Isoflavones in legumes as functional forages and feeds in ruminant grazing systems.

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Keywords: Legume; red clover; isoflavone; fescue toxicosis; antimicrobial

Abstract. Legumes are often utilized in livestock grazing systems to improve diet quality, diversity, and consequently animal performance. In addition to their nutritional utility, legumes contain phytoestrogenic isoflavones that have selective antimicrobial activity in the rumen and once absorbed can cause arterial vasodilation. These added bioactive functions of isoflavones suggest that legumes could be strategically utilized in grazing systems as functional forages to improve ruminant performance and health. The objective of this presentation will be to discuss past and current research on the application of isoflavones via legumes as an antimicrobial for growth promotion and as a mitigation strategy for fescue toxicosis in grazing ruminants.

Introduction

The term "functional foods" has been coined in human nutrition and defined as foods or food ingredients that contain bioactive compounds that provide health benefits beyond basic nutrition (Domínguez Díaz *et al.* 2020). The concept of "functional feeds," has also been extended to applications in animal health and nutrition specifically as it relates to increasing efficiencies of animal production (Harmon 2020). Functional feeds often comprise a relatively small proportion of the diet but offer potential benefits for animal health, production, and the environment beyond what can be achieved with conventional feedstuffs. While functional feeds in animal nutrition are often non-traditional feed sources (or derived extracts; Lillehoj *et al.* 2018), more common animal feedstuffs or forages may also contain bioactive phytochemicals that would provide similar benefits (Hoste *et al.* 2015; Harlow *et al.* 2020).

Plants, including those present in ruminant grazing systems, can produce a plethora of plant secondary metabolites that confer several necessary functions to the plant itself including those related to defense (herbivory, pathogens, and other plants), attraction/stimulation (pollination, seed dispersal, nutrient acquisition), and protections (UV, drought, temperature, water holding capacity; Hartmann, 1996; Clemensen *et al.* 2020). When consumed, plant secondary metabolites in turn can have both positive and negative effects on grazing animals. For example, alkaloids can be toxic to grazing ruminants (Strickland *et al.* 2011), but when consumed in combination with either condensed tannin (Villalba *et al.* 2011) or isoflavone secondary metabolites (Aiken *et al.* 2016; Harlow *et al.* 2021) those negative impacts can be mitigated. Gaining a better understanding of the phytochemical landscape and diversity present in pastures and plant secondary metabolite complentaries is essential for not only improving livestock health and performance but also ensuring sustainability of grazing systems.

Legumes are conventionally incorporated in livestock productions systems to improve diet quality and diversity. Legumes can also produce isoflavones (most notably Biochanin A) that once consumed act as selective rumen antimicrobials improving animal growth performance (Harlow *et al.* 2017, 2020). After absorption, isoflavones can also have phytoestrogenic activity that cause arterial vasodilation via nitric oxide synthase activation (Teede *et al.* 2003; Aiken *et al.* 2016). These diverse benefits of isoflavones on animal physiology and efficiency suggest that legumes could be considered "functional forages." The objective of this presentation will be to discuss past and current research on the potential applications of isoflavones via legumes as functional forages and feeds in grazing ruminant production systems.

Legumes as Selective Rumen Antimicrobials

Dietary protein supplementation is often used to enhance the nutritional status and overall performance of grazing ruminant livestock. Grazing ruminants require some rumen degradable protein to maximize the ability of their rumen microbial community to catabolize cellulose, but when production exceeds utilization, excess ammonia is wasted via the urine (Satter and Slyter 1978). Excess loss of ammonia is predominately caused by the activity of hyper ammonia-producing bacteria (HAB), which break down dietary amino acids and convert them into ammonia (Chen and Russell 1989). Therefore, strategies to selectively reduce HAB,

minimize rumen protein degradation and in turn increase rumen bypass protein available to the animal for the production of milk and meat are of interest. There are several methods that can be used to increase rumen bypass protein includng feed processing and the use of selective rumen antimicrobials (*e.g.*, ionophores; Russell and Strobel 1989). Feed additive ionophores (e.g., monensin, virginiamycin, and lasalocid) are often employed in ruminant livestock production to reduce methane, increase rumen bypass protein by inhibiting HAB, and consequently improve feed efficiency and growth performance (Potter *et al.* 1976; Wedegaertner and Johnson 1983; Russell and Strobel 1989). Despite these benefits, there is increasing social and legal pressure to ban and impose greater regulation on growth promoters that are categorized as antibiotics (*i.e*., an antimicrobial of microbial origin) to reduce the spread of antimicrobial resistance. In addition to regulatory hurdles, another marked disadvantage of conventional feed antimicrobials is that they have a broad spectrum of activity that can include rumen cellulolytic bacteria that catabolize fiber and are essential for utilization of high forage diets. For these reasons, discovery or development of novel sources of selective antimicrobials for use in grazing ruminant systems specifically are of great interest (Russell and Houlihan 2003).
Many plant species, including forage species in pastures produce selective antimicrobial, plant secondary metabolites, that can have beneficial effects on rumen fermentation (Evans and Martin 2000; Flythe 2009; Flythe and Kagan 2010). Biochanin A, an isoflavone produced by forage legume red clover (*Trifolium pratense*), has been shown to selectively inhibit HAB and promote cellulolytic bacteria and fiber fermentation *in vitro* and *ex vivo* (Flythe and Kagan 2010; Flythe *et al.* 2013; Harlow *et al.* 2017; Harlow *et al.* 2018). These selective rumen antimicrobial impacts have been demonstrated to improve growth performance in steers grazing mixed grass pastures when supplemented as either purified biochanin A (Harlow *et al.* 2017) or red clover hay (Harlow *et al.* 2020), with 15% w/w inclusion in the diet being most effective (+25 – 30% average daily gain). Similar results were observed when incorporating red clover hay into finishing diets of ram lambs including reduced HAB, increased cellulolytic bacteria, and consequently a 25% increase in feed efficiency at both 15 and 7.5% w/w inclusion rates (Weinert-Nelson *et al.* in review). Future research is needed to evaluate lower levels of isoflavone supplementation in grazing ruminants to identify optimal supplementation strategies.

Legumes as Vasodilators

Tall fescue (TF) is the most prevalent cool-season perennial grass in the US (Thompson *et al.* 2001). The majority (>90%) of TF is infected with an ergot alkaloid-(predominantly ergovaline; Lyons *et al.* 1986) producing fungal endophyte (E+ TF) that when chronically consumed result in a syndrome known as fescue toxicosis. Ergot alkaloids bind vascular receptors causing persistent vasocontriction to peripheral tissues that can result in reduced feed intake, weight gain, reproductive performance, milk production, and hyperthermia (Strickland *et al.*, 2011). It is speculated that fescue toxicosis contributes to over $2 billion in annual economic losses to US livestock industries (Kallenbach *et al.* 2015).
Including legumes in E+ TF grazing systems either by inter/over-seeding (e.g., clovers) or feed supplementation (e.g., soyhulls, soybean meal), has long been recommended to mitigate performance losses and the negative health impacts of fescue toxicosis. Historically, these benefits have been attributed to improvement of diet intake and quality or "dilution" reducing the total consumption of ergot alkaloids (Ball 1984; Fribourg *et al.* 1981; Roberts and Andrae 2004). In addition to nutritional benefits, legumes contain biologically active isoflavones that can induce arterial vasodilation post-absorption, preventing and reversing fescue toxicosis (Aiken *et al.* 2016). Several routes of legume supplementation have been successfully utilized to mitigate fescue toxicosis in ruminants including: overseeding clovers, stored forages, legume-based feeds, and by-products (e.g., soybean meal; soyhulls; Carter *et al.* 2010; Shappell *et al.* 2015; Harlow *et al.* 2021, 2022). It has also been demonstrated that conventional loose mineral amended with red clover leaf (20% w/w) is readily consumed by cattle and can ameliorate and prevent the adverse effects of fescue toxicosis, most notably peripheral vasoconstriction and prolactin depression in both pen (Davis *et al.* accepted) and field studies (Davis unpublished). When heifers grazing E+ TF pastures were supplemented with red clover leaf containing loose mineral (20% w/w) they had reduced hair coat retention/re-growth, vasoconstiction, and heat stress (as evidenced by ~15% lower respiration rates). Furthermore, despite the phytoestrogenic activity of isoflavones, red clover leaf containing mineral had no apparent negative effects on heifer conception rates.

Conclusions and/or Implications

Low levels of isoflavone supplementation can produce ruminant livestock health and production benefits consistent with categorization of red clover and other legumes as functional forages or feeds in addition to their traditional use as feedstuffs. Isoflavones have demonstrated utility as a superior alternative to conventional antimicrobial growth promoters and as vasodilatory agents in the treatment/prevention of fescue toxicosis. Future research is required to determine the optimum concentrations and composition of isoflavones required to elicit these physiological and production benefits across multiple species and grazing systems. Although, it is tempting to claim isoflavones are a panacea; a natural all-curing medicine for a variety of maladies and challenges faced by grazing ruminants, there is another possible perspective to consider. Throughout their evolutionary history ruminant species have migrated and grazed a variety of diverse plants and it is reasonable to assume that they were exposed to a phytochemical landscape that is comparatively underrepresented in modern diets. Therefore, it should not surprise us that plant secondary metabolites, like isoflavones, play undiscovered roles in ruminant nutrition.

Acknowledgements

The authors acknowledge the technical assistance of Jessica Ferrell, the assistance with sample analyses and characterization provided by John May, Jack Goodman and Huihua Ji, and the animal husbandry assistance of Matthew Hamilton, LeeAnn Jacks, and Derrick Wise. The authors would also like to acknowledge the collaboration and support of this research by Kentucky Beef Network, Eden Shale Farm, and Burkmann Nutrition.

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