# EFFECT OF CONSORTIUM *Trichoderma viride, Trichoderma reesei, Aspergillus oryzae* and *Rhizopus oligosporus,* AND DOSE ON INOCULANT VIABILITY, REDUCING SUGAR AND NUTRITIONAL VALUE OF CORN COBS

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ABSTRACT

This study aims to determine the influence of a consortium of fungus Trichoderma viride, *Trichoderma reesei*, Aspergillus oryzae, and Rhizopus oligosporus, the dose of inoculum on the viability of inoculant, reducing sugar content and nutrient content of corn cobs. The experimental design used was completely randomized design factorial with three factors, namely type of consortium, the dose of inoculum, and fermentation time. Type a consortium consisting of (1) *Trichoderma viride* and *Trichoderma reesei* (2) of *Trichoderma reesei* and *Rhizopus oligosporus* (3) Trichoderma viride, *Trichoderma reesei, Aspergillus oryzae* and Rhizopus oligosporus. Inoculum dose was 1%, 2% and 3% of the weight of the substrate. Fermentation carried out for 8 days. The method used in this research is descriptive and experimental methods. Descriptive method used to analyze the nutritional quality of corn cobs that includes crude protein, crude fiber and crude fat. Experimental methods used to analyze the growth of fungi (TPC : Total Plate count), pH, temperature, moisture content), and content of reducing sugar during the 8-day of fermentation process. Data were analyzed with ANOVA and further test used was Duncan's Multiple Range Test. The results showed that the consortium of Trichoderma viride, *Trichoderma reesei, Aspergillus oryzae* and *Rhizopus oligosporus* showed viability and the highest reducing sugar production and increasing the nutritional quality of corn cob best. Fermentation with consortium *Trichoderma viride, Trichoderma reesei, Aspergillus oryzae* dan *Rhizopus oligosporus*, inoculums dosage 3% and 6 days

fermentation resulted the highest crude protein (12.48%) but the lowest crude fiber (16.56%).

**Keyword :** *consortium, Trichoderma viride, Trichoderma reesei, Aspergillus oryzae, Rhizopus oligosporus, inoculum dosage, corn cobs, reducing sugars, crude protein, crude fiber, crude fat, fermentation.*

## INTRODUCTION

Feed is one important factor that will determine the success of the breeding business. The biggest cost of procurement of feed in the livestock business when compared with other production costs that can reach 60-80% (Hardiyanto 2002). The greater production costs incurred for the rations will be even greater if the price of feed raw materials increasingly menigkat (Mirzah, 1998). Some ways to reduce the cost of rations, have been conducted among others by making use of feed materials from the agricultural industries that use does not compete with human needs (Suparjo and Handoko, 2003). Corn is one of many agricultural products produced in Indonesia.

Corn cob which is agricultural waste corn plants reach about 30% of the total weight of the fruit of corn (Koswara, 1991; MoA, 2004). Utilization of corn cobs in Indonesia is still small, so far only corn cobs discarded or burned, but cobs can be used as an alternative feed mainly during the dry season. The main problem using corn cobs as feed is the high content of crude fiber, so we need a method that could utilize the waste corn. Fermentation technique is the proper way to treat waste corn with a consortium of fungi. This is expected to improve the nutritional quality of corn cobs to serve as an alternative livestock feed. Consortium fungi used in this study consisted of three groups, namely *Trichoderma viride* and Aspergillus oryzae, *Trichoderma reesei* and Rhizopus oligosporus, and mixtures of these four fungi.

## MATERIAL AND METHODE

The method used in this research is descriptive-experimental method. Descriptive method is used to describe the results of analysis of nutrient content of corn cobs include crude protein, crude fiber and crude fat after fermentation for 2, 4, 6 and 8 days. Experimental methods used to analyze the statistical data of the measured parameters, including pH, temperature, water content, reducing sugars, and total microbes (TPC). Experimental method using Completely Randomized Design (CRD) consisting of 3 factors, namely a consortium of fungal species (K), inoculum dose (D) and the duration of fermentation (W). Factor type consortium consists of three levels, namely (1) a consortium of *Trichoderma viride* and Aspergillus oryzae, (2) of *Trichoderma reesei* and Rhizopus oligosporus, and (3) consortium combined Trichoderma viride, *Trichoderma reesei,* Aspergillus oryzae, and Rhizopus oligosporus. Factors inoculum dose was 1%, 2%, and 3%. While the fermentation duration factor consists of 1, 2, 3, 4, 5, 6, 7 and 8 days. Each treatment was repeated three times.

## RESULTS AND DISCUSSION

1. Sugar Reducer Concentration (mg / L) at Various Corn Cob Consortium During Fermentation.

The most optimum time for the formation of reducing sugars is the sixth day of fermentation. Whether treatment with a consortium of k1, k2, and k3, the highest reducing sugar formation productivity achieved on day six. The addition of the next fermentation time resulted in decreasing the amount of reducing sugars. While the consortium of the most productive species yield of reducing sugar is a consortium of Trichoderma viride, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* (k3). The consortium consists of four fungi with

different enzyme activities that reshuffle corn cobs as substrate run more effectively than any other consortium. Degradation undertaken by a consortium of Trichoderma viride, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* (k3) on corn cob substrate that is rich in cellulose in the end will produce glucose. Glucose is ready to be used to support the growth of fungi during the fermentation process takes place.

2. Reducing Sugar Concentration (mg / L) at type of Consortium and Inoculum Dose During Fermentation

The highest reducing sugar produced in the fermentation with a consortium of Trichoderma viride, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* (k3) and 3% inoculum dose. Meanwhile, the optimum time is day 5 fermentation. Reducing sugar levels at the beginning of fermentation tended to increase and decrease at the end of fermentation. Use of high inoculum doses it will provide greater opportunities for fungal growth on the substrate. Inoculum dose of less may decrease the effectiveness of the fermentation process so that the formation of reducing sugar is slow. Addition of fermentation time after reaching peak production will not continue to increase the reducing sugar, but on the contrary, the more reducing sugars decreased as the number of fungi that continues to diminish as the death phase of the fungus.

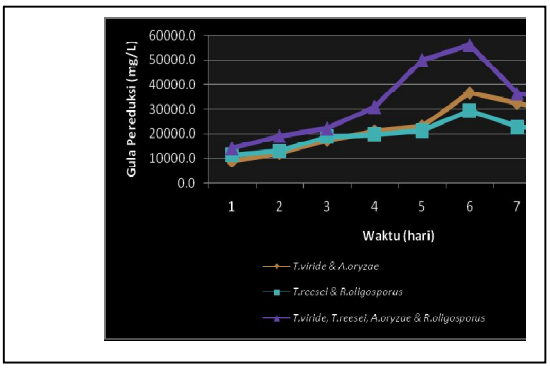


Fig. 1. Graph of reducing sugar (mg / L) corn cob with the influence of type of the consortium during the fermentation.

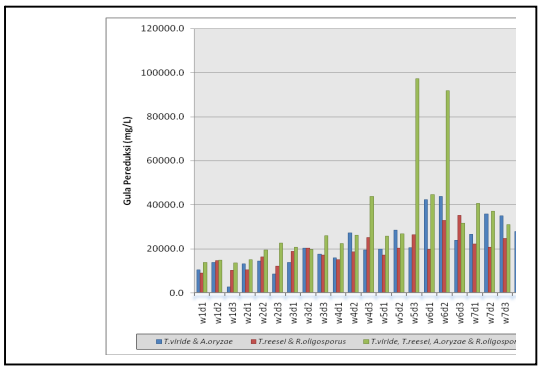


Fig. 2. Reducing sugar content (mg / L) at type of consortium, and the dose of inoculum.

3. The effect of the Consortium and Dose Inoculum on Nutrient Corn Cob

Fermentation plays a role in improving the nutritional value of low-quality feed ingredients such as nutrient availability, decreased levels of antinutrisi and repair efficiency of protein (Kompiang et al., 1994). Fermentation can alter the complex organic materials such as carbohydrates, proteins and fats into molecules more simple and easily digestible, adds flavor and aroma that is not preferred to be likeable and adds durability storage (Shurtleff and Aoyagi, 1979). Fermentation is series of the process of organic materials degradation by the enzymatic activity of microorganisms in order to obtain the desired component. Some changes may occur in the substrate due to the enzyme activity of microorganisms, including their chemical composition. In this study, analysis of chemical changes in corn cob substrate includes crude protein (CP), crude fiber (SK) and crude fat (LK). Chemical analysis of corn cob through proximate analysis conducted at the Laboratory of Livestock Ruminant Nutrition and Food Chemistry Faculty of Animal Husbandry, Padjadjaran University.

4. The effect of the Fungi Consortium, and Dose Inoculum on Levels of Crude Protein Corn Cob.

Crude protein content of fermented corn cob by proximate analysis are presented in Table1:

**Table 1.** Levels of Crude Protein (%) resulted by type fungi consortium, at various Dose Inoculum.

| Days | Dose | Type Fungi Consortium | | | Control |
|---|---|---|---|---|---|
| | | *T. viride* & *A. oryzae* | *T. reesei* & *R. oligosporus* | Konsorsium 4 Jamur | |
| 2 | 1% | 6.12 | 7.03 | 8.46 | 3.95 |
| | 2% | 6.6 | 7.03 | 8.63 | |
| | 3% | 5.33 | 7.66 | 9.96 | |

| | | | | | |
|---|---|---|---|---|---|
| 4 | 1% | 6.97 | 7.51 | 10.01 | 4.09 |
| | 2% | 7.63 | 7.74 | 10.17 | |
| | 3% | 5.82 | 7.25 | 10.35 | |
| 6 | 1% | 7.89 | 8.04 | 11.99 | 4.1 |
| | 2% | 7.91 | 8.34 | 12.02 | |
| | 3% | 7.38 | 8.59 | 12.48 | |
| 8 | 1% | 6.91 | 6.38 | 8.35 | 3.53 |
| | 2% | 7.87 | 7.78 | 9.01 | |
| | 3% | 6.43 | 7.84 | 9.17 | |

Based on descriptive analysis, in general, the addition of a consortium of three types of fungus on corn cobs to increase crude protein (CP). Increased levels of crude protein varied from 2:57% before fermentation to 5:33% -12.48% after fermentation. The highest crude protein content reaches 12.48% after fermentation, while the control only reached 4.1%.

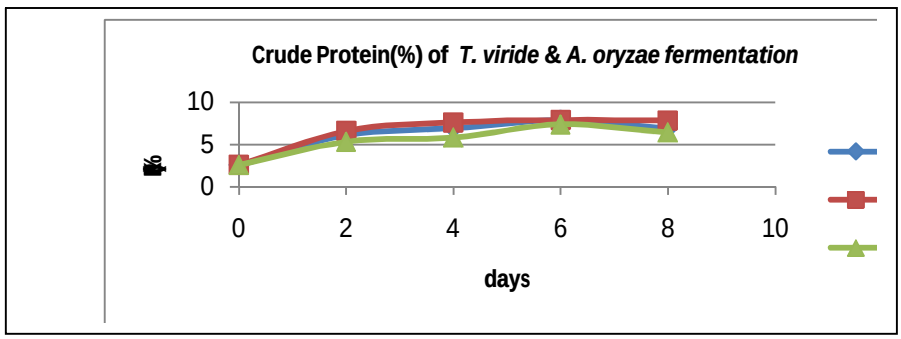


**Figure 3.** Crude Protein (%) resulted by the consortium of T. viride and A. oryzae fermentation.

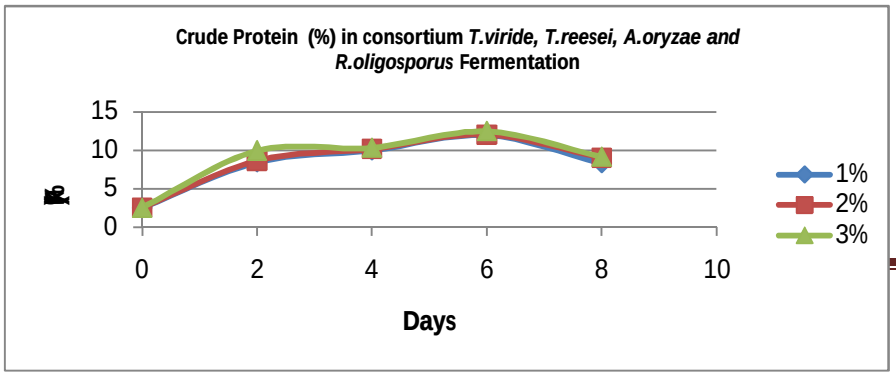

**Figure 4.** Crude Protein (%) resulted by the consortium of T. viride, T. reesei, A. oryzae, and R.oligosporus fermentation.

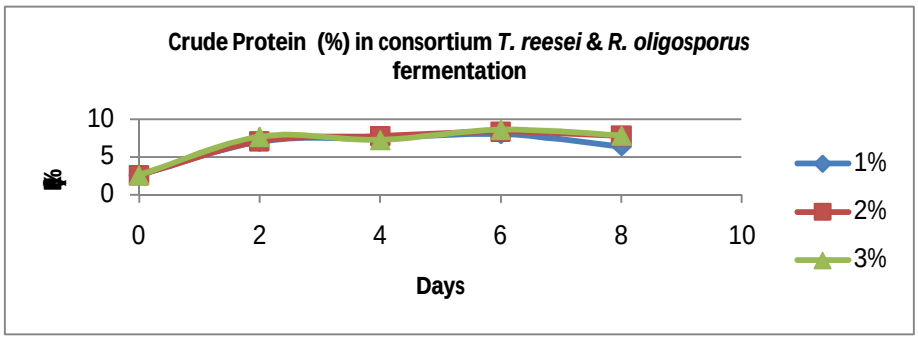


**Figure 5.** Crude Protein resulted by the consortium of T. reesei and R. oligosporus fermentation.

Consortium fungus Trichoderma viride, *Trichoderma reesei, Aspergillus oryzae* and *Rhizopus oligosporus* (k3) with a dose of 3% produced the highest crude protein content increased with the highest increase. Increasing crude protein content of fermented corn cob is related to the enzymatic activity of the fungus and the growing number of yeast cells during the fermentation process takes place (Tangendjaya, 1993). More growth of fungi, the crude protein content of the substrate will increase. The presence of *Aspergillus oryzae* and *Rhizopus oligosporus* in a consortium fungus Trichoderma viride, *Trichoderma reesei, Aspergillus oryzae* and *Rhizopus oligosporus* (k3) have a high proteolytic activity. *Rhizopus oligosporus* to produce protease enzyme that is able to describe the protein into amino acids and peptides (Wang et al., 1979). *Rhizopus oligosporus* protease causing an increase of dissolved nitrogen that comes from the decomposition of protein (Suliantari and Rahayu, 1990).

The increase of dissolved nitrogen that characterize the solubility of proteins increased as well. Meanwhile, *Aspergillus oryzae* produces active enzymes and proteolytic amilolitik that can improve nutrient digestibility of food (Lee et al.,

2006). *Aspergillus oryzae* encourage the decomposition of proteins into other forms. This resulted in nitrogen content and total amino acids increased during fermentation. The highest crude protein content of corn cobs was obtained at the treatment of fermentation with the fungus *Trichoderma viride* consortium, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* (k3) with a dose of 3%. This shows that the higher the dose the more inoculum fungi populations and in turn more and more the mycelium is formed Addition of inoculum dose causing fungus population density in a certain unit of weight substrate, so that microbial protein derived from the mushroom bodies increase the crude protein content of the substrate. In this case, the fungus acts as a source of single cell protein (Muhidin et al., 2001).

Increasing crude protein levels are also due to decrease of starch content or carbohydrate and fat which is supported by the proliferation of fungal growth that contains enough protein. Besides dihasilakan enzyme is also produced extracellular enzyme protein and protein metabolism of the fungus causing the increase in crude protein content. Increased levels of crude protein on the second day after fermentation is higher when compared with crude protein levels on day four, six or eight. This is apparently related to the consortium of fungal enzymes that are secreted whose activity was higher in the second day of fermentation compared to the fourth day, six or eight. Crude protein content on the eighth day of each treatment has decreased, it is due to decreasing availability of nutrients for use during fermentation substrate for fungal growth.

The number of fungi increased with increasing time of fermentation causes the energy needs for the fungus has been increasing. The fungus will degrade proteins that have been previously synthesized to meet energy needs. The results are consistent with research Sulaiman (1988) which states that the crude protein of fermented product will have increased in line with the long fermentation time, and then will decrease again. Consortium fungus Trichoderma viride, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* (k3) has the best potential to

improve crude protein corn cobs when compared with other consortia. The highest crude protein was achieved at a dose of 3% and in 2 days.

5. Effect of the Consortium Fungus, Dose Inoculum On Crude Fiber of Cob Corn.

Crude fiber content of corn cobs decreased over increasing fermentation time. Crude fiber content decreased from 29.14% before fermentation to 16:56% - 24.28% after fermentation. The lowest crude fiber content after fermentation is 16:56% 26.41% compared to controls. Fermentation treatment with 3 types of mushrooms consortium have significant decrease in crude fiber content is different. So is the length of fermentation time which affects the decrease in crude fiber content along with increasing time of incubation. While the use of inoculum dose for every type of consortium on the same fermentation time, seemed not so different.

Consortium fungus Trichoderma viride, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* (k3) with a dose of 3% gave the highest crude fiber content decreased by long fermentation period of two days. Crude fiber content of fermented corn cobs decreased. This is apparently due to the growth of fungi that require some nutrients, such as crude fiber as a substrate. In fermentation, the medium serves as a source of carbon, nitrogen and energy. Decrease in crude fiber fermentation product can also be caused by tercernanya the crude fiber by the fungus. Crude fiber content of corn cobs which decreases show that there is pendegradasian corn cob fiber bonding complex into a simpler form of bond (oligo and monosaccharides). Components of crude fiber in feed ingredients to increase kecernaanya with the help of fiber degrading enzymes produced by *Trichoderma viride* (Ramli et al 2005). This is in line with the growth of mycelium which at the same time degraded cellulose and hemicellulose. These events closely related to the role of the fungus *Trichoderma viride* and *Trichoderma reesei* in the production of cellulase enzyme complex that can degrade components of crude fiber by fermentation process.

Cellulase activities produced by the fungus *Trichoderma viride* and *Trichoderma reesei* able to break the bonds of polysaccharides in corn cob so kecernaannya increases. Digestibility increased mean metabolizable energy value also increases. The results in line with the results of research and Abou-Zeid Abou-Zeid (1991) who suggested that the cellulase enzyme activity reached maximum on day 6. When the changes both in quantity and quality of organic elements and then the enzyme activity and changes in organizational components are relatively stable. Fermentation in 2 days a consortium believed to be the optimum time the fungus Trichoderma viride, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* (k3) in the lower crude fiber content, because on the eighth day of crude fiber content increased again. The increase in crude fiber content in the media due to the addition of coarse fiber that comes from the body of the fungus. Fungal mycelium and sporangia walls mainly consist of substance chitin, which is a compound that has a function similar to cellulose in plant cells. Meanwhile, according Suparjo et al., (2003), this is presumably because of changes in organic materials such as starch can cause shrinkage of the substrate. The fermentation process of carbohydrate components will lead to depreciation of the basic ingredients so that the proportion of crude fiber increased. In addition, crude fiber can be derived from cell walls ofmicroorganisms (Kompiang et al., 1994).

6. The effect of the Consortium of Fungus, Dose Inoculum on Levels of Crude Fat of Corn Cob.

Crude fiber content of fermented corn cob through proximate analysis are presented in Table 6. Crude fat content decreased from 5:52% before fermentation to 1.08% -3.81% after fermentation. The lowest crude fat content after fermentation is 1.08% compared with the control 2:54%. The reduced value of the fat content due to the enormous population of fungi. More and more population fungus that grows the more fat that reorganized into free fatty acids are then used by the fungus for the purpose of his life. Overhaul of fat into the simpler form

used by fungi to obtain energy sources. This is in line with the opinion Shurtleff and Aoyagi (1979), who explained that occur revamp of fat in the fermentation process using *Rhizopus oligosporus* about 35% hydrolyzed neutral fat and the emergence of free fatty acids, namely linoleic acid which are 40% used by fungi for life.

Decline in crude fat content during the fermentation process to prove that Trichoderma viride, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* in addition to utilizing carbohydrates also utilize fat for growth and breeding. In line with what was raised Wagenknecht (1961) who cited Darana (1995) that during the fermentation process, the fungus can utilize fat as an energy source. The number of fungi increases with increasing time of fermentation. The use of fat as an energy source by the fungi associated with fungus secreted lipase enzyme to break down fat substrate. *Aspergillus oryzae* and *Rhizopus oligosporus* produces lipase enzyme known to degrade components of fat, as well as Trichoderma viride. Lipase enzyme plays a role in the synthesis of various organic materials and about 20% reshuffle undertaken involving fungal lipase. When considering the length of fermentation, the second day is the highest crude fat content decreased. This is caused by the fungus was located at a phase of rapid growth (exponential phase), ie when the mycelium grows rapidly, which in turn leads to increased energy needs, on the fourth day and sixth the utilization of fat for growth and development of the fungus began to decline. While on the eighth day, crude fat levels back up. This is thought to occur because of increased fungal cell mass media raising the fat content. Yeast cells have potential as a source of fat, because the yeast cells containing 4.9% crude fat dry matter.

Table 2. Crude fat content (%) Corn Cob Fermentation Resulted By Type Consortium Mushrooms, Dose

Inoculum .

| Day | Dose | Type of Concortium | | | Control |
|---|---|---|---|---|---|
| | | T. viride & A. oryzae | T. reesei & R. oligosporus | Konsorsium 4 Jamur | |
| 2 | 1 % | 3.72 | 2.99 | 3.69 | 4.69 |
| | 2 % | 3.81 | 3.34 | 3.13 | |
| | 3 % | 3.6 | 3.76 | 2.69 | |
| | 1 % | 3.37 | 2.83 | 2.69 | |

Kadar lemak kasar tongkol jagung menurun seiring bertambahnya waktu fermentasi.

## CONCLUSION

Based on research on the effect of inoculation of the fungus *Trichoderma viride* consortium, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* on the nutritional quality of corn cobs can be concluded as follows:

Consortium fungus Trichoderma viride, *Trichoderma reesei*, *Aspergillus oryzae* and *Rhizopus oligosporus* has a high lignocellulosic degradation activity increased in line with fermentation time and inoculum dose. Inoculum dose of 3% and the fermentation time of 6 days is proven to increase levels of crude protein (12:48%), lower levels of crude fiber (16:56%) and crude fat content of corn cobs (1:18%).

## REFERENCE

Hardiyanto, R., D. E. Wahyono, C. Anam. Suyatmo, G. Kartono, dan S. R. Soemarsono. 2002. *Kegiatan Teknologi Pakan Lengkap (Complete Feed) Sebagai Peluang Agribisnis Bernilai Komersil di Pedesaan*. Makalah Seminar dan Ekspose Teknologi Spesifik. Badan Litbang Pertanian Jakarta.

Mirzah. 1998. Peningkatan Kualitas Nilai Gizi Tepung Limbah Udang Melalui Pengolahan Dengan Uap Panas. Jurnal Penelitian Andalas No. 26 Tahun X.

Suparjo, Y. dan Heru, H. 2003. *Peningkatan Nilai Nutrisi Dedak Padi Melalui Proses Biokonversi Menggunakan Kapang* Aspergillus niger. Jurnal Ilmiah Ilmu-Ilmu Peternakan. Vol. VI. No. 4.

Koswara, J. 1991. *Budidaya Jagung*. Jurusan Budidaya Pertanian. Fakultas Pertanian. IPB, Bogor.

Departemen Pertanian. 2004. *Statistika Pertanian Jagung Indonesia*. http// www.deptan.go.id.

Kompiang, P., J. Dharma., T. Purwadaria., E. Sinurat, dan Supriyati. 1994. *Protein Enrichment Study, Cassava Enrichment Melalui Proses Biologi Untuk Ternak Monogastrik*. Laporan Hasil Pertanian. Balai Penelitian dan Pengembangan Pertanian Ciawi. Bogor.

Shurtleff, W. dan A. Aoyagi. 1979. *A Super Soy Food From Indonesia*. The Book of Tempeh. Harper and Row. New York.

Tangendjaya, B. 1993. *Bungkil Inti Sawit Dan Pollard Gandum Yang Difermentasi dengan Rhizopus oligosporus untuk Ayam Pedaging*. Jurnal Ilmu Peternakan , Vol.6. No.2: 34-38.

Wang, H.L., E.W. Swain. And C.W. Hesseltine. 1979. *Mass Production of* Rhizopus oligosporus. Spores and Their Application in Tempeh Fermentation. Journal Food Science, 40: 168.

Suliantari, dan W. P. Rahayu. 1990. *Teknologi Fermentasi Umbi-Umbian dan Biji-Bijian*. Pusat Antar Universitas Pangan dan Gizi. IPB. Bogor.

Lee, K. W., S. K. Lee and B. D. Lee. *Aspergillus oryzae as Probiotic in Poultry - A Review*. International Journal of Poultry Science 5 (1): 01-03, 2006.

Muhidin, N. H., Juli, N., Aryantha, I. N. P. 2001. Peningkatan Kandungan Protein Kulit Umbi Kayu Melalui Proses Fermentasi. JMS 6 (1): 1-12.

Ramli, N., R.A. Haryadi dan D. G. Dinata. 2005. *Evaluasi Kualitas Nutrien Dedak Gandum Hasil Olahan Enzim Yang Diproduksi* Aspergillus niger *dan Trichoderma viride Pada Ransum Ayam Broiler.* Jurnal Media Peternakan. Vol. 28. No. 3. hlm: 124-129.