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Evaluation of the Relationship Between Condensed Tannin Content and Rumen Protein Degradability in Sainfoin (*Onobrychis viciifolia*)

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Abstract

Condensed tannins, naturally occurring polyphenolic compounds present in certain legumes such as sainfoin (*Onobrychis viciifolia*), can bind dietary proteins at ruminal pH levels. These tannin-protein complexes resist microbial degradation in the rumen but dissociate in the acidic environment of the abomasum, thereby enhancing amino acid absorption in the small intestine. This study aimed to evaluate the relationship between condensed tannin content and ruminal protein degradability in sainfoin, with a focus on the potential role of condensed tannins in protecting dietary proteins from microbial breakdown. Twenty-five sainfoin samples, harvested as green forage from various regions of Türkiye at 50–100% flowering stage, were used in this study. Crude protein content was determined according to the AOAC (2003) method 954.01, while condensed tannin content was measured using the hydrochloric acid (HCl)-butanol assay. Effective protein degradability of the samples was assessed using the *in situ* nylon bag technique, following the method of Ørskov and McDonald (1979). The effective protein degradability values, calculated at a ruminal outflow rate of k = 0.06 h^{-1}, ranged from 53.7% to 62.5%, indicating variability in protein degradation characteristics among the samples. Correspondingly, condensed tannin content ranged from 34.2 to 50.9 g kg^{-1} dry matter. Regression analysis revealed a strong negative linear relationship between condensed tannin concentration and effective protein degradability, with a coefficient of determination (R^2) of 0.80. These findings suggest that condensed tannins play a significant role in reducing ruminal protein degradation by forming stable tannin-protein complexes, thereby enhancing the potential for feed proteins to bypass the rumen and be absorbed in the small intestine as undegraded protein.

Key Words: *Sainfoin, Condensed tannins, Protein, Rumen degradability*

Introduction

Tannins are phenolic compounds known for their strong ability to bind proteins and other macromolecules, forming stable complexes (Mangan, 1988). By protecting dietary proteins from microbial breakdown, these complexes reduce both the rate and extent of protein degradation in the rumen (McSweeney et al., 1988; Min et al., 2001). Tannins bind forage proteins at the neutral pH of the rumen, forming complexes that resist microbial enzymatic activity. These complexes then dissociate in the acidic environment of the abomasum, releasing proteins for digestion and thus enhancing nitrogen utilization efficiency (Jones & Mangan, 1977; Waghorn & Shelton, 1995; Hudson, 2001).

Tannins are typically classified into two main groups: hydrolysable tannins (HT) and condensed tannins (CT). Condensed tannins, also known as proanthocyanidins, are polymers of flavan-3-ols that yield colored anthocyanidins when treated with acidified alcohol (Mueller-Harvey & McAllen, 1992). While HT can be toxic to ruminants, CT are generally considered non-toxic or less harmful due to their poor absorption in the gastrointestinal tract.

Forage legumes containing CT have been shown to stabilize dietary proteins in the rumen by reducing proteolysis, thereby allowing more intact protein to bypass the rumen and reach the abomasum (Kingston-Smith et al., 2004). This bypass protein, also known as rumen undegraded protein (RUP), is then digested and absorbed in the small intestine. Differences in protein degradation rates among legume species have been used to assess the impact of tannins on ruminal proteolysis (Broderick & Albrecht 1994). In CT-containing forage plants, protein–tannin complexes are formed during mastication, limiting microbial access to plant proteins. These complexes resist microbial degradation in the rumen and pass intact to the abomasum, where they dissociate at low pH, making the proteins available for digestion and absorption in the small intestine (Woodward, 2000).

Sainfoin (*Onobrychis viciifolia*) is among the most prominent CT-rich forage legumes. Due to its CT content, sainfoin typically shows lower ruminal protein degradability than other forages, such as alfalfa (Van Soest, 1994). In sainfoin, CT are distributed across most plant tissues except for the cotyledons and roots, with the highest concentrations found in the leaves (Less et al., 1993; 1995). Concentrations of CT ranging from 5% to 8% of dry matter have been reported to reduce protein solubility and limit degradation in the rumen (Waghorn et al., 1998).

Tannin-rich legumes reduce ruminal protein degradation and improve nitrogen utilization efficiency (Reid et al., 1974; Egan & Ulyatt, 1980). For example, CT in sainfoin and birdsfoot trefoil have been associated with improved protein metabolism and digestibility in ruminants (Reed, 1995). Thomson et al. (1971) reported greater protein utilization from dried sainfoin compared to alfalfa, attributing this to its tannin content. As such, sainfoin is

recognized as a valuable source of bypass protein. Its low ruminal protein degradability enables more protein to reach the small intestine intact for digestion and absorption (Wilkins & Jones, 2000).

Multiple studies have demonstrated that CT in sainfoin reduce ruminal protein breakdown, lower ammonia concentrations, and increase the flow of bypass protein and amino acids to the duodenum (Hart & Sahlu, 1993; Broderick & Albrecht, 1997; McMahon et al., 2000; Broderick, 2001; Wang et al., 2003). The inhibitory effect of CT on ruminal protein degradation increases proportionally with tannin concentration (Broderick & Albrecht, 1997), and has been associated with residual dry matter (DM) and crude protein (CP) after incubation (Julier et al., 2003). Protein degradability in buffer and *in vitro* systems has shown a strong negative correlation with tannin levels (Hedqvist, 2004). In sainfoin silage, higher CT levels have significantly reduced total tract nitrogen digestibility and *in situ* nitrogen degradability in sheep, especially in early-flowering silages (Theodoridou et al., 2012). A significant negative relationship between CT content and effective protein degradability (DE) has also been reported (Azuhnwi et al., 2012), with CT concentrations being negatively correlated with rapidly degradable fraction (a) and degradation rate of the slowly degradable fraction (c) (Aufrère et al., 2014). Similarly, increased CT levels in early-stage sainfoin were found to reduce ruminal CP degradability and nitrogen digestibility (Özbilgin, 2019).

Accordingly, the aim of this study was to evaluate the relationship between CT content and DE in sainfoin samples collected from different regions of Türkiye during the same vegetative stage. In this context, the hypothesis of the study was that naturally varying levels of CT in sainfoin samples grown in different regions would have a significant effect on ruminal protein degradability, and that increasing tannin content would lead to decreased protein degradability.

Materials and Methods

Experimental forages and chemical analyses

In the present study, twenty-five samples sainfoin (*Onobrychis viciifolia*) were harvested as green forage from various regions of Türkiye, were used. The samples were harvested at 50-100% flowering period. Forage samples collected were dried in an air-forced oven at 60°C for at least 72 hours until a constant weight was achieved, then ground to pass through a 1-mm screen using a Retsch mill (Retsch GmbH, Haan, Germany) and analyzed for DM (930.15), and CP (954.01) according to AOAC (2003) methods. The CT content was determined by the hydrochloric acid (HCl)-butanol assay (Reed, 1986).

In situ disappearance in the rumen

The ruminal degradation of CP was evaluated using the nylon bag technique. Dacron bags (Ankom Co., Macedon, NY, USA), with a pore size of 53 ± 15 µm and an internal surface area of 5 × 11 cm, were heat-sealed after being filled with approximately 4 g of air-dried sainfoin samples (1 mm mesh) collected at the 50–100% flowering stage. These bags were incubated in the rumen of three cows maintained on a diet consisting of 70% hay and 30% concentrate (DM basis). *In situ* degradation was measured at incubation times of 4, 8, 16, 24, 48, 72, and 96 h. Dehydrated lucerne samples, used as a standard, were incubated in duplicate in the rumen of each cow to monitor potential changes in ruminal degradation activity throughout the experiment. After removal from the rumen, the bags were thoroughly rinsed and stored at −20°C until further analysis. Prior to analysis, they were thawed and washed in a household washing machine with cold water (three cycles of 10 minutes each) until the rinse water became clear. Finally, the bags were dried at 60°C for a duration of 72 hours.

In situ CP rumen disappearance curves of samples were fitted to the model of Ørskov & McDonald (1979) using a non-linear regression procedure (SAS, 2000):

$$P = a + b\,(1 - e^{-ct})$$

where P is the potential degradable, a is the rapidly degradable fraction, b is the slowly degradable fraction, e is the base of the natural logarithm, c is the degradation rate of the slowly degradable fraction, and t is the incubation time. The residual fraction was calculated as $(100 - a - b)$.

The effective protein degradability (DE) was estimated using the following equation:

$$DE = a + bc / (c + kp)$$

where kp, the fractional passage rate, was assumed to be 0.06/h.

Statistical analysis

A simple linear regression analysis was conducted using SAS Version 8.0 Statistical Package (SAS Institute Inc., Cary, NC, USA) to determine the relationship between content of CT and DE.

Results and Discussion

The distribution of DE values of sainfoin samples is presented in Figure 1. The effective protein degradability values of samples, calculated at a ruminal outflow rate of k = 0.06 h^{-1}, ranged from 53.7% to 62.5%, with a mean of 58.1%. The majority of the values were clustered within the 56% to 60% range, indicating that the DE of sainfoin was minimally affected by environmental factors.

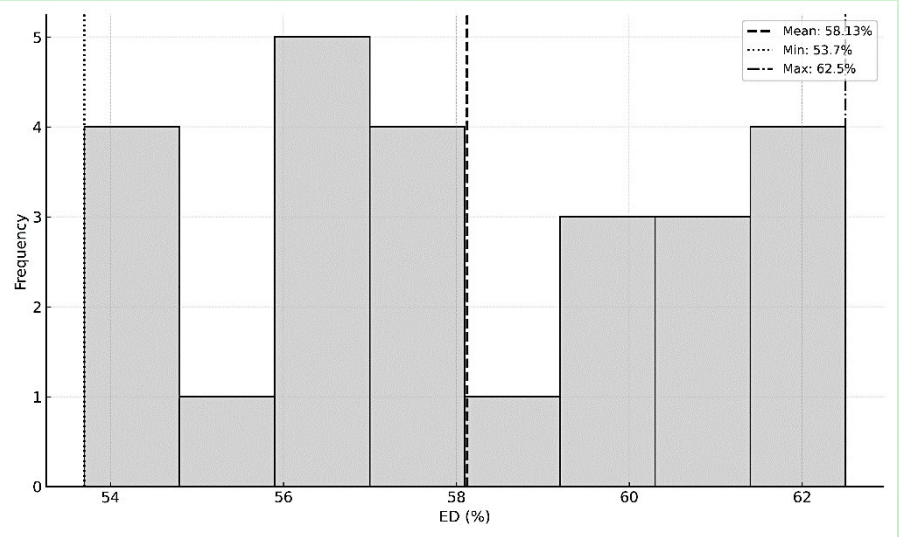

Figure 1. Distribution of effective protein degradability values of sainfoin

These results indicated that sainfoin generally exhibited a moderate level of ruminal protein degradability. In various studies, the DE of sainfoin has typically been reported within the range of 53% to 61%, which is lower than that of more commonly used forage legumes such as alfalfa (Ocak, 1997; Sarıçiçek & Kılıç, 2002; Hanoglu, 2004, Azuhnwi et al., 2012; Aufrère et al., 2014). This difference is primarily attributed to the presence of CT in sainfoin, which are known to form stable complexes with proteins. These complexes limit microbial proteolysis in the rumen and promote the transfer of undegraded protein to the abomasum and small intestine, thereby enhancing bypass protein availability (Jones & Mangan, 1977; Waghorn et al., 1987; McSweeney et al., 1988). Aufrère et al. (2014) reported that tannin-rich forages such as sainfoin exhibit significantly lower DE values compared to tannin-free species, a difference attributed to the interaction between CT and the molecular structure of plant proteins.

Effective protein degradability can be influenced by various factors such as plant species (Turgut & Yanar, 2004), growth stage and harvest time (Aufrère et al., 2014), and forage structure and chemical composition (Broderick & Albrecht, 1997). However, since all sainfoin samples used in this study belonged to the same cultivar and were harvested at similar developmental stages, the DE values exhibited only limited variation.

The distribution of CT content in sainfoin is presented in Figure 2. Condensed tannin concentrations varied between 34.2 and 50.9 g·kg^{-1} DM, averaging 43.0 g·kg^{-1} DM. The data demonstrate a narrow variability, suggesting that most samples had similar CT levels within the given range. Tannin levels in plants can differ substantially not only between species but also among tissues and developmental stages within a species. Their concentrations are modulated by various environmental factors such as drought, soil nutrient status, pH, herbivore pressure, ozone exposure, and atmospheric CO_2 levels (Kraus et al., 2003). In line with this, CT levels in sainfoin have also been shown to vary depending on the plant variety, the location where it is grown, and the timing of harvest (Azuhnwi et al., 2011).

Azuhnwi et al. (2012) reported that CT contents in different sainfoin varieties ranged from 26.6 to 57.3 g·kg^{-1} DM, with this variation being largely attributed to genetic differences, while environmental influences were considered limited. Aufrère et al. (2014) reported CT contents ranging from 35.5 to 48.6 g·kg^{-1} DM across three sainfoin cultivars grown under the same conditions, suggesting that variation was primarily attributable to genetic differences rather than environmental factors. Theodoridou et al. (2012) reported CT concentrations ranging from 33.1 to 51.4 g·kg^{-1} DM in sainfoin silages, and found that both the CT content and its biological activity were affected by the phenological stage at harvest. Özbilgin (2019) reported that CT content in sainfoin increased with advancing maturity, with the highest levels observed at full flowering and the lowest at the budding stage.

The relationship between CT content and DE in sainfoin samples is presented in Figure 3. The derived linear regression equation is:

$y = -0.54x + 81.42$

where y is effective protein degradability, x is condensed tannin content.

This equation indicates that for every 1 g·kg^{-1} DM increase in CT content, the DE value decreases by approximately 0.54 percentage points. This result highlights the inhibitory effect of tannin compounds on protein degradability. The coefficient of determination was calculated as $R^2 = 0.80$, $P = 0.001$, suggesting that 80% of the variation in DE can be explained by differences in CT levels. This high R^2 value demonstrates that the regression model provides a strong fit to the data. Furthermore, the distribution of data points closely around the regression line supports the appropriateness of a linear model. These findings confirm a strong and statistically meaningful inverse relationship between CT content and DE in sainfoin.

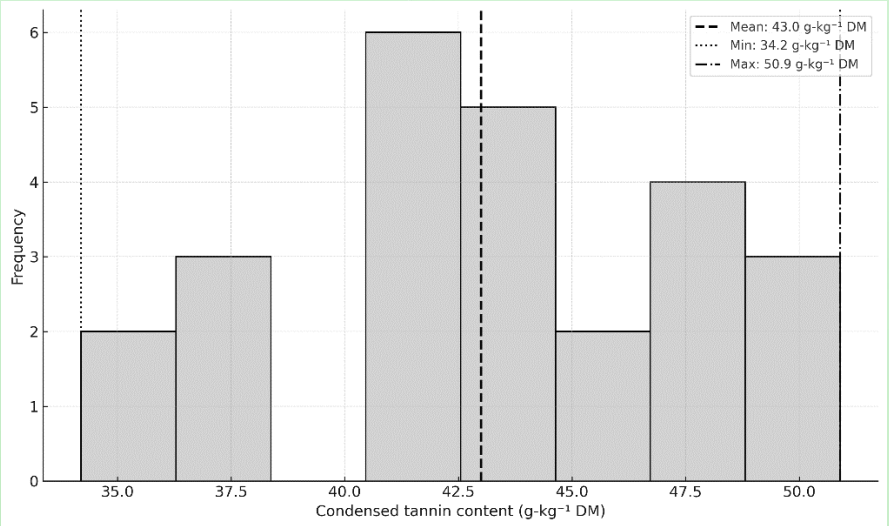

Figure 2. Distribution of condansed tannin content of sainfoin

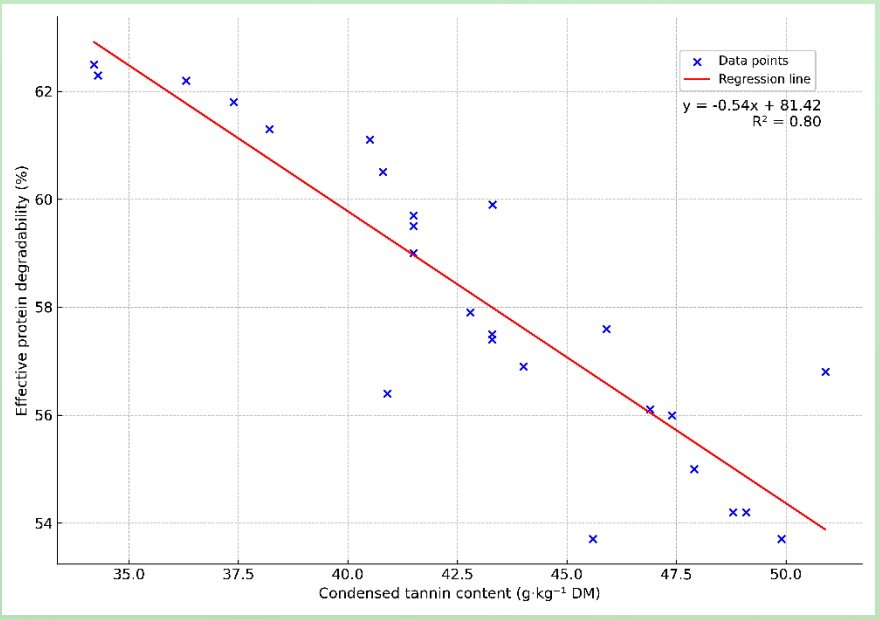

Figure 3. Relationship between condensed tannin content and effective protein degradability in sainfoin

Similarly, the literature clearly demonstrates that CT have a direct inhibitory effect on protein degradability in the rumen. Tannins form strong complexes with proteins under rumen pH, making them resistant to microbial degradation; these complexes then dissociate in the acidic environment of the abomasum, rendering the proteins digestible and thereby increasing the proportion of by-pass protein (Barry et al., 1986; Mangan, 1988; McSweeney et al., 1988; Waghorn et al., 1987, 1994; McNabb et al., 1996; Beck, 2001; Min et al., 2001, 2002).

Azuhnwi et al. (2012) reported that as the CT content increased in sainfoin, the DE significantly ($R^2 = 0.36$, $P = 0.003$) decreased. They noted that this relationship was generally linear and that the reduction in protein degradability was more pronounced in samples from the second harvest. Aufrère et al. (2014) reported a strong negative correlation ($r = -0.81$) between ruminal nitrogen degradability and CT content. Condensed tannins was shown to inhibit both the rapidly degradable nitrogen fraction (a) and the degradation rate of the slowly degradable fraction (c). These results indicate that CT can reduce the rate of protein breakdown in the rumen and potentially enhance the proportion of by-pass protein. Similar findings were reported by Theodoridou et al. (2012), clearly demonstrating that CTs in sainfoin silage have a direct inhibitory effect on protein digestibility.

Conclusion

This study revealed a statistically significant negative relationship between CT content and DE in sainfoin. The increase in CT levels was associated with reduced ruminal protein degradability, indicating that sainfoin can be considered a valuable forage source in terms of bypass protein. However, excessive levels of condensed tannins

may have adverse effects on animal performance and should be carefully evaluated. Therefore, as long as CT content remains within nutritionally appropriate limits**,** sainfoin not only enhances protein utilization efficiency in ruminant nutrition but also stands out as a promising forage crop that supports sustainable livestock production systems thanks to its drought tolerance and adaptability to environmental stress under the challenges of climate change.

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