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The Effect of Feed Fermented Rubber Seeds with *Rhizopus oligosporus* and *Neurospora stiophila* on the Percentage of Abdominal Fat and Blood Profile of Native Chickens

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ABSTRACT

The purpose of this study was to explore feed ingredients from rubber seed plantation waste and their effect on the percentage of abdominal fat and blood profile due to the high content of rubber seed oil and the presence of anti-nutrients (HCN). The concentrations of *Rhizopus oligosporus* and *Neurospora stiophila* inoculum were 10^9 CFU/g each. This study was subjected to a Nested Completely Randomized Design. The first factor was the type of microbial used. Factor II was inoculum dose (0%, 0.4%, 0.8%, 1.6%, and 3.2%). The best concentration of inoculum was used in the making of fermented rubber seeds to be given to native chickens. The application on native chickens used statistical analysis Completely Randomized Design consisting of 3 treatments, namely P1: feed without using fermented rubber seeds; P2: feed using 5% Neurospora fermented rubber seed; and P3: feed using 5% Rhizopus fermented rubber seed. Significantly different results continued with Duncan's test. The results showed that the type of fungi had no significant effect on the treatment, but increasing the dose of fungi inoculum up to 3.2% had a significant effect on decreasing the content of dry matter, HCN, and crude fiber in rubber seeds. Administration of Rhizopus and Neurospora inoculums had a significant effect on increasing abdominal fat when compared to control. The conclusion of this study was that the processing method before fermentation and the amount of fungal inoculum used during fermentation affected the nutritional and anti-nutritional content of HCN rubber seeds. The presence of fermented rubber seeds which had either *Rhizopus oligosporus* or *Neurospora stiophila* increased the percentage of abdominal fat. Native chickens which were given fermented rubber seeds with Neurospora showed a higher level of stress marked by increased blood lymphocytes.

Keywords: Rubber seeds, Native chicken, Abdominal fat, Blood profile

Introduction

Rubber seed is a rubber plantation waste that can be utilized because it has several chemical contents. The content of rubber seeds based on SNI 01-3930-2006 is water content <14%, ash content <8%, crude fat <7.4%, crude fiber <6%, protein >19%, and phosphate >0.4% (SNI, 2006). The protein consists of threonine, cystine, lysine, and isoleucine (Nwokolo and Smartt, 2012). Indonesia is also the largest rubber producer in the world after Thailand. Statistical data shows that Indonesia's rubber production in 2020 was 3,037,448 tons with a rubber plantation area of 3,726,173 ha (Direktorat Jendral Perkebunan, 2020). One hectare of land can produce around 2,253 up to 3 million seeds/year (Sultan *et al*., 2018). This shows that rubber seed is very potential as a poultry feed ingredient.

The problem is that rubber seeds also contain the anti-nutritional substance Cyanide Acid (HCN) which is formed enzymatically from the precursor compound (potential poison) linamarin (Abdullah *et al.*, 2013). Decreasing the HCN of rubber seeds through fermentation begins with boiling. The increased temperature due to the boiling process can detoxify poisons by reducing HCN and enriching nutrients (Agbai *et al.*, 2021). HCN levels were lowered by 80.27% through a fermentation process (Yatno *et al.*, 2015). The microbes commonly used for fermenting grains which are proven to be safe for consumption are *Rhizopus oligosporus* (tempe fungi) and *Neurospora stiophila* (red oncom fungi).

Both of these fungi can degrade cell wall due to the presence of cellulase and hemicellulase enzymes. Damage to the cell wall of the rubber seed will damage the linamarin (which will produce HCN) and prevent the formation of HCN. According to Yerizam *et al.* (2018) that the cyanogenic glycogen derivative in the form of linamarin (2-β-D-glucopyranosyloxy-2- methylpropanenitrile) which is the precursor for the formation of HCN will be damaged through the fermentation process. That is to prevent the formation of HCN. The HCN that has been formed will also evaporate due to the heat generated by the fermentation (Mushollaeni, 2019). This is because HCN is volatile (Yerizam *et a*l., 2018).

The use as an alternative feed ingredient for native chicken, in addition to the anti-nutritional problem of HCN, also contains quite high rubber seed oil, which is around 40-50% (Hakim and Mukhtadi, 2017). The high oil content and the presence of anti-nutrients in the form of HCN in the rubber seeds, so this study was carried out to determine its impact on abdominal fat and blood profile of native chickens that consumed it. The aim of this study was to explore feed ingredients from rubber seed as feed ingredients and their effect on the percentage of abdominal fat and blood profile due to the high content of rubber seed oil and the presence of anti-HCN nutrients.

Materials and Methods

The rubber seeds used were seeds that had been removed from the skin by breaking the shell. That rubber seeds obtained were washed, soaked overnight, then then the soaking water was discarded. Then they were boiled for one hour after the water boiled (100ºC). The next process was removing the epidermis and steaming it for one hour. Rubber seeds were ready to be inoculated by first airing them until the seeds are warm.

The concentrations of the *Rhizopus oligosporus* and *Neurospora stiophila* inoculum were 10^9 CFU/g each. The dose of inoculum given was in accordance with the treatment (0%, 0.8%, 1.6%, 3.2%, and 6.4%). The inoculated rubber seeds were put in a plastic bag with a few small holes, then ripened in an incubator. Fermentation time was 48 hours at 30˚C.

Parameters observed were nutrient contents, including: dry matter (DM), crude fiber (CP), ether extract/fat (AOAC, 2005), as well as anti-nutritional content, namely HCN (Arianto *et al.*, 2014). The best concentration of fungal inoculum dose was used in the making of fermented rubber seeds to be given to native chickens to observe their abdominal fat and blood profile.

A total of 90 native chicken with strain *Kampung Unggul Balitbangtan* (KUB) aged 2 months with an average initial weight of 1.020 g/head (P0); 981 g/head (P1); and 942 g/head were used in this study. The native chickens were not differentiated by sex (unsexed) and had previously been given basal feed containing fermented rubber seeds according to the treatment for five weeks. Fermented rubber seeds with the selected dose of fungal inoculum were dried in the sun until they contained ≤15% moisture content. Then they were grinded to form powder and mixed with other ingredients such as laying hen concentrate, corn, fine bran until iso protein and iso energy were reached with a nutritional content of CP = 19% and EM = 2,900 kcal/kg (Supriadi, 2014) (Table 1). The parameters observed were carcass presentation, percentage of abdominal fat, and blood profile (Onunkwo *et al.*, 2022).

Data analysis

To determine the effect of rubber seed fermentation using *Rhizopus oligosporus* or *Neurospora stiophila* on the nutritional content of rubber seed and its HCN content, Nested Completely Randomized Design was carried out. The first factor was the type of microbial used. Factor II was inoculum dosage (0%, 0.4%, 0.8%, 1.6%, and 3.2%). Each treatment was repeated twice. Significantly different results were followed by Duncan's multiple range test to determine differences between treatments (Astuti, 2007).

The effect of rubber seed administration on the percentage of carcass and abdominal fat was analyzed using a Completely Randomized Design (CRD) consisted of 3 treatments; each treatment consisted of 5 replications. Each replication consisted of 6 native chickens. P1 = basal feed (without using fermented rubber seed); P2 = feed using Neurospora fermented rubber seed 5%; and P3 = feed using Rhizopus fermented rubber seed 5%. Data were observed for 5 weeks, and the data of the previous week was used for adaptation. Significantly different results were followed by Duncan's test to determine differences between

Table 1. Composition of native chickens fed with fermented rubber seeds

Ingredients	Composition		
	P0	P1	P2
Milled corn (%)	52.0	46.5	50.0
Broiler chicken concentrate (%)	33.0	31.0	31.0
Fermented rubber seed (Rhizopus) (%)		5.0	
Fermented rubber seed (Neurospora) (%)			5.0
Fine rice bran (%)	12.0	16.5	13.0
Palm oil (%)	2.0		
Premix (%)	1.0	1.0	1.0
T o t a l (%)	100.0	100.0	100.0
Nutritional contents (calculated or proximate analysis) (%DM)			
Crude protein (CP) (%)	19.1	19.3	19.3
Ether extract/ fat (%)	5.4	8.6	8.3
Crude fiber (CF) (%)	7.1	7.4	7.0
EM (Kkal/kg)	2923.5	2923.0	2915.0
Ca (%)	1.0	1.1	1.0
P (%)	0.4	0.4	0.4

P0 = Chickens fed with no fermented rubber seed; P1 = Chickens fed feed containing Rhizopus fermented rubber seeds; P2 = Chickens fed feed containing Neurospora fermented rubber seeds.

treatments (Astuti, 2007). Specifically for the blood profile, 3 native chickens of the same sex were taken, in this case females, so that their body weight was uniform. Each native chicken was also used as replicates.

Results and Discussion

The contents of dry matter (DM), HCN, crude fiber and ether extract (fat) from fermented rubber seeds

The results showed that increasing the dose of the fungi would further reduce the dry matter content of the rubber seeds (Table 2). This is caused by the increasing number of degradation activities by fungi. According to Suwignyo *et al.* (2015) that the chemical composition of nutrients, such as dry matter, decreased after the fermentation process because during the fermentation process, the chemical structure of the substrate changed from complex compounds to simpler compounds and also removed anti-nutrients.

The results of the analysis showed that both *Rhizopus oligosporus* and *Neurospora sitophila* had the potential to reduce HCN doses. The more doses of fungi used, the lower the dose of HCN. The lowest HCN content was obtained by fermentation by using fungi as much as 6.4% for 2 days with fungi concentration of 10^9 CFU/g. This is because the heat generated by boiling rubber seeds before fermentation and the heat generated during the fermentation process can evaporate the HCN content. Described by Qin *et al.* (2021) that most of the linamarin has been hydrolyzed into volatile HCN due to its low boiling point after the steaming and fermentation processes. The lowest dose of HCN from the fermentation process here was still quite high, namely 12.94% (12.94 g/kg rubber seed), while the dose was safe for consumption at concentrations of less than 50 mg/kg rubber seed. This is because the average HCN content of rubber seeds that have been processed before being fermented was still quite high, namely 35.45 (0% dose). This is possible during the processing of rubber seeds (before they were fermented), that is periodically changed the water when steaming without soaking. According to Mushollaeni *et al.* (2019) that the reduction of HCN by immersion means that the enzymatic hydrolysis process occurs in the cyanide bonds. One property of HCN is that it dissolves easily in water.

Based on the results of the analysis, there was no interaction effect between the type of fungi and the dose of fungal inoculum (concentration 10^9 CFU/g) on crude fiber content. However, doses of up to 6.4% fungal inoculum had a significant effect ($p<0.05$) on the crude fiber content in fermented rubber seeds. The decrease in crude fiber content was indicated by the increasing dose of fungal inoculum for rubber seed fermentation. The dose of 6.4% fungal inoculum for rubber seed fermentation was significantly different from other treatments with lower dose of fungal inoculum. This is because the higher the dose of fungi, the greater the number of microbes that degrade fiber.

Suningsih *et al.* (2019) explained that in fermentation, cellulolytic microbes have a good ability to decompose and break down chemical bonds when adding a starter compared to without adding a starter. Increasing the dose of fungi for fermentation can speed up the process of breaking down complex compounds into simpler ones. According to Malianti *et al.* (2019) that the decrease in crude fiber can occur due to the formation of enzymes produced by microbes during the fermentation process, so that they can break down complex bonds into simple ones. Thus, they are easily digested. Nursiwi *et al.* (2018) crude fiber during the fermentation process decreased even in small amounts due to microbial activities during the fermentation process.

Rubber seed fermented with *Rhizopus oligosporus* has a higher crude fat content than *Neurospora sitophila*. This shows that each fungal inoculum's ability to utilize fat as an energy source is different. Wattiheluw (2012) explained that *Rhizopus oligosporus* is able to degrade fat substrates after using carbohydrates as an energy source for metabolic processes, so that the crude fat content becomes lower. In this case, the ability of *Neurospora sitophila* to utilize carbohydrates as an energy source to be used in degrading crude fat was greater than that of *Rhizopus oligosporus*.

Based on the ability to reduce the HCN content without significantly reducing the nutritional content, a dose of 3.2% fungal inoculum was used for fermentation (concentration 10^9 CFU/g) to be tested on native chickens to determine the effect on carcass fat and blood profile.

Table 2. Dry matter (DM) content of fermented rubber seeds (%)

	Fungi types	Doses (%)					Mean
		0	0.4	0.8	1.6	3.2	
Dry matter (DM)	*Rhizopus oligosporus*	98.36	98.26	98.10	97.50	96.93	97.83
	Neurospora sitophila	98.36	98.33	97.80	97.36	97.11	97.79
	Mean	98.36^c	98.30^c	97.95^{bc}	97.43^{ab}	97.02^a	
HCN	*Rhizopus oligosporus*	35.45	19.95	18.15	15.25	11.88	20.14
	Neurospora sitophila	35.45	21.95	20.45	17.55	14.00	21.88
	Mean	35.45^c	20.95^b	19.30^b	16.40^{ab}	12.94^a	
Crude fiber	*Rhizopus oligosporus*	2.89	2.81	3.37	2.89	1.61	2.71
	Neurospora sitophila	2.89	3.57	2.76	2.59	1.54	2.67
	Mean	2.89^b	3.19^b	3.06^b	2.74^b	1.57^a	
Fat	*Rhizopus oligosporus*	59.61	61.69	59.74	61.40	62.80	61.05
	Neurospora sitophila	59.61	60.26	60.36	58.95	57.74	59.39
	Mean	59.61	60.98	60.05	60.17	60.27	

^{a,b,c} Different superscripts on the same line show a significant difference ($p<0.05$).

Percentage of carcass and abdominal fat of native chickens fed with fermented rubber seeds

The percentage of carcass and abdominal fat of native chickens fed with fermented rubber seed with *Rhizopus oligosporus* (P1) and *Neurospora sitophila* (P2), and without rubber seed (P0) is presented in Table 3. The results of statistical analysis showed no significant differences in the effect of feeding containing fermented rubber seeds (Rhizopus sp. and Neurospora) with a control. The percentage uses of carcasses for P0, P1, and P2 were 65.17%, 67.10%, and 61.51% respectively. The carcass' percentage value was for female native chickens. This condition is in accordance with the statement of Hidayat *et al.* (2021) that the carcass' performance of all types of native chickens in Indonesia is not much different, namely 62% for females and 71% for males. There was no significant effect showed of giving fermented rubber seeds as much as 5%. This showed that it was still at a safe level, and the carcass' percentage itself was mainly influenced by genetic factors. The quality of poultry meat results from a complex interaction between genotype, age, and sex of the poultry, as well as the management system including the quality of the feed given. It is further stated that genotype had a strong effect on slaughter weight and carcass, but has little effect on the fatty acid profile (Tavaniello *et al.*, 2022).

Tabel 3. Percentage of carcass and abdominal fat of native chickens fed with fermented rubber seeds

Parameters	Treatments		
	Control	P1	P2
Carcass^{ns} (%)	65.17	67.10	61.51
Abdominal Fat (%)	1.28^{b}	1.86^{a}	1.88^{a}

P0 = Chickens fed with no fermented rubber seed; P1 = Chickens fed feed containing Rhizopus fermented rubber seed; P2 = Chickens fed feed containing Neurospora fermented rubber seeds.

^{a,b} Different superscripts on the same line show significant differences (p<0.05).

The results of the analysis showed that there was a significant difference between the treatments in the percentage of abdominal fat (p<0.05). Chickens fed fermented rubber seed (both using Rhizopus and Neurospora) had a higher percentage of abdominal fat compared to control. This was due to the excessive fat content of the rubber seed even though it had been fermented (Table 2), namely 8.6% for P1 and 8.3% for P2 compared to P0 which was 5.4%. This caused a high feed fat content in the provision of fermented rubber seeds of 5% which had an effect on the abdominal fat of chickens that consumed it.

The use of oil and fat in the ration will affect the production performance and characteristics of the poultry carcass. The degree of saturation of fat from feed affects the accumulation of body fat. It was further stated that the provision of saturated fat (using beef fat) in poultry showed higher body fat storage compared to poultry that was given unsaturated fat in its ration (Mardhotillah *et al.*, 2020). It is possible that the high fat content of the ration containing rubber seed could increase the body's abdominal fat content compared to the control. In addition to containing unsaturated oils (oleic acid and linoleic acid), rubber seeds also contain 17% - 22% saturated fat (palmitic, stearic acid, arachidic acid) (Hakim and Mukhtadi, 2018).

Blood profile of native chicken fed fermented rubber seed

The blood profile of native chickens fed fermented rubber seed with *Rhizopus oligosporus* (P1) and *Neurospora sitophila* (P2), and without rubber seed (P0) is presented in Table 4. The results showed that there was no significant difference between the treatments on red blood cells such as hemoglobin and erythrocytes (both in control and native chickens fed fermented rubber seeds) (Table 4). This shows that the dose of fermented rubber seeds as much as 5% in the feed with HCN content of 20.4 mg/100g (fermented with *Rhizopus oligosporus*) and 21.88 mg/100g (fermented with *Neurospora stiophila*) did not affect the number of erythrocytes and hemoglobin (HB) content, thus supporting the adequacy of oxygen supply for the body's metabolic processes. Normal standards for hemoglobin and erythrocyte native chicken levels are 7.0-13.0 g/dL and 2.3-3.5 x 10^6/mm^3 (Alfian *et al.*, 2017); Baudouin *et al.*, 2021). According to Aguihe *et al.* (2017) that a high HB means a high oxygen carrying capacity, while if it is below normal, it indicates a low oxygen carrying capacity, so that the animal will experience respiratory pressure. It was further explained that the effect of rubber seed HCN poisoning is inhibition of cytochrome oxidase, the terminal enzyme of the mitochondrial electron transport

Table 4. Blood profile of native chickens given 5% fermented rubber seeds

Laboratory results	Treatment		
	P0	P1	P2
Haemoglobine (g/dl)	14.9	14.6	15.2
Erytrocyte (RBC) x10^6	2.5	2.5	2.3
HCT	31.8	31.1	32.3
MCV (Mean corpuscular volume) (FL)	126.5	124.4	123.2
MCH (Mean corpuscular hemoglobin) (pg)	59.3	58.5	57.8
MCHC (Mean corpuscular hemoglobin concentration) (%)	46.8	47.1	47.0
Leucocyte (1 x 10^3 µL)	70.2	59.0	61.3
Neutophil (1 x 10^3 µL)	3.2^{ab}	4.0^{a}	2.0^{b}
Lymphocyte (1 x 10^3 µL)	89.4^{ab}	88.4^{b}	90.7^{a}
Monocit (1 x 10^3 µL)	7.5	7.6	7.2
Trombosit	6.7	6.0	0

^{a,b} Different superscripts on the same line show a significant difference (p<0.05).

chain by binding to heme. In the end, it will affect the process of erythrocytes as well, causing low red blood cell synthesis.

The results showed that the blood profile of native chickens fed with 5% fermented rubber seed in their diet and without rubber seed showed significantly different results for lymphocytes and neutrophils. Both of these components are part of leukocytes that function in the body's defense system.

The P2 treatment showed the highest lymphocyte value, which indicated that the P2 treatment chickens experienced the most impact due to consuming rubber seeds with HCN content above the safe limit. According to Moenek *et al.* (2019) that lymphocytes play a role in responding to antigens (foreign bodies) by forming antibodies and developing immunity. The normal limit for lymphocytes is 45-70 x 10^3/µL. Overall, the blood lymphocyte levels of native chickens from the study results exceeded the normal limit, namely 88.4 – 90.7 x 10^3/µL. Other factors that can affect the number of leukocytes besides nutrition are environmental conditions, such as uncomfortable conditions when taking blood samples which may also cause chickens to experience stress, which is possibly the cause of high leukocytes in control chickens.

Neutrophils at P2 did not experience an increase because the role of neutrophils here was to fight bacteria directly when an infection occurs. In this case, it was a problem of anti-HCN nutrition, so that it did not affect the neutrophil value. According to (Napirah, 2013) that the function of neutrophils is as the first line of defense against bacterial infections; when there is no infections, the neutrophils are not affected. The percentage of neutrophils will increase when there is a bacterial infection in the body.

Conclusions

The method of processing before fermentation and the amount of fungal inoculum used during fermentation affect the nutritional and anti-nutritional content of HCN rubber seeds. Giving fermented rubber seeds using both *Rhizopus oligosporus* (tempe fungi) and *Neurospora stiophila* (red oncom fungi) increased the percentage of abdominal fat, and native chickens that were given fermented rubber seeds with *Neurospora stiophila showed* a higher level of stress marked by the increased amount of blood lymphocytes.

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