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Efficacy of Clove Leaves, Mangosteen Peel Extract and Liquid Smoke as Feed Additives for Native Chickens

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ABSTRACT

Feed additives are commonly supplemented in poultry feed to improve the animal health status and performance. Previous *in vitro* studies about feed additives showed that coconut shell liquid smoke (CSLS), mangosteen peel extract (MP), and clove leaf extract (CL) had a similar antibacterial activity with the AGP. According to those insights, an *in vivo* study was designed to explore the effect of those combinations as feed additives on the slow-growth chickens' performances. A total of 588-one-day-old unsexed chicks were distributed into 42 pens with 14 birds/pen and reared for 84 days. They were fed each of six different diets, The formulated diets are standard diet without feed additives as Negative Control (NC); NC + Virginiamycin as Positive Control (PC); NC + CSLS; NC + mixtures of CSLS + CL; NC + mixture of CSLS, CL, and MP in low-, medium-, and high- doses. Results showed no significant effects (P>0.05) of diets on body weight gain, feed consumption, and FCR during starter and whole periods. The treatments also showed no significant (P>0.05) effects on carcass yield, liver, spleen, and thymus weight. However, supplementation of a low-dose bioactive mixture produced a higher (P<0.05) abdominal fat, and supplementation of CSLS + CL or medium doses of CSLS + CL + MP mixtures produced a heavier (P<0.05) bursa weight than other treatments. In conclusion, the low-dose mixture of CSLS, CL, and MP was recommended based on a similar effectiveness trend to AGP in potentially improving the FCR.

Key words: antibiotic growth promoters; feed additive; plants' bioactive; sensi-1 agrinak

INTRODUCTION

Feed additives, such as antioxidant, antibacterial, antifungal, prebiotic, probiotic, essential oils, and organic acids, are commonly supplemented in poultry feed to improve animal health status, performance, and feed quality. Recently, these supplementations have been questionable due to increasing consumer concerns related to human health, which resulted in abandoning antibiotic growth promoters (Salim et al. 2018). That leads to an increasing necessity for alternative feed additives to replace antibiotic growth promoters in poultry feed.

Previous studies have reported several alternative feed additives from plants' bioactive. They are liquid smoke, mangosteen peel extract (MP), and clove leaf extract (CL). Liquid smoke is a lignocellulose pyrolysis product produced by collecting rising smokes into a cool pipe (Winarni et al. 2021). This liquid smoke is known for its antibacterial activities. According to Widodo et al. (2021), coconut shells liquid smoke (CSLS) combined with 0.5% formic acids perform a better antibacterial activity than 0.1% zinc bacitracin (commercial antibacterial).

MP was also known as a feed additive for chickens. Its inclusion in the feed improves the FCR of broilers and Sentul-laying chickens (Herawati et al. 2020; Widjastuti et al. 2021). The MP contains both antioxidants and antibacterial. The antioxidant activity of the MP was 0.05µL/mL when the extraction ratio was 11:100 (Pasaribu et al. 2021).

Other plant materials reported as natural antibacterial were clove leaves. The clove were extracted in sterile distilled water (60-100%) and had a similar antibacterial activity with 18 ppm of Chloramphenicol (Pasaribu et al. 2021).

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Furthermore, a combination approach of plants' bioactive was suggested as an alternative feed additive to replace the AGP. This alternative feed additive can improve poultry performances and combat antibiotic microorganism resistance. A previous *in vitro* study showed that the combination of CSLS, MP, and CL had a similar antibacterial activity with 30 ppm of Chloramphenicol and 40 ppm of Virginiamycin (Pasaribu et al. 2021). Subsequently, the effect of CSLS, MP, and CL combinations as feed additives (antibacterial and antioxidant) through *in vivo* study must be observed on the poultry performances.

MATERIALS AND METHODS

Bioactive extract preparation

The extraction was prepared according to the methods described by Pasaribu et al. (2021). In brief, clove leaves or mangosteen peel were extracted with 100% methanol (1:9), stirred in a shaker incubator for 4 hours, and stored overnight. The filtered supernatant was evaporated at 50ºC using a rotary evaporator. The CSLS is produced through the pyrolysis process and obtained from a home industry in Bogor. The CSLS was diluted with distilled water in proportion 3:1 (mL/mL), while the CL was in proportion 1:4. Diluted CSLS and CL mixture was made in proportion 1:1, While CSLS, CL, and MP mixture in proportion 1:1:1 before mixed into the experimental diets.

Animals and management

This research was conducted with the approval of the animal ethics clearances (Balitbangtan/ Balitnak/A/07/2020).

A total of 588-one-day-old unsexed chicks of slow-growth chickens (SenSi-1 Agrinak) were distributed randomly into 42 pens with 14 birds/pen. Each pen measured 300cm x 150cm, and the floor was covered with rice hulls as litter, equipped with a light bulb as a heater during the starter period (1–28 d), a feeder, and a drinker. The standard starter and grower feeds were formulated to meet the nutrient requirements of local chickens recommended by Sinurat et al. (2022). The chicks were reared and fed a standard starter diet from 1 to 28 d, followed by a standard grower diet from 29 to 84 d old. The starter feed was made in crumble, while the grower feed was in pellet forms. Table 1 shows the compositions and nutrient contents of the diets. The chickens freely accessed feed and drinking water during all observation periods.

This research conducted seven dietary treatments in different combinations of the bioactive extract and their concentrations. A negative control (NC) is a control diet without feed additives addition. A positive control diet (PC) is the NC supplementing with 50 ppm virginiamycin (AGP). CLSL treatment is NC supplementing with 75.25mL of CLSL/100kg NC diet. CSLS and CL treatment is NC supplementing with 37.625mL of CSLS/100kg and 37.625mL of CL/100kg NC diet. Mixture Treatments is NC supplementing with a combination of CSLS, CL, and MP in 3 different concentrations, i.e., a low concentration by supplementing 20mL of each CSLS, CL, and MP/100kg NC diet, a medium concentration by supplementing 30mL of each CSLS, CL, and MP/100kg NC diet, and a high concentration by supplementing 43mL of each CSLS, CL, and MP/100kg NC diet. The total phenolic of plant bioactive addition in NC+CSLS, NC+CSLS+CL, NC+low dose, NC+medium dose, and NC+high dose is 0.09mg, 0.47mg, 0.41mg, 0.62mg, and 0.88mg every 100kg of diet, respectively. Each dietary treatment was given to 6 pens of chickens from day old to the end of the experiment (84 days old) and each pen was considerated a replicate.

Table 1: Ingredients composition and nutrient contents of the starter and grower diets.

Ingredient	Level in the diet (% As fed)	
	Starter	Grower
Corn	50.6	56.1
Wheat pollard	10	10
Palm kernel cake	5	7
Soybean meal 44	26.52	20.77
Vegetable oil	2.03	0.24
Meat and bone meal CP 50%	4.3	4.35
CaCO3	0.86	0.89
L-Lysine	0	0
Mono calcium phosphate	0	0
DL-Methionine	0.19	0.11
NaCl	0.2	0.2
Vitamin, mineral, premix	0.31	0.31
Total	100	100
Nutrient content (by Calculation)		
Crude Protein, %	20.5	18.5
Metabolizable Energy, kcal/kg	2900	2800
Lysine, %	1.000	0.900
Methionine, %	0.500	0.428
Methionine + Cystine, %	0.800	0.700
Threonine, %	0.750	0.674
Tryptophan, %	0.236	0.210
Calcium, %	0.900	0.900
Available phosphorous %	0.350	0.350

Parameters and data analysis

This study observes the bird's performance, i.e., live body weight gain, feed consumption, and mortalities. The FCR was calculated by dividing the feed consumption by the body weight. At the end of the study, two chickens per pen (one male and one female) were used to determine the carcass percentage, abdominal fat content, the weight of organs related to digestion (liver weight, gizzard weight, intestine length), and the weight of organs related to immune systems (spleen-, thymus-, and bursa weight).

This research uses a completely randomized design. Duncan's multiple range test is used to identify differences between treatments if the ANOVA showed significance at $P<0.05$.

RESULTS

Performances of the chickens

Data on the chickens' performances during the starter period is shown in Table 2. The dietary treatments have no significant effect ($P>0.05$) on body weight gain, feed consumption, FCR, and mortality. All treatments showed a trend of improvement in the FCR compared to the NC. The trend of improvement in the FCR for PC, CLSL treatment, CSLS+CL treatment, low dose, medium dose, and high dose is 6.42, 2.64, 4.30, 5.73, 5.08, and 4.51%, respectively.

The performances of the chickens during the whole period of the experiment (age 1 to 84 d) are presented in Table 3. The body weight gain, feed consumption, FCR, and mortality of the chickens were not significantly (P>0.05) affected by the supplementation of the AGP or plant extracts. All treatments showed various trends of FCR improvement compared to the NC. The trend of FCR improvement for PC, low dose, and medium dose are 1.26, 0.75, and 0.17%, respectively. Meanwhile, the three other treatments show no trend of FCR improvement compared to the NC.

The mortalities of chickens in all treatments, including the NC, were very low in the starter and throughout the period. This indicated that all birds were exposed to a minimum infection of pathogenic organisms or had high immunity to pathogens.

Carcass and organs related to the immune system

Data on carcass percentage, abdominal fat levels, and internal organs weight of the chickens are presented in Table 4. Results of the experiment showed that the carcass percentage and liver weight were not influenced by the treatments significantly (P>0.05). However, the treatments significantly (P<0.05) influenced the abdominal fat levels. Supplementation of the low dose of bioactive mixtures produced a higher (P<0.05) abdominal fat level than the other treatments.

The treatments did not significantly (P>0.05) influence the relative weights of the spleen and thymus. However, the treatments significantly (P<0.05) affected the relative weight of bursa Fabricius. The CSLS + CL treatment or medium dose produced a heavier (P<0.05) bursa weight than PC, CSLS treatment, and low dose. The relative weight of bursa Fabricius of chickens fed with the PC treatment (AGP supplementation) or other bioactive extracts (CSLS treatment, CSLS+CL treatment, low, medium, and high dose) was not significantly (P>0.05) different compared to the NC.

Digestive tract of the chickens

The effect of feed additive supplementation on the digestive tracts (relative gizzard weight, duodenum length, jejunum length, and ileum length) is presented in Table 5. None of the digestive tracts observed in this study were significantly (P>0.05) affected by supplementing plant bioactive or AGP.

Table 2: Performances of Slow-growth chickens during the starter period (1 – 28 d) as affected by feed additive supplementation.

Treatment	BWG (g)	Feed Consumption (g)	FCR	Mortality (%)
Negative control (NC)	230±24	506±17	2.180±0.255	0
Positive control (NC+AGP)	247±10	512±10	2.077±0.114	0
CSLS treatment (NC+CSLS)	236±12	510±21	2.161±0.125	0
CSLS + CL treatment (NC+CSLS+CL)	249±11	508±12	2.124±0.084	0
low dose (NC+CSLS + CL+MP)	238±17	497±23	2.093±0.076	0
medium dose (NC+CSLS + CL+MP)	240±17	504±15	2.107±0.142	1.19
high dose (NC+CSLS + CL+MP)	240±16	506±9	2.120±0.169	0
*P-value	1.00	0.53	0.853	0.44

BWG=Body Weight Gain; FCR=Feed Conversion Ratio; AGP=Antibiotic growth promoters; CSLS=Coconut shells liquid smoke; CL=Clove leaves extract; MP= mangosteen peel extract.

Table 3: Performances of slow-growth chickens during the whole period (1 – 84 d) as affected by feed additive supplementation.

Treatment	BWG (g)	Feed Consumption (g)	FCR	Mortality (%)
Negative control (NC)	1201±44	4274±69	3.562±0.128	0.00
Positive control (NC+AGP)	1226±37	4308±50	3.515±0.117	0.00
CSLS treatment (NC+CSLS)	1192±48	4290±40	3.603±0.152	0.00
CSLS + CL treatment (NC+CSLS+CL)	1215±57	4356±163	3.593±0.230	2.38
low dose (NC+CSLS + CL+MP)	1210±33	4274±87	3.533±0.101	0.00
medium dose (NC+CSLS + CL+MP)	1226±49	4351±120	3.554±0.172	2.38
high dose (NC+CSLS + CL+MP)	1208±55	4338±46	3.597±0.185	1.19
*P-value	0.89	0.74	0.707	0.49

BWG=Body Weight Gain; FCR=Feed Conversion Ratio; AGP=Antibiotic growth promoters; CSLS=Coconut shells liquid smoke; CL=Clove leaves extract; MP= mangosteen peel extract.

Table 4: Carcass percentage and organ weight affected by AGP and bioactive supplementation.

Treatment	Dressing carcass percentage (%LBW)	Liver weight (%LBW)	Abdominal fat weight (%LBW)*	Immune organs		
				Spleen weight (%LBW)	Bursa of Fabricius weight (%LBW)*	Thymus weight (%LBW)
Negative control (NC)	69.4±2.0	1.86±0.24	$0.60±0.36^{b}$	0.14±0.01	$0.27±0.09^{abc}$	0.25±0.07
Positive control (NC+AGP)	69.4±1.3	1.78±0.21	$0.64±0.36^{b}$	0.16±0.02	$0.21±0.09^{bc}$	0.27±0.11
CSLS treatment (NC+CSLS)	68.6±1.7	1.83±0.15	$0.38±0.08^{b}$	0.17±0.05	$0.20±0.07^{c}$	0.24±0.14
CSLS + CL treatment (NC+CSLS+CL)	67.9±1.6	1.99±0.25	$0.46±0.20^{b}$	0.20±0.06	$0.33±0.09^{a}$	0.26±0.11
low dose (NC+CSLS + CL+MP)	68.5±3.2	1.81±0.25	$1.31±0.77^{a}$	0.17±0.02	$0.22±0.06^{bc}$	0.35±0.49
medium dose (NC+CSLS + CL+MP)	68.6±1.9	1.76±0.30	$0.53±0.21^{b}$	0.18±0.02	$0.30±0.12^{a}$	0.26±0.15
high dose (NC+CSLS + CL+MP)	68.9±1.8	1.80±0.25	$0.69±0.30^{b}$	0.18±0.05	$0.29±0.10^{ab}$	0.26±0.15
*P value	0.58	0.28	<0.001	0.165	0.003	0.913

LBW=Live Body Weight; AGP=Antibiotic growth promoters; CSLS=Coconut shells liquid smoke; CL=Clove leaves extract; MP= mangosteen peel extract; *P value=Columns show the significant difference in α=0.05 analyzed using Duncan's test while means within a column with different superscripts are significantly different.

Table 5: Relative- weight of gizzard and length of digestive tracts of slow-growth chickens affected by AGP and bioactive supplementation.

Treatment	Gizzard weight (%LBW)	Duodenum length (cm/100g LBW)	Jejenum length (cm/100g LBW)	Ileum length (cm/100g LBW)
Negative control (NC)	2.69±0.72	1.90±0.38	3.63±0.84	3.41±0.88
Positive control (NC+AGP)	2.78±0.38	1.93±0.42	3.83±0.62	3.54±0.63
CSLS treatment (NC+CSLS)	2.94±0.42	1.74±0.26	3.58±0. 86	3.45±0.57
CSLS + CL treatment (NC+CSLS+CL)	2.98±0.40	1.85±0.29	3.66±0.72	3.48±0.46
low dose (NC+CSLS + CL+MP)	2.86±0.54	1.68±0.34	3.98±0.77	3.63±0.59
medium dose (NC+CSLS + CL+MP)	2.84±0.51	1.68±0.29	3.64±1.01	3.22±0.50
high dose (NC+CSLS + CL+MP)	2.64±0.49	1.76±0.27	3.63±0.75	3.29±0.49
*P value	0.62	0.51	0.89	0.68

LBW=Live Body Weight; AGP=Antibiotic growth promoters; CSLS=Coconut shells liquid smoke; CL=Clove leaves extract; MP= mangosteen peel extract.

DISCUSSION

Performances of the chickens

Statistical analyses showed no significant effect of the treatment on all performance parameters observed in this experiment, including the FCR. Nevertheless, the FCR is in the normal range according to Windisch et al. (2008). Based on a meta-analysis, supplementation of AGP in broiler feed improved the FCR by 2.7% during the starter period (Cardinal et al. 2019). The present study showed that the AGP supplementation has trends to improve the FCR by 6.42%, and the bioactive supplementation varies from 2.64 to 5.73% during the starter period.

Some studies have been conducted on the effectiveness of bioactive extract from single- or mixed plants as feed additives for broilers. Some modes of action of plant bioactive in improving the performance of poultry have been proposed, i.e., by suppressing the harmful microorganisms, stimulating the beneficial microorganism population in the digestive tracts, and improving the immune system (Seidavi et al. 2021; Rahman et al. 2022).

One of the ingredients tested in the present study was CSLS. Supplementation of CSLS in broiler feed increased the population of beneficial bacteria (*Lactobacillus*) and reduced the population of pathogenic bacteria (*Salmonella* sp. and *E. coli*) in the ileal digesta (Ardilla et al. 2021). The pattern of these responses was similar when the AGP was supplemented in broiler diets (Baurhoo et al. 2009). It is expected that the CSLS could be used as a feed additive to replace the AGP. However, the present study showed that supplementation of 75.25mL CSLS/100kg feed did not affect the body weight but had trends to improve the FCR by 2.64% during the starter period. This result was lower when compared to the effect of the AGP, which has trends to improve the FCR by 6.42% during the same period. However, when the CSLS combined with the CL and MP, especially in low concentration, the potential improvement was higher, i.e., the FCR potentially improved by 5.730%. This effect was almost similar to the potential AGP supplementation. It may be related to the properties of the CSLS, CL, and MP mixture, which have anti-bacterial and antioxidant properties (Pasaribu et al. 2021). A similar performance improvement was reported when broilers were fed soybean bioactive peptides (Sa'adoon and Abbas 2023) or curcumin (Yadav et al. 2020).

The present study showed no significant difference between the treatments. Despite that, higher trends of FCR improvement were found in PC, low-dose, and medium-dose treatments. Nowadays, the price of bioactive is more expensive than the AGP. Therefore, from a practical point of view, it is beneficial to use the bioactive mixture at the lowest concentration as long as it potentially improves the performance of the chickens. So, the low-dose mixture of CSLS, CL, and MP was recommended based on similar effectiveness with the potential supplementation effect of AGP to improve the FCR without affecting the chicken performance.

A higher performance improvement is also affected by the time of additive inclusion in feed. Paul et al. (2022) reported a higher performance improvement in the early stage of life due to AGP supplementation. The immune status of broilers at an early age was low and decreased until 13 days old (Song et al. 2021). Supplementation of feed additives may benefit the immune status and performance (feed utilization efficiency) improvement at the early stages of growth. Related to those, this study also shows a higher FCR improvement potential at the early stage than the whole experiment period. Another reason may be related to the amounts of phytochemicals consumed. As feed consumption increased with age increased, the phytochemicals consumed also increased and may accumulate in the bodies of the birds. However, excess amounts of some phytochemical compounds consumption may cause some acute and chronic toxic effects (Suntar and Yakinci 2020). In this case, the supplementation of the bioactive compounds should be given during the starter period. Another possibility is the dose given in the grower diet should be less than in the starter diet.

Carcass and organs related to the immune system

Supplementation of an AGP in a broiler (fast-growing chickens) diet activates fat synthesis in the liver and then promotes abdominal fat accumulation (Li et al. 2020). However, the present study did not show a similar result. That may indicate different responses between the slow- and fast-growing chickens since the chickens used in this study were native-breed chickens with slow-growth characteristics. The highest abdominal fat level was found in low-dose treatment. High intramuscular fat levels may positively impact the tenderness and flavor of meat (Bonny et al. 2015). Meanwhile, high levels of abdominal fat negatively impact consumers' preferences (Ramiah et al. 2014). However, the abdominal fat levels in this study are lower than broilers, i.e., 2 to 5% of body weight (Leng et al. 2016; Li et al. 2020).

The liver, spleen, thymus, and bursa fabricius are organs related to the immune status of chickens. The weights of these organs (Cheng et al. 2021; Czajka and Skomorucha 2021; Osowe et al. 2023) and liver tissue architecture (Jasim and Al-Taee 2023) were observed when feeding bioactive compounds containing antioxidants to chickens. The present study showed that bursa fabricius was significantly affected by the treatments. Supplementation of CSLS + CL mixtures or medium dose of CSLS + CL + MP mixtures in the diet produced a larger bursa fabricius. Chickens in this treatment may have lower stress conditions compared to others since depletion of bursa fabricius has been known as an indication of stress conditions (Fascina et al. 2017). A report showed an increase in the weight of bursa fabricius as the effect of plant bioactive inclusion from lotus leaf extract (Cheng et al. 2021). A short-term (13 weeks) inclusion of dried pomage of black chokeberry or black currant in a laying hen diet did not affect the relative weight of bursa fabricius, but prolonged (20 weeks) inclusion increased the weight significantly (Czajka and Skomorucha 2021). Previous studies show similar results to this study that plant bioactive supplementation did not affect the liver-, spleen-, and thymus weight (Cheng et al. 2021; Czajka and Skomorucha 2021; Osowe et al. 2023). That may indicate that the bursa fabricius is the most sensitive organ in the immune status of slow-growth chickens.

Digestive tract of the chickens

The relative size of digestive organs or tracts is related to their capability to digest feed. The higher the size, the more active the organs digest the feed. Hence, the more nutrients are digested and available for metabolism. The present study showed no significant effect of AGP or the plants' bioactive supplementation on the weight of the gizzard and the length of the duodenum, jejunum, and ileum. A similar result was also reported by Hernández et al. (2004) in the digestive tract of broilers. This result indicates that the bioactive mixture may not affect chickens' performance by altering the digestive tracts.

Conclusion

The mixtures of coconut shell liquid smoke, mangosteen peel extract, and cloves leaf extract could be used as feed additives to substitute the AGP for chickens. A low-dose use of these mixtures is recommended due to a similar effect with the antibiotic growth promoters for potentially improving the FCR of the slow-growth chickens.

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Author's contribution

Tuti Haryati, Agustin Herliatika, and Arnold Parlindungan Sinurat designed the concept of this study, searched for funding, and conducted field and laboratory work. Elizabeth Wina, Maijon Purba, and Wisri Puastuti collected and analyzed the data. Tuti Haryati, Agustin Herliatika, and Arnold Parlindungan Sinurat drafted the manuscript. All authors have read and approved the final manuscript.

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