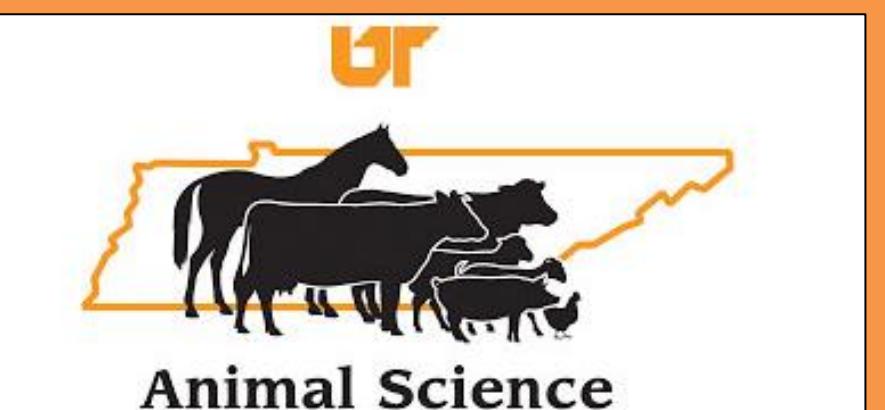


# Rumen fluid metabolites influenced by endophyte-infected tall fescue seed and red clover isoflavones in beef steers



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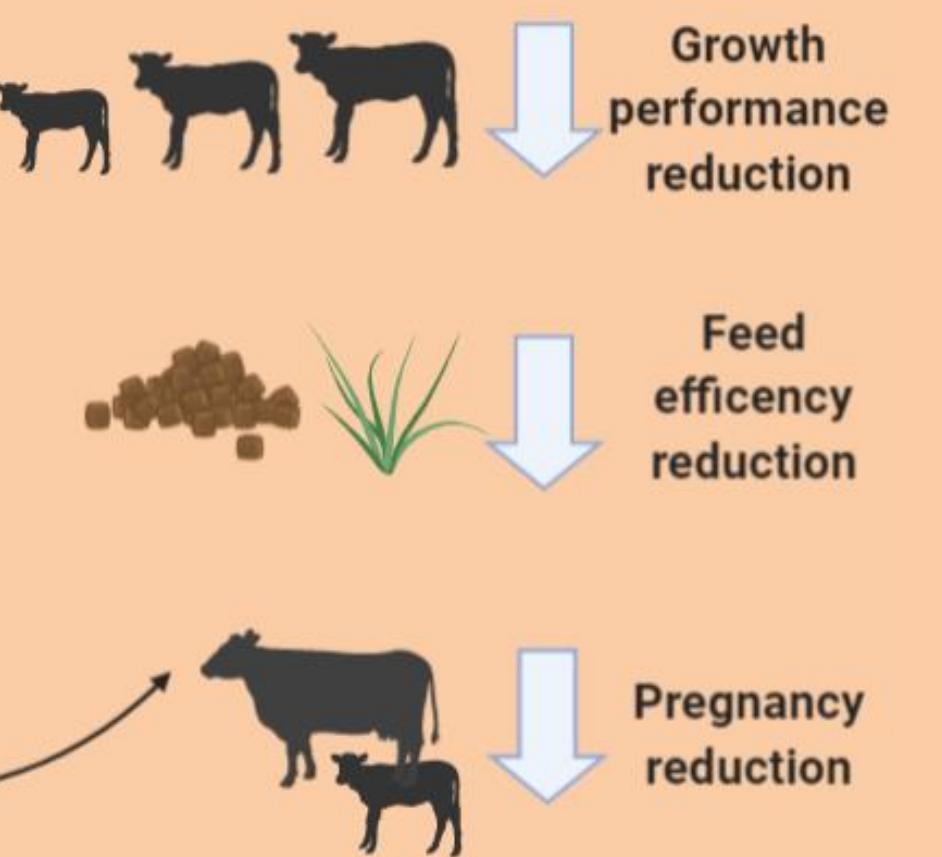
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## Introduction

- Substantial financial losses to the beef industry due to the negative effects of fescue toxicosis



- Reproduction, growth, and feed efficiency are severely affected[1]
- Researches have demonstrated that the negative effects of fescue toxicosis may be mitigated by supplementation of red clover isoflavones[2]

## Methodology

### Animals:

- 36 Angus steers weighing  $250 \pm 20$  kg[3]

### Treatments:

- 21-day trial feeding isoflavones (943 mg) daily via bolus per animal
- Diet supplemented with or without fescue seed ( $0.011 \text{ mg} \times \text{kg of body weight}^{-1} \times \text{d}^{-1}$ )[4]
- Blocked on DRD2 genotype and treatments assigned in a  $2 \times 2$  factorial arrangement
  - Two types of fescue seed (E+ or E-) and
  - Isoflavone treatment (Promensil or none)

### Sample Collection and Analysis:

- Rumen content collected on final day of 21-day trial
- Rumen metabolites analyzed on UHPLC-MS
- Statistical analysis by MIXED procedure in SAS 9.4

## Results

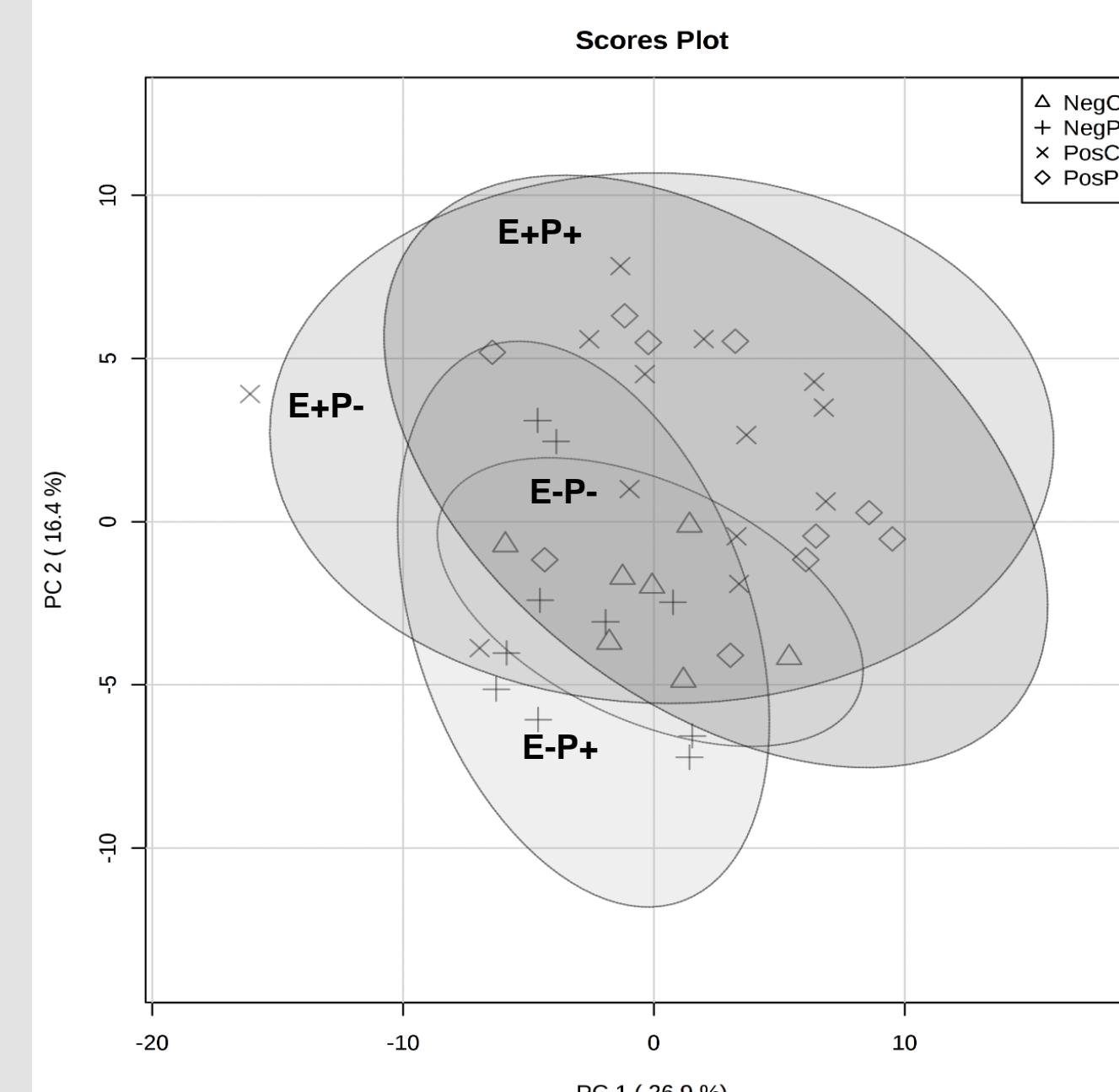
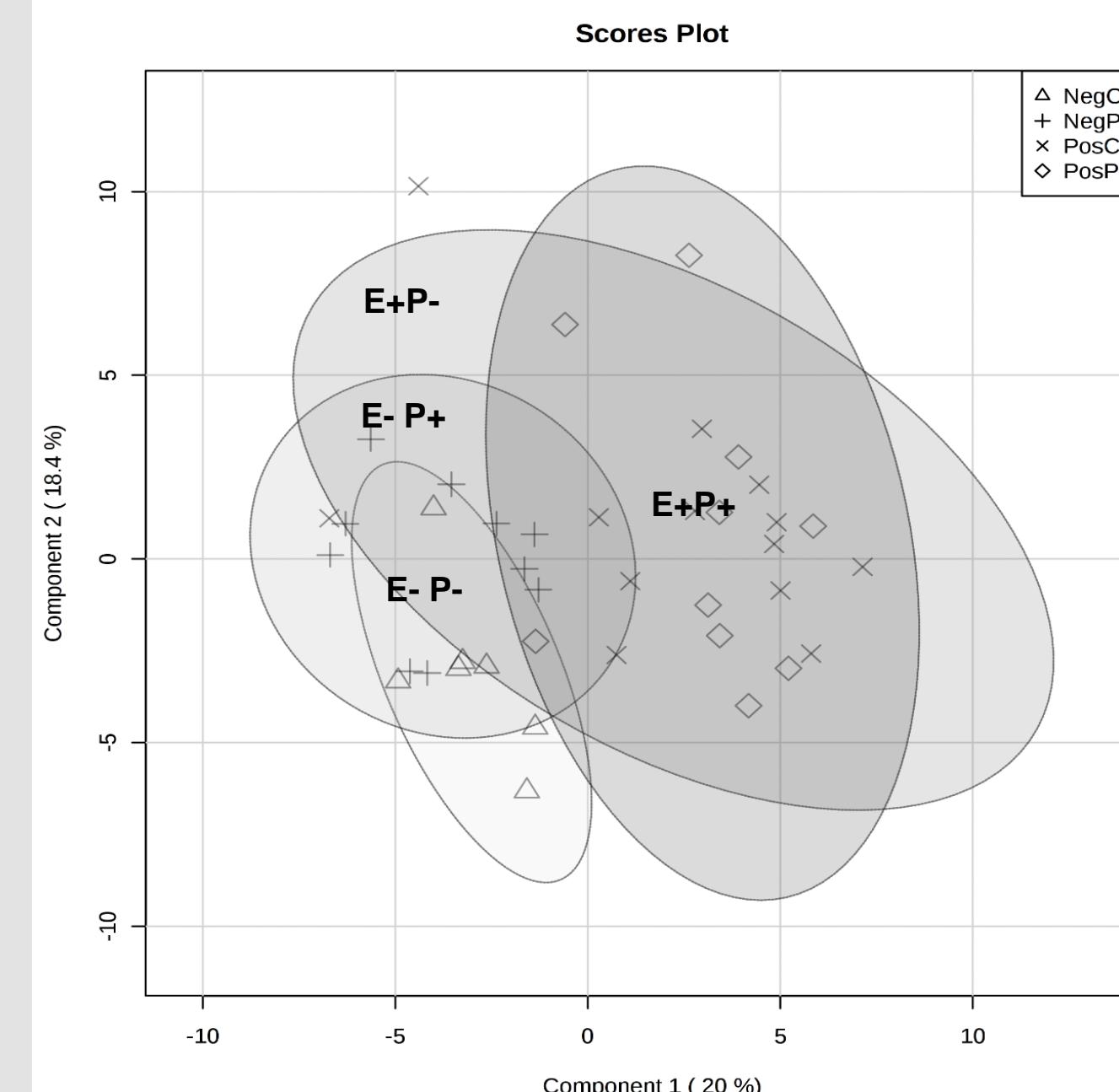


Figure 1. Principal components analysis of the rumen fluid metabolome by all treatment combinations

▲	endophyte-free w/o red clover isoflavone
+	endophyte-free w/ red clover isoflavones
X	endophyte-infected w/o red clover isoflavones
◆	endophyte-infected w/ red clover isoflavones.

Table 1. Rumen fluid metabolites that significantly differed by seed type

Metabolite	Seed Type		P Value
	E+	E-	
Dihydroxybenzoate	$5.90 \times 10^7 \pm 5.35 \times 10^6$	$8.43 \times 10^7 \pm 6.26 \times 10^6$	0.05
Adenine	$2.30 \times 10^7 \pm 1.13 \times 10^7$	$6.84 \times 10^7 \pm 1.32 \times 10^7$	0.02
CMP	$9.17 \times 10^5 \pm 7.64 \times 10^5$	$3.17 \times 10^6 \pm 8.95 \times 10^5$	0.04
Deoxyuridine	$8.04 \times 10^5 \pm 2.71 \times 10^5$	$1.74 \times 10^6 \pm 3.18 \times 10^5$	0.02
Glutamate	$7.18 \times 10^7 \pm 2.17 \times 10^7$	$1.57 \times 10^8 \pm 2.54 \times 10^7$	0.05
Guanosine	$3.00 \times 10^5 \pm 1.44 \times 10^5$	$8.63 \times 10^5 \pm 1.69 \times 10^5$	0.05
Homoserine/threonine	$1.02 \times 10^7 \pm 8.90 \times 10^5$	$6.65 \times 10^6 \pm 7.60 \times 10^5$	0.05
Hypoxanthine	$4.40 \times 10^7 \pm 1.66 \times 10^7$	$1.17 \times 10^8 \pm 1.94 \times 10^7$	0.01
Uracil	$5.76 \times 10^7 \pm 1.19 \times 10^7$	$1.08 \times 10^8 \pm 1.39 \times 10^7$	0.02
Xanthine	$1.79 \times 10^8 \pm 4.34 \times 10^7$	$3.48 \times 10^8 \pm 5.09 \times 10^7$	0.01
Xylose	$3.63 \times 10^6 \pm 1.05 \times 10^6$	$8.69 \times 10^6 \pm 1.23 \times 10^6$	0.01

Table 2. Rumen fluid metabolic pathways impacted by seed type

Pathway Name	FDR
Purine metabolism	$7.16 \times 10^{-4}$
beta-Alanine metabolism	$7.16 \times 10^{-4}$
Pentose and glucuronate interconversions	$7.16 \times 10^{-4}$
Ubiquinone and other terpenoid-quinone biosynthesis	$7.48 \times 10^{-4}$
Pyrimidine metabolism	$7.48 \times 10^{-4}$
Phenylalanine, tyrosine and tryptophan biosynthesis	$7.48 \times 10^{-4}$
Aminoacyl-tRNA biosynthesis	$8.04 \times 10^{-4}$
Phenylalanine metabolism	$8.04 \times 10^{-4}$
Arginine and proline metabolism	$1.33 \times 10^{-3}$
Porphyrin and chlorophyll metabolism	$1.36 \times 10^{-3}$
Pentose phosphate pathway	$3.57 \times 10^{-3}$
Glutathione metabolism	$3.57 \times 10^{-3}$
Pantothenate and CoA biosynthesis	$3.89 \times 10^{-3}$
Nitrogen metabolism	$4.74 \times 10^{-3}$
Histidine metabolism	$4.80 \times 10^{-3}$
Alanine, aspartate and glutamate metabolism	0.01
Arginine biosynthesis	0.01
Butanoate metabolism	0.01
Tyrosine metabolism	0.01
Glyoxylate and dicarboxylate metabolism	0.02
D-Glutamine and D-glutamate metabolism	0.03
Glycine, serine and threonine metabolism	0.04

## Conclusions

- Rumen metabolome appears to be more affected by seed type
- A total of 11 rumen metabolites were affected by seed type
- Rumen metabolites are not affected for the interaction of isoflavone x fescue seed type
- Rumen metabolic pathways were not affected by isoflavones treatment

## Acknowledgments

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## References

- 1 Aiken, G. E., M. D. Flythe, I. A. Kagan, H. Ji, and L. P. Bush. 2016. Mitigation of ergot vasoconstriction by clover isoflavones in goats (*Capra hircus*). *Front. Vet. Sci.* 3:17. doi:10.3389/fvets.2016.00017.
- 2 Hannah, S. M., J. A. Paterson, J. E. Williams, M. S. Kerley, and J. L. Miner. 1990. Effects of increasing dietary levels of endophyte-infected tall fescue seed on diet digestibility and ruminal kinetics in sheep. *J. Anim. Sci.* 68:1693–1701. doi:10.2527/1990.6861693x
- 3 Harlow, B. E., M. D. Flythe, and G. E. Aiken. 2017a. Effect of biochanin A on corn grain (*Zea mays*) fermentation by bovine rumen amylolytic bacteria. *J. Appl. Microbiol.* 122:870–880. doi:10.1111/jam.13397.
- 4 Melchior, E. A., J. K. Smith, L. G. Schneider, J. T. Mulliniks, G. E. Bates, M. D. Flythe, J. L. Klotz, H. Ji, J. P. Goodman, A. R. Lee, J. M. Caldwell, and P. R. Myer. 2018. Effects of endophyte-infected tall fescue seed and red clover isoflavones on rumen microbial populations and physiological parameters of beef cattle1,2. *Translational Animal Science* 3(1):315-328. doi: 10.1093/tas/txy147