

# Promote Growth and Animal Health with Isoflavones in Red Clover and other Legumes

**Michael Flythe & Glen Aiken**  
**USDA-ARS Forage-Animal Production Research Unit**  
**Lexington, KY**

## Introduction

The field of nutrition has continued to expand since the 18<sup>th</sup> century. We once thought that the only important components in foods and feeds were proteins, carbohydrates, fats and salts. Evidence was slowly pieced together to show that certain minor components were essential for life, and the vitamins were discovered. Like the doctors that first suspected vitamins were essential, some cattlemen have long noted advantages in animal performance and health on certain diets in ways that cannot be explained by a simple forage analysis. Today, we are learning the roles that phenolic plant secondary metabolites, sometimes called polyphenols, play in both human and animal nutrition. In particular, our USDA-ARS unit is conducting research on a group of polyphenols called isoflavones, which are found in clovers and other legumes.

Isoflavones prevent damage by ultraviolet light in plants. They are also a chemical defense against infection by bacteria and fungi. It has long been recognized that isoflavones also have biological effects on animals that consume the plants. They are antioxidants and estrogens. The estrogenic effects of legumes, well known in ruminants, are due to isoflavones. Much of the early research on isoflavones in ruminant diets is about their negative effects on reproduction. However, new research is showing there are benefits to cattle that consume isoflavones. In this article, we will explore two recently discovered benefits of isoflavones: 1) improved dietary nitrogen efficiency, and 2) improved blood flow during fescue toxicosis.

## Isoflavones as growth promoters

Red clover (*Trifolium pratense*) is a common forage legume in Kentucky and other regions. It is well known that it has high protein content, but red clover is also rich in isoflavones. Recent research has shown that red clover isoflavones can influence the way that protein is utilized. An isoflavone called biochanin A affects digestion in the rumen, but we must first include some background information on rumen function to understand how isoflavones can act as growth promoters.

The rumen is the special digestive organ that allows cattle and other ruminants to utilize feeds that other mammals cannot digest. The bacteria and other microorganisms in the rumen can digest fiber in grasses and other forages, which allows the animal to get energy from this otherwise indigestible feed component. However, the rumen bacteria also have first access to all the other nutrients in the feed. Protein can be broken down to its amino acid-building blocks. Then particular group of bacteria, called Hyper Ammonia-Producing Bacteria, or HAB, can convert the amino acids to ammonia. Some ammonia gets recycled into microbial protein that can be used by the animal, but much of it goes into the blood and is lost in the urine.

The red clover isoflavone, biochanin A, selectively kills the HAB. When the HAB are suppressed, fewer amino acids are converted to ammonia and more of the original feed protein is available for the animal to absorb. Amino acids are a versatile nutrient for cattle. Amino acids can be used for energy, but they are most important as the building blocks for animal tissue. When more of the essential amino acids are available, growth is promoted and the gain-to-feed ratio improves. Growth promotion by suppressing HAB is a common effect of the antibiotic growth promoters that were used in finishing cattle for many years. Thus, biochanin A could be considered an antimicrobial growth promoter, but unlike other growth promoters, it is a natural compound from a well-known forage.

We first discovered the effects of biochanin A on HAB in the laboratory. Since then we have conducted supplemented pasture experiments with purified biochanin A. The supplemented pasture experiments were conducted in the spring (5 May to 7 July) and fall (2 September to 2 November) of 2015. The pastures were cool-season grasses with no legumes. The steers (36 per trial) were on one of three treatments: pasture only, pasture + dried distiller's grains, or pasture + dried distillers grains with biochanin A. The average daily weight gain of calves that received distiller's grains without biochanin A was 13% greater than the pasture only control. The average daily gain of calves that received distiller's grains with biochanin A was 29% greater than the pasture only groups.

Ongoing experiments are exploring a number of questions such as: How do we deliver the biochanin A (e.g. red clover hay, grazing)? Will the isoflavones of other legumes (e.g. white clover, soy) have the same effect? How much biochanin A is required to promote growth? This latter question of dose is critical, and future results will include a precise dose-response. For now, we can say that the amount of biochanin A used in the pasture trials was less than what the calves would receive if the diet were 1/3 red clover. It would be possible for cattle to consume that level of biochanin A from grazing a 20-40% stand of red clover.

### **Isoflavones can promote blood flow**

We now know that isoflavones have immediate effects on rumen bacteria, but what happens to these compounds next? Isoflavones are chemically altered by the rumen bacteria, and then absorbed by the animal. Fortunately, there is human medical research on the effects and fates of isoflavones once absorbed by animals. The antioxidant and estrogenic effects are well studied in humans and in the model species typically used by medical researchers. Medical researchers also showed that biochanin A and other isoflavones can act as vasodilators, *i.e.*, they can cause arteries to increase diameter, which increases blood flow. Medical researchers are interested in vasodilation by isoflavones for a number of reasons ranging from hypertension to migraine headaches, but those of us in agriculture have reasons to be excited about vasodilation, too.

It is possible that the vasodilation properties of isoflavones can mitigate fescue toxicosis. Tall fescue, the predominant cool-season grass in Kentucky and surrounding States, is well known for a fungal endophyte that lives in its tissues. The endophyte harbored in the common tall fescue cultivar (Kentucky 31) produces ergot alkaloids, which cause vasoconstriction, the constriction of blood vessels. Vasoconstriction impedes blood flow, heat dissipation and leads to the health problems and production slumps called fescue toxicosis. Because isoflavones have been

indicated to dilate arteries in human, we believed that the isoflavones in clover could explain the long-standing observation that the effects of toxic tall fescue are not as severe when there is clover in the pasture.

Goats in a controlled environment were used to test the interaction of toxic ergot alkaloids and isoflavones. The goats (6) were dosed through rumen cannula with an ergot alkaloid-rich fescue seed extract. The fescue extract caused vasoconstriction in the carotid artery and the interosseous artery (in the forelimb). When the red clover extract was also added through the rumen cannula, artery diameters returned to normal in less than 4 days. Another experiment was performed in which goats received both red clover extract and fescue seed extract. The arteries did not constrict. In fact, the carotid arteries were dilated relative to normal by the 3<sup>rd</sup> day of treatment. The red clover extract was withdrawn, but the goats continued to receive fescue seed extract. Both types of arteries were constricted in less than 2 days after the clover extract was withdrawn.

These experiments show that red clover isoflavones can reverse and prevent vasoconstriction from toxic tall fescue in a controlled laboratory setting. Moreover, it explains the effect of clover in toxic fescue pastures that has previously been attributed entirely to “dilution” of the alkaloids by giving the cattle another forage to selectively graze. It is still unclear which isoflavones are most active in vasodilation because the red clover extract was used rather than a purified compound, like biochanin A. It is also important to determine if isoflavones from other legumes can be used to mitigate fescue toxicosis. In previous experiments, soy hull supplementation improved performance of cattle on toxic tall fescue. The improvement could be due to isoflavones in the soy hulls, particularly because performance was improved even more when soy hull supplementation was combined with estradiol ear implantation. The estrogenic activity in the blood was also greatest when steers received both the estradiol and the isoflavone-containing soy hulls.

## **Final Thoughts**

Our research is providing evidence that isoflavones can inhibit HAB in the rumens of cattle that are responsible for breaking down amino acids. As a result, the utilization of protein is improved for more efficient weight gain. Further, the isoflavone, biochanin A, was determined to improve blood flow in ruminants exposed to toxic ergot alkaloids and, therefore, mitigate the effects of fescue toxicosis. More research is needed to further verify the benefits of biochanin A produced by red clover, and to evaluate the impacts of other isoflavones on cattle weight gain performance and well-being.

## References

Semba, R.D. The Discovery of the Vitamins. *Int. J. Vitam. Nutr. Res.*, 82 (5), 2012, 310 – 315

Flythe, M. and Kagan, I. Antimicrobial effect of red clover (*Trifolium pratense*) phenolic extract on the ruminal hyper ammonia-producing bacterium, *Clostridium sticklandii*. *Current Microbiology*. 61:125-131. 2010.

Flythe, M.D., Harrison, B., Kagan, I.A., Klotz, J.L., Goff, B.M. and Aiken, G.E. Antimicrobial activity of red clover (*Trifolium pratense* L.) extract on caprine hyper ammonia-producing bacteria. *Agricultural, Food & Analytical Bacteriology*. 3 (3):176-185. 2013.

Harlow, B.E., Flythe, M.D., Kagan, I.A., Aiken, G.E. Biochanin A, an isoflavone produced by red clover, promotes weight gain of steers grazed in mixed grass pastures and fed dried-distillers' grains. *Crop Science*. 57:1-9

Aiken, G.E., Flythe, M.D., Kagan, I.A., Ji, H. and Bush, L.P. Mitigation of ergot vasoconstriction by clover isoflavones in goats (*Capra hircus*). *Frontiers in Veterinary Science*. 3:17:1-10. 2016.

Shappell N.M., Flythe M.D. and Aiken G.E. The effects of steroid implant and dietary soybean hulls on estrogenic activity of sera of steers grazing toxic endophyte-infected tall fescue pasture. *Frontiers in Veterinary Science*. 2:30. 2015.