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# Impact of the inclusion of calcium soap in feed on intestinal histology and blood lipid profile of native chickens

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## Abstract

The aim of this study was to examine the impact of the inclusion of calcium soap in feed on the intestinal histology and blood lipid profile of native chickens. This study used 192 native chickens aged 6 weeks with homogeneous body weight which were randomized into 4 types of treatment and 6 replications and each replication used 48 chickens with homogeneous body weight. The four types of treatment were: Group of chickens fed without the addition of Ca-PFAD as a control (chicken Group A); chicken feed with the addition of 2% Ca-PFAD (chicken Group B); chicken feed with the addition of 4% Ca-PFAD (chicken Group C); and chicken feed with the addition of 6% Ca-PFAD (chicken Group D). The results showed that the height of the jejunal villi in chicken groups C and D was significantly ($P<0.05$) higher than the control (chicken group A). Likewise, the depth of the jejunum crypts of Group C and D chickens was significantly ($P<0.05$) deeper than that of Group A chickens. The inclusion of 2-6% Ca-PFAD in the feed significantly ($P<0.05$) improved the blood lipid profile of native chickens. It was concluded that administration of calcium soap (Ca-PFAD) in feed can increase the height of the villi and the depth of the crypts of the intestinal jejunum. On the other hand, at the 4% level, giving calcium soap in feed can reduce cholesterol and Low Density Lipoprotein (LDL) levels, as well as increase triglyceride and High Density Lipoprotein (HDL) levels in native chicken.

**Keywords:** Calcium soap; Chicken; Cholesterol; Crypt depth; Villi height

## 1. Introduction

Native chickens have slower growth than broilers, so their feed efficiency will also be lower [1]. This is influenced by the feed digestion process, which is related to the histological condition of the intestine and the possibility of histological differences in the digestive organs. The intestine's ability to utilize nutrients is determined by the development of the digestive tract organs. The development of digestive tract organs, especially the intestines, is correlated with the growth rate of chickens.

There are several ways that can be taken to optimize the efficiency of nutrient absorption in the digestive tract of native chickens, one of which is by using calcium soap from palm oil waste. The Indonesian Central Statistics Agency (BPS) noted that Indonesia produces 45.58 million tonnes of palm oil, so the palm oil waste it produces is high. Palm oil waste is rich in energy or fat and has a high digestibility value, reaching 85.4%, which is equivalent to an energy supply of 8,030 Kcal/kg [2]. Processing palm oil into cooking oil will produce a by-product in the form of palm oil fatty acid distillate or Palm Fatty Acid Distillate (PFAD). The abundant availability of PFAD has great potential as an animal feed ingredient. Apart from the cheap price, the use of PFAD also does not compete with food ingredients, such as palm oil [3].

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The mineral Ca influences bone growth and metabolic processes [4]. Vitamin E in PFAD can act as an antioxidant and has an effect on reducing lipid oxidation in meat and adipose tissue of livestock [5].

Using PFAD directly in large quantities into feed is difficult, because its liquid form can affect the structure of the feed, making it sticky and lumpy. Such a form can affect the technique of mixing and administering it to livestock. The way to overcome this is to turn palm oil waste into calcium soap. Calcium soap is made by reacting palm oil waste in such a way with CaO, thus producing calcium soap [6]. Using palm oil waste in the form of calcium soap can make it easier to mix feed.

Compounds in calcium soap in the form of tocotrienols can optimize the function of the digestive tract and metabolism, so that feed use becomes more efficient [7]. In addition, this compound can stimulate the walls of the gallbladder to secrete bile, thereby facilitating fat metabolism. Tocotrienol compounds can increase the height of the villi and the depth of the crypts of the small intestine [7]. This means increasing the intestinal surface area and expanding the absorption area, thus the ability to absorb nutrients will increase.

Calcium soap can significantly improve performance, nutrient digestibility and carcass quality, as well as produce antibiotic residue-free meat that is high in protein and minerals, but low in fat and cholesterol [8].

This study aims to examine the impact of adding calcium soap to feed on intestinal histology, meat chemistry and the blood lipid profile of native chickens.

## 2. Material and methods

### 2.1. Animal treatments and experimental design

This research took place for eight weeks located at Farm Sesetan, Faculty of Animal Husbandry, Udayana University which is located on Jalan Raya Sesetan, Gang Markisa, Denpasar, Bali and has been approved by the Animal Ethics Commission, Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia. The ration given to chickens is commercial ration 511B produced by PT. Charoen Pokphand, Indonesia and Calcium Soap (Ca-PFAD) produced by the Chemical Engineering Study Program, Faculty of Mathematics and Natural Sciences, Bandung Institute of Technology, West Java, Indonesia.

**Table 1** Nutrient composition in commercial diet 511B for native chickens aged 6-12 weeks

| **Nutrient** | **Calcium Soap (Ca-PFAD) level in feed (%)** | | | | **Standard** [9] |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | |
| Metabolizable energy (kcal/kg) | 2900 | 2946 | 2990 | 3032 | 2900 |
| Crude protein (%) | 20 | 19.62 | 19.26 | 18.91 | 18-22 |
| Ether extract (%) | 5 | 5.51 | 6.01 | 5.94 | 5 |
| Water content (%) | 13 | 12.88 | 12.78 | 12.68 | 13 |
| Calcium (%) | 0.90 | 0.94 | 0.98 | 1.02 | 0.80 – 1.10 |

This study used 192 native chickens aged 6 weeks with homogeneous body weight which were randomized into 4 types of treatment and 6 replications and each replication used 48 chickens with homogeneous body weight. The four types of treatment were: Group of chickens fed without the addition of Ca-PFAD as a control (chicken Group A); chicken feed with the addition of 2% Ca-PFAD (chicken Group B); chicken feed with the addition of 4% Ca-PFAD (chicken Group C); and chicken feed with the addition of 6% Ca-PFAD (chicken Group D). All feed was in crumble form and given *ad libitum* throughout the research period (6-12 weeks of age).

Feeding of chickens is carried out *ad libitum*, which means unlimited. Feeding is given at ¾ of the capacity of the feeder to avoid spillage. Drinking water were given to chickens *ad libitum* and replaced so that it remains clean and fit to drink. The nutrient composition of commercial feed 511B is presented in Table 1.

At the end of the study (12 weeks old), all chickens were slaughtered to observe blood lipid profiles and intestinal histology. Sampling for chemical analysis of meat will be carried out by slaughtering the chicken at the neck by cutting

the carotid artery and jugular vein. After being slaughtered and the blood has come out completely, the chicken was placed in hot water with a temperature of 50-55$^0$ C for 10-15 minutes. After that, the hair was removed, the internal organs were removed and the neck and legs were cut. The meat to be analyzed was taken from the chest and stored in airtight plastic. The meat was then put in the refrigerator.

### 2.2. Histology of the jejunum

Villi and crypt samples were taken by slaughtering one KUB chicken in each experimental unit. Villous samples were taken by cutting 4-5 cm in the ileum and then stored in film pots containing formalin with a concentration of 10%. The villi samples are then taken to the laboratory for preparation.

Histology preparations of the jejunal intestine were made by cutting a section of the jejunal lumen 4 μm thick using a microtome and placing it on a slide for staining using the Hematoxylin-eosin method [10]. The finished villi preparations were then taken to the laboratory to measure the height of the villi and the depth of the crypts. Villous height was measured using the Image Raster application which has been calibrated according to the magnification of the microscope [11]. Crypt depth was measured from the base of the lamina propria to the base of the villi [12].

### 2.3. Blood Lipid Profile

Checking cholesterol levels uses the enzymatic colorimetric method with the method required in accordance with WHO/IFCC standards. The principle of examining total cholesterol levels using the emzymatic colorimetric method is that cholesterol esters were broken down into cholesterol and fatty acids using the enzyme cholesterol esterase. The cholesterol formed was then converted into cholesterol-3-one and hydrogen peroxide by the enzyme cholesterol oxidase. The hydrogen peroxide formed along with phenol and 4-aminophenazone was converted by perioxidase into a red substance, the color intensity formed was proportional to the total cholesterol concentration and was read at a wavelength of 500 nm [13]. Blood cholesterol levels were carried out using the Cholesterol Oxsidase p-aminophenazone (CHOD-PAP) method [14]. HDL examination uses the precipitation method by adding phosphotungstic acid and magnesium ions, after desincentrifuging HDL in the supernatant was measured using the same reagent kit as CHOD-PAP total cholesterol measurement [14].

## 3. Results and discussion

### 3.1. Villi height and jejunum crypt depth

The impact of giving Ca-PFAD in native chicken feed from 6-12 weeks of age turned out to have a significant effect ($P<0.05$) on villi height and jejunal crypt depth (Table 2). The average villus height in chicken Group C (4% Ca-PFAD) and chicken Group D (6% Ca-PFAD), namely: 15.61% and 13.89% significantly ($P<0.05$) higher compared to chicken Group A (0% Ca-PFAD). Chickens that received feed with the addition of 4% Ca-PFAD (chicken Group C), namely: 56.27% significantly different ($P<0.05$) higher than chicken Group B. Likewise with chicken Group D, namely: 53.96% significant ($P<0.05$) was higher than in Group B chickens, while the height of the jejunal villi in Chicken Group C was not significantly different ($P>0.05$) compared to Chicken Group D.

The average jejunum crypt depth of chickens given 4% Ca-PFAD (chicken Group C) and 6% Ca-PFAD (chicken Group D) was: 63.09% and 95.70% significantly ($P<0.05$) higher than control chickens (chicken group A). The depth of jejunal crypts in Group B chickens was: 39.92% significantly ($P<0.05$) lower than control (A). The crypt depth of chicken Group C was: 171.46% significantly ($P<0.05$) higher than that of chicken Group B. The crypt depth of chicken Group D was: 225.73% significant ($P<0.05$) higher than chicken Group B, while chicken Group C had a jejunal depth that was: 19.99% significantly ($P<0.05$) lower than chicken group D. More details are presented in Table 2 and Fig. 1.

**Table 2** Impact of giving calcium soap in native chicken feed at 6-12 weeks of age on villi height and into the intestinal jejunum crypts

| Variable | Calcium soap (Ca-PFAD) levels in feed (%) | | | | SEM |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | |
| Villi height(μm) | 950.85$^{b2)}$ | 703.41$^{c}$ | 1099.25$^{a}$ | 1082.94$^{a}$ | 16.73 |
| Crypt depths(μm) | 69.99$^{c}$ | 42.05$^{d}$ | 114.15$^{b}$ | 139.97$^{a}$ | 0.901 |

Note: $^{a,b,c,d}$ Different letters on the same row are significantly different ($P<0.05$); SEM = Standar Error of the Treatmen Mean.

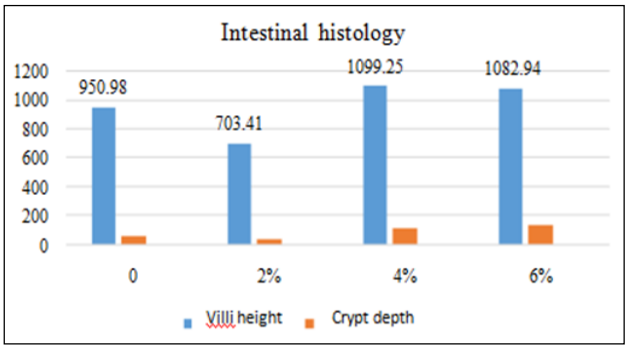


**Figure 1** Villi height and depth of jejunal crypts in chickens fed with different Ca-PFADs

The addition of calcium soap (Ca-PFAD) in commercial feed at a level of 4% (chicken Group C) gave the best results on villus height and crypt depth in the jejunum. Calcium soap functions as an antioxidant, anti-atherogenic, and contains tocopherol, tocotrienol, phytosterol, vitamin E, inflammatory squalene, immunomodulatory compounds, and has hypocholesterolemic properties. Other components of PFAD are: phytosterols, squalene, coenzyme Q10, polyphenols, phospholipids, quinones, ubiquinones, thiols, coumarins and amino acids [6].

Palm oil contains 50% saturated fatty acids, 40% monounsaturated fatty acids, and 10% polyunsaturated fatty acids, as well as trace amounts of beta-carotene, tocopherol, and tocotrienol, all of which are natural antioxidants. Natural antioxidants and polyunsaturated fatty acids can reduce the growth of pathogenic bacteria, reduce inflammatory processes in the intestinal mucosa, and increase nutrient absorption [15]. The ability to digest and absorb nutrients is influenced by the surface area of the intestine, the number of folds, the number of villi and microvilli. The longer the small intestinal villi and the depth of the crypts, the greater the effectiveness of nutrient absorption through the small intestinal epithelium [16]. Awad et al. [17] also stated that increasing the height and width of the villi in the chicken intestine is closely related to improving digestive function and increasing the area for nutrient absorption.

The chicken digestive tract epithelium is composed of villi and crypts to provide maximum mucosal surface area for nutrient absorption. Therefore, nutrient absorption and absorption efficiency depend on the integrity of the intestinal mucosa[18]. Overall, longer villi provide more mucosal surface, whereas deeper crypts indicate more rapid enterocyte turnover [19]. Thus, long villi and deep crypts indicate that the intestinal mucosa can be well differentiated with a maximum absorption surface. According to research by [7] that calcium soap supplementation in the diet can increase the length of the small intestine in ducks. In addition,[20] who stated that liver oil supplementation could increase the length of the villi and the depth of the duodenal crypts in rabbits.

### 3.2. Blood lipid profile

In Table 3, the blood lipid profile of native chickens that have received feed containing calcium soap (Ca-PFAD) is presented. The addition of Ca-PFAD to the feed turned out to have a significant effect ($P<0.05$) on the blood profile of native chickens.

Total cholesterol levels in chicken groups B and C were: 3.64% and 6.36% not significant ($P>0.05$) lower compared to chicken group A, while chicken group D was: 17.43% significant ($P< 0.05$) lower than chicken Group A. Cholesterol levels of chickens fed commercial rations with 4% calcium soap (chicken Group C) were: 2.83% not significantly ($P>0.05$) lower than the addition of 2% calcium soap (chicken group B). Chickens that received the addition of 6% calcium soap (chicken Group D), namely: 14.31% significantly ($P<0.05$) lower than chicken Group B. In chickens that received 6% calcium soap (chicken Group D) cholesterol levels, namely: 11.82% significantly ($P<0.05$) lower than Group C chickens.

Triglyceride levels in chicken groups B, C, and D, were: 21.29; 27.16; and 26.25% significantly ($P<0.05$) higher compared to chicken Group A. Hight density lipoprotein (HDL) levels in chicken Groups B, C, and D were: 13.61%; 17.10%; and 21.99% significantly ($P<0.05$) higher than chicken Group A. Low density lipoprotein (LDL) levels in chicken Groups B, C, and D were: 4.56%; 4.26%; and 10.80% was not significantly ($P>0.05$) lower than the blood LDL levels of chickens in Group A (control). In more detail the effect of giving Ca-PFAD on the blood lipid profile of native chickens is presented in Table 3.

**Table 3** Effect of adding calcium soap (Ca-PFAD) to native chicken feed at 6-12 weeks of age on the blood lipid profile of chickens

| Variable | Calcium soap (Ca-PFAD) levels in feed (%) | | | | SEM |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Total cholesterol (mg/dl) | 110.00[a2)] | 106.00[a] | 103.00[ab] | 90.83[c] | 1.6850 |
| Trygliserides (mg/dl) | 90.83[b] | 110.17[a] | 115.50[a] | 114.67[a] | 4.1858 |
| HDL (mg/dl) | 95.50[c] | 108.50[ab] | 111.83[a] | 116.50[a] | 2.5353 |
| LDL (mg/dl) | 109.67[a] | 105.00[a] | 104.67[a] | 97.83[ab] | 2.6763 |

Note: [a,b,c,] Different letters on the same row are significantly different (P<0.05);SEM = Standar Error of the Treatmen Mean.

Providing calcium soap in the ration at levels of 2%, 4% and 6% can reduce cholesterol levels. This is a result of the squalene, vitamin E and phytosterol content in PFAD which act as antioxidants to ward off or neutralize free radicals and inhibit free radical oxidation of total cholesterol, so that the ability to increase fecal excretion of bile acids causes a decrease in cholesterol levels. The addition of Ca in calcium soap as a mineral source of coenzymes, functions to activate the work of enzymes in the metabolic process.

The energy formed in the metabolic process is used for chicken growth, so that little energy is stored in the form of fat, resulting in a decrease in cholesterol. Loganathan et al.[21] reported that total blood cholesterol decreased because calcium soap contains the bioactive compound squalene which has anticancer and cholesterol-lowering properties. Squalene can lower cholesterol by reducing cholesterol synthesis by inhibiting the activity of the acly-CoA cholesterol aclytransferase (ACAT) enzyme found in HepG2 cells which has the function of reducing cholesterol esterification in the intestines and liver, as well as inhibiting the activity of the 3-hydroxy 3-metyglutaryl-CoA enzyme which causes inhibition of cholesterol synthesis. Reported by [22] that adding 1-3% calcium soap to the ration can reduce cholesterol levels in male Bali ducks. According to [23], total blood cell cholesterol in poultry is between 125-200 mg/dl. Based on this opinion, the cholesterol levels from the results of this study are classified as good, ranging between 90.83-110 mg/dl.

The results of the study showed that the provision of calcium soap in the ration had a significant effect on the chicken triglyceride variable. The addition of 4% calcium soap in the ration showed the highest results (115.50 mg/dl), then decreased in chicken groups D, B, and the lowest in chicken group A. This is thought to be because the administration of calcium soap in each treatment was different, so the intake and the amount of nutritional content in the calcium soap consumed in each treatment is also thought to be not the same. Bariyah [24] added that the formation of triglycerides in the liver will increase if the ration consumed contains excessive carbohydrates.This is because the liver converts carbohydrates into fatty acids, and then forms triglycerides. Apart from the factors above, the squalene content in calcium soap up to 2% is sufficient to influence lipid metabolism, especially triglycerides. Citrawidi et al.[25] said that blood triglyceride levels are greatly influenced by feed carbohydrate levels and the circulation of free fatty acids in the body.

Triglyceride levels are influenced by changes in the synthesis of fatty acids originating from the feed consumed by chickens. The higher the fatty acids produced from the lipogenesis process of carbohydrates and proteins, as well as amino acids, the triglycerides synthesized in the liver also increase and this directly affects the concentration of triglycerides in the blood serum. Apart from fat, carbohydrate content is also a material for lipogenesis to occur which produces fatty acids and glycerol. A similar opinion was expressed by [26,27] that triglycerides do not only come from dietary fat (saturated and unsaturated fatty acids), but also from foods containing carbohydrates (simple and complex). Based on this research, the average chicken triglyceride level is still within the normal range, namely: 90.83-115.50 mg/dl. This is in accordance with the statement by [28] that normal chicken triglyceride levels range between 19-150 mg/dl.

The research results showed that the addition of calcium soap in the ration had a significant effect on High Density Lipoprotein (HDL) of chickens. The highest mean HDL value was in treatment D, namely 116.50 mg/dl, while the lowest value was in treatment A, namely 95.50 mg/dl. The results of this study are the same as those reported by [22] that giving calcium soap at a level of 1-3% in the ration increases HDL levels in male Bali ducks. Hasanudin et al. [29] stated that HDL has a positive correlation with LDL and both are influenced by cholesterol levels in the blood.

High and low HDL in the blood is related to cholesterol levels, as well as the activity of synthesizing steroid compounds and bile salts [30]. Increased blood HDL cholesterol levels can be caused by the influx of cholesterol from lipoproteins with low cholesterol potential (LDL), to cell membranes, and the use of HDL for the synthesis of steroid compounds such as hormones or bile salts in the liver. HDL is a lipoprotein that functions as a means of transporting cholesterol from peripheral cells to liver cells and other body glands [30]. HDL plays a role in transporting cholesterol from the blood vessels back to the liver for disposal, thus preventing blood vessels from thickening or preventing the process of atherosclerosis, so it can be categorized as a good type of cholesterol. Sunita [31] states that HDL takes cholesterol and phospholipids from the liver and hands the cholesterol over to other lipoproteins to be transported back to the liver and recirculated or excreted from the body.Based on the results of this study, HDL levels are still in the normal range, namely 95.50-116.50 mg/dl. This is in accordance with the statement of [32] who stated that the normal value for chicken blood is >22 mg/dl. Mustikaningsih [33] added that the normal level of HDL in chickens is >60 mg/dl.

The research results showed that giving calcium soap in the ration showed a significant effect on Low Density Lipoprotein (LDL) of chickens. Chicken group A showed the highest results, followed by chicken group C; B; and D. Compounds in PFAD play a role in reducing LDL, such as phytosterols, vitamin E, aqualene, tocopherols and tocotrienols which can reduce cholesterol by increasing the production of bile acids and eliminating them for excretion with feces, so that the liver tries to secrete bile acids in the body which are lost with feces. In producing bile salts, the liver requires cholesterol and if the reserves are inadequate, the liver will send a signal to the brain and the brain will respond by sending a signal to HDL in the liver to secrete cholesterol in the form of unused LDL and stored in the blood vessels of the tissue to be carried to the blood vessels. liver and is used in metabolic processes that occur in the liver.

Blood LDL levels are also influenced by the feed consumed, the speed of the cholesterol biosynthesis process in the blood and the heredity (genetics) of the livestock. Tanuwiria et al.[27,34] stated that lipid levels, including lipid transport, such as LDL in the blood, can be influenced by the type of food and water consumed by livestock. Furthermore [30] stated that the derivatives and fatty acid content in the feed consumed can also influence LDL levels in the blood. The LDL levels in this study ranged from 97.83-109.67 mg/dl, which is smaller than that reported by [32] who stated that the normal LDL level in chickens is <130 mg/dl. The results of this study are equivalent to those reported by [22] that giving 3% calcium soap in commercial rations can reduce LDL levels in Bali ducks. Bidura [8] stated that the addition of 3% calcium soap in the ration can reduce broiler LDL levels.

## 4. Conclusion

It was concluded that administration of calcium soap (Ca-PFAD) in feed can increase the height of the villi and the depth of the crypts of the intestinal jejunum. On the other hand, at the 4% level, giving calcium soap in feed can reduce cholesterol and Low Density Lipoprotein (LDL) levels, as well as increase triglyceride and High Density Lipoprotein (HDL) levels in native chicken.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of ethical approval*

The Animal Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia has approved this research.

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