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NUTRIENT CONTENT AND QUALITY OF SOYBEAN MEAL WASTE FERMENTED BY *Aspergillus ficuum* AND *Neurospora crassa*

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ABSTRACT: Present research aimed to increase soybean meal waste quality and nutrient by fermentation using different ratio of mixed fungus inoculum (*Aspergillus ficuum* and *Neurospora crassa*) and fermentation time. The primary materials were soybean meal waste (SMW), fungus *Aspergillus ficuum* and *Neurospora crassa*. The experiment applied a completely randomized design (CRD) with a 3 × 3 factorial pattern and three replications. Two treatments were given in this study, factor A (combination of *A. ficuum* and *N. crassa*), comprising of A1 (3:1), A2 (3:2), and A3 (3:3). Factor B (fermentation time) comprising of B1 (5 days), B2 (7 days), and B3 (9 days). The variance analysis exposed a highly significant interaction between factor A and factor B, and those factors also exposed a highly significant effect. The correlation between SMW crude protein and broiler nitrogen retention showed a positive trend, contrary SMW crude fiber content negatively affected crude fiber digestibility. In conclusion, the combination of *A. ficuum* and *N. crassa* (3:2) and seven days fermentation period showed optimal results as seen from 28.25% crude protein, 13.77% crude fibre, 61.16 nitrogen retention and 58.76% crude fibre digestibility of fermented SMW.

Keywords: *Aspergillus ficuum*, Crude protein, Digestibility, Fermentation, *Neurospora crassa*.

INTRODUCTION

The availability of soybean meal waste (SMW) is abundant in line with the mushrooming of the home industry for making soy milk due to the high public awareness of healthy living. Soy milk has high protein content and contains isoflavone compounds is a beneficial foodstuff for healthy nutrition (Jang et al., 2021; Chitisankul et al., 2022). Along with the increasing demand for soy milk, the availability of SMW is also increasing, so it is the potential to be utilised as a source of animal feed, especially for poultry feed. Nutrient content of SMW is quite high, such as crude protein 27.62%, crude fat 2.95%, non-nitrogen free extract 52.66%, crude fiber 13.81% and ash 2.96%, Ca 0.09%, P 0.04% (Mirnawati, 2011). Although the protein content of SMW is quite high, the benefits are limited. Only 6.2% are possible to be applied in broiler rations. It is due to the low palatability and quality of rations which can be seen from nitrogen retention that is also lower (Mirnawati, 2012).

Based on the previous study, it was found in the broiler that supplemented with fermented soybean milk by *Aspergillus ficuum* resulted in higher feed intake, body weight gain and feed conversion compared to control (Ciptaan et al., 2021). But it is still limited to only being used up to 25% of rations. The limiting factor of using SMW in poultry rations since it has high crude fibre content and phytic acid. Furthermore, Ciptaan and Mirnawati (2015) stated that fermented SMW with *Neurospora sitophila* gave better results than the of *Neurospora crassa* and *Neurospora* sp. saw from crude protein content and nitrogen retention are 37.68% and 55.77%. Although the crude protein content is comparatively high, nitrogen retention is still low because of the presence of phytic acid which will reduce the digestibility of the protein so that the protein becomes unavailable. *In vitro* evaluation of *N. crassa* as an oligosaccharide inoculum toward soybean pulp extract showed a safer fermentation profile with probiotic properties (Zhou et al., 2019). Biotechnology research on cellulolytic fungi and phytates to produce cellulases and phytases to degrade cellulose and phytic acid are to improve the SMW quality (Yang et al., 2019; Chen et al., 2021). So fermented soybean milk is of high quality, namely low crude fibre and phytic acid can be utilized more in poultry rations. One of the fungus that produce high cellulase and phytase is *A. ficuum*. Phytase production from potato waste fermentation using *A. ficuum* reached 5.17 U/g ds (Tian and Yuan, 2016).

Based on the above description, a study to determine the combination of *A. ficuum* with *N. crassa* with fermentation period to increase the SMW quality as local feed ingredients that can replace or reduce the imported feed ingredients in poultry rations is necessary. Besides that, it also determines of fermented SMW that can be applied in the poultry industry to provide low cholesterol meat and eggs.

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MATERIALS AND METHODS

Materials

The materials applied to conduct the study include SMW (Figure 1), *A. ficuum* (Figure 2), *N. crassa* (Figure 3), Potato Dextrose Agar (PDA), experimental chicken, distilled water, rice bran, standard mineral solution comprising of $(NH_4)_2SO_4$ 0.14%, KH_2PO_4 0.2%, $MgSO_4$ $7H_2O$ 0.03%, urea 0.03%, $CaCl_2$ 0.03%, $FeSO_4$ $7H_2O$ 0.0005%, $MnSO_4$ H_2O 0.00016%, $ZnSO_4$ $7H_2O$ 0.00014%, $CoCl_2$ 0.0002%, pepton 0.075%, ADS solution, alcohol, aceton and H_2SO_4 72%. The equipment used is an autoclave to sterilize tools and materials for 1 kg size plastic bags (polypropylene), analytical scales, incubator cabinets, electric ovens, oasis needles, bunsen, test tube, cotton, aluminium foil, and trophy cups, a set of laboratory equipment for analysis.

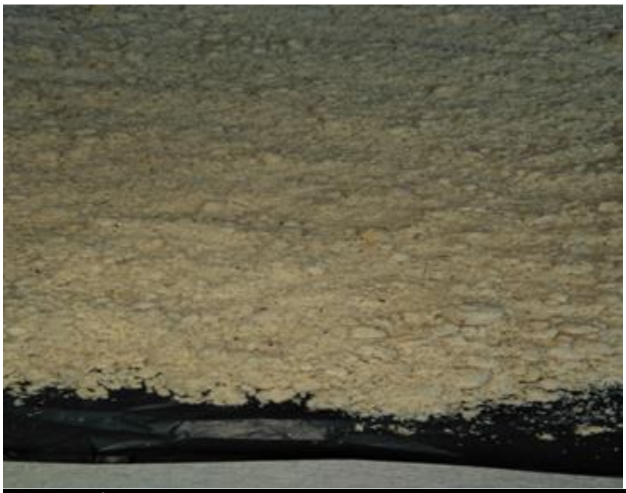

Figure 1 - Soybean meal waste (SMW)

Figure 2 - *Aspergillus ficuum*

Figure 3 - *Neurospora crassa*

Figure 4 - Fermented SMW

Methods

Soybean meal waste fermentation was processed by adding rice bran in a ratio (80:20) as a substrate while the inoculum is *A. ficuum* and *N. crassa* as much as ration treatment then placed in plastic bags as an incubator for several days. The factors for the treatment were: Factor A (ratio of inoculum): A1=3:1, A2=3:2, A3=3:3; Factor B (duration of fermentation): B1=5 day, B2=7 day, B3=9 day.

Fermented SMW was harvested (Figure 4) and dried at a temperature of 60°C and ready to be given as rations.

Determination of crude protein and crude fibre of SMW using proximate analysis

In 100 broilers, Lohmann strain at five weeks of age was prepared for nitrogen retention and crude fibre digestibility measurement. Five birds per unit cage (80 × 80 × 60 cm) supplied with *ad libitum* drinking water. Nitrogen retention and crude fibre digestibility were measured following Sibbald (1980) and Mirnawati et al. (2019). For comparison, we performed nutritional analysis and measurements of nitrogen retention unfermented SMW (Table 1). A completely randomized design applied in this experimental research with factorial patterns (3 × 3) and three replications. The data obtained will be analyzed for variance. If treatment has a significant effect on variables, then proceed with Duncan's Multiple Range Test. Pearson correlation was performed to determine the relationship between the variables using MedCalc software (version 20.110).

Table 1 - Proximate analysis and nitrogen retention of unfermented SMW

Nutritional value (%)	Soybean meal waste
Crude protein	27.62
Ether extract	2.95
Non-nitrogen free extract	52.66
Crude fiber	13.81
Ash	2.96
Ca	0.09
P	0.04
Nitrogen retention	40

RESULTS AND DISCUSSION

Crude protein content

The results showed that there were interactions that had a highly significant effect (P<0.01) between Factor A (composition of inoculum) and Factor B (duration of fermentation) on crude protein. Each factor A (composition of inoculum) and factor B (duration of fermentation) also showed a similar effect (P<0.01) on crude protein. The interaction between inoculum composition and fermentation period is due to the mixed inoculum nutrition availability and the proper duration of fermentation.

In Table 2 the composition of the treatment inoculum A2 (3:2), it is better than A1 (3:1) and A3 (3:3). A1 (3:1) was higher than A3 (3:3). The composition of the inoculum is optimal in A2 (3:2) thus each inoculum can perform a synergistic effect that enhances enzymatic activity. *A. ficuum* can produce phytase at 37.46 U/ml for seven day incubation period (Pazla et al., 2021). Meanwhile, fermentation of soybean meal by *N. crassa* can hydrolyze protein more efficiently and increase amino acids (Li et al., 2019). *Neurospora* can produce protease enzymes that digest proteins into amino acids and lipases that digest fat, and triglycerides become free fatty acids (Kurniati, 2012). In addition, the highest crude protein content in A2B2 is because of the prominent contribution of the microbes population. Yousufi (2012) stated that the elevated in protein substance was because of the proteins supplement contributed by microbial cells as single cell protein source.

Table 2 - Nutrient content, nitrogen retention, and crude fiber digestibility of SMW.

Variables	A (ratio of inoculum)	B (duration of fermentation)		
		B1 (5 day)	B2 (7 day)	B3 (9 day)
Crude protein (%)	A1 (3:1)	21.08[c]	25.39[a]	23.26[b]
	A2 (3:2)	22.51[c]	28.25[a]	25.18[b]
	A3 (3:3)	19.11[c]	24.47[a]	22.04[b]
Crude fiber (%)	A1 (3:1)	18.81[a]	16.71[c]	17.90[b]
	A2 (3:2)	17.25[a]	13.77[c]	15.97[b]
	A3 (3:3)	20.54[a]	18.40[c]	19.24[b]
Nitrogen retention (%)	A1 (3:1)	45.64[c]	55.56[a]	51.48[b]
	A2 (3:2)	51.25[c]	61.16[a]	56.43[b]
	A3 (3:3)	42.57[c]	49.56[a]	47.52[b]
Crude fibre digestibility (%)	A1 (3:1)	45.32[c]	55.28[a]	50.53[b]
	A2 (3:2)	49.50[c]	58.76[a]	53.11[b]
	A3 (3:3)	39.60[c]	49.30[a]	45.23[b]

Different letters in different lines and uppercase letter show a highly significant effect (P <0.01)

Crude fibre content

The results of the analysis showed that treatment had a highly significant interaction (P<0.01) between factor A (composition of inoculum) and factor B (duration of fermentation) on crude fibre content. More specifically, each factor showed an eminent effect (P<0.01). The lowest crude fibre content is found in A2B2 (13.77%). Li et al. (2014) stated that *N. crassa* has peptidase enzyme activity, endoglucanase, exoglucanase, and cellobiose dehydrogenase. It is an extracellular enzyme that plays a role in the hydrolysis of cellulose and hemicellulose. Fermentation inoculum using *N. crassa* can improve nutritive value in agriculture waste and might enhance poultry production (Liu et al., 2016). This result is close to the crude fibre content of fermented palm kernel cake with *Bacillus subtilis* plus humic acid treatment with a value range of 18.05-19.96% (Mirnawati et al., 2022). In addition, the application of fermentation to palm kernel cake using *Sclerotium rolfsii* can replace commercial concentrate in quail ration without significantly affecting crude fiber content and egg production (Ciptaan et al., 2022).

Nitrogen retention

The finding has proven that there were high significant interactions (P<0.01) between factor A (composition of inoculum) and factor B (duration of fermentation) on nitrogen retention. Each factor also showed a high significant effect

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(P<0.01) on a variable. As shown in Table 2 that the highest nitrogen retention is found in A2B2 (61.16%). Since the crude protein content in A2B2 is higher than in the other treatments. In addition, during fermentation, *A. ficuum* produces a protease enzyme resulting in better nutrition quality for SMW. Malomo et al. (2013) showed that broiler nitrogen retention is positively related to dietary protein content. This also has an impact on increasing the protein efficiency ratio in diet substituted with 15% SMW (Dono et al., 2017). If the protein content is low, it will impact the animal production. However, Brink et al. (2022) showed that higher crude protein levels did not elevate broiler nitrogen retention.

Crude fibre digestibility

The variance analysis exposed that there were interactions between factors A and B that had a highly significant effect (P<0.01). Each factor also contributes to the digestibility of crude fibre SMW with a prominent effect (P<0.01). Table 2 showed the A2B2 highest crude fibre digestibility (58.76%). Since the crude fibre content in A2B2 is lower than in other treatments. Crude fibre components are degraded during the fermentation process that animals could easily digest. *N. crassa* has high cellulolytic activity and produces cellulase enzymes that enhance crude fibre digestibility and also protein availability (Mirnawati et al., 2017). Compared with the combination of *Phanerochaete chrysosporium* and *N. crassa* (4:1), the digestibility of palm oil sludge fibre was 57.66% for an incubation period of 13 days. A mixture of *A. ficuum* and *N. crassa* showed SMW in better crude fibre digestibility with a shorter incubation period (Mirnawati et al., 2019).

Relationship between nutritional value

Fermented SMW crude protein content has a highly positive correlation with broiler nitrogen retention (r=0.95, P=0.0001, Figure 5). Meanwhile, fermented SMW crude fiber and broiler crude fiber digestibility showed a highly negative correlation (r=-0.95, P=0.0001, Figure 6). The effect of reducing crude protein on a diet (maize-soybean meal) showed a downward trend in broiler nitrogen retention, but it was not significant (Chrystal et al., 2020). This difference may be due to physicochemical profile of feed ingredients. The relationship between crude fiber content and crude fiber digestibility is in accordance with the results of Ginindza et al. (2017) in indigenous breed chickens, where increased CF levels decreased feed intake as well as digestibility but this was compensated by adaptation in intestinal villi. Promising nutritional profile of fermented SMW is expected to support broiler production performance.

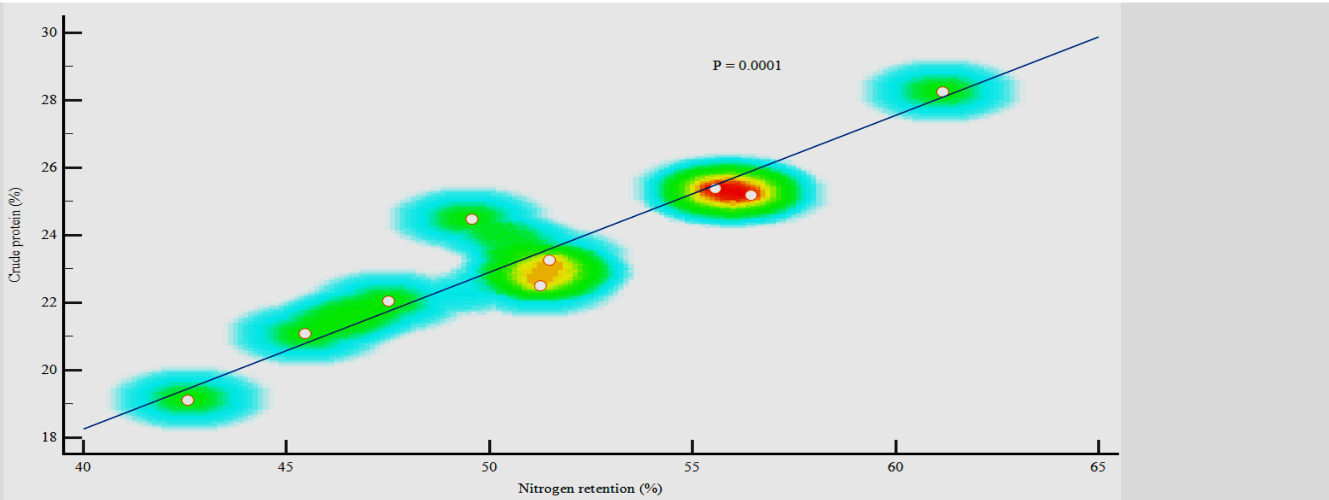

Figure 5 - Correlation between SMW crude protein and broiler nitrogen retention

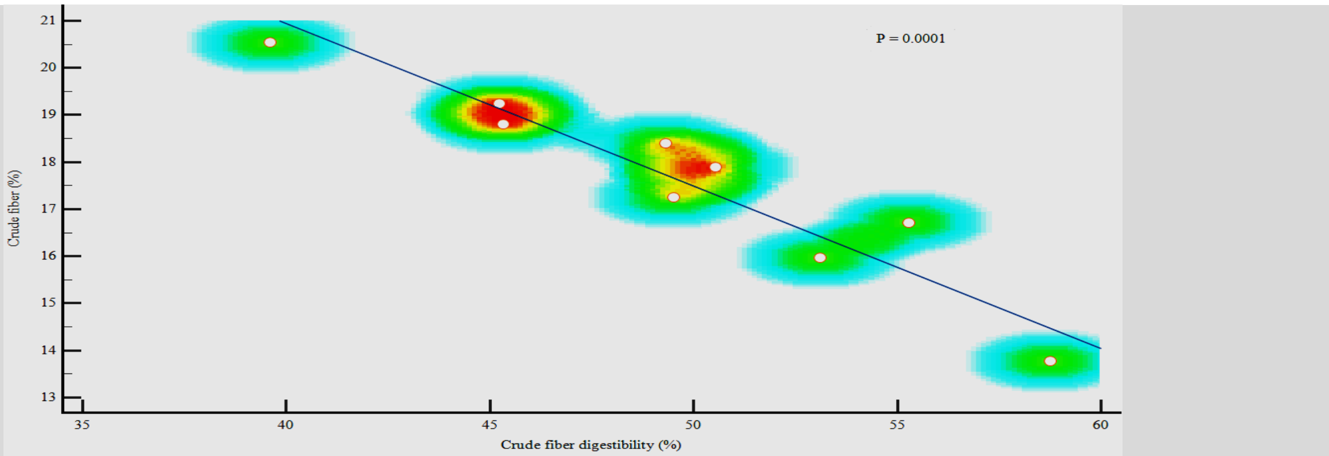

Figure 6 - Correlation between SMW crude fiber and broiler broiler crude fiber digestibility

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CONCLUSION

The mixed inoculum *A. ficuum* plus *N. crassa* (3:2) with seven days of fermentation session showed optimal results. That is shown by increased crude protein, nitrogen retention, crude fibre digestibility, and reduced crude fibre of soybean meal waste. Early indications have signified that fermented soybean meal waste has promised to be an alternative protein source for poultry feed. Further studies focused on the amino acid quality of fermented soybean meal waste.

DECLARATIONS

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Authors' contribution

Ciptaan G, Mirnawati M, Aini Q, Makmur M contribute to experiment, analysis and writing manuscript

Conflict of interests

The authors have not declared any conflict of interests.

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