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Effects of Inoculum Concentration and Soaking of Acacia Pods and Seeds in Ashed Rice Husk Filtrate on Chemical Compunds and In-Vivo Digestibilityof Fermented and Unfermented Acacia

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Abstract
A study was conducted to determine the effect of concentration of inoculum (*Saccharomyces cerevisiae*) and soaking acacia (seeds and pods) with ashed rice husk filtrate (ARHF) on nutrient and anti-nutrient content of fermented and unfermented acacia and their effect on *in-vivo* digestibilities of dry matter and organic matter. A total of 24 local goats were study. The animals were placed in individual pen. The goats were fed with corn stovers and concentrate. The concentrate consisted of 20% corn, 10% full fat soybean meal, 30% rice bran and 40% acacia seeds and pods. The experimental diets were four levels of inoculum (0, 5, 10 and 15%) and two levels of soaking (without and with ARHF). The feeds and water were available ad-libitum. A randomised block factorial design was used with the inoculum concentration (*Saccharomyces cerevisiae*) as a first factor, levels of soaking as a second factor and three replications. The results indicated that inoculum concentration produced significant effect ($P<0.05$) on crude protein, crude fibre and saponint contents of fermented acacia. Dry matter and organic matter digestibilities was also affected ($P<0.05$) due to inoculum concentration. The effects of soaking acacia with ARHF on crude protein, crude fibre, saponin contents, dry matter and organic matter digestibilities were statistically significant ($P<0.05$). There was an interaction between inoculum concentration and soaked acacia with ARHF on tannin conecntration of fermented acacia. In conclusion, the use of inoculum (*Saccharomyces cerevisiae*) improved nutritive value of acacia and soaking the acacia with ashed rice husk filtrate prior to fermentation increased the nutritive value. There was an interaction between inoculum concentration and soaked acacia with ashed rice husk filtrate on acacia quality.
Keywords: Acacia, fermentation, ashed rice husk filtrate, goats.

1. Introduction

One of the problems in small ruminant production in the tropical region, particularly during dry season is the inadequacy and poor quality of feedstuffs. Searching for locally available feedstuffs which are nutrionally and economically viable has been a main concern over the last decades by ruminant nutritionists. During dry season, the quality and quantity of pastures in some parts of the tropical countries is poor for a number of months. Accordingly, the use of trees and shrubs has been considered important in some parts of Indonesia to meet the nutreint requirements of grazing and browsing animals, such as goat.

Acacia (*Acacia nilotica* (L.) Willd. ex Del), one of the among promising legume trees, was widely available in arid and semi-arid regions of eastern Indonesia. This tree has great potential for feeding ruminants, particularly during the drought season when the pastures were scarcely available. A comprehensive study of the nutritive value of acacia seeds and pods in Indonesia has been reported by Djufri (2002). The seeds and pods contained 12.3% protein and 13.9% crude fibre with 67.2% organic matter digestibility. Although the nutrient contents of this feedstuff is relatively favourable for ruminants, its use in the ruminant diets is still limited due to anti-nutrient content of tannin. This polyphenolic substance of tannin had inhibitory effect on protein digestibility (Anganga *et al*., 1998).

Efforts to reduce the negative impact of using acacia seeds and pods in ruminant diets have been made through soaking in polyethilene glycol, crushing and soaking in wood ash and boiling in water (Uguru *et al*., 2014). The above methods of processing acacia pods and seeds need technical skill and these methods only foccus on the severe effect of tannins. A comprehensive processing technology is needed to not only to minimize the anti nutrient effect of tannins but also increase the quality of the nutrients.

Fermentation technology has long been practiced to increase the nutritive value of a low quality feedstuff. This technology accomodates proliferation of microbes which can induce microbial enzyme for breaking down glycosidic bond of dietary fibre or other substances, such as tannins. There is an ample evidence that fermentation increases nutritive value of feedstuff and decrease the negative effect of tannins (Oseni and akindahunsi, 2011; Abang and Shittu, 2015). *Saccharomyces cerevisiae* has been used to ferment feedstuffs to improve the nutritive value (Rui-yang *et al*., 2013) and soaking the substrate in alkaline solution also could weaken the lignocellulosic bonds (Ryu, 1989).

2. Material and methods

2.1. Preparation of rice husk filtrate

A method of Sutrisno et al. (1986) was used to produce ashed rice husk filtrate. Rice husk was collected from local farmers and then ashed. A total of 100 g ashed rice husk was diluted in a liter of water at room temperature. The solution was stirred until all the ash was solved. The slurry was then left for 24 hours. After the solution become clear, the liquid was then filtered through a cloth bag and measured for the pH. The filtrate was oven-dried at 60^{o}C for 48 hours. The dried acacia seeds and pods were then finely ground for chemical analysis.

2.2. Fermentation of acacia seeds and pods

Fine ground acacia seeds and pods were mixed with cassava cake with the ratio of 3:1. The mixture was then steamed for 20 minutes. The steamed substrate was placed on the plastic tray with 3 cm thick. The substrate was allowed to cool at room temperature. *Saccharomyces cerevisiae* with the concentration of 14.26 X 10^9 sel/ml (5, 10 and 15%) was evenly placed onto the substrate. The substrate was incubated at 26 – 29^{o}C for 72 hours with 28 – 72% humidity. The fermented substrate was air dried to keep the yeast alive, so that the probiotic property of yeast can be maintained. The fermented substrate was added onto the diet.

2.3. Animals and Diet

A total of 24 local goats were used as experimental animals. The animals were placed in individual pen. The animals were fed with corn stovers and concentrate. The concentrate consisted of 20% corn, 10% full fat soybean meal, 30% rice bran and 40% acacia seeds and pods. The animals were fed 8 diferrent experimental diets (Table 1)

Table 1. Nutrients content of the experimental feeds.

	Inoculum concentration	Soaking with ARHF	Protein content	Crude fibre	TDN
SC_0A_0	0	Without	14.03	17.00	62.94
SC_0A_1	0	With	14.21	18.51	62.15
SC_5A_0	5%	Without	14.08	15.69	61.87
SC_5A_1	5%	With	14.23	15.32	63.12
$SC_{10}A_0$	10%	Without	14,09	14.77	62,25
$SC_{10}A_1$	10%	With	14.38	15.31	63.62
$SC_{15}A_0$	15%	Without	14.54	14.18	62.56
$SC_{15}A_1$	15%	With	14.89	14,51	62,34

ARHF: Ashed rice husk filtrate; TDN: Total digestible nutrient.; SC_0A_0, no inoculum and ; SC_0A_1: no inoculum and soaked acacia with ARHF prior to fermentation ; SC_5A_0: 5% inoculum and without soaking; SC_5A_1:5% inoculum and soaked acacia with ARHF prior to fermentation; $SC_{10}A_0$: 10% inoculum and without soaking; $SC_{10}A_1$: 10% inoculum and soaked acacia with ARHF prior to fermentation; $SC_{15}A_0$: 15% inoculum and without soaking; $SC_{15}A_1$: 15% inoculum and soaked acacia witht ARHF prior to fermentation.

2.4. Parameters measured and statistical anlysis

Parameters measured in this study were : (1) crude protein, crude fibre, tannin and saponin contens of either unfermented or fermented acacia (a mixture between acacia seeds and pods) with Saccharomyces cereviseae, dry matter digestibility and organic matter digestibility. A randomized block factorial design with four concentration of inoculum (*saccharomyces cerevisiae*) either with / without soaking in ashed rice husk filtrate (ARHF) and three replications. Data were analysed by analysis of variance. The significance of difference between treatment means were tested by Tukey test (Steel and Torrie, 1980).

3. Results and discussions

Data on the effect of concentration of inoculum (*Saccharomyces cerevisiae*) and the effect of soaking acacia with ARHF on chemical compounds of fermented acacia, *in-vivo* dry matter digestibility and organic matter digestibility were shown in Tables 2, 3, 4 and 5. The effect of interaction between inoculum concentration and soaking acacia with ARHF on nutrient and anti-nutrient content of fermented and unfermented acacia was shown in Table 6. Different concentration of inoculum produced significant effect on crude protein content, crude fibre, dry matter digestibility and organic matter digestibility. The effect soaking acacia with ARHF on crude protein content, crude fibre, dry matter digestibility and organic matter digestibility was also significantly different, while interaction between concentration of inoculum and soaking acacia was not significant.

Table 2. The effect of inoculum concentration on nutrient and anti-nutrient content of fermented acacia

Cemical compounds (%)	Treatments				Significance
	Concentration of *S. cereviseae*				
	SC-0	SC-5	SC-10	SC-15	
Crude protein	12.73[a]	12.81[a]	13.01[a]	14.21[b]	**
Crude fibre	21.94[a]	18.65[b]	16.31[c]	14.74[d]	**
Saponin	1.13[a]	1.07[b]	1.07[b]	0.93[c]	**
Tannin	6.90	5.61	4.82	4.32	ns

** very significant; ns: non-significant

Table 3. The effect of soaking acacia with ashed rice husk filtrate (ARHF) on nutrient and anti-nutrient content of acacia

Cemical compounds (%)	Soaking with ARHF		Significance
	With ARHF	Without ARHF	
Crude protein	12.89[a]	13.49[b]	**
Crude fibre	18.41[a]	17.41[b]	**
Saponin	1.08[a]	1.03[b]	**
Tannin	4.32	6.51	ns

Table 4. The effect of inoculum concentration on nutrient and anti-nutrient content of fermented and unfermented acacia

Digestibilities (%)	Treatments				Significance
	Doses of S. cereviseae				
	SC-0	SC-5	SC-10	SC-15	
Dry matter	68.0[b]	69.3[ab]	70.0[ab]	70.9[a]	*
Organic matter	52.4[b]	53.5[ab]	54.4[ab]	56.2[a]	*

Table 5. The effect of soaking acacia with ashed rice husk filtrate (ARHF) on nutrient and anti-nutrient content of acacia

Digestibilitiess (%)	Soaking with ARHF		Significance
	With ARHF	Without ARHF	
Dry matter	68.5[b]	70.6[a]	*
Organic matter	52.3[b]	55.9[a]	*

Table 6. The effect of interaction between inoculum concentration and soaking acacia with ARHF on protein and anti-nutrient content

Inoculum	Soaking with ARHF	Saponin	Tanins	CrudeProtein	Crude fibre
SC- 0	without	1.13	7.57[e]	12.49	22.38
SC- 0	with	1.12	6.33[a]	12.96	21.49
SC-5	Without	1.09	6.08[f]	12.62	19.11
SC-5	With	1.05	5.11[b]	13.00	18.19
SC-10	without	1.09	5.51[g]	12.65	16.81
SC-10	with	1.05	4.14[c]	13.37	15.81
SC-15	Without	0.99	4.74[h]	13.78	15.32
SC-15	With	0.87	3.85[d]	14.64	14.16
Significance		ns	*	ns	ns

Crude protein content of fermented acacia with 5 and 10% of *Saccharomyces cerevisiae* was the same as the unfermented (0%) acacia. However, when the concentration of *Saccharomyces cerevisiae* was increased to 15%, protein content of fermented acacia was significantly higher. The increase in protein content of fermented acacia in 15% inclusion of inoculum was probably due to the decrease in other fractions, such as: lipid and carbohydrates, particularly crude fibre. The carbohydrate components might underwent bioconversion into heat through metabolic process of the yeast *Saccharomyces cerevisiae*. The decrease in other components either in percentage or quantity could increase the percentage of protein. Another possibility of the increase in protein content of fermented substrate was due to the addition of microbial single cell protein of yeast *per se* as this yeast had high protein content.

Crude fibre of fermented acacia was decreased linearly ($Y=-0.478x + 21.5$; $r^2 = 0.974$) over the increased concentration of inoculum. Saponin concentration in the fermented acacia was also decreased due to the increased concentration of inoculum. These findings might indicate that fermentation can cause changes in the nature of the feedstuffs as a results of breakdown of feed substances by the activity of the enzyme produced by microbes or yeast (Winarno et al., 1980). Accordingly, it can be said that *Saccharomyces cerevisiae* used as inoculum could break down crude fibre and saponin into simpler form.

Soaking acacia with ARHF increased protein percentage by about 4.7% and decreased crude fibre and saponin content in the fermented acacia by 5.4% and 4.6% respectively. Since using ARHF could make the solution alkaline, Tsao *et al.* (1978) stated that alkaline solution could weaken or break lignocellulosic bond or lignoprotein of plant cell walls and that could release other important nutrients such as protein. Accordingly, soaking the acacia with ARHF prior to fermentation in this current study improved produced significant increase in the digestibilities of dry matter and organic matter.

Digestibilities of dry matter and organic matter increased significantly when the animals were offered the feeds containing fermented acacia with 15% inoculum, compared to the unfermented feed. This improvement might be through two mechanisms. First, yeast, particularly *Saccharomyce cervisiae* plays an important role in changing rumen condition. According to Dawson (1993), *Saccharomyces cereviseae* could absorb oxygen in the rumen to make the rumen anaerobic. This could optimize fibre-digesting bacteria to utilize fibrous material and thus increased digestibility. Second, fermentation process could break down the fibre fraction and other complex substances into simpler form. Accordingly, when the fermented feeds were offered into the animals, the nutrients presenting in the simpler form are easily absorbed. However, the additon of acacia seeds with less then 15% inoculum (5 and 10%) could not produced significant effect on the digestibility. The results of the study also indicate that feeding the goats with Soaked the acacia with ARHF improved dry matter and organic matter digestibilities by about 3.1 % and 6.9 % respectively.

4. Conclusions

Concentration of inoculum (*Saccharomyces cereviseae*) increased crude protein percentage in fermented acacia and decreased crude fibre, lipid and saponin saponin contents. Soaking the acacia with ashed rice husk filtrate increased protein content and decreased crude fibre, lipid and saponin content. There was an interaction between inoculum concentration and soaking acacia with ashed rice husk filtrate in tannin content of acacia.

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