The effect of leguminous leaf meal substitution as a tannin source in complete feed on Rumen Degradable Protein (RDP), Rumen Undegradable Protein (UDP), and Digestible Energy (DE) using *in vitro* analysis

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Abstract. This study aimed to assess the impact of substituting concentrate with leguminous leaf meal as a source of tannins in complete feed on Rumen Degradable Protein (RDP), Rumen Undegradable Protein (UDP), and Digestible Energy (DE) content in vitro. The complete feeds were formulated using ingredients such as corn stover, soybean meal, copra meal, cassava meal, rice bran, pollard, molasses, minerals, calliandra meal, gamal meal, and indigofera meal. The experiment included ten treatments, each replicated three times. The treatments were as follows: T1 complete feed (60% corn stover + 40% concentrate), T2 complete feed (60% corn stover + (35% concentrate + 5% calliandra meal), T3 complete feed (60% corn stover + (35 % concentrate) + 5% indigofera meal), complete feed T4 (60% corn stover + (35% concentrate + 5% gamal meal), T5 complete feed (60% corn stover + (30% concentrate + 10% calliandra meal), T6 complete feed (60% corn stover + (30% concentrate + 10% indigofera meal), complete feed T7 (60% corn stover + (30% concentrate + 10% gamal meal), complete feed T8 (60% corn stover + (25) % concentrate + 15% calliandra meal), complete feed T9 (60% corn stover + (25% concentrate + 15% indigofera meal), complete feed T10 (60% corn stover + (25% concentrate + 15% gamal meal). The conclusion of this study reveals that the addition of 15% calliandra meal reduced RDP by 46.56% and increased UDP by 53.44%. Furthermore, the inclusion of 15% gliricidia meal led to an increase in DE value by 3.46 MJ/KgDM.

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1 Introduction

Ruminant livestock are typically provided with forage, which is animal feed characterized by low protein content, ranging from 7-12% (Sutardi, 1981; Ensminger, 2001), but it contains a high level of crude fiber exceeding 18%. Corn plantations are among the forages suitable for animal feed, encompassing stems, leaves, and other parts of the corn plant with elevated water content (Ahmad et al., 2020). Athori (2023) notes that corn kernels exhibit substantial nutritional value, including 9.84% crude protein, 25.2% crude fiber, and 3.86% crude fat, accompanied by a Nitrogen-Free Extract content of approximately 59.9%. While providing forage alone is insufficient to fulfill the nutritional requirements of ruminant livestock, it is essential to supplement their diet with foods rich in protein to meet both production and breeding demands, such as concentrates. Concentrates serve as a valuable source of energy and protein, offering easily digestible nutrients that contribute to enhancing livestock productivity. Nevertheless, breeders often encounter the challenge of the relatively high cost associated with concentrate feeds. As a result, the expense of maintaining ruminant livestock is predominantly driven by the expenditure on feed, constituting approximately 70%. This challenge can be addressed by adopting alternative local feeds, with legume leaves emerging as a viable protein source.

According to Kushartono and Iriani (2004), legumes boast an average protein content of 22%. Additionally, livestock require legumes to uphold the stability of microbes in the rumen due to their high fiber content. Some legumes suitable for animal feed include *Calliandra*, *Indigofera*, and *Gliricidia*. These legumes encompass secondary metabolic compounds known as tannins. Tannins possess protein-binding properties and play a crucial role in the rumen, serving as protein protectors in the digestion of feed by ruminants. This compound has the ability to safeguard proteins in the feed, and feeding high-protein content without tannin protection can diminish feed efficiency as the protein is susceptible to degradation into ammonia (NH3) by rumen microbes. Tannins also function to control parasites, particularly nematodes, in the digestive tract of livestock, contributing to overall animal health and increased productivity.

Tannins act as protein protectors by binding feed ingredients into complex compounds that resist degradation by proteolytic bacteria. These protected proteins undergo hydrolysis in the abomasum under low pH conditions. Proteins passing through the abomasum are then enzymatically digested in the intestine into amino acids, serving as a nutritional supply for the host. Proteins escaping microbial degradation in the rumen are referred to as by-pass proteins. Tannins effectively convert Rumen Degradable Protein (RDP) into Undegradable Protein (UDP), subsequently enhancing metabolizable protein (MP). This means that the protein is absorbed by the small intestine (Jayanegara, et al., 2019).

The aim of protein protection is to enhance the quantity of protein entering the digestive tract post-rumen. Substituting concentrate feed with legume flour is anticipated to positively impact this research, as the tannin content in legume plants is capable of shielding protein from rumen microbial activity. This protection allows the protein to undergo enzymatic digestion in the small intestine, and the crude protein that evades digestion by rumen microbes (by-pass protein) can be efficiently utilized by the small intestine.

2 Materials and Methods

2.1 Location

This research was carried out in the Animal Nutrition and Feed Laboratory, Faculty of Animal Science, Universitas Brawijaya.

2.2 Materials

The materials utilized in this research include corn stover, a concentrate comprising rice bran, pollard, cassava flour, copra meal, soybean meal, molasses, minerals, urea, leaf flour, and legume leaf flour, specifically from tannin-rich sources such as *Calliandra*, *Indigofera*, and *Gliricidia*. The tools and materials employed in the production of complete feed include those for obtaining rumen fluid, proximate analysis, in vitro analysis (following the method outlined by Tilley and Terry in 1963), as well as the analysis of Rumen Degradable Protein (RDP), Undegradable Protein (UDP), and Digestible Energy (DE) protein.

2.3 Methods

The experiment included ten treatments, each replicated three times. The treatments were as follows: T1 complete feed (60% corn stover + 40% concentrate), T2 complete feed (60% corn stover + (35% concentrate + 5% *Calliandra* meal), T3 complete feed (60% corn stover + (35 % concentrate) + 5% *Indigofera* meal), complete feed T4 (60% corn stover + (35% concentrate + 5% gamal meal), T5 complete feed (60% corn stover + (30% concentrate + 10% *Calliandra* meal), T6 complete feed (60% corn stover + (30% concentrate + 10% *Indigofera* meal), complete feed T7 (60% corn stover + (30% concentrate + 10% gamal meal), complete feed T8 (60% corn stover + (25) % concentrate + 15% *Calliandra* meal), complete feed T9 (60% corn stover + (25% concentrate + 15% *Indigofera* meal), complete feed T10 (60% corn stover + (25% concentrate + 15% gamal meal).

2.4 Statistical Analysis

The data obtained in this research were analyzed using a Randomized Block Design comprising 10 treatments with 3 replications, employing analysis of variance (ANOVA). Additionally, in cases where there are substantial and statistically significant differences between treatments, Duncan's multiple range test will be conducted to ascertain the specific variations among each treatment (Steel and Torrie, 1993).

3 Results and discussion

3.1 Chemical composition of feeds used in this study

The feed is a combination of feed ingredients consumed by livestock, providing essential nutrients such as energy and protein to meet the animals' dietary requirements over a 24-hour period (Parakkasi, 1999). Prior to being formulated into complete feed, the individual ingredients undergo proximate testing at the Animal Nutrition and Feed Laboratory. The nutrient composition of these feed ingredients is outlined in Table 1 and includes corn stover, legume leaves (*calliandra*, *indigofera*, and *gliricidia*), and concentrate ingredients (rice bran, pollard, soybean meal, copra meal, cassava, molasses, and urea).

Based on the proximate analysis results, corn stover exhibits the following composition: DM 24.52%, OM 93.32%, CP 8.68%, CF 26.29%, and EE 1.36%. In comparison, the CP content in this study is higher than that reported by Mustika and Hartutik (2021), who found for corn stover: DM 31.2%, OM 80.3%, ash 7.43%, CP 7.8%, CF 23.55%, and EE 2.34%. This discrepancy is attributed to variations in the cutting age of corn stover, where an increase in age leads to a decrease in protein content and an increase in crude fiber (Indriani et al., 2022). The detailed nutritional content analysis is presented in Table 1.

Table 1. Nutritional Content of Feed

	DM(1) (%)	OM(1)	Ash(1)	CP(1) (% BK)	CF(1)	EE(1)	Tannin(2)
Pollard	87,85	94,09	5,91	15,05	7,44	3,47	
Gaplek	87,13	95,29	4,71	2,98	6,97	0,42	
Rice Bran	90,81	80,06	19,94	10,12	31,16	6,46	
Copra Meal	90,39	92,44	7,56	20,49	23,22	3,48	
Soybean Meal	90,85	92,23	7,77	43,59	5,18	2,72	
Molasses	76,02	92,82	7,28	4,26	0,86	0,22	
Urea	99,89	99,85					
Mineral	99,82						
Corn Sover	24,52	93,32	6,68	8,68	26,29	1,36	
Concentrate	90,68	89,52	10,48	12,88	13,79	2,08	
Calliandra Flour	97,35	92,72	7,28	21,32	10,77	2,15	3,06
Gliricidia Flour	95,37	90,22	9,78	22,53	11,8	5,79	6,83
Indigofera Flour	94,21	90,18	9,82	24,28	10,34	5,69	4,06

Note:
(1) Analysis results from The Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Universitas Brawijaya.
(2) Tannin analysis From The Laboratory of Chemistry and Biochemistry of Food and Agricultural Products, Universitas Brawijaya

3.2 The RDP, RUP and DE levels in diet

Referring to Table2, it can be seen that the treatment has a highly statistically significant effect ($P < 0.01$) on RDP and RUP, whereas it demonstrates a significant effect on the DE value.

Table 2. The RDP, RUP and DE levels in diet

Treatments	RDP (%)	RUP (%)	DE (MJ/KgBK)	TDN (%)	Tannin (%)
T1	$59,49 \pm 1,54^{c}$	$40,51 \pm 1,54^{a}$	$3,33 \pm 0,14^{bc}$	$75,57 \pm 3,27^{cd}$	0,00
T2	$59,75 \pm 1,51^{c}$	$40,25 \pm 1,51^{a}$	$3,25 \pm 0,19^{bc}$	$73,61 \pm 44,21^{bc}$	0,15
T3	$58,38 \pm 2,86^{c}$	$41,62 \pm 2,86^{a}$	$3,35 \pm 0,05^{bc}$	$76,03 \pm 2,95^{cd}$	0,20
T4	$55,44 \pm 0,98^{bc}$	$44,56 \pm 0,98^{ab}$	$3,37 \pm 0,13^{bc}$	$76,49 \pm 1,74^{cd}$	0,34
T5	$49,52 \pm 1,05^{ab}$	$50,48 \pm 1,05^{ab}$	$3,14 \pm 0,08^{b}$	$71,15 \pm 1,74^{ab}$	0,30
T6	$53,49 \pm 3,14^{abc}$	$46,51 \pm 3,14^{ab}$	$3,35 \pm 0,19^{bc}$	$76,00 \pm 4,39^{cd}$	0,40
T7	$55,20 \pm 1,27^{bc}$	$44,80 \pm 1,27^{ab}$	$3,31 \pm 0,06^{bc}$	$75,07 \pm 1,29^{cd}$	0,68

T8	46,56 ± 1,75[a]	53,44 ± 1,75[c]	2,83 ± 0,07[a]	65,25 ± 1,69[a]	0,45
T9	52,89 ± 0,55[abc]	47,11 ± 0,55[abc]	3,30 ± 0,10[bc]	74,90 ± 2,22[cd]	0,60
T10	54,91 ± 2,82[bc]	45,09 ± 2,82[ab]	3,46 ± 0,08[c]	78,51 ± 1,75[d]	1,02

The research results indicated that Kaliandra flour was added in treatments P2, P5, and P8, Indigofera flour in treatments P3, P6, and P9, and Gliricidia flour in treatments P4, P7, and P10. The addition of three different legume flours, combined with varying tannin concentrations (5%, 10%, and 15%), led to a reduction in the RDP value. This reduction can be attributed to the inverse relationship between tannin concentration and RDP value. Specifically, lower tannin concentrations resulted in higher RDP values, indicating that the proteins present in *Caliandra*, *Indigofera*, and *Gliricidia* were not maximally protected by tannin. Consequently, rumen microbes degraded the proteins, leading to an increase in N-NH3 levels.This study aims to assess and evaluate feed protein based on rumen degradable protein (RDP) and undegradable protein. RDP, constituting part of the crude protein in the feed, is degraded by rumen microbes into peptides and ammonia, with a significant portion converting into microbial crude protein. The availability of RDP in the rumen serves to increase ammonia nitrogen for the synthesis of microbial protein (Iswanto et al., 2022). Suryani et al. (2014) proposed that the high composition of microbial protein in the rumen is directly proportional to the protein composition in the feed. Since crude protein is a crucial component for microbial protein synthesis, it signifies the availability of nitrogen elements for rumen microbes. However, an excess of N-NH3 production does not confer positive benefits for microbial protein production; instead, it may reduce protein efficiency values as a substantial amount of nitrogen is excreted through urine and feces (Afzalani, 2021).

In this experiment, RUP varied among treatments but constantly ranged from 40,25% to 53,44%. These results concur with a previous study, which stated that the RUP digestibility varied considerably from 25% to 60% in ruminant. According to research conducted by Pangestuti et al. (2023), the consistently highest average UDP value was observed in P4 at 47.66%. The addition of legume flour with varying levels of tannin concentration resulted in a higher UDP value compared to P1, which served as the control and contained complete feed without tannin protection treatment. In this study, the highest UDP results were obtained from the P8 treatment, involving the addition of 15% calliandra flour, with an average value of 53.44%. This outcome is attributed to the fact that calliandra contains condensed tannin, as indicated by Setyawati's research (2014), reporting a condensed tannin content of 7.43 g/100 g and a protein content of 23.24 g/100 g in calliandra leaf flour.

The presence of condensed tannin in calliandra does not interfere with rumen microbial activity; instead, it inhibits nutrient degradation by rumen microbes. The addition of tannin protection demonstrated an ability to increase the UDP value, suggesting that tannin can form protein bonds and create insoluble complex compounds that reduce rumen microbial degradation. As the tannin content in the feed increases, the protein content in the feed becomes protected from rumen microbes, subsequently passing to the feed by-pass where the protein is reabsorbed to enhance feed efficiency.

The data shows that the addition of legume leaves to the feed tends to increase DE values. The data indicates that feed treatment tends to result in increased DE content values. This suggests that substituting concentrate with legumes in complete feed has no significant effect on increasing DE content. However, in the legume substitution treatment at T5, T8, and T10, there are significant differences ($P<0.01$) in DE values. This suggests that adding legumes at these concentrations leads to decreased DE values, thereby impacting a reduction in DE content. This is distinct from the treatment at P5, P8, and P10, where there are very significant differences ($P<0.01$) in DE values. DE values in the treatments involving the addition of calliandra flour at T2, T5, and T8 (5%, 10%, and 15% concentrations), *Indigofera* flour to treatments T3, T6, and T9 (5%, 10%, and 15% concentrations), and *Gliricidia* flour to treatment T4, T7, and T10 (5%, 10%, and 15% concentrations) tend to decrease alongside decreasing TDN values.

4 Conclusion

The conclusion of this study reveals that the addition of 15% calliandra meal reduced RDP by 46.56% and increased RUP up to 53.44%. Furthermore, the inclusion of 15% gliricidia meal led to an increase in DE value by 3.46 MJ/KgDM

The results from this research provide additional information on how rumen degradable protein and rumen undegradable protein can impact nutrient digestion in ruminants consuming leguminous meals.

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