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Research Article
Improving the Quality of the Leaves and Seeds of Rubber Trees (*Hevea brasilliensis*) for Poultry Feed Through Various Types of Microbial Biotechnology

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Abstract
Objective: This study aims to determine the ability of some microbes to reduce the nutrients and crude fiber from the leaves and seeds of the rubber tree (*Hevea brasilliensis*) at various temperatures after a long fermentation. **Methodology:** This study was a laboratory experiment to measure the effects of multiple microbial fermentation times and temperatures on the nutrient content of the leaves and seeds of the rubber tree. The design used was a completely randomized $3\times3\times3$ factorial design. The first factor is the type of microbe (*Trichoderma spiralis*, *Rhizopus oligosporus* and *Neurospora sitophila*). The second factor is the temperature (24, 34 and 44°C). The third factor is the fermentation time (2, 5 and 8 days). The best of the substrate proximate in the inoculum is then analyzed and calculated to determine the metabolizable energy and protein. **Results:** The nutrients of the leaves and seeds were compared after processing via rubber processing technology using fermentation with *Trichoderma* with other treatments and without fermentation. **Conclusion:** The quality of its nutritional value can be improved, namely, through an increase in the crude protein content of 60.67%, amounting to 32.58% nitrogen retention and 36.67% energy metabolism and a decline in the crude fiber content of 39.02%.

Key words: Leaves and rubber seeds, microbes, dose, fermentation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the intensive poultry farm businesses, nutritional needs should be met by the breeder. In this system, the feed is the largest component of the production costs. Laying chicken feed costs are 80% of the variable costs[1], for broiler chickens they are 73%[2], for duck they are 53%[3] and for laying ducks they are 61.6%[4]. High feed prices are causing losses to the poultry farm businesses in Indonesia. One cause of the high price of poultry feed is the high price of the raw materials of the feed, which are mainly imported, such as fish meal, soybean meal and corn.

Soybean meal is one of the feed materials that makes up the vegetable protein constituent of poultry feed. It contains high protein and amino acids that are more complete than those of other vegetable feed ingredients. However, the availability of soybean meal is sometimes limited. The need for raw materials such as soybean meal is always increasing along with the development of poultry farms. Almost 100% of the need for more soybean meal is met by imports, which constituted approximately 1.26204 million tons[5] in 2014. To reduce dependence on imported feed ingredients such as soybean meal, it is necessary to find raw materials that can replace feed soybean meal with alternative feed ingredients. One such ingredient that has not been widely used is the leaves and seeds of the rubber tree (*Hevea brasilliensis*). This tree has high adaptability and can be grown at altitudes of 0-1500 m. The rubber tree (*Hevea brasilliensis*) has become a plantation crop. According to the Central Bureau of Statistics[5], extensive rubber plantations in Indonesia take up 5,487,305 ha, which makes Indonesia home to the largest set of rubber tree plantations in the world. Each hectare of a rubber plantation contains approximately 400-500 trees.

The low nutrient content and presence of anti-nutritive substances has led to the use of the rubber tree in rations not being maximized. An analysis of the leaves and seeds of the rubber tree showed from 15.70-18.62% crude protein, 10.89% crude lipid, metabolizable energy of 1762.95-2301.64, nitrogen retention of 53.42-71.19 and fiber roughage[6] of 15.73-18.62. According to Oluyemi and Roberts[7], the metabolizable energy of the leaves and seeds of the rubber tree is approximately 4,835 kcal kg^{-1} and leaf and rubber seeds also contain various amino acids, such as aspartic acid 10.25, glutamic acid 14.73, lysine 2.55, arginine 7.23, methionine 0.92 and trionin[8] 2.65. The main obstacle in the use of rubber leaves and seeds as animal feed is the high levels of cyanide (HCN) they contain. According to Giok *et al*.[9], the HCN content of the fresh leaves and seeds of the rubber tree is 263 mg/100 g. According to Syahruddin and Rita[10], the HCN content of the leaves and seeds of the rubber tree can be reduced or eliminated by the process of storage, extraction, drying, immersion in water or boiling. The leaves and seeds of the rubber tree were soaked for 24 h to reduce the levels of HCN. According to Toh and Chia[11], rubber tree leaves and seeds boiled at a temperature of 160°C can eliminate toxic HCN. Syahruddin and Rita[12] showed that fresh rubber tree leaves and seeds added to the livestock rations of broiler chickens in excess of 9% in the diet can reduce weight gain and feed intake. By anticipating processing needs, the quality of the leaves and seeds of the rubber tree can be increased. It is necessary to determine the efficiency of fermenting rubber tree leaves with *Trichoderma* microbes for increasing the percentage of crude protein to 23.98%.

To realize the productivity and potential benefits of rubber plantations for exporting raw materials, the use of the leaves and seeds of the rubber tree as poultry feed material also needs to be studied. Based on the above data, rubber tree leaves and seeds used as fodder have great potential and serve to reduce cholesterol production.

MATERIALS AND METHODS

Material research: The leaves and seeds were obtained from small holder rubber tree plantations in the district of Abai Siat Dharmasraya. The inoculum used was culture obtained from the Laboratory Institute for Agricultural Technology Sukarami Solok. yaitu: *Trichoderma spiralis*, *Rhizopus oligosporus* and *Neurospora sitophila*.

Research methods: The leaves and seeds of rubber trees were fermented with microbes by the solid culture method. The design used was completely randomized and factorial, with three factors ($3\times3\times3$ with three replications)[13]. The first factor is the 3 types of microbes: *Trichoderma spiralis. Rhizopus oligosporus* and *Neurospora sitophila.* The second factor is the temperature (24, 34 and 44°C). The third factor is the fermentation time of 2, 5 or 8 days. After fermentation, the content of protein, amino acids, nitrogen retention, energy and fiber metabolism were analyzed.

RESULTS AND DISCUSSION

Effect of fermentation treatment on the crude protein content of rubber tree (*Hevea brasilliensis*) leaves and seeds: The mean crude protein content of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation is shown in Table 1. From the analysis presented in Table 1, it appears that the administration of the three

Table 1: Average crude protein content of leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation

	Fermentation duration		
Treatments	L2	L5	L8
M1S24	26.873^{Ec}	27.352^{Eb}	28.088^{Ba}
M1S34	27.113^{Db}	27.838^{Eb}	28.203^{Ba}
M1S44	27.528^{Dc}	28.873^{Db}	29.614^{Aa}
M2S24	27.114^{Db}	29.478^{Ca}	27.773^{Cb}
M2S34	30.803^{Aa}	29.054^{Cb}	27.424^{Cc}
M2S44	29.058^{Bb}	30.828^{Ba}	27.663^{Cc}
M3S24	28.730^{Ca}	28.118^{Da}	24.383^{Eb}
M3S34	29.383^{Bb}	31.963^{Aa}	25.773^{Dc}
M3S44	28.723^{Ca}	27.398^{Eb}	23.512^{Fc}

^{A,B}Means with different capital letters in the same column show a statistically significant difference (p<0.05), ^{a,b}Means with different small letters on the same line show a statistically significant difference (p<0.05)

Table 2: Average crude fiber content of leaves and seeds of rubber tree (*Hevea brasilliensis*) after fermentation

	Fermentation duration		
Treatments	L2	L5	L8
M1S24	14.945^{Aa}	14.626^{Aa}	14.049^{Aa}
M1S34	13.430^{Ba}	13.306^{Ba}	12.832^{Cb}
M1S44	13.829^{Ba}	13.521^{Ba}	13.305^{Ba}
M2S24	12.890^{Cb}	11.971^{Cc}	13.339^{Ba}
M2S34	11.116^{Db}	11.710^{Cb}	12.327^{Ca}
M2S44	11.636^{Db}	11.048^{Cb}	12.378^{Ca}
M3S24	12.236^{Cb}	11.829^{Cc}	13.016^{Ba}
M3S34	11.540^{Da}	9.349^{Ec}	10.117^{Db}
M3S44	11.340^{Db}	10.555^{Dc}	12.270^{Ca}

^{A,B}Means with different capital letters in the same column show a statistically significant difference (p<0.05), ^{a,b}Means with different small letters on the same line show statistically significant differences (p<0.05)

levels of long fermentation has a significantly different effect (p<0.05) on the protein content. The protein content in the real-time of 8 days (p<0.05) is higher than for 2 and 5 days at S24 and it decreases with longer incubation. At 2 day, the protein content increased with increasing incubation time except in S44, whereas, after 8 days, the protein content increased from S24-S34 and decreased at S44. This occurred because the fermentation time is closely related to the growth and activity of the mold that will influence the product. This result is in accordance with the opinion of Alexopoulus and Mims[14] that a fermentation time exceeding the optimum time of mold growth can cause damage to proteins and DNA that play an important role in metabolism and cell growth. Differences in the growth of this fungus will influence the protein content of the product, increasing the protein due to the contribution of protein from the mycelium of the fungi. In accordance with Saono's[15] opinion, approximately 31-50% of the body mold is made up of proteins and fermentation also produces an enzyme that is itself a protein.

On the 2 day of the fermentation period, the protein content of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) obtained the highest fermentation at a temperature of 44°C with mold M3 (M3S44) and the lowest at a temperature of 24°C with mold M1 (M1S24). Duncan's multiple range test showed that increasing the incubation time from 2-5 and 8 days with molds Ml and M3 boosted the protein content. This result is in accordance with the opinion of Aidoo *et al.*[16] that the protein content proportionally increased in line with the length of time of the incubation to a certain extent and then decreased. However, as the long incubation of mold M2 increases from 2-5 days, the protein content significantly increases and then declines at 8 days. The high protein content in M2 at 5 days indicates that in these conditions, the growth of mold M2 is optimum. Its growth is closely related to the reduction in the growth of mold M2 at 5 days and 8 days.

After fermentation at S24 and S44, the protein content of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) was significantly higher for mold M3 than for molds M2 and Ml, contrary to the S34 M2 protein content being higher than that of the M3 and M1.

At the fermentation time of 5 days, increasing the dose of S24-S34 markedly increased the protein content, but it declined again at S44. This situation is no different from the long fermentation of 2 day for all three types of mold. Judging from the type of mold, the highest protein content was obtained at S34 for the three types of fungi. At the fermentation period of 8 days, increasing the incubation time will lower the protein content of Ml, M2 and M3. This effect is due to the high temperature causing the faster the activity of mold, so that optimum growth was achieved in a relatively short time. High temperatures will speed up chemical reactions so that the availability of more nutrients will quickly spur growth. This occurs because the growth is the result of a series of chemical reactions that are strongly influenced by temperature[16].

Viewed as a whole, the highest protein content was obtained from a fermentation of 8 days for S44, the length of fermentation of 5 days gave the best result for S34 and a fermentation period of 8 days obtained at the best result for S34. This shows that at low temperatures, obtaining a high protein content requires a longer time and at high temperatures, only a short incubation time is needed. The highest protein content was obtained for mold M3 (*Trichoderma spiralis*) with S34 and a fermentation time of 5 days, it increased from 23.512-31.963%.

Effect of fermentation treatments on the crude fiber content of the leaves and seeds of the rubber tree (*Hevea brasilliensis*): The average of the crude fiber content of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation can be seen in Table 2.

The results of an analysis of variance showed an interaction between the types of molds, temperatures and incubation periods affecting the crude fiber content of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation. The results of a Duncan test showed that the content of the crude fiber of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after a long fermentation at 2 day were significantly lower compared to the fermentation time of 5 days and 8 days, but for S34 and S44, the content of crude fiber was higher than for the length of fermentation at 2 and 5 days. This result occurred because at high temperatures, the enzyme activity was reduced due to the destruction of the enzyme and the substrate water content being much reduced. Consequently, the raw fiber is reshuffled into sugars.

On the first day of the fermentation period, the increase in the incubation time will lower the crude fiber content of the leaves and seeds of the rubber tree (*Hevea brasilliensis*), whether M1, M2 or M3 is used. The decline in crude fiber from S24-S34 is higher than the drop in crude fiber from S34-S44. Similar results were also obtained in the fermentation time at 5 and 8 days. This change is closely related to the growth of mold and cellulase enzyme activity. With increasing incubation time, some cellulase enzymes may overhaul the higher crude fiber so that the fiber content decreases. According to Aidoo *et al.*[16] the longer the incubation time, the more nutrients are overhauled. Judging from the type of mold in S24, S34 and S44, the content of crude fiber in the M3 was significantly lower compared to M1 and M2 on a long fermentation of 2, 5 and 8 days. However, this was not true for S24 with a fermentation time of 8 days, S34 with 2 day of fermentation or S44 with a fermentation time of 8 days. This pattern is closely related to the activity of the cellulase enzymes in the crude fiber remodel. This result shows that there are differences in the activity of the cellulase enzyme on the three types of molds for all treatment combinations. The crude fiber content of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation is low with *Trichoderma spiralis* and *Rhizopus oligosporus* showing that the cellulase enzyme activity in crude fiber remodels the rubber seed meal better than *Neurospora sitophila*.

From Table 2, it can be concluded that the overall decline in the crude fiber content of the leaves and seeds of the rubber tree *(Hevea brasilliensis*) after fermentation. The highest decline in crude fiber content was obtained from *Trichoderma* fungi with a change from 14.945-9.349% in the 5 day condition of fermentation at a temperature of 34°C, followed by *Rhizopus oligosporus* and *Neurospora sitophila*.

Table 3: Mean protein digestion of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation

Treatments	Incubation time L2	L5	L8
M1S24	68.870^{Ec}	70.990^{Ea}	69.520^{Cb}
M1S34	67.770^{Fa}	67.760^{Ga}	67.145^{Eb}
M1S44	70.805^{Db}	71.500^{Da}	69.815^{Cc}
M2S24	70.930^{Db}	72.315^{Ca}	70.170^{Bc}
M2S34	71.300^{Ca}	69.745^{Fb}	66.475^{Fc}
M2S44	74.370^{Aa}	73.300^{Bb}	72.710^{Ac}
M3S24	72.365^{Ba}	69.935^{Fb}	68.100^{Dc}
M3S34	70.590^{Db}	76.775^{Aa}	65.120^{Gc}
M3S44	70.680^{Db}	73.200^{Ba}	68.465^{Dc}

^{A,B}Means with different capital letters in the same column show statistically significant differences (p<0.05), ^{a,b}Means with different small letters on the same line show statistically significantly differences (p<0.05)

Effect of treatment on the digestion of protein *in vitro* for the leaves and seeds of rubber tree (*Hevea brasilliensis*) after fermentation: The effect of treatment of the protein digestibility *in vitro* of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation can be seen in Table 3.

The results of an analysis of variance showed that there is an interaction between the types of mold, temperature and the incubation time on the digestibility of the proteins *in vitro*. The test results further indicate that the three levels of temperature influence were significantly different (p<0.05) in their effects on the digestibility of the crude protein *in vitro* of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation.

The digestibility of the crude protein in *Trichoderma spiralis* is significantly (p<0.05) higher than the digestibility of the crude protein in *Rhizopus oligosporus* and *Neurospora sitophila*. This difference is closely related to the activity of the fungi in overhauling proteins into amino acids, wherein the protease enzyme activity in the fungus *Trichoderma spiralis* is higher than that of the two other fungi. It is also evident from the amino acid content in the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation with *Trichoderma spiralis*, which is higher than the amino acids in *Rhizopus oligosporus* and *Neurospora sitophila*. Another factor that causes the high protein digestibility is the fact that *Trichoderma spiralis* is rough on the low crude fiber content after fermentation. The decrease in fiber will improve the digestibility of nutrients, including the digestibility of the crude protein. In *Neurospora sitophila*, increasing the incubation time at a temperature of 24°C significantly increases the digestibility of the crude protein, but at a temperature of 34 and 44°C, an increase in the incubation time from 2-5 days improves the digestibility of the crude protein, which decreases again on day 8 of incubation.

Table 4: Mean metabolic energy content of and nitrogen retention of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation compared with the leaves and seeds of the rubber tree without fermentation

		Deuteronomy						
Quality nutrition	Treatments	1	2	3	4	5	Total	Mean
Metabolizable energy (kcal kg^{-1})	Without calculated	1602.54	1564.41	1567.78	1526.41	1593.99	7834.96	1570.98
	L8S24-N	2001.10	2172.71	2126.99	2023.98	2117.82	10523.8	2108.8
	L5S34-T	2185.06	2078.05	2132.17	2118.53	2166.28	10680.8	2136.1
	L2S44-R	1941.77	2010.72	2077.98	2185.36	2059.96	10275.8	2055.24
	Without calculated	37.91	38.31	39.25	38.17	38.72	180.23	38.47[a]
Nitrogen retention (%)	L8S24-N	53.47	55.64	53.79	53.55	52.67	256.99	53.82[b]
	L5S34-T	57.32	55.89	56.52	57.03	58.27	285.03	57.01
	L2S44-R	54.58	51.97	52.45	53.14	52.58	264.72	52.94

Metabolic energy content and nitrogen retention in the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation compared to leaves and seeds without fermentation: The average content of the metabolizable energy and nitrogen retention in the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation compared with gum leaves and seeds without fermentation can be seen in Table 4. The results of an analysis of variance showed that the treatment has a statistically significantly effect ($p<0.05$) on the content of metabolizable energy and the nitrogen retention. Further test results show that the content of metabolizable energy and the nitrogen retention in the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation is significantly higher ($p<0.05$) than that of the leaves and seeds of the rubber tree without fermentation and treatment with *Trichoderma spiralis* showed the highest metabolic energy content.

Similar results were also seen for nitrogen retention. The treatment of the leaves and seeds of the rubber tree (*Hevea brasilliensis*) after fermentation with *Trichoderma spiralis* with a fermentation time of 5 days and a temperature of 34°C resulted in significantly more nitrogen retention than no treatment and a fermentation time of 2 day and a temperature of 24°C or a fermentation time of 5 days and a temperature of 24°C. Increased metabolizable energy content and higher nitrogen retention was obtained for the treatment with a 5 days of fermentation time and a temperature of 34°C, i.e., 1570.9-2136.1 kcal kg^{-1} and 38.47-57.01%. Improving the quality of the nutritional value is an increase of 60.67% for crude protein, crude fiber decrease of 30.88%, metabolizable energy of 26.46% and nitrogen retention of 32.52%.

CONCLUSION

Processing the leaves and seeds of the rubber tree (*Hevea brasilliensis*) using fermentation technology with the fungus *Trichoderma spiralis* and incubating for 5 days at a temperature of 34°C improves the nutritional content compared to the results from leaves and seeds treated with other fermentations or without fermentation. In terms of quality, we can improve the nutritional value, with an increase in the crude protein content of 60.67% and a reduction in the crude fiber content of 30.88%, nitrogen retention of 32.58% and metabolizable energy of 36.63%.

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