The Secret Ingredients of Clover: Biochanin a and Isoflavonoids

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Functional Feeds

It is well known that the value of clover is in nitrogen. Clovers fix their own nitrogen so that an input of nitrate fertilizer is not required. Furthermore, they are rich in protein-nitrogen, and can be used to meet the protein requirements of ruminants. Clovers also contain a class of chemicals called isoflavones, and we have recently discovered evidence that the isoflavones positively influence the way that ruminants digest protein.

Many members of the legume family (*Fabaceae*) produce natural products called isoflavones. They are well known for being biologically active because they are chemically similar to the hormones called estrogens. Most of the older research on isoflavones has been about the negative effects on reproduction in specific circumstances. For example, a large proportion of red clover in the diet has interfered with the estrous cycle in sheep, and reduced lambing rates, in some cases. This same estrogenic property has been exploited in red clover supplements that reduce the symptoms of menopause in humans. Soy isoflavones are also of interest in human medicine. There is evidence that hypertension can be reduced by the isoflavones in soy-based foods.

Human nutritionists call foods with medicinal value, like soy, “functional foods”. We can borrow that term to consider whether legume forages, such as red clover, have value as “functional feeds”. Cattle often receive drugs of different types at different stages of the production system. Vaccines and anthelmintics are given to young calves, while steroid implants, beta-agonists and antibiotics can be used in backgrounding and finishing. These drugs have revolutionized modern production systems, but they have been criticized. The use of antibiotics for growth promotion has been especially criticized because of possible contributions to antibiotic resistance in bacteria. Therefore, we explored the idea that red clover could be used as a functional feed to achieve the same benefit as antibiotic growth promoters.

Antimicrobial Growth Promoters and a Plant-Based Antimicrobial

To evaluate chemicals from clover as antimicrobials, we first need to understand how antibiotic growth promoters work. Chemicals like tylosin, lasalocid and monensin are antibiotics, that is, they are made by microorganisms to kill other microorganisms. The rumen is densely populated with many types of bacteria and other microorganisms. Some bacteria, like those that break down fiber, are very important to the animal. Others carry out wasteful processes, such as

methane production and protein degradation. The steps in rumen protein degradation are shown in Figure 1. Plant protein is consumed; it is broken down into amino acids by one group of microorganisms; then another converts the amino acids into ammonia. Some of the ammonia gets recaptured into microbial protein by beneficial bacteria, but much of it is transported into the blood and is lost in the urine. Protein and amino acids that survive the rumen are called "bypass protein", and can be absorbed by the animal in the lower digestive tract. A group of rumen bacteria called the Hyper Ammonia-producing Bacteria (HAB) convert most of the feed amino acids into ammonia. Antibiotics like monensin kill HAB, which increases bypass protein, feed efficiency and weight gain.

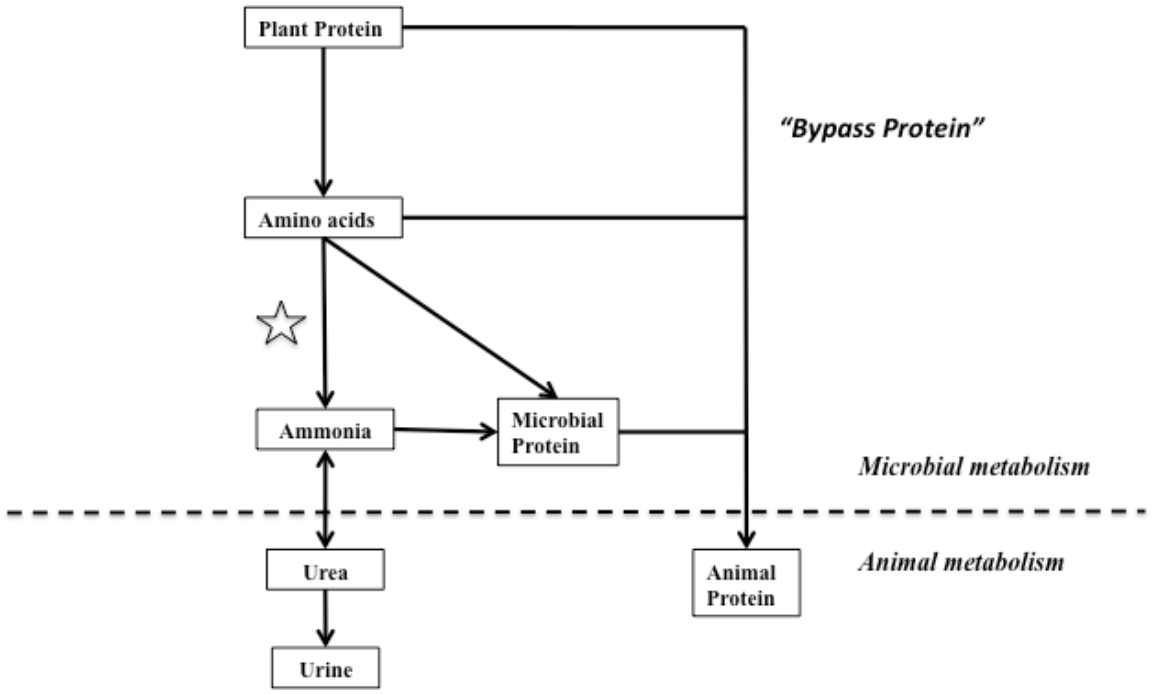


Figure 1. Protein Metabolism in the Rumen. The steps carried out by the ruminant animal are below the dotted line. The steps carried out by the rumen microorganisms are above the dotted line. The star shows the activity of the Hyper Ammonia-producing Bacteria (HAB), which are inhibited by both antibiotic growth promoters and by the red clover isoflavone, biochanin A.

We began by extracting a mixture of isoflavones and similar natural products from red clover (cultivar Kenland). The extract was added to pure cultures of rumen HAB. It was determined that the extract could prevent the growth and ammonia production by the HAB. This result indicated that red clover contained at least one compound that had the desired antimicrobial property. The natural products in the extract were chemically separated and screened for activity using the pure HAB culture. The compound that prevented growth of the HAB was identified as an isoflavone called biochanin A.

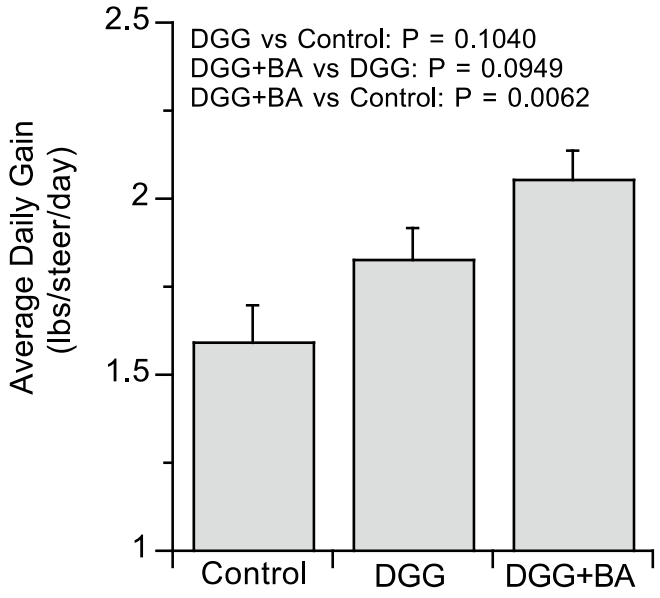
Next we tested both the red clover extract and pure biochanin A on natural rumen bacteria. Instead of using a pure HAB culture, rumen fluid was collected from fistulated goats. When the

bacteria from the rumen fluid were fed amino acids, ammonia was produced. Either the clover extract or biochanin A decreased ammonia production. These results showed that the antimicrobial had the desired effect on a natural mix of rumen bacteria, not just a pure culture, and that biochanin A was the active component.

Field Testing Biochanin A as a Growth Promoter

Once it was determined that the red clover isoflavone, biochanin A, could reduce ammonia production from rumen bacteria by killing the same bacteria as antibiotic growth promoters, then the next step was to test its ability to promote growth. Two feeding trials were carried out (spring and fall 2015). In each trial, 48 Angus cross steers were put on pasture (clover-free, novel endophyte fescue) in one of three groups: pasture only, pasture plus dry distillers' grains or pasture plus dry distillers' grains with added biochanin A. The biochanin A was given at 7 g/head/day, which would be equivalent to the amount of biochanin A in a diet that was approximately 1/3 red clover. The average daily gains were calculated the end of the 63- and 61-day trials (Figure 2). In both cases, the addition of biochanin A improved average daily gain.

Figure 2. Effect of biochanin A (red clover isoflavone) on average daily gain of steers on pasture. There was no effect of season, and the trials were analyzed together. The error bars show standard error. Control is pasture only. DGG is pasture supplemented with dry distiller's grains. DGG+BA is pasture supplemented with dry distiller's grains and biochanin A.



The Unexpected Bonus: Clover Isoflavones to Treat Fescue Toxicosis

The results of the feeding trials can be explained solely by the effect of biochanin A on the activity of rumen bacteria. However, the effects of the isoflavone on the physiology of the animal itself must also be studied. As previously mentioned, the isoflavones are estrogenic, which could affect ruminants in a number of ways. A survey of research done on humans and non-ruminant animals revealed that isoflavones were also used to treat hypertension. Previous researchers showed that biochanin A could dilate a blood vessel (the aorta) in rats. This made us consider the common wisdom about clovers in tall fescue pastures. Fescue toxicosis is caused by toxic alkaloids produced by a fungus that lives in the grass. The alkaloids cause the blood vessels to constrict, which causes many of the negative health effects associated with tall fescue. It has long been held that clovers in the pasture can "dilute" the concentration of alkaloids in the diet; that is, the animals will consume less tall fescue because they also have clover. However, if isoflavones could relax blood vessels in ruminants, then clover might directly counteract blood vessel constriction by fescue alkaloids.

Two experiments were conducted with goats to explore the interaction of fescue alkaloids and clover isoflavones. In the first experiment, six goats were administered toxic tall fescue seed and red clover extract together for 4 days. The carotid artery, in the throat, and left recurrent interosseous artery, in the forelimb, were observed with ultrasonography. The luminal areas (size of cross-section of the vessel) were not different from goats receiving only orchardgrass hay. Then the red clover extract was taken away, and the goats only received fescue seed. The blood vessels constricted within 48 hours. In the second experiment, the goats received only fescue seed initially, and the vessels were very constricted. When the goats began receiving red clover extract with the fescue seed, the vessels began to open up and were normal by 5 days of treatment. These results demonstrate that clover isoflavones can counteract the toxic effect of tall fescue on blood vessels.

Conclusions

These results may explain many of the benefits seen in animals grazing clovers. It is well known that clovers fix their own nitrogen and convert the nitrogen to valuable protein for grazing ruminants. Now there are reasons to believe that the isoflavones in clover also improve utilization of the protein and promote weight gain by influencing rumen bacteria in a manner similar to antibiotic growth promoters. The discovery that clover isoflavones can counteract at least one of the negative effects of toxic tall fescue gives us another reason to maintain clovers in the regions where this forage grass is prevalent. Isoflavones are estrogenic, which means that they have to be carefully used. For example, different feeding levels might be recommended for backgrounding and finishing beef versus pre-weaning or in dairy production. There are also special considerations for sheep, which are believed to be particularly sensitive to reproductive effects of plant estrogens. Clearly, more research is needed on the biologically active chemicals made by forage legumes, as well as how to best utilize them as "functional feed" in each industry supported by forage. However, the above results indicate that this research is well worth pursuing.

References

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