Effects of a blend of mannan and glucan on growth performance, apparent nutrient digestibility, energy status, and whole-blood immune gene expression of beef steers during a 42-d receiving period

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ABSTRACT: We examined the effects of dietary supplementation of a blend of mannan and glucan on the growth performance, energy status, and whole-blood immune gene expression of newly weaned beef steers during a 42-d receiving period. Forty-eight newly weaned Angus crossbred steers (2-d post-weaning; 199 ± 13 kg of initial body weight [BW]) from a single source were stratified by BW and randomly assigned to one of the two treatments: basal diet with no additive (CON; n = 24) or a basal diet top-dressed with 5 g of a blend of mannan and glucan (MANGLU; n = 24). Average daily gain (ADG) and feed efficiency (FE) from days 1 to 14, 15 to 42, and 1 to 42 were calculated from daily dry matter intake (DMI) and weekly BW. Blood samples were collected on days 0, 14, and 42 for measurement of plasma glucose and nonesterified fatty acids (NEFA). Blood samples collected on days 14 and 42 were composited for each steer for untargeted carbonyl-metabolome analysis (measurement of carbonyl-containing metabolites). Expression of 84 immune-related genes was analyzed on blood samples collected on day 42. Beginning on days 37 to 42, total mixed ration, refusals, and fecal samples were collected once daily to determine apparent total tract digestibility of DM, CP, NDF, and ADF using indigestible NDF as an internal marker. Over the 42-d feeding trial, supplemental MANGLU tended to increase final BW (P = 0.07) and ADG (P = 0.06). Compared to CON, beef steers fed supplemental MANGLU had greater (P = 0.01) DMI during the first 14 d, greater DM digestibility (P = 0.03), and tended to have greater NDF digestibility (P = 0.09). No treatment effects (P > 0.10) on plasma glucose and NEFA on days 14 and 42 were detected; however, carbonyl-metabolome analysis revealed increased (FDR ≤ 0.05) plasma concentrations of galactose and glyceraldehydes, and altered (FDR ≤ 0.05) concentrations of some microbiome-derived metabolites in beef steers fed MANGLU. Compared with CON, MANGLU increased (P ≤ 0.05) the expression of five immune-related genes involved in recognition of and mounting immune defense against microbial pathogens. In conclusion, the results of this study demonstrated that supplemental MANGLU enhances beef cattle immunocompetence and productivity during feedlot receiving period.

Key words: beef cattle, immune gene, microbial pathogens, plasma carbonyl-metabolome

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INTRODUCTION

Transitioning newly weaned calves to feedlot is a critical time because this period, known as the receiving period, is characterized by several stressors caused by separation from the mother, commingling, transportation stress, exposure to pathogens, vaccination, and changes in diet and environment (Arthington et al., 2013). These stressors cause suppression of the immune system which is exacerbated by reduced feed intake during the first few days following weaning (Galyean and Hubbert, 1995). Thus, in recent years, several nutritional strategies, including the use of microbial feed additives, have been employed to optimize the intake and health of beef cattle during the first few days of receiving period.

Saccharomyces cerevisiae is a species of yeast that is commonly supplemented to animals during a period of stress to improve their performance and health (Cheng et al., 2014). Yeast products are commonly supplemented in the diet as a direct-fed microbial, prebiotics (yeast cell wall [YCW] polysaccharides), or both (Broadway et al., 2015). The major components of YCW are glucan and mannan which have been demonstrated to improve the gastrointestinal tract (GIT) health of animals by interacting directly with immune cells and bind pathogens and toxins (Oyofo et al., 1989; Diaz et al., 2004). A recent study demonstrated that dietary supplementation of a hydrolyzed mannan- and glucan-rich yeast fraction to newly received feedlot cattle did not influence the performance but improved the immune response of the beef steers (Pukrop et al., 2018). In addition, other reports have shown that beef cattle receiving YCW products experienced increased dry matter intake (DMI) and improved immune response when challenged with bacterial endotoxins (Burdick Sanchez et al., 2013, 2014; Finck et al., 2014); however, inconsistent responses to supplemental microbial-based products in animal production and continuing development of different microbial products highlight the need for more research studies to understand their mechanisms of action. In addition, most of the previous studies that evaluated the use of YCW products were performed using a few immune-related parameters. Comprehensive analysis of immune-related gene expression profile of beef cattle during the receiving period is needed to better understand how mannan and glucan supplementation improves the health and performance of beef cattle. We hypothesized that supplementing a blend of mannan and glucan would improve the performance and alter the whole-blood immune gene expression of newly weaned beef steers during the first 42 d in the feedlot. The objective of this study was to determine the effects of dietary supplementation of a blend of mannan and glucan during a 42-d receiving period on intake, performance, nutrient digestibility, energy status, and mRNA expression of 84 immune-related genes in the whole blood of beef steers.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committees of Kentucky State University approved all research procedures (17-001).

Animals, Housing, and Feeding

A total of 48 newly weaned Angus crossbred steers (2-d post-weaning; 199 ± 13 kg of body weight [BW]) were obtained from a single source, transported approximately 200 miles to the research feedlot barn, and immediately placed on a corn silage-based diet. On the day of arrival, the beef steers were ear-tagged and administered vaccines and a dewormer (Dectomax, Pfizer, New York, NY). The vaccination protocol consisted of three vaccinations (Ultrabac 7/Somubac, Zoetis Inc., Kalamazoo, MI, Titanium 5, Elanco Animal Health, Greenfield, IN, and PMH IN, Merck Animal Health, Summit, NJ). The steers received booster shots of Ultrabac 7/Somubac and Titanium 5 on day 16 of the experimental period.

On the third day after arrival (representing day 0 of the experiment), the beef steers were weighed after 12 hours of feed withdrawal, stratified by BW into three weight blocks. Within each weight block, the beef steers were assigned randomly to one of the two treatments and housed in individual slatted floor pens (2.44 × 14.63 m^2; 24 pens per treatment) in an open barn for 42 d. Treatments were (1) a basal diet with no additive (CON; n = 24) and (2) a basal diet top-dressed with 5 g of fulfill feed additive (MANGLU; n = 24) according to the manufacturer's recommendation. Fulfill (PMI, Arden Hills, MN) is a prebiotic polysaccharide formulated to contain 18% crude protein, 20–26% β-glucan, 20–26% mannan, and trace amounts of galactan. The basal diet was fed as a total mixed ration (TMR) and the beef steers were fed ad libitum daily at 0800 hours to achieve at least 10% ort. The feed ingredients and chemical composition of the basal diet are shown in Table 1. The additive was top-dressed in the morning daily during the time

Table 1. Ingredient and chemical composition of the basal diet[a]

Ingredient (%DM)	% of dietary DM
Corn silage	79.5
Dehydrated distillers grain	9.11
Soybean meal	9.83
Limestone	0.42
Deccox[b]	0.03
Vitamin and mineral premix[c]	1.11
Nutrient analysis[d]	
DM, %	45.9
CP, %	13.9
aNDF, %	37.8
ADF, %	22.0
Ca, %	0.67
P, %	0.65
TDN, %	70.2
NE_m, Mcal/kg	1.63
NE_g, Mcal/kg	1.03

[a]Chemical composition of complete diets calculated from analysis of individual ingredients at a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY).

[b]Contains 6% decoquinate for the prevention of coccidiosis (Zoetis Inc.).

[c]Guaranteed analysis: 15% Ca; 7.5% P; 20% salt; 1% Mg; 1% K; 3,600 mg/kg Mn; 12 mg/kg Co; 1,200 mg/kg Cu; 3,600 mg/kg Zn; 27 mg/kg Se; 60 mg/kg I; 660,000 IU/kg vitamin A; 660 IU/kg vitamin E; and 66,000 IU/kg vitamin D.

[d]aNDF, neutral detergent fiber (amylase-treated); EE, ether extract; TDN, total digestible nutrients; NE_m, net energy of maintenance; NE_g, net energy of gain.

the animals were actively eating to ensure it was completely consumed.

Sample Collection and Measurements

Dry matter intake. The quantity (as fed) of feed offered to and refused by each steer was recorded daily. Daily samples of diets refused and offered were dried in a forced-air oven at 56 °C for 48 hours. Daily DMI was calculated by subtracting the daily DM refused from the daily DM offered. In addition, samples of feed ingredients and TMR were collected once weekly, dried for 48 hours at 56 °C in a forced-air oven, ground using a 1-mm screen (Wiley Mill; Arthur H. Thomas Co.) and sent to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for chemical composition analysis. The analyses included DM (method 930.15; AOAC International, 2000), N (method 990.03; AOAC International, 2000), ADF (method 989.03; AOAC International, 2000), NDF (Van Soest et al., 1991), ether extract using diethyl ether (method 2003.05; AOAC International, 2000), and Ca and P (Sirois et al., 1994).

Body weight. Body weights of the steers were obtained before morning feeding after 12 hours of feed withdrawal on days 0, 14, and 42. Values of average daily gain (ADG) from days 1 to 14 and 1 to 42 were determined by subtracting the initial weight on day 0 from the weights obtained on days 14 and 42, respectively, and then dividing by the number of days. Additionally, ADG from days 15 to 42 was determined by subtracting the weight obtained on day 14 from the weight obtained on day 42 and then dividing by the number of days. Feed efficiency (FE) was determined by dividing ADG by DMI.

Apparent total tract digestibility measurements. Beginning on days 37 to 42, TMR, refusals, and fecal samples were collected daily to determine apparent total tract digestibility of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) using indigestible neutral detergent fiber (iNDF) as the digestibility marker (Cole et al., 2011; Krizsan and Huhtanen, 2013). TMR samples were collected once daily immediately after feeding and immediately stored at −20 °C. Approximately, 40 g of fecal samples were collected from each steer three times daily at 0800, 1200, and 1600 hours from the ground, inside the pen, within a few minutes after the animal defecated and were immediately stored at −20 °C. At the end of the experiment, TMR, refusals, and fecal samples were dried (60 °C for 48 hours in a forced-air oven), ground through a 2-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA), pooled within steer, and sent to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) for analysis of DM, CP, ADF, NDF, and iNDF. Total iNDF consumed was corrected for refusals, and total feces output (kg) was calculated as total iNDF consumed (g/d) divided by fecal iNDF concentration (g/kg). Digestibility was calculated as: (nutrient intake − fecal output of nutrient)/nutrient intake.

Blood sample collection. On days 0, 14, and 42, blood was collected from each steer before the morning feeding from the coccygeal vessels in 10-mL evacuated tubes containing sodium heparin (BD Vacutainer, Franklin Lakes, NJ), immediately placed on ice. A subsample of the whole blood (500 µL) was transferred into RNA-protect tubes (Cat. No. 76554; Qiagen) containing a reagent that lyses blood cells and stabilizes intracellular RNA. These samples were stored at −80 °C until RNA extraction and expression analysis of innate and adaptive immune-related genes. The remaining whole-blood samples were subsequently centrifuged for 15 min at 4,000 ×

g at 4 °C to harvest the plasma. The plasma samples were thereafter stored at −20 °C until glucose, nonesterified fatty acids (NEFA), and carbonyl-metabolome analysis.

Whole-Blood Immune Gene Expression Analysis

Total RNA was extracted from the whole-blood samples collected on days 0 and 42 using RNeasy Protect Animal Blood kit (Cat. No. 73224; Qiagen) following strictly the manufacturer's instructions. All samples had >100 ng/µL total RNA. RNA concentration was measured using a NanoDrop 2000 spectrophotometer with an A260:A280 ratio from 1.8 to 2.0 (Thermo Fisher Scientific, Waltham, MA, USA). After evaluation of RNA purity and final concentration, cDNA was synthesized through reverse transcription (RT) using the RT2 First Strand Kit (Cat. No. 330401; Qiagen) following the manufacturer's instructions. Briefly, GE buffer (2 µL) was added to RNA samples (25 ng) and nuclease free for a total volume of 10 µL to eliminate genomic DNA. Then, the samples were incubated for 5 min at 42 °C and placed immediately on ice for at least 1 min. After incubation, RT mix (10 µL) was added to samples and samples were incubated at 42 °C for 15 min. Reaction was stopped by incubating at 95 °C for 5 min and RNase-free water (91 µL) was added to each reaction. Samples were stored at −80 °C until qPCR procedures.

Alterations in mRNA expressions of 84 genes related to innate and adaptive immunity were determined using a RT2 Profiler cow innate and adaptive immune responses PCR Array (PABT-052ZA; Qiagen) by using a QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Briefly, cDNA samples were mixed with RT2 SYBR Green ROX qPCR Mastermix (Cat. No. 330523; Qiagen) and RNase-free water. Then, 25 µL of the PCR mix was placed into each well of the 96-well plate PCR array. The PCR array contained 84 adaptive and innate immune-related genes (Supplementary Table S1), five housekeeping genes (β-actin, glyceraldehyde-3-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase 1, TATA box-binding protein, and tyrosine 3-monooxygenase), three RT, three positive PCR controls, and one genomic DNA control.

Glucose and NEFA Measurements

Plasma samples collected on days 0, 14, and 42 were analyzed for glucose and NEFA concentrations in duplicate using hexokinase method and NEFA-C kit (Wako Diagnostics Inc., Richmond, VA) on a Konelab 20XTi Clinical Chemistry Analyzer (Thermo Fisher Scientific, Vantaa, Finland). The intra- and interassay coefficients of variation for glucose were 4.21% and 6.92%, respectively, while those for NEFA were 6.75% and 9.43%, respectively.

CIL/LC-MS-Based Untargeted Carbonyl-Metabolomics Analysis

Prior to metabolomics analysis, the plasma samples collected on days 14 and 42 were pooled for each steer. Relative quantification of metabolites containing a carbonyl chemical group (carbonyl-metabolome) was done using a chemical isotope labeling (CIL)/liquid chromatography–mass spectrometry (LC-MS)-based technique. The CIL/LC-MS-based metabolomics technique uses a differential ^{12}C- and ^{13}C-dansylhydrazine labeling to alter the properties (chemical and physical) of carbonyl-containing metabolites to enable their separation by LC and ionization by MS (Zhao et al., 2017). Sample amount normalization was done by quantification of dansyl-labeled metabolites using liquid chromatography–ultraviolet (LC-UV) (Wu et al., 2012), and relative quantification of the metabolites was done using an Agilent 1100 LC system (Palo Alto, CA) connected to a Bruker Impact HD quadrupole time-of-flight (QTOF) MS (Billerica, MA). Detailed information of sample preparation methods, dansylation protocol, LC-UV normalization, and LC-MS setup and running conditions have been previously reported (Zhao et al., 2017; Ogunade et al., 2020).

Data and Statistical Analysis

Variables, such as growth performance data (ADG, BW, DMI, and FE), apparent digestibility, and plasma glucose and NEFA concentrations, were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC), with fixed effect of treatment and random effects of pen and block. The pen served as the experimental unit. Values of initial weight of the steers were included as a covariate for the final BW, and values on day 0 were included as covariates for plasma glucose and NEFA for days 14 and 42. Data are reported as least square means. Significant effects were declared at $P \leq 0.05$ and tendencies for significance were declared at $0.05 < P \leq 0.10$.

Analysis of the CIL/LC-MS-based carbonyl-metabolomics data was done using Metaboanalyst 4.0 (metaboanalyst.ca). Prior to analysis, the data were exported to IsoMS Pro 1.0.10 for data quality check and processing according to procedures described by Mung and Li (2017). Metabolites were identified by searching against the CIL Library [DnsHz (ketone and aldehyde)] based on accurate mass and retention time and using linked identity library (LI Library) based on accurate mass and predicted retention time matches (Li et al., 2013; Huan and Li, 2015). All identified metabolite data were then exported to Metaboanalyst 4.0. The data were first normalized by median, log-transformed, and autoscaled. Partial least squares discriminant analysis (PLS-DA) scores plot was generated to visualize the distributions of the carbonyl-metabolome by treatment. Univariate analysis was performed with a volcano plot using fold change (FC; MANGLU/CON) of each metabolite against false discovery rate (FDR). Metabolites with FC ≥ 2.0 or ≤0.5 having FDR ≤ 0.05 were considered to be differentially increased or decreased relative to CON, respectively.

Analysis of the immune-related gene expression data was performed using a GeneGlobe Data Analysis Center (Qiagen, Valencia, USA) using the delta-delta-Ct (ΔΔCt) method [$(CT_{\text{gene of interest}} - CT_{\text{housekeeping genes}})_{\text{MANGLU}} - (CT_{\text{gene of interest}} - CT_{\text{housekeeping genes}})_{\text{CON}}$] with normalization of the raw data using the arithmetic mean of the five housekeeping genes. Genes with FC ≥ 1.2 or ≤0.83 having *P*-value ≤ 0.05 were considered to be differentially upregulated or downregulated, respectively, relative to CON.

RESULTS

Growth Performance and Apparent Nutrient Digestibility

The effects of MANGLU supplementation on growth performance are shown in Table 2. Over the 42-d feeding trial, supplemental MANGLU tended to increase final BW ($P = 0.07$) and ADG ($P = 0.06$), but no treatment effects were observed for DMI ($P = 0.33$) and FE ($P = 0.27$). During the first 14 d, there were no effects on ADG ($P = 0.32$) and FE ($P = 0.81$), but beef steers fed supplemental MANGLU had greater ($P = 0.01$) DMI. No treatment effects on DMI, ADG, and FE ($P > 0.05$) were found from days 15 to 42. Compared with CON, beef steers fed supplemental MANGLU had greater apparent total tract DM digestibility ($P = 0.03$) and tended to have greater NDF digestibility ($P = 0.09$); however, digestibilities of ADF and CP were similar ($P > 0.05$) between treatments (Table 3).

Whole-Blood Immune Gene Expression Analysis

Out of the 84 immune-related genes analyzed, none was altered on day 0 (FC < 1.2 or > 0.83; $P > 0.05$; Supplementary Table S2), whereas a total of five genes were differentially expressed on day 42 (Table 4). Compared to CON, the mRNA expressions of all the five differentially expressed genes, chemokine receptor 5 (CCR5), chemokine receptor 3 (CXCR3), myeloid differentiation primary response protein 88 (MYD88), transcription factor T-bet (TBX21), and toll-like receptor 2 (TLR2) were upregulated (FC > 1.2; $P < 0.05$) in beef steers fed supplemental MANGLU.

Table 2. Effects of a blend of glucan and mannan on the performance of steers during a 42-d receiving period[a]

Item	CON	MANGLU	SEM	*P*-value
Initial weight, kg	231	230	5.03	0.86
Final weight, kg	298	304	3.25	0.07
Days 1–42				
ADG, kg/d	1.60	1.75	0.08	0.06
DMI, kg/d	6.65	6.83	0.18	0.33
Feed efficiency (kg/kg)	0.24	0.26	0.01	0.27
Days 1–14				
ADG, kg/d	1.69	1.88	0.19	0.32
DMI, kg/d	5.62	6.48	0.22	0.01
Feed efficiency (kg/kg)	0.31	0.30	0.04	0.81
Days 15–42				
ADG, kg/d	1.56	1.68	0.08	0.17
DMI, kg/d	7.06	7.18	0.21	0.56
Feed efficiency (kg/kg)	0.22	0.24	0.01	0.27

[a]CON, control; MANGLU, a blend of mannan and glucan fed at 5 g/steer/d (PMI, Arden Hills, MN).

Table 3. Effects of a blend of glucan and mannan on apparent nutrient digestibility in beef steers during a 42-d receiving period[a]

	CON	MANGLU	SEM	*P*-value
Dry matter, %	66.7	69.6	1.32	0.03
Crude protein, %	61.5	63.1	1.44	0.42
Acid detergent fiber, %	57.2	59.3	1.27	0.16
Neutral detergent fiber, %	60.4	63.3	1.21	0.09

[a]CON, control; MANGLU, a blend of mannan and glucan fed at 5 g/steer/d (PMI, Arden Hills, MN).

Table 4. Effects of a blend of glucan and mannan on whole-blood immune gene expression in beef steers on day 42 of the receiving period

Gene name	Gene symbol	FC[a]	*P*-value
Chemokine (C–C motif) receptor 5	CCR5	1.30	0.04
Chemokine (C-X-C) receptor 3	CXCR3	1.23	0.03
Myeloid differentiation primary response protein 88	MYD88	1.22	0.03
Transcription factor T-bet	TBX21	1.70	0.01
Toll-like receptor 2	TLR2	1.27	0.04

[a]Fold change (relative to control) = 2−ΔΔCt = [(CTgene of interest – CTreference gene)$_{MANGLU}$ – (CTgene of interest – CTreference genes)$_{CON}$]. Only genes with both fold change ≥1.2 or ≤0.83, relative to control, and $P \leq 0.05$ are shown.

Energy Status and Carbonyl-Metabolome Analysis

There were no treatment effects ($P > 0.05$) on plasma concentrations of glucose and NEFA on days 14 and 42 (Table 5). Carbonyl-metabolome analysis revealed a total number of 37 identified metabolites in all samples (Supplementary Table S3). The PLS-DA scores plot showed separations between the two treatments (Figure 1), indicating that supplemental MANGLU altered the carbonyl-metabolome of the beef steers. Volcano plot analysis (Figure 2) showed that relative to CON, the plasma concentrations of 12 metabolites, including galactose, two isomers of glyceraldehyde, S-2-aceto-2-hydroxybutanoate, hippuric acid, 3-methylindolepyruvate, and nutriacholic acid were increased (FC ≥ 2.0, FDR ≤ 0.05) whereas plasma concentrations of two metabolites (γ-oxalocrotonate and 2-dehydro-3-deoxy-L-arabinonate) were decreased (FC ≤ 0.05, FDR ≤ 0.05; Table 6).

DISCUSSION

Responses of beef cattle to yeast-based additives, including mannan and glucan, have been inconsistent across studies, with more studies reporting no effects, possibly due to differences in

Table 5. Effects of a blend of glucan and mannan on plasma glucose and NEFA concentrations in beef steers during a 42-d receiving period[a]

Item	CON	MANGLU	SEM	*P*-value
Day 14				
Glucose, mM	68.3	71.4	1.94	0.15
NEFA, mM	0.29	0.31	0.08	0.74
Day 42				
Glucose, mM	79.2	77.9	1.21	0.52
NEFA, mM	0.23	0.29	0.04	0.61

[a]CON, control; MANGLU, a blend of mannan and glucan fed at 5 g/steer/d (PMI, Arden Hills, MN).

sources, strain, concentrations, processing, and doses as well as physiological status and health conditions of animals (Desnoyers et al., 2009; Aragon et al., 2016; Plaizier et al., 2018). Consistent with the results of this study, Lei et al. (2013) reported increased ADG receiving beef steers fed 2 g/kg DM of YCW product. In contrast, several studies have reported no effects of supplemental YCW products in the diets of beef cattle during the receiving period. Pukrop et al. (2018) reported no effect on the performance of newly received feedlot cattle fed diet supplemented with a hydrolyzed mannan- and glucan-rich yeast fraction. Finck et al. (2014) reported no effects on the growth performance of beef heifers fed diet supplemented with a YCW product during the receiving period. In a similar study, Burdick Sanchez et al. (2014) reported no effects of dietary supplementation of 2.5 g/d of YCW product on the growth performance of newly received crossbred beef heifers. It is important to note that in addition to mannan and glucan, supplemental MANGLU also contained trace amounts of galactan, a polysaccharide consisting of polymerized galactose with an immune system-modulating activity (Delattre et al., 2011; Totté et al., 2015), which might have contributed to its growth-promoting effects observed in this study.

The effect of supplemental MANGLU on intake was more evident during the first few days in the feedlot as greater DMI was observed only in the first 14 d. The benefits of feeding *S. cerevisiae*-based products such as supplemental mannan and glucan in animals have been reported to be more pronounced under stress conditions and majorly mediated via improvement in GIT health (Cole et al., 1992; Salinas-Chavira et al., 2017; Oh et al., 2019) which often supports improved feed intake and nutrient utilization. During the first few days in the feedlot, cattle experience depressed feed intake due to stressors such as separation from

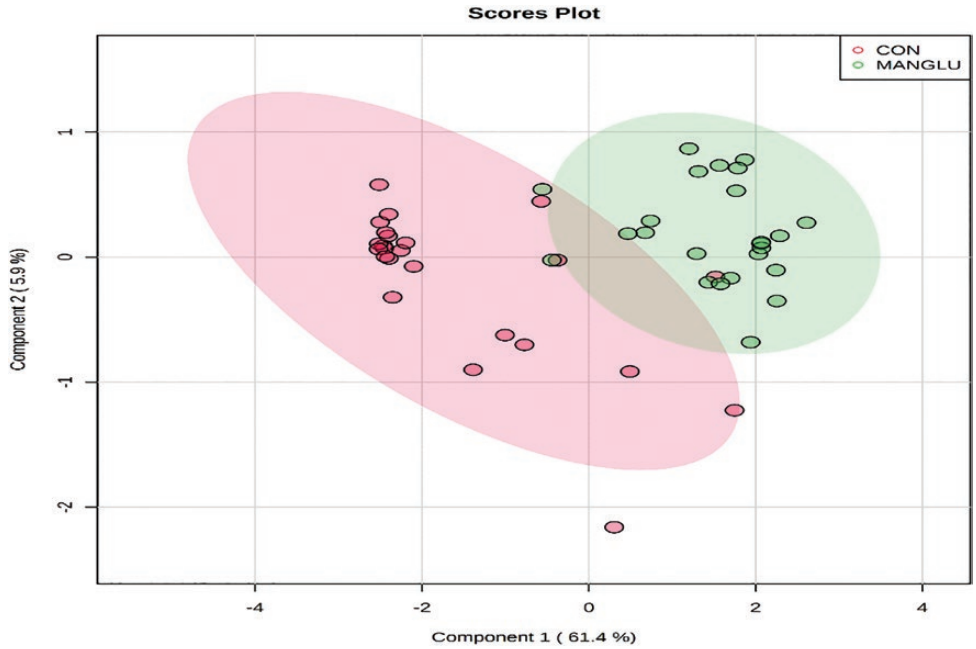

Figure 1. Partial least squares discriminant analysis (PLS-DA) scores plot of the plasma carbonyl-metabolome of the beef steers fed diets supplemented with a blend of mannan and glucan. CON, control; MANGLU, a blend of mannan and glucan fed at 5 g/head/d (PMI, Arden Hills, MN).

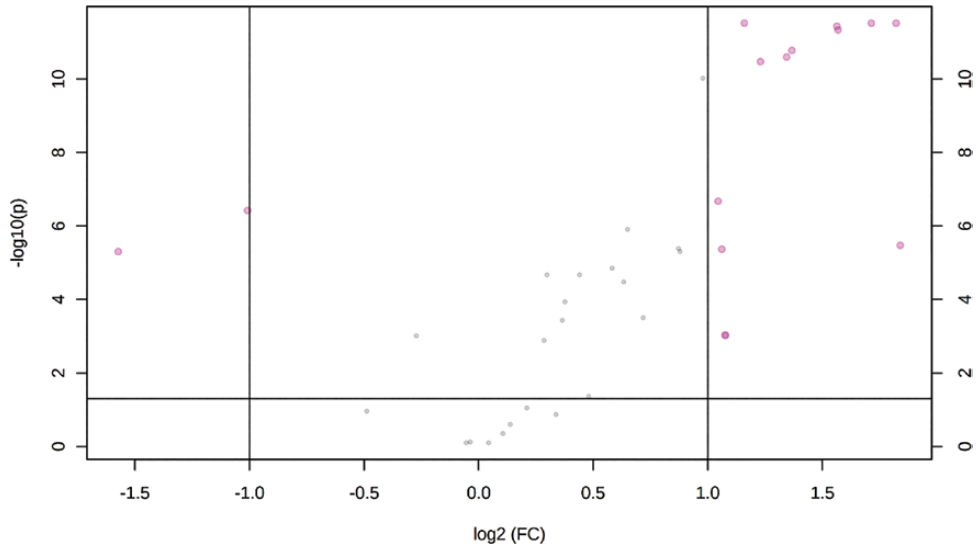

Figure 2. Volcano plot showing the differential plasma carbonyl-containing metabolites in beef steers fed diets supplemented with a blend of mannan and glucan (5 g/head/d; PMI, Arden Hills, MN).

mother, commingling, transportation, exposure to pathogens, vaccination, and changes in diet and environment (Arthington et al., 2013). These stressors, in addition to low nutrient intake, weaken the immune system and make the animals more susceptible to diseases (Bernhard et al., 2012). Thus, improved feed intake during the first few days of the receiving period is very essential to the overall wellbeing of beef cattle in feedlot.

Greater digestibilities of DM and NDF in beef steers fed supplemental MANGLU is possibly a reflection of an improved GIT health, which in part explains the improved growth performance of the beef steers. Previous studies have demonstrated that dietary supplementation of mannan and/or glucan improved the integrity, and digestive and absorptive function of the digestive tract by interacting directly with gastrointestinal immune cells, binding toxins, and blocking colonization of pathogens (Diaz et al., 2004; Broadway et al., 2015). Diaz et al. (2004) observed reduced aflatoxin concentration in milk of dairy cows

Table 6. Plasma carbonyl-containing metabolites that were altered by dietary supplementation of a blend of mannan and glucan on day 42 of the receiving period

Metabolites[a]	FC[b]	FDR
3-methylindolepyruvate	3.58	<0.01
S-2-aceto-2-hydroxybutanoate	3.54	<0.01
Nutriacholic acid	3.28	<0.01
Isomer of S-2-aceto-2-hydroxybutanoate	2.97	<0.01
Galactose	2.58	<0.01
3-fumarylpyruvate	2.54	<0.01
1-deoxy-D-xylulose 5-phosphate	2.36	<0.01
5-oxopentanoate	2.23	<0.01
Glyceraldehyde	2.12	<0.01
Isomer of glyceraldehyde	2.11	<0.01
Phenylacetylglycine	2.09	<0.01
Hippuric acid	2.06	<0.01
γ-Oxalocrotonate	0.50	<0.01
2-dehydro-3-deoxy-L-arabinonate	0.34	<0.01

[a]Only metabolites with either FC ≥ 2.0 or ≤0.50, relative to control, and FDR ≤ 0.05 are shown. [b]FC: fold change relative to control.

fed an esterified glucomannan suggesting that the additive binds and prevents absorption of aflatoxin in the GIT. In beef cattle, feeding 2 g/kg of YCW reduced lipopolysaccharide (LPS) concentrations in the blood from 0.96 to 0.66 EU/mL (Lei et al., 2013). Similar result was observed in sheep-fed high grain diets (Diaz et al., 2018). In addition, several in vitro studies have demonstrated that YCW products can bind a wide range of toxins (Yiannikouris et al., 2003, 2004; Shetty and Jespersen, 2006). Mannan has the ability to prevent the colonization of bacterial pathogens in the GIT by selectively binding to bacterial mannan-binding lectin (Oyofo et al., 1989). Control of pathogenic bacterial adhesion to the intestinal epithelial surface and removal of toxins improve the integrity of the GIT, thereby, increasing the efficiency of nutrient digestion and absorption (Spring et al., 2015).

The mechanism for improved performance response of animals to MANGLU supplementation is believed to be mediated via immuno-stimulatory properties of mannan and glucan, as well as galactan (Spring et al., 2000; Thanissery et al., 2010; Totté et al., 2015). Immune status of beef cattle during the receiving period is critical to their overall wellbeing because they are in constant exposure to stress and health challenges that negatively affect their health and productivity throughout the feedlot period. Mannan and glucan are active biological response modifiers that can directly or indirectly interact with immune cells and activate various immunological responses (Korolenko et al., 2019; Yin et al., 2019). Although the effects of YCW products on the immune status of animals have been extensively studied, this study represents the first to comprehensively analyze mRNA expressions of 84 immune-related genes in the whole blood of beef cattle fed YCW products. In addition, unlike several previous studies (Burdick Sanchez et al., 2013, 2014; Finck et al., 2014), the animals were not artificially challenged with pathogens or toxins (Adeyemi et al., 2019), thus, the effects observed in this study represent the response to their natural environmental stressors.

Corroborating the immunomodulatory properties of mannan, glucan, supplemental MANGLU increased the expressions of TLR2 and MYD88, compared with CON. For immune cells to effectively mount an efficient host defense against microbial pathogens, specific molecular patterns from invading microbial pathogens must first be recognized (Calich et al., 2008). Toll-like receptors are one of the several pattern recognition receptors that play a crucial role in the recognition of microbial pathogens (Takeda et al., 2003; Kawai and Akira, 2007) and TLR2 is known to be the major receptor for molecular patterns of gram-positive bacteria and several microbial components, including fungal cell wall components (glucan and mannan), peptidoglycan, and LPS (Turner, 2003). Myeloid differentiation primary response protein 88 is a downstream adaptor protein of all TLRs except TLR3 and is involved in signal transduction of the TLR signaling pathway (Deguine and Barton, 2014). Mannan is known to be a strong ligand for mannose-binding lectin (MBL), a pattern recognition molecule of the innate immune system (Estabrook et al., 2004; Nigou et al., 2008). MBL is a liver-derived protein that can recognize several infectious agents, including bacteria, yeasts, parasites, viruses, and mycobacteria, by binding to sugars such as *N*-acetyl-D-glucosamine, mannose, *N*-acetyl-mannosamine which are expressed on their surfaces (Neth et al., 2000; Turner, 2003). Ip et al. (2008) evaluated innate immune response to *Staphylococcus aureus* in mice and demonstrated that MBL forms a complex with TLR2 to upregulate its responses, indicating that microbial recognition mediated by MBL and TLR2-MYD88 signaling is functionally linked. This probably explains the increased mRNA expressions of TLR2 and MYD88 observed in beef steers fed MANGLU.

Compared to CON, there were greater (FC ≥ 1.2, $P \leq 0.05$) mRNA expressions of two chemokine receptors (CCR5 and CXCR3) and TBX21 in beef

steers fed supplemental MANGLU. The ability of immune cells to move to the sites of infection or inflammation is essential for effective defense and clearance of invading pathogens (Dawson et al., 2000). Chemokines are chemotactic cytokines that orchestrate the movement of leukocytes to the sites of infection or inflammation (Luster, 1998; Zweemer et al., 2014), and their receptors are expressed on the surfaces of many immune cells (Proudfoot, 2002; Chaplin, 2010). C-C chemokine receptor type 5 is a protein expressed on the surface of white blood cells (de Roda Husman et al., 1999), while CXCR3 is expressed on several cell types including T cells and dendritic cells (Hancock et al., 2000; Liu et al., 2005). T-bet (TBX21) is the master regulator for development of T-helper 1 cells, which promote cell-mediated inflammatory response by producing proinflammatory cytokines against intracellular pathogens, including viral infections (Luckheeram et al., 2012). In agreement with the results of this study, Kozłowska et al. (2020) provided evidence that mannan and glucan can activate peripheral blood mononuclear cells to express CCL3 at protein and transcript levels and CXCL8 at mRNA level. Taken together, these results demonstrate that beef steers fed supplemental MANGLU had a better ability than CON to quickly recognize and mount an efficient defense against invading microbial pathogens.

Despite the differences in DMI (during the first 14 d) and DM digestibility, no treatment effects were detected for plasma glucose and NEFA on either day 14 or 42. Lack of difference in blood glucose and NEFA, which are often considered indicators of energy status in animals (Adewuyi et al., 2005), is a reflection that the energy requirement of the steers was met by the diet fed. In addition to using enzyme-based assays, which are limited to a few metabolites, to assess the energy status of the animals, we analyzed several intermediate products of energy metabolism in the plasma of the animals. Advances in metabolomics have provided the opportunity to analyze multiple metabolites in biofluids (Nicholson and Wilson, 2003; Tomita and Kami, 2012). CIL/LC-MS is a powerful metabolomics technique for in-depth analysis of metabolites based on the presence of common functional groups such as amine, carbonyl, hydroxyl, and carboxyl (Guo and Li, 2010; Bueschl et al., 2013). Some common intermediates of energy metabolism in the body, such as ketones and aldehydes, contain carbonyl chemical group and their concentrations in the blood may reflect the energy status of animals (Zhao et al., 2019). Additionally, some microbiome-derived metabolites contain carbonyl functional group, and their alterations in blood may possibly suggest changes in GIT microbial population.

In agreement with enzyme-based technique, results of carbonyl-metabolome analysis showed no difference in plasma glucose between treatments; however, the plasma concentrations of three monosaccharides (two isomers of glyceraldehyde and galactose) were greater in beef steers fed MANGLU. Greater plasma galactose concentration observed in beef steers fed supplemental MANGLU is possibly due to hydrolysis of galactan, a constituent of supplemental MANGLU, in the rumen. Galactan is a soluble fiber consisting of polymerized galactose that can be hydrolyzed by ruminal microbial enzymes into galactose units (McCann et al., 2014). Galactose can serve as an energy substrate via conversion into a metabolite of glucose, glucose-6-phosphate in the liver (Berg et al., 2002). Glyceraldehyde is a triose monosaccharide that can be converted to glyceraldehyde-3-phosphate, a high-energy intermediate of glycolysis, by the enzyme glyceraldehyde kinase (Clough, 2005). Thus, greater plasma concentrations of glyceraldehydes and galactose in beef cattle fed supplemental MANGLU possibly indicate an improved energy status.

Several microbiome-derived plasma metabolites were altered, suggesting alterations in ruminal (or hindgut) microbial composition of the beef steers. 1-deoxy-D-xylulose 5-phosphate is a bacterial metabolite involved in de novo biosynthesis of pyridoxal phosphate (Bartee and Meyers, 2018). Hippuric acid is a gut-derived phenolic metabolite formed by the conjugation of benzoic acid (Graefe and Veit, 1999; Clifford et al., 2000). Phenylacetylglycine is formed by the conjugation of phenylacetate by microbial metabolism (Jones, 1982). 3-methylindolepyruvate is a derivative of 3-methylindole, a compound produced in the rumen from anoxic degradation of tryptophan by bacteria (Yokoyama and Carlson, 1979). 2-dehydro-3-deoxy-L-arabinonate is a metabolite formed from L-arabinonate by the action of a bacterial L-arabinonate dehydratase (Rahman et al., 2017). S-2-aceto-2-hydroxybutanoic acid is an intermediate metabolite in biosynthesis of branched-chain amino acid metabolism in microorganisms (and plants) via acetolactate synthase (Gedi and Yoon, 2012). Several studies have demonstrated that dietary supplementation of *S. cerevisiae*-based products can modulate ruminal fermentation and

microbiota (Kumar et al., 1997; Thrune et al., 2009; Poppy et al., 2012; Ogunade et al., 2019); however, the significance of changes in the plasma concentrations of these aforementioned metabolites is unknown because rumen microbial composition and fermentation was not measured in this study.

CONCLUSIONS

This study demonstrated that dietary supplementation of MANGLU improved DMI during the first 14 d and tended to improve the growth performance of beef steers over a 42-d receiving period. The increased growth performance of the beef steers was supported by increased DM and NDF digestibilities and increased mRNA expressions of immune-related genes involved in recognition of and promoting immune defense against microbial pathogens. Results of plasma carbonyl-metabolome analysis showed increased concentrations of nonglucose monosaccharides, an indication of better energy status and alterations in gut-derived metabolites, an indication of altered GIT microbiota. Overall, the results of this study demonstrated that, under the condition of this experiment, supplemental MANGLU enhances beef cattle immunocompetence and productivity during feedlot receiving period.

SUPPLEMENTARY DATA

Supplementary data are available at *Translational Animal Science* online.

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