

## Background

- Oral antibiotics alter the mammalian gut microbiome and metabolome (González-Arancibia et al., Psychopharmacol. 236:1611–1622; 2019).
- The gut microbiome produces neurotransmitters (Galland, J. Med. Food 17:1261-1272, 2014; Mazzoli & Pessione, Front. Microbiol. 7; 2016, Strandwitz et al., Nat. Microbiol. 4:396–403, 2019).
- Monensin, an oral ionophore antibiotic, reduces the acetate:propionate ratio in the rumen of beef cattle (Moseley et al., J. Anim. Sci. 45:961-968, 1977).

## Objective

An untargeted analysis was conducted to determine changes in metabolites in the rumen of temperate and tropically-adapted beef cattle resulting from dietary monensin, with an emphasis on neurotransmitters.

## Materials and Methods

- After a 21-d adaptation period, 5 Angus, 5 Brahman, and 5 BrahmanXAngus (F1) steers (matched by age, BW, temperament) were studied in two 21-day feeding periods.
- During Period 1, steers were group-fed Tifton bermudagrass hay and libitum within breed group.
- During Period 2 steers were individually fed hay and libitum plus supplement with monensin (220mg·steer<sup>-1</sup>·day<sup>-1</sup>).
- An untargeted analysis of rumen fluid collected after Periods 1 and 2 was performed using Liquid Chromatography-Mass Spectroscopy (LCMS). Short chain fatty acid (SCFA) and monensin content were determined by Gas Chromatography and LCMS, respectively.
- Defined compounds within breed were annotated using Compound Discoverer v3.1 for changes (P<0.05) due to dietary monensin (Fig 1).

## Results

- Monensin was not detected pre-treatment but averaged 363±24 ng/mL in post-treatment rumen fluid.
- Ruminant acetate:propionate ratio was decreased (P<0.05) by monensin (Fig 2) in Angus (17%), Brahman (18%) and F1 (10.5%) steers.
- Various potential neurotransmitter-related molecules were increased/decreased (P<0.05) among the three breeds (Table 1).

Fig 1. Volcano Plots of Compounds from Untargeted Analysis for Angus (A), Brahman (B) and F1 Cross (C) Steers

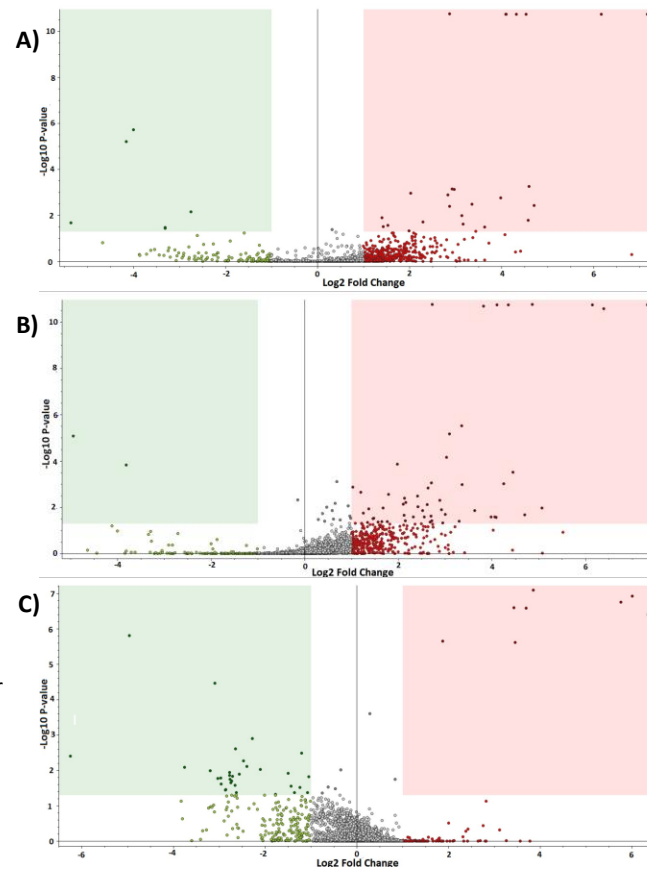


Fig 2. Acetate:Propionate Ratios Before and After Dietary Monensin in All Breeds

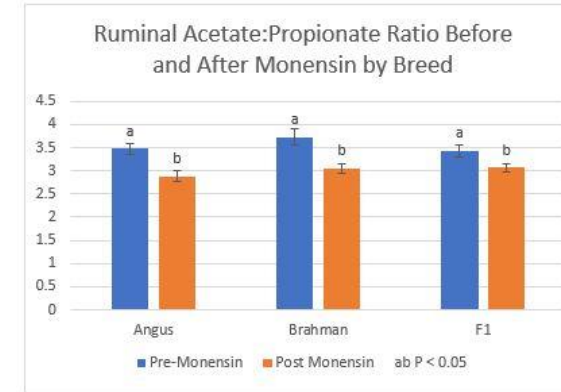


Table 1. Potential Neurotransmitters and Neurotransmitter-related Metabolites Affected by Dietary Monensin by Cattle Breed

Breed	Neurotransmitter Group	Metabolite	Post/Pre Ratio	P Value*
Angus	Acetylcholine	4-Oxo-4-(3-pyridinebutanal)	↑ 7.906	0.002
	GABA	Pyridoxamine	↑ 7.869	0.001
	GABA	Oxiracetam	↑ 2.895	0.027
	GABA	Pregabalin	↑ 2.643	0.013
	Dopamine	Dopamine	↓ 0.149	0.007
Brahman	GABA	Pyridoxamine	↑ 8.551	6.9E-06
	GABA	Benzotriazole	↑ 5.726	0.014
	Serotonin	5-Hydroxyindoleacetic acid	↑ 4.389	0.004
F1	Acetylcholine	Acetylcholine	↓ 0.391	0.043
	Dopamine	Dopamine	↓ 0.206	0.001
	Dopamine	6-methyltetrahydropterin	↓ 0.435	0.003

\*P-values were calculated by Tukey's HSD test, following ANOVA and controlling for false discovery rate (Benjamini-& Hochberg, J. Royal Stat. Soc. 57:289-300; 1995).

Table 2. Roles of Neurotransmitters

Neurotransmitter	Neurological Role
Acetylcholine (ACh)	Modulates cation channels; releases intracellular calcium; increases attentiveness and responsiveness.
Dopamine	Increased dopamine is associated with depression, hyperactivity, and anxiety.
γ-aminobutyric acid (GABA)	Exerts excitatory effects. Increased GABA associated with calming and anti-anxiety effects.
Serotonin	Modulates sleep, arousal, and sexual behavior. Decreased serotonin is associated with depression, posttraumatic stress disorder, and anxiety.

## Summary

- Ruminant acetate:propionate ratio was similarly reduced by dietary monensin in the Angus, Brahman and F1 steers.
- Neurotransmitter-related compounds in the rumen were differentially influenced by monensin in Angus, Brahman and F1 (Table 1).
- Angus had increases in ACh and GABA-related compounds but a decrease in dopamine. Brahman had increases in serotonin and GABA-related compounds. The F1 had decreases in ACh and dopamine compounds.
- Whether an oral ionophore antibiotic (i.e., monensin) results in ruminal concentrations of neuroactive substances adequate to affect communication between the gut and brain, remains unknown.

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