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The Effect of *Chrysonilia crassa* Additive on Duodenal & Caecal Morphology, Bacterial & Fungal Number, and Productivity of Ayam Kampung

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Abstract: Fungi is a microorganism that can live in gastrointestinal tract of chicken. One type of fungi is multicellular or filamentous fungi. *C. crassa* is a species of filamentous fungi that has been isolated in the earlier study and it showed the best probiotic potency in vitro. The objective of this research was to study the effect of addition of dried culture of *C. crassa* in feed on intestinal & caecal morphology, bacterial & fungal number, and productivity of indigenous chicken (ayam kampung). Research used completely randomized design with four treatments. The treatments were the level of dried culture in basal diet (0%, 0.25 %, 0.50 % and 0.75 %). Each treatment was replicated 5 times and each replicate consists of 10 chickens. The parameters observed were : villi morphology, number of bacteria and fungi in the duodenum and cecum of chickens aged 1, 21 and 35 days and productivity i.e. feed intake, final body weight and feed conversion. The results showed that 0.50% dried culture of *C. crassa* could increase the duodenal villi width, decreased the number of bacterial and fungal colonies in duodenum and caecum, but it did not increase productivity. The conclusion : *C. crassa* could stimulate the duodenal villi development and decreased the number of the bacteria and fungi in the gastrointestinal tract, yet it has no positive impact on the chicken productivity

Keywords : *Chrysonilia crassa*, gastrointestinal tract, productivity, indigenous chicken.

I. INTRODUCTION

There are two types of fungi and they can be found in the gastrointestinal tract of chicken (Kompang et al., 2006; Lumpkins et al., 2010, Yudiarti et al., 2012). We previously have found fifty isolates of filamentous fungi from the gastrointestinal tract of indigenous chicken (Yudiarti et al. (2012). In vitro test of this fungi as a probiotic has been done and *C. crassa* showed the most potential species to be a probiotic.

Probiotic has been used as feed additives as it can improve the microbial balance of the digestive tract, improve the development of the small intestine, and increase the productivity of chicken (Fuller, 1992, Daud et al., 2007, Samli et al., 2007, Slizewska et al., 2008, Awad et al., 2009). However there is still little studies on the use of filamentous fungi as probiotic in vivo. Therefore, in this study *C. crassa*

was given as feed additive and the effect on duodenal & caecal morphology, bacterial & fungal number, and productivity of indigenous chicken was examined.

II. RESEARCH METHODS

Dried culture of *C. crassa* was grown in corn and rice brand medium. The dried culture was mixed in to basal diet. Two hundreds day old chickens (DOC) were taken from a chicken farm which was located in Yogyakarta. They were given the basal diet (Table 1) that has been added with dried culture *C. crassa* (1 g dried culture = 2,5 10² cfu). Drinking water was given *ad libitum*. The collected data were taken from the chickens aged 1, 21 and 35 days. The variables observed were: the height and width of the villi and the total number of bacteria and fungi in duodenum and cecum, feed intake, final body weight and feed conversion. To calculate the total number of bacteria in duodenum and caecum Nutrient Agar (NA) was used, whereas to count total fungi Potato Dextrose Agar was used (Fardiaz, 1993). To measure the height and width of the villi, histological preparation of the duodenum and caecum were made

Table 1.
Composition of the Basal Diet

Components	%
Corn	41,792
Rice brand	22,3
Soybean meal	29,8
Meat Bone meal	1,5
Fish meal	1,47
Lysine	0,106
Premix	3,0
NaCl	0,032
Nutrient content :	
ME (kkal)	2750
Protein	20,9
Lipid	6,26
Crude fibre	4,45
Calcium (C)	1,1
Phosphor (P)	0,4
Lysine	1,05
Methionine	0,396

Research used completely randomized design with four treatments. The treatments were the level of dried culture of

C. crassa in basal diet (0%, 0.25 %, 0.50 % and 0.75 %). Each treatment was replicated 5 times and each replicate consists of 10 chickens. Data were analyzed by analysis of variance and Duncan's Multiple Range Test.

III. RESULTS AND DISCUSSION

The number of bacteria and fungi in duodenum and caecum

The total number of bacteria and fungi in duodenum is shown in Table 2. Statistical analysis showed there was an effect of *C. crassa* addition on the number of bacteria and fungi in duodenum. Duncan test showed that there were significant difference ($P < 0,05$) between all treatments (Table 2).

Table 2.
 Effect of Addition of *C. crassa* Dried Culture on The Colonies Number of Bacteria and Fungi in Duodenum

Level (%)	Bacteria			Fungi		
	1 day	21 days	35 days	1 day	21 days	35 days
0.00	8.9x10 ^{5a}	4.8x10 ^{7a}	7.4 x10 ^{8a}	1.9x10 ^{4ab}	3.0x10 ^{5a}	5.6x10 ^{5a}
0.25	1.1x10 ^{6a}	2.6x10 ^{7b}	3.0 x10 ^{8a}	1.3x10 ^{4b}	1.1x10 ^{5b}	3.3x10 ^{5b}
0.50	1.8x10 ^{6a}	2.1x10 ^{7c}	6.8 x10 ^{7c}	2.9 x10 ^{4a}	1.0x10 ^{5b}	1.3x10 ^{5c}
0.75	1.2x10 ^{6a}	1.7x10 ^{7d}	1.6 x10 ^{7d}	2.1x10 ^{4ab}	8.5x10 ^{4b}	6.0x10 ^{4d}

Different superscripts within the same column indicate significantly different ($P < 0,05$);
 Cc : *C. crassa*

Table 2 showed that as the chicken getting older, the colonies number of bacteria and fungi in duodenum for all treatments increased. As the level of *C. crassa* addition increase the colony number of bacteria and fungi in duodenum decreased. This indicated that the addition *C. crassa* reduce the total number of bacteria and fungi in duodenum.

In the gastrointestinal tract of chicken there are natural indigenous fungi and bacteria (Yegani and Krover, 2008). Nutrients in the gastrointestinal tract are usually available only for the indigenous microbes. However since there are external microbial populations (*C. crassa*), some nutrients will also be used by the external microbes. When there are two or more microbes growing together in the same place, they will compete for nutrients for their growth (Fuller, 1992). Thus, by increasing the level of *C. crassa*, there in an increase in the competition among the microbes. The microbes which do not get the nutrients, will not survive and therefore their number will be reduced.

The total number of bacteria and fungi in caecum are shown in Table 3. Statistical analysis showed that there was an effect of dried culture *C. crassa* on the number of bacteria and fungi in caecum. Duncan test showed there were significant difference ($P < 0,05$) among treatments (Table 3).

Data in Table 3 are similar to the data in Table 2 which showed that the addition of *C. crassa* also reduced the number of bacteria and fungi in caecum. This condition was the same as the condition in duodenum whereby increasing the level of *C. crassa* the competition among the microbes became stronger and it reduced the number of microbial colonies.

Table 3.
 Effect of Addition of *C. crassa* Dried Culture on The Colonies Number of Bacteria and Fungi on Caecum

Level (%)	Bacteria			Fungi		
	1 day	21 days	35 days	1 day	21 days	35 days
0.00	6.9x10 ^{6a}	4.1x10 ^{8a}	7.3x10 ^{8a}	6.9x10 ^{4a}	3.8x10 ^{5a}	1.3x10 ^{6a}
0.25	5.5x10 ^{6ab}	3.0x10 ^{8a}	7.0x10 ^{8a}	2.4x10 ^{4b}	1.3x10 ^{5b}	4.3x10 ^{5b}
0.50	3.0x10 ^{6bc}	2.0x10 ^{8b}	2.9x10 ^{8b}	2.1x10 ^{4b}	8.9x10 ^{4bc}	1.4x10 ^{5b}
0.75	1.3x10 ^{6c}	1.1x10 ^{8c}	7.8x10 ^{7c}	4.9x10 ^{4a}	7.9 x10 ^{4c}	8.6x10 ^{4c}

Different superscripts within the same column indicate significantly different ($P < 0,05$)

Table 2 and 3 also showed that the total number of bacterial colonies was larger than fungal colonies. This because, bacterial growth is faster than fungal growth (Gabriel et al., 2006), Yegani and Krover, 2008)

Morphology of Duodenum and Caecum

The measurement of height and width of the villi in duodenum is shown in Table 4. Statistical analysis showed that there was an effect of dried culture *C. crassa* on height and width of duodenal villi. Duncan test showed there was significant difference ($P < 0,05$) between 0.50 % *C. crassa* and control (Table 4).

Table 4.
 Effect of Addition of *C. crassa* Dried Culture on Morphology Development of Duodenum

Level (%)	Villi height			Villi width		
	1 day	21 days	35 days	1 day	21 days	35 days
0.00	40.56 ^a	68.17 ^a	81.23 ^a	9.68 ^a	10.69 ^a	9.98 ^b
0.25	37.72 ^a	73.72 ^a	84.32 ^a	10.07 ^a	10.45 ^a	12.10 ^{ab}
0.50	44.08 ^a	44.46 ^b	95.15 ^a	8.35 ^a	14.54 ^a	14.03 ^a
0.75	36.10 ^a	72.11 ^a	94.29 ^a	8.17 ^a	10.55 ^a	11.32 ^{ab}

Different superscripts within the same column indicate significantly different ($P < 0,05$)

Table 4 showed that duodenal villi width of 0.50 % *C. crassa* addition was significantly different ($P < 0.05$) than control, but it was not significantly different to other treatment. This indicated that dried culture *C. crassa* increased the villi morphology in particular duodenal villi width.

According to Jin *et al.* (1998) that in the early first week after hatching, the chicken gastrointestinal tract grows faster than other organs and the most rapid growth is found in duodenum. Normally, young chickens that consume a good feed, the gastrointestinal tract will grow well. In this research, beside the basal diet there was also added *C. crassa*. In vitro test, showed that *C. crassa* has a potency as a probiotic (Yudiarti et al, 2012) and according to Linberg *et al* (1982) that the fungi can produce protease enzyme. This enzyme can help in the digestion process of proteins to amino acids. The amino acids are the basic component of protein synthesis and protein is the main substances in promoting growth. Therefore, by increasing amino acids, the protein synthesis or growth will increase. Because in young chicken, the initial growth occurs in the duodenum (Jin *et al.*, 1998), it is in accordance with result of this experiment where duodenum width was wider than control. The same results obtained by Pelicano *et al.* (2005) that used fungal *Aspergillus oryzae* and

some bacteria for 21 days could increase the development of duodenal villi. Research by Awad *et al.* (2009) also showed that chickens fed bacterial *Lactobacillus sp* until 5 weeks could increase the development of duodenal and ileal villi.

The measurement of villi like projection of caecum is shown in Table 5. Statistical analysis showed that there was no effect of dried culture of *C. crassa* on morphological development of caecum (Table 5).

Table 5.
Effect of Addition of *C. crassa* Dried Culture on Morphology Development of Caecum

Level (%)	Villi -like projections		
	1 day	21 days	35 days
0,00	16.82	28.31	29.26
0,25	11.21	19.86	21.97
0,50	10.07	22.14	21.85
0,75	7.98	20.71	27.93

. Adding fungal dried culture in the chicken basal diet would increase the fungal population in the caecum. On the other hand, there are many bacteria in caecum (Table 3) most of which are decaying bacteria. This condition is not favorable for fungal growth including *C. crassa*. Consequently, the number of *C. crassa* would be lower and less enzyme was produced and less contribution for growth.

A other possibility is that in this study, the basal diet contained low crude fiber (4,45%). Fibre is a source of nutrients for the growth of fungi (Fisher, 2003, Lan et al., 2005). As the level of fibre in the basal diet was low, it provided less substrate for fungal growth and hence less protease production. In the end, this situation can not contribute to the development of the caecal morphology.

Productivity of the chicken

Body weight gain, feed consumption, and feed conversion ratio are shown in Table 6. Statistical analysis showed that there was no effect of dried culture of *C. crassa* on productivity of the chicken (Table 6).

Table 6.
Effect of Addition of *C. crassa* Dried Culture on Productivity of Indigenous Chicken

Level (%)	Feed Consume (g/bird/week)	Body weight gain (g/bird/week)	Feed Conversion Ratio
0,00	164.57	39.38	4.29
0,25	153.97	37.88	3.94
0,50	153.80	41.75	3.65
0,75	141.30	37.87	3.69

As it has been explained that addition of 0.50% dried culture *C. crassa* increased the duodenal villi width (Table 4). But, it did not effect the productivity of the chickens, as seen in Table 6. *C. crassa* has never been tested as probiotic. This was an initial experiment to use the filamentous fungi *C. crassa* in vivo indigenous chicken as a feed additive. The causal factor is even though *C. crassa* has a potency as a probiotic *in vitro* (Yudiarti et al., 2012), they need support from other microbes. Each kind of probiotic has a special function and if more than one probiotics are mixed, they can provide synergistic action. This is supported by a study using

a mixture of microbes (starbio) which can result in body weight gain (Gunawan and Sundari, 2003). Pelicano *et al.* (2005) also used multi strain probiotics, (a mixture of filamentous *Aspergillus oryzae* and bacteria), that could increase in the development of villi of duodenum, jejunum and ileum.

Increase productivity of the chicken has not been attained, because the potency of *C. crassa* has not been fully examined. Some characters which need to be further examined are the length of residence within gastrointestinal tract, attachment to intestinal epithelial cells, and production of antimicrobial substance against pathogen (Ocana and Elena Nader-Macias, 2004, Sukrisni, 2007; Murwani, 2008). Therefore there is possibility that *C. crassa* is excreted /washed out from the body together with secretion product. However *C. crassa* appears to be safe to be used for chicken.

IV. CONCLUSION

1 Filamentous fungal *C. crassa* 0.50% could stimulate duodenal villi development and decrease the number of bacteria and fungi in duodenum and caecum, yet it has no positive impact on the productivity of chicken.

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