Research Article

Encapsulated of Cassava, Skim Milk and Malto Dextrin Symbiotic Characteristic and their Synergitic Effects against *Salmonella* sp. Inhibition

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Abstract | Antibiotics are the most widely used feed additives in broiler feed, but dependence on antibiotics has now begun to be reduced when it is known that antibiotics use has produced harmful residuals on both livestock and human health. The study produced synbiotics from the fungus *Trichoderma sp*. developed in local materials such as cassava and then encapsulated using skim milk and dextrousmalto. Design research in this study experimental design using a factorial Completely Randomized Design (CRD) pattern of three replications. The research concluded most excellent concentration of dried cassava flour was 100% and supplemented with 4% *Trichoderma koningii*. The greatest number of synbiotics spores 416.2, pH ranged 4.40-5.80, glucose test 0.30%. Result of proximate analysis synbiotics, which have already been encapsulated using maltodkestrin and skim milk contain dry matter 89.11%, crude protein 3.21%, Crude fat 0.73%, Crude fiber 2.87% and Ash 1.25%. The inhibition of encapsulated synbiotics to *Salmonella sp* shows that the resulted synbiotics would be encapsulated using ratio maltodextrin:skim is 1:10.

Keywords | Synbiotics, *Trichoderma koningii*, Broiler, Encapsulation, Proximate

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INTRODUCTION

The use of feed additive in breeding broiler is prevalent due to feed additive has contributed in increasing poultry digestive efficiency that has impact on improvement of the poultry production appearance. Antibiotic is feed additive, which is mostly used for broiler feeds, but dependency on antibiotic nowadays has been reduced when the fact shows that antibiotic remains residue, which is harmful for poultry and human health (Emma et al., 2013). Residue effect from antibiotic had triggered European Union society to forbid the application of various antibiotics on January 1st, 2006 (based on regulation number 1831/2003) as concern of the society in the world who pay more attention on health.

The phenomenon has insisted the experts of poultry feed nutrition to find feed additive as alternatives as substitute for antibiotics, such as the application of probiotics, prebiotics, and synbiotics. Probiotics will affect the physiological function of the intestines, directly and indirectly, by modulating intestinal microflora and mucosal immune system, particularly gastrointestinal mucosa. *Trichoderma* is one of probiotics from mold that produces extracellular cellulose enzyme, in which high cellulose content of the material may produce carbon microorganism growth through bioconversion process (Rifai, 1969; Mandels, 1982; Enari and Fogarty, 1983; Pelczar and Chan, 1988; Fardiaz, 1992) One of the most cellulase-producing microorganisms is *Trichoderma sp.* in the solid substrate fermentation process such as *gaplek* (dried cassava), rice

bran or corn substrate (Bardant et al., 2013). *Trichoderma koningii* may increase the renewal of the organic materials, loosen and break lignin and cellulose bond, as well as ferment them without creating detrimental effects, which are caused by indecomposable organic materials.

Salmonella sp. is a microbe that cause salmonellosis to humans and animals that cause illness and death. One of the efforts to improve performance and balance microbes in digestion and reduce unwanted microbes, it is necessary to give probiotics and prebiotic. Prebiotics as nutrition is useful for microbial development, and combination between probiotics and prebiotics are called synbiotics (Haryati, 2011). In general, prebiotic limitations are indigestible food ingredients and have a beneficial influence on the host through growth stimulation and/or selective activities against one or more beneficial microbes in the digestive system. A combination of probiotics and prebiotis is so-called synbiotics. The application of synbiotics on poultry feeds should not be more than 2% because it will form residue (Wijayanti et al., 2008). Based on Luoma et al. (2017) using symbiotic can have an impact lesser of *Salmonella* colonization very well. Symbiotic also influence the intestinal microflora by increasing beneficial bacteria and decreasing pathogenic bacteria within the intestines due to competitive exclusion and production of antimicrobials.

The development of *Trichoderma koningii* probiotic, which are bred in local material, such as *gaplek* (dried cassava) in Indonesia, is expected to provide an opportunity to the farmers to make synbiotics by themselves, which would be mixed in the feeds. But, the storability of synbiotic should be considered whereas it may be damaged in probiotic activities if it is stored for a long time. Encapsulation method is the appropriate method to lengthen the storability of a product. Encapsulation is a coating process of core material using specific encapsulation material, which is beneficial to maintain its viability and to protect probiotic from any damage as a result of detrimental environment, as well as to increase the durability (Feng et al., 2000; Pacifico et al., 2014). To produce good synbiotic, several things must be done in the research, such as analyzing the spore characteristics, colony density, glucose level, pH, dry matter, protein, fat, and ash levels using proximate analysis.

MATERIALS AND METHODS

Making and characterization of synbiotics

Trichoderma koningii will was growing optimal under humidity 80 – 90% and water content should not be less than 14–15% on cereals or dry foods (Rakhmani, 2005). The growth process of *Trichoderma* in this study occurred at 80% humidity. In order to obtain humidity level of 80%, humidity of the planting media of *Trichoderma* must be counted by calculating weight of the dried cassava (1000 g) x dry matter (87%) x expected humidity (80%) and resulted 70ml/100g aquadest/sample. Furthermore, the media of the dried cassava flour was weighted 100 g and 50 g, in which each of them consisted of 24 experimental units. Dried cassava media was sterilized using autoclave for 20 minutes at 121°C, under pressure 15 psi and 5 minutes using low heat to stabilize temperature. Dried cassava media was set apart for 1 x 24 hours, and then it would be isolated with *Trichoderma* in 8 treatments based on Completely Randomized Design (CRD) scheme on each sample in *enkas*. Then, the media was incubated for 3 weeks at room temperature.

Calculation for number of the colony spore

Mold of *Trichoderma* sp., which has been inoculated for 3 weeks, was diluted 3 times using 10 ml aqudest/1gr. Hemocytometer test was done to find out the number of spores that grow under light microscope by 4x magnification up to 10x magnification, and hand counter was used to count the number of spores. Calculation of *Trichoderma* sp. colony is based on *Standard Plate Count* (SPC) Following the procedure/formula of Bacteriological Analytical Manual: Number of population (cfu/gr).

$$\text{number of colony} \times \frac{1}{dilution\ factor} \times \frac{1}{sample\ weight\ factor}$$

Synbiotic encapsulation

Prepare the whole feeds that have been treated with *Trichoderma koningii* Prepare the used materials as coating or encapsulation material. The materials include Maltodextrin and skim milk (10%) (1:1), Maltodextrin and skim milk (20%) (1:2) and Maltodextrin and skim milk (30%) (1:3) and each is put into 500 ml water. The use of 1% maltodextrin is the maximum limit when used as an encapsulation material. The result of the study Harimurti et al. (2017) shows that the whole power of inhibition of probiotics decreased after microencapsulation using maltodextrin is 1%. The prepared encapsulation materials were evenly mixed in accordance with each ratio until it was completely homogenous. For the feed component has conformed to the design of the arranged research. After the encapsulation materials were homogenous, they would be sprayed on the feed materials. Each of the feed material was sprayed with the percentage of Maltodextrin and skim milk, and then evenly mixed until it was completely homogenous using feed mixing molen. After that, the homogenous mixture was sun-dried until component of each material was ready to be mixed with other component.

pH test

pH test was done to measure the enzymatic activity by finding out the glucose level using Somogy-Nelson's method (Hatanaka and Kobara, 1980).

pH test carried out on each treatment using litmus paper on the isolation of *Trichoderma koningii* mold on cassava flour which has been diluted 3 times using 10ml aqudes/1g. Then the pH is observed based on the colors that appear and are adjusted to the manual pH meter standard. pH test was done to measure the enzymatic activiy with the results glucose level measurement by finding out the glucose level using Somogy-Nelson's method (Hatanaka and Kobara, 1980).

Glucose level measurement

The absorbant is measured using spectrophotometer with wave length 540 nm. Value of the obtained absorbant was put into standard curve of glucose, so that value for sample of the glucose would be obtained (Indrayati et al., 2017).

Synbiotics inhibition test on *Salmolla* sp.

The applied method of antibacterial effect test in the research used Kirby-Bauer'method (disc diffusion). The test used Mueller Hinton Agar (MHA) media that was two petri dishes and 10 whatman paper discs. Whatman paper is made using perforator in order to form like a disc of 6 mm in diameter. Before the bacteria are planted in Mueller Hinton Agar (MHA) media, the front of a petri dish is divided into four parts and coded with label. The cotton bud is dipped into the bacterial suspension in Nutrient Broth (NB) media and pressed a little on the tube wall and then scratched on Mueller Hinton Agar (MHA) media. The diluted synbiotics were put into the tube, and the whatman paper disc was dipped into the synbiotic liquid and then, put it on surface of the Mueller Hinton Agar (MHA) media by a little pressure so that the whatman paper disc will be well attached, and after that it will be incubated at 37°C for 24 hours.

Synbiotics proximate test

Proximate test refers to the research by (Ferris et al., 1995) over several nutritive elements, such as: dry matter, protein, fat, and ash levels.

Data analysis

The collected data was subjected to ANOVA using SPSS version 17 with level of trust 95%. Further Treatments mean differences were separated by using Duncan test.

RESULTS AND DISCUSSION

Making and characterization of synbiotics

The growth of *Trichoderma koningii* in media of dried cassava flour 50% and 100% is presented in Figure 1. The Figure 1 describes optimal growth of *Trichoderma koningii* in media of dried cassava flour 100g in level 4%. It shows that the less or the more *Trichoderma koningii* given during the isolation is not good for its growth, as occurred by the addition of 1% or 6% *Trichoderma*, in which the graphic tends to decline. It is presumed that the average decline of colony was due to nutrition availability in media tends to decrease. One of the causes that bacteria/mold has declining phase or death is less nutrition availability. As described that number of *Trichoderma koningii* has correlation, which is inversely proportional to the nutrition of the media, whereas the higher colony of *Trichoderma koningii*, the lesser nutrition in the media would be left. It was supported by Rakhmani (2005) in his research, in which *Trichoderma* will grow optimal at 25°C–30°C and grow well at 35°C–37°C. According to Islamiyati and Asriany (2020) the best duration of incubation is 2 weeks and 5% *Trichoderma* on corn cob. Research showed that population of multiantagonist *Streptomyces* sp., *Gliocladium* sp. and *Trichoderma harzianum* could be increased by formula pellet of glutinous rice flour with storability of 3 weeks. The given optimal percentage is 2%–4% on weight of the used media because *Trichoderma koningii* will be able to reproduce its colony optimally along with total distribution and balanced area.

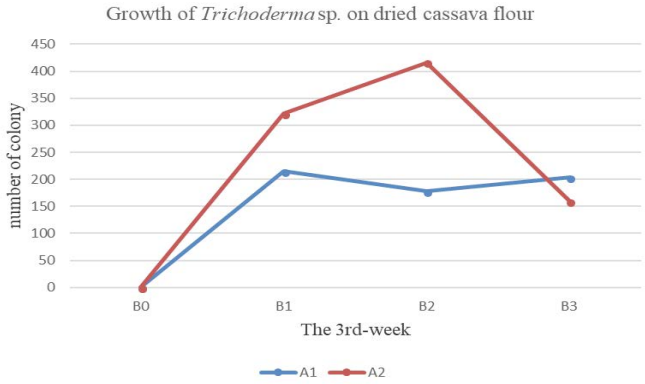

Figure 1: Graphic for *Trichoderma koningii* growth in dried cassava flour media.
Notes: A1(Dried cassava/*Gaplek* 50%), A2 (Dried cassava/*Gaplek* 100%), B0 (*Trichoderma* 0%), B1 (*Trichoderma* 2%), B2 (*Trichoderma* 4%), B4 (*Trichoderma* 6%).

Carbohydrate in a substrate of the dried cassava flour will be degraded by *Trichoderma koningii* activity, which is used as energy for its growth, therefore carbohydrate proportion will decrease while protein proportion will increase. So that it will produce high protein due to the microorganism growth, in which properties of the microbe is contributing protein. It is supported in Akbar et al, (2014) who reported that mold contains raw protein 31– 50%, so that the mold growth automatically increases raw protein. Mold is a protein source of single-cell (Fardiaz, 1992; Akbar et al., 2014). According to Pamungkas et al. (2011), the increase protein of the fermented feed is contribution of single cell protein from *Trichoderma koningii* cell during fermentation.

Number of synbiotic spore colony

Trichoderma koningii mold, which has been inoculated with substrate of the dried cassava flour, has significant effect on its viability (Table 1). On factorial correlation test, it has interaction between media weight and percentage of *Trichoderma koningii* increase at significance level 5%.

Table 1 shows significant different numbers of spore colony between the increase percentage of *Trichoderma koningii* 4% and 6%. Meanwhile, the media weights of 50g and 100g do not show significant interaction against number of the resulted spore colonies. It has been supported by result of DMRT test, which indicates that the best treatment was from 4% increase of *Trichoderma* from total used media. Therefore, it can be concluded that media weight does not affect number of the spore resulted by *Trichoderma koningii*. According to Gandjar and Rifai (1999), the fungi growth is affected by some factors, such as substrate, humidity, temperature, acidity of the substrate (pH), and chemical compounds in the environment. As a whole, results of hemocytometer test generate 10^8-10^9 cfu/g, which means it is highly possible that *Trichoderma* sp. produces cellulase enzyme during fermentation of dried cassava flour. It is supported by research from Ezekiel et al. (2010), which reported that pile (*onggok*) fermentation using *Trichoderma* to produce cellulase by inoculums concentration of 10^7 spore/g, pH 5 with each cellulase 0.168 and 0.072 µmol/minute/ml for carboxymethyl cellulase and Filter paper–ase. According to Indrayati et al. (2017), conversion of sago wastes using *T. harzianum* mold may produce propagule 1.6 x 10^9 cfu/gr with amylase activity 1.420 unit/g, cellulase activity 0.427 unit/gr, glucose level 36.150 µg/g, and pH 4.20.

Trichoderma koningii grows faster in 50g dried cassava flour media, in comparison with in 100g dried cassava flour, as shown by darker green in 50g media at the 14th-day. Green is one of physical indicators to determine whether *Trichoderma koningii* has grown or not. According to Gandjar and Rifai (1999), *Trichoderma koningii* has white, yellow, light green, and dark green colonies. Cell arrangement of *Trichoderma koningii* has many cells that lined up and form fine thread, which is so-called hypha. *Trichoderma koningii* is able to reproduce colony faster on small area of the media. Spore density that has been observed on hemocytometer test showed spore density of *Trichoderma koningii* during 3 weeks incubation, which indicated significant difference on weight of both media (Figure 2).

Note: 100gr dried cassava flour at the 21st-day by 4x magnification

Note: 50gr dried cassava flour at the 21st-day by 4x magnification

Figure 2: Density of *Trichoderma koningii* colony in different media weights.

Observation by light microscope showed that the greatest colonies was found in 100g dried cassava flour even though, in physical observation, it took relatively longer to observe its growth in comparison with its growth in 50g dried cassava flour. Flour media, such as dried cassava flour, rice polish, and rice bran have solid texture in comparison with texture of the corn rice formula, which tends to be more crumbly. Therefore, it possibly inhibits the growth of *Trichoderma* in more solid media. It is said that inhibition of fungi growth is due to the increase consistency of the medium and inhibition of air and water diffusion.

Calculation of pH synbiotics

Results of pH synbiotics are presented in Table 2. Data shows significant difference of pH levels among the increa-

Table 1: Number of *Trichoderma koningii* spore colony in dried cassava flour media.

Weight of dried cassava flour media	Percentage of *Trichoderma koningii*				Mean
	0%	2%	4%	6%	
50 g	0	214.800±62.9	177.800±57.2	203.400±47.4	198.67
100 g	0	321.800±146.9	416.200±153.8	159.600±59.8	299.20
Mean	0	268.300^{ab}	297.000^{b}	181.500^{a}	248.93

Notes: Different superscripts show significant differences at level 0,05.

Table 2: Results for calculation of pH synbiotics cassava flour media.

Weight of dried cassava flour media	Percentage of *Trichoderma*				Mean
	0%	2%	4%	6%	
50 gr	5.40±0.55	5.20±0.45	4.40±0.55	6.80±0.45	5.45
100 gr	7.00±0.00	4.40±0.55	4.40±0.55	4.80±0.45	5.15
Mean	6.20^{b}	4.80^{a}	4.40^{a}	5.80^{b}	5.30

Notes: Different superscripts show significant difference at level 0,05.

-se percentages of *Trichoderma koningii* 0%, 2%, 4%, and 6%. For 0% increase of *Trichoderma koningii*, the resulted pH was 6.20, on average, or tended to be neutral. Meanwhile, in media of dried cassava flour that had been inoculated with *Trichoderma koningii* during the incubation period, pH decrease, as a whole, to be acid in which average pH was 4.40 – 5.80. It was because of *Trichoderma* sp. activities in fermenting the dried cassava flour and evaporated the resulted acids during the fermentation process. It conforms to the natural properties of *Trichoderma koningii,* in which it could live in wide interval of pH that range 2.0 – 8.5 even though mold prefer to live in acidic condition or pH below 7,00 (Rakhmani, 2005). Jaelani (2007) reported that ideal pH for *Trichoderma koningii* growth is 5-6, and results of the research showed average pH 5.84 as counted since fermentation in the first day to the 14th-day.

Trichoderma sp produces endoglucanase and exoglucanase up to 80%, but its β glucosidase is lower, so that the main product of its hydrolysis was not glucose, but cellobiose (Anita, 2013). The formation of acidic atmosphere in dried cassava media indicated that cellulose in dried cassava has been well consumed by *Trichoderma* through fermentation process and produce high cellulase enzyme. The higher enzyme level is, the more acidic media will be grown by *Trichoderma* (Anita, 2013). Suyandra (2007) suggested that the more active of the microbe in fermentation process, the higher products will be resulted. These resulted acids will decrease pH of the media.

Glucose level of synbiotics

Enzymatic activities can be found out by observing total glucose level, in which the higher absorbance is resulted, the more reducing glucose will be contained. Glucose value was taken in accordance with spectrophotometer test in 100g media using 2 samples for each treatment based on result of the hemocytometer test, the greatest and the least number of spore colonies. Data for result of the reducing glucose test is presented in Table 3.

Table 3 shows that 0% *Trichoderma koningii* has higher reduction glucose than media, which is inoculated with *Trichoderma koningii.* It is due to dried cassava media, which is not inoculated with *Trichoderma koningii* and does not show enzymatic activity that is reinforced by no change of pH to be acid in pH test. Therefore, the contained starch would not be completely hydrolyzed. The more the starchs are hydrolyzed, the higher reduction glucose will be obtained. On the contrary, low reduction glucose indicates more amylose and amylopectin polymer that are not degraded in starch (Sun et al., 2017).

The whole dried cassava flour that has been incubated by *Trichoderma koningii* show significant decrease against total reduction glucose. The highest decrease of total reduction glucose is on the combination level 4% addition of *Trichoderma koningii* for about 0.39%. It is supported by Gandjar and Rifai (1999) which suggested that in substrates of rice, cassava, and potato, the fungi should be able to excrete α-amylase enzyme to process amylum to be glucose. And then, the glucose will be reabsorbed by fungi. So that it would cause the greatest colonies of spore decrease the highest reduction glucose on percentage of 4% *Trichoderma koningii*.

In accordance with results of hemocytometer test and pH test, percentage of 4% *Trichoderma koningii*, they show the greatest spore colonies for about 3.0 x 10^9 cfu/g, on average, and decrease the lowest acidic pH, 4.40. Therefore, it is possible that the increase 4% *Trichoderma koningii* has degraded amylose and amylopectin polymer. It is supported by research from Mountzouris et al. (2010) that the ability is derived as a result of specific enzymes belong to organisms to break the bond. The fraction of complex molecules into simple molecules will facilitate absorption by digestive tracts of human and animals.

Result of synbiotics proximate test on dried cassava

Results of the synbiotics proximate test on dried cassava are presented in Table 4. The highest DM was derived from encapsulated synbiotics by ratio of maltodkestrin and skim milk (1:20), in which DM reached 89.11%. Results of ANOVA test against DM level of each treatment showed significant difference P<0.05. The highest CP was derived from encapsulated synbiotics by ratio maltodkestrin and skim milk (1:10), in which DM reached 3.21%. Results of ANOVA test against DM level of each treatment showed significant difference P<0.05. The highest EE was derived from encapsulated synbiotics by ratio mattodekstrin and skim milk (1:20) was 0.73; but based on results of ANOVA test, EE values of all treatments did not show significant difference P>0.05. For ratio of CF values, the results did not show significant difference P>0.05; but the highest CF values were derived from encapsulation of maltodextric and skim milk by ratio (1:20) and the value was 2.87%. Result of final proximate test was ash level, in which the lowest ash was derived from encapsulation using maltodextrin and skim milk by ratio (1:10) and the value was 1.06%, ANOVA test from various treatments concluded that Ash values did not significant difference P>0.05. From the whole variables of proximate analysis, it can be concluded that the encapsulated synbiotics using maltodextrin and skim milk were derived from ratio (1:10) and (1:20).

Table 3: Test results of glucose level in synbiotics.

Treatment	Reduction glucose (%)
Media of dried cassava flour 100 g + *Trichoderma* 0%	7.57
Media of dried cassava flour 100 g + *Trichoderma* 2% (+)	0.75
Media of dried cassava flour 100 g + *Trichoderma* 2% (-)	0.97
Media of dried cassava flour 100 g + *Trichoderma* 4% (+)	0.49
Media of dried cassava flour 100 g + *Trichoderma* 4% (-)	0.30
Media of dried cassava flour 100 g + *Trichoderma* 6% (-)	0.69
Media of dried cassava flour 100 g + *Trichoderma* 6% (+)	0.31

Notes: Superscript (+) for the greatest number of spores and (–) for the least spore

Table 4: Results of synbiotics proximate analysis on dried cassava.

Encapsulation (%)	Result of synbiotics proximate test of dried cassava				
	Dry matter	Crude protein	Either extract	Crude fibers	Ash
Without encapsulation	83.85±2.13	3.20±0.45	0.61±0.08	2.62±0.23	64.22±5.18
Malto:Skim (1:10)	86.24±1.42	3.21±0.21	0.52±0.11	2.87±0.15	1.06±0.19
Malto:Skim (1:20)	89.11±1.21	2.94±0.17	0.73±0.05	2.20±0.11	1.25±0.23
Malto:Skim (1:30)	87.94±2.53	3.20±0.31	0.57±0.14	2.48±0.34	1.22±0.12

The decrease of DM was due to *Trichoderma koningii* activities during fermentation process, in which it live well in media that contains high starch and glucose. Starch and glucose, which were derived from maltodextrin and dextrim, were used as food reserves in the form of energy from *Trichoderma koningii* to produce enzyme, particularly cellulase enzyme that could degrade the feed ingredients (Harman, 2006). Besides the dry matter decreased significantly, it indicated that the fermentation process has run well.

The fermentation process occurred through a series of chemical reactions that change dry matters BIS into energy (heat), water molecule (H_2O), and CO_2; this process may decrease dry matter of the used substrate (Ginting and Krisnan, 2006). Santoso (2001) reported that the increase of dry matter related to ability of the accelerator, which was inoculated in material that could decrease pH and inhibit the bacterial growth of *clostridia*, and then suppress the nutrient degradation, so that dry matter, which has been supplemented with accelerator, will be higher than without accelerator. Relatively high dry matter in feed ingredients is directly proportional to storability, in which the higher level of dry matter, the storability will be longer.

The decrease of CP (crude protein) as a result of *Trichoderma koningii* is able to produce starch breaker enzyme, for example, cellulase enzyme that may change chemical composition of the material, such as carbohydrate and fats, which were decreased due to the increased protein as a result of starch breaker enzyme. *Trichoderma koningii* is able to produce enzyme, particularly cellulase, which could degrade (Udding et al., 2014).

Results of the proximate analysis indicated that crude fibers decrease, on average, along with the treatment of enkapsulasi Malto:Skim (1:30) except on synbiotics of rice bran that did not show any decrease of crude fibers. It was presumed that such decrease was due to the change of crude fibrous components in synbiotics of dried cassava and corn during fermentation process because *Trichoderma koningii* is able to decompose crude fibers into more simple and soluble compound. According to Hood et al. (2003), crude fibers may decrease as a result of decomposition process on fibrous components by fungi. Most of crude fibers are derived from the plant cell wall and contain cellulose, hemicelluloses, and lignin.

Trichoderma koningii is able to degrade fibrous components because it produces enzyme, which could degrade lignin, and *Trichoderma koningii* is also able to produce enzyme, which degrades cellulose. Crude fiber is the main component that mostly contains carbohydrate as energy source for fungi/mold, besides extract ingredients without nitrogen (BETN). Therefore, a part of crude fiber fraction is used as energy source for the growth of *Trichoderma koningii* fungi, particularly for mycelium growth by degrading crude fibers using the resulted cellulase enzyme. As a result, the crude fibers decrease in the substrate, which is used as inoculation media (Udding et al., 2014).

Ashes are remains of the feed burning in furnace at 500 – 600°C, so that the whole organic materials will be burned out. Ashes are part of burning remains in furnace at 400-600°C that comprise of inorganic substances or minerals. Results of this research conformed to results of the research by (Hartadi et al., 1990; Zuprizal, 2000), which suggested that amount of ashes from rice bran was 10%-20% (Hartadi et al., 1990; Zuprizal, 2000).

Result from the inhibition test of the encapsulated synbiotics on *Salmonella* sp.

Synbiotic is microbial feed that could increase microbial balance in livestock digestive tract, and one of the potential microbes, used as synbiotic, is *Trichoderma* sp. *Trichoderma koningii* is potential to produce enzymatic secondary metabolite as antibiotic, such as viridin and trichomidin. Viridin and Trichomidin may inhibit the growth or even exterminate other fungi or bacteria. Data for synbiotic inhibition in dried cassava is presented in Table 5, *Salmonella* inhibition from the encapsulated synbiotic of dried cassava using maltodextrin and skim milk with diverse ratio resulted significant differences (P<0,05). Result for the highest inhibition was derived from synbiotics of dried cassava, which was encapsulated with maltodextrin and skim milk by ratios (1:10) In which the inhibition values were 4.91 mm.

Table 5: Test results for synbiotic inhibition.

Encapsulation	Diameter of inhibition
	mm
Without encapsulation	3.5
Malto:Skim (1:10)	4.91
Malto:Skim (1:20)	4.49
Malto:Skim (1:30)	2.4

Trichoderma koningii is able to secrete proteins and enzymes that can reduce and act as capping on the growth of *Salmonella* typhimurium (Tripathi et al., 2013). The group of proteins capable of showing antibiotic activity against bacteria and fungi of *Trichoderma* species is the peptaibols group (Shi et al., 2010). The microcapsule wall consisting of two encapsulated materials was able to provide better protection against probiotics, compared to one encapsulation. Lactose in skim milk can provide good protection against drying effects. Maltodextrin as an oligosaccharide derivative can work well as a prebiotic. However, the higher the concentration of skim can reduce the activity of probiotics, because the suspension becomes thick and makes the atomization process difficult. In addition, coatings that are too high cause puffing and particle cracking which will reduce the retention of synbiotic.

The inhibition of encapsulated synbiotics shows that the resulted synbiotics would be used as anti-microbe particularly to exterminate *Salmonella* in the intestines of broiler. The decrease population of *Salmonella* sp will produce healthy chickens and meat that is safe for consumption. Pathogenic microbe of *Salmonella* will cause salmonellosis on chickens. Foods, which contain pathogenic bacteria of *Salmonella* if it is consumed by human may cause *Salmonellosis* that attract the digestive system organs, small intestines and colon (Alipin et al., 2016). Based on WHO (2002) *Salmonella* all are regarded as risk of producing disease in humans body like enteric infectious disease from food. However, foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human salmonellosis.

CONCLUSIONS AND RECOMMENDATIONS

The research concluded most excellent concentration of dried cassava flour was 100% and supplemented with 4% *Trichoderma koningii.* The greatest number of synbiotics spores 416.2, pH ranged 4.40-5.80, glucose test 0.30%. Result of proximate analysis synbiotics, which have already been encapsulated using maltodkestrin and skim milk contain dry matter 89.11%, crude protein 3.21%, Crude fat 0.73%, Crude fiber 2.87% and Ash 1.25%. The inhibition of encapsulated synbiotics to *Salmonella sp* shows that the resulted synbiotics would be encapsulated using ratio maltodextrin: skim is 1:10.

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NOVELTY STATEMENT

The development of Trichoderma probiotics that are bred in local ingredients such as bran, corn and cassava which are available in Indonesia is expected to provide opportunities for farmers to be able to make their own synbiotics mixed in feed, so as to achieve independence in the provision of feed additives to increase the availability of healthy animal protein.

AUTHOR'S CONTRIBUTION

The authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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