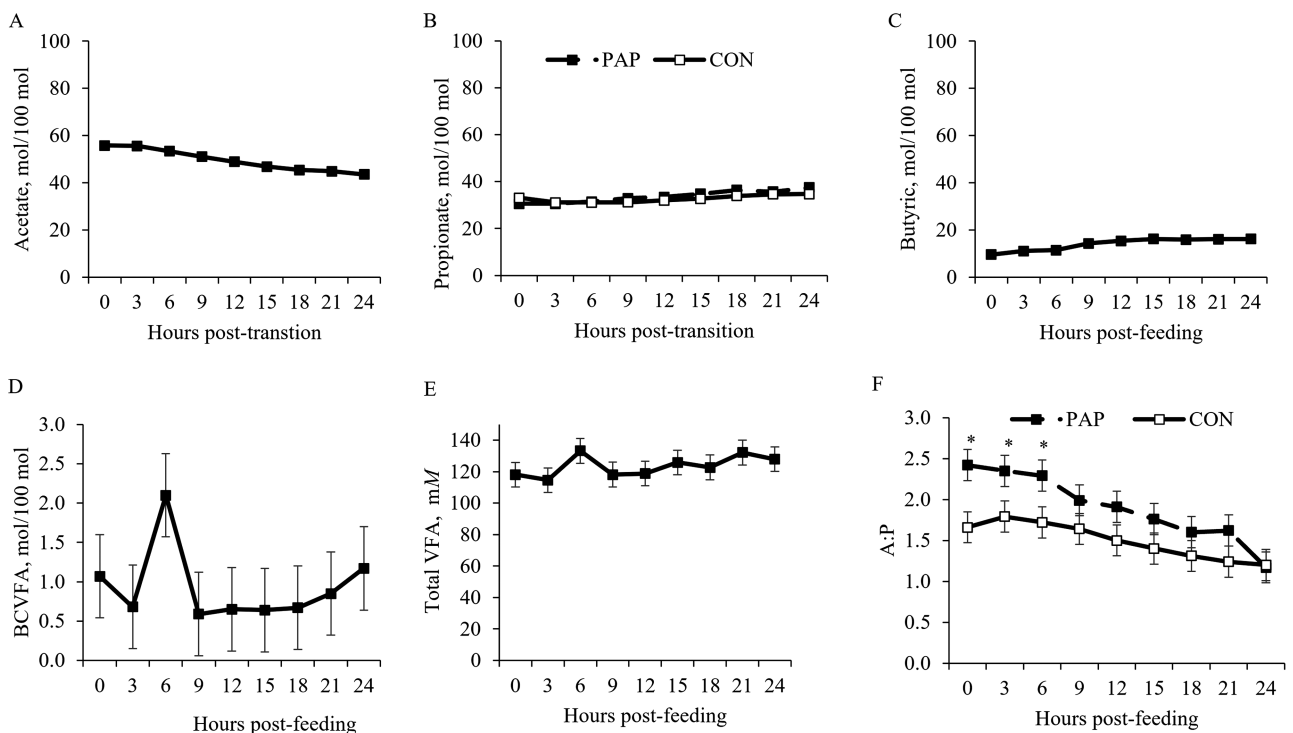


Figure 5. Ruminal volatile fatty acids within the first 24-h post-diet transition of Angus crossbred steers fed free-choice step-up diets that contained increased concentrations of cracked corn (35%, 60%, and 82%; STEP1 [d 14–21], STEP2 [d 21–28], and STEP3 [d 28–35], respectively) plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively) during the STEP2. Treatment \times hour interaction observed for propionate (B; $P < 0.01$; SEM = 0.7) and A:P ratio (F; $P < 0.01$; SEM = 0.17). Effect of hour post-transition on ruminal VFA [Acetate (A); $P < 0.001$; SEM = 1.7; and Butyrate (C); $P = 0.003$; SEM = 1.9, but not for BCVFA (D); $P = 0.52$; SEM = 0.53) and total (E); $P = 0.44$; SEM = 5.6). Within hours, means with an asterisk differ ($P \leq 0.05$).

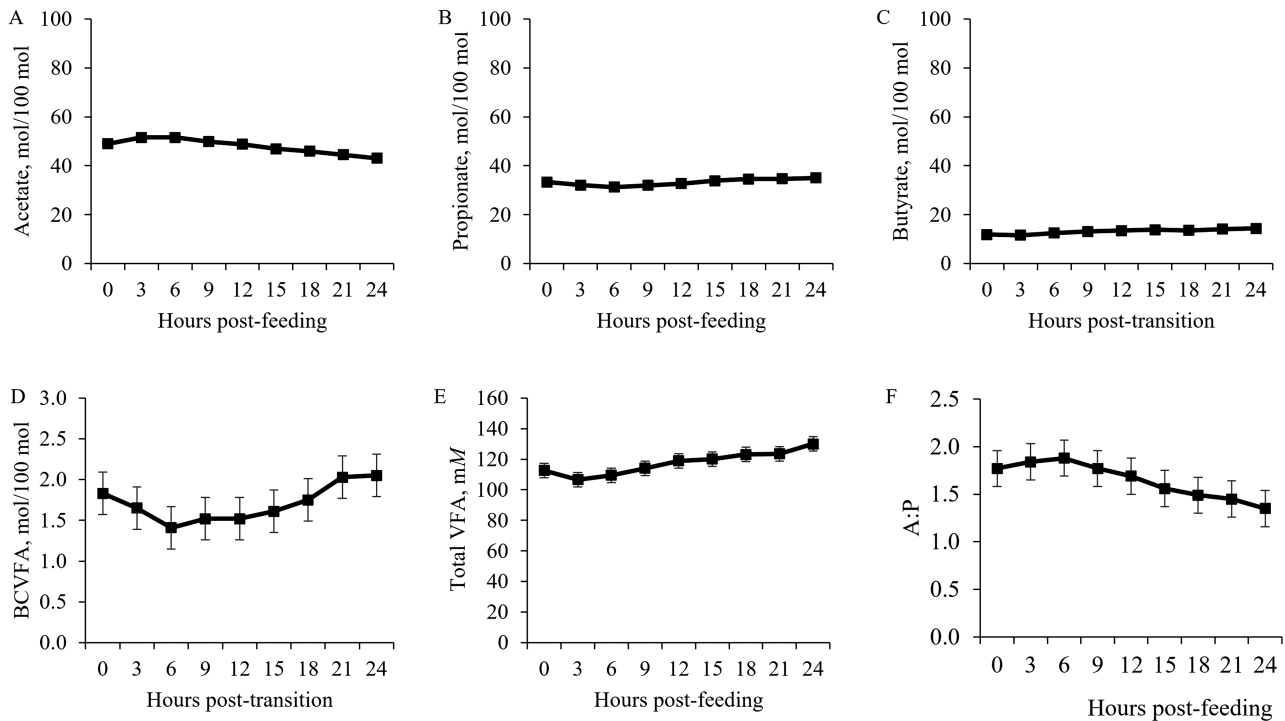


Figure 6. Ruminal volatile fatty acids within the first 24-h post-diet transition of Angus crossbred steers fed free-choice step-up diets that contained increased concentrations of cracked corn (35%, 60%, and 82%; STEP1 [d 14–21], STEP2 [d 21–28], and STEP3 [d 28–35], respectively) plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively) during the STEP3. Effect of hour posttransition on ruminal VFA [Acetate (A); $P < 0.01$; SEM = 1.71; Propionate (B); $P < 0.01$; SEM = 1.77; BCVFA (D); $P < 0.01$; SEM = 0.26; Total (E); $P < 0.01$; SEM = 4.7; and A:P (F); $P < 0.001$; SEM = 0.19] but not treatment \times hour ($P \geq 0.11$) or treatment ($P \geq 0.28$) were detected. No effects of hour, treatment, or treatment \times hour interaction was observed for butyrate (C; $P \geq 0.10$).

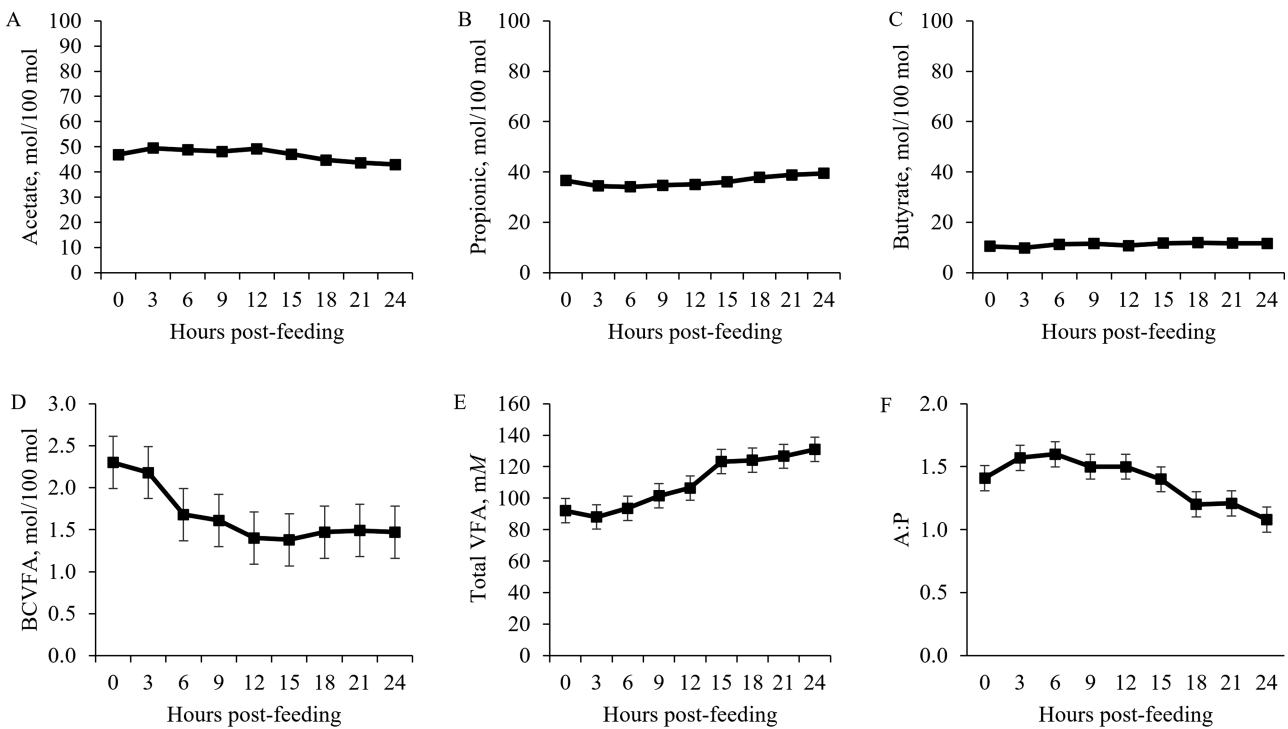


Figure 7. Ruminal volatile fatty acids within the first 24-h post-diet transition of Angus crossbred steers fed free-choice step-up diets that contained increased concentrations of cracked corn (35%, 60%, and 82%; STEP1 [d 14–21], STEP2 [d 21–28], and STEP3 [d 28–35], respectively) plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively) during the STEP3-7d. Effect of hour posttransition on ruminal VFA [Acetate (A); $P < 0.01$; SEM = 1.0; Propionate (B); $P < 0.01$; SEM = 1.6; BCVFA (D); $P < 0.01$; SEM = 0.31; Total (E); $P < 0.01$; SEM = 7.7; and A:P (F); $P < 0.01$; SEM = 0.10] but not treatment \times hour ($P \geq 0.21$) or treatment ($P \geq 0.23$) were detected. No effects of hour, treatment, or treatment \times hour interaction was observed for butyrate (C; $P \geq 0.14$).

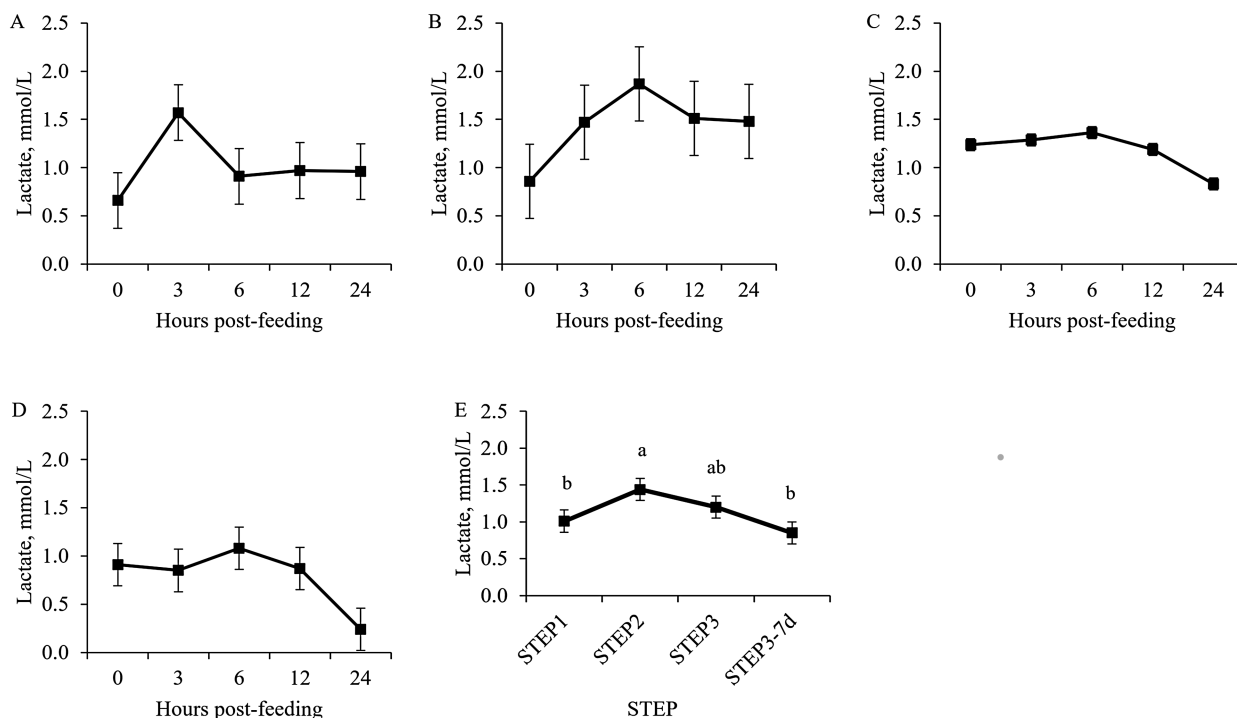


Figure 8. Ruminal lactate concentrations (mmol/L) within the first 24-h post-diet transition of Angus crossbred steers fed free-choice step-up diets that contained increased concentrations of cracked corn (35%, 60%, and 82%; STEP1 [d 14–21], STEP2 [d 21–28], and STEP3 [d 28–35], respectively) plus 0.45 kg of molasses that was individually fed containing or not 3 g of polyclonal antibody preparations against *S. bovis*, *F. necrophorum*, and lipopolysaccharides (40%, 35%, and 25% of the preparation, respectively) during STEP1, STEP2, STEP3, and STEP3d-7 (A–D). Effect of hour post-transition on ruminal lactate was observed for STEP2, STEP3, and STEP3d-7 ([B]; $P = 0.02$; SEM = 0.384; [C]; $P < 0.01$; SEM = 0.06; [D]; $P = 0.04$; SEM = 0.220). Effect of STEP was observed for ruminal concentrations of lactate ([E]; $P = 0.04$; SEM = 0.151). Within hours or STEP, means without a common superscript (a–b) differ ($P \leq 0.05$). No effect of hour was observed for STEP1 ([A]; $P = 0.24$; SEM = 0.289).

DISCUSSION

Previous studies have reported that feeding PAP increased DMI by 8.1% in backgrounding beef cattle (Silva et al., 2022) or had no impact on DMI of beef steers (DiLorenzo et al., 2008; Blanch et al., 2009). In the current study, DMI was not affected by the inclusion of PAP throughout the study; however, DMI gradually reduced with corn inclusion. Diet type (e.g., forage- vs. grain-based diets) and fermentability can influence DMI of cattle through primarily metabolic signals (Allen et al., 2009). The signal to terminate meals is most likely caused by propionate as its flux to the liver is increased (Allen, 2000) when grain diets are fed.

There were no effects of PAP feeding on total VFA concentrations, which agrees with other reports in the literature (DiLorenzo et al., 2008; Blanch et al., 2009; Marino et al., 2011; Bastos et al., 2012; Barros et al., 2019). However, during STEP2, A:P ratio was greater in the first 6 h postfeeding for steers receiving PAP compared with control. Similar results were reported by Marino et al. (2011) when feeding PAP to cannulated Holsteins cows. In addition, butyrate molar proportions were lesser when PAP was provided. In the rumen, lactate is metabolized mainly to acetate, propionate, and butyrate with the molar proportions of the fermentation products from lactate being influenced by ruminal pH (Nagaraja and Lechtenberg, 2007). Acetate and butyrate production from lactate fermentation are proven to be pH dependent (Satter and Esdale, 1968), with butyrate production maximized at lower ruminal pH, whereas acetate production predominates at a higher pH (Coe et al., 1999). Hence, the

increased molar proportions of butyrate in the control steers may indicate lactate fermentation under lowered ruminal pH (Coe et al., 1999), while the greater A:P ratio and decreased butyrate molar proportions in steers fed PAP may be associated with the increased ruminal pH.

Ruminal pH can reach values as low as 4.5 when lactic acid accumulates, which is a result of increased lactic acid production and its decreased fermentation to VFA (Nagaraja and Lechtenberg, 2007). Under normal conditions, lactate in the rumen does not accumulate at concentrations above 5 mM (Owens et al., 1998), and when it does, concentrations rarely exceed 10 mM (Hristov et al., 2001). In the current study, the concentration of lactate was within the normal range throughout the experiment and was not impacted by PAP feeding. Coe et al. (1999) adapted steers in 6 d to a diet containing 65% cracked corn and 25% cracked wheat (DM basis) from a diet containing 100% roughage, applying two transitional diets, with average ruminal lactate concentrations not exceeding 0.4 mM. The gradual adaptation to high-grain diets, as performed in this experiment, was important to possibly adjust the ruminal microbiota and avoid an accumulation of lactate.

Provision of PAP during the step-up transition increased mean ruminal pH and reduced the time and area with pH below 5.2, as described by other studies (DiLorenzo et al., 2006, 2008; Blanch et al., 2009; Marino et al., 2011; Barros et al., 2019). In the present study, ruminal pH below 5.6 was considered an indicator of subacute acidosis, whereas a pH lower or equal to 5.2 of acute ruminal acidosis (Owens et al.,

1998; Bevans et al., 2005). The lower ruminal pH for steers in the control treatment for longer periods may indicate a greater risk of encountering acidosis compared with steers fed PAP. Lactate production by *S. bovis* is one of the reasons why ruminal pH declines rapidly, and explosive growth of *S. bovis* occurs during the step-up period when the availability of fermentable carbohydrate is high (Nagaraja and Lechtenberg, 2007). Feeding PAP against *S. bovis* reduced target bacteria counts (DiLorenzo et al., 2006), which likely contribute to increased ruminal pH (Owens et al., 1998). Even though lactate concentrations were similar within treatments, the pathway to minimize lactate accumulation was likely different. The reduction in the time pH was below 5.2 for PAP-fed steers could be a consequence of decreased *S. bovis* ruminal counts, thereby reducing lactate production. Moreover, a greater ruminal pH could allow an active population of lactate-utilizing bacteria to ferment lactate faster to VFA (Mackie and Gilchrist, 1979). At ruminal pH greater than 5.5, fermentation of lactate to VFA typically exceeds the rate of lactate production (Asanuma and Hino, 2005), but when ruminal pH is lesser than 5.5, ruminal lactate-utilizing bacteria growth is compromised, allowing lactate to accumulate (Owens et al., 1998). Likely control steers needed more time for lactate-utilizing bacteria to ferment lactate, which explains the greater amount of time control steers were with ruminal pH below 5.2. Hence, PAP effectively reduced the incidence of acidosis during the step-up transition likely by reducing the *S. bovis* counts and maintaining mean ruminal pH above 5.5, which allows the growth of lactate-utilizing bacteria.

Increasing the inclusion of cracked corn in the diet reduced average ruminal pH and increased the time and area of ruminal pH below 5.6. The observed reduction in pH during concentrate feeding could be explained by the increased VFA concentration (Coe et al., 1999). Increased VFA concentrations were observed from STEP1 to STEP3 diets and were associated with a reduction in A:P ratio and increased molar proportions of propionate. Consumption of highly fermentable carbohydrates can impact ruminal pH, as an increased rate of VFA production overcomes VFA clearance rates, resulting in accumulation which generally reduces mean ruminal pH to between 5.6 and 6.2 (Schwartzkopf-Genswein et al., 2002).

Ruminal $\text{NH}_3\text{-N}$ concentrations were greater for steers receiving PAP compared with control steers during STEP1. Conversely, no effect of PAP on $\text{NH}_3\text{-N}$ concentration was described by DiLorenzo et al. (2008), Blanch et al. (2009), and Marino et al. (2011). Starch-utilizing bacteria such as *S. bovis* can use $\text{NH}_3\text{-N}$ as the only source of N for growth, and it still grows rapidly (Russell, 2002). Therefore, the lesser concentrations of $\text{NH}_3\text{-N}$ in control steers can potentially be attributed to greater uptake for *S. bovis* growth. Furthermore, when ruminal pH declines and VFA accumulates, urea cycling is compromised, reducing urea influx and $\text{NH}_3\text{-N}$ concentrations in the rumen (Aschenbach et al., 2011). Available ruminal $\text{NH}_3\text{-N}$ is an essential source of N for microbial growth. When $\text{NH}_3\text{-N}$ exceeds microbial needs, it immediately binds H^+ to form NH_4^+ in the ruminal content, and only the efflux of NH_4^+ to the blood stream can remove a proton from the system, then, serving as a potent buffer (Aschenbach et al., 2011). Therefore, increased concentration of $\text{NH}_3\text{-N}$ in the rumen content of PAP fed steers promoted greater opportunities to increase ruminal pH and maintain ruminal homeostasis.

In conclusion, feeding 3 g/d of polyclonal antibody preparation against *S. bovis*, *F. necrophorum*, and lipopolysaccharides was effective in increasing ruminal pH, contributing to mitigate the risks of ruminal acidosis in beef steers during the step-up transition from forage to high-grain diets.

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Conflict of interest statement

None declared.

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