

Effect of incubation time on chemical compositions and *in vitro* digestibility of treated extracted gambir leaf waste (*Uncaria gambir roxb*) with mix *Rhizopus sp* and *Aspergillus niger* as animal feed

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Introduction In Indonesia, the agricultural by-products are considered as stable source of ruminant feeds and now a days interest in their effective utilization is increasing all over the world due to economical factors and pollution. Shortage in animal feeds has been found to have a negative impact on the development of animal production. Non traditional feed sources such as extracted gambir leaf waste (*Uncaria Gambir Roxb*) are poor in nutrients such as protein content and vitamins and they are rich in fibers with low digestibility, or palatability and high lignin contents. The degree of lignifications is relatively more important in controlling hydrolysis rate in animal digestive tract (Fan *et al.*, 1981). Therefore, biological treatment is used for increasing the nutritional value of gambir leaf extracted waste, because microbial conversion of these wastes can improve their nutritional value and transforming them into animal feed with high quality (Villas-Boas *et al.*, 2002). Many efforts have been employed to remove the lignin and/or to break up the linkages between lignin and carbohydrates and to increase their feed values by biological treatments (Abo-Eid *et al.*, 2007). The main objectives of this study were to evaluate the effect of biological treatments of extracted gambir leaf waste with two fungal (F) strains (*Rhizopus sp* and *Aspergillus niger*) for 5 to 20 days of incubation on chemical composition, and cell wall constituents as well as on the *in vitro* dry and organic matter digestibility.

Material and method The objective of this study was to evaluate the effect of biological treatment with three fungal strains for extracted gambir leaf waste on chemical composition, cell wall constituents and *in-vitro* digestibility. The extracted gambir leaf waste was chopped (approximate 1-3 cm) and each one was divided into 6 times of incubations treatment. The tested fungi were obtained from the extracted gambir leaf waste decay. The strains obtained were *Rhizopus sp* and *Aspergillus niger*. The strains were maintained on potato dextrose agar medium (PDA), grown at 24 - 28°C for 48 - 72 hrs, and then stored at 4°C. The medium used for the maintenance of the fungi consists of coconut water and 2.5 gram sugarcane /L. The pH value was adjusted to 5.6 before autoclaving at 121°C for 20 minutes. Precultures of the mix fungal strains were prepared by inoculating 1500 mL conical flasks containing 1000 ml coconut water (Gusmanizar, 2008) with mycelial discs of 7 days old culture. The inoculated flasks were incubated at 28°C for 7 days. The mycelia of growing fungi were diluted 10 times and used to inoculate the extracted gambir leaf waste at 10% (V/W). Two hundred and fifty grams of extracted gambir leaf waste were packed in plastic bags for each and then inoculated with the above prepared inoculums (moisture content 60 - 65%). Data were analyzed according to Statistical Analysis System user's Guide (SAS, 1998) for one way analysis of variance. Separations among means were carried out by using Duncan's (1955) multiple range test.

Results Table 1 illustrates the effects of time of incubation on the chemical composition and *in vitro* digestibility of treated extracted gambir leaf waste. Except the OM CF, all nutrients and *in vitro* digestibility were significantly (P < 0.01) affected by time of incubations.

Table 1 Time of incubation effect on chemical composition and digestibility of treated extracted gambir leaf waste (%).

Time of incubation (days)	OM	CF	CP	CP Degradation	ADF	NDF	ADF Degradation	NDF Degradation
5	95.38 ^a	30.53 ^a	13.02 ^b	45.72 ^b	29.89 ^b	57.20 ^c	12.15 ^a	11.59 ^a
10	95.29 ^a	31.37 ^a	14.52 ^{ab}	48.25 ^c	31.82 ^b	59.65 ^c	17.68 ^b	19.94 ^b
15	94.88 ^a	31.55 ^a	14.87 ^a	46.03 ^b	34.10 ^b	60.85 ^b	10.35 ^a	11.76 ^a
20	94.82 ^a	30.49 ^a	14.68 ^{ab}	43.00 ^a	36.77 ^a	63.44 ^a	13.66 ^a	13.21 ^a
SE	0.25	0.3	0.5	2.1	2.3	1.9	2.2	2.1

These results show that times of incubation influence the crude protein, ADF and NDF, and their digestion *in vitro*.

Conclusions Biological treatment of extracted gambir leaf waste with the mix *Rhizopus sp* and *Aspergillus niger*,) can improve chemical compositions and nutritive values at 10 days of incubation.

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