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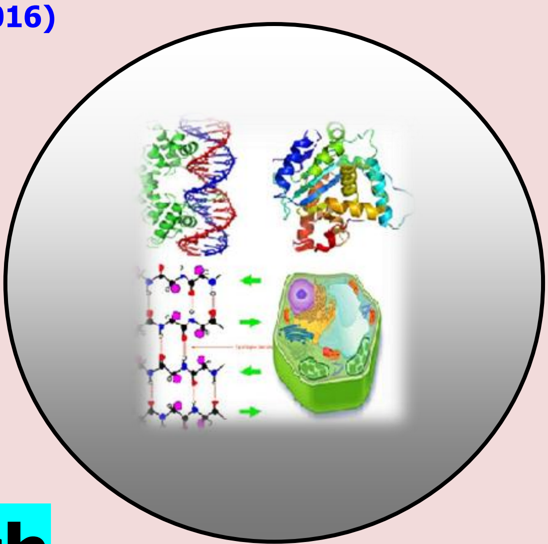

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RESEARCH PAPER

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ABSTRACT

A 56-day feeding trial involving 144, 8-week old female native chickens was carried out in a completely randomized design to evaluate the performance and cacrcass of native chickens fed fermentation cassava (Manihot esculenta) leaf meal at dietary levels of 0%, 5%, and 10%, respectively. Feed intake, body weight gain, feed conversion ratio, and carcass weight of birds on (5%) and (10%) fermented leaf meals were significantly superior (P<0.05) to the group on the control (0% fermented leaf meal). The abdominal fat were decreased at 5 and 10% (P<0.05) groups of the fermented cassava leaf meal. It is suggested that 5 to 10% inclusion of fermented cassava leaf meal by probiotic Saccharomyces sp. could be used in native chicken diets without any deleterious effects.
Key words: Cassava Leaves, Carcasses, Abdominal Fat and Native Chickens.

INTRODUCTION

Many traditional ingredients used in poultry diets are forecast to be in short supply mainly as a result of the increase in human population and unfavourable climatic conditions (Hasanuddin *et al*., 2017). The growing demand for traditional energy and protein feed ingredients as food by the ever-growing world's human population and other industrial uses has increased research interest in alternative cheaper ingredients for poultry feeding. Actually, feed accounts for 70-75% of the production cost of poultry, becouse the bulk of the feed cost arises from protein concentrates such as fish meal and soybean meal. Prices of these conventional protein sources have soared so high in recent times that it is becoming uneconomical to use them in poultry feeds.

There is need therefore for interesting for locally available and cheap sources for feed ingredients, particularly those that do not attract competition in consumption between humans and livestock, espcecialy cassava leaves. Diara and Devi (2015) concluded that efficient use of cassava by-products will reduce feed cost of poultry production and provide additional source of income to cassava farmers and processors. The cassava leaves meal protein content was high and comparable with some rich conventional protein sources of plant and animal origins used in monogastric feed formulation. The mineral content was high particularly Ca, Zn, Ni and K (Fasuyi, 2005).

Cassava leaves are a by-product of root production and comprise the leaves and petioles of cassava plant. Cassava, manioc (*Manihot esculenta*), a root crop cultivated mainly in tropical and sub-tropical regions of the world, tolerant to poor soils, diseases and drought, can yield between 25 to 60 tons ha^{-1} and the importance of the leaves becomes obvious when one considers that, depending on the variety, as much as 10-30 tons ha^{-1} of leaves would be obtained in a year and that this amount is usually wasted or used as manure (Bokanga, 1994). Cassava leaves, moderate to good protein contents, have been used as protein supplements. Dietary recommendations of cassava by-products for poultry have varied considerably. The major factors limiting the efficient utilization of these byproducts in poultry diets include the high fibre and low energy contents and likely presence of antinutritional factors, mainly hydrocyanic acid (HCN) in the bitter variety of cassava (Diara and Devi, 2015). Several technologies have been used to improve the utilization of cassava by-products by poultry.

Some feed technologies used to enhance the utilization of these by-products by poultry are also highlighted. The high fibre, high moisture and likely presence of HCN are however, major limitations to their efficient utilization by poultry. Following appropriate processing, the inclusion rates of these byproducts could be maximized to further reduce production cost (Diara and Devi, 2015). The cassava leaves (*Manihot esculanta*) in the poultry ration need processing because its low quality (Zalán *et al*., 2015). That's because of the high crude fiber in the form of manan content, while poultry do not have fiber and manan enzyme in digestive tract. It is necessary for processing prior to improve the quality cassava leaves is through biotechnology and fermentation by cellulolytic and mananolitik of fungi (Meryandini *et al*., 2008) will be able to decrease of crude fiber and manan content so quality of cassava leaves will increased that it can replace soybean meal in poultry rations. Wizna *et al*. (2009) stated that chicken's digestive tolerance to crude fiber was very low and the limit of crude fiber content in broiler chicken feed was 2-5%. A feedstuff with high crude fiber content, should be processed before using in chicken's ration to decreased the crude fiber content.

Several studies (Yang *et al*., 2007; Song *et al*., 2008; Bidura *et al*., 2012; Bidura *et al.,* 2016) reported that fermentation process efficiently eliminates antinutritive compounds and improves nutritional value of feed. Fermentation has been widely used to increase the bioavailability of nutrients (Hotz and Gibson, 2007) and reduce the levels of antinutritional factors (Egounlety and Aworh, 2003) of soybean. The fermentation process is facilitated by the use of a mold or a bacterium. The conditions and nutritional quality of the fermented feeds thus produced can vary depending on the type of microorganism used.

The peptides content and fibrinolytic enzyme activity increased and the anti-nutrition factors reduced after fermentation effectively (Amadou *et al*., 2010a). The efficiency of solid state fermentation in improving nutritional quality and reducing the anti-nutritional factors were ascertained by the works of Amadou *et al.* (2010a, b). Fermentation with fungi also successfully reduces the amount of stachyose and raffinose in soybean meal (Cervantes-Pahm and Stein, 2010). Apart from degrading the anti-nutritional factors, fungal fermentation increases the nutritional value of feed by increasing the crude fat, crude ash, dry matter, and protein contents (Hong *et al*., 2004; Feng *et al*., 2007a, b; Bidura *et al*., 2012). The increases in protein and fat contents may partially be attributed to the decrease in carbohydrate content during fermentation. Hasan *et al*. (2016) reported that that using wet fermented feed with 1 and 2 g of prepared probiotic caused significant improvement in the production performance of native chicken especially in the live body weight and growth rate traits.

Pervious work demonstrated that using a dry form of fermented feed with probiotic had highly significant improvement on the production performance parameters of chicken (Lokman *et al*., 2015). So, present research was planned to investigate the effects of fermented cassava leaves with probiotic (*Saccharomyces sp.*) in a solid form on the production performance and carcass weight of local Timor Lorosae chickens.

MATERIAL AND METHODS

Experimental birds and design of the experiment: One hundred and fourty four famale native chickens eight weeks old were used for this study. The birds were divided into three treatment groups identified as A, B, and C consisting of 48 birds per group. Each treatment group was further replicated 6 times with 8 birds per replicate. Based on the result of the chemical analysis four finisher chicken diets were formulated to contain fermented cassava leaf meal at 0% (A), 5% (B), and 10% (C) and fed to the birds in a Completely Randomized Design (CRD). The treatment diets were isoenergetic (2750 kcal/kg) and isonitrogenous (CP: 17%). The birds were were housed in pens whose floors were covered with wood shavings. Each cage was 120×100×50 cm and was equipped with a feeding and drinking trough as well as lamps for heating and lighting. Feed and water were provided *ad libitum.* The necessary routine management, vaccinations and medications were were provided. Feed intake was recorded daily and the birds were weighed weekly. Other routine poultry management practices were maintained. The feeding trial lasted for 56 days.

Preparation of the cassava leaf meal: The cassava leaves used for this study were harvested at the Lautem District farms, Timor Leste Province. The leaves were chopped for faster and effective drying on a cement floor. The chopped leaves were sun-dried for three days until they become crispy while still retaining the greenish colouration. The leaves were turned regularly to prevent uneven drying and possible decay of the leaves. The dried leaves were then milled using a hammer mill to produce leaf meal. A sample of the leaf meal was subjected to proximate analysis according to AOAC (1995), while gross energy was determined with a Gallenkamp adiabatic calorimeter.

Fermented of the cassava leaf meal: The isolate of *Saccharomyces spp.Sb-6* which has been passed approved from bile salt test and *in vitro* test on poultry digestive tract, could assimilate cholesterol, and both as probiotics agent and has CMC-ase activity too. The isolate has passed the test and has been considered as potential probiotic according to our previous study (Bidura *et al*., 2014). Fermentation of cassava leaves meal was prepared as follows: cassava leaves meal was added approximately 0.40% (5.31×10^7 spores) *Saccharomyces spp.Sb-6* into the 100 g of steamed cassava leaves meal. Then, water was added until the content of water up to 35% and for 2 days for incubations. After that, cassava leaves meal fermented was dried at 45^0C temperature for six hours and then were prepared for analysis or mix in rations.

Carcass characteristics: At day 56, eighten birds from each replicate with body weight close to the mean of the group were selected and tagged. The birds were then starved but given ample supply of drinking water 12 h prior to slaughtering. Each bird was weighed separately and sacrificed, then properly bled. The slaughtered birds were scalded at 70^0C for 2 min and manually defeathered. After five minutes of bleeding, each bird was scalded, defeathered, and eviscerated after removal of head, neck, and legs. The carcass without giblets was weighed, expressed as a percentage of its live weight and considered as the carcass yield. Live weight, bled weight, defeathered weight, carcass weight, eviscerated weight were recorded respectively. The eviscerated carcass was then carefully cut into parts and their weight also recorded. The weight of the gastrointestinal tract, abdominal fat was carefully removed from gizzard and abdominal region weighed and recorded respectively. Recorded weights of parts were expressed as percentage of the respective live body weight.

Statistical analysis: Data were analyzed using an analysis of variance (ANOVA) from the Statistical Package for the Social Sciences (SPSS version 21.0). A Duncan's multiple range test was applied to determine differences among treatments. Differences were considered significant at the 5% level.

RESULTS

The results of the current study also indicated that the inclusion of cassava leaves meal by probiotics of *Saccharomyces sp.* in the diets of local native chickens were affect significantly different (P<0.05) on final body weight, live weight gains, Feed conversion ratio (g feed/g gain), dry matter digestibility, and organic matter digestibility (Table 1).

The mean final body weight of chicken fed with cassava leaves as control (A) was 692.83 g head^{-1} (Table 1). The average final weight of chicken fed with 5% cassava leaves flour was fermented by *Sacharromyces sp.* (B) and 10% fermented cassava leaf with yeast *Sacharromyces sp.* (C) in feed, were: 22.78% and 27.91%, each significantly different (P<0.05) were higher than control. The use of 5-10% fermented cassava leaves in feed were significant (P<0.05) to increase live weight gains compared to control (without cassava leaves). The live weight gains of chickens on B and C treatments was higher: 59.15% and 73.32%, respectively than controls (A).

Table 1. Influence of use of cassava leaves (*Manihot esculanta*) fermented by probiotics *Saccharomyces sp*. in diet on performance and carcass weight in local native chickens aged 8-16 weeks.

Variable	Treatment[1)]			SEM[2)]
	A	B	C	
Final body weight (g head^{-1})	692.83b	850.67a	886.17a	4.458
Live weight gains (g head^{-1} 56 days^{-1})	262.33b	417.50a	454.67a	4.717
Feed consumption (g head^{-1} 56 days^{-1})	1179.92b	1570.32a	1653.08a	27.862
Feed conversion ratio (g feed/g gain)	4.50a	3.76b	3.64b	0.068
Dry matter digestibility (%)	68.20b	71.61a	71.93a	0.432
Organic matter digestibility (%)	69.02b	72.35a	72.53a	0.418

Note:

1. Feed without cassava leaves as control (A); rations using 5% cassava leaves fermented by Sacharromyces sp (B); and rations that use 10% cassava leaves fermented by yeast Sacharromyces sp (C).
2. *Standard Error of the Treatment Means*
3. Means with different superscripts within raws are significantly different (P<0.05)

The increase in final weight and live weight gains of chicken were caused by fermented cassava leaves by *Sacharromyces sp*. were coused that khamir *Saccharomyces sp.* which is used as fermentation inoculant has passed the test as probiotic and yeast degradation agent of crude fiber (Bidura *et al*., 2014; Bidura *et al*., 2016; Candrawati *et al*., 2014) in the digestive tract able to improve the digestibility of feed. Probiotics in the gastrointestinal tract of chicken can increase the absorption of nutrients so as to increase weight gain, and can increase the absorption of nitrogen and phosphorus (Piao *et al*., 1999). The same thing was also reported by Wu *et al.* (2005) and Huang *et al*. (2004) that *Aspergillus xlanase* supplementation in bran-based wheat ration can improve broiler performance. Hasan *et al.* (2016a) reported that using wet fermented feed with 1 and 2 g of prepared probiotic caused significant improvement in the production performance of chicken especially in the live body weight and growth rate traits. Hasan *et al.* (2016b) reported that used of solid state fermented feed with and without prepared probiotic were increased live body weight, weight gain, feed intake, and feed conversion ratio of a local Malaysian chicken.

In this study, the inclusion of 5-10% fermented cassava leaves in the ration can significantly increase feed intake. The average feed intake for eight weeks of observation in control chickens was 1179.92 g head^{-1} 56 days^{-1} (Table 1). Average feed intake in treated chickens B and C, were 33.09% and 40.10%, each significantly (P<0.05) higher than control.

Increased feed intake in chickens treated B and C caused due to the change in body weight is higher than the control chicken (A). The occurrence of weight gain will have an impact on the increased need for nutrients, especially energy and protein, so chicken increases the consumption of feed. The same thing was reported by Bidura *et al*. (2012) and Bidura *et al.* (2016).

The average value of Feed conversion ratio (g feed/g gain) during eight weeks of observation in control chickens was 4.50 head^{-1} (Table 5.1). The use of 5% and 10% cassava leaves fermented by *Saccharomyces sp.* significantly increased feed efficiency by 16.44% and 19.11%, each more efficient than control (A). The value of FCR is an indicator to assess the efficiency of ration usage. The lower the FCR value, indicating the higher the use efficiency of the feed. Conversely, the higher the FCR value indicates the lower the feed efficiency. Increased efficiency of feed use in chickens given cassava leaves fermented by probiotics *Saccharomyces sp.*, caused by fermentation process of cassava leaves will occur breakdown of cell wall structure of plants, making it more easily digested by the digestive enzymes of the chicken. In addition, the fermentation process can overhaul complex compounds into simple compounds that are easily soluble and easily digested by chicken digestive enzymes (Bidura *et al*., 2012). Provision of feed containing probiotics can stimulate the improvement of feed metabolism in the digestive process (Nurhayati, 2008). Fermentation of wheat bran increased the protein content from 20.35% to 21.65% for wheat brans and carbohydrates percentage increased from 45.09 to 47.4% in fermented wheat bran (Hassan *et al*., 2008).

Hasanudin *et al*. (2017) reported that the inclusion of fermented feed by-products in the basal diet significantly affected feed intake, body weight gain (BWG), and feed conversion ratio (FCR). Moreover bran contains phytic acid which acts as an antinutrient due to its chelation of various metals and its binding of protein, therefore, diminishing the bioavailability of protein and nutritionally important minerals (Zalan *et al.,* 2015). The extensive use of antibiotics in poultry with the purpose of promoting growth rate, increasing feed efficiency and for the prevention of intestinal infections have led to an imbalance of the beneficial intestinal flora and the appearance of resistant bacteria (Mahfuz *et al*., 2017). Han *et al*. (1999) stated that supplementation of *Aspergillus oryzae* and *S.cerevisiae* in basal ration at level of 0.15% and 0.30% can increase the activity of amylolytic and proteolytic enzymes in the chicken gastrointestinal tract, thereby increasing the metabolizable energy and protein digestibility of the ration. Increased protein digestibility and metabolizable energy rations can have an impact on improving feed efficiency and increasing chicken growth. Supplementation of probiotics in poultry can increase feed metabolism in the poultry digestive tract (Nurhayati, 2008).

The results showed that the digestibility of dry matter and organic material of feed given cassava leaves fermented by yeast *Saccharomyces sp*. increased compared with control rations (Table 1). The use of 5% (treatment B) and 10% (treatment C) of cassava leaves meal fermented by yeast probiotics *Saccharomyces sp*. ($P < 0.05$) could improve the dry matter digestibility of each ration: 5.00% and 5.47% compared with control (A). Similarly, organic matter digestibility (KCBO) experienced a marked increase ($P<0.05$) each: 4.82% and 5.09% higher than control (treatment A). The increase in the digestibility of the dry matter and organic matter is due to the probiotic Saccharomyces sp. which is used in the fermentation process, as well as in the chicken intestines after consuming (Mulyono *et al.,* 2009). Yeast probiotics can produce amylase and protease enzymes, so its presence in the gastrointestinal tract of chicken will increase the activity of the enzyme, and increase the breakdown of nutrients into a simpler form and easily absorbed by the digestive tract. Carbohydrates and crude fiber components can be used by yeasts for their growth (microbial protein synthesis) in the chicken's digestive tract, especially in the cecum and colon sections.

The results of the study Chen *et al*. (2005) reported that the addition of 0.20% of complex probiotics (*L.acidophilus* and *S.cerivisae*) in the diet significantly improved dry matter digestibility.

The results of Bidura *et al*. (2012) and Candrawati *et al*. (2014) showed that fermentation of feed by using yeast inoculant (*Saccharomyces spp*) significantly increased the digestibility of dry matter, organic matter, crude protein, and crude fiber from feed compared to fermented feed. The same thing was reported by Jaelani *et al.* (2008) that fermented feeding material (palm kernel meal) with Trichoderma reesei can increase the metabolized energy and crude protein feed ingredients. The extracellular peroxidase enzyme acts actively on lignolysis activity, resulting in the lignocellulosic bond terminating, and the lignin fraction breaking down into CO_2. Hong *et al*. (2004) reported, feed fermentation using *Aspergilus oryzae* significantly improved the digestibility of dry matter and crude protein in the diet.

Table 2 shows that sloughter weight of chicken in this study increased significantly (P<0.05) with fermented cassava leaves by *Sacharromyces sp* in rations (treatment B and C), higher 4.82% and 5.09%, each compared to control (A). Chicken sloughter weight is increased because the cassava leaves are given first fermented by *Sacharromyces sp*. that serves as a source of probiotics. Reported by Bidura *et al*. (2012) that the process of feed fermentation prior to use in the ration will be able to increase the nutrient content and digestibility of feed itself, so that more can be absorbed by the chicken body (Bidura *et al.,* 2016).

Fermentation of cassava leaves by *Saccharomyces sp* can significantly increase the sloughter weight of chickens. This is due to the presence of microbial probiotics in the chicken intestinal tract will be able to increase enzyme activity, absorption of nutrients, and increase protein and energy retention in the chicken body. As Yi *et al.* (1996), that probiotic microbial supplementation into real rations can improve nitrogen retention in broilers, the fermentation process will break down proteins and carbohydrates into amino acids, nitrogen and dissolved carbon needed for the synthesis of body proteins. The results showed that carcass weight of chickens at treated B and C significantly (P<0.05) were higher: 25.78% and 30.48% than carcass weight in control chick (A). According to Rasyaf (2004), carcass weight is positively correlated with sloughter weight. The higher the sloughter weight, it will be followed by the higher carcass weight. Carcass weight is strongly influenced by the weight of non carcass, especially the digestive tract and abdominal fat. The increase in carcass weight of chicken cassava leaves fermented by *Saccharomyces sp.* were caused because yeast *Saccharomyces sp.* which is used as fermentation inoculant is potential yeast as a source of probiotics, and has the ability to degrade crude fiber (Bidura *et al*., 2014). Several studies have shown that the use of probiotic microbes in rations can significantly increase carcass weight (Bidura *et al*., 2015; Bidura *et al*., 2016; Candrawati *et al*., 2014). Reported by Tang *et al*. (2007) that an increase in protein and lysine consumption in broiler chickens will increase the percentage of carcass and the amount of breast meat compared with lower protein and lysine intake. Foods containing high protein can increase the meat component in the carcass.

Table 2. Effect of cassava leaves (*Manihot esculanta*) fermented by probiotics *Saccharomyces sp.* in rations on sloughter weight and carcass weight in local native chickens up to eight weeks of age.

Variable	Treatment[1)]			SEM[2)]
	A	B	C	
Sloughter weight (g $head^{-1}$)	690,17c[3)]	850,00b	878,83a	3,986
Carcass weight (g $head^{-1}$)	435,83c	548,17b	568,67a	3,150
Carcass percentage (%)	63,08b	64,49a	64,71a	0,169
Abdominal fat (% body weight)	2,13a	1,49b	1,50b	0,110

Note:

1. Feed without cassava leaves as control (A); rations using 5% cassava leaves fermented by Sacharromyces sp (B); and rations that use 10% cassava leaves fermented by yeast Sacharromyces sp (C).

2. *Standard Error of the Treatment Means*
3. Means with different superscripts within raws are significantly different (P<0.05)

The amount of abdominal chicken fat in this study decreased significantly (P<0.05) in the presence of fermented cassava leaves by *Sacharromyces sp.* in rations (treatment B and C), were: 30.05% and 29.58%, respectively compared to control (A). The decrease in abdominal fat is due to the probiotic role of *Sacharromyces sp.* in the fermentation process of cassava leaves before being given to chickens. Lipids derived from the food eaten, then in the intestine will be digested by enzymes released by the pancreas and emulsified by bile salts into micelles or kilomikron. Micelles are then absorbed by the body as a source of energy and basic ingredients cholesterol-forming, then deposited on the organs of the body as fat and cholesterol. The reduction of body fat (abdominal fat) by yeast *Saccharomyces sp.* as a source of probiotics in the diet can increase the amount of lactic acid bacteria (BAL) that will affect the digestion and absorption of fat in the digestive tract of poultry. This result is supported by Nurhayati (2008) who reported that the mixture of fermented feed by *A. niger* at level 10-30% significantly reduced the amount of abdominal fat.

Mahmud *et al*. (2008) reported that the highest abdominal fat percentage value was recorded for birds fed the control diet, while the lowest value was recorded for birds fed the probiotics supplemented diet. No clear mechanisms have been reported to be responsible for the reduction of lipid synthesis by probiotic. It might in part be due to increasing beneficial bacteria such as *Lactobacillus* that decrease the activity of acetyl-CoA carboxylase, which is the rate-limiting enzyme in fatty acids synthesis (Astuti *et al*., 2017); Ristiani *et al*., 2017). Probiotics, even blood cholesterol decrease (Ooi and Liong, 2010; Ristiani *et al*., 2017; Bidura *et al*., 2016). Furthermore, fermented feed by-products significantly reduced the fat and cholesterol content of the breast meat, but improve growth performance and the quality of the breast meat (Hasanudin *et al*., 2017)

CONCLUSION

It can be concluded that the use of 5-10% cassava leaves fermented by probiotics *Saccharomyces sp.* in the diet can improve performance and carcass weight, otherwise decrease the amount of abdominal fat in female local native chickens up to eight weeks of age.

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