Synergistic Work of Encapsulated *Acalypha australis* L. Leaf Extracts and Chitosan in Improving Production Performance of Broilers

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Summary

The study aimed to investigate the effect of encapsulated *Acalypha australis* L. leaf extract (EALE), chitosan (CHIT) or blend of both on production parameters, internal organ weight, carcass traits and meat colour of broilers. A number of 280 broiler chicks were arranged randomly into four groups of seven replicates. The groups were control (CNTL, basal feed without additives), basal feed supplemented with 0.01% EALE (EALE), basal feed supplemented with 0.01% CHIT (CHIT), and basal feed supplemented with 0.01% EALE and 0.01% CHIT (EACH). The dietary treatment was applied from day 1 to 36. Body weight (BW), feed intake and feed conversion ratio (FCR) were weekly recorded, whereas internal organ weight, carcass traits and meat colour were determined at harvest. Compared to control, EALE, CHIT or a combination of both improved ($P < 0.05$) FCR of broilers throughout the rearing period. Unlike EALE or CHIT alone, blend of EALE and CHIT resulted in greater ($P < 0.05$) BW gain compared to control. Broilers receiving CHIT or blend of EALE and CHIT had higher ($P < 0.05$) production efficiency index (PEI) compared to control. Liver relative weight of broilers given a combination of EALE and CHIT was lower ($P < 0.05$) compared to control. The thymus was heavier ($P < 0.05$) in broiler receiving EALE than that of control. There was no significant ($P > 0.05$) effect of treatments on eviscerated carcass and commercial cuts of broilers. Contrast analysis showed that the lightness values were lower ($P < 0.05$) in CHIT and EALE than in CNTL birds. In conclusion, dietary supplementation with EALE, CHIT, or a combination of both improved FCR of broilers. In comparison to other treatments, the combination of EALE and CHIT was more effective in increasing broiler chicken growth and PEI.

Key words

broiler, carcass, chitosan, growth, herbs, synergy

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Introduction

The ban on the use of antibiotic growth promoters (AGP) has encouraged farmers to look for alternatives to AGP that are safe for consumers and ensure the sustainability of broiler chicken production. Several alternatives to AGP have been widely applied to broiler production, one of which is herbal-based additives. Herbal products are widely known to contain various active components that can be employed as anti-stress, immunomodulators and antibacterial for broiler chickens (Sapsuha et al., 2021). One of the herbal-based additives that has been applied to broiler chickens is *Acalypha australis* L. leaf extract. Several active components are present in *A. australis* leaf extract, including gallic acid which has antibacterial characteristics (Xiao et al., 2013). Alkaloids, saponins and flavonoids which can function as antioxidants and immunomodulators are also abundant in *A. australis* leaf extract (Kim et al., 2020; Kasrina and Zukmadini, 2021). However, in a recent study by Sugiharto et al. (2023), the use of *A. australis* leaf extract did not have a beneficial impact on growth performance, immune response and bacterial populations of broilers.

During storage, the light, oxygen and heat can easily cause the active components in herbal substances to be degraded (Sugiharto and Ayasan, 2023). This may lead the herbal extracts to lose their potency as the alternative to AGP for broilers (Sugiharto et al., 2023). Another classic problem that hampers the effectiveness of herbal extracts is the low bioavailability of herbal extracts for the host (Kikusato, 2021). This is related to the poor absorption of herbal extracts, low bio-transformation, fast metabolism and fast excretion (Kikusato, 2021; Sugiharto and Ayasan, 2023). Encapsulation is a process of coating the bioactive compounds using a coating material that can protect these compounds from damage (Sugiharto and Ayasan, 2023). Sugiharto (2021) states that compared to non-encapsulated herbal extracts, encapsulated herbal extracts have a better effect on the growth performance of broiler chickens. In agreement with this, Natsir et al. (2017) reported that the use of encapsulated herbs had a better effect on bacterial populations in the small intestine, intestinal morphology and serum protein profiles in broiler chickens. In this case, encapsulation can be an effective method for preventing the rapid breakdown of herbal extracts into their metabolites and increasing the absorption of herbal extracts in the intestine of broilers (Sugiharto and Ayasan, 2023).

Another substitute for AGP, which is also frequently used in broiler chicken feed, is chitosan (CHIT). CHIT is a compound derived from chitin, and is widely produced from the exoskeleton shells of arthropods (crabs and shrimp). Several studies have reported the effectiveness of CHIT in increasing growth rates, improving intestinal histomorphology, intestinal ecosystem and physiological conditions of broiler chickens (Nuengjamnong and Angkanaporn, 2018; Ayman et al., 2022). CHIT is an intriguing substance since it can increase absorption and the host's ability to absorb active compounds derived from feeds (Tiyaboonchai, 2003). In the present study, *A. australis* leaf extract will be encapsulated and combined with CHIT in the hope of getting a synergistic effect from the two ingredients. Considering their synergistic and complementary effects, the combination of encapsulated *A. australis* leaf extract (EALE) and CHIT is therefore expected to have a better effect on the growth and health of broiler chickens compared to those receiving only EALE or CHIT individually. The purpose of this study is to investigate the effect of EALE, CHIT or a combination of both on production parameters, internal organ weight, carcass traits and meat colour of broilers.

Materials and Methods

Encapsulated *A. australis* Leaf Extract Preparation

After having been harvested from the garden nearby the campus, the leaves were spread out on a tray and placed in a room away from direct sunlight. The air-drying process lasted two days, and the leaves were turned at regular intervals. After drying, the leaves were ground into a fine powder using a hammer mill. The leaf powder was then soaked in 70% ethanol (1:6, g : mL) for 72 hours at room temperature. Using a vacuum rotary evaporator, the filtrate was concentrated at a maximum temperature of 60 °C to produce the paste-like extract of *A. australis* leaves (Agusetyaningsih et al., 2022). Encapsulation of *A. australis* leaf extract was carried out based on the freeze-drying method as described by Agusetyaningsih et al. (2022). Maltodextrin was used as a coating material for encapsulation. Maltodextrin was dissolved in distilled water at a ratio of 1:3 (g:mL) before mixing with *A. australis* leaf extract. The mixture of *A. australis* leaf extract and dissolved maltodextrin (1:1.25; g: mL) was freeze-dried to produce EALE powder. The EALE was stored in the refrigerator until used in *in vivo* trials.

In vivo Study

The trial was carried out on 280 Cobb (mixed-sex) broiler chicks using a fully randomized arrangement. The chicks were weighed individually (average BW 42.97 ± 1.64 g) and then divided into four groups. Each group had seven replicates/pens with 10 chickens. The groups were (1) control (CNTL, basal feed without additives), (2) basal feed supplemented with 0.01% EALE (EALE), (3) basal feed supplemented with 0.01% CHIT (CHIT), and (4) basal feed supplemented with 0.01% EALE and 0.01% CHIT (EACH). The dietary supplementation dosage of EALE was based on Jafari et al. (2021), who used 100 or 200 $mg \cdot kg^{-1}$ of *Otostgia persica* (Burm.f.) Boiss. leaf extract, and Lan et al. (2023), who used 600 $mg \cdot kg^{-1}$ of broiler feed containing CHIT oligosaccharide. From the time of arrival until day 7, the chicks were fed commercial pre-starter feed that contained (per the feed label) 22-24% crude protein, <5% crude fiber, 5% crude fat, and 7% ash. From days 8 to 36, the chicks were given *ad libitum* starter and finisher feeds (Table 1). Throughout the trial period (days 1-36), the additives were supplemented to the feed.

The chicks were raised in a broiler house with open sides and a litter of rice husks. The experiment involved continuous usage of the lighting program. Soon after hatching, the chicks were immunized using the spraying technique against the infectious bronchitis virus (IBV) and the Newcastle disease virus (NDV). At the age of 11 days, the chicks received the Gumboro (infectious bursal disease virus/IBDV) vaccination (Medivac Gumboro B vaccine), and on the 18th day, the chickens received the NDV vaccination (Medivac ND La Sota) through drinking water. BW, feed consumption and feed conversion ratio (FCR) were measured on a weekly basis. Throughout the study period, production efficiency index (PEI) of broilers was calculated as = (daily weight gain (kg) × livability/feed conversion ratio) × 100 (Lorençon et al., 2007). At the day 36, one chick per replicate (seven chicks per treatment group) was slaughtered (according to Islamic rulings on animal slaughter), eviscerated, internal organs were collected and weighed, and the commercial cuts of chickens were determined. For meat color determination, meat samples were collected from the breast and thigh of broiler chickens, placed in the sample containers and brought to laboratory.

With Mac OS X's digital color meter set to CIE Lab (illuminant C), the color of the broiler meat was determined. In order to depict the color of the broiler meat, the values of L* (brightness), a* (redness), and b* (yellow) were employed. In fiveplicate, a color analysis was done. During the study, a standard white ceramic tile was used to calibrate the colorimeter.

Table 1. Broiler feed compositions

Ingredients (%, unless otherwise specified)	Starter (day 8-21)	Finisher (day 22-36)
Yellow maize	54.1	62.7
Palm oil	2.10	2.10
Soybean meal	40.0	31.7
DL-methionine	0.19	0.19
Bentonite	0.75	0.75
Limestone	0.75	0.75
Monocalcium phosphate	1.30	1.05
Premix[1]	0.34	0.34
Choline chloride	0.07	0.07
Salt	0.40	0.40
Nutritional compositions		
ME (kcal/kg)[2]	2,900	3,004
Crude protein, %	22.0	19.0
Crude fibre, %	5.50	5.62
Ca, %	1.04	0.96
P (available), %	0.57	0.54
Met, %	0.52	0.48
Lys, %	1.18	0.98

[1] Each kg of feed contained 1,100 mg Zn, 1,000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1,225 mg K, 1,225 mg Mg, 1,250,000 IU vit A, 250,000 IU vit D_3, 1,350 g pantothenic acid, 1,875 g vit E, 250 g vit K_3, 250 g vit B_1, 750 g vit B_2, 500 g vit B_6, 2,500 mg vit B_{12}, 5,000 g niacin, 125 g folic acid and 2,500 mg biotin

[2] ME (metabolizable energy) was calculated based on formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen − free extract] + 2.5}

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA, SPSS version 16.0). Duncan's multiple analysis was run when the treatments resulted in a significant effect. To evaluate the mean comparison between control group and the treated groups, planned contrast was used (SPSS version 16.0). A P value of <0.05 was considered a significant.

Results

Production Parameters of Broilers

During the day 1-21, feed intake was the highest ($P < 0.05$) in CNTL and the lowest in EALE chickens. Based on the planned contrast analysis, FCR of EALE and EACH was different ($P < 0.05$) from that of CNTL chickens. During the day 22-36, the FCR of chickens was higher ($P < 0.05$) in CNTL than that of treated chickens. Contrast analysis showed that at day 22-36, the BW gain was higher ($P < 0.05$) in EACH than that of CNTL broilers. During the day 1-36, dietary treatments resulted in lower ($P < 0.05$) FCR compared to control. Moreover, contrast analysis indicated that the BW gain was higher ($P < 0.05$) in EACH than that of CNTL broilers throughout the study period (Table 2).

The production efficiency index (PEI) was higher ($P < 0.05$) in EACH and CHIT than that in CNTL, but it did not vary from EALE chickens (Fig. 1).

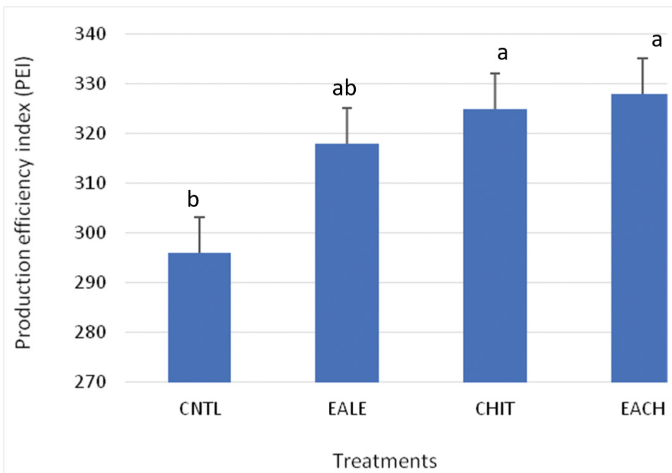

Figure 1. Production efficiency index (PEI) of broilers. CNTL: basal feed without additives, EALE: basal feed supplemented with 0.01% EALE, CHIT: basal feed supplemented with 0.01% CHIT, EACH: basal feed supplemented with 0.01% EALE and 0.01% CHIT. [a,b] Bars with various superscripts shows different means ($P < 0.05$)

Internal Organ Weight of Broilers

Analysis of variance did not show any significant impact ($P > 0.05$) of dietary treatments on the relative weight of internal organs (i.e., heart, liver, proventriculus, gizzard, pancreas, abdominal fat, duodenum, jejunum, ileum, caecum, spleen, thymus, and *bursa of Fabricius*) of broilers (Table 3). Yet, planned contrast analysis showed the different ($P < 0.05$) liver relative weight between CNTL and EACH. Also, the relative weight of thymus was greater ($P < 0.05$) in EALE compared to CNTL birds.

Table 2. Production performance of broilers

Treatments	Day 1-21			Day 22-36			Day 1-36		
	BWG (g/bird)	FI (g/bird)	FCR	BWG (g/bird)	FI (g/bird)	FCR	BWG (g/bird)	FI (g/bird)	FCR
CNTL	701	913[a]	1.23	1020	1983	1.95[a]	1754	2895	1.65[a]
EALE	693	871[c]	1.20	1075	1958	1.82[b]	1795	2828	1.57[b]
CHIT	717	901[b]	1.20	1074	1953	1.82[b]	1825	2854	1.56[b]
EACH	715	888[bc]	1.18	1118	2066	1.85[b]	1866	2954	1.58[b]
SEM	5.60	4.60	0.01	71.2	89.3	0.02	85.8	92.0	0.01
ANOVA	0.39	0.01	0.05	0.07	0.05	0.04	0.08	0.05	0.01
Contrast statements									
CNTL *vs.* EALE	0.31	0.01	0.04	0.13	0.57	0.01	0.34	0.14	0.01
CNTL *vs.* CHIT	0.64	0.27	0.07	0.15	0.50	0.01	0.11	0.36	0.01
CNTL *vs.* EACH	0.38	0.03	0.01	0.01	0.07	0.04	0.01	0.19	0.02

[a,b,c] Means within the similar row with divergent superscripts different significantly ($P < 0.05$)

CNTL: basal feed without additives, EALE: basal feed supplemented with 0.01% EALE, CHIT: basal feed supplemented with 0.01% CHIT, EACH: basal feed supplemented with 0.01% EALE and 0.01% CHIT, BWG: BW gain, FI: feed intake, FCR: feed conversion ratio, SEM: standard error of the means, ANOVA: analysis of variance

Table 3. Relative internal organ weights of broilers (%BW)

Treatments	Heart	Liver	Proventriculus	Gizzard	Pancreas	Abdominal fat	Duodenum	Jejunum	Ileum	Caecum	Spleen	Thymus	*Bursa of Fabricius*
CNTL	0.45	2.46	0.50	1.61	0.29	0.95	0.63	1.24	1.11	0.57	0.12	0.16	0.12
EALE	0.45	2.11	0.49	1.57	0.27	0.78	0.61	1.26	1.12	0.56	0.12	0.23	0.12
CHIT	0.46	2.20	0.42	1.57	0.25	0.82	0.58	1.22	0.96	0.54	0.12	0.20	0.10
EACH	0.48	2.04	0.48	1.65	0.27	1.04	0.55	1.24	0.99	0.55	0.09	0.17	0.09
SEM	0.01	0.07	0.02	0.03	0.01	0.09	0.03	0.03	0.04	0.02	0.01	0.01	0.01
ANOVA	0.92	0.12	0.51	0.83	0.79	0.75	0.71	0.97	0.44	0.97	0.55	0.12	0.65
Contrast statements													
CNTL *vs.* EALE	0.35	0.06	0.78	0.73	0.58	0.51	0.83	0.80	0.97	0.98	0.97	0.03	0.88
CNTL *vs.* CHIT	0.78	0.16	0.16	0.72	0.32	0.63	0.51	0.81	0.21	0.66	0.95	0.15	0.55
CNTL *vs.* EACH	0.50	0.03	0.64	0.65	0.59	0.75	0.29	0.99	0.33	0.79	0.24	0.65	0.34

CNTL: basal feed without additives, EALE: basal feed supplemented with 0.01% EALE, CHIT: basal feed supplemented with 0.01% CHIT, EACH: basal feed supplemented with 0.01% EALE and 0.01% CHIT, BW: body weight, EC: eviscerated carcass, SEM: standard error of the means, ANOVA: analysis of variance

Commercial Cuts and Meat Colors of Broilers

Table 4 shows the data on commercial cuts of broiler chickens. Typically, analysis of variance did not show any significant ($P > 0.05$) effect of dietary treatments on eviscerated carcass and commercial proportions of broiler chickens. However, contrast analysis showed the difference ($P < 0.05$) between CNTL and EALE in term of eviscerated carcass of broilers.

The data of broiler breast and thigh meat colours are presented in Table 5. Analysis of variance did not show any substantial effect ($P > 0.05$) of the treatments on the values of L*, a* and b* of breast and thigh of broiler chickens. However, contrast analysis showed that the L* values were higher ($P < 0.05$) in CHIT than that in CNTL birds.

Table 4. Commercial cuts of broilers

Treatments	Eviscerated carcass (%BW)	Breast (% EC)	Thigh (% EC)	Drumstick (% EC)	Wings (% EC)	Backs (% EC)
CNTL	65.8	36.1	16.0	15.9	11.2	22.0
EALE	69.3	35.5	15.0	16.0	10.8	23.0
CHIT	68.0	35.9	15.8	15.6	11.9	21.1
EACH	65.5	36.0	16.2	15.2	11.0	21.3
SEM	0.63	0.34	0.29	0.22	0.15	0.36
ANOVA	0.09	0.94	0.38	0.63	0.05	0.27
Contrast statements						
CNTL *vs.* EALE	0.04	0.54	0.19	0.86	0.31	0.33
CNTL *vs.* CHIT	0.18	0.78	0.82	0.69	0.08	0.37
CNTL *vs.* EACH	0.87	0.85	0.75	0.31	0.68	0.52

CNTL: basal feed without additives, EALE: basal feed supplemented with 0.01% EALE, CHIT: basal feed supplemented with 0.01% CHIT, EACH: basal feed supplemented with 0.01% EALE and 0.01% CHIT, BW: body weight, EC: eviscerated carcass, SEM: standard error of the means, ANOVA: analysis of variance

Table 5. Colour of broiler meats

Treatments	Breast meats			Thigh meats		
	L*	a*	b*	L*	a*	b*
CNTL	38.9	4.36	10.6	41.9	5.27	9.25
EALE	43.0	4.49	13.0	43.5	4.22	11.4
CHIT	43.4	2.49	12.2	43.2	5.69	12.0
EACH	42.5	4.36	12.7	46.4	6.83	11.1
SEM	0.78	0.78	0.59	1.13	0.89	0.97
ANOVA	0.15	0.79	0.49	0.57	0.80	0.79
Contrast statements						
CNTL *vs.* EALE	0.06	0.96	0.36	0.62	0.69	0.45
CNTL *vs.* CHIT	0.04	0.42	0.17	0.69	0.88	0.35
CNTL *vs.* EACH	0.10	0.96	0.22	0.18	0.56	0.52

CNTL: basal feed without additives, EALE: basal feed supplemented with 0.01% EALE, CHIT: basal feed supplemented with 0.01% CHIT, EACH: basal feed supplemented with 0.01% EALE and 0.01% CHIT, L*: lightness value, a*: redness value, b*: yellowness value, SEM: standard error of the means, ANOVA: analysis of variance

Discussion

Supplementation of EALE and CHIT in feed was expected to promote the growth performance of broiler chickens as formerly performed by the AGP. The results in this study showed that when compared with control, supplementation of EALE, CHIT or a combination of both had a substantial impact on reducing the FCR of broiler chickens during the 36 days of rearing. In line with our current finding, Agusetyaningsih et al. (2022) previously reported that encapsulated *Cosmos caudatus* leaf extract improved broiler chicken feed efficiency. Some bioactive compounds in the leaf extract, such as phenols, ascorbic acid, and anthocyanins, were likely to promote broiler growth performance by improving intestinal (digestive) functions, physiological conditions, antioxidant status, and immune responses (Sugiharto and Ayasan, 2023). In regard to CHIT, Nuengjamnong et al. (2018) noticed that broilers' FCR was improved by dietary supplementation of CHIT. In such case, the latter author proposed that CHIT could improve intestinal morphology, microbial populations, and protein utilization, resulting in increased growth rate and improved feed conversion in broilers. An interesting finding was observed in the BW gain of broilers, where in rearing for 36 days chickens given a combination of EALE and CHIT showed a higher BW gain compared to control chickens. Such increase in the BW was not observed in chickens receiving EALE or CHIT alone. Taken all these conditions together, this fact may indicate a synergistic effect between EALE and CHIT in improving the growth of broilers. In accordance with this, the combination of phytobiotic (Artichoke extract) and probiotic (Protexin) had a synergistic effect on improving the growth rate, antioxidant capacity, lipid profile, and liver function of broiler chickens (Gholami-Ahangaran et al., 2022). In our case, it was most likely that CHIT increased the capability of chickens in absorbing and utilizing the active compounds contained in *A. australis* leaf extract (Tiyaboonchai, 2003), hence improving the physiological conditions and growth of broiler chickens. The planned contrast analysis further showed that the BW gains appeared different between control chickens and chickens receiving a combination of EALE and CHIT during the grower period (day 22-36), but such difference was not found between control chickens and chickens given EALE or CHIT alone. In contrast to the grower period, differences in BW gain among the treatment groups were not observed during the starter period (day 1-21). Typically, the grower period is characterized by stressful conditions for the chickens due to overcrowding (related to the increased BW) condition in the pen/cage. The improvement in growth performance of chickens given the blends of EALE and CHIT during the grower period indicated that the antioxidants contained in *A. australis* leaf extract could be maximally utilized by chickens in dealing with stress. In this regard, CHIT may improve the chickens' ability in absorbing active compounds derived from *A. australis* leaf (Tiyaboonchai, 2003).

It was apparent that broilers receiving CHIT alone or blends of EALE and CHIT had a higher PEI compared to control broilers. Unlike the CHIT and EACH groups, the PEI did not differ between the chickens receiving EALE and control chickens. The exact reason for the latter condition is still unclear. In most cases, long-term use of phytogenic materials may exert a prooxidant effect (Rajashekar, 2023), resulting in compromised physiological condition and health, as well as an increase in broiler mortality. Besides daily weight gain and FCR, livability influences PEI according to the formula described by Lorençon et al. (2007). Thus, long-term use of EALE, which has the potential to cause prooxidant effects, appeared to reduce livability (CNTL: 100%, EALE: 97.33%, CHIT: 100% and EACH: 100%), and hence lowering the PEI value of broiler chickens in this current study. After being combined with CHIT, the EALE resulted in a higher PEI. This may suggest the complementary effect of CHIT on EALE in improving the PEI of broiler chickens. In this case, it was most likely that CHIT could inhibit prooxidant activity (due to the long-term use of EALE) and the production of reactive oxygen species (Ivanova and Yaneva, 2020). As a result, the excessive increased prooxidant activity in the broiler body could be avoided.

It was observed in the current study that the liver relative weight of broilers given a combination of EALE and CHIT was lower compared to control chickens. The exact explanation for such condition has not yet been known, but the synergistic work of herbal ingredients (Sugiharto, 2021) and CHIT (Ayman et al., 2022) in reducing fatty acid biosynthesis and fat deposition in the liver was very likely to cause the relative weight of chicken liver to be lower. The reducing effect of phytobiotics on fatty acid biosynthesis and fat deposition has also been demonstrated in rabbits, in which dietary supplementation with blends of turmeric, garlic, ginger and clove powder reduced liver relative weight (Okanlawon et al., 2024). Likewise, Wang et al. (2003) found that CHIT supplementation decreased the liver weight of broiler chickens. However, the lowering effect of phytobiotics and CHIT on lipid biosynthesis should be interpreted with caution, as neither treatment had a significant effect on broiler abdominal fat content. There are several factors that influence the abdominal fat content of broilers, one of which is the dietary energy level (Fouad and El-Senousey, 2014). Rosa et al. (2007) found that when broiler chickens were fed diets with metabolizable energy (ME) of 2,950 and 3,200 kcal/kg during the finisher phase, their abdominal fat content was 1.9% BW and 2.6% BW, respectively. In the current study, the abdominal fat content of broilers fed a diet containing ME at 3,004 kcal/kg (during the finisher phase) ranged from 0.78 to 1.04% BW across treatment groups. Such abdominal fat content was significantly lower than that reported by Rosa et al. (2007). Given that abdominal fat can serve as a stored energy source, it is especially important during periods of high activity or low dietary intake (Fouad and El-Senousey, 2014). In this regard, it seemed unnecessary for phytobiotics and CHIT to reduce the abdominal fat content of broilers during this current study because their abdominal fat content was already lower than normal. In this work, the relative weight of the thymus, which is the primary lymphoid organ, was higher in EALE compared with control group. In line with our findings, feeding a polyherbal mixture increased immune organ indexes, particularly the thymus in broiler chickens (Liu et al., 2023). In such case, Sugiharto (2021) suggested that various active ingredients such as triterpenoids, steroids, saponins, alkaloids, terpenoids, quinones, simple phenolic compounds, polysaccharides, peptides, glycoproteins and nucleotides contained in herbal products were very beneficial in improving the development of immune tissues and organs of broiler chickens.

In this study, there was no significant effect of treatments on eviscerated carcass and commercial cuts of broilers. However, on the basis of planned contrast analysis, eviscerated carcass in chickens receiving EALE was greater when compared to that of control. In line with our findings, Sangilimadan et al. (2020) reported an increase in broiler eviscerated carcass following dietary supplementation with ginger powder, garlic paste, or turmeric powder. Typically, increased eviscerated carcass results from muscle growth and minimal fat deposition in the chicken body. Indeed, phytobiotics can promote muscle growth (Kikusato, 2021) and reduce fat (Sugiharto, 2021), resulting in greater eviscerated broiler carcasses.

According to the analysis of variance, dietary interventions had no appreciable impact on the colours of the broiler breast and thigh meats in the current study. The broiler breast and thigh meats can typically be divided into three categories based on their lightness values, i.e., ($L^* < 56$), normal ($56 \leq L^* \leq 62$) and pale ($L^* > 62$) (Lee et al., 2022). The broiler breast and thigh meats from our investigation can be categorized as dark meats in this regard. The exact cause of the broilers' dark meats in the current study remains unknown, but a number of factors, including diets and post-slaughter processing techniques, may affect the colour of broiler meat (Wideman et al., 2016). Contrast analysis revealed that dietary interventions, particularly chitosan, enhanced the lightness values of breast meats. This result was in contrast to Lan et al. (2023) who found that broiler meats' lightness values decreased with chitosan. Given that the broiler meats in this study were categorized as dark meats, the increasing lightness values of chitosan on the broiler breast meats in this study may actually have a positive effect, which is elevating the colour into the normal range.

Conclusions

Dietary supplementation with EALE, CHIT, or a combination of both improved broiler chicken FCR. In comparison to other dietary treatments, the combination of EALE and CHIT was more effective in increasing broiler chicken growth and PEI. The use of EALE in feed increased broiler chickens' immune organ index (as indicated by increased thymus weight) and carcass yield. Overall, a combination of EALE and CHIT is preferred to maximize broiler chicken growth performance.

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Ethical Approval

The experiment was approved by the Faculty of Animal and Agricultural Sciences' Animal Ethical Committee, Universitas Diponegoro, Semarang, Central Java, Indonesia under approval number 59-07a/A-16/KEP-FPP.

CRediT authorship contribution statement

Yuki Zulpa: Performed experiment, did laboratory analysis, analyzed the data and drafted the manuscript. **Ikania Agusetyaningsih, Endang Widiastuti, Hanny Indrat Wahyuni, Turrini Yudiarti, Tri Agus Sartono:** Performed experiment, did laboratory analysis, analyzed the data and revised the manuscript. **Sugiharto Sugiharto:** Conceptualized and supervised the experiment, obtained the research grant and revised the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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