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To cite this article: T D Nova *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **757** 012055

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The Effect Levels of Cassava Leaf and Palm Kernel Meal Fermented Used *Bacillus amylolicofaciens* in Laying Duck Feed

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Abstract. This study aims to determine the level of administration of a mixture of cassava leaves and palm kernel meal fermented with *Bacillus amylolicofaciens* in rations on egg index, shell thickness, shell strength and color index of duck egg yolk. This study used 160 laying ducks aged 14 months and weighing \pm 1500g. This research method is an experimental method using a completely randomized design (CRD), with 4 treatments and 4 replications. The treatment is feed that uses a mixture of cassava leaves and fermented palm kernel cake (CLPKCF). The treatments were Ration A 0% CLPKCF, Ration B 6% CLPKCF, Ration C 12% CLPKCF and Ration D 18% CLPKCF, each experimental unit consisted of 10 laying kamang ducks. The results of the analysis of diversity showed that the application of the CLPKCF mixture in the ration of laying ducks had no significant effect ($P > 0.05$) on the egg index, shell thickness, shell strength and color index of duck egg yolk. The conclusion of this research is the use of CLPKCF ration up to 18% level in the ration can maintain the quality of duck eggs. In this condition, the egg index was 0.80, the shell thickness was 0.28, the shell strength was 4.73 kg / mm and the egg yolk color index was 14.83.

Keywords: CLPKCF, laying duck, cassava leaf, palm kernel meal, external egg quality

1. Introduction

The availability of feed affects the success of a poultry farm. Poultry feed that has good quality can ensure the fulfilment of the nutrients needed for growth, production and reproduction. Good quality feed is usually relatively expensive because the feed ingredients used come from imports. One of the efforts to save production costs is by utilizing raw materials that are cheap, easy to obtain and have good quality and nutritional content. Alternative raw materials for poultry other than corn and soybeans can use products from agricultural waste such as cassava leaves (CL) and palm kernel meal (PK).

Cassava leaf flour (CLF) has been widely studied as a mixture of poultry ration feed to replace a portion of soybean meal because cassava leaves contain high crude protein. According to [1], the crude protein content of cassava leaves ranges from 17 - 28% depending on whether the leaves are young or old, at the base or at the tip, and mixed with the stalk or leaves only. Crude fiber content ranges from 11-21% ([1] and [2]). Efforts to improve the nutritional content and quality of the use of cassava leaf meal (CL) in poultry rations, it is necessary to find a mixture of ingredients in processing this CL by fermentation so that it can be used more in poultry rations. One way is to combine CL with other feed



ingredients such as rice cake palm kernel (CPK). Through the combination with CPK it is hoped that the complementary nutrients for life and microbial growth will occur in the fermentation process.

Palm kernel meal is a by-product in the extraction of palm kernel oil obtained by chemical and mechanical processes [3]. Furthermore, he explained that in this oil palm processing, 45-46% of CPK was produced. This waste has a great opportunity to be used as poultry feed, namely as a source of vegetable protein. To increase the value of CPK benefits, the processing is carried out using fermentation technology. The results of research by [4] and [5] showed an increase in crude protein and a decrease in crude fiber in CPK fermented by *Bacillus amyloliquefaciens* bacteria at a dose of 6% and fermentation time of 6 days. According to [6] CPK can be used up to 10% or replace 40% of soybean meal in broiler chicken rations. [7] Reported that CPK can only be used as much as 10% in duck rations, this is due to the low palatability and efficiency of protein and high crude fiber.

Based on the description of the research results above, it is known that CL and CPK still have the opportunity to improve their quality before being used in the poultry ration mix. Cassava leaves and Palm kernel cake have high crude fiber so it needs to be fermented, for example by *Bacillus amyloliquefaciens*. *Bacillus amyloliquefaciens* can produce several enzymes such as alpha-amylase, alpha acetolactase, decarboxylase, beta-glucanase, hemicellulase, maltogenic amylase, protease, xylanase [8], phytase [9], mannanase [10], β mannanase [11], chitinase [12] lipase. [13], and endoglucanase [14] and phytase enzymes and extracellular enzymes, cellulases and hemicellulase ([8]; [9];[15]). [15] said that *Bacillus amyloliquefaciens* is cellulolytic and has a strong ability to degrade crude fiber because it produces extracellular enzymes cellulase and hemicellulase. . The enzymes produced from *Bacillus amyloliquefaciens* play a role in fermentation that converts complex molecules into simpler molecules.

Phytase enzymes produced from *Bacillus amyloliquefaciens*. Some researchers report that the addition of microbial phytase to poultry rations can increase protein and energy utilization [16] and other researchers reported an increase in the bioavailability of minerals in the ration as a result of the addition of microbial phytase which includes P ([17];[18] ; [19]) and other minerals such as Ca, Mg, Cu, Zn, Fe, and K which are bound to phytate [20]. This indicates that *Bacillus amyloliquefaciens* can be added to the ration to contribute to eggshell formation and eggshell resistance and thickness.

[21], found that the best combination of cassava leaf and palm kernel cake mixture in fermentation by *Bacillus amyloliquefaciens* is a ratio of 80%: 20%, then the best inoculum dose and fermentation time of this mixture is 8% for 8 days. According to [22] in the results of this fermentation, there was a decrease in crude fiber from 16.3% to 7.2% and an increase in crude protein from 19.2% to 22.8%. The results of this fermentation obtained beta-carotene content of 49.5 mg / kg while beta-carotene in corn had a beta-carotene content of 33 mg / kg or 3.3 mg / 100g [23]. This shows that CL can partially replace beta-carotene in corn. Addition of feed containing beta-carotene can increase the amount of pigment and vitamin A content in egg yolks. Increased carotene pigments can increase the yellow colour of eggs.

According to [24] the shape of the egg is an expression of the protein content of the feed. Feed protein will affect the internal quality of the eggs, which in turn can affect the egg index. This shows that the mixture of CL and CPK fermentation can be included in the ration to contribute to the egg index. The results of the fermented CL and CPK research have not been tested on poultry. For this reason, a research will be carried out on the use of a mixture of CL and CPK (80: 20%) fermented with *Bacillus amyloliquefaciens* in ducks, with the title "the effect of giving a mixture of cassava leaves and palm kernel cake fermented with *Bacillus amyloliquefaciens* in rations on egg index, thickness shell, shell strength and colour index of duck egg yolk "

2. Material and methods

2.1. Experimental design

This study used an experimental method designed with a completely randomized design (CRD) with 4 treatments and 4 replications. The treatment was the ration using the level of use of the CLPKCF mixture. The treatments of these rations were: Ration A (0% CLPKCF), Ration B (6% CLPKCF), Ration C (12% CLPKCF), Ration D (18% CLPKCF).

The mathematical model and design used in this study based on [25] are:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Information:

Y_{ij} = Results of observations on treatment i and repetition j

i = Treatment (1, 2, 3, 4 and 5)

j = Deuteronomy (1,2,3 and 4)

μ = Common mean

τ = Treatment Effect

ε_{ij} = The residual (random) effect of the j th receiving treatment i

2.2. Material methods and design

This study used 160 laying ducks aged 14 months with an average daily egg production of 65% and an average body weight of \pm 1500 g.

The cage used in this study is a dry cage. Each cage unit contains 10 ducks measuring 1 x 3m, equipped with a place to feed and drink. To weigh the feed ingredients for the ration, a Camry scale of 15 kg is used. The tools used in this study include callipers, screw micrometres, Egg Force Reader and Egg Yolk Colour Fan.

The rations used in this study were prepared based on isoprotein (18%) and energy (2650 kcal / kg) and drinking water was given ad libitum [26] The rations were prepared by them and provided with ingredients consisting of commercial rations, coconut oil, and a mixture of cassava leaves and palm kernel cake fermented with *Bacillus amyloliquefaciens* (80: 20%). The content of food substances and metabolic energy of the ingredients of the ration is presented in Table 1. The composition of the ingredients of the ration and the nutritional content and metabolic energy of the research ration are presented in Table 2. The content of food substances and energy of the research ration can be seen in Table 3

Table 1. The Content of Food Substances (%), Metabolic Energy (Kcal / kg) and total carotenoids (mg / 100g) of the ingredients of the ration.

Feed ingredients	CP	CL	Fibre	Ca	P	Met ^c	Lys ^c	ME ^c	Total carotenoids ^d
Complete Feed ^a	18	7	8	3	0,60	0,50	1	2600	6010
Coconut ^b									
Oil	-	100	-	-	-	-	-	8600	2338
DUKBISF ^c	22.83	5.64	7,23	0.29	0.34	0.13	1.1	1505	7705

Explained:

^a: Label Commercial feed N544 Production PT.Charoen Pokphand Indonesia (2015)

^b: [22]

^c: [27]

^d: Instrument Laboratory Test of the Faculty of Agricultural Technology, Unand
CLPKCF : CL and PKC Fermentation

Table 2. The content of feed substances and energy of the research ration.

Feed Ingredients	RA	RB	RC	RD
Complete ration	99,50	92,50	85,00	78,00
Coconut oil	0,50	1,50	3,00	4,00
CLPKCF	0,00	6,00	12,00	18,00

Table 3. The content of food substances in the research ration

Feed ingredients	RA	RB	RC	RD
CP (%)	17,91	18,02	18,04	18,15
CF (%)	7,47	8,31	9,63	10,48
Fibbre(%)	7,96	7,83	7,67	7,54
Ca(%)	2,99	2,79	2,58	2,39
P available(%)	0,60	0,58	0,55	0,53
Met(%)	0,50	0,47	0,44	0,41
Lys(%)	1,00	0,99	0,98	0,98
ME (kcal/kg)	2630	2624	2649	2643
Total carotenoid (mg/100g)	6010	6056	6103	6150

Explained: Calculated Table 3 based on Table 1 and Table 2.

2.3. Observed Variables

1. Egg Index

The egg index can be calculated based on the ratio of egg width to egg length, which is measured using a calliper [28].

Egg Index

2. Eggshell Thickness

Measuring eggshell thickness using a micrometre in [29] study:

- Clean the surface of the measuring object and the mouth of the micrometre.
- Checked alignment of point "0" and calibrated before using the micrometre.
- opened the measuring mouth until it slightly exceeds the dimensions of the measuring object (eggshell)
- Hold the measuring object (eggshell) with the left hand and the micrometre in the right hand with the micrometre frame placed on the palm of the hand and held by the little finger, ring finger and middle finger
- when measuring, do not press too hard on the measuring shaft
- Clamped the micrometre on the holder, then rotated the thimble towards the object (eggshell) being measured and rotated the retched stopper until it touched the spindle then rotated the stopper 2 to 3 times so that the emphasis was sure, then read.
- Repeat the measurement several times so that the error in the measurement is as small as possible.

3. Eggshell Strength

The measurement of shell strength is done by weighing the eggs and after weighing the eggs are placed in a vertical position on the surface of the intron plate, continue to be pressed using compression until the eggs break. The graph shown can be used to calculate the strength of the eggshell kg / mm². The greater the value in kg / mm² of each measured egg the higher the strength [30] Eggshell strength is measured using the Egg Force Reader.

4. Egg Yolk Colour Index

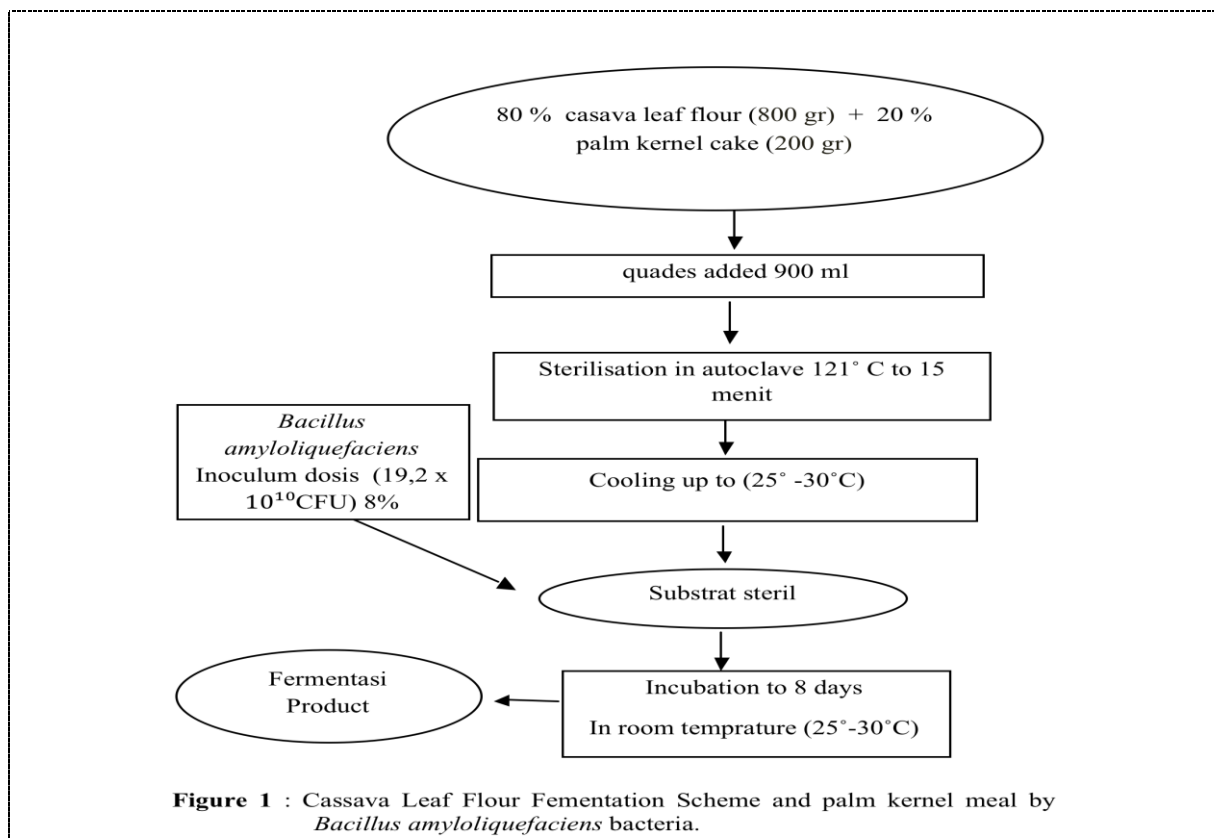
Determination of the colour of the egg yolk is done visually, namely by matching it with the standard colour of the colour fan (Roche yolk colour fan) with a score of 1-15 from the standard colour from pale to dark orange (dark), based on measurements with this tool, the yolk colour is both are in the range 9-12 [31].

2.4. Research Implementation Procedure

In this research, the activities carried out include making a mixture of Cassava Leaf (CL) and Palm Kernel PKC fermentation, composing rations and preparing cages.

2.4.1. Preparation of fermented CL and mixture

CL waste is collected from farmers' fields in several places in West Sumatra. Then dry in the sun to dry, and then grind CL until it forms flour. CL that has become flour is then mixed with PKC, the ratio of CL: PKC is 80: 20%, add 900ml of distilled water, then sterilize by autoclave for 15 minutes then cool and ferment by *Bacillus amyloliquefaciens* at a dose of 8% (19.2×10^{10} Cpu) and fermentation time of 8 days [21]. The results of fermentation are dried to moisture content of up to 14% and ground until smooth into CL and PKC flour. The process of preparing CL and PKC flour mixture can be seen in Figure 1.



2.4.2.. Feeding and Drinking

Feeding and drinking are carried out adlibitum, and cleaning the feed and drinking water containers is carried out every day.

2.5. Data analysis

All data obtained were processed statistically by analysis of variability according to the completely randomized design pattern (CRD) used. Analysis of variance can be seen in Table 5 if there are differences between treatments tested by the Duncans Multiple Range Test (DMRT) according to the procedure according to [25]

3. Results and discussion

3.1. The effect of treatment on egg index, thickness, shell strength and egg yolk color.

The mean of research on egg index, thickness, shell strength and egg yolk colour can be seen in Table 4.

Table 4. Average Egg Index, thickness, shell strength and egg yolk colour

Treatment	Egg index ^{ns}	Shell thickness (mm)	Shell strength (kg/mm ²)	Egg yolk colour
A (0% CLPKCF)	0,78	0,30	5,00	14,50
B (6% CLPKCF)	0,78	0,29	4,85	14,67
C (12% CLPKCF)	0,79	0,27	4,67	14,75
D (18% CLPKCF)	0,80	0,28	4,73	14,83
SE	0,006	0,0143	0,19	0,1250

Explained: ns = non-significant ($P > 0,05$); SE = Standard Error

3.1.1. The effect of CLPKCF on the egg index

The shape of the egg is proportional, neither too oval nor too round. The results of this study obtained the egg index in treatment D (up to the level of 18% CLPKCF) with an average of 0.80 in Table 4.

This figure is consistent with research from [32] index of duck eggs with the provision of katuk leaf flour (*Sauropus androgynus*) in the ration on the quality of Mojosari duck eggs at 7.25% in rations, namely 0.80. The egg index in this study was still in the normal range, as stated by [33] which confirmed that the normal duck egg index ranged from 63.3 to 81.70%. According to [24] egg shape is an expression of feed protein content. Cassava leaves are agro-industrial waste which contains high crude protein, so that after fermentation by *Bacillus amyloliquefaciens*.

There was an increase in protein in the mixed CLPKCF ration so that the use of CLPKCF could be increased in livestock rations.

3.1.2. The effect of CLPKCF on the egg shell thickness

The addition of a mixture of CL and PKC fermented by *Bacillus amyloliquefaciens* (CLPKCF) in the ration had an insignificant difference ($P > 0.05$) on the thickness of the shells from the analysis of variability. In Table 4

According to [34] the thickness of the shell normally ranges from 0.3 to 0.5 mm. The results of this study resulted in shell thickness ranging from 0.27 to 0.29 mm. The results of this study were due to the low Ca content in the mixed ration of CLPKCF under the provisions stipulated in [35] that the Ca content required for laying ducks was 2.75% compared to the research results of [36] who used shrimp waste flour in rations resulted in a duck egg shell thickness of 0.35 by providing a Ca content in the ration of 2.76%, because shrimp is a mineral source, it can increase the thickness of the eggshell.

It was further explained that in addition to the Ca content, according to [37] it is stated that egg shells are influenced by genetic traits, nutrition in feed, environment and management. The ability of ducks to produce good quality shellfish depends on the calcium in the ingested feed and reserves in the bones.

3.1.3. The effect of CLKPCF on the egg shell strength

The addition of a mixture of CL and PKC fermented by *Bacillus amyloliquefaciens* (CLPKCF) in the ration gave not significant ($P > 0.05$) on the strength of the shells from the diversity analysis results in Table 4

In this study, the obtained duck egg shell strength ranged from 4.58–5.00 kg / mm². The use of the CLPKCF mixture was influenced by the content of Ca and P in the ration that was almost the same as it also caused the formation of shell thickness and strength of duck egg shells which were not much different. This is supported by the statement of [38], that that the main nutritional factors related to shell quality are calcium, phosphorus, and vitamin D. The decrease in eggshell quality is in line with increasing age, genetics, and feed, especially Ca and P balance [39]. The strength of the shells is related to the supply of calcium and phosphorus obtained during the shell formation process [40]

3.1.4. The effect of the CLPKCF on the egg colour yolk

The addition of a mixture of CL and PKC fermented by *Bacillus amyloliquefaciens* (CLPKCF) in the ration had not significant ($P > 0.05$) on the index of egg yolk colour based on diversity analysis in Table 4

The quality and colour of egg yolks, were influenced by carotenoid levels and increased levels of pigment in the ration so that it will affect the pigmentation process [27]. Carotenoids as colour pigments that cannot be synthesized by poultry but must be available in feed [41]. In this study the control ration (A) which was the commercial ration used during the study produced a good egg yolk colour index and when the use of the control ration was reduced by its use then it was replaced with a CLPKCF mixture which produced a better egg yolk colour index than the control ration due to the carotenoid levels there is also high treatment.

The colour of the egg yolk is closely related to vitamin A in the feed so that the more carotene that will be deposited in the yolk, which in turn will affect the colour of the yolk [42].

The results of this study obtained the egg yolk colour index in treatment D (up to the level of 18% CLPKCF) with an average of 14.83. This figure is high from the study of [43] colour index of duck egg yolk with increasing quality of duck eggs by feeding fermented lamtoro leaf meal (*Leucaena leucochepala*) with *Bacillus laterosporus* and *Trichoderma viride* level of 30% in rations, namely 12.67.

4. Conclusions

Based on the results of the study, it can be concluded that giving a mixture of cassava leaves and palm kernel cake fermented by *Bacillus amyloliquefaciens* (CLPKCF) in rations up to a level of 18% in the ration can maintain the quality of duck eggs. In this condition, the egg index was 0.80 cm, the shell thickness was 0.28 mm, the shell strength was 4.73 kg / mm and the egg yolk colour index was 14.83.

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