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Effect of *Candida lipolytica* and *Rhizopus microsporus* Consortium Inoculum Dosage on Local Soybean Fermentation on Crude Protein Content, Crude Fiber, and Non-Nitrogen Extract Ingredients

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Abstract

Soybeans are a type of local feed that has high potential to be used as a material for quality poultry feed because the nutrient content in soybeans is quite complete. This study aims to determine the inoculum dose of the microbial consortium *Rhizopus microsporus* and *Candida lipolytica* in local soybean fermentation on the content of crude protein, crude fiber, and non-nitrogen extract (NNE). The method used was experimental, using a complete random design with four treatments and five replicates as follows: D1 = Dose of *Rhizopus microsporus* and *Candida lipolityca* microbial consortium 0.5%, D2 = Dose *of Rhizopus microsporus* and *Candida lipolityca* microbial consortium 1%, D3 = Dose of *Rhizopus microsporus* and *Candida lipolityca* microbial consortium 1.5 %, and D4 = Dose of a consortium of *microbes, Rhizopus microsporus* and *Candida lipolityca* 2 %. The data obtained was followed by a follow-up test of the Smallest Value Difference. The results showed that the content of crude protein, crude fiber, and NNE was D1 41.14%; 3,49%; 31.62%, D2 42.58%; 3.7%: 29.49%, D3 44.7%; 2,63%; 22.41%, D4 46.35%; 3,11%; 24,55%. The dose with the best results on crude protein, crude fiber, and NNE content was shown in the D4 treatment with a dose of 2%.

Keywords: Local soybeans; Fermentation; Consortium; *Candida lipolytica; Rhizopus microsporus;* Nutrients.

1. Introduction

Feed is one of the factors that can affect the success of poultry rearing. About 60-70% of the production cost is spent on feed. The soaring feed price caused the poultry industry to experience a downturn. One of the causes of this is the availability of poultry feed ingredients that must be imported. It is necessary to find alternative ingredients as a substitute for imported materials so that the cost of poultry feed can be minimized. 42% of the feed materials used in the preparation of feed rations come from imports. This is in accordance with government policy, that there needs to be further research and development on alternative feed ingredients that can be produced locally and can replace feed ingredients that are still imported, such as soybean meal, so that feed costs can be reduced.

Local soybeans are one of the ingredients that can be used as an ingredient for poultry feed as a source of energy and protein [1], [2]. The use of alternative feed ingredients in the form of soybeans can be used as an alternative solution to the problem of feed sources. Giving rations in the form of whole soybean flour can have an impact on chicken growth. Soybean flour contains 35.9% protein, 29.9% carbohydrates, 20.6% fat, and other nutrients such as vitamins, minerals, and fiber, so that it can meet the needs of livestock [3], [4]. The use of soybeans as a feed ingredient has a weakness, namely the existence of one of the antinutrient substances in the form of antitrypsin. This substance can cause impaired

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performance of the trypsin enzyme, which functions to digest proteins perfectly. This will result in low protein benefits in feed [5]. The reduction of antitrypsin in soybeans can be done by physical, chemical, and biological processes [4].

Fermentation is a series of processes that transform complex substances into simpler substances with the help of microbes that produce enzymes. Fermentation is a biological process that can lower antitrypsin levels [1], [6]. Fermentation is carried out to improve nutritional quality and reduce or eliminate the effects of nutrients on certain feed ingredients [1], [7], [8], [9]. Microorganisms will overhaul the soybean substrate in the form of dry matter, consisting of organic substances, inorganic substances, and water. Organic substances in dry matter consist of crude fiber, non-nitrogen extract (NNE), crude fat, and crude protein [10], [11]. During the fermentation process, all these organic components will change because of enzyme activity. These changes are changes in quality and quantity, changes in quality change the structure of the substrate to be simpler so that the antitrypsin in soybeans becomes inactive, while the change in quantity will cause changes in the components in the substrate caused by microbes during the fermentation process [12], [13].

Fermentation in soybeans can use fungi or bacteria. Mold and yeast are microbes that can be used in the soybean fermentation process. One type of yeast used for soybean fermentation is *Candida lipolytica*. *Candida lipolytica* looks more competitive than the non-oleaginous yeast*, S. cerevisiae,* because it has a co-translational pathway that is the main pathway, like mammalian cells [14]. The pumping system in *Candida lipolytica* serves for the depletion of alkanes, which increases the tolerance to alkanes. This leads to many heterologous proteins that have been produced by *Candida lipolytica* [8]. In addition, one type of mold used in soybean fermentation is *Rhizopus microsporus. Rhizopus microsporus* produces enzymes that act to break down complex proteins into peptides and free amino acids that have a low molecular weight so that they are more easily dissolved [15], [16]. In addition, *Rhizopus microsporus* also plays a role in producing lipase enzymes, which play a role in breaking down triacylglycerol into free fatty acids so that fat levels are lower [17].

The combination of yeast and mold can also be utilized as fermented microorganisms. This merger is called a consortium. A microbial consortium is a group of microbes that work in a group so that they have more ability to degrade an organic compound [8]. Microbes in the consortium have a great chance of gaining energy and survival because they can take advantage of coenzymes or exoenzymes that are excreted by other microbes, in addition to other microbes that can decompose substrates that have been previously degraded by a microbe. The use of microbial consortiums when compared to the use of single isolates showed superior results because microbial activities complemented each other to produce enzymes that function to optimize nutrient utilization in the growth medium [18]

In the consortium, there are interactions that are beneficial and detrimental between microorganisms. Beneficial interactions between organisms indicate that the microbial pool has compatibility [19]. Compatibility is a group of microbes that work together to form a cooperative, commensal, and mutualistic relationship. So that it is more successful in decreasing single isolated compounds [20]. The use of consortiums must also be considered for the dosage. The dose level influences the size of the microbial population, which affects how quickly microbes produce enzymes to break down the substrate and ultimately impacts the final product [21]. Therefore, it is necessary to know the optimal dosage level to produce the best nutrient content. Fermentation of black soybean tempeh using different inoculum (*Rhizopus Oligosporus, Rhizopus stolonifera,* and *Rhizopus Oryzae*) with a dose of 0.1% of 1 kg showed the best results, where crude protein increased by more than 18% from 45.93%, rising to 63.01% [22]. Fermentation in soybeans in combination with roay beans using *yeast* with a dose of 2.5% showed the best results, where the crude protein content was 31.45% [1].

2. Research Materials and Methods

2.1. Research Materials

The ingredients used and fermented in this study are local soybeans with a moisture content of 9.25% (Lab. Nutrition of Non-Ruminant Poultry and Animal Food Industry, 2023). The number of soybeans used is 20 kg, obtained from soybean farmers in Cicalengaka, Bandung Regency, Indonesia. The microbes used in this research on local soybean fermentation are the molds *Rhizopus microsporus* and *Candida lipolytica.*

2.2. Research Methods

Experimental research was conducted to determine the content of crude protein, crude fiber, and extracts without nitrogen in fermented soybeans.

2.2.1. Soybean Fermentation Stage

- Mash the soybeans.
- Then weigh using an analytical scale of 100 g, and then put it in a heat-resistant plastic bag.
- It is then sterilized using a 121 °C autoclave for 20 minutes.
- After that, drip the bacterial inoculum according to the treatment into a plastic containing soybeans using a micropipette.
- After that, tie and solidify, then put it in a jar and close it tightly.
- Fermentation is carried out according to the treatment, then harvested by sterilizing using a 121 °C autoclave for 20 minutes, then dried in an oven with a temperature of 105 °C for 6 hours, and mashed using a blender.

2.2.2. Crude Protein Analysis

- Weighing the sample that has been done is 1 g.
- Filling the sample into a Kjeldahl flask.
- Weighing 7 g of K2SO4 and 3 g of CuSO4.
- The addition of 7 g of K2SO4 and 3 g of CuSO4 to the Kjeldahl flask containing the sample, and the input of boiling stones.
- The addition of H2SO4 solution as much as 12 ml is carried out by an acid cabinet.
- The destruction process is carried out in an acid chamber by heating the sample in the Kjeldahl flask; by letting it sit for 20 minutes.
- Refrigerate the kjeldahl pumpkin by letting it sit for 20 minutes.
- Add 25 mL of aqueduct to the Kjeldahl flask containing the sample.
- Addition of 50 ml of 40% NaOH and a few boiling stones to the Kjeldahl flask containing the sample.
- The addition of 30 ml of H_3BO_3 4% to the Erlenmeyer with the addition of a 7-drop BCG-MR indicator to capture the distillate from the distillation results.

2.2.3. Distillation tool device.

- Distillates are obtained from distillation using a standard solution of HCl 0.1 N until the color of the solution changes to pale pink.
- Perform the same procedure to calculate the % N blank (the sample is replaced with aqueducts).

$$\% \text{ N} = \frac{ml\ HCl\ (sample - blanco)}{sample\ weight\ (g)\ x\ 1000} x\ HCl\ x\ 14{,}008\ x\ 100\%$$

Crude protein % = % N x 5,75 (Protein conversion factor)

2.2.4. Rough Fiber Analysis

- Weigh the sample by 0.3 grams.
- Insert into the reflux tube
- Input of H2SO4 0.3 N as much as 30 ml
- Fill the measuring cup with water, then place the reflux that has filled the sample with H2SO4
- Heat on the stove
- Count for 30 minutes starting when boiling
- After that, add 25 ml of NaOH 1.5 N
- Simmer for 30 minutes
- Rinse with 30ml hot water
- Rinse with
  - Rinse with H_2SO_4 0.3 N to 25 ml
  - Rinse with hot water 50 ml
  - Rinse with 25 ml of ethanol
  - Rinse with hexane 25 ml
- The filtered fiber residue is transferred into the cup
- The residue is aired for 30 minutes and then put in an oven at a temperature of 105 °C for 3 hours, after which it is cooled in a desiccant for 15 minutes
- Then it was weighed, and it was carried out at a temperature of 600 °C for 3 hours. After that, it is put into a desiccant for 30 minutes and weighed.

Crude fiber% % = $\frac{Residue - Cawan}{sample}$

2.2.5. Analysis of Non-Nitrogen Extracts

The NNE content can be obtained from the following calculations:

NNE (%) = 100% - ash (%) – crude protein (%) – extract ether (%) – crude fiber (%).

2.3. Statistical Analysis

This study used a Complete Random Design consisting of 4 treatments and 5 replicates. The treatment used is as follows:

- D1: A consortium of microbes, *Rhizopus microsporus* and *Candida lipolityca* 0.5 %.
- D2: Dose of a consortium of microbes, *Rhizopus microsporus* and *Candida lipolityca* 1 %.
- D3: A consortium of *Rhizopus microsporus* and *Candida lipolityca* microbes 1.5 %.
- D4: A consortium dose of *Rhizopus microsporus* and *Candida lipolityca* 2 %.

The data obtained will be analyzed with ANOVA with a significance level of 5%. If there is an influence in the treatment, then proceed with the 5% BNT test.

3. Results and Discussion

3.1. Effect of Treatment on Crude Protein Content

The results of the study on the effect of the inoculum dose of *the Candida lipolityca* and *Rhizopus microsporus* consortium on crude protein content are presented in Table 1.

Table 1 Results of Analysis of the Effect of Treatment on Crude Protein Content

Test	**Dosage (Treatment)**			
.....(%).....				
	D1	D2	D3	D4
1	41.14	42.58	44.70	46.35
2	46.08	40.78	45.06	45.62
3	41.14	42.58	44.70	46.35
4	36.20	44.38	44.34	47.08
5	41.14	42.58	44.70	46.35
Average	41.14[a]	42.58[ab]	44.70[bc]	46.35[c]

Information: D1: Dose of the microbial *consortium of C. lipolityca* and *R. microsporus* 0.5%; D2: Microbial consortium dose *of C. lipolityca* and *R. Microsporus* 1%; D3: Microbial consortium dose *of C. lipolityca* and *R. Microsporus* 1.5%; D4: Dose of the microbial consortium *of C. lipolityca* and *R. Microsporus* 2%

Based on the research that has been conducted, crude protein data were obtained from fermentation results using the inoculum of *the consortium of Candida lipolityca* and *Rhizopus microsporus*. The results of the study showed an average of between 41.14% to 46.35%. The results of the variety-fingerprint test showed that the use of the consortium type was significantly different ($P<0.05$) for the crude protein content. The crude protein content with the dose of consortium inoculum D4 and D3 was significantly different ($P<0.05$), higher than the use of consortium inoculum than the use of the D1 dose. The best treatment that produces the highest crude protein content is with a D4 dose of 46.35%.

The protein content of local soybeans in this study was higher than the content of fresh local soybeans before fermentation, which was 41.13 – 46.34%. The crude protein content of local soybeans increased due to the activity of protease enzymes during the fermentation process using a consortium of microbes, *Rhizopus microsporus* and *Candida lipolytica,* that can hydrolyze soybean protein. This is in line with the opinion [15] that *Rhizopus microsporus* can produce protease enzymes that are effective in the hydrolysis of soy protein. This enzyme breaks down proteins into

amino acids that are easier to digest, thereby increasing crude protein levels [23], [24]. The activity of consortium microorganisms can increase crude protein levels during the fermentation process [8]. These microorganisms form mycelium, which increases protein levels in line with the increase in the length of incubation time in the biodegradation process. Protein levels rose in this study because the consortium's microbes produced protease enzymes that hydrolyzed soy protein effectively. This enzyme breaks down proteins into amino acids that are easier to digest, thereby increasing crude protein levels [25].

3.2. Effect of Treatment on Crude Fiber Content

The results of the study on the effect of the inoculum dose of *the Candida lipolityca* and *Rhizopus microsporus consortium* on the content of Crude Fiber are presented in Table 2.

Table 2 Results of Analysis of the Effect of Treatment on Crude Fiber Content

Test	**Dosage (Treatment)**			
.....(%).....				
	D1	D2	D3	D4
1	3.49	3.70	2.63	3.11
2	3.11	4.22	2.56	2.45
3	3.07	3.12	2.60	3.13
4	4.29	3.75	2.72	2.74
5	3.49	3.70	2.36	3.11
Average	3.49^{bc}	3.70^{cd}	2.63^{a}	3.11^{ab}

Information: D1: Dose of the microbial *consortium of C. lipolityca* and *R. microsporus* 0.5%; D2: Microbial consortium dose *of C. lipolityca* and *R. Microsporus* 1%; D3: Microbial consortium dose *of C. lipolityca* and *R. Microsporus* 1.5%; D4: Dose of the microbial consortium *of C. lipolityca* and *R. Microsporus* 2%

Based on the research that has been conducted, crude fiber data were obtained from fermentation using a consortium inoculum *of Candida lipolityca* and *Rhizopus microsporus*. The results of the study showed an average of between 2.63% to 3.70%. The results of the variegated fingerprint test showed that the use of the type of consortium was significantly different ($P<0.05$). The lowest average of crude fiber with the best dose was found in the 3rd dose treatment with a dose of 1.5% a result of 2.63% (Table 2). The crude fiber content with the dose of consortium inoculum D3 was significantly different ($P<0.05$) lower than the use of consortium inoculum than the use of D1 and D2 doses. The best treatment that produces the lowest crude fiber content is with a D3 dose of 2.62%.

The crude fiber content of local soybeans in this study was lower than the crude fiber content of fresh local soybeans before fermentation, which was 2.65 – 3.69%. The decrease in crude fiber content after the fermentation process occurs due to the hydrolysis of the fibers by the enzyme cellulase produced by *the microbes Rhizopus microsporus.* This is in line with the opinion [15], which mentions that the enzyme cellulase can degrade cellulose into a simpler form. Cellulose is an organic compound that cannot be dissolved in water. The decrease in the crude fiber content of feed caused by cellulolytic microbes through the fermentation process must be in accordance with the available source of nutrients so that competition between microbes will not occur [16]. This can cause microbes to grow optimally, so that the cellulose degradation process resulting from microbial activity will also be more optimal. High crude fiber can cause poultry to feel full and can reduce feed consumption [26].

3.3. Effect of Treatment on NNE

Preferences The results of the study on the effect of the inoculum dose of *the Candida lipolityca* and *Rhizopus microsporus consortium* on the content of non-nitrogen extract (NNE) ingredients are presented in Table 3.

Table 3 Results of Analysis of the Effect of Treatment on NNE

Test	Dosage (Treatment)			
.....(%).....				
	D1	D2	D3	D4
1	31.62	29.49	22.41	24.55
2	27.22	30.66	23.39	25.86
3	31.62	29.49	22.41	24.55
4	36.02	28.32	21.43	23.25
5	31.62	29.49	22.41	24.55
Average	31.62^{cd}	29.49^{c}	22.41^{a}	24.55^{ab}

Information: D1: Dose of the microbial *consortium of C. lipolityca* and *R. microsporus* 0.5%; D2: Microbial consortium dose *of C. lipolityca* and *R. Microsporus* 1%; D3: Microbial consortium dose *of C. lipolityca* and *R. Microsporus* 1.5%; D4: Dose of the microbial consortium *of C. lipolityca* and *R. Microsporus* 2%

Based on the research that has been conducted, NNE data were obtained from the fermentation results using a consortium inoculum *of Candida lipolityca* and *Rhizopus microsporus*. The results of the study showed an average of between 22.41% to 31.52%. The results of the variegated fingerprint test showed that the use of the type of consortium was significantly different ($P<0.05$). The highest average of NNE with the best dose was found in treatment with a dose of 0.5%, with a result of 31.62% (Table 3). The content of NNE with the doses of consortium inoculum D1 and D2 was significantly different ($P<0.05$) from the use of consortium inoculum than from the use of D3 and D4 doses. The best treatment that produces the highest NNE content is with a D1 dose of 31.62%.

The content of local soybean NNE in this study increased and decreased compared to the content of fresh local soybean NNE before fermentation, which was 22.41% – 31.62%. The content of BETN increased at doses D1 and D2 but decreased at doses D3 and D4. This is because of the enzyme activity produced by the consortium of microbes *Candida lipolytica* and *Rhizopus microspores,* and the activity of these enzymes is influenced by the dose given; the more doses given, the more microbes can produce enzymes during the fermentation process. The content of NNE after fermentation has increased due to the decrease in crude fiber content because carbohydrates in local soybeans are hydrolyzed by cellulase enzymes. This is in line with the statement [24] that a decrease in the crude fiber content of a material will increase the content of NNE.

The content of NNE has decreased due to the consortium of microbes *Candida lipolytica* and *Rhizopus microsporus* used in the fermentation process, which can be very active in degrading substrates. This microbial activity can lead to a decrease in NNE content because microbes use NNE as an energy source [27], [28]. The addition of various fermentation starters can increase microbial activity and substrate degradation processes. This can lead to a decrease in NNE content as microbes degrade the substrate more [29].

4. Conclusion

Based on the results of the research and discussion, the following conclusions can be drawn: The dosage treatment of a consortium of *different microbes, Candida lipolytica* and *Rhizopus microspores,* affects the content of crude protein, crude fiber, and non-nitrogen extract ingredients. The dose treatment of a consortium of microbes, *Candida lipolytica* and *Rhizopus microspores,* with a dose of D4 (dose 2%) showed the best results for crude protein, crude fiber, and NNE content of 46.35, 3.11, and 24.55%, respectively.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest is to be disclosed

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