J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian
kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
49(4):286-296, December 2024
DOI: 10.14710/jitaa.49.4. 286-296

Effect of encapsulated Tahongai (*Kleinhovia hospita* l.) leaf extract on growth performance, intestinal condition and antioxidative status of broilers raised in high stocking density pens

R. Yusuf[1,2]*, V. D. Y. B. Ismadi[1], S. Kismiati[1] and S. Sugiharto[1]
[1]*Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java, Indonesia*
[2]*Department of Animal Science, Faculty of Agriculture, Mulawarman University, Samarinda, East Borneo, Indonesia*
**Corresponding Email: E-mail: roosenayusuf@gmail.com*

Received August 25, 2024; Accepted October 21, 2024

ABSTRACT

The objective of the present study was to investigate the effect of *Kleinhovia hospita* L. extract (KE) on growth performance, intestinal condition and antioxidative status of broilers raised in high stocking density pens. A total of 370-day-old broiler chicks were randomly grouped into five groups with five replicates. The groups were T0 (chicks raised in normal density, 10 birds/m^2; as a negative control), KE0 (chicks raised in high density, 16 birds/m^2, without KE supplementation; as a positive control), KE0.25, KE0.5 and KE1 (chicks raised in high density with KE supplementation of 2.5, 5 and 10 g/kg, respectively). Based on the completely randomized design, the data were treated. Results showed that KE1 chicks had the highest ($p<0.05$) body weight (BW) at day 21 and 28. The T0, KE0 and KE2.5 chicks consumed more ($p<0.05$) feed than the other treatment groups. The KE0.25, KE0.5 and KE1 showed lower ($p<0.05$) FCR than the KE0 group. The KE0 chicks showed lower ($p<0.05$) carcass yield than the other groups. The KE0 had the highest ($p<0.05$) heart relative weight of all groups. The KE1 had the highest ($p<0.05$) small intestinal weight, cecum, colon and abdominal fat of any treatment group. Among the groups, crypt depth of the duodenum in KE0 was the lowest ($p<0.05$). There was no substantial effect of the treatments on the counts of coliform and lactic acid bacteria in the ileum of broilers. The superoxide dismutase (SOD) levels in KE0.5 and KE1 were higher ($p<0.05$) than those in T0, KE0 and KE0.25 groups. In conclusion, stocking in high density pens negatively affected the carcass yield of broiler chickens. Dietary KE supplementation was beneficial in improving FCR and antioxidant status of broiler chickens.
Keywords: Antioxidant, Carcass, Growth, Intestine, Phytogenic, Stress

INTRODUCTION

Poultry production is crucial for supplying human needs with high-quality protein and other micronutrients, as well as for promoting economic expansion and increasing farmer earnings (Nkukwana, 2018; Oni *et al*., 2024). Over the past ten years, the amount of chicken meat consumed in emerging nations has consistently outpaced its production (Enahoro *et al*., 2021). Hence, broiler chicken production needs to be increased to meet consumer needs.

High stocking density (HSD) rearing has commonly been applied in broiler production to optimize the space area of broiler house in order to meet market demand and achieve high efficiency (Sugiharto, 2022). In tropical countries, a maximum stocking density of 16 chickens/m^2 is allowed as long as appropriate rearing management is implemented. In broiler production, the HSD is typically determined by the weight of birds per square meter as well as the number of chicks per square meter (Sapsuha *et al*., 2021). Apart from the efficiency reason, HSD can have detrimental effects on chickens, such as insufficient space for living and feeding, production of heat, obstruction of airflow, decline in health and welfare, reduced intake of feed and water, and lowered feed efficiency as a consequence of stress and poor air quality (Feddes *et al*., 2002). Indeed, stress may cause an excess of reactive oxygen species (ROS), which would inhibit the activities of antioxidant enzymes. This latter circumstance may result in gut damage, high broiler mortality, declining meat quality and poor growth performance (Estévez, 2015).

Antibiotic growth promoters (AGPs) at a subtherapeutic dosage are the most often used additives that might improve intestinal ecology and functions, feed conversion, growth rate and chicken's health (Gadde *et al*., 2017; Lourenco *et al*., 2019). Previous studies have highlighted concerns regarding the use of antibiotics to boost broiler performance because of the resistance exhibited by human microbial pathogens, which has eventually led to the prohibition of AGP use in animal feeding (Gheisar and Kim, 2018). Yet, this prohibition results in declining chicken performance and an increase in the frequency of pathogenic illnesses, which increases production costs and financial loss (Cardinal *et al*., 2019). On this background, it is necessary to search alternatives to AGPs in order to maintain the productivity of poultry while keeping production cost-efficient.

The utilization of phytogenic feed additives (PFA) has garnered attention in recent times owing to their perceived naturalness, affordability, safety, lack of residue, non-toxicity, and reasonable effectiveness (Gheisar *et al*., 2015; Yitbarek, 2015; Abudabos *et al*., 2018). The botanical products known as PFA are derived from herbs, spices, essential oils, or oleoresins. Common uses of PFA include whole plants and the constituent parts of herbs and spices. The biological function of essential oils, which are secondary metabolites derived from odoriferous plants, is generally higher than that of their raw materials (Yitbarek, 2015). With regard to poultry, the bioactive components of PFA have been demonstrated in several studies to have the potential to improve chicken performance and reduce stress (Sugiharto *et al*., 2021; Sapsuha *et al*., 2021).

Kleinhovia hospita Linn (*K. hospita*), known as Paliasa or Tahongai in Indonesia, is widely utilized for the therapeutic uses. The phytochemicals component from this plant includes tannins, flavonoids, and saponins (Hanum and Maesen, 1997). Furthermore, the natural compounds found in *K. hospita* leaves, cycloartane triterpenoid alkaloids, are rich in flavonoid glycosides that have been shown to be powerful anti-inflammatory agents (Soromouo *et al*., 2012) and effective hepatoprotective agents (Kleinhospitines A-D). This compound act as a protection for liver cells from H_2O_2 oxidative stress (Gan *et al*., 2009; Zhou *et al*, 2013). Likewise, previous research demonstrated that the majority of the bioactive compounds derived from the *K. hospita* plant had effective antioxidant capabilities properties (Hanum and Maesen, 1997; Arung *et al*., 2012; Soromouo *et al*., 2012).

At present, an investigation on the use of *K. hospita* L. extract to increase the broilers performance are still very limited. The objective of the present study was therefore to investigate the effect of *K. hospita* L. extract (KE) on growth performance, intestinal condition and antioxidative status of broilers raised at high stocking density pens.

MATERIALS AND METHODS

Ethical Clearance

The Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 60-02/A-05/KEP-FPP) gave approval for the current *in vivo* study.

Preparation of Phytogenic Feed Additives

The *K. hospita* L. fresh leaves were collected and prepared using the previous specified procedure (Solihah *et al*., 2019). The samples were gained from Samarinda, East Borneo, Indonesia. The fresh leaves were cleaned up and dried naturally in shade for around five days to achieve a uniform weight, then ground into a fine powder.

Table 1. Ingredients and Nutrient Composition of Broiler Basal Diets in Finisher Period

Items	Grower Feed (15-35 days)	
Feed ingredients		
Corn	58.54	
Palm oil	2.96	
Soybean meal	34.7	
DL-methionine	0.19	
Bentonite	0.75	
Limestone	0.75	
Calcium monophosphate	1.30	
Premix[1]	0.34	
Chlorine chloride	0.07	
Salt	0.40	
Nutritional Compositions	Calculated	Analysed
ME[2] (kcal/kg)	3,000	3,240
Crude protein	20.0	20.7
Crude fibre	5.51	4.91
Ether extract	4.41	3.83
Ca	1.02	0.74
P (available)	0.58	0.54

[1]Vitamins and mineral composition per kg premix: 50.000 IU Vit. (D3); 0.5 mg Vit. (B12); 32.5% of Calcium (Ca); 1% of Phosphor (P); 6 g of Iron (Fe); 4 g Manganese (Mn); 0.075 g Iodine (I); 0.3 g Copper (Cu); 3.75 g of Zinc (Zn).
[2]ME (metabolizable energy) was calculated according to formula: 40.81 {0.87 (crude protein + 2.25 crude fat + nitrogen-free extract) + 2.5}

The gradual maceration method was applied in extraction process. One kilogram of dried leaves was soaked in 5 L of 96% ethanol for 48 h at room temperature, and stirred twice a day, then filtered using Whatman No. 2 filter paper. Extract was evaporated using a rotary evaporator. The greenish brown oil was obtained after complete removal and prepared to dry in freeze dryer. Maltodextrin was used to create coatings for microencapsulated processes. The coating process was started with 15% maltodextrin that was dissolved in distilled water to create a suspension. The *K. hospita* L. extract (KE) was added to the maltodextrin solution at a ratio of 1: 5. The suspension was microencapsulated using freeze dryer (Menezes *et al*., 2018). The drying powder was weighed and kept at room temperature until use.

Animals, Diets and Experimental Design

During the 35 days research, 370 Lohmann day-old broiler chicks (mixed-sex) with an initial average body weight of 43.49±1.52 g were used. The chicks were randomly grouped into five treatments from day 1 to 35 days, and each treatment had 5 replicates. The chickens were raised at open-sided broiler houses with floor pen layered with rice husk as a litter. The temperature ranged between 26 and 33°C, whereas humidity was about 70% throughout the study period. The chicks were grouped into T0 (negative control with normal density, 10 birds/m^2), KE0 (positive control with high density, 16 birds/m^2 without KE supplementation), KE2.5, KE5 and KE10 with a high density of 16 birds/m^2 and KE supplementation at 2.5, 5, and 10 g/kg, respectively.

Broilers were fed with commercial starter feed (1 to 14 d of age), and formulated finisher diets (15 to 35 d of age). The commercial starter feed contained crude protein of minimum 20%, crude fibre of maximum 5%, Calcium of 0.8-1.1%, and Phosphorus of 0.5% (based on feed label). The formulated grower feed is presented in Table 1. The KE was supplemented to feed since the day 15 as much as 2.5, 5, and 10 g/kg for KE2.5, KE5 and KE10, respectively. The groups of T0 and KE0 were given basal diet without KE. There was no antifungal, antibacterial, antiprotozoal, or enzymes in the formulated grower feed. For 35 days of experiment, feed and water were provided *ad libitum*. The birds were vaccinated with Newcastle Disease (ND) using spray technique following hatching. On day 12, the commercial Gumboro vaccine was given to each chicken (through drinking water), and on day 18 the ND vaccination was conducted *via* drinking water.

Data Collection and Analysis

The body weight (BW), weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) of all chickens were recorded weekly. One chick from each replication was randomly taken, slaughtered, and de-feathered on day 35 to obtain the weight of carcass. The internal organs were removed after evisceration and weighed. In order to avoid gender bias, one male chick representing the average body weight of each experimental unit was selected at the end of the experiment to have blood drawn from the brachial vein. Three mL of blood were placed in tube (without anticoagulants) to produce serum. The same chicks used for the blood sample were subsequently slaughtered. The chicken's intestines were removed right away after it was slaughtered. The segment of duodenum (roughly 2 cm) was obtained and immersed in 10% buffered formalin (Leica Biosystems Richmond, Inc., Richmond, USA) for the duodenal morphology measurement. The digesta were taken from ileum and placed in sterile sample pot for selected bacterial population counts.

The concentration of malondialdehyde (MDA) in serum was determined by reacting MDA with thiobarbituric acid (TBA) (Sigma-Aldrich, St. Louis, USA). The MDA concentration was measured spectrophotometrically at 532 nm. The serum superoxide dismutase (SOD) concentration was determined using SOD kits (Sigma-Aldrich, St. Louis, USA) and an indirect assay method based on xanthine oxidase. The SOD concentration in serum was measured using a spectrophotometer (absorbance at 550 nm). Haematoxylin and eosin-dyed 5 µm sections of the duodenum were used to histologically examine the duodenal morphology. Using an optical microscope connected to a digital camera (Leica Microsystems GmbH, Germany), the height of villus and depth of crypt were measured for each segment. For each sample, the mean values of crypt depth and villus height were calculated using five measurements. The total plate count procedures were used to determine the bacterial counts in the ileal and caecal digesta. After an aerobic 24-hour incubation at 38°C, coliforms were counted as red colour on MacConkey agar (Merck KGaA, Germany). Lactic acid bacteria (LAB) were counted on de Man, Rogosa, and Sharpe (MRS; Merck KGaA) agar following a 48-hour anaerobic incubation period at 38°C.

Statistical Analysis

This experiment was carried out based on a completely randomized design. The SPSS version 26.0 was used to analyse the collected data. Following ANOVA test, the differentiation

Table 2. Effect of KE on Growth Performance of Broilers Raised in High Stocking Density Pens

Variables	Treatment Groups (Means±SE)					p value
	T0	KE0	KE2.5	KE5	KE10	
Body weight (g/bird)						
Day 14	485±6.88	485±6.09	485±4.10	483±4.36	485±4.72	0.99
Day 21	851±1.10^{d}	849±1.41^{d}	855±1.21^{c}	862±1.00^{b}	867±0.91^{a}	<0.01
Day 28	1364±7.81	1361±4.62	1366±2.76	1374±4.79	1380±5.99	0.05
Day 35	1773±13.9	1772±8.07	1782±7.48	1785±10.6	1793±9.91	0.59
Cumulative feed intake (g/bird)						
Day 21	1057±6.27^{a}	1060±3.57^{a}	1051±4.32^{ab}	1040±6.63^{b}	1040±2.57^{b}	0.02
Day 28	2151±9.04^{a}	2153±7.65^{a}	2128±7.62^{a}	2096±12.8^{b}	2080±11.4^{b}	<0.01
Day 35	3344±12.9^{a}	3364±16.5^{a}	3343±8.05^{a}	3295±18.0^{b}	3271±15.6^{b}	<0.01
Feed conversion ratio (FCR)						
Day 21	1.38±0.01^{ab}	1.38±0.01^{a}	1.36±0.01^{b}	1.34±0.01^{c}	1.33±0.01^{c}	<0.01
Day 28	1.58±0.00^{ab}	1.58±0.01^{a}	1.56±0.01^{b}	1.53±0.01^{c}	1.52±0.01^{c}	<0.01
Day 35	1.89±0.01^{ab}	1.91±0.01^{a}	1.88±0.00^{b}	1.85±0.01^{c}	1.82±0.01^{c}	<0.01

T0: chicken raised in normal density pens (10 birds/m^2), KE0: chickens raised in high density pens (16 birds/m^2) without KE supplementation, KE2.5, KE5 and KE10: chickens raised in high density pens and provided respectively with KE for 2.5, 5, and 10 g/kg of feed, SE: standard error of the means

$^{a, b,c,d}$ Distinct superscripted letters in the same row indicate a noticeably different

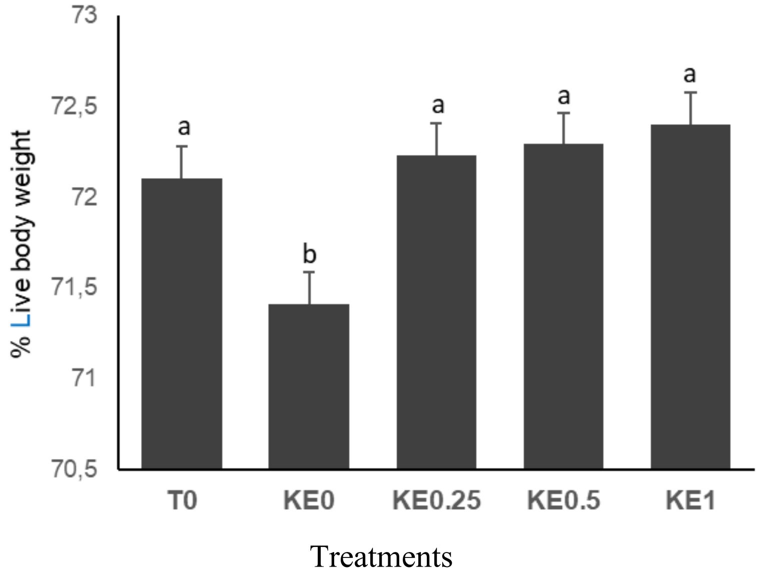

Figure 1. Carcass yield of broiler raised in high stocking density pens.
(T0: chicken raised in normal density pens (10 birds/m^2), KE0: chickens raised in high density pens (16 birds/m^2) without KE supplementation, KE2.5, KE5 and KE10: chickens raised in high density pens and provided respectively with KE for 2.5, 5, and 10 g/kg of feed)

among groups were tested by the Least Significant Difference (LSD) test, with p<0.05 as the significant level.

RESULTS AND DISCUSSION

Performance of Broilers

During the course of the study, all birds remained healthy. The data on dietary KE supplementation affected BW, WG, FI, and FCR are shown in Table 2. The BW varied considerably by the end of day 21. The KE10 group had the highest (p<0.05) BW at the end of day 21 and 28. At day 35, there was no significant difference in BW among the groups of chickens. During the study period, feed intake was significantly different among treatment groups, in which T0, KE0 and KE2.5 chicks consumed more (p<0.05) feed than the other treatment groups (KE5 and KE10). With regard to FCR, the groups of KE2.5, KE5 and KE10 showed lower (p<0.05) FCR than the KE0 group during the study period. Yet, the FCR of KE2.5 did not significantly differ from T0 group. The carcass yields of broilers chickens are presented in Figure 1. It was apparent that KE0 chicks showed lower (p<0.05) carcass yield as compared to the other groups of chicks.

It was possible in this present study that the chickens raised in high density pens underwent less stress, resulting in the negligible differences in body weight and FCR between T0 and KE0 in the current study. In KE0 group, the average bird's total body weight per square meter was approximately 28,344 g, or less than 33 kg/m^2. According to Sugiharto (2022), only broilers housed in pens with a total body weight of more than 33 kg/m^2 will suffer from high density stress. In term of FCR, the birds raised in high density pens and received KE showed better FCR than those raised in high density pens receiving no additive. The latter circumstances indicated better feed efficiency in chicks receiving KE. Other studies conducted by Richards and Proszkowiec (2007), Sapsuha *et al*. (2021) and Cho *et al*. (2023) supported our findings, showing that phytogenic supplementation improved feed efficiency and nutrient digestibility of broilers raised in high stocking density pens. The abundance of bioactive compounds found in phytogenic materials is well known to improve intestinal ecology and functions, which in turn improves digestion and nutrient utilization (Cho *et al*., 2023). With regard particularly to KE, Dey *et al*. (2017) confirmed that the terpenoid present in *K. hospita* L. has the ability to act as an antibacterial agent that is essential in maintaining the

Table 3. Effect of KE on Internal Organ Relative Weight of Broilers Raised in High Stocking Density Pens

Variables (g/100 g BW)	Treatment Groups (Means±SE)					p value
	T0	KE0	KE2.5	KE5	KE10	
Heart	0.45±0.00[d]	0.48±0.00[a]	0.47±0.00[b]	0.46±0.00[c]	0.45±0.00[d]	<0.01
Liver	1.77±0.04	1.65±0.10	1.80±0.12	1.53±0.11	1.69±0.11	0.35
Gizzard	1.49±0.05	1.67±0.09	1.67±0.10	1.54±0.07	1.63±0.08	0.37
Proventriculus	0.47±0.01	0.51±0.04	0.51±0.03	0.51±0.02	0.54±0.03	0.53
Pancreas	0.17±0.03	0.22±0.02	0.24±0.02	0.18±0.02	0.22±0.04	0.36
Abdominal fat	1.95±0.02[d]	1.98±0.02[cd]	2.06±0.05[bc]	2.12±0.03[b]	2.22±0.04[a]	<0.01
Spleen	0.06±0.01	0.07±0.01	0.08±0.01	0.08±0.01	0.07±0.01	0.27
Thymus	0.14±0.04	0.11±0.03	0.13±0.04	0.11±0.02	0.10±0.03	0.91
BF	0.10±0.01	0.12±0.02	0.16±0.02	0.10±0.02	0.10±0.02	0.12
Duodenum	1.37±0.04[e]	1.69±0.01[c]	1.78±0.02[b]	1.52±0.02[d]	1.86±0.01[a]	<0.01
Jejunum	0.64±0.02[e]	0.78±0.02[c]	0.85±0.01[b]	0.72±0.02[d]	0.94±0.02[a]	<0.01
Ileum	3.64±0.02[e]	4.09±0.05[c]	4.21±0.01[b]	3.77±0.04[d]	4.35±0.04[a]	<0.01
Cecum	1.03±0.01[e]	1.21±0.01[c]	1.26±0.01[b]	1.12±0.01[d]	1.35±0.00[a]	<0.01
Colon	0.83±0.01[d]	0.91±0.02[c]	0.93±0.01[b]	0.87±0.01[c]	0.96±0.01[a]	<0.01

BW: body weight, BF: *Bursa of Fabricius*, T0: chicken raised in normal density pens (10 birds/m^2), KE0: chickens raised in high density pens (16 birds/m^2) without KE supplementation, KE2.5, KE5 and KE10: chickens raised in high density pens and provided respectively with KE for 2.5, 5, and 10 g/kg of feed, SE: standard error of the means

[a, b,c,d] Distinct superscripted letters in the same row indicate a noticeably different

intestinal bacterial balance during stress. Other than antibacterial properties, the bioactive compounds within KE exhibit hepatoprotective, immunomodulatory, and anti-inflammatory properties (Hafeez *et al*., 2016; Dey *et al*., 2017). Moreover, antioxidant compounds such as flavonoids found in KE have the potential to mitigate the deleterious effects of free radicals, which may disrupt cell metabolism and living cells (Dey *et al*., 2019). Additionally, these compounds can offer substantial protection against oxidative stress (Diniyah and Lee, 2020). All these circumstances eventually lead to better physiological conditions, which would then improve nutrient allocation and utilization as well as broiler chicken growth performance. The KE5 and KE10 birds consumed less feed during the study than the other groups of chickens. Indeed, the FCR was better in the KE5 and KE10 chickens as their body weights were the same as those of the other chickens. The reason behind the KE5 and KE10 groups' lower feed consumption in comparison to other groups is uncertain. However, given the potent taste, strong odour, and bitter taste of phytogenic compounds, it is highly likely that feed palatability may be impacted by phytogenic supplementation (Amad *et al*., 2011; Zhai *et al*., 2018). In agreement, Mehala *et al*. (2021) observed a decline in broiler feed consumption when fed *panchagavya* and phytogenic feed additives. They went on to confirm that the bitterness of the phytogenic compounds added to the broiler diets was probably the cause of the birds' decreased feed intake.

Earlier study confirmed that raising broiler chickens in high stocking density pens resulted in lower carcass weight (Nasr *et al*., 2021). In line with this study, our current finding also showed that broilers (receiving no additive) stocked at high density pens had lower carcass yield as compared to the broilers raised at normal density pens. Interestingly, the KE supplementation was attributed to the enhanced carcass yield of broilers raised at high density pens in the present study. In agreement to our findings, Puvača *et al.* (2016) and Toson *et al.* (2023) noted that the administration of phytogenic extract increased carcass yield of broilers. They further confirmed that phytogenic components improved nutrient digestion and absorption, thus promoting muscle deposition and increasing carcass yield in broilers.

Internal Organ Relative Weights of Broilers

Table 3 shows the impact of KE supplementation on the weight of certain internal organs of broilers. Weights of immune organs (spleen, thymus, *bursa of Fabricius*) and internal organs (liver, gizzard, proventriculus, and pancreas) did not differ (p>0.05) among treatment groups. The KE0 had the highest (p<0.05) heart relative weight of all the treatment groups. Moreover,

Table 4. Effect of KE on Duodenum Morphology and Ileum Bacterial Counts of Broilers Raised in High Stocking Density Pens

Variables	Treatment Groups (Means±SE)					p value
	T0	KE0	KE2.5	KE5	KE10	
VH and CD of duodenum						
VH (µm)	1163±68.1	1165±114	1172±54.5	1118±69.9	1173±70.7	0.99
CD (µm)	232±13.5^{ab}	201±12.8^{b}	257±12.4^{a}	258±14.8^{a}	239.6±12.2^{ab}	0.04
VH:CD ratio	5.01±0.60	5.81±0.93	4.57±0.19	4.34±0.44	4.89±0.25	0.31
Bacterial counts of ileum (log cfu/g)						
Coliform	3.25±1.43	4.97±1.18	3.16±0.73	3.41±0.85	2.96±1.14	0.71
LAB	4.17±1.29	2.98±0.50	5.21±1.36	5.08±1.65	3.80±1.03	0.69

VH: villus height, CD: crypt depth, LAB: lactic acid bacteria, T0: chicken raised in normal density pens (10 birds/m^2), KE0: chickens raised in high density pens (16 birds/m^2) without KE supplementation, KE2.5, KE5 and KE10: chickens raised in high density pens and provided respectively with KE for 2.5, 5, and 10 g/kg of feed, SE: standard error of the means
^{a,b} Distinct superscripted letters in the same row indicate a noticeably different

Table 5. Effect of KE on Antioxidant Status of Broilers Raised in High Stocking Density Pens

Variables	Treatment Groups (Means±SE)					p value
	T0	KE0	KE2.5	KE5	KE10	
MDA (nmol/mL)	12.5±1.58	15.5±1.96	15.9±2.11	11.9±0.94	14.6±0.98	0.33
SOD (U/mL)	7.52±0.87^{b}	8.05±0.79^{b}	8.75±1.17^{b}	11.8±0.91^{a}	12.1±0.26^{a}	0.002

MDA: malondialdehyde, SOD: superoxide dismutase, T0: chicken raised in normal density pens (10 birds/m^2), KE0: chickens raised in high density pens (16 birds/m^2) without KE supplementation, KE2.5, KE5 and KE10: chickens raised in high density pens and provided respectively with KE for 2.5, 5, and 10 g/kg of feed, SE: standard error of the means
$^{a, b}$ Distinct superscripted letters in the same row indicate a noticeably different

KE10 had the highest ($p<0.05$) small intestinal weight, cecum, colon and abdominal fat of any treatment group.

Present results showed that rearing broiler chickens in high stocking density pens resulted in greater relative heart weight as compared to those reared in normal density pens. This finding was in accordance with Sahin *et al*. (2002) reporting an increased heart relative weight of broilers with heat stress. It was very possible that stress may increase heart rate in pumping the blood for the thermoregulatory process (Gogoi *et al*., 2021). Such increased heart rate may therefore cause cardiac muscle hypertrophy. Interesting results were observed in the present study, in which KE supplementation resulted in decreased heart relative weight of broilers. The antioxidant properties of KE seemed to ameliorate the stress effects in broilers due to high density rearing in this present study. In line with our report, Dalólio *et al*. (2021) revealed that chromium-methionine (rich in antioxidants) supplementation could alleviate heat stress and thereby decreased relative weight of heart of broiler chickens.

Previous study revealed that broilers raised in high stocking density pens showed decreased the relative weight of duodenum, jejunum, ileum caecum, and colon weights (Thema *et al*., 2022). In this regard, stress induced by high density pens compromised the development of intestine of broilers. Unlike the above-mentioned study, our present study showed that the broilers raised in high density pens had greater relative weight of duodenum, jejunum, ileum caecum, and colon. To date, the definite explanation for the latter discrepancies remains unsolved. With regard particularly to the KE effect, the KE supplementation (especially for KE10) was attributed to the greater relative weights of small intestines, cecum and colon of broilers as compared to those of other birds. Ahsan *et al*. (2018) reported that dietary phytogenic supplementation increased height and diameter of villus, as well as increased goblet cell number. The latter condition may therefore enhance the relative weight of intestine and the capacity of digestion and absorption in broilers. Indeed, our inference was supported by the fact that KE supplementation resulted in better FCR of broilers.

Our current finding showed that raising

broilers in high density pens did not induce the change in abdominal fat content. In accordance with our result, Zuowei *et al*. (2011) showed no significant effect of stocking density on the abdominal fat content of broiler chickens. With regard to phytogenic effect, the dietary KE supplementation increased abdominal fat yield of broilers in the current study. This finding was in contrast to Sugiharto *et al*. (2020) reporting the reduced abdominal fat content in broiler chickens with acidified turmeric supplementation. There is no exact explanation for the latter circumstance. However, phytogenic supplementation seemed to promote faster development in broilers resulting in an earlier change from muscle to fat deposition (Hossain *et al*., 2013). The increased energy availability in the body as a consequence of the phytocomponents' effects may also increase fat synthesis and deposition (Vase-Khavari *et al*., 2019).

Intestinal Conditions of Broilers

Data on villus height, crypt depth and selected bacterial counts of broilers raised in high stocking density pens are presented in Table 4. Among the treatment groups, crypt depth of the duodenum was the lowest ($p<0.05$) in KE0. However, there was no significant effect of the treatments on the villus height and villus height to crypt depth ratio. There was no substantial effect of the treatments on the counts of coliform and LAB in the ileum of broilers.

It was apparent in the present study that crypt depth was lower in KE0 chicks than that in the other groups. According to Sholeha *et al*. (2023), efficient nutrient absorption and better growth performance in broilers were linked to decreased crypt depth. However, data in our current study showed the opposite condition, in that the chickens of KE0 group had higher FCR (lower feed efficiency) than that of KE2.5, KE5 and KE10 groups. Indeed, the villus height to crypt depth ratio seems to be more important in nutrient absorption than simply crypt depth. In this respect, Awad *et al*. (2009) confirmed that one of the most important factors linked to the absorption of nutrients, feed efficiency and weight gain of broiler chickens is the ratio of villus height to crypt depth. They further confirmed that improvements in growth performance of broiler chickens were linked to increases in the villus height:crypt depth ratio.

It has been shown in the present study that there was no change in coliform and LAB counts in the ileum of broilers with different stocking density pens. This finding was in accordance with Ningrum *et al*. (2022) showing no difference in coliform and LAB numbers in the ileum of broilers raised in either normal or high-density pens. Indeed, the treatment with KE did not affect the numbers of coliform and LAB in the ileum of broilers in the current study. This actually was different from Ningrum *et al*. (2022) as they reported the efficacy of the encapsulated *Cosmos caudatus* leaf extract in reducing and enhancing the counts of coliform and LAB in the ileum and caecum of broilers, respectively. Different antimicrobial activities between *C. caudatus* leaf extract and KE may be responsible for the above divergent results.

Antioxidant Status of Broilers

The levels of SOD were higher ($p<0.05$) in KE5 and KE10 as compared to T0, KE0 and KE2.5 chicken groups. However, there was no significant effect of the treatments on the MDA levels in the serum of broilers (Table 5).

In the present study, the effect of stocking density did not have a substantial effect on the antioxidant status of broilers as indicated by the levels of MDA and SOD in the serum. This finding was in line with other parameters measured in the current study particularly growth performance and FCR of broilers as discussed in the previous section. Interesting results were shown in the present study, in which broilers raised in the high stocking density pens receiving KE (especially at 5 and 10 g/kg of feed) had higher serum SOD levels. This indicated that dietary KE supplementation was beneficial to improve the antioxidant status of broilers stocked in high density pens. Indeed, the antioxidative compounds in the KE (Hanum and Maesen, 1997; Arung *et al*., 2012; Soromouo *et al*., 2012) was most likely to contribute for improving the antioxidative status of broilers particularly during stress conditions.

CONCLUSION

Stocking in high density pens negatively affected the carcass yield of broiler chickens. Dietary KE supplementation was beneficial to improve FCR and antioxidant status of broiler chickens as indicated by the SOD levels. Nevertheless, caution must be taken when using KE because excessive use of KE can make the feed less palatable.

REFFERENCES

Abudabos, A.M., A.H. Alyemni, Y.M. Dafalla and R.U. Khan. 2018. The effect of phytogenics on growth traits, blood biochemical and intestinal histology in broiler chickens exposed to *Clostridium perfringens* challenge. J. Appl. Anim. Res., 46(1):691-695

Ahsan, U., E. Kuter, I. Raza, B. H. Köksal, O. Cengiz, M. Yıldız, P. K. Kızanlık, M. Kaya, O. Tatlı, and O. Sevim. 2018. Dietary supplementation of different levels of phytogenic feed additive in broiler diets: the dynamics of growth performance, caecal microbiota, and intestinal morphometry. Br. J. Poult. Sci., 20(4):737-746.

Amad, A.A., K. Männer, K. R. Wendler, K. Neumann and J. Zentek. 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poult. Sci. 90(12):2811-2816.

Arung, E. T., I. W. Kusuma, Y. U. Kim, K. Shimizu K, R. Kondo. 2012. Antioxidative compounds from leaves of Tahongai (*Kleinhovia hospita*). J. Wood Sci., 58:77-80.

Awad, W.A., K. Ghareeb, S. Abdel-Raheem, and J. Böhm. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult. Sci., 88(1):49-56.

Bilal, R. M., F. U. Hassan, M. R. Farag, T. A. Nasir, M. Ragni, H. A. Mahgoub, and M. Alagawany. 2021. Thermal stress and high stocking densities in poultry farms: potential effects and mitigation strategies. J. Therm. Biol., 99:102944.

Cardinal, K. M., M. Kipper, I. Andretta, and A. M. L. Ribeiro. 2019. Withdrawal of antibiotic growth promoters from broiler diets: Performance indexes and economic impact. Poult. Sci., 98(12):6659-6667

Dalólio, F. S., L. F. T. Albino, A. K. B. A. T. Fireman, A. M. Burin, M. Busanello, A. A. Calderano, V. Ribeiro and H. S. Rostagno. 2021. Effect of chromium-methionine supplementation on meat quality of broilers reared under heat stress. Arq. Bras. Med. Vet. Zootec., 73(4):995-999.

Dey, M. C., R. N. Roy, R.N. and A. Sinhababu, 2017. Fatty acid composition and antibacterial activity of the leaf oil of *Kleinhovia hospita* Linn. Int. J. Chem. Tech Res., 10 (3):1102-1105.

Dey, M. C., S. Ukil and A. Sinhababu. 2019. Physico-chemical characterization, fatty acid constituents, and antibacterial and antioxidant activities of *Kleinhovia hospita* seed oil. J. Chem. Nat. Comp., doi:10.1007/s10600-019-02621-x

Diniyah, N. and S. H. Lee. 2020. Phenolic composition and antioxidant potential of legumes - A review. Jurnal Agroteknologi. 14 (1):91-102.

Enahoro, D., S. Bahta, C. Mensah, S. Oloo, and K. M. Rich. 2021. Current and future trade in livestock products. Rev. Sci. Tech. Off. Int. Epiz., 40(2):2-6.

Estévez, M., 2015. Oxidative damage to poultry: from farm to fork. Poult. Sci. 94(6):1368-1378.

Feddes, J. J., E. J. Emmanuel, M. J. Zuidhoft. 2002. Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. Poult. Sci., 81:774-779.

Gadde, U., W.H. Kim, S.T. Oh, and H.S. Lillehoj. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev., 18:26-45

Gan, L. S, G. Ren, J. X. Mo, X. Y. Zhang, W. Yao, and C. X. Zhou. 2009. Cycloartane triterpenoids from *Kleinhovia hospita*. J. Nat. Prod., 72:1102-5.

Gheisar, M. and I. H. Kim. 2018. Phytobiotics in poultry and swine nutrition–a review. Italian J. Anim. Sci., 17(1):92-99.

Gheisar, M. M. and Kim, I. H., 2018. Phytobiotics in poultry and swine nutrition–a review. Italian J. Anim. Sci., 17(1):92-99.

Gheisar, M., M. A. Hosseindoust, and I. H. Kim. 2015. Evaluating the effect of microencapsulated blends of organic acids and essential oils in broiler chicken diet. J. Appl. Anim. Res., 24:511-519

Gogoi, S., G. Kolluri, J. S. Tyagi, G. Marappan, K. Manickam, and R. Narayan. 2021. Impact of heat stress on broilers with varying body weights: Elucidating their interactive role through physiological signatures. J. Therm. Biol., 97:102840.

Hafeez, A., K. Männer, C. Schieder, and J. Zentek. 2016. Effect of supplementation of phytogenic feed additives (powdered vs. encapsulated) on performance and nutrient digestibility in broiler chickens. Poult. Sci., 95(3):622-629.

Hanum, F. and L. J. G. Maesen. 1997. Plant resources of South-East Asia. Backhuys Publisher. Leiden. p:166-168

Hassan, W. H., M. M. Mustafa and R. H. Isa. 2023. Effect of herbal extracts as alternatives to antibiotics in the first week of age on broiler performance, serum biochemistry, and intestinal morphology under commercial farm conditions. S. Afr. J. Anim. Sci., 53(3):455-465.

Hossain, M. A., A. F. Islam and P. A. Iji. 2013. Growth responses, excreta quality, nutrient digestibility, bone development and meat yield traits of broiler chickens fed vegetable or animal protein diets. S. Afr. J. Anim. Sci., 43(2):208-218

Lourenco, J. M., D. S. Seidel, and T. R. Callaway. 2019. Antibiotics and gut function: historical and current perspectives. In: Improving Gut Health in Poultry (S. C. Ricke, ed). Francis Dodds Science Publishing, Cambridge. p:172-189

Mehala, C., D. Kannan, M. Moorthy. 2021. Production performance of broiler chicken fed with *panchagavya* and phytogenic feed additives. Pharm. Innov. J. 10(8): 432-436.

Menezes, A. L. A., C. A. Matias de Almeida, N. M. D. S. Mattarugo, E. A. V. Ferri, P. R. S. Bittencourt, E. Colla, and D. A. Drunkler. 2018. Soy extract and maltodextrin as microencapsulating agents for *Lactobacillus acidophilus*: a model approach. J. Microencapsul., 35(7-8):705-719.

Nasr, M.A., A. Q. Alkhedaide, A.A. Ramadan, E. H. Abd-El Salam, and M. A. Hussein. 2021. Potential impact of stocking density on growth, carcass traits, indicators of biochemical and oxidative stress and meat quality of different broiler breeds. Poult. Sci., 100(11):101442.

Ningrum, D. M., H. I. Wahyuni, T. Yudiarti, E. Widiastuti, T. A. Sartono, and Sugiharto, S. 2022. Encapsulated *Cosmos caudatus* K. leaf extract improved feed conversion and intestinal bacterial population of broilers stocked at different density-induced stress. Acta Vet. Eur., 48(3): 183-188.

Nkukwana, T. T. 2018. Global poultry production: current impact and future outlook on the South African poultry industry. S. Afr. J. Anim. Sci., 48(5):869-884.

Oni, A. I., O. O. Adeleye, T. O. Adebowale, and O. E. Oke. 2024. The role of phytogenic feed additives in stress mitigation in broiler chickens. J. Anim. Physiol. Anim. Nutr., 108(1):81-98.

Puvača, N. M., L. M. Kostadinović, O. M. Duragić, D. B. Ljubojević, B. M. Miščević, T. L. Könyves, S. J. Popović, J. D. Lević, and N. B. Nikolova. 2016. Influence of herbal drugs in broiler chicken nutrition on primal carcass cuts quality assessements. Food Feed Res., 43(1):43-49.

Richards, M. P. and M. Proszkowiec-Weglarz. 2007. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. Poult. Sci., 86:1478-90.

Sahin, K., N. Sahin, and S. Yaralioglu. 2002. Effects of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites, and mineral concentrations of laying hens reared at high ambient temperature. Biol. Trace Elem. Res., 85(1):35-45.

Sapsuha, Y., E. Suprijatna, S. Kismiati, and S. Sugiharto. 2021. The effect of nutmeg flesh (*Myristica fragrans Houtt*) extract on growth performance, internal organ and carcass of broiler chickens raised at high stocking density. Livest. Res. Rural Dev., 33(6):1-7.

Solihah, I., H. Herlina, R. S. P. Rasyid, T. Suciati and K. Khairunnisa. 2019. A Cytotoxic Activity of Tahongai (*Kleinhovia hospita* Linn.) leaves extracts using brine shrimp lethality test. Scie. Technol. Indon., 4(3):60-63.

Soromouo, L. W, N. Chen, L. Jiang, M. Huo, M. Wei, X. Chu, F. M. Millimouno, H. Feng,

Y. Sidime, and X. Deng. 2012. Astragalin attenuates lipopolysaccharide-induced inflammatory responses by down-regulating NF-κB signalling pathway. Biochem. Biophysic. Res. Com., 419(2):256-261.

Sugiharto, S. and T. Ayasan. 2023. Encapsulation as a way to improve the phytogenic effects of herbal additives in broilers-an overview. Ann. Anim. Sci., 23(1):53-68.

Sugiharto, S., 2022. Dietary strategies to alleviate high-stocking-density-induced stress in broiler chickens-a comprehensive review. Arch. Anim. Breed., 65(1):21-36.

Thema, K.K., C. M. Mnisi, and V. Mlambo. 2022. Stocking density-induced changes in growth performance, blood parameters, meat quality traits, and welfare of broiler chickens reared under semi-arid subtropical conditions. Plos One., 17(10): e0275811.

Toson, E., M. Abd El Latif. A. Mohamed, H. S. Gazwi, M. Saleh, D. Kokoszynski, S. S. Elnesr, W. N. Hozzein, M. A. Wadaan, and H. Elwan. 2023. Efficacy of licorice extract on the growth performance, carcass characteristics, blood indices and antioxidants capacity in broilers. Animal., 17(1):100696.

Vase-Khavari, K., S. H. Mortezavi, B. Rasouli, A. Khusro, A. Z. Salem, and A. Seidavi. 2019. The effect of three tropical medicinal plants and superzist probiotic on growth performance, carcass characteristics, blood constitutes, immune response, and gut microflora of broiler. Trop. Anim. Health Prod., 51:33-42.

Yitbarek, M. B. 2015. Phytogenics as feed additives in poultry production: A review. Int. J. Ext. Res., 3:49-60

Zhai, H., H. Liu, S. Wang, J. Wu and A. M. Kluenter. 2018. Potential of essential oils for poultry and pigs. Anim. Nutr., 4(2):179-186.

Zhou, C. X., L. Zou, L. S. Gan, Y. L. Cao. 2013. Kleinhospitines A-D, new cycloartane triterpenoid alkaloids from *Kleinhovia hospita*. Org. Lett., 15:2734-2737

Zuowei, S., L. Yan, L.Yuan, H. Jiao, Z. G. Song, Y. Guo and H. Lin. 2011. Stocking density affects the growth performance of broilers in a sex-dependent fashion. Poult. Sci., 90:1406-1415.