Improved broiler growth, carcass and meat yields by phyto-additive Chromanone Deamine (ChD) from Aegle marmelos (L) Correa fruits

by Sri Kismiati

Improved broiler growth, carcass and meat yields by phyto-additive Chromanone Deamine (ChD) from *Aegle marmelos* (L) Correa fruits

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Abstract

Aegle marmelos(L) Correa an indigenous plant, contains various functional phytochemical and has been used as phytomedicine in human. In the present study, isolated components of the *A. marmelos,* Chromanone deamine (ChD), were tested as a natural phyto-additive to improve broiler performance. Six hundred two-days old broilers (Cobb-CP 707) were randomly assigned into six groups receiving 1) 0 (ChD_0), 2) 0.025 ($ChD_{0.025}$), 3) 0.05 ($ChD_{0.05}$), 4) 0.075 ($ChD_{0.075}$), 5) 0.10 ($ChD_{0.1}$) and 6) 0.125 ($ChD_{1.25}$) mL ChD per Kg live weight. Each group consisted of five replicates having 20 birds in each replicate. Administration of ChD was carried out via drinking water for five weeks, and feed intake, live weight, FCR were recorded. On day 35, broiler samples were sacrificed, and the carcass and meat were weighed and recorded. The results revealed that all levels of ChD increased feed intake and growth, and lowered FCR significantly (p=0.01). Administration of $ChD_{0.075}$ improved feed intake from 2.82 Kg to 3.40 Kg (20.6% increase) per bird, live weight from 1.74 Kg to 2.15 Kg (23.6% increase) per bird, but lowered FCR from 1.62 to 1.58. The same effect was also shown in carcass and meat yield. Administration of $ChD_{0.075}$ increased significantly (p=0.01) carcass weight from 1.21 Kg to 1.50 Kg per bird (24% increase), and meat weight from 845 g to 1.08 Kg (28.2% increase) per bird. The present study suggests that ChD from *A. marmelos* can be used as a natural phyto-additive to improve broiler performance.

Keywords: *body weight, bael fruit, buah mojo*

Introduction

Broiler meat is commonly considered as a high-quality protein-source for consumers. The meat, being inexpensive, is easily available at an affordable price; besides, it can also be produced within a short span of time, which increases its demand in the market. Over the last decade, broiler production has increased rapidly from 987 million tonnes in 2010 (BPS 2011), to 3.25 billion tonnes in 2019 (BPS 2020). However, production costs per kg live weight decreased from US $ 1.19 in 2012 (USAID 2012) to US $ 1.02 in 2019 (Commodity Index 2020). The latest production cost is still more expensive than the average supplier in the international market i.e., US $ 0.92 or 10%, nonetheless, it has decreased significantly compared to 2012 which was 42% at that time. The reduction in the production costs could be attributed to cutting down of the import components of chicken production, especially feed by the Indonesian Government, which reached 43% of the production cost structure in 2012 (USAID, 21012).

Antibiotics as growth promoters in broilers have been widely used to suppress harmful bacteria in the digestive tract so that the feed conversion ratio is better. Many studies revealed the negative consequences of in-feed antibiotics after tens of years of practice and followed by subsequent banned in many countries better (Murwani 2008a, Murwani and Bayuardhi 2010). Various strategies have been developed to maintain or enhance broiler performance without antibiotics since then. Some studies used feed ingredients or indigenous fruits or herbs that naturally contain functional phytochemicals (Murwani 2008b; Murwani and Murtini 2009; Murtini et al 2010; Tanod et al 2015; Marlani et al 2017; Kusumanti and Murwani 2018). Some other studies attempted to use additives such as prebiotics, probiotics, and phytogenic additives (Murwani et al 2011). To provide affordable and safe animal protein-source-food for the consumers, the Indonesian Government has prohibited using antibiotics in livestock cultivation since 2017 (The Ministry of Agriculture Regulation 2017).

Fruit pulp of bael fruit *Aegle marmelos (L) Correa*) locally called “buah mojo” has various active components with numerous biological activities and has been used for generation as indigenous herbal medicine for humans in Indonesia, India, Sri Lanka, and Thailand (Pathirana et al 2020; Akbar 2020). We have isolated one component from *A. marmelos* pulp and obtained the active compound chromanone deamine (ChD). Our early studies regarding ChD use as feed additive have shown considerable improvement in the quality of chicken meat (Handria et al 2015) as well as an increase in the broiler protein levels (Widjaya et al 2015). Recent study has found that ChD at 0.05 ml/kg combined with feed containing antibiotics have resulted in a chicken live weight of 1.974 kg at 28 days of age, and an FCR of 1.58 (Sumardi 2016). However, there have been no studies on the graded level of ChD in broiler. Hence, this present study aimed to determine the effects of the administration of graded level of ChD via drinking water on the performance characteristics of broilers that were fed antibiotic-free diet.

Materials and methods

ChD was prepared by deaminating chromanone amine (ChA) contained in the pulp of bael fruit (*A. marmelos*) (Figure 1a). ChA was prepared by drying the fresh pulp including small seeds scattered throughout the flesh (Figure 1b) and milled into a fine powder (Figure 1c). The powder was extracted by 70% ethanol, and the extract was then fermented with *Aspergillus niger* to yield a liquid form. The liquid containing ChA was filtered. A spinner assisted this filtration in separating ChA from the rest of the mixture (to remove cellulose, hemicellulose, glycoproteins and pectin from the seed in the pulp). The ChA was then converted to ChD using *Photorhabdus luminescens*, and the resulting mixture was freeze-dried, and ChD was separated from bacterial mass and spores by a spinner. The frozen ChD was [illegible] room temperature to form a thick brown colored liquid (Figure 1d) with a mass weight of 1.25. This liquid ChD was confirmed

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for its structure based on infra-red spectra from FTIR spectrometer (Shimadzu Buck M500). ChD content was determined according to Korany et al (2014) using HPLC, where each mL of ChD was equivalent to 0.876 mg of 4-chromanone standard (Aldrich, 122351-10G). Based on this concentration, ChD dosage was prepared via drinking water.

Six hundred two-days old broilers (Cobb CP-707) in a uniform weight of around 40 g consisted of 300 males and 300 females were obtained from a commercial supplier, PT. Charoen Pokphand Indonesia. They were allocated randomly into six groups of six levels of ChD administration, namely: Control (ChD_0); 0.025 mL ($ChD_{0.025}$); 0.05 mL ($ChD_{0.05}$); 0.075 mL ($ChD_{0.075}$); 0.1 mL ($ChD_{0.1}$); and 0.125 mL ($ChD_{0.125}$) per Kg of live weight. Each group consisted of five replicates, with 20 birds in each replicate (10 males and 10 females).

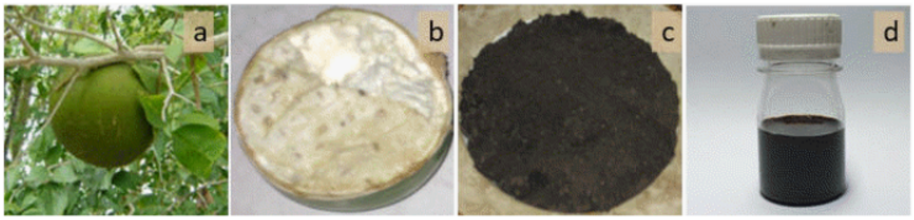

Photo 1. Mature bael fruit (a), bael fruit pulp (b), the powder of bael fruit pulp (c) and ChD (d)

An antibiotic-free complete feed with the nutritional composition as shown in Table 1 was used. From the brochures, this commercial feed was produced from the following raw materials: corn (40%), rice bran (12-18%), molases (2-4%), rice brand (8-12%), pollard (5 - 8%), soybean meal (7 - 9%), cocoa shell (1-3%), cassava flour (12-15%), fish meal together with bone meal (<3%) and amino acids (<1 %) mainly lysine, methionine and tryptophan. The feed ingredients and complete feed are available in the market and use by poultry farmers. The complete feed was also analyzed independently (proximate analysis) and the results were equal to nutritional composition as shown in Table 1. Drinking water containing ChD was given to each group for 3 hours per day as per their drinking needs. In an attempt to ensure that all ChD was consumed, broilers were prohibited from drinking for 30 minutes prior to ChD administration. The drinking water was then returned to normal water without ChD. Moreover, feed and drink were given *ad libitum* according to daily live weight of each replicate chickens.

Table 1. Nutritional composition of antibiotic-free feed for experimental broilers

Nutritional composition	Contents
Moisture (%)	13
Protein (%)	21.5-23.5
Crude Fiber (%)	5
Fat (%)	5
Ash (%)	7
Ca (%)	0,9
P (%)	0,6
Metabolic energy (kcal)	3000-3100

*Source PT Charoen Phokpand *(2019). The proximate contents were independently evaluated and the results were similar*

Feed intake, live weight, carcass and meat yields were measured and recorded. Feed intake data were obtained from the daily live weight of sample broilers per replicate. Live weight of chicken samples was recorded on day 35 by randomly choosing two broilers from each replicate, one male and one female. On day 35, they were sacrificed, and the carcass and meat parts were then separated and weighed. The data for each replicate of each group was the average weight of male and female broilers. Feed conversion ratio (FCR) was calculated from feed intake and live weight. Carcass and meat yield were calculated as the percentage from live weight.

Data of feed intake, live weight, FCR, both the weight of carcass and meat weight and the percentage were analyzed by using one-way analysis of variance (Anova) at $p<0.05$. Differences between means of ChD administration were tested by using two tails Duncan Multi-Range Test at $p<0.05$. Second order polynomial was employed to determine the optimal dose of ChD administration.

Results and discussion

Feed intake, live weight, dan FCR from first to fifth week of rearing are presented in Table 2. Table 2 showed that ChD administration increased ($p=0.01$) both the feed intake and live weight, and consequently lowered FCR significantly. Administration of $ChD_{0.075}$ produced the highest ($p=0.01$) (Table 2) feed intake to 3.4 Kg/bird, while control with no ChD was 2.83 Kg per bird (a 580 g or 20.6% increase), the live weight at 2.15 Kg compared to control with no ChD only produced 1.75 Kg (410 g increase or 23.5%). As a consequences of increase feed intake and live weight, administration of $ChD_{0.075}$, significantly lowered ($p=0.01$) (Table 2) the FCR to 1.58. These results were similar to previous studies that used 0.05 ml ChD per bird with concomitant feeding of in-feed antibiotics, and consequently, at the age of 28 days the live weight achieved was 1.97 kg (Sumardi 2016).

Table 2. Feed intake, live weight and FCR per bird broilers under 6 level of ChD administration for 35 days of rearing

Parameters		ChD_0	$ChD_{0.025}$	$ChD_{0.05}$	$ChD_{0.075}$	$ChD_{0.1}$	$ChD_{0.125}$	SEM	*p*
Feed	kg	2.83	3.22	3.36	3.40	3.31	3.33	0.0291	0.01*
Weight gain	kg	1.74	2.00	2.11	2.15	2.08	2.07	0.0416	0.01*
FCR		1.62	1.61	1.60	1.58	1.59	1.60	0.0774	0.01*

*n=5 with 2 sample birds for each replicate. * is significant at p<0.05*

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Furthermore, the result on the FCR of 1.58 by the administration of ChD $_{0.075}$, was lower than the supplementation of 1 mL multivitamin per L of drinking water, which yielded an FCR of 1.59 at 35 days of harvest but with a much lower live weight of 1.47 kg (Islam and Nishibori 2017). The FCR at $ChD_{0.075}$ was also lower than the addition of combined prebiotics and probiotics during six weeks of rearing, resulting in live weight at 1.71 kg and 1.73 kg respectively and the FCR at 1.63 (Sarangi et al 2016). Several other studies that used comparable levels of metabolite energy in feed, i.e. 3,007.65 Kcal with the addition of 2% virgin coconut oil, 0.9% lysin and 0.37% methionine, at the age of 35, have shown that the live weight was 1.53 kg / bird, although with an FCR of 1.57. Therefore, the results of the current study demonstrate that administration of ChD $_{0.075}$ offers an option to improve the performance of broilers raised without in-feed antibiotic.

Feed conversion is determined by feed intake that can be converted into live weight efficiently. Higher feed intake provides higher available nutrient to be used for growth or body weight gain, and when body weight gain is greater than the increase in feed intake, the feed is utilized more efficiently as shown in our results. Broiler genetically has high appetite; therefore, ChD may not be likely to affect appetite. Studies of some phytogenic additives have demonstrated that they can improve the performance, nutrient digestibility, carcass and meat quality, gut health and overall health of broilers. However, the observed performance improvement mechanism is far from clear (Puvaca et al 2013, Puvaca et al 2020, Saracila et al 2020). As ChD administration produced a significant increase in feed intake, live weight, and carcass, we could only speculate that there is a better utilization of absorbed nutrient from improved feed intake. Nonetheless, how ChD can improve nutrient utilization is homework that needs further studies.

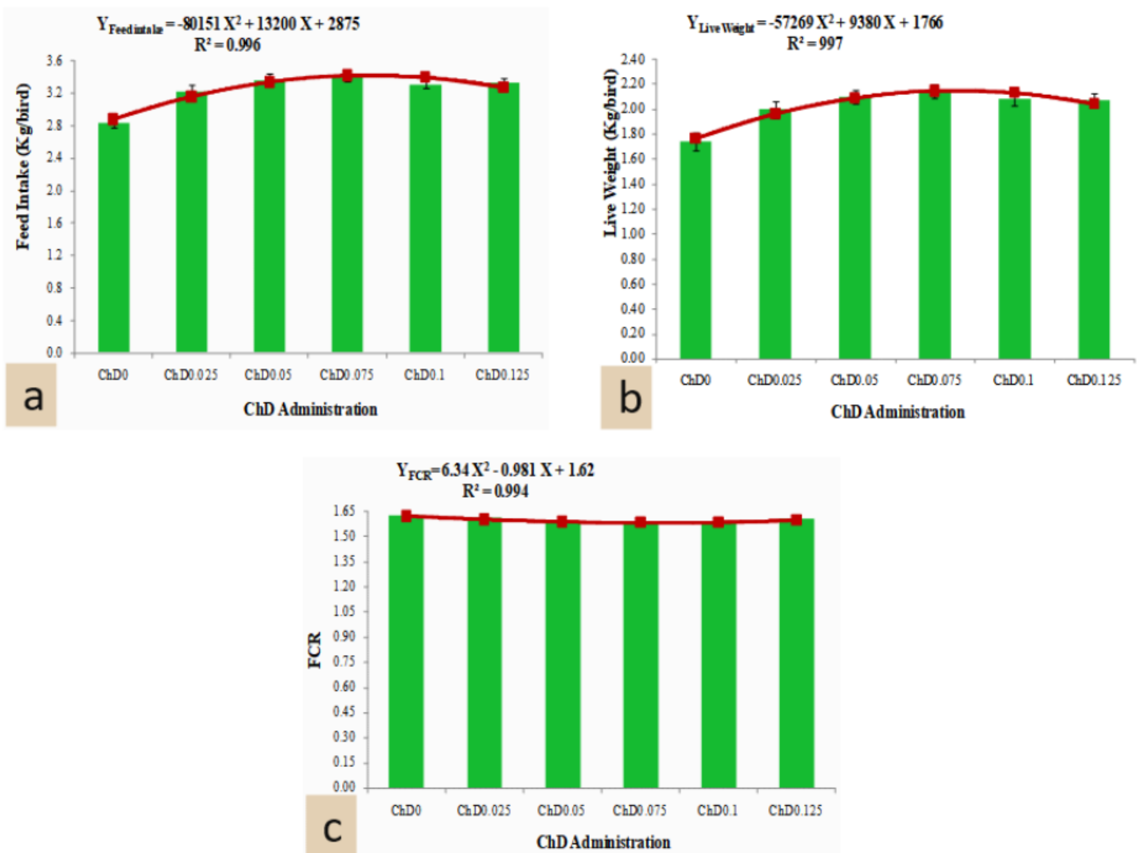

Figure 1. Feed intake (Kg/bird) (a), live weight (Kg/bird) (b) and FCR (c) of broilers under 6 levels of ChD administration for 35 days (5 weeks) of rearing (green) together with the 2-nd order of polynomial trendlines (red line) and the equation of the lines. The vertical lines on each histogram bar indicates the standard deviation of the mean of each bar, with n = 5, with two bird samples per replicate

Figure 2 shows the comparison of broiler performances in term of feed intake (Figure 2 a), live weight (Figure 2 b) and FCR (Figure 2 c). An increase in feed intake and live weight, and a decrease in FCR under ChD administration follows the 2-nd order of polynomial trendline with its optimum dose (dY / dX) of ChD administration are 0.0823 mL, 0.0819 mL and 0.0773 mL per Kg live weight respectively. In term of broiler production FCR is the main indicator to determine the efficient of livestock business, therefore 0.0773 mL ChD would be the optimum dose. At this dose the broiler would have a feed intake of 3.42 kg per bird, and the live weight would be 2.15 kg per bird, and FCR at 1.58. Referring to the polynomial equations, the control (ChD_0) would have 2.88 kg feed, producing 1.77 kg live weight, and having 1.62 FCR. This means that at the optimum dose of ChD administration of 0.0773 mL per kg live weight, would save 37.9 g of feed per Kg live weight.

Both the weight and percentages of carcass and meat observed on day 35 are listed in Table 3. The table shows that all doses of ChD administration increased the weight and percentage of carcass and meat. The highest results (p=0.01) (Table 3) were obtained at $ChD_{0.075}$ administration. Further, compared to ChD_0, administration of ChD $_{0.075}$ increased carcass weight from 1.21 Kg to 1.50 Kg per bird (24% increase), and meat weight from 845 g to 1.08 Kg (28.2% increase) per bird. The $ChD_{0.075}$ administration also produced higher carcass Go to Top ge of 70.07% compared to ChD0 of 69.2% (0.86 % increase), and meat percentage from 70.1% to 72.1% (2% increase).

Table 3. The weight and percentage of carcass and meat per bird under different level of ChD administration on day 35 of rearing

Parameters		ChD_0	$ChD_{0.025}$	$ChD_{0.05}$	$ChD_{0.075}$	$ChD_{0.1}$	$ChD_{0.125}$	SEM	*p*
Carcass	kg	1.21	1.39	1.47	1.5	1.45	1.44	0.0262	0.01*
	%	69.2	69.5	69.8	70.1	69.9	69.9	0.102	0.01*
Meat	kg	0.84	0.98	1.04	1.08	1.04	1.04	0.0167	0.01*
	%	70.1	70.4	70.9	72.1	71.8	71.5	0.178	0.01*

*n=5 with 2 sample birds for each replicate. * is significant at p<0.05*

The comparison of carcass and meat percentages between ChD doses is shown in more detail in Figure 3. The figure confirms that administration of ChD $_{0.075}$ is the optimum dose for the formation of carcass and meat. Administration of ChD below the optimum dose increased the percentage of both carcass and meat, however administration with a higher dose than the optimum i.e. $ChD_{0.1}$ and $ChD_{0.125}$, declined the percentage of carcass and meat (Table 3).

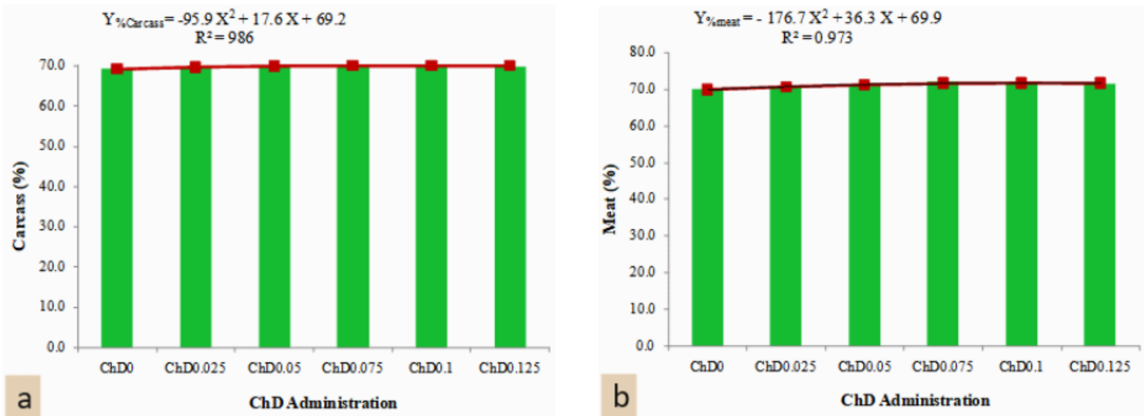

Figure 2. The percentage of carcass (a) and meat (b) of broilers under 6 levels of ChD administration on day 35 of rearing, together with the 2-nd order of polynomial trendlines (red line) and the equation of the lines. The vertical lines on each histogram bar indicates the standard deviation of the mean of each bar, of n = 5, with two bird samples per replicate

The administration of $ChD_{0.025}$ significantly increased (p=0.01) the percentage of carcass (Table 3 and Figure 3 a) and meat (Table 3 and Figure 3 b). Administration of $ChD_{0.05}$ resulted in a high increase in carcass percentage and therefore it was not significantly different (p=0.01) (Table 3) from those produced by $ChD_{0.1}$. On the contrary, the effect on the meat was lower, and consequently, the percentage of meat produced was significantly lower (p=0.01) than both $ChD_{0.1}$ and $ChD_{0.125}$ (Table 3 and Figure 3 b). These differences indicated that the administration of ChD below the optimum dose was significantly effective in increasing carcass weight, however, with a lower meat weight.

Referring to the established polynomial equations (Figure 3), carcass production without ChD (Control) was 69.21% or 1.22 Kg per bird, which resulted from 2.89 Kg of feed. At optimum ChD administration (0.0773 mL ChD) the percentage of carcass was 70% (1,50 Kg per bird), which was produced from 3,42 Kg of feed. Then to produce 1 Kg of carcass without ChD (Control) broilers need 2.35 Kg of feed, compared to optimum ChD administration (0.0773 mL ChD) that needs 2.27 Kg feed; more efficient 81.03 grams. Furthermore, the control broiler produces 69.87% (853.94 g) meat per bird, which comes from 2.88 kg of feed. The ChD administration at optimum dose resulted in 71.6% (1.08 Kg) of meat per bird, which produced from 3.42 Kg of feed. These figures indicated that to produce 1 kg of meat, the control broiler required 3.37 kg of feed, while at the optimal administration of ChD required less feed i.e 3.17 kg, which means that the efficiency of feed utilization was 195 g.

Since proteins have the greatest mass weight among the macromolecular compounds that make up meat, a higher meat weight in broilers produced by high doses of ChD administration ($ChD_{0.075}$, $ChD_{0.1}$ and $ChD_{0.125}$) compared to low doses ($ChD_{0.025}$ and ChD $_{0.05}$) is generally considered to contain high levels of protein in the produced meat. Our prior study found that ChD at a dose of 0.05 mL per live weight increased protein levels by 3.72% (Widjaya et al 2015).

ChD is 3,6,7-chromanone deamine which is produced by deaminating 3,6,7-chromanone amine of *A. marmelos* pulp. Our previous study has established that ChD treatment at a dose of 0.05 mL reduced the total volatile nitrogen excreta in chicken from 243 mg/100 g to 95 mg/100 g (Handria et al 2015). This finding suggests that the free nitrogen existed as $–NH_2^-$ ion of which is normally wasted as NH_3 in the excreta could be bound by the 3,6,7-chromanone deamine into 3,6,7-chromanone amine. The bound amine may provide available pool of amine for synthesis of amino acids. The more amine is bounded, the more amino acids would be formed either by the thickening of an existing protein fraction or forming a new fraction. Therefore, higher dosage of ChD administration provides higher available deamine to bind more –NH2 $^-$ ion and hence more protein or meat yield. Further investigation is underway to elucidate this possible mechanism.

Conclusion

- Administration of $ChD_{0.075}$ via drinking water produced 20.6% increase in feed intake, 23.6% increase in live weight per bird, and lowered FCR from 1.62 to 1.58. It also increased carcass weight by 24%, and meat weight by 28.2% per bird. Administration $ChD_{0.075}$ can be used as a natural phyto-additive to improve broiler performance on feed with antibiotic-free rations.

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- The optimum of ChD administration is 0.0773 mL per Kg live weight and it increases the feed efficiency of 37.9 g per Kg live weight, 81 g of feed per Kg carcass and 195 g of feed per Kg of meat.

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