Impacts of polyclonal antibody preparations from avian origin on nutrient digestibility and performance of backgrounding beef cattle

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ABSTRACT

This study evaluated the effects of feeding an avian-derived polyclonal antibody preparation (PAP; CAMAS, Inc.) against Streptococcus bovis, Fusobacterium necrophorum, and lipopolysaccharides (LPS; 40%, 35%, and 25% of the preparation, respectively) on growth performance (Exp. 1) and apparent total tract digestibility of nutrients (Exp. 2) of beef cattle consuming a backgrounding diet. In Exp. 1, Angus crossbred heifers (n = 70; 360 ± 24 kg of initial body weight; BW) and steers (n = 20; 386 ± 24 kg of BW) were used in a generalized randomized block design. Heifers and steers were allocated to 1 of 18 concrete-surfaced pens (6 pens per treatment) to receive a common ad libitum diet (35% cottonseed hulls, 34% dry-rolled corn, and 20% corn gluten pellets; 15.9% crude protein on a dry matter [DM] basis, 1.58 Mcal/Kg DM of net energy [NE] of maintenance, and 0.98 Mcal/Kg DM of NE of gain) and 1 of the 3 treatments consisting of feeding 1 (PAP1), 3 (PAP3), or 0 g (CON) of PAP per day for 56 d. Feed intake was recorded daily and BW was obtained on days −1, 0, 14, 28, 42, and 56 to assess average daily gain (ADG), dry matter intake (DMI), and gain:feed (G:F). Plasma concentrations of glucose and haptoglobin were measured on days 0, 14, 28, 42, and 56. In Exp. 2, 25 Angus crossbred steers (390 ± 24 kg BW) were used in a completely randomized design to receive the same diet and treatments from Exp. 1 (CON: n = 8; PAP1: n = 9; and PAP3: n = 8). Following a 14-d adaptation to diets, feed and fecal samples were collected to determine apparent total tract nutrient digestibility. In Exp. 1, overall BW, DMI, ADG, G:F, and plasmatic measurements did not differ among treatments over the 56-d period (P > 0.16). However, from days 0 to 14, a quadratic effect was observed for ADG, in which cattle receiving PAP1 had greater (P = 0.04) ADG compared with CON. In Exp. 2, no difference in DMI was observed (P = 0.88), yet DM, organic matter, neutral and acid detergent fiber, and starch digestibility were least (P ≤ 0.05) for PAP3, whereas digestibility of neutral detergent fiber was greatest (P < 0.01) for PAP1. In summary, feeding 1 g/d of a PAP against S. bovis, F. necrophorum, and LPS improved growth performance in the first 14 d and increased fiber digestibility of beef cattle consuming a backgrounding diet. Further research is needed to understand the impaired responses on nutrient digestibility when greater doses are provided.

Key words: beef cattle, Fusobacterium necrophorum, lipopolysaccharides, Streptococcus bovis

INTRODUCTION

Backgrounding of beef calves after weaning is a common practice (Hall et al., 2018) that emphasizes body weight (BW) gain rather fattening (Peel, 2003), and weight gain is achieved by providing either forage- (Vaage et al., 1998) or high grain-based rations (Ametaj et al., 2009). Young backgrounding beef cattle may require little, if any, forage and can be grown efficiently on concentrate diets (Peel, 2003). When grain prices are low compared with forages, high-energy diets for growing cattle can be more economical (NASEM, 2016). However, diets rich in rapidly fermentable nonstructural carbohydrates increase the risk of acidic events (NASEM, 2016), resulting in disruption of the adequate ruminal environment and fiber digestion (Russell and Wilson, 1996). Ruminal bacteria, such as Streptococcus bovis and Fusobacterium necrophorum, respond to increased availability of starch and sugars by increasing their growth rates in grain-fed animals (Nagaraja and Tigemeyer, 2010). As a result of the reduced ruminal pH from high grain diets and subsequent lysis of Gram-negative bacteria, ruminal concentration of free lipopolysaccharide (LPS) were reported to increase as well as translocation of ruminal LPS into the bloodstream causing further inflammatory responses (Gozho et al., 2005; Nagaraja and Lechtenberg, 2007). Ruminal disturbances are linked to the suboptimal performance of growing cattle (Ametaj et al., 2009) as nutrients are diverted from supporting growth to support immunity (Johnson, 1997).

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). Nevertheless, new technologies are emerging, such as polyclonal antibody preparations (PAP), as possible tools to ameliorate the effects of high grain diets in cattle health and performance. Previous research using PAP against S. bovis and F. necrophorum was effective in increasing the rumen pH in beef steers (DiLorenzo et al., 2006; Silva et al., 2019), heifers (Blanch et al., 2009), and Holstein cows (Marino et al., 2011) fed high grain diets. Feed efficiency of
feedlot beef steers was improved when PAP against *S. bovis* was fed (DiLorenzo et al., 2008) and milk production of dairy cows enhanced when PAP against LPS was provided even though cow health status was not changed (Ibarbia et al., 2014). To our knowledge, the effects of PAP against *S. bovis*, *F. necrophorum*, and LPS on performance, innate immunity, and apparent total tract nutrient digestibility of beef cattle consuming a backgrounding diet containing dry-rolled corn and non-forage fiber sources (cottonseed hulls, corn gluten pellets, and cottonseed meal) have yet to be investigated.

We hypothesize that high grain backgrounding diets lead to metabolic disturbances, which reduces nutrient digestibility, initiates systemic inflammation, and impairs growth performance of growing beef cattle. Therefore, our objective was to evaluate the effects of feeding PAP against *S. bovis*, *F. necrophorum*, and LPS on performance, nutrient digestibility, and innate immunity in backgrounding beef cattle consuming high grain-based 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).

**Experimental Design, Animals, and Treatments**

**Experiment 1.** The experiment was conducted at the University of Florida, Feed Efficiency Facility (FEF). Angus crossbred heifers (*n* = 70; 360 ± 24 kg of initial BW; 470 ± 26 d of age) and steers (*n* = 20; 386 ± 24 kg of BW; 465 ± 30 d of age) were used in a generalized randomized block design, using initial BW as blocking factor (6 blocks with 1 pen per treatment per block). Heifers and steers were stratified by sex, with each group blocked by BW and allocated to 1 of 18 concretesurfac ed pens (108 m²; 6 pens per treatment) that were randomly assigned to the 3 treatments. Cattle received a common total mixed ration (TMR, 15.9% crude protein [CP] on a dry matter [DM] basis, 1.58 Mcal/kg DM of net energy of maintenance [NEm], and 0.98 Mcal/kg DM of NE of gain [NEg]) containing a formulated premix at 0%, 0.42%, or 1.27% (DM basis) of PAP against *S. bovis, F. necrophorum,* and *LPS from E. coli* and *S. bovis* respectively (40%, 35%, and 25% of the preparation, respectively) are produced under patented and proprietary procedures (Cams Inc., Le Center, MN); thus, refer to DiLorenzo et al. (2006) for limited disclosure of the production process. The powder preparation used in the current study comprised of the whole egg (egg white and yolk) and contained immunoglobulin Y (IgY), immunoglobulin M, and immunoglobulin A. The PAP1 and PAP3 premix were analyzed before the start of the trials by specific ELISA test plates (Corning Inc., Corning, NY) to monitor antibody concentration. Concentration of IgY was 0.068 and 0.130 mg/g in PAP1 and PAP3 premix, respectively.

**Polyclonal Antibody Preparations**

The PAP against *S. bovis* (ATCC 9809), *F. necrophorum* (ATCC 27852), and LPS from *E. coli* O157:H7 and bacteria from the genus Salmonella (40%, 35%, and 25% of the preparation, respectively) are produced under patented and proprietary procedures (Cams Inc., Le Center, MN); thus, refer to DiLorenzo et al. (2006) for limited disclosure of the production process. The powder preparation used in the current study comprised of the whole egg (egg white and yolk) and contained immunoglobulin Y (IgY), immunoglobulin M, and immunoglobulin A. The PAP1 and PAP3 premix were analyzed before the start of the trials by specific ELISA test plates (Corning Inc., Corning, NY) to monitor antibody concentration. Concentration of IgY was 0.068 and 0.130 mg/g in PAP1 and PAP3 premix, respectively.

**Performance and Blood Metabolites (Exp. 1)**

Initial BW was calculated as the 2-d average of full BW on days −1 and 0, while final BW was the average of weights on days 55 and 56. Additional BW measurements were obtained every 14 d, which corresponds to days 14, 28, and 42 of the study. Changes in BW, ADG, dry matter intake (DMI), and gain:feed (GF) were analyzed. Blood samples (approximately 10 mL) were collected via jugular venipuncture into sodium-heparin containing tubes (158 USP; Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for the collection of plasma on days 0, 14, 28, 42, and 56. Blood samples were immediately placed on ice following collection and then centrifuged for 15 min at 4,000 × g at 4 °C. After centrifugation, plasma was transferred into polypropylene vials (12 × 75 mm; Fisherbrand;
Thermo Fisher Scientific Inc., Waltham, MA), and stored at −20 °C for further analysis.

Plasma concentration of haptoglobin was determined in duplicate samples using a biochemical assay evaluating the haptoglobin–hemoglobin complex by the estimation of differences in peroxidase activity (Cooke and Arthington, 2013). Plates were read at 450 nm in a microplate spectrophotometer (Multiskan Go, Thermo Fisher Scientific). Inter- and intra-assay coefficients of variation of Hp were 8.2% and 10.0%, respectively. Glucose was determined in duplicate samples using quantitative colorimetric kit G7521 (Pointe Scientific Inc., Canton, MI) and a microplate spectrophotometer at 520 nm (Multiskan Go, Thermo Fisher Scientific). Intra- and inter-assay coefficients of variation for glucose were 3.0% and 3.7%, respectively.

**Apparent Total Tract Digestibility of Nutrients (Exp. 2)**

Determination of apparent total tract digestibility of DM, organic matter (OM), CP, neutral detergent fiber (NDF), acid detergent fiber (ADF), and starch was performed using indigestible NDF (iNDF) as an internal marker. Concentration of iNDF in feed and fecal samples was determined as described by Cole et al. (2011) with modifications proposed by Krizsan and Huhtanen (2013). Dietary and fecal samples were collected beginning on days 13 and 14, respectively, for 4 consecutive days. Feed and fecal samples were collected twice per day, at 0800 and 1700 hours. Following collection, samples were stored at −20 °C until further processing and analyses at the Animal Laboratory, NFREC. Feed and fecal samples were dried at 55 °C for 48 h in a forced-air oven, ground in a Willey mill (Thomas Scientific, Swedesboro, NJ) to pass a 2-mm sieve, and pooled within steer on an equal weight basis to determine nutrient and marker concentration. For the determination of sample DM and OM, approximately 0.50 g of sample was weighed in duplicate, dried in a forced-air oven at 100 °C for 24 h and ashed at 550 °C for 6 h. To determine the fibrous portions, 0.50 g of dry sample was weighed in duplicate into F57 bags (Ankom Technology Corp.), incubated in the rumen of a cannulated steer for 288 h, and the residue analyzed for NDF. Apparent total tract digestibility of DM, OM, CP, NDF, and ADF were calculated using the following formula:

\[
100 - \frac{\text{marker concentration in feed}}{\text{marker concentration in feces}} \times \frac{\text{nutrient concentration in feces}}{\text{nutrient concentration in feed}}
\]
Statistical Analyses

In Exp. 1, the data were analyzed as a generalized randomized block design using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.4). For performance parameters, heifers and steers were used as the experimental unit, and block and sex were used as random effects. Level of inclusion of PAP was used as the fixed effect in Exp. 1 and 2. Plasma measurements were analyzed as repeated measures and tested for fixed effects of treatment, day of the study, and the treatment × day interaction, using animal within treatment as the subject. The intake and digestibility data (Exp. 2) were analyzed as a complete randomized design using the GLIMMIX procedure of SAS. Steer was the experimental unit, and steer within treatment was a random effect. Proc IML function of SAS was used in both studies to determine the coefficients of orthogonal regression comparisons involving unequal number of observations among treatments and unequally spaced levels of inclusion of PAP. Significance was declared at \( P \leq 0.05 \), and tendencies considered when \( 0.05 < P \leq 0.10 \).

RESULTS

Diet composition and nutritive value for Exp. 1 and 2 are presented in Table 1. In Exp. 1, from days 0 to 14, a quadratic effect of treatment was detected for ADG (Table 2) where PAP1 fed cattle had greater \( (P = 0.04) \) gain compared with those fed CON, and cattle fed PAP3 did not differ from those fed PAP1 or CON (1.19, 0.84, and 1.01 kg/d for PAP1, CON, and PAP3, respectively). Additional effects of PAP on ADG were not further observed \( (P \geq 0.20) \). A quadratic effect of treatment was detected for DMI from days 0 to 28 \( (P = 0.05) \), and a tendency from days 0 to 42 \( (P = 0.08) \). From days 0 to 28, heifers and steers in the PAP1 treatment had greater \( (P = 0.03) \) DMI compared with those in CON, whereas intake by PAP3 counterparts was intermediate \( (P = 0.49) \). Additional effect of PAP on DMI during the 56-d backgrounding period was not observed \( (P = 0.16) \). Treatment did not affect final BW, BW change, or G:F \( (P \geq 0.27; \text{Table 3}) \).

Plasma concentrations of haptoglobin and glucose were not affected \( (P = 0.58) \) by treatment, nor was a treatment × day interaction observed \( (P \geq 0.16) \); however, an effect of day \( (P \leq 0.04; \text{Figures 1 and 2}) \) was detected. Haptoglobin concentrations were greater on day 42 and lowest on day 56 \( (0.102 \text{ and } 0.018 \text{ mg/mL, respectively}) \), whereas plasma glucose concentration was the greatest on day 14 \( (83.2 \text{ mg/dL}) \).

In Exp. 2, intake of DM, OM, CP, NDF, ADF, starch, or DMI as a percentage of BW, did not differ among treatments \( (P \geq 0.18; \text{Table 4}) \). An effect of treatment was observed \( (P \leq 0.01) \) for apparent total tract digestibility of DM, OM, NDF, ADF, and starch, where PAP3 steers had lowered digestibility of those nutrients compared with CON and PAP1 treatments (Table 4). A linear decrease \( (P = 0.03) \) in apparent total tract digestibility of CP was observed as PAP dose increased from 0 to 3 g/d. Steers fed PAP3 had the lowest, PAP1 intermediate, and CON steers had the greatest CP digestibility \( (48.3\%, 46.7\%, \text{ and } 37.9\% \text{ for CON, PAP1, and PAP3, respectively}) \). A quadratic effect of PAP dose was observed \( (P < 0.01) \) on the apparent total tract digestibility of NDF, where PAP1 steers had the greatest digestibility of NDF, followed by reduced digestibility in CON and then PAP3 steers \( (32.6\%, 46.2\%, \text{ and } 23.1\% \text{ for NDF digestibility in CON, PAP1, and PAP3 steers, respectively}) \).

**Table 2.** BW of Angus crossbred heifers and steers (Exp. 1), receiving PAP against *S. bovis*, *F. necrophorum*, and LPS during a 56-d backgrounding phase.

| Item     | Treatment\(^a\) | SEM\(^b\) | \( P\)-value |
|----------|------------------|-----------|--------------|
|          | CON   | PAP1  | PAP3  | Treatment | Linear | Quadratic |
| ADG, kg  |       |       |       |          | \( P\)  | \( P\)    | \( P\)    |
| d 0–14   | 0.84\(^a\) | 1.19\(^a\) | 1.01\(^b\) | 0.12 | 0.10 | 0.50 | 0.04 |
| d 0–28   | 1.50  | 1.52  | 1.52  | 0.07 | 0.68 | 0.51 | 0.57 |
| d 0–42   | 1.43  | 1.51  | 1.53  | 0.05 | 0.37 | 0.22 | 0.48 |
| d 0–56   | 1.50  | 1.57  | 1.50  | 0.05 | 0.43 | 0.81 | 0.20 |
| DMI, kg/d|       |       |       |          | \( P\)  | \( P\)    | \( P\)    |
| d 0–14   | 10.1  | 11.0  | 10.7  | 0.40 | 0.21 | 0.35 | 0.13 |
| d 0–28   | 10.2\(^a\) | 11.1\(^a\) | 10.9\(^b\) | 0.36 | 0.08 | 0.25 | 0.05 |
| d 0–42   | 10.3\(^a\) | 11.2\(^a\) | 11.0\(^b\) | 0.32 | 0.09 | 0.20 | 0.08 |
| d 0–56   | 9.8   | 10.5  | 10.3  | 0.30 | 0.16 | 0.27 | 0.12 |
| G:F      |       |       |       |          | \( P\)  | \( P\)    | \( P\)    |
| d 0–14   | 0.07  | 0.09  | 0.08  | 0.01 | 0.46 | 0.87 | 0.22 |
| d 0–28   | 0.11  | 0.11  | 0.11  | 0.01 | 0.90 | 0.91 | 0.66 |
| d 0–42   | 0.12  | 0.12  | 0.12  | 0.01 | 0.89 | 0.88 | 0.65 |
| d 0–56   | 0.12  | 0.12  | 0.12  | 0.01 | 0.63 | 0.35 | 0.82 |

\(^a\)Limestone-based premix containing 0%, 0.42%, or 1.27% (DM basis) of PAP to deliver either 0, 1, or 3 g of PAP per day, respectively. CON = 0 g, PAP1 = 1 g, and PAP3 = 3 g daily.

\(^b\)Pooled standard error of treatment means.

\(^\text{within a row, means without a common superscript differ} (P \leq 0.05)\).

\(^\text{within a row, means without a common superscript tend to differ} (0.05 < P \leq 0.10)\).
Polyclonal antibodies for growing cattle

Table 3. Growth performance of Angus crossbred heifers and steers (Exp. 1) receiving PAP against *S. bovis*, *F. necrophorum*, and LPS during a 56-d backgrounding phase

| Item† | Treatment‡ | SEM§ | P-value |
|-------|------------|------|---------|
|       | CON | PAP1 | PAP3 |
| BW, kg |     |      |       |
| −14   | 308 | 316  | 307   | 24 | 0.59 | 0.75 | 0.33 |
| 0     | 362 | 367  | 356   | 24 | 0.50 | 0.44 | 0.39 |
| 14    | 375 | 385  | 371   | 24 | 0.32 | 0.51 | 0.18 |
| 28    | 402 | 409  | 398   | 25 | 0.52 | 0.56 | 0.32 |
| 42    | 423 | 432  | 421   | 24 | 0.52 | 0.67 | 0.30 |
| 56    | 445 | 455  | 439   | 24 | 0.27 | 0.38 | 0.18 |
| change | 84  | 88   | 84    | 3  | 0.44 | 0.82 | 0.21 |

†Limestone-based premix containing 0%, 0.42%, or 1.27% (DM basis) of PAP to deliver either 0, 1, or 3 g of PAP per day, respectively. CON = 0 g, PAP1 = 1 g, and PAP3 = 3 g daily.
‡Individual BW was obtained on days −1, 0, 14, 28, 42, 55, and 56. Initial and final BW were averaged over 2 consecutive days.
§Pooled standard error of treatment means.

Discussions

Performance and Blood Metabolites (Exp. 1)

Ruminal bacteria, such as *S. bovis* and *F. necrophorum*, respond to an increased availability of starch by boosting growth rates in grain-fed animals (Nagaraja and Lechtenberg, 2007), contributing to health problems and impaired productivity in cattle. Furthermore, diets rich in rapidly fermentable carbohydrates trigger systemic inflammation in cattle through translocation of immunogenic compounds into circulation, such as LPS (Horadagoda et al., 1999; Kvidera et al., 2017). The efficacy of PAP against *S. bovis* to increase feed efficiency of finishing beef cattle was reported by DiLorenzo et al. (2008). However, to our knowledge, growth performance of beef cattle consuming a backgrounding diet formulated with non-forage fiber sources and the addition of a blend of PAP against *F. necrophorum*, *S. bovis*, and LPS has not yet been documented. In the current study, overall ADG, BW, and G:F from days 0 to 56 were not affected by the inclusion of PAP, whereas ADG in the first 14-d of feeding was positively influenced by addition of PAP at 1 g/d. The positive effects in ADG for PAP1 fed cattle observed in the first 14 d of Exp. 1 may be potentially explained by increased fiber digestibility as reported in Exp. 2. Backgrounding cattle consuming 1 g of PAP daily gained 0.35 kg/d more than animals not fed PAP. However, those results were not sufficient to increase final BW.

DMI from days 0 to 42 was 8.1% greater for PAP1 compared with CON heifers and steers, whereas PAP3 was intermediate. Previous studies testing the effects of PAP did not report differences in DMI for beef steers (DiLorenzo et al., 2008), cannulated Holstein cows (Marino et al., 2011), or yearling bulls (Barducci et al., 2013) consuming high grain diets. The differences between the previous reports in literature and the current experiment can be potentially explained by the type of diet used (finishing vs. backgrounding diets, respectively) and the slightly variation in PAP formulation of each study (*F. necrophorum* or *S. bovis*; *S. bovis*, *F. necrophorum*, *Clostridium aminophilum*, *Clostridium sticklandii*, and *Peptostreptococcus anaerobius*; and *S. bovis*, *F. necrophorum*, *Lactobacillus*, and LPS for DiLorenzo et al., 2008, Marino et al., 2011, and Barducci et al., 2013, respectively), which could change the magnitude of results when PAP is provided. The increased DMI in Exp. 1 for PAP1 heifers and steers compared with CON could be explained by greater NDF digestibility observed in Exp. 2, as fiber digestibility may influence feed intake. Increasing fiber digestibility enhances passage rate, which contributes to clearance of fiber from the rumen and allow for additional feed consumption (Dado and Allen, 1996).
Ruminal bacteria, such as *S. bovis* and *F. necrophorum*, respond to increased availability of starch and sugars by increasing growth rates in grain-fed animals where animals are not adapted to the diet, but after adaptation occurs, numbers of *S. bovis* decline (Nagaraja and Titgemeyer, 2010). The additional gain for PAP1 fed cattle can be explained by the lack of adaptation to the diet within the first 14-d of feeding; however, possible adaptation to the diet throughout the study may potentially explain the lack of differences observed in final ADG and BW gain with PAP feeding.

High grain diets are associated with systemic inflammation and may elicit an acute phase response with increased concentration of plasmatic haptoglobin in cattle (Gozho et al., 2005; Silva et al., 2021). To cause an inflammatory response, circulating LPS must combine with LPS binding protein, and the subsequent compound should bind to immune cell receptors (Tomlinson and Blikslager, 2004), promoting the secretion of pro-inflammatory cytokines that will further stimulate the synthesis of Hp in hepatocytes (Plazier et al., 2018). The rise of LPS binding protein in cattle occurs significantly 6 h poststimulus, reaching a maximum at 24 h (Schroedl et al., 2001), whereas Hp concentrations remained greater than 0.11 mg/mL for at least 13 d in cattle receiving high grain diets (Silva et al., 2021). Therefore, for this experiment, Hp was used as an indicator of inflammation. Steers fed a backgrounding diet (45% barley grain-based concentrate and 55% barley silage on a DM basis) had a peak in plasmatic hp concentration of 1.7 mg/mL after 9 wk of the beginning of the feeding period (Ametaj et al., 2009). While an acute phase response and related proteins are an important defense mechanism, nutrients such as amino acids and glucose are shifted from supporting growth to the increased demand of the immune system (Reeds and Jahoor, 2001). Consequently, mobilization of muscle and fat tissues occurs (Jahoor et al., 1999), causing an overall reduction in DMI and gain (Moriel et al., 2015). Therefore, it was hypothesized that feeding PAP could reduce the amount of free LPS translocating from the rumen to the bloodstream and subsequently decrease the effects of diet on immune responses and ultimately BW gain. Despite our expectations, plasma concentrations of haptoglobin and glucose were within the normal range for beef cattle (≥0.11 mg/mL, Touloumoussis et al., 2004; and approximately 87 mg/dL for yearling steers, Doornenbal et al., 1988, respectively) throughout the study, which indicates that the type of diet provided was not sufficient to generate an inflammatory response. In this context, feeding PAP against LPS to beef cattle consuming the backgrounding diet provided in the current study, did not provide any advantages to mitigate inflammation as it did not occur, and therefore, the increase in gain during the first 14-d of feeding for the PAP1 treatment is unlikely to be caused by the effect of decreased translocation of LPS into circulation.

### Apparent Total Tract Digestibility of Nutrients (Exp. 2)

When comparing performance results from Exp. 1 with the results of apparent total tract digestibility of nutrients in Exp. 2, the differences in ADG during the first 14-d of the experiment may be attributed to increased NDF digestibility for PAP1 compared with feeding CON and PAP3. Steers consuming 1 g of PAP daily had 13.6% and 23.1% greater NDF digestibility when compared with CON and PAP3, respectively. Greater NDF digestibility was observed by Barros et al. (2019) when feeding 20 mL/d of liquid PAP (46% of antibodies against *S. bovis*, 23% against *F. necrophorum*, 16% against *E. coli* O157:H7, and 15% against LPS) to cannulated cattle.

### Table 4. Intake and apparent total tract of Angus crossbred steers (Exp. 2) receiving PAP against *S. bovis, F. necrophorum*, and LPS during a 56-d backgrounding phase

| Item               | Treatment | SEM | P-value |
|--------------------|-----------|-----|---------|
|                    | CON      | PAP1| PAP3    | Treatment | Linear | Quadratic |
| N                  | 8        | 9   | 8      | 0.4       | 0.88   | 0.62      | 0.96     |
| DMI, % of BW       | 3.5      | 3.5 | 3.7    | 0.08      | 0.97   | 0.80      | 0.97     |
| Intake, kg/d       |          |     |        |           | 0.95   | 0.76      | 0.95     |
| DM                 | 15.3     | 15.5| 15.8   | 0.97      | 0.65   | 0.99      | 0.36     |
| OM                 | 14.3     | 14.6| 14.9   | 0.95      | 0.67   | 0.40      | 0.81     |
| NDF                | 7.1      | 7.8 | 7.2    | 0.64      | 0.71   | 0.88      | 0.42     |
| ADF                | 5.2      | 4.9 | 4.7    | 0.43      | 0.71   | 0.88      | 0.42     |
| CP                 | 1.9      | 1.9 | 1.7    | 0.22      | 0.71   | 0.88      | 0.42     |
| Starch             | 5.3      | 4.5 | 5.6    | 0.43      | 0.18   | 0.37      | 0.11     |
| Digestibility, %   |          |     |        |           | 0.18   | 0.37      | 0.11     |
| DM                 | 49.2     | 55.5| 42.6   | 2.35      | <0.01 | 0.02      | <0.01    |
| OM                 | 50.9     | 57.3| 44.1   | 2.28      | <0.01 | 0.01      | <0.01    |
| NDF                | 32.6     | 46.2| 23.1   | 2.88      | <0.01 | <0.01     | <0.01    |
| ADF                | 36.9     | 38.9| 18.3   | 2.70      | <0.01 | <0.01     | 0.02     |
| CP                 | 48.3     | 46.7| 37.9   | 3.31      | 0.08   | 0.03      | 0.65     |
| Starch             | 93.2     | 93.8| 89.7   | 1.08      | 0.03   | 0.02      | 0.18     |

1Feed samples were collected twice daily for 4 d; feed intake was measured using the GrowSafe System Ltd., Airdrie, Alberta, Canada.
2Limestone-based premix containing 0%, 0.42%, or 1.27% (DM basis) of PAP to deliver either 0, 1, or 3 g of PAP per day, respectively. CON = 0 g, PAP1 = 1 g, and PAP3 = 3 g daily.
3Pooled standard error of treatment means.
4Within a row, means without a common superscript differ (P ≤ 0.05).
Holstein cows. Nevertheless, Barros et al. (2019) did not observe differences in NDF digestibility when PAP was fed in the powder form at 7 g/d. In the experiment conducted by Barros et al. (2019) where NDF digestibility was enhanced by PAP addition, rumen pH was greater with PAP feeding at 20 mL/d compared with control cows (6.62 vs. 6.57, respectively). Other researchers using PAP formulation that contained antibodies against S. bovis, reported that ruminal pH was greater compared with animals not receiving additives (DiLorenzo et al., 2006, 2008; Blanch et al., 2009; Marino et al., 2011; Silva et al., 2019). During grain-feeding, lactic acid production by S. bovis contributes to a decrease in ruminal pH, which inhibits growth rates of most ruminal bacteria (Nagaraja and Lechtemberg, 2007), especially fiber-digesting bacteria. Previous data showed that PAP formulated against S. bovis was successful in decreasing ruminal counts of target bacteria and increasing ruminal pH (DiLorenzo et al., 2006). Ruminal pH was not measured in the current study, but the increase in digestibility of NDF may potentially be explained by the reduction of target ruminal bacteria and, consequently, an increase in ruminal pH, as it has been observed previously with these type of additives (DiLorenzo et al., 2006; Blanch et al., 2009).

In contrast to the beneficial effects of providing PAP at 1 g/d on NDF digestibility and ADG on the first 14-d of the experiment, increasing the doses of PAP to 3 g/d negatively affected digestibility of DM, OM, NDF, ADF, CP, and starch. Bastos et al. (2012) evaluated four doses (0, 1.5, 3, and 4.5 g/d) of PAP (26% S. bovis, 12% F. necrophorum, 48% against the proteoletic bacteria [C. aminophilum, P. anaerobius, and C. sticklandii], and 14% E. coli O157:H7) on digestibility of Holstein cows fed high concentrate diets with no treatment effects being observed on nutrient digestibility. Marino et al. (2011) observed only a reduction in starch digestibility for Holstein cows consuming high grain diets with the addition of PAP at 10 mL/d compared with cows consuming the control treatment (95.3 or 96.8, respectively). Streptococcus bovis counts in forage-fed animals is minimal, but it can increase with the availability of starch substrate, thus enabling S. bovis an advantage due to its rapid growth rate (Nagaraja and Lechtemberg, 2007). Despite the ability of many ruminal bacteria to utilize starch as a substrate, starch digestibility seems to be impaired if counts of S. bovis are reduced, which potentially explains the reduction in digestibility of starch when PAP is fed.

Based on the nutrient digestibility results from the current experiments, the benefits of using PAP as a feed additive seem to be dose dependent as the animal responses diverged within doses. In the experiments conducted by Bastos et al. (2012), the greatest PAP dose was 4.5 g/d that were fed to cows weighing 567 ± 104 kg, whereas Barros et al. (2019) fed 7 g/d to 747 ± 90 kg Holstein cows. Those experiments provided 7.9 and 9.4 mg of PAP/kg of BW, respectively. The increased NDF digestibility observed in Exp. 2 was obtained with a dose of 3.2 mg of PAP daily per kg of BW, much lower than the doses used in the studies described above where no effect on nutrient digestibility reported. The greatest dose in the current study (9.8 mg/kg of BW; PAP3), which caused reduction in nutrient digestibility, was greater than the doses used in previous research (Bastos et al., 2012, and Barros et al., 2019). This substantiates the importance of doses formulated based on BW instead of a standard dose. Lower doses may potentially be beneficial as observed in the current study, whereas greater doses may either result in no effect or potentially negative effects with regard to nutrient digestibility. Although DiLorenzo et al. (2006) did not observe cross-reactivity of PAP within S. bovis and F. necrophorum, indicating some specificity of avian antibodies, cross-reactivity of PAP with other species has not been tested, especially for other Gram-negative bacteria (that presents LPS in the outer membrane). Cross-reactivity could be one explanation for reduced nutrient digestibility when greater doses are fed.

In conclusion, providing 1 g/d of a PAP against S. bovis, F. necrophorum, and LPS was effective in improving growth performance in the first 14-d of the feeding period and increased apparent total tract digestibility of the NDF in beef cattle consuming a backgrounding diet. The exact mechanism responsible for negative effects on nutrient digestibility when greater doses of PAP are used is not known, and further research is needed to understand this relationship, as well as the possible cross-reactivity of PAP with other ruminal bacteria.

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**Conflict of interest statement**

The authors declare no conflict of interest.

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