EFFECT OF *LACTOBACILLUS SALIVARIUS* L28 USED AS A FEED ADDITIVE ON THE ANTIMICROBIAL RESISTANCE OF COMMENSAL ORGANISMS OF FEEDLOT CATTLE

Authors: Andrea R. English[*1], Alejandro Echeverry[1], Jhones O. Sarturi[1], Tosha L. Opheim[1], Kendra K. Nightingale[1], Markus. F. Miller[1], Mindy M. Brashears[1]

[1]Texas Tech University, Lubbock, Texas, 79409-2141, USA
*Corresponding author email: andrea.english@ttu.edu

I. INTRODUCTION

Antibiotic usage over decades, to improve animal and human health, could be a contributing factor to the increase and emergence of antimicrobial resistance globally [1]. There is evidence that direct-fed microbials (DFM) are an effective alternative to animal growth promoters (AGPs) to improve cattle gain, feed efficiency and increased milk production in dairy cows [2], while also reducing the shedding of pathogenic bacteria, such as *Escherichia coli* O157:H7 in the feces [3]. The objective of this study was to monitor the antimicrobial resistance profiles of generic *Escherichia coli* and *Enterococcus* spp. during the feeding period.

II. MATERIALS AND METHODS

A total of three dietary treatments based on conventional high concentrate diets were fed to finish cattle for harvest: CONTROL containing tylosin (88 mg/hd/d of diet dry matter (DM)), and monensin (330 mg/hd/d of diet DM); MONPRO containing a newly isolated DFM, *L. salivarius* L28, at a feeding rate of 10^6 CFU/hd/d, monensin (330 mg/hd/d of diet DM), and no tylosin; and BASE which had no DFM, tylosin or monensin. Fecal samples were collected on 0, 56, and 140 d by rectal grab. Days 0 and 56 fecal samples were collected from 3 animals per pen and formed into one composite sample (n=36/day). On 140 d, one fecal sample per animal was collected (n=144). Samples were weighed, diluted, and plated onto MacConkey (MAC) agar for isolation of generic *E. coli*, and Kenner Fecal (KF) *Streptococcus* agar for *Enterococcus* spp. From each MAC and KF *Streptococcus* agar plate, three typical colonies were selected from 0 and 56 d, and one colony from 140 d. Colonies were streaked onto 5% sheep blood agar plates and subjected to antibiotic susceptibility testing using the micro-broth dilution (Sensititre™) susceptibility minimum inhibitory concentration plates, following the National Antimicrobial Resistance Monitoring System protocol. Resistance and susceptible breakpoints were determined from the Clinical and Laboratory Standard Institute.

III. RESULTS AND DISCUSSION

Enterococcus was susceptible (no resistance detected) across day and treatment to chloramphenicol, gentamicin, kanamycin, and tigecycline. *Enterococcus* antimicrobial resistance increased over time for all treatments. With the highest rates of resistance to lincomycin for all treatments over time (Table 1). At 140 d all treatments expressed resistance to vancomycin; base (24.4%), control (18.6%) and MonPro (22.5%). Vancomycin resistant enterococci is of significant concern due to its difficulty to treat in hospital acquired infections [4]. The World Health Organization (2017) [5] classified vancomycin resistant *Enterococcus faecium* as a pathogen of high priority for the development of new antimicrobial treatment.

Table 1. *Enterococcus* isolates susceptibility profile to individual antimicrobials.

			% of isolates resistant to[*]:										
DOF[1]	TRT[2]	No. of Isolates	CIPR	DAPT	ERYT[3]	LINC	LINE	NITR	PENI[3]	SYNE	TETR[3]	TYLO	VANC[3]
0 d	Base	35	5.7	14.3	11.4	42.9	0	0	2.9	0	37.1	42.9	0
	Control	35	2.9	11.4	11.4	65.7	0	0	0	0	54.3	54.3	0
	MonPro	32	3.1	9.4	6.3	43.8	0	0	0	0	37.5	31.3	0
56 d	Base	35	0	17.1	11.4	94.3	0	2.9	0	0	34.3	0	0
	Control	36	0	25	27.8	100	0	11.1	0	0	66.7	0	0
	MonPro	36	0	13.9	16.7	94.4	0	2.8	0	0	47.2	0	0
140 d	Base	45	0	26.7	35.6	86.7	24.4	0	22.2	24.4	57.8	64.4	24.4
	Control	43	0	20.9	32.6	100	18.6	0	18.6	20.9	51.2	72.1	18.6
	MonPro	40	2.5	30	37.5	92.5	22.5	0	22.5	22.5	45	77.5	22.5

[*]CIPR-Ciprofloxacin, DAPT-Daptomycin, ERYT-Erythromycin, LINC-Lincomycin, LINE-Linezolid, NITR-Nitrofurantoin, PENI-Penicillin, SYNE-Quinupristin/dalfopristin, TETR-Tetracycline, TYLO-Tylosin tartrate, VANC-Vancomycin. [1]DOF-Days on feed. [2]TRT-Treatment. [3]Antimicrobials that are used in animal production and in human medicine.

E. coli isolates were susceptible to amikacin, ciprofloxacin, gentamicin, sulfisoxazole, and trimethoprim across time and treatment. The MonPro treatment had the highest percentage of susceptible isolates on 0 d (88.9%), 56 d (66.7%) and 140 d (87.5%), while also having the least multi-drug resistance (MDR; resistance to 3 or more drugs) on all days (0 d (0%), 56 d (5.6%), and 140 d (2.1%).

Table 2. *Escherichia coli* isolates susceptibility profile to individual antimicrobials.

			% of isolates resistant to[*]:								
DOF[1]	TRT[2]	No. of Isolates	AMOC	AMPI	CEFO	CEFT	CEFTR	CHLO	NALA	STRE	TETR
0 d	Base	36	0	0	0	0	0	8.3	0	38.9	47.2
	Control	36	8.3	8.3	8.3	8.3	8.3	16.7	8.3	16.7	47.2
	MonPro	36	0	0	0	0	0	0	0	8.3	11.1
56 d	Base	36	0	2.8	0	0	2.8	19.4	0	50	55.6
	Control	36	8.3	8.3	8.3	8.3	8.3	19.4	8.3	25	50
	MonPro	36	0	5.6	0	0	0	0	0	19.4	30.6
140 d	Base	46	0	2.2	0	0	0	13	2.2	15.2	19.6
	Control	48	0	0	0	0	0	4.2	0	10.4	18.8
	MonPro	48	0	0	0	0	0	2.1	0	4.2	12.5

[*]AMOC-Amoxicillin, AMPI-Ampicillin, CEFO-Cefoxitin, CEFT-Ceftriaxone, CEFTR-Cefiofur, CHLO-Chloramphenicol, NALA-Nalidixic Acid, STRE-Streptomycin, TETR-Tetracycline. [1]DOF-Days on feed. [2]TRT-Treatment.

IV. CONCLUSION

Enterococcus spp. regardless of diet had large percentages of antibiotic resistance and MDR during the entire feeding period. Enterococci is known for its ability to transfer genes and harbor multiple resistances [6]. Bacteria have the ability to mobilize and distribute AMR genes, thus expanding the resistome even in the absence of antimicrobials this could be the cause of the resistance found in the base treatment group [7]. Thus, this makes it important to monitor the antimicrobial resistance in these commensal organisms that could potential enter the food supply. However, for *E. coli* these results suggest the use of DFM-supplemented diet could be effective at reducing the resistance of *E. coli* isolates shed by feedlot cattle.

ACKNOWLEDGEMENTS

Funding was provided by the Texas Beef Council and Texas Tech Universities, International Center for Food Industry Excellence.

REFERENCES

1. Laxminarayan, R., Van Boeckel, T., and Teilant, A. (2015) The Economic Costs of Withdrawing Antimicrobial Growth Promoters from the Livestock Sector. OECD Food, Agriculture and Fisheries Papers No. 78.
2. Krehbiel, C. R., Rust, S. R., Zhang, G., & Gililand, S. E. (2003) Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci. 81(E. Suppl. 2): E120-E132.
3. Brashears, M. M., Galyean, M. L., Loneragan, G. H., Mann, J. E., and Killinger-Mann, K. (2002) Prevalence of Escherichia coli O157:H7 and Performance by beef feedlot cattle given Lactobacillus Direct-Fed Microbials. Journal of Food Protection, Vol 66, No. 5, 2003, Pages 748-754.
4. Gilmore, M.S., F. Lebreton, and W. V. Schaik. (2013) Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. Current Opinion in Microbiology 2013, 16:10-16
5. World Health Organization. (2017) Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discover, and Development of New Antibiotics. Retrieved: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1
6. Jackson, C. R., Lombard, J. E., Dargatz, D. A., and Fedorka-Cray, P. J. (2010) Prevalence species distribution and antimicrobial resistance of enterococci isolated from US dairy cattle. Letter in Applied Microbiology. 52, 41-48.
7. Wright, G. D. The antibiotic resistome: the nexus of chemical and genetic diversity. 2007. Nature Reviews: Microbiology. Vol. 5. Doi: 10.1038/nmicro1614.