Dear Editor,

I am writing in response to the major revision request for my paper titled "**Corn Substitution Through Na-Glutamate and Neurospora Species Supplementation in Cassava and Tofu Dregs**," which I submitted to your esteemed journal Advances in Animal and Veterinary Sciences (ISSN: 2307-8316). I would like to express my gratitude to you and the reviewers for providing constructive feedback, which has greatly improved the quality of my paper.

After carefully considering the reviewers' comments and suggestions, I have made significant revisions to the content of the manuscript as follows:

1. The title has been revised as suggested by the reviewer: "**Corn Substitution Through Na-Glutamate and Neurospora Species Supplementation in Cassava and Tofu Dregs**."
2. In the abstract, we have simplified complex sentences and rephrased ambiguous phrases to improve clarity. We have incorporated additional examples and explanations to further illustrate key concepts and clarified the research methodology and results to improve the overall understanding of the research. In addition, the keywords have been arranged alphabetically.
3. In the introduction section, we have divided it into only three paragraphs. The first provides the background of the study, the second discusses the significance of the study, and the third outlines the aim of the study.
4. In the methods section, we have stated the approval of the current experiment and provided the source of each material used.
5. We have fixed the scientific name writing style.
6. We have made some modifications to enrich and enhance the readability and understanding of the text in the results, discussion, and conclusion sections.
7. In the references, we have revised them to include updated and relevant research.

I am confident that these revisions have substantially improved the manuscript and addressed all the concerns raised by the reviewers. I would like to thank the reviewers for their time and effort in providing constructive feedback, and I hope that the revised manuscript meets their expectations.

Thank you for considering my revised manuscript, and I look forward to hearing from you soon.

Sincerely,

Munasik

Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, 53122, Indonesia

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LETTER OF ACCEPTANCE

Munasik[1*], Titin Widiyastuti[1], Caribu Hadi Prayitno[1]
[1]Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, 53122, Indonesia

[*]Corresponding author email : munasik@unsoed.ac.id

It's my pleasure to inform you that, after the peer review, your paper
(manuscript ID: MH20230316080332-R1):

"**Corn Substitution Through Na-Glutamate and Neurospora Species Supplementation in Cassava and Tofu Dregs**"

has been ACCEPTED to publish with Advances in Animal and Veterinary Sciences, (ISSN: 2307-8316). The journal is publishing original research articles and reviews including wide ranging issues on: Agricultural and Biological Sciences (Animal Science and Zoology) and Veterinary ((Veterinary (miscellaneous)).
It will be published in upcoming issue of **Volume 11, Issue 6 (2023)**.

ACCEPTED	~~REVISIONS REQUIRED~~	~~REJECTED~~

Please do not hesitate to contact me if you have any further questions. Sincerely,

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Dr Mohammed Rohaim
Editor-in-Chief of Advances in Animal and Veterinary Sciences

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I have addressed all of the errors with comments that were highlighted in the proofreading file, and I have provided response the changes and let me know if there are any further adjustments required.

I appreciate all the hard work and effort that the Editorial Office has put into this process, and I look forward to seeing the fina

Thank you for this opportunity.

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Research Article

Corn Substitution Through Na-Glutamate and Neurospora Species Supplementation in Cassava and Tofu Dregs

Munasik*, Titin Widiyastuti, Caribu Hadi Prayitno

Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, 53122, Indonesia.

Abstract | This research aims to increase the sustainability of feed availability by creating feed independence. Corn is the primary energy source for livestock, but when the price of corn rises and its supply declines, it becomes essential to find alternative ingredients to use in animal feed. This study explores the use of cassava (tapioca by-products) and tofu dregs as substitutes for corn through fermentation technology using *Neurospora* sp. This produces alternative feed ingredients with nutrient levels that are in balance with corn. To improve the quality of the cassava-tofu dreg mixture, this study used red oncom mushrooms enriched with Na-glutamate during the fermentation process. The research used a completely randomized factorial design, where two factors were considered: the addition level of Na-glutamate (0%, 0.5%, 1%, and 1.5%), and the addition level of red oncom mushrooms (0%, 5%, 10%, and 15%). Each treatment was repeated twice, followed by an orthogonal polynomial test. The variables observed were nutrient contents, including moisture, crude fat, crude protein, crude fiber, NFE, calcium and phosphor, and anti-nutritional phytic acid. The results showed that the fermentation process using red oncom mushrooms enriched with Na-glutamate improved the nutritional level of the cassava-tofu dreg mixture. Although the NFE content decreased, the crude fiber content increased, and the amounts of calcium, phosphorus, and protein significantly increased ($P < 0.05$). Furthermore, fermentation reduced the phytic acid content to 90.83% at 1.5% Na-glutamate and 15% red oncom mushroom level. In conclusion, this research demonstrates that the quality of cassava-tofu dreg mixture can be enhanced through fermentation using red oncom mushrooms enriched with Na-glutamate. This approach can increase the sustainability of feed availability and reduce the dependence on corn as the primary energy source for livestock.

Keywords | Alternative feed, Cassava dreg, Fermentation, Red *oncom* mushrooms, Tofu dreg

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Citation | Munasik, Widiyastuti T, Prayitno CH (2023). Corn substitution through na-glutamate and neurospora species supplementation in cassava and tofu dregs. Adv. Anim. Vet. Sci. 7(s1):XXX-XXX.
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ISSN (Online) | 2307-8316

INTRODUCTION

Corn is a primary source of energy for livestock and a major raw material in poultry feed, accounting for up to 50% of the ration. Yellow corn is particularly suitable for poultry feed because it contains essential amino acids, high energy content, and is a source of provitamin A, specifically carotenoids (Edi, 2021; Gupta *et al*., 2019). However, the use of corn as food and biofuels competes fiercely with its usage as animal feed, making it one of the most expensive commodities in the market. Therefore, it is necessary to identify alternative feed ingredients that are readily available, cost-effective, and have nutritional profiles nearly identical to corn.

Local feed ingredients from agro-industrial waste, such as cassava and tofu dregs, have great potential as poultry feed ingredients but are rarely employed in Indonesia (Khasanah *et al*., 2022). Cassava has high energy content similar to corn but low crude protein and high crude fiber, while tofu dregs have high crude protein but lack carotene, an essential nutrient for poultry (Riswandi *et al*., 2020; Zohari *et al*., 2020). Fermentation using red fermented peanut cake yeast can provide the carotene that is missing in the cassava-tofu dregs mixture since it contains *Neurospora* sp., a fungal species that produces carotene and grows easily on a (Gmoser *et al*., 2018; Zohari *et al*., 2020). Thus, the fermentation of cassava and tofu dregs using *Neurospora* sp. is expected to improve the nutritional value of these agro-industrial wastes and provide a viable alternative to expensive corn-based poultry feed.

The aim of this study is to determine the nutritional and antinutrient contents of fermented cassava and tofu dregs using *Neurospora* sp. as a substitute for corn-based poultry feed. The fermentation process is expected to increase the mineral content of the mixture, particularly calcium and phosphorus, and reduce the phytic acid content, which inhibits the bioavailability of minerals in monogastric animals (Feizollahi *et al*., 2021). The use of a balanced nutrient content, including the addition of Na-glutamate, a non-essential amino acid and salt compound, is expected to optimize the growth of *Neurospora* sp. and increase carotene production during fermentation (Rahayu *et al*., 2019). Ultimately, the findings of this study could provide valuable insights into the potential of cassava and tofu dregs as alternative feed ingredients for the poultry industry, which could contribute to sustainable livestock production in Indonesia.

MATERIALS AND METHODS

Research materials

The study was conducted in the Laboratory of Feedstuff Science, the Faculty of Animal Science, Jenderal Soedirman University, Purwokerto. The use of material in this study has been approved by the Animal Ethics Committee for Laboratory of Feedstuff Science, the Faculty of Animal Science, Jenderal Soedirman University, Purwokerto.

The materials employed in this study were cassava dreg, red *oncom* mushrooms containing *Neurospora* sp., tofu dreg, coconut water, distilled water, PDA media, and chemicals for analysis of calcium, phosphor, and phytic acid. Cassava dregs were obtained from tapioca processing industries in the Purwokerto area, while red *oncom* mushrooms containing *Neurospora* sp. were obtained from a local market in Purwokerto. Tofu dregs were obtained from a tofu processing industry in the Purwokerto area, and coconut water was obtained from young coconuts purchased at a local market in Purwokerto. Distilled water was obtained from the laboratory's water purification system, and PDA media used in the study were purchased from a commercial supplier.

The tools utilized was an autoclave, analytical balance, furnace, oven, and tools for analysis of Ca, P, and phytic acid from Animal Feedstuff Science Laboratory, Faculty of Animal Science, Jenderal Soedirman University.

A fermented mixture of cassava dreg and tofu dreg

Cassava dreg was added up to 75 g, 25 g of tofu dreg (a protein source), 15 ml of coconut water, and 200 ml of distilled water. The ingredients were mixed and sterilized by autoclaving at 121°C/15 min. After cooling, sprinkle the mixture with red *oncom* mushrooms containing *Neurospora* sp. according to treatment, transferred to a covered tray with a hole in the bottom, and incubated at 30°C for three days. The fermented material was dried for two days at 60°C, mashed, and taken as a sample for observation.

Research design and data analysis

The study was conducted using a completely randomized design with a factorial pattern i.e. factor (A) was the addition level of Na-glutamate; A1 (0%), A2 (0.5%), A3 (1%), A4 (1.5 %), while factor (B) was the addition level of red *oncom* mushrooms; B1 (0%), B2 (5%), B3 (10%), and B4 (15%), each treatment was repeated twice. The variables were nutrient contents (moisture content, crude fat, crude protein, crude fiber, BETN, calcium, and phosphorus) and phytic acid.

The research data obtained were analyzed using analysis of variance using Microsoft Excel ver. 2010. If the treatment has a significant effect, then proceed with the Orthogonal Polynomial Test (Maizar *et al*., 2022).

RESULTS AND DISCUSSION

The results showed that the fermentation of cassava-tofu dregs with red *oncom* mushrooms at a level of 5-15% which was enriched with Na-glutamate at a level of 0-1.5% resulted in changes in varying levels of nutrients (Table 1).

Moisture contents

Post-fermentation of cassava and tofu dregs showed a range of the lowest moisture contents in A3B3 to the highest in A3B4. The results showed that the interaction had no significant effect on moisture content. The addition of the Na-glutamate level had no significant effect, while the mushroom level had a significant effect ($P < 0.05$), with the orthogonal polynomial test results showing a very significant ($P < 0.05$) cubic response with the equation Y= 6.84 + 4.8 X - 0.87X2 + 0.038X3. The coefficient of determination R^2= 27.04%, indicating the effect of the treatment on the moisture contents of 27.04%. The treatment showed the highest moisture content at an inflection point 1 = (3.63, 14.63) at which 3.63% mushroom

Table 1: Nutrient Levels of post fermented cassava dreg-tofu dreg.

Treatment	Moisture	DM	Ash	CF	FC	CP	NFE
				(%)			
A1B1	6.25 ± 0.35	93.75 ± 0.35	4.53 ± 0.38	26.14 ± 0.86	1.81 ± 0.23	5.32 ± 0.16	55.96 ±1.53
A1B2	9.75 ± 9.55	90.25 ± 9.55	3.82 ± 1.17	26.36 ± 0.83	4.29 ± 0.05	6.41 ± 0.13	56.65 ± 0.87
A1B3	0[illegible]35	94.75 ± 0.35	3.17 ± 0.76	27.18 ± 0.47	4.15 ± 0.09	8.04 ± 0.42	52.23 ± 1.91
A1B4	5.75 ± 1.06	94.25 ± 1.06	4.78 ± 1.56	27.59 ± 0.31	5.41 ± 0.75	9.09 ± 0.35	47.38 ± 2.53
A2B1	10.35 ± 3.04	89.65 ± 3.04	4.81 ± 0.47	27.33 ± 0.13	4.68 ± 0.40	6.24 ± 0.34	46.61 ± 3.71
A2B2	18.00 ± 12.02	82.00 ± 12.02	6.44 ± 0.51	29.06 ± 1.24	5.17 ± 1.90	8.88 ± 0.25	32.47 ± 12.11
A2B3	6.50 ± 0.00	93.50 ± 0.00	4.82 ± 0.76	29.15 ± 0.38	6.51 ±0.22	9.27 ± 0.13	43.77 ± 1.22
A2B4	6.00 ± 0.71	94.00 ± 0.71	5.05 ± 1.09	29.53 ± 0.60	6.69 ± 0.04	10.62 ± 0.60	42.13 ± 0.79
A3B1	5.50 ± 0.71	94.50 ± 0.71	3.96 ± 0.34	27.51 ± 1.29	3.82 ± 0.73	7.32 ± 0.19	51.90 ± 1.46
A3B2	14.00 ± 9.90	86.00 ± 9.90	4.91 ± 0.67	30.78 ± 0.57	5.90 ± 2.4	9.69 ± 0.39	34.74 ± 6.68
A3B3	4.50 ± 1.41	95.50 ± 1.41	5.24 ± 0.08	30.88 ± 0.28	5.06 ± 1.89	10.54 ± 0.81	43.79 ± 3.91
A3B4	21.25 ± 3.89	78.75 ± 3.89	5.42 ± 0.71	32.40 ± 0.70	4.15 ± 0.67	13.25 ± 0.81	23.55 ± 6.79
A4B1	5.25 ± 0.35	94.75 ± 0.35	3.17 ± 0.73	27.97 ± 0.85	3.61 ± 0.94	6.28 ± 0.23	53.74 ± 0.70
A4B2	13.75 ± 6.01	86.25 ± 6.01	6.41 ± 1.26	29[illegible]38	4.61 ± 2.5	10.59 ±0.12	35.65 ± 5.74
A4B3	8.00 ± 2.12	92.00 ± 2.12	5.98 ± 0.14	30.98 ± 0.06	6.31 ± 0.61	10.45 ±0.83	38.29 ± 0.77
A4B4	14.75 ± 9.55	85.25± 9.55	5.27 ± 0.23	33.50 ± 1.26	5.87 ± 0.38	11.63 ±2.46	28.99 ± 13.41

was used with 14.63% water content. The moisture content then experienced the lowest decrease at an inflection point 2 = (11.57, 5.087) at which 11.57% mushroom was used with a moisture content of 5.087%.

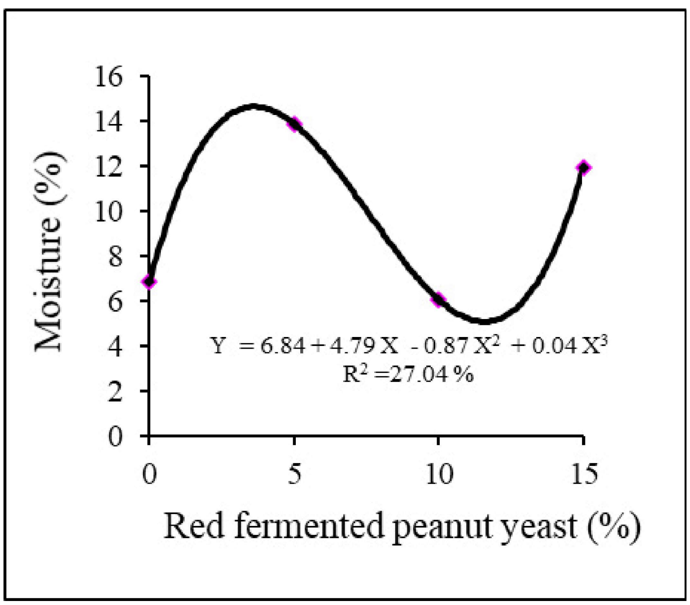

Figure 1: Moisture of post fermented cassava-tofu dreg.

The effect of adding red *oncom* mushrooms on the moisture and dry matter content of post-fermented cassava dregs - tofu dregs is shown in Figures 1 and 2.

Dry matter

Determining the DM content of feed provides a measure of the amount of a particular feed that is required to supply a set amount of nutrients to the animal (Singh, 2019). The dry matter content of post-fermented cassava dreg-tofu dregs showed the lowest range in A3B4 or the addition of 1% Na-glutamate and or 15% level of *oncom* mushrooms. The highest dry matter content in A3B3 treatment or the addition of 1% Na-glutamate level and 10% level of red *oncom* mushrooms. The interaction had no significant effect and it was also found that the addition of Na-glutamate during the fermentation process had no significant effect on dry matter content. Meanwhile, the addition of red *oncom* mushrooms had a significant effect on the dry matter content of post-fermented cassava-tofu dregs. Differences in fungal inoculum levels cause differences in dry matter levels caused by differences growth of red [illegible]om mushroom mycelium. This is in line with the report of Hasanuddin and Aidah (2021) that *Neurospora* sp. showed highest growth at 96-hour incubation at 10% concentration. The longer the incubation time, the more feed ingredients that can be overhauled by mold so that at the end of fermentation the dry matter will increase. Increases or decreases in feed DM content in the result in over or under feeding of nutrients. The results of the orthogonal polynomial test are cubic ($P < 0.01$) with the equation Y = 93.163 - 4.795 X + 0.87X2 - 0.038 X3. The coefficient of determination R^2 = 27.0394 %, Inflection point 1 = (3.63, 85.37), Inflection point 2 = (11.57, 94.91).

Crude fiber

The crude fiber content of post-fermented cassava dregs-tofu dregs showed the lowest in the A1B1 treatment and reached the highest in the A4B4 treatment. This showed that the higher the addition of Na-glutamate and mushrooms, the higher the crude fiber contents. The interaction had a significant effect ($P < 0.05$). The addition of red *oncom* mushroom levels had a significant linear effect on Na-glutamate levels of 0% ($P < 0.05$), 0.5% ($P < 0.05$), 1% ($P < 0.051$), and 1.5% ($P < 0.01$) on the crude fiber content of cassava-tofu dregs. With the coefficient of determination respectively R^2 = 58.9879 %, R^2 = 57.3036 %, R^2 = 77.8662 %, R^2 = 90.5948 %. The effect of the highest treatment was the addition of 15% mushroom level and 1.5% Na-glutamate. The higher the level of Na-glutamate supplementation and the level of red *oncom* mushrooms, the higher the crude fiber content. The high crude fiber is thought to be due to the relatively high mass of microbial cells, this is evidenced by the thicker growth of fungi and hyphae. This result is different from other studies, which state that *Neurospora* sp. has activity in reducing crude fiber (Matitaputty, 2018). The effect of red *oncom* mushroom addition on the crude fiber of post-fermented cassava-tofu dreg is shown in Figure 3.

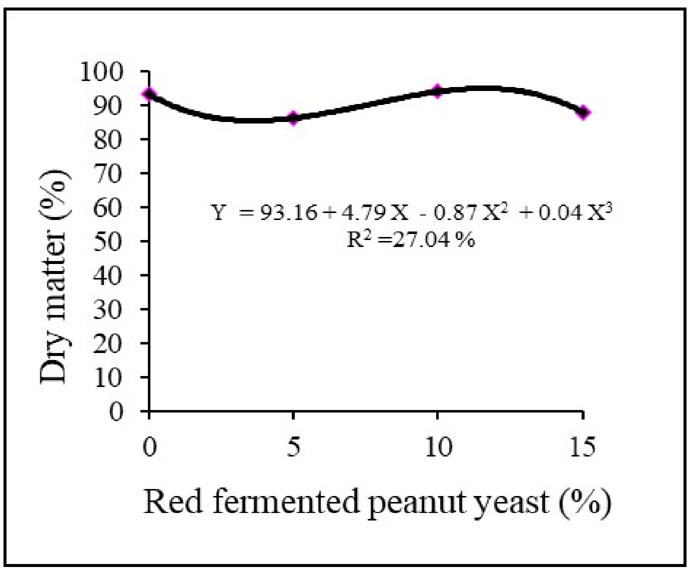

Figure 2: Dry matter of post fermented cassava-tofu dreg.

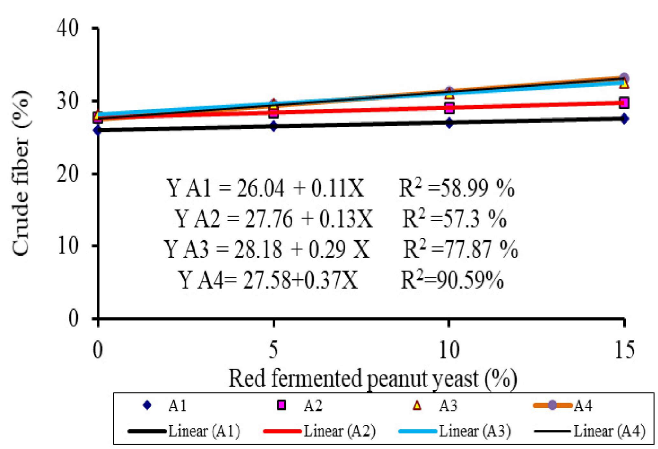

Figure 3: Effect of red fermented peanut cake yeast addition on the crude fiber of post fermented cassava-tofu dreg.

Crude fat

The results showed that the lowest fat content of post-fermented cassava-tofu dregs in A1B1 to the highest in A2B4. The interaction had no significant effect, while the addition of Na-glutamate had a significant ($P < 0.05$) effect on the fat content of cassava dregs-tofu dregs. Meanwhile, the level of red *oncom* mushroom had a highly significant ($P < 0.01$) effect on the fat contents of cassava-tofu dregs. The results of the orthogonal polynomial test showed that the effect of Na-glutamate is cubic with the equation $Y = 3.9125 + 9.41 X - 14.288 X2 + 5.693 X3$. With a coefficient of determination R^2 = 20.80%, this shows that the effect of Na-glutamate is only 20.80% on the fat content of post-fermented cassava-tofu dregs. The effect of adding Na-glutamate and red *oncom* mushroom to crude fat in post-fermented cassava-tofu dregs can be seen in Figures 4 and 5.

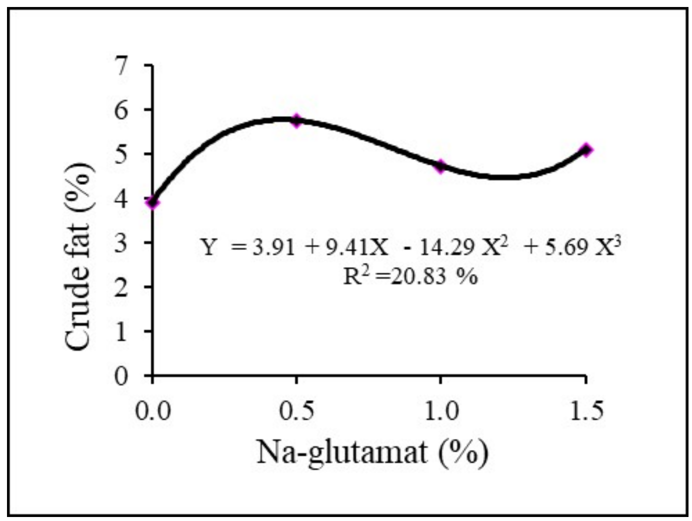

Figure 4: Effect Na-glutamate on the crude fat.

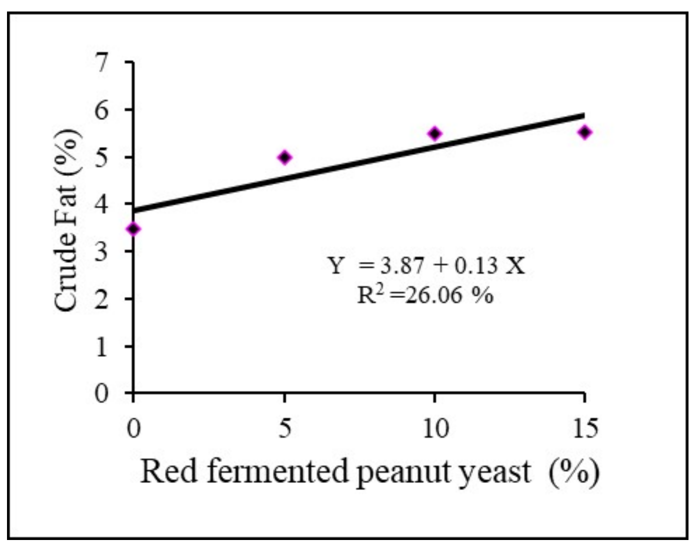

Figure 5: Effect of red fermented peanut on the crude fat.

While the effect of red *oncom* mushroom level is linear ($P < 0.01$), with the equation $Y = 3.8732500 + 0.13340000 X$, and the coefficient of determination R^2 = 26.0594% means that the effect of red oncom mushroom level on the fat content of post-fermented cassava-tofu dregs is by 26.06%, the effect outside the treatment was 73.96%.

Ash content

The results showed that the ash contents of post-fermented cassava-tofu dregs were the lowest in A4B1 up to the highest in A2B2. The interaction had a significant ($P < 0.05$) effect on the ash content of cassava-tofu dregs. The treatment response had a significant effect only on A4 which was quadratic, with the equation $Y = 3.3345 + 0.7114 X - 0.03960000 X2$ and the coefficient of determination $R^2 = 77.1736$ %, with the Maximum point (8.98, 6.53). This showed that the highest ash content was produced by red *oncom* mushrooms at a Na-glutamate level of 1.5%. The effect of adding red *oncom* mushrooms to the ash content of post-fermented cassava dregs-tofu dregs can be seen in Figure 6. High ash (mineral) content can be used by livestock for tissue growth, egg production, lactation and skeleton.

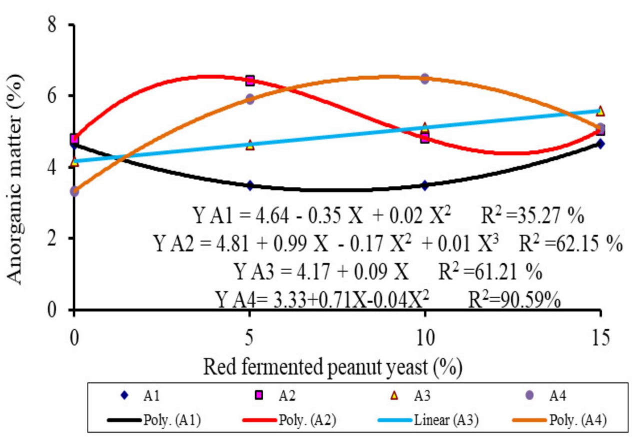

Figure 6: Effect of red fermented peanut cake yeast addition on the inorganic matter of post fermented cassava waste-tofu dreg.

Crude protein contents

Fermented cassava and tofu dregs have the lowest protein content in A1B1 and the highest in A3B4. Fermentation using red *oncom* mushrooms enriched with Na-glutamate has been shown to increase the crude protein content of the substrate. The protein content increases during the fermentation, due to the fact that the proteolytic activities of enzymes produced by microorganisms which increases the bioavailability of amino acids (Hasanuddin and Aidah, 2021). The sharpest increase of up to 149% was shown in fermentation using 1% Na-glutamate and 15% red oncom mushrooms. The results of the analysis of variance showed that the interaction had no significant effect on the crude protein content of post-fermented cassava-tofu dregs. Meanwhile, supplementation with Na-glutamate had a highly significant effect ($P < 0.01$) on increasing crude protein levels with a quadratic response, with a quadratic equation $Y = 7.12 + 4.80 X - 1.999 X2$. The coefficient of determination $R^2 = 25.71$%, meaning that the effect of Na-glutamate on increasing the crude protein of fermented cassava dregs and tofu dregs is 25.71%. The maximum point (1.20, 10.004) indicated that the optimum level of addition of Na-glutamate was 1.20% with a crude protein content of 10.004%. This treatment has an impact on improving the quality of feed ingredients. Changes in the feed from feed energy sources to protein sources have the potential to reduce feed prices. Hasanuddin and Aidah (2021) report that the increase in crude protein occurs due to the addition of inoculum capable of using the substrate for the growth and formation of microbial proteins during the fermentation process perfectly and the increase in crude protein substrate occurs as a result of nitrogen supplementation in the form of urea which is added when fermentation is carried out. In this study, the addition of Na-glutamate has the function of being a source of N for red oncom mushroom to multiply cells. The effect of adding Na-glutamate and fermented red *oncom* mushrooms on the crude protein of post-fermented cassava tofu dreg is shown in Figures 7 and 8.

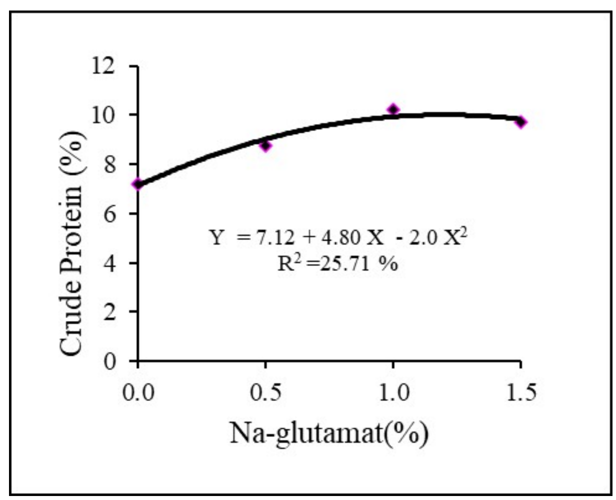

Figure 7: Effect of Na-glutamate on the crude protein.

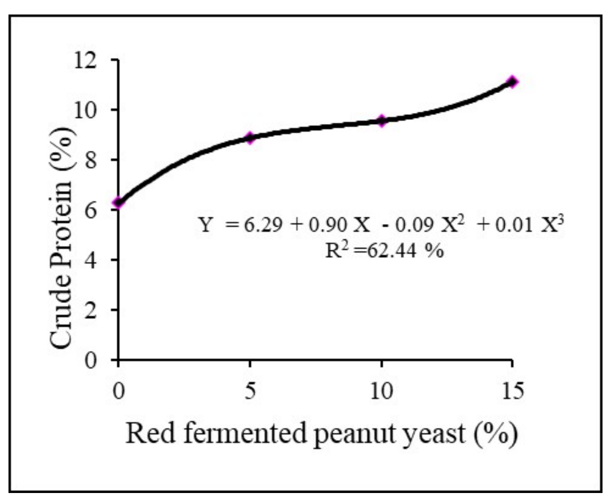

Figure 8: Effect of red fermented peanut cake yeast on the crude protein.

Meanwhile, the level of red *oncom* mushroom had a highly significant ($P < 0.01$) effect on the protein content of cassava-tofu dregs. With the test results of the cubic orthogonal polynomial with the equation Y = 6.2862500 + 0.89879167 X - 0.09440000 X2 + 0.00373833 X3, with the coefficient of determination R^2 = 62.44 % and inflection point 1st inflection point = (4.68, 8.81), 2nd inflection point = (12.014, 9.94). The addition of red *oncom* mushrooms had an effect of 62.44% with a pattern of changes in protein content increasing at the mushroom level of 4.68% with a crude protein content of 8.81% then decreasing and increasing again at the level of red *oncom* mushrooms of 12.014% with an optimum protein content of 9 .94%.

Nitrogen free extract (NFE) content

NFE levels of post-fermented cassava and tofu dregs were the lowest in A3B4 to the highest in A1B2. The decrease in NFE levels occurred along with the increase in Na-glutamate and red *oncom* mushrooms. This is presumably because the increase in several post-fermentation nutrients causes a decrease in NFE levels. The increase in crude fiber is primarily the most significant cause of the decrease in NFE. The interaction had no significant effect on NFE levels. Meanwhile, Na-glutamate levels had a highly significant ($P < 0.01$) effect on NFE levels in fermented cassava-tofu dregs with a quadratic response with the equation Y= 52.771562 - 27.609375 X + 12.483750 X2, Coefficient of determination R^2= 31.5056% and Minimum Point = (1.11, 37.51). This showed that the Na-glutamate level has an effect of 31.5056% on the NFE levels in the fermented cassava-dregs tofu dregs; with the optimum level of giving Na-glutamate 1.11% with the lowest NFE level of 37.51%. The effect of Na-glutamate and red *oncom* mushroom addition on the NFE of post-fermented cassava-tofu dreg is shown in Figures 9 and 10.

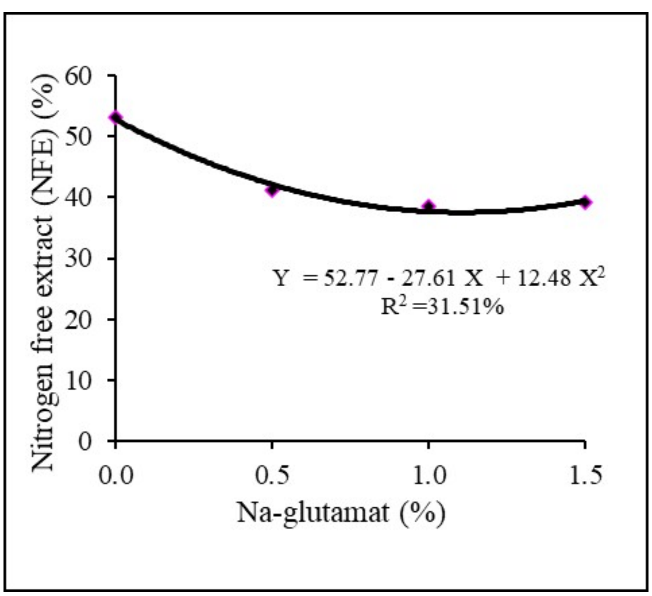

Figure 9: Effect of Na-glutamate on the NFE.

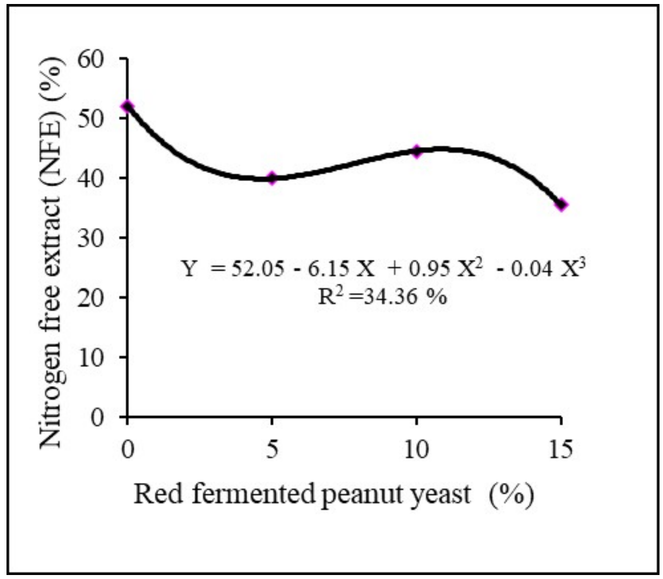

Figure 10: Effect of red fermented peanut cake yeast addition on the NFE.

Meanwhile, the addition of red *oncom* mushroom levels in the fermentation process had a highly significant ($P < 0.01$) effect on NFE levels in fermented cassava-tofu dregs, the results of the orthogonal polynomial test showed a cubic response with the equation Y = 52.05-6.15X + 0.95 X2 - 0.041 X3, coefficient of determination R^2 = 34.3596 %, Inflection point 1 (4.63, 39.83), Inflection point 2 = (10.89, 44.79).

Calcium, phosphor, and phytic acid contents

The results showed that the calcium content in the fermented cassava-tofu dregs was the lowest in A1B1 and the highest in A4B4, phosphor levels recorded low levels in A1B1 and highest levels in A4B4, while the levels of phytic acid were the lowest in A1B1 and the highest in A4B4. The results revealed an increase in calcium and phosphorus levels with increasing levels of red *oncom* mushrooms and Na-glutamate, but there was a decrease in phytic acid contents. The lowest levels of phytic acid were shown in A4B4. The interactions had a highly significant ($P < 0.01$) effect on increasing Ca and P levels and decreasing phytic acid. This showed that there is an increase in the availability of phosphorus, with a decrease in the content of phytic acid along with increasing levels of red *oncom* mushrooms and Na-glutamate supplementation. The contents of calcium, phosphor, and phytic acid in fermented cassava-tofu dreg with red *oncom* mushroom and Na-glutamate supplementation are shown in Table 2.

The lowest calcium levels were shown in A1B1 and the highest levels were with the addition of 1.5% Na-glutamate and 15% red *oncom* mushrooms. Fermentation of cassava-tofu dregs with red *oncom* mushrooms and Na-glutamate supplementation can increase calcium content by 62.12%. The interaction has a highly significant ($P < 0.01$) effect on increasing calcium. The highest response was the addition of 1.5% Na-glutamate and 15% red *oncom* mushrooms with the equation line YA4 = 0.464575 + 0.05665 X + 0.0855 X2 (R^2 = 99.6 %). The coefficient of determination showed that the effect of Na-glutamate supplementation and red *oncom* mushroom treatment of 15% is 99.6%. This means that fermentation is very effective in increasing calcium levels of post-fermented cassava-tofu dregs. Increased levels of calcium are caused by the breakdown of phytic acid which chelates calcium, where phytic acid is a strong chelate (mineral binding compound) that can bind divalent metal ions to form phytate complexes so that minerals cannot be absorbed by the body. These minerals are Ca, Zn, Cu, Mg, and Fe. Calcium concentrations from post-fermented cassava-tofu dregs with levels of Na-glutamate (A) and levels of red *oncom* mushrooms (B) can be seen in Figure 11.

Table 2: Content of calcium, phosphor, and phytic acid in fermented cassava dreg-tofu dreg with red oncom mushrooms and Na-glutamate supplementation.

Treatment	Calcium (%)	Phosphor (%)	Phytic acid (%)
A1 B1	0.28 ± 0.01	0.14 ± 0.01	0.60 ± 0.01
A1 B2	0.32 ± 0.01	0.16 ± 0.00	0.51 ± 0.01
A1 B3	0.39 ± 0.01	0.20 ± 0.01	0.44 ± 0.01
A1 B4	0.46 ± 0.01	0.26 ± 0.01	0.35 ± 0.01
A2 B1	0.34 ± 0.01	0.16 ± 0.01	0.52 ± 0.01
A2 B2	0.38 ± 0.01	0.18 ± 0.00	0.42 ± 0.01
A2 B3	0.45 ± 0.01	0.22 ± 0.01	0.36 ± 0.01
A2 B4	0.52 ± 0.00	0.26 ± 0.01	0.25 ± 0.01
A3 B1	0.38 ± 0.00	0.19 ± 0.01	0.43 ± 0.00
A3 B2	0.46 ± 0.01	0.24 ± 0.01	0.35 ± 0.02
A3 B3	0.53 ± 0.00	0.29 ± 0.01	0.25 ± 0.00
A3 B4	0.60 ± 0.01	0.33 ± 0.01	0.15 ± 0.01
A4 B1	0.45 ± 0.01	0.24 ± 0.00	0.36 ± 0.01
A4 B2	0.54 ± 0.01	0.34 ± 0.05	0.24 ± 0.00
A4 B3	0.65 ± 0.00	0.48 ± 0.01	0.14 ± 0.01
A4 B4	0.74 ± 0.01	0.58 ± 0.01	0.06 ± 0.01

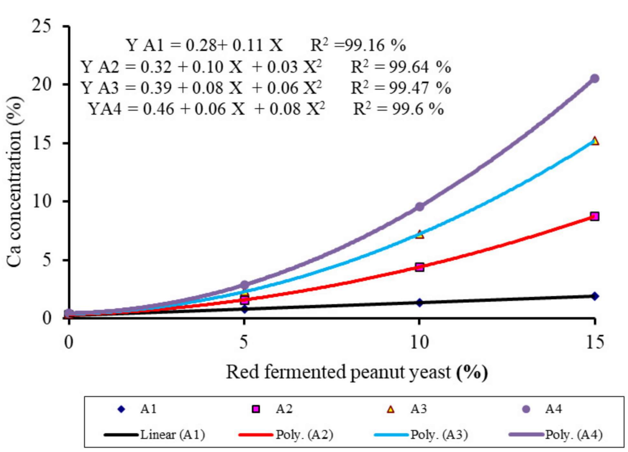

Figure 11: Calcium concentration of post-fermented cassava waste-tofu dreg with Na-glutamate level (A) and red fermented peanut cake yeast level (B).

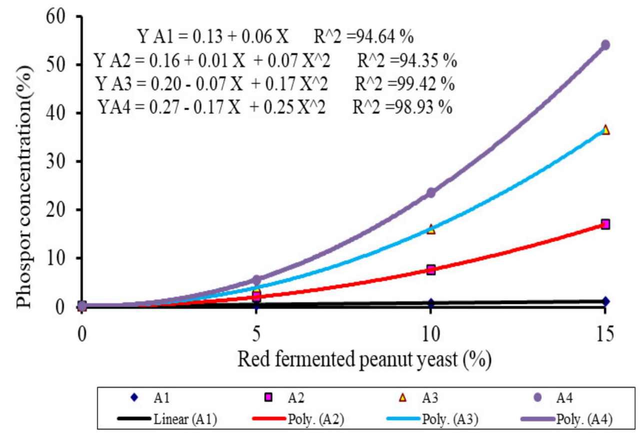

Figure 12: Phosphor concentration of post-fermentation cassava waste tofu dreg with Na-glutamate level (A) and red fermented peanut cake yeast level (B).

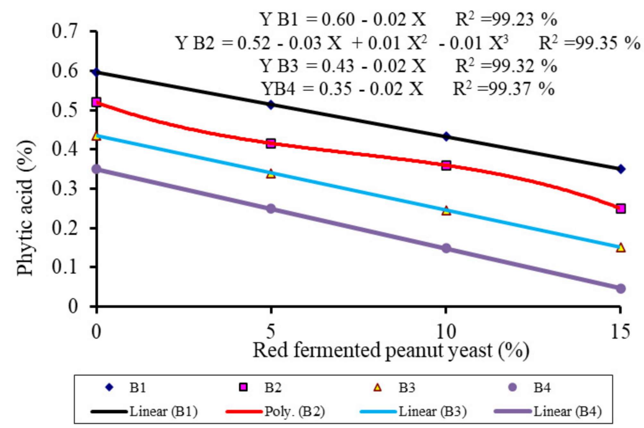

Figure 13: Cassava waste-tofu dreg phytic acid content post-fermentation with different levels of Na-glutamate (A) and level of red fermented peanut cake yeast (B).

The interaction had a highly significant effect on the phosphor content of post-fermented cassava and tofu dregs ($P < 0.01$). The results of the orthogonal polynomial test showed a quadratic response with the equation YA4 = 0.26775 - 0.168 X + 0.25 X2, the coefficient of determination is R^2 = 98.93% indicating that the treatment has an effect of 98.93%. The highest increase in phosphorus levels obtained post-fermentation was 75.77%. An increase in phosphorus levels indicated that the fermentation process has succeeded in changing the bound phosphorus to become more available due to the presence of the phytase enzyme produced by the mushrooms. *Neurospora* sp. that grows on red *oncom* mushrooms has the potential to produce phytase enzymes (Kanti and Sudiana, 2018; Umboh and Rampe, 2019; Wikandari *et al.*, 2022). The addition of Na-glutamate can also provide a source of sodium and non-essential amino acids in the growth of red oncom mushrooms.

The highest decrease in phytic acid was shown in the interaction of 1.5% Na-glutamate and 15% red *oncom* mushrooms with a linear response YB4 = 0.34985 - 0.02018 X, (R^2 = 99.37%). This showed that the higher the Na-glutamate level and the red *oncom* mushrooms in the fermentation, the higher the decrease in phytic acid. The decrease in phytic acid indicated that fermentation with *oncom* mushrooms supplemented with Na-glutamate was effective in breaking down mineral-phytic acid bonds. Phytic acid is an anti-nutritional substance because it can bind to minerals which causes the solubility of these minerals to decrease so that the availability of minerals becomes low. Under natural conditions, phytic acid will form bonds with both divalent minerals (Ca, Mg, and Fe) and proteins into compounds that are difficult to dissolve. This negatively influences the absorption of minerals and proteins. Therefore, phytic acid is considered an anti-nutritional food ingredient (Jatuwong *et al.*, 2020). The results showed that fermentation using red *oncom* mushrooms could reduce phytic acid by up to 90.83% (A1B1) to 0.0550 ± 0.0099 % (A4B4). The high decrease in phytic acid indicated the high phytase enzyme produced by the red *oncom* mushroom during fermentation. As stated by Wikandari *et al.* (2022) that in fermentation with tofu dregs substrate, *Neurospora sitophila* produced the highest phytase enzyme activity compared to *Aspergillus niger* and *Rhizopus oligosphorus*.

CONCLUSION AND RECOMMENDATION

Fermentation using red oncom mushrooms enriched with Na-glutamate has been found to improve the quality of cassava-tofu dregs. Although there is a decrease in some nutrient levels, such as NFE, the crude fiber content increases. On the other hand, there are significant increases in protein content, calcium, and phosphorus levels. Additionally, the fermentation process reduces phytic acid levels by up to 90.83% at 1.5% Na-glutamate level and 15% red oncom mushroom level.

To optimize these research findings, further studies are necessary to evaluate the *in-vivo* bioavailability of cassava and tofu dregs in both poultry and ruminants. This will help to determine if fermented cassava and tofu dregs with red oncom mushrooms can serve as a viable substitute for conventional carbohydrate sources like corn or bran. Such an alternative approach could improve the sustainability of feed availability and reduce the reliance on corn as the primary energy source for livestock.

ACKNOWLEDGEMENTS

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NOVELTY STATEMENT

The present study shows that the sustainability of feed ingredients for livestock (chicken and cow) can be realized by using other feed ingredients that are cheaper and widely available besides corn, namely using a mixture of cassava dregs - tofu dregs fermented with Na-glutamate and red oncom mushrooms containing *Neurospora* sp.

AUTHOR'S CONTRIBUTION

M and TW designed the concept, conducted data analysis, and wrote the manuscript. CHP reviewed the paper. All authors participated in the experimental design, and read, and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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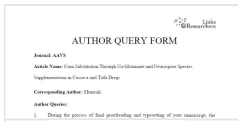

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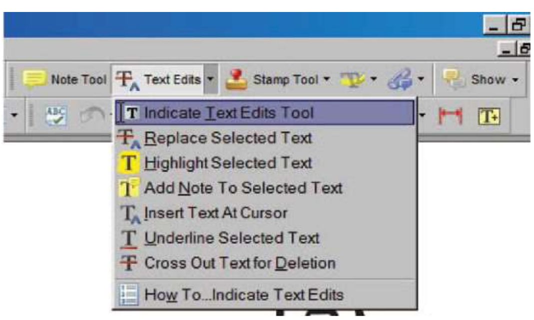

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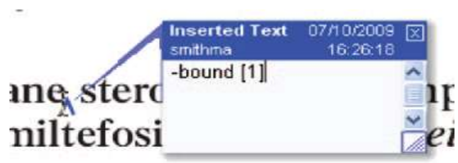

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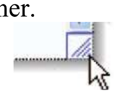

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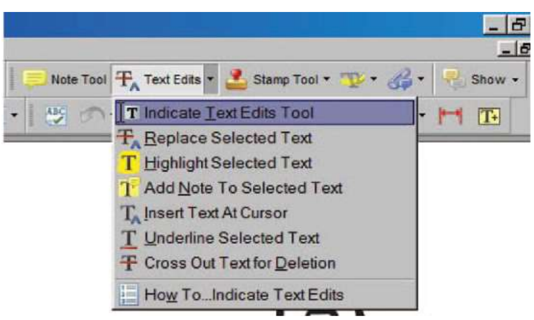

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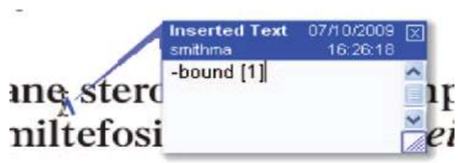

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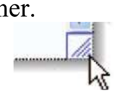

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Research Article

Corn Substitution Through Na-Glutamate and Neurospora Species Supplementation in Cassava and Tofu Dregs

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Abstract | This research aims to increase the sustainability of feed availability by creating feed independence. Corn is the primary energy source for livestock, but when the price of corn rises and its supply declines, it becomes essential to find alternative ingredients to use in animal feed. This study explores the use of cassava (tapioca by-products) and tofu dregs as substitutes for corn through fermentation technology using *Neurospora* sp. This produces alternative feed ingredients with nutrient levels that are in balance with corn. To improve the quality of the cassava-tofu dreg mixture, this study used red oncom mushrooms enriched with Na-glutamate during the fermentation process. The research used a completely randomized factorial design, where two factors were considered: the addition level of Na-glutamate (0%, 0.5%, 1%, and 1.5%), and the addition level of red oncom mushrooms (0%, 5%, 10%, and 15%). Each treatment was repeated twice, followed by an orthogonal polynomial test. The variables observed were nutrient contents, including moisture, crude fat, crude protein, crude fiber, NFE, calcium and phosphor, and anti-nutritional phytic acid. The results showed that the fermentation process using red oncom mushrooms enriched with Na-glutamate improved the nutritional level of the cassava-tofu dreg mixture. Although the NFE content decreased, the crude fiber content increased, and the amounts of calcium, phosphorus, and protein significantly increased ($P < 0.05$). Furthermore, fermentation reduced the phytic acid content to 90.83% at 1.5% Na-glutamate and 15% red oncom mushroom level. In conclusion, this research demonstrates that the quality of cassava-tofu dreg mixture can be enhanced through fermentation using red oncom mushrooms enriched with Na-glutamate. This approach can increase the sustainability of feed availability and reduce the dependence on corn as the primary energy source for livestock.

Keywords | Alternative feed, Cassava dreg, Fermentation, Red *oncom* mushrooms, Tofu dreg

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INTRODUCTION

Corn is a primary source of energy for livestock and a major raw material in poultry feed, accounting for up to 50% of the ration. Yellow corn is particularly suitable for poultry feed because it contains essential amino acids, high energy content, and is a source of provitamin A, specifically carotenoids (Edi, 2021; Gupta *et al*., 2019). However, the use of corn as food and biofuels competes fiercely with its usage as animal feed, making it one of the most expensive commodities in the market. Therefore, it is necessary to identify alternative feed ingredients that are readily available, cost-effective, and have nutritional profiles nearly identical to corn.

Local feed ingredients from agro-industrial waste, such as cassava and tofu dregs, have great potential as poultry feed ingredients but are rarely employed in Indonesia (Khasanah *et al*., 2022). Cassava has high energy content similar to corn but low crude protein and high crude fiber, while tofu dregs have high crude protein but lack carotene, an essential nutrient for poultry (Riswandi *et al*., 2020; Zohari *et al*., 2020). Fermentation using red fermented peanut cake yeast can provide the carotene that is missing in the cassava-tofu dregs mixture since it contains *Neurospora* sp., a fungal species that produces carotene and grows easily on a (Gmoser *et al*., 2018; Zohari *et al*., 2020). Thus, the fermentation of cassava and tofu dregs using *Neurospora* sp. is expected to improve the nutritional value of these agro-industrial wastes and provide a viable alternative to expensive corn-based poultry feed.

The aim of this study is to determine the nutritional and antinutrient contents of fermented cassava and tofu dregs using *Neurospora* sp. as a substitute for corn-based poultry feed. The fermentation process is expected to increase the mineral content of the mixture, particularly calcium and phosphorus, and reduce the phytic acid content, which inhibits the bioavailability of minerals in monogastric animals (Feizollahi *et al*., 2021). The use of a balanced nutrient content, including the addition of Na-glutamate, a non-essential amino acid and salt compound, is expected to optimize the growth of *Neurospora* sp. and increase carotene production during fermentation (Rahayu *et al*., 2019). Ultimately, the findings of this study could provide valuable insights into the potential of cassava and tofu dregs as alternative feed ingredients for the poultry industry, which could contribute to sustainable livestock production in Indonesia.

MATERIALS AND METHODS

Research materials

The study was conducted in the Laboratory of Feedstuff Science, the Faculty of Animal Science, Jenderal Soedirman University, Purwokerto. The use of material in this study has been approved by the Animal Ethics Committee for Laboratory of Feedstuff Science, the Faculty of Animal Science, Jenderal Soedirman University, Purwokerto.

The materials employed in this study were cassava dreg, red *oncom* mushrooms containing *Neurospora* sp., tofu dreg, coconut water, distilled water, PDA media, and chemicals for analysis of calcium, phosphor, and phytic acid. Cassava dregs were obtained from tapioca processing industries in the Purwokerto area, while red oncom mushrooms containing *Neurospora* sp. were obtained from a local market in Purwokerto. Tofu dregs were obtained from a tofu processing industry in the Purwokerto area, and coconut water was obtained from young coconuts purchased at a local market in Purwokerto. Distilled water was obtained from the laboratory's water purification system, and PDA media used in the study were purchased from a commercial supplier.

The tools utilized was an autoclave, analytical balance, furnace, oven, and tools for analysis of Ca, P, and phytic acid from Animal Feedstuff Science Laboratory, Faculty of Animal Science, Jenderal Soedirman University.

A fermented mixture of cassava dreg and tofu dreg

Cassava dreg was added up to 75 g, 25 g of tofu dreg (a protein source), 15 ml of coconut water, and 200 ml of distilled water. The ingredients were mixed and sterilized by autoclaving at 121°C/15 min. After cooling, sprinkle the mixture with red *oncom* mushrooms containing *Neurospora* sp. according to treatment, transferred to a covered tray with a hole in the bottom, and incubated at 30°C for three days. The fermented material was dried for two days at 60°C, mashed, and taken as a sample for observation.

Research design and data analysis

The study was conducted using a completely randomized design with a factorial pattern i.e. factor (A) was the addition level of Na-glutamate; A1 (0%), A2 (0.5%), A3 (1%), A4 (1.5 %), while factor (B) was the addition level of red *oncom* mushrooms; B1 (0%), B2 (5%), B3 (10%), and B4 (15%), each treatment was repeated twice. The variables were nutrient contents (moisture content, crude fat, crude protein, crude fiber, BETN, calcium, and phosphorus) and phytic acid.

The research data obtained were analyzed using analysis of variance using Microsoft Excel ver. 2010. If the treatment has a significant effect, then proceed with the Orthogonal Polynomial Test (Maizar *et al*., 2022).

RESULTS AND DISCUSSION

The results showed that the fermentation of cassava-tofu dregs with red *oncom* mushrooms at a level of 5-15% which was enriched with Na-glutamate at a level of 0-1.5% resulted in changes in varying levels of nutrients (Table 1).

Moisture contents

Post-fermentation of cassava and tofu dregs showed a range of the lowest moisture contents in A3B3 to the highest in A3B4. The results showed that the interaction had no significant effect on moisture content. The addition of the Na-glutamate level had no significant effect, while the mushroom level had a significant effect ($P < 0.05$), with the orthogonal polynomial test results showing a very significant ($P < 0.05$) cubic response with the equation Y= 6.84 + 4.8 X - 0.87X2 + 0.038X3. The coefficient of determination R^2= 27.04%, indicating the effect of the treatment on the moisture contents of 27.04%. The treatment showed the highest moisture content at an inflection point 1 = (3.63, 14.63) at which 3.63% mushroom

Table 1: Nutrient Levels of post fermented cassava dreg-tofu dreg.

Treatment	Moisture	DM	Ash	CF	FC	CP	NFE
				(%)			
A1B1	6.25 ± 0.35	93.75 ± 0.35	4.53 ± 0.38	26.14 ± 0.86	1.81 ± 0.23	5.32 ± 0.16	55.96 ±1.53
A1B2	9.75 ± 9.55	90.25 ± 9.55	3.82 ± 1.17	26.36 ± 0.83	4.29 ± 0.05	6.41 ± 0.13	56.65 ± 0.87
A1B3	0.35	94.75 ± 0.35	3.17 ± 0.76	27.18 ± 0.47	4.15 ± 0.09	8.04 ± 0.42	52.23 ± 1.91
A1B4	5.75 ± 1.06	94.25 ± 1.06	4.78 ± 1.56	27.59 ± 0.31	5.41 ± 0.75	9.09 ± 0.35	47.38 ± 2.53
A2B1	10.35 ± 3.04	89.65 ± 3.04	4.81 ± 0.47	27.33 ± 0.13	4.68 ± 0.40	6.24 ± 0.34	46.61 ± 3.71
A2B2	18.00 ± 12.02	82.00 ± 12.02	6.44 ± 0.51	29.06 ± 1.24	5.17 ± 1.90	8.88 ± 0.25	32.47 ± 12.11
A2B3	6.50 ± 0.00	93.50 ± 0.00	4.82 ± 0.76	29.15 ± 0.38	6.51 ±0.22	9.27 ± 0.13	43.77 ± 1.22
A2B4	6.00 ± 0.71	94.00 ± 0.71	5.05 ± 1.09	29.53 ± 0.60	6.69 ± 0.04	10.62 ± 0.60	42.13 ± 0.79
A3B1	5.50 ± 0.71	94.50 ± 0.71	3.96 ± 0.34	27.51 ± 1.29	3.82 ± 0.73	7.32 ± 0.19	51.90 ± 1.46
A3B2	14.00 ± 9.90	86.00 ± 9.90	4.91 ± 0.67	30.78 ± 0.57	5.90 ± 2.4	9.69 ± 0.39	34.74 ± 6.68
A3B3	4.50 ± 1.41	95.50 ± 1.41	5.24 ± 0.08	30.88 ± 0.28	5.06 ± 1.89	10.54 ± 0.81	43.79 ± 3.91
A3B4	21.25 ± 3.89	78.75 ± 3.89	5.42 ± 0.71	32.40 ± 0.70	4.15 ± 0.67	13.25 ± 0.81	23.55 ± 6.79
A4B1	5.25 ± 0.35	94.75 ± 0.35	3.17 ± 0.73	27.97 ± 0.85	3.61 ± 0.94	6.28 ± 0.23	53.74 ± 0.70
A4B2	13.75 ± 6.01	86.25 ± 6.01	6.41 ± 1.26	29 ± 0.38	4.61 ± 2.5	10.59 ±0.12	35.65 ± 5.74
A4B3	8.00 ± 2.12	92.00 ± 2.12	5.98 ± 0.14	30.98 ± 0.06	6.31 ± 0.61	10.45 ±0.83	38.29 ± 0.77
A4B4	14.75 ± 9.55	85.25± 9.55	5.27 ± 0.23	33.50 ± 1.26	5.87 ± 0.38	11.63 ±2.46	28.99 ± 13.41

was used with 14.63% water content. The moisture content then experienced the lowest decrease at an inflection point 2 = (11.57, 5.087) at which 11.57% mushroom was used with a moisture content of 5.087%.

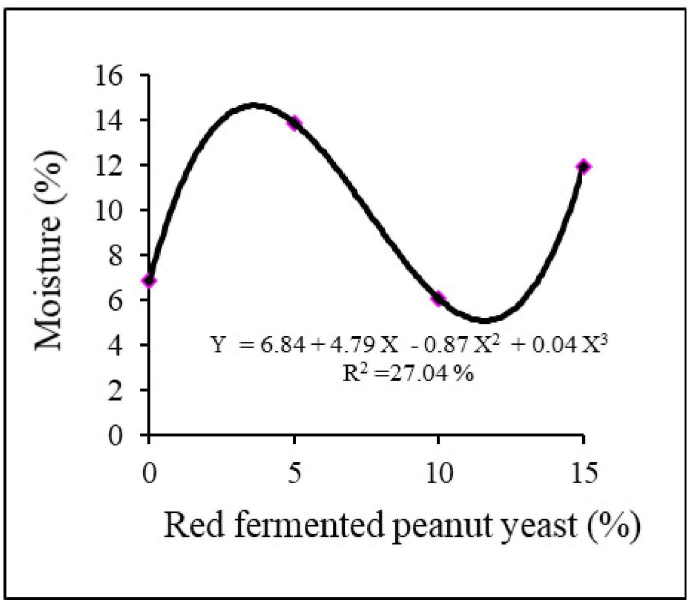

Figure 1: Moisture of post fermented cassava-tofu dreg.

The effect of adding red *oncom* mushrooms on the moisture and dry matter content of post-fermented cassava dregs - tofu dregs is shown in Figures 1 and 2.

Dry matter

Determining the DM content of feed provides a measure of the amount of a particular feed that is required to supply a set amount of nutrients to the animal (Singh, 2019). The dry matter content of post-fermented cassava dreg-tofu dregs showed the lowest range in A3B4 or the addition of 1% Na-glutamate and or 15% level of *oncom* mushrooms. The highest dry matter content in A3B3 treatment or the addition of 1% Na-glutamate level and 10% level of red *oncom* mushrooms. The interaction had no significant effect and it was also found that the addition of Na-glutamate during the fermentation process had no significant effect on dry matter content. Meanwhile, the addition of red *oncom* mushrooms had a significant effect on the dry matter content of post-fermented cassava-tofu dregs. Differences in fungal inoculum levels cause differences in dry matter levels caused by differences growth of red oncom mushroom mycelium. This is in line with the report of Hasanuddin and Aidah (2021) that *Neurospora* sp. showed highest growth at 96-hour incubation at 10% concentration. The longer the incubation time, the more feed ingredients that can be overhauled by mold so that at the end of fermentation the dry matter will increase. Increases or decreases in feed DM content in the result in over or under feeding of nutrients. The results of the orthogonal polynomial test are cubic ($P < 0.01$) with the equation Y = 93.163 - 4.795 X + 0.87X2 - 0.038 X3. The coefficient of determination R^2 = 27.0394 %, Inflection point 1 = (3.63, 85.37), Inflection point 2 = (11.57, 94.91).

Crude fiber

The crude fiber content of post-fermented cassava dregs-tofu dregs showed the lowest in the A1B1 treatment and reached the highest in the A4B4 treatment. This showed that the higher the addition of Na-glutamate and mushrooms, the higher the crude fiber contents. The interaction had a significant effect ($P < 0.05$). The addition of red *oncom* mushroom levels had a significant linear effect on Na-glutamate levels of 0% ($P < 0.05$), 0.5% ($P < 0.05$), 1% ($P < 0.051$), and 1.5% ($P < 0.01$) on the crude fiber content of cassava-tofu dregs. With the coefficient of determination respectively R^2 = 58.9879 %, R^2 = 57.3036 %, R^2 = 77.8662 %, R^2 = 90.5948 %. The effect of the highest treatment was the addition of 15% mushroom level and 1.5% Na-glutamate. The higher the level of Na-glutamate supplementation and the level of red *oncom* mushrooms, the higher the crude fiber content. The high crude fiber is thought to be due to the relatively high mass of microbial cells, this is evidenced by the thicker growth of fungi and hyphae. This result is different from other studies, which state that *Neurospora* sp. has activity in reducing crude fiber (Matitaputty, 2018). The effect of red *oncom* mushroom addition on the crude fiber of post-fermented cassava-tofu dreg is shown in Figure 3.

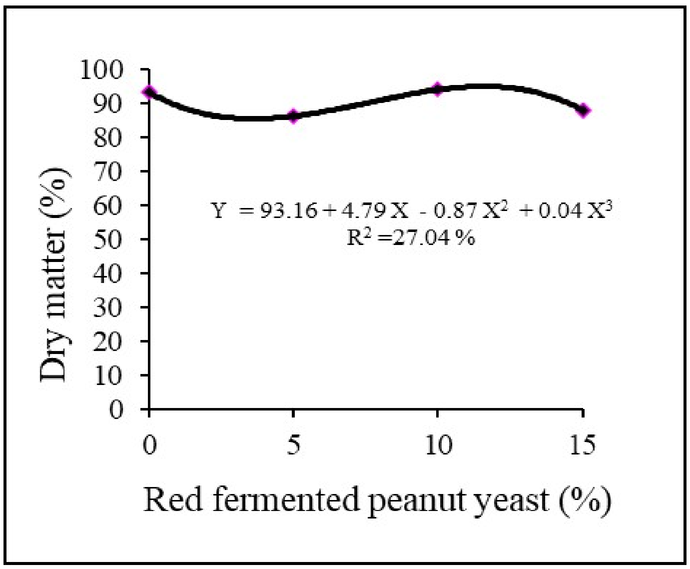

Figure 2: Dry matter of post fermented cassava-tofu dreg.

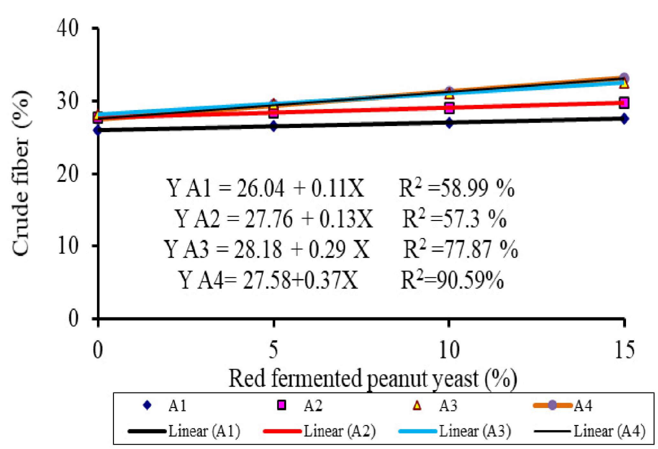

Figure 3: Effect of red fermented peanut cake yeast addition on the crude fiber of post fermented cassava-tofu dreg.

Crude fat

The results showed that the lowest fat content of post-fermented cassava-tofu dregs in A1B1 to the highest in A2B4. The interaction had no significant effect, while the addition of Na-glutamate had a significant ($P < 0.05$) effect on the fat content of cassava dregs-tofu dregs. Meanwhile, the level of red *oncom* mushroom had a highly significant ($P < 0.01$) effect on the fat contents of cassava-tofu dregs. The results of the orthogonal polynomial test showed that the effect of Na-glutamate is cubic with the equation $Y = 3.9125 + 9.41 X - 14.288 X2 + 5.693 X3$. With a coefficient of determination R^2 = 20.80%, this shows that the effect of Na-glutamate is only 20.80% on the fat content of post-fermented cassava-tofu dregs. The effect of adding Na-glutamate and red oncom mushroom to crude fat in post-fermented cassava-tofu dregs can be seen in Figures 4 and 5.

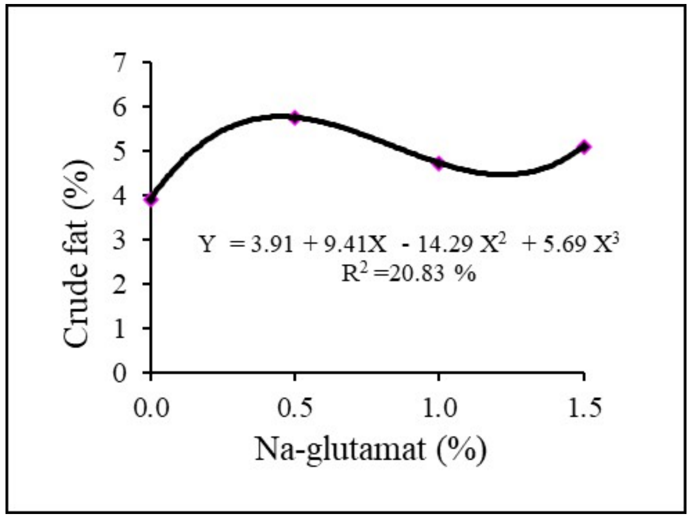

Figure 4: Effect Na-glutamate on the crude fat.

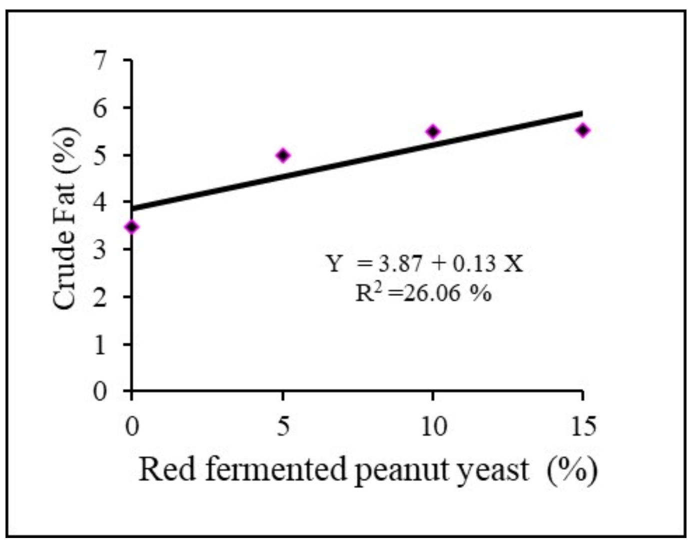

Figure 5: Effect of red fermented peanut on the crude fat.

While the effect of red *oncom* mushroom level is linear ($P < 0.01$), with the equation $Y = 3.8732500 + 0.13340000 X$, and the coefficient of determination R^2 = 26.0594% means that the effect of red oncom mushroom level on the fat content of post-fermented cassava-tofu dregs is by 26.06%, the effect outside the treatment was 73.96%.

Ash content

The results showed that the ash contents of post-fermented cassava-tofu dregs were the lowest in A4B1 up to the highest in A2B2. The interaction had a significant ($P < 0.05$) effect on the ash content of cassava-tofu dregs. The treatment response had a significant effect only on A4 which was quadratic, with the equation Y = 3.3345 + 0.7114 X - 0.03960000 X2 and the coefficient of determination R^2 = 77.1736 %, with the Maximum point (8.98, 6.53). This showed that the highest ash content was produced by red *oncom* mushrooms at a Na-glutamate level of 1.5%. The effect of adding red *oncom* mushrooms to the ash content of post-fermented cassava dregs-tofu dregs can be seen in Figure 6. High ash (mineral) content can be used by livestock for tissue growth, egg production, lactation and skeleton.

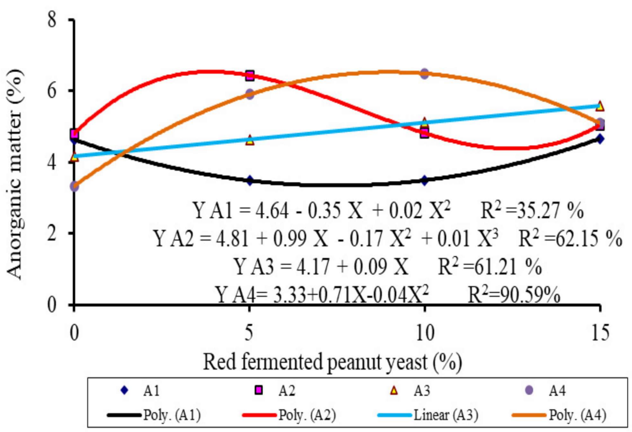

Figure 6: Effect of red fermented peanut cake yeast addition on the inorganic matter of post fermented cassava waste-tofu dreg.

Crude protein contents

Fermented cassava and tofu dregs have the lowest protein content in A1B1 and the highest in A3B4. Fermentation using red *oncom* mushrooms enriched with Na-glutamate has been shown to increase the crude protein content of the substrate. The protein content increases during the fermentation, due to the fact that the proteolytic activities of enzymes produced by microorganisms which increases the bioavailability of amino acids (Hasanuddin and Aidah, 2021). The sharpest increase of up to 149% was shown in fermentation using 1% Na-glutamate and 15% red oncom mushrooms. The results of the analysis of variance showed that the interaction had no significant effect on the crude protein content of post-fermented cassava-tofu dregs. Meanwhile, supplementation with Na-glutamate had a highly significant effect ($P < 0.01$) on increasing crude protein levels with a quadratic response, with a quadratic equation Y = 7.12 + 4.80 X - 1.999 X2. The coefficient of determination R^2 = 25.71%, meaning that the effect of Na-glutamate on increasing the crude protein of fermented cassava dregs and tofu dregs is 25.71%. The maximum point (1.20, 10.004) indicated that the optimum level of addition of Na-glutamate was 1.20% with a crude protein content of 10.004%. This treatment has an impact on improving the quality of feed ingredients. Changes in the feed from feed energy sources to protein sources have the potential to reduce feed prices. Hasanuddin and Aidah (2021) report that the increase in crude protein occurs due to the addition of inoculum capable of using the substrate for the growth and formation of microbial proteins during the fermentation process perfectly and the increase in crude protein substrate occurs as a result of nitrogen supplementation in the form of ureaw hich is added when fermentation is carried out. In this study, the addition of Na-glutamate has the function of being a source of N for red oncom mushroom to multiply cells. The effect of adding Na-glutamate and fermented red *oncom* mushrooms on the crude protein of post-fermented cassava tofu dreg is shown in Figures 7 and 8.

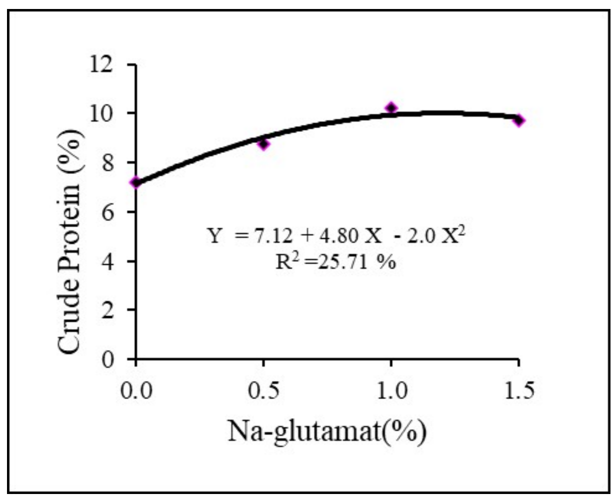

Figure 7: Effect of Na-glutamate on the crude protein.

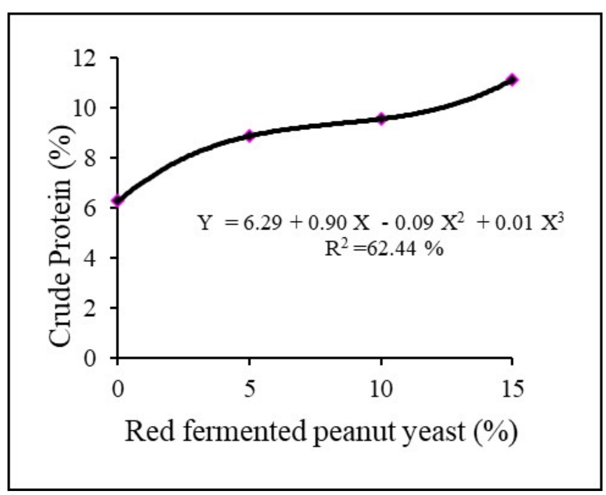

Figure 8: Effect of red fermented peanut cake yeast on the crude protein.

Meanwhile, the level of red *oncom* mushroom had a highly significant ($P < 0.01$) effect on the protein content of cassava-tofu dregs. With the test results of the cubic orthogonal polynomial with the equation Y = 6.2862500 + 0.89879167 X - 0.09440000 X2 + 0.00373833 X3, with the coefficient of determination R^2 = 62.44 % and inflection point 1st inflection point = (4.68, 8.81), 2nd inflection point = (12.014, 9.94). The addition of red *oncom* mushrooms had an effect of 62.44% with a pattern of changes in protein content increasing at the mushroom level of 4.68% with a crude protein content of 8.81% then decreasing and increasing again at the level of red oncom mushrooms of 12.014% with an optimum protein content of 9 .94%.

Nitrogen free extract (NFE) content

NFE levels of post-fermented cassava and tofu dregs were the lowest in A3B4 to the highest in A1B2. The decrease in NFE levels occurred along with the increase in Na-glutamate and red *oncom* mushrooms. This is presumably because the increase in several post-fermentation nutrients causes a decrease in NFE levels. The increase in crude fiber is primarily the most significant cause of the decrease in NFE. The interaction had no significant effect on NFE levels. Meanwhile, Na-glutamate levels had a highly significant ($P < 0.01$) effect on NFE levels in fermented cassava-tofu dregs with a quadratic response with the equation Y= 52.771562 - 27.609375 X + 12.483750 X2, Coefficient of determination R^2= 31.5056% and Minimum Point = (1.11, 37.51). This showed that the Na-glutamate level has an effect of 31.5056% on the NFE levels in the fermented cassava-dregs tofu dregs; with the optimum level of giving Na-glutamate 1.11% with the lowest NFE level of 37.51%. The effect of Na-glutamate and red *oncom* mushroom addition on the NFE of post-fermented cassava-tofu dreg is shown in Figures 9 and 10.

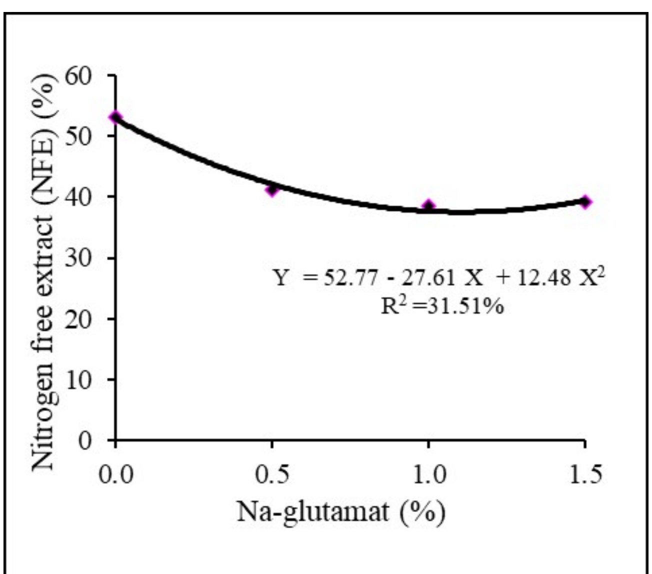

Figure 9: Effect of Na-glutamate on the NFE.

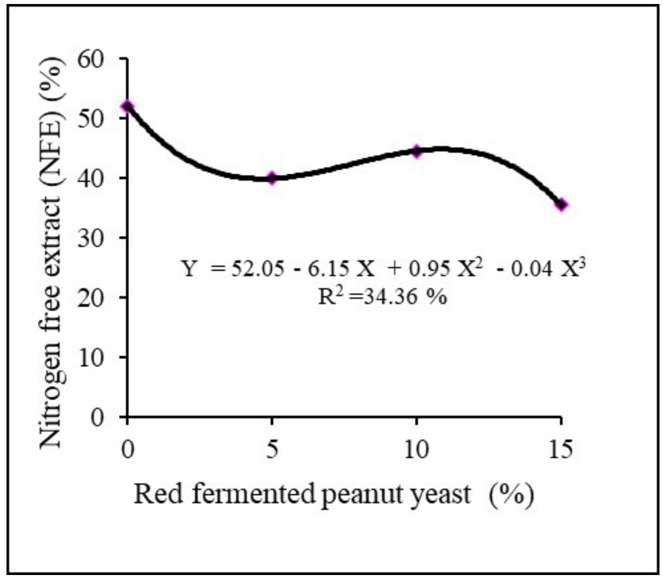

Figure 10: Effect of red fermented peanut cake yeast addition on the NFE.

Meanwhile, the addition of red *oncom* mushroom levels in the fermentation process had a highly significant ($P < 0.01$) effect on NFE levels in fermented cassava-tofu dregs, the results of the orthogonal polynomial test showed a cubic response with the equation Y = 52.05-6.15X + 0.95 X2 - 0.041 X3, coefficient of determination R^2 = 34.3596 %, Inflection point 1 (4.63, 39.83), Inflection point 2 = (10.89, 44.79).

Calcium, phosphor, and phytic acid contents

The results showed that the calcium content in the fermented cassava-tofu dregs was the lowest in A1B1 and the highest in A4B4, phosphor levels recorded low levels in A1B1 and highest levels in A4B4, while the levels of phytic acid were the lowest in A1B1 and the highest in A4B4. The results revealed an increase in calcium and phosphorus levels with increasing levels of red *oncom* mushrooms and Na-glutamate, but there was a decrease in phytic acid contents. The lowest levels of phytic acid were shown in A4B4. The interactions had a highly significant ($P < 0.01$) effect on increasing Ca and P levels and decreasing phytic acid. This showed that there is an increase in the availability of phosphorus, with a decrease in the content of phytic acid along with increasing levels of red *oncom* mushrooms and Na-glutamate supplementation. The contents of calcium, phosphor, and phytic acid in fermented cassava-tofu dreg with red *oncom* mushroom and Na-glutamate supplementation are shown in Table 2.

The lowest calcium levels were shown in A1B1 and the highest levels were with the addition of 1.5% Na-glutamate and 15% red *oncom* mushrooms. Fermentation of cassava-tofu dregs with red *oncom* mushrooms and Na-glutamate supplementation can increase calcium content by 62.12%. The interaction has a highly significant ($P < 0.01$) effect on increasing calcium. The highest response was the addition of 1.5% Na-glutamate and 15% red *oncom* mushrooms with the equation line YA4 = 0.464575 + 0.05665 X + 0.0855 X2 (R^2 = 99.6 %). The coefficient of determination showed that the effect of Na-glutamate supplementation and red *oncom* mushroom treatment of 15% is 99.6%. This means that fermentation is very effective in increasing calcium levels of post-fermented cassava-tofu dregs. Increased levels of calcium are caused by the breakdown of phytic acid which chelates calcium, where phytic acid is a strong chelate (mineral binding compound) that can bind divalent metal ions to form phytate complexes so that minerals cannot be absorbed by the body. These minerals are Ca, Zn, Cu, Mg, and Fe. Calcium concentrations from post-fermented cassava-tofu dregs with levels of Na-glutamate (A) and levels of red *oncom* mushrooms (B) can be seen in Figure 11.

Table 2: Content of calcium, phosphor, and phytic acid in fermented cassava dreg-tofu dreg with red oncom mushrooms and Na-glutamate supplementation.

Treatment	Calcium (%)	Phosphor (%)	Phytic acid (%)
A1 B1	0.28 ± 0.01	0.14 ± 0.01	0.60 ± 0.01
A1 B2	0.32 ± 0.01	0.16 ± 0.00	0.51 ± 0.01
A1 B3	0.39 ± 0.01	0.20 ± 0.01	0.44 ± 0.01
A1 B4	0.46 ± 0.01	0.26 ± 0.01	0.35 ± 0.01
A2 B1	0.34 ± 0.01	0.16 ± 0.01	0.52 ± 0.01
A2 B2	0.38 ± 0.01	0.18 ± 0.00	0.42 ± 0.01
A2 B3	0.45 ± 0.01	0.22 ± 0.01	0.36 ± 0.01
A2 B4	0.52 ± 0.00	0.26 ± 0.01	0.25 ± 0.01
A3 B1	0.38 ± 0.00	0.19 ± 0.01	0.43 ± 0.00
A3 B2	0.46 ± 0.01	0.24 ± 0.01	0.35 ± 0.02
A3 B3	0.53 ± 0.00	0.29 ± 0.01	0.25 ± 0.00
A3 B4	0.60 ± 0.01	0.33 ± 0.01	0.15 ± 0.01
A4 B1	0.45 ± 0.01	0.24 ± 0.00	0.36 ± 0.01
A4 B2	0.54 ± 0.01	0.34 ± 0.05	0.24 ± 0.00
A4 B3	0.65 ± 0.00	0.48 ± 0.01	0.14 ± 0.01
A4 B4	0.74 ± 0.01	0.58 ± 0.01	0.06 ± 0.01

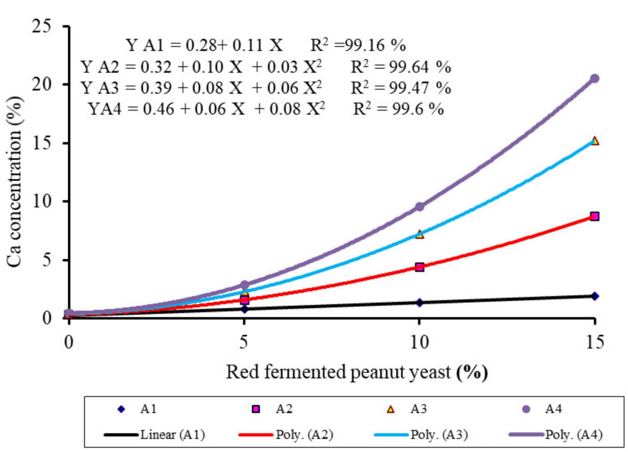

Figure 11: Calcium concentration of post-fermented cassava waste-tofu dreg with Na-glutamate level (A) and red fermented peanut cake yeast level (B).

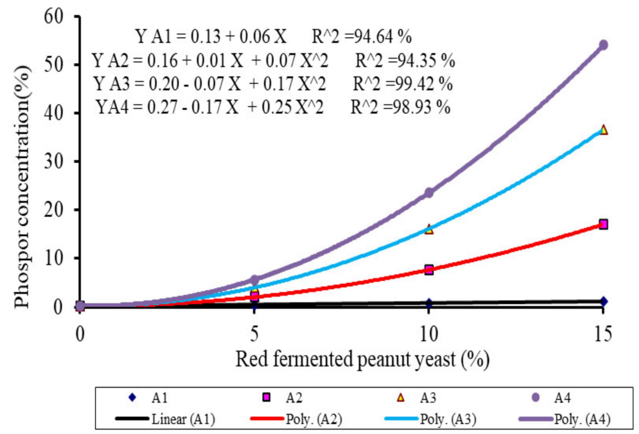

Figure 12: Phosphor concentration of post-fermentation cassava waste tofu dreg with Na-glutamate level (A) and red fermented peanut cake yeast level (B).

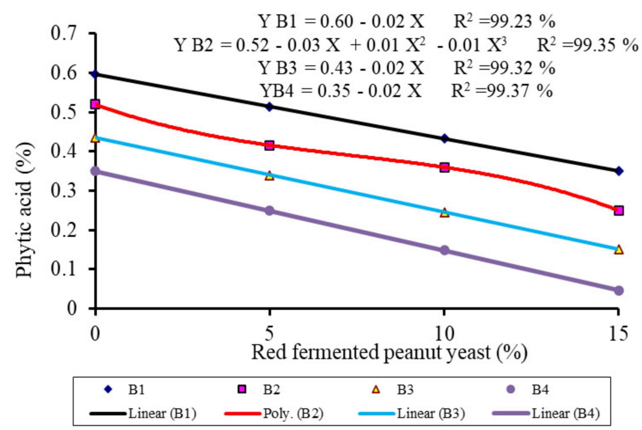

Figure 13: Cassava waste-tofu dreg phytic acid content post-fermentation with different levels of Na-glutamate (A) and level of red fermented peanut cake yeast (B).

The interaction had a highly significant effect on the phosphor content of post-fermented cassava and tofu dregs ($P < 0.01$). The results of the orthogonal polynomial test showed a quadratic response with the equation YA4 = 0.26775 - 0.168 X + 0.25 X2, the coefficient of determination is R^2 = 98.93% indicating that the treatment has an effect of 98.93%. The highest increase in phosphorus levels obtained post-fermentation was 75.77%. An increase in phosphorus levels indicated that the fermentation process has succeeded in changing the bound phosphorus to become more available due to the presence of the phytase enzyme produced by the mushrooms. *Neurospora* sp. that grows on red *oncom* mushrooms has the potential to produce phytase enzymes (Kanti and Sudiana, 2018; Umboh and Rampe, 2019; Wikandari *et al.*, 2022). The addition of Na-glutamate can also provide a source of sodium and non-essential amino acids in the growth of red oncom mushrooms.

The highest decrease in phytic acid was shown in the interaction of 1.5% Na-glutamate and 15% red *oncom* mushrooms with a linear response YB4 = 0.34985 - 0.02018 X, (R^2 = 99.37 %). This showed that the higher the Na-glutamate level and the red *oncom* mushrooms in the fermentation, the higher the decrease in phytic acid. The decrease in phytic acid indicated that fermentation with *oncom* mushrooms supplemented with Na-glutamate was effective in breaking down mineral-phytic acid bonds. Phytic acid is an anti-nutritional substance because it can bind to minerals which causes the solubility of these minerals to decrease so that the availability of minerals becomes low. Under natural conditions, phytic acid will form bonds with both divalent minerals (Ca, Mg, and Fe) and proteins into compounds that are difficult to dissolve. This negatively influences the absorption of minerals and proteins. Therefore, phytic acid is considered an anti-nutritional food ingredient (Jatuwong *et al*., 2020). The results showed that fermentation using red *oncom* mushrooms could reduce phytic acid by up to 90.83% (A1B1) to 0.0550 ± 0.0099 % (A4B4). The high decrease in phytic acid indicated the high phytase enzyme produced by the red *oncom* mushroom during fermentation. As stated by Wikandari *et al*. (2022) that in fermentation with tofu dregs substrate, *Neurospora sitophila* produced the highest phytase enzyme activity compared to *Aspergillus niger* and *Rhizopus oligosphorus*.

CONCLUSION AND RECOMMENDATION

Fermentation using red oncom mushrooms enriched with Na-glutamate has been found to improve the quality of cassava-tofu dregs. Although there is a decrease in some nutrient levels, such as NFE, the crude fiber content increases. On the other hand, there are significant increases in protein content, calcium, and phosphorus levels. Additionally, the fermentation process reduces phytic acid levels by up to 90.83% at 1.5% Na-glutamate level and 15% red oncom mushroom level.

To optimize these research findings, further studies are necessary to evaluate the *in-vivo* bioavailability of cassava and tofu dregs in both poultry and ruminants. This will help to determine if fermented cassava and tofu dregs with red oncom mushrooms can serve as a viable substitute for conventional carbohydrate sources like corn or bran. Such an alternative approach could improve the sustainability of feed availability and reduce the reliance on corn as the primary energy source for livestock.

ACKNOWLEDGEMENTS

We thank the Rector of Universitas Jenderal Soedirman for funding the applied research scheme.

NOVELTY STATEMENT

The present study shows that the sustainability of feed ingredients for livestock (chicken and cow) can be realized by using other feed ingredients that are cheaper and widely available besides corn, namely using a mixture of cassava dregs - tofu dregs fermented with Na-glutamate and red oncom mushrooms containing *Neurospora* sp.

AUTHOR'S CONTRIBUTION

M and TW designed the concept, conducted data analysis, and wrote the manuscript. CHP reviewed the paper. All authors participated in the experimental design, and read, and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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Corn Substitution Through Na-Glutamate and Neurospora Species Supplementation in Cassava and Tofu Dregs

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Abstract: ~~The long-term goal of the research is to create feed independence by increasing the sustainability of feed availability. The primary energy source for livestock is corn. When corn price soars and supply declines, it becomes vital to diversify the ingredients used in feed as a corn substitute. This study is using cassava (tapioca by-products) and tofu dregs as alternatives to replace corn through fermentation technology using *Neurospora* sp. to produce alternative feed ingredients with nutrient levels that are in balance with corn. We used a completely randomized factorial design, where (A) was the addition level of Na-glutamate; A1 (0%), A2 (0.5%), A3 (1%), and A4 (1.5%), while (B) was the addition level of red *oncom* mushrooms; B1 (0%), B2 (5%), B3 (10%), and B4 (15%). Each treatment was repeated twice, followed by an orthogonal polynomial test. The variables observed were nutrient contents (moisture, crude fat, crude protein, crude fiber, NFE, calcium and phosphor, and anti-nutritional phytic acid). The quality of cassava - tofu dreg mixture can be improved by fermentation using red *oncom* mushrooms, which are enriched with Na-glutamate. The nutritional level of NFE contents dropped, but the crude fiber contents rose. The amounts of Ca, P, and protein significantly (P < 0.05) increased. Fermentation also reduced phytic acid content to 90.83% at 1.5% Na-glutamate and 15% red oncom mushrooms level.~~
This research aims to increase the sustainability of feed availability by creating feed independence. Corn is the primary energy source for livestock, but when the price of corn rises and its supply declines, it becomes essential to find alternative ingredients to use in animal feed. This study explores the use of cassava (tapioca by-products) and tofu dregs as substitutes for corn through fermentation technology using *Neurospora* sp. This produces alternative feed ingredients with nutrient levels that are in balance with corn. To improve the quality of the cassava-tofu dreg mixture, this study used red oncom mushrooms enriched with Na-glutamate during the fermentation process. The research used a completely randomized factorial design, where two factors were considered: the addition level of Na-glutamate (0%, 0.5%, 1%, and 1.5%), and the addition level of red oncom mushrooms (0%, 5%, 10%, and 15%). Each treatment was repeated twice, followed by an orthogonal polynomial test. The variables observed were nutrient contents, including moisture, crude fat, crude protein, crude fiber, NFE, calcium and phosphor, and anti-nutritional phytic acid. The results showed that the fermentation process using red oncom mushrooms enriched with Na-glutamate improved the nutritional level of the cassava-tofu dreg mixture. Although the NFE content decreased, the crude fiber content increased, and the amounts of calcium, phosphorus, and protein significantly increased (P < 0.05). Furthermore, fermentation reduced the phytic acid content to 90.83% at 1.5% Na-glutamate and 15% red oncom mushroom level. In conclusion, this research demonstrates that the quality of cassava-tofu dreg mixture can be enhanced through fermentation using red oncom mushrooms enriched with Na-glutamate. This

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approach can increase the sustainability of feed availability and reduce the dependence on corn as the primary energy source for livestock.

Keywords: Alternative feed, Cassava dreg, Fermentation, Red *oncom* mushrooms, Tofu dreg.

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INTRODUCTION

Corn is the main source of energy for livestock, which until now has not been replaced by other ingredients. As the main raw material for poultry feed, it takes up to 50% of the ration. Edi (2021)Suarni and Widowati (2006) revealed that yellow corn is a suitable energy source since it contains several nutrients, including important amino acids for poultry, 8.68.1.% crude protein, 2.5 2.6 % low crude fiber, 3500 3356 kcal of high energy, and 81.9% total digestible nutrient. In addition, Nuraini and Latif (2008) argue that yellow corn has 33 mg/kg of carotene, a precursor of vitamin A.

The use of corn as food and biofuels compete fiercely with its usage as animal feed. With IDR 7000/kg, corn is currently one of the most expensive commodities on the market. Due to its roles as a primary source of energy, a supply of vital amino acids, and a source of provitamin A, specifically carotenoids, corn is extremely important in feed. Therefore, it is necessary to use different alternative feed ingredients in place of corn in a readily available, cost-effective poultry feed with nutritional profiles nearly identical to corn.

Although widely available in Indonesia, local feed ingredients from agro-industrial waste are rarely employed for poultry feed. Cassava and tofu dregs are two wastes that have a great deal of potential as feed ingredients. Yohanista et al. (2014) reported that cassava is an ingredient with high energy contents, i.e., 3000-3500 kcal, equivalent to that of corn, but has a low crude protein and high crude fiber, while tofu dreg is an ingredient that has a high crude protein of 25.15% (Nuraini and Latif, 2008) so that combined cassava and tofu dreg can be used as a substitute for corn. However, the mixture of cassava and tofu dregs does not contain carotene, so it is still far from the yellow corn. The availability of carotene in the cassava-tofu dreg mixture was obtained through fermentation using red fermented peanut cake yeast. Priatni (2014) remarked that red fermented peanut cake yeast contains *Neurospora* sp, one of the fungal species reported to produce carotene (Rebecca et al., 2018). It grows easily on a substrate, has a short generation time, and the mycelium are branching, rising hyphae, easily recognized by their orange color condition (.

Neurospora sp. requires a substrate for the sources of carbohydrates (glucose), nitrogen, vitamins, and minerals (Nuraini and Latif, 2008). Nuraida et al. (1996) and Dorsam et al (2017) claimed that cassava has the potential to be used as a carbon source in mold fermentation systems, while coconut water is a good growth medium because it contains glucose, vitamins, and minerals. Hsieh and Yang (2003) added that tofu dreg could be a nitrogen source in solid media fermentation. The study from Hsieh and Yang (2003) was the reason for this study using 75% cassava dreg, 25% tofu dreg, and 15% coconut water as substrates providing a source of carbon, glucose, nitrogen, vitamins, and minerals for the growth of *Neurospora* sp. Na-glutamate is a non-essential amino acid and salt compound that can be used as a source of nutrients, namely minerals and nitrogen available to *Neurospora* sp., which can initiate growth during fermentation. Fermentation media with a balanced nutrient content is needed to support the *Neurospora* sp. more optimally in producing carotene so that a fermented product rich in carotenes is produced (Nuraini and Latif, 2008).

Minerals are nutrients that are needed by livestock, especially those that grow and undergo a process of bone formation. Minerals are also used by livestock in milk and egg production (shell formation). So, the presence of minerals must be fulfilled. The availability of minerals in feed ingredients determines the quality of these feed ingredients. It is necessary to reduce the presence of bonds with specific compounds that reduce the availability of minerals. Phytic acid is the primary type of phosphor stored in grains, legumes, oil seeds, and nuts. Because humans lack the phytase enzyme in their digestive systems, phytic acid is a dietary inhibitor that chelates micronutrients and inhibits them from being bioavailable for monogastric animals. Several techniques have been created to lower the amount of phytic acid in food and enhance the nutritional value of grain, which is degraded by such antinutrients (Gupta *et al*., 2015).

The most significant phosphor storage form in grains and legumes is phytic acid. In its normal state, phytic acid interacts with proteins and divalent minerals (Ca, Mg, and Fe) to create compounds that are difficult to dissolve. It prevents the body from absorbing minerals and proteins or makes them less digestible.

Feed ingredients' nutritional and antinutrient contents can be used to determine their quality profile. Fermentation of a mixture of cassava and tofu dregs using *Neurospora sitophila* is expected to improve the mineral contents, especially calcium and phosphorus, as well as phytic acid content. As a corn substitute, the nutrient profile of post-fermented cassava-tofu dregs needs to be studied, to what extent cassava-tofu dregs can be categorized as an alternative to substitute corn.

Corn is a primary source of energy for livestock and a major raw material in poultry feed, accounting for up to 50% of the ration. Yellow corn is particularly suitable for poultry feed because it contains essential amino acids, high energy content, and is a source of provitamin A, specifically carotenoids (Edi, 2021; Gupta et al., 2019). However, the use of corn as food and biofuels competes fiercely with its usage as animal feed, making it one of the most expensive commodities in the market. Therefore, it is necessary to identify alternative feed ingredients that are readily available, cost-effective, and have nutritional profiles nearly identical to corn.

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Local feed ingredients from agro-industrial waste, such as cassava and tofu dregs, have great potential as poultry feed ingredients but are rarely employed in Indonesia (Khasanah et al., 2022). Cassava has high energy content similar to corn but low crude protein and high crude fiber, while tofu dregs have high crude protein but lack carotene, an essential nutrient for poultry (Riswandi et al., 2020; Zohari et al., 2020). Fermentation using red fermented peanut cake yeast can provide the carotene that is missing in the cassava-tofu dregs mixture since it contains *Neurospora* sp., a fungal species that produces carotene and grows easily on a (Gmoser et al., 2018; Zohari et al., 2020). Thus, the fermentation of cassava and tofu dregs using *Neurospora* sp. is expected to improve the nutritional value of these agro-industrial wastes and provide a viable alternative to expensive corn-based poultry feed.

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The aim of this study is to determine the nutritional and antinutrient contents of fermented cassava and tofu dregs using *Neurospora* sp. as a substitute for corn-based poultry feed. The fermentation process is expected to increase the mineral content of the mixture, particularly calcium and phosphorus, and reduce the phytic acid content, which inhibits the bioavailability of minerals in monogastric animals (Feizollahi et al., 2021). The use of a balanced nutrient content, including the addition of Na-glutamate, a non-essential amino acid and salt compound, is expected to optimize the growth of *Neurospora* sp. and increase carotene production during fermentation (Rahayu et al., 2019). Ultimately, the findings of this study could provide valuable insights into the potential of cassava and tofu dregs as alternative feed ingredients for the poultry industry, which could contribute to sustainable livestock production in Indonesia.

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RESEARCH METHODS

Research materials

The study was conducted in the Laboratory of Feedstuff Science, the Faculty of Animal Science, Jenderal Soedirman University, Purwokerto. The use of material in this study has been approved by the Animal Ethics Committee for Laboratory of Feedstuff Science, the Faculty of Animal Science, Jenderal Soedirman University, Purwokerto.

The materials employed in this study were cassava dreg, red *oncom* mushrooms containing *Neurospora* sp., tofu dreg, coconut water, distilled water, PDA media, and chemicals for analysis of calcium, phosphor, and phytic acid. Cassava dregs were obtained from tapioca processing industries in the Purwokerto area, while red oncom mushrooms containing *Neurospora* sp. were obtained from a local market in Purwokerto. Tofu dregs were obtained from a tofu processing industry in the Purwokerto area, and coconut water was obtained from young coconuts purchased at a local market in Purwokerto. Distilled water was obtained from the laboratory's water purification system, and PDA media used in the study were purchased from a commercial supplier. The ~~equipment~~ tools utilized was an autoclave, analytical balance, furnace, oven, and tools for analysis of Ca, P, and phytic acid from Animal Feedstuff Science Laboratory, Faculty of Animal Science, Jenderal Soedirman University.

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A fermented mixture of cassava dreg and tofu dreg

Cassava dreg was added up to 75 g, 25 g of tofu dreg (a protein source), 15 ml of coconut water, and 200 ml of distilled water. The ingredients were mixed and sterilized by autoclaving at 121°C/15 min. After cooling, sprinkle the mixture with red *oncom* mushrooms containing *Neurospora* sp. according to treatment, transferred to a covered tray with a hole in the bottom, and incubated at 30°C for three days. The fermented material was dried for two days at 60ºC, mashed, and taken as a sample for observation.

Research design and data analysis

The study was conducted using a completely randomized design with a factorial pattern i.e. factor (A) was the addition level of Na-glutamate; A1 (0%), A2 (0.5%), A3 (1%), A4 (1.5 %), while factor (B) was the addition level of red *oncom* mushrooms; B1 (0%), B2 (5%), B3 (10%), and B4 (15%), each treatment was repeated twice. The variables were nutrient contents (moisture content, crude fat, crude protein, crude fiber, BETN, calcium, and phosphorus) and phytic acid.

The research data obtained were analyzed using analysis of variance using Microsoft Excel ver. 2010. If the treatment has a significant effect, then proceed with the Orthogonal Polynomial Test (Maizar et al., 2022)~~(Steel and Torrie, 1991)~~.

RESULTS and DISCUSSION

The results showed that the fermentation of cassava-tofu dregs with red *oncom* mushrooms at a level of 5 - 15% which was enriched with Na-glutamate at a level of 0 - 1.5% resulted in changes in varying levels of nutrients (Table 1).

Moisture Contents

Post-fermentation of cassava and tofu dregs showed a range of the lowest moisture contents in A3B3 to the highest in A3B4. The results showed that the interaction had no significant effect on moisture content. The addition of the Na-glutamate level had no significant effect, while the mushroom level had a significant effect ($P < 0.05$), with the orthogonal polynomial test results showing a very significant ($P < 0.05$) cubic response with the equation Y = 6.84 + 4.8 X - 0.87X2 + 0.038X3. The coefficient of determination R2 = 27.04%, indicating the effect of the treatment on the moisture contents of 27.04%. The treatment showed the highest moisture content at an inflection point 1 = (3.63, 14.63) at which 3.63% mushroom was used with 14.63% water content. The moisture content then experienced the lowest decrease at an inflection point 2 = (11.57, 5.087) at which 11.57% mushroom was used with a moisture content of 5.087%.

Table 1. Nutrient Levels of post fermented cassava dreg-tofu dreg

The effect of adding red *oncom* mushrooms on the moisture and dry matter content of post-fermented cassava dregs - tofu dregs is shown in Figures 1 and 2.

Figure 1. The moisture of post-fermented cassava-tofu dreg

Figure 2. Dry matter of post-fermented cassava-tofu dreg

Dry Matter

Determining the DM content of feed provides a measure of the amount of a particular feed that is required to supply a set amount of nutrients to the animal (Singh, 2019)~~(Singh, 2019).~~ The dry matter content of post-fermented cassava dreg-tofu dregs showed the lowest range in A3B4 or the addition of 1% Na-glutamate and or 15% level of *oncom* mushrooms. The highest dry matter content in A3B3 treatment or the addition of 1% Na-glutamate level and 10% level of red *oncom* mushrooms. The interaction had no significant effect and it was also found that the addition of Na-glutamate during the fermentation process had no significant effect on dry matter content. Meanwhile, the addition of red *oncom* mushrooms had a significant effect on the dry matter content of post-fermented cassava-tofu dregs. Differences in fungal inoculum levels cause differences in dry matter levels caused by differences growth of red oncom mushroom mycelium. This is in line with the report of ~~(~~Hasanuddin & Aidah (~~,~~2021)~~Hasmida et al. (2021)~~ that ~~Neurospora~~*Neurospora* sp. showed highest growth at 96-hour incubation at 10% concentration. The longer the incubation time, the more feed ingredients that can be overhauled by mold so that at the end of fermentation the dry matter will increase. Increases or decreases in feed DM content in the result in over or under feeding of nutrients. The results of the orthogonal polynomial test are cubic ($P < 0.01$) with the equation $Y = 93.163 - 4.795X + 0.87X^2 - 0.038X^3$. The coefficient of determination $R^2 = 27.0394$ %, Inflection point 1 = (3.63, 85.37), Inflection point 2 = (11.57, 94.91).

Crude Fiber

The crude fiber content of post-fermented cassava dregs-tofu dregs showed the lowest in the A1B1 treatment and reached the highest in the A4B4 treatment. This showed that the higher the addition of Na-glutamate and mushrooms, the higher the crude fiber contents. The interaction had a significant effect ($P < 0.05$). The addition of red *oncom* mushroom levels had a significant linear effect on Na-glutamate levels of 0% ($P < 0.05$), 0.5% ($P < 0.05$), 1% ($P < 0.051$), and 1.5% ($P < 0.01$) on the crude fiber content of cassava-tofu dregs. With the coefficient of determination respectively $R^2 = 58.9879$ %, $R^2 = 57.3036$ %, $R^2 = 77.8662$ %, $R^2 = 90.5948$ %. The effect of the highest treatment was the addition of 15% mushroom level and 1.5% Na-glutamate. The higher the level of Na-glutamate supplementation and the level of red *oncom* mushrooms, the higher the crude fiber content. The high crude fiber is thought to be due to the relatively high mass of

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microbial cells, this is evidenced by the thicker growth of fungi and hyphae. This result is different from other studies, which state that *Neurospora* sp. has activity in reducing crude fiber (Matitaputty, 2018)~~(Nurfaizin and Matitaputy 2015)~~. The effect of red *oncom* mushroom addition on the crude fiber of post-fermented cassava-tofu dreg is shown in Figure 3.

Figure 3. Effect of red *oncom* mushrooms addition on the crude fiber of post-fermented cassava-tofu dreg

Crude Fat

The results showed that the lowest fat content of post-fermented cassava-tofu dregs in A1B1 to the highest in A2B4. The interaction had no significant effect, while the addition of Na-glutamate had a significant ($P < 0.05$) effect on the fat content of cassava dregs-tofu dregs. Meanwhile, the level of red *oncom* mushroom had a highly significant ($P < 0.01$) effect on the fat contents of cassava-tofu dregs. The results of the orthogonal polynomial test showed that the effect of Na-glutamate is cubic with the equation Y = 3.9125 + 9.41 X - 14.288 X2 + 5.693 X3. With a coefficient of determination R2 = 20.80%, this shows that the effect of Na-glutamate is only 20.80% on the fat content of post-fermented cassava-tofu dregs. The effect of adding Na-glutamate and red oncom mushroom to crude fat in post-fermented cassava-tofu dregs can be seen in Figures 4 and 5.

Figure 4. Effect Na-glutamate on the crude fat

Figure 5. Effect of red *oncom* mushroom on the crude fat

While the effect of red *oncom* mushroom level is linear ($P < 0.01$), with the equation Y = 3.8732500 + 0.13340000 X, and the coefficient of determination R2 = 26.0594% means that the effect of red oncom mushroom level on the fat content of post-fermented cassava-tofu dregs is by 26.06%, the effect outside the treatment was 73.96%.

Ash Content

The results showed that the ash contents of post-fermented cassava-tofu dregs were the lowest in A4B1 up to the highest in A2B2. The interaction had a significant ($P < 0.05$) effect on the ash content of cassava-tofu dregs. The treatment response had a significant effect only on A4 which was quadratic, with the equation Y = 3.3345 + 0.7114 X - 0.03960000 X2 and the coefficient of determination R2 = 77.1736 %, with the Maximum point (8.98, 6.53). This showed that the highest ash content was produced by red *oncom* mushrooms at a Na-glutamate level of 1.5%. The effect of adding red *oncom* mushrooms to the ash content of post-fermented cassava dregs-tofu dregs can be seen in Figure 6. High ash (mineral) content can be used by livestock for tissue growth, egg production, lactation and skeleton.

Figure 6. Effect of red fermented peanut cake yeast addition on the inorganic matter of post-fermented cassava dreg -tofu dreg

Crude protein contents

Fermented cassava and tofu dregs have the lowest protein content in A1B1 and the highest in A3B4. Fermentation using red *oncom* mushrooms enriched with Na-glutamate has been shown to increase the crude protein content of the substrate. The protein content increases during the

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Formatted: Space After: 10 pt, Line spacing: Multiple 1,15 li fermentation, due to the fact that the proteolytic activities of enzymes produced by microorganisms which increases the bioavailability of amino acids (Hasanuddin & Aidah, 2021) ~~(Tufa et al., 2016)~~. The sharpest increase of up to 149% was shown in fermentation using 1% Na-glutamate and 15% red oncom mushrooms. The results of the analysis of variance showed that the interaction had no significant effect on the crude protein content of post-fermented cassava-tofu dregs. Meanwhile, supplementation with Na-glutamate had a highly significant effect (P < 0.01) on increasing crude protein levels with a quadratic response, with a quadratic equation Y = 7.12 + 4.80 X - 1.999 X2. The coefficient of determination R2 = 25.71%, meaning that the effect of Na-glutamate on increasing the crude protein of fermented cassava dregs and tofu dregs is 25.71%. The Maximum point (1.20, 10.004) indicated that the optimum level of addition of Na-glutamate was 1.20% with a crude protein content of 10.004%. This treatment has an impact on improving the quality of feed ingredients. Changes in the feed from feed energy sources to protein sources have the potential to reduce feed prices. ~~(~~Hasanuddin & Aidah (~~,~~2021)~~Hasmida et al. (2021)~~ report that The increase in crude protein occurs due to the addition of inoculum capable of using the substrate for the growth and formation of microbial proteins during the fermentation process perfectly and the increase in crude protein substrate occurs as a result of nitrogen supplementation in the form of ureaw hich is added when fermentation is carried out. In this study, the addition of Na-glutamate has the function of being a source of N for red oncom mushroom to multiply cells. The effect of adding Na-glutamate and fermented red *oncom* mushrooms on the crude protein of post-fermented cassava tofu dreg is shown in Figures 7 and 8.

Figure 7. Effect of Na-glutamate on the crude protein

Figure 8. Effect of red oncom mushrooms on the crude protein

Meanwhile, the level of red *oncom* mushroom had a highly significant (P < 0.01) effect on the protein content of cassava-tofu dregs. With the test results of the cubic orthogonal polynomial with the equation Y = 6.2862500 + 0.89879167 X - 0.09440000 X2 + 0.00373833 X3, with the coefficient of determination R2 = 62.44 % and inflection point 1st inflection point = (4.68, 8.81), 2nd inflection point = (12.014, 9.94). The addition of red *oncom* mushrooms had an effect of 62.44% with a pattern of changes in protein content increasing at the mushroom level of 4.68% with a crude protein content of 8.81% then decreasing and increasing again at the level of red oncom mushrooms of 12.014% with an optimum protein content of 9 .94%.

Nitrogen Free Extract (NFE) content

NFE levels of post-fermented cassava and tofu dregs were the lowest in A3B4 to the highest in A1B2. The decrease in NFE levels occurred along with the increase in Na-glutamate and red *oncom* mushrooms. This is presumably because the increase in several post-fermentation nutrients causes a decrease in NFE levels. The increase in crude fiber is primarily the most significant cause of the decrease in NFE. The interaction had no significant effect on NFE levels. Meanwhile, Na-glutamate levels had a highly significant (P < 0.01) effect on NFE levels in fermented cassava-tofu dregs with a quadratic response with the equation Y = 52.771562 - 27.609375 X + 12.483750 X2, Coefficient of determination R2 = 31.5056% and Minimum Point = (1.11, 37.51). This showed

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that the Na-glutamate level has an effect of 31.5056% on the NFE levels in the fermented cassava-dregs tofu dregs; with the optimum level of giving Na-glutamate 1.11% with the lowest NFE level of 37.51%. The effect of Na-glutamate and red *oncom* mushroom addition on the NFE of post-fermented cassava-tofu dreg is shown in Figures 9 and 10.

Figure 9. Effect of Na-glutamate on the NFE

Figure 10. Effect of red *oncom* mushrooms addition on the NFE

Meanwhile, the addition of red *oncom* mushroom levels in the fermentation process had a highly significant ($P < 0.01$) effect on NFE levels in fermented cassava-tofu dregs, the results of the orthogonal polynomial test showed a cubic response with the equation Y = 52.05-6.15X + 0.95 X2 - 0.041 X3, coefficient of determination R2 = 34.3596 %, Inflection point 1 (4.63, 39.83), Inflection point 2 = (10.89, 44.79).

Calcium, Phosphor, and Phytic Acid contents

The results showed that the calcium content in the fermented cassava-tofu dregs was the lowest in A1B1 and the highest in A4B4, phosphor levels recorded low levels in A1B1 and highest levels in A4B4, while the levels of phytic acid were the lowest in A1B1 and the highest in A4B4. The results revealed an increase in calcium and phosphorus levels with increasing levels of red *oncom* mushrooms and Na-glutamate, but there was a decrease in phytic acid contents. The lowest levels of phytic acid were shown in A4B4. The interactions had a highly significant ($P < 0.01$) effect on increasing Ca and P levels and decreasing phytic acid. This showed that there is an increase in the availability of phosphorus, with a decrease in the content of phytic acid along with increasing levels of red *oncom* mushrooms and Na-glutamate supplementation. The contents of calcium, phosphor, and phytic acid in fermented cassava-tofu dreg with red *oncom* mushroom and Na-glutamate supplementation are shown in Table 2.

Table 2. Content of calcium, phosphor, and phytic acid in fermented cassava dreg-tofu dreg with red oncom mushrooms and Na-glutamate supplementation

The lowest calcium levels were shown in A1B1 and the highest levels were with the addition of 1.5% Na-glutamate and 15% red *oncom* mushrooms. Fermentation of cassava-tofu dregs with red *oncom* mushrooms and Na-glutamate supplementation can increase calcium content by 62.12%. The interaction has a highly significant ($P < 0.01$) effect on increasing calcium. The highest response was the addition of 1.5% Na-glutamate and 15% red *oncom* mushrooms with the equation line YA4 = 0.464575 + 0.05665 X + 0.0855 X2 (R2 = 99.6 %). The coefficient of determination showed that the effect of Na-glutamate supplementation and red *oncom* mushroom treatment of 15% is 99.6%. This means that fermentation is very effective in increasing calcium levels of post-fermented cassava-tofu dregs. Increased levels of calcium are caused by the breakdown of phytic acid which chelates calcium, where phytic acid is a strong chelate (mineral binding compound) that can bind divalent metal ions to form phytate complexes so that minerals cannot be absorbed by the body. These minerals are Ca, Zn, Cu, Mg, and Fe. Calcium concentrations from post-fermented cassava-tofu dregs with levels of Na-glutamate (A) and levels of red *oncom* mushrooms (B) can be seen in Figure 11.

Figure 11. Calcium concentration of post-fermented cassava dreg - tofu dreg with Na-glutamate level (A) and red oncom mushrooms level (B)

The interaction had a highly significant effect on the phosphor content of post-fermented cassava and tofu dregs ($P < 0.01$). The results of the orthogonal polynomial test showed a quadratic response with the equation YA4 = 0.26775 - 0.168 X + 0.25 X2, the coefficient of determination is R2 = 98.93% indicating that the treatment has an effect of 98.93%. The highest increase in phosphorus levels obtained post-fermentation was 75.77%. An increase in phosphorus levels indicated that the fermentation process has succeeded in changing the bound phosphorus to become more available due to the presence of the phytase enzyme produced by the mushrooms. *Neurospora* sp. that grows on red *oncom* mushrooms has the potential to produce phytase enzymes (Kanti, 2017; Kanti and Sudiana, 2016; Kanti and Sudiana, 2018). The addition of Na-glutamate can also provide a source of sodium and non-essential amino acids in the growth of red oncom mushrooms.

Figure 12. Phosphor concentration of post-fermentation cassava dreg - tofu dreg with Na-glutamate level (A) and red oncom mushrooms level (B)

The highest decrease in phytic acid was shown in the interaction of 1.5% Na-glutamate and 15% red *oncom* mushrooms with a linear response YB4 = 0.34985 - 0.02018 X, (R2 = 99.37 %). This showed that the higher the Na-glutamate level and the red *oncom* mushrooms in the fermentation, the higher the decrease in phytic acid. The decrease in phytic acid indicated that fermentation with *oncom* mushrooms supplemented with Na-glutamate was effective in breaking down mineral-phytic acid bonds. Phytic acid is an anti-nutritional substance because it can bind to minerals which causes the solubility of these minerals to decrease so that the availability of minerals becomes low. Under natural conditions, phytic acid will form bonds with both divalent minerals (Ca, Mg, and Fe) and proteins into compounds that are difficult to dissolve. This negatively influences the absorption of minerals and proteins. Therefore, phytic acid is considered an anti-nutritional food ingredient (Jatuwong et al 2020). The results showed that fermentation using red *oncom* mushrooms could reduce phytic acid by up to 90.83% (A1B1) to 0.0550 ± 0.0099 % (A4B4)). The high decrease in phytic acid indicated the high phytase enzyme produced by the red *oncom* mushroom during fermentation. As stated by Kanti (2017) that in fermentation with tofu dregs substrate, *Neurospora sitophila* produced the highest phytase enzyme activity compared to *Aspergillus niger* and *Rhizopus oligosphorus.*

Figure 13. Phytic Acid Levels of Post-Fermented Cassava Dregs-Tofu Dregs with Different Levels of Na-Glutamate (A) and Red Oncom Mushrooms (B)

CONCLUSION AND RECOMMENDATION

~~The quality of cassava-tofu dregs can be improved by fermentation using red *oncom* mushrooms which are enriched with Na-glutamate. A decrease in some nutrient levels occurs at NFE levels, in which crude fiber increases. Significant increases occurred in protein content, Ca, and P levels. Fermentation also succeeded in reducing phytic acid levels up to 90.83% at 1.5% Na-glutamate level and 15% red *oncom* mushroom level.~~

~~Further studies are needed on the *in-vivo* bioavailability of cassava and tofu dregs in both poultry and ruminants, to optimize research results so that fermented cassava and tofu dregs with red *oncom* mushrooms can be used as a substitute for conventional carbohydrate sources such as corn or bran.~~

Fermentation using red oncom mushrooms enriched with Na-glutamate has been found to improve the quality of cassava-tofu dregs. Although there is a decrease in some nutrient levels, such as NFE, the crude fiber content increases. On the other hand, there are significant increases in protein content, calcium, and phosphorus levels. Additionally, the fermentation process reduces phytic acid levels by up to 90.83% at 1.5% Na-glutamate level and 15% red oncom mushroom level.

To optimize these research findings, further studies are necessary to evaluate the in-vivo bioavailability of cassava and tofu dregs in both poultry and ruminants. This will help to determine if fermented cassava and tofu dregs with red oncom mushrooms can serve as a viable substitute for conventional carbohydrate sources like corn or bran. Such an alternative approach could improve the sustainability of feed availability and reduce the reliance on corn as the primary energy source for livestock.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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NOVELTY STATEMENT

The present study shows that the sustainability of feed ingredients for livestock (chicken and cow) can be realized by using other feed ingredients that are cheaper and widely available besides corn, namely using a mixture of cassava dregs - tofu dregs fermented with Na-glutamate and red oncom mushrooms containing ~~Neurospora~~*Neurospora* sp.

AUTHOR'S CONTRIBUTION

M and TW designed the concept, conducted data analysis, and wrote the manuscript. CHP reviewed the paper. All authors participated in the experimental design, and read, and approved the final manuscript.

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