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EFFECT OF DIETARY SUPPLEMENTATION OF ETHANOL EXTRACT OF CHIVES AND GINGER ON PRODUCTIVITY AND HEALTH PERFORMANCE OF CHICKEN BROILERS

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Abstract. One hundred eighty-one-day male Egyptian broiler chicks were randomly assigned to six dietary treatments consisting of three replicates of 10 chicks each. In the control group's diet (CT), neither ginger nor chive preparation was introduced. The diets of birds in the five trial groups (CE1, CE2, GE1, GE2 and CG) were supplemented with 0.5% CE, 1% CE, 0.5% GE, 1% GE, and 0.5% CE and 0.5% GE preparations, respectively (CE is the chive extract; GE is the ginger extract; CG is their combination). At seven-week-old age, the bodyweight gain (g/bird) was significantly ($p < 0.05$) higher in the CE2 than in the other groups, meanwhile the feed conversion rate (FCR) of the CG group chicks was significantly ($p < 0.05$) lower than in the control one. In general, the supplementation of CE and GE did not considerably affect feed consumption, mortality, performance efficiency index (PEI), and the respiratory syndrome in chickens. However, the herb extract preparations could reduce the incidence of diarrhoea in broiler chickens during five- to ten-week age periods. Microscopic examination of small intestinal mucosa shows that the heights of villi and the depths of crypts of chicks in the CE2 treatment and the diameters of villi of chicks in the CG treatment were significant ($p < 0.05$) higher than in the other groups. In general, supplementation of 1% CE and the combined 0.5% CE and 0.5% GE preparations improved the growth performance of broilers, and the combination can be an alternative to antibiotics as growth promoters for chicken.

Keywords: broiler chicken, respiratory, diarrhoea, chive, ginger

1 Introduction

Feed is the major component of total costs of poultry ventures as 80% of the total expenditure is on the procurement of feed [9]. Feed additives are a group of nutrient and non-nutrient compounds, improving the efficiency of feed utilization and thus reducing the cost of feed. The introduction of feed-additive antibiotics as a growth promoter has been actually used for some decades. However, nowadays, antibiotics are banned in many countries due to numerous reasons, such as antibiotic resistance [12] and reducing the effectiveness of antibiotics used for human medical purposes [7]. In addition, new pathogens have emerged over time, some of which are zoonotic, possibly due to inappropriate antibiotic use [2]. For replacing antibiotics,

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natural growth promoters, such as prebiotics, probiotics, synbiotics, enzymes, and plant extracts, can be used to feed the broilers [15, 24].

Among potential candidates for this purpose are ginger and chives, which are common agricultural products in the hilly and sandy areas in the central provinces of Vietnam. Chives (*Allium schoenoprasum*) belong to the genus Allium, a garlic–onion family. Various bioactive substances, such as allicin, diallyl disulfide, ajoene, organosulfur, polyphenols, saponins, fructans, and fructooligosaccharides, produced in chives [22], have antibacterial and antioxidant activities and stimulate the immunity of animals [20]. Ginger (*Zingiber officinale*) is commonly used for different purposes like antiemetics, throat antiseptic, gastrointestinal stimulants, treatments of stomach pain, indigestion, common colds, and cough [5]. Our previous research shows that ethanol extracts from chive and ginger bulbs have antibacterial activities [10, 11].

So far, these herbs have often been practically used as a raw powder or freshly squeezed juice in broiler production. However, it is difficult to quantify and qualify substances in the herbs, determine the dosage, and control the pharmacological effect. Besides, for chive and ginger bulbs, the biggest drawback when used directly is their pungent and unpleasant taste, affecting the palatability of animals. Moreover, hot and humid monsoon climate and poor storage conditions deteriorate herb officinal quality. To solve this problem for a broader use, we have tested chive and ginger ethanol extract preparations previously tested *in vitro* on broilers' growth and health performance.

2 Materials and methods

Time and location

The experiment was conducted in Thuy An Laboratory Practice Centre, Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, Hue University, from November 2019 to February 2020. The temperature ranged from 15 to 31°C, and the average rainfall was 3,210 mm.

Solidified herbal extract

Fresh bulbs of ginger (12–14 months old from A Luoi, Thua Thien Hue) and fresh chives (4–5 months old from Hai Lang, Quang Tri) were used. Both ginger and chive herbs were determined genetically as *Zingiber officinale* and *Allium scordoprasum*, as DNA sequences of PCR-amplified ITS1-4 gene products of the two plants showed related to those species' data stored in GenBank. Herbs extracts were prepared with the cool extraction with ethanol, as described in our previous studies [10, 11]. The yield of solid-liquid extraction (moisture content of ~15%) obtained from fresh material was 10.55% for chives and 9.60% for ginger. The extracts were

Table 1. Quantitative results of chives and ginger ethanol extract

Items	Ginger extract	Chive extract
Carotenoid	+	+
Essential oil	+	+
Alkaloid	+	–
Coumarin	+	+
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Terpenoids	–	–
Organic acids	–	–
Reducing agent	–	–

(–) Unavailable; (+) Available

quantitatively analyzed with the method reported by Sofowora [21]; the main active ingredients in the extracts are shown in Table 1. The amorphous solid extracts were pre-dried by mixing with an amount of desiccant Aerosil® 200, equal to 20% of the main materials and then dried to under 5% moisture. The coarse granular products were then finely ground and mixed with three volumes of tapioca flour to form "chive and ginger extract preparations" as the main materials for the clinical trials.

Experimental animals

One-day-old Egyptian breed male chickens purchased from the National Institute of Animal Science (Thuy Phuong, Tu Liem, Hanoi) with an average body weight of 35.21 ± 0.12 g were raised on a floor (10 chickens/m^2) for 12 weeks.

Table 2. Nutrient composition of feed used on the experiment

No.	Composition, as fed	Unit	Mean
1	Dry matter	%	92.29
2	Gross energy	cal/kg	4064.83
3	Crude protein	%	21.30
4	Ash insoluble in hydrochloric acid	%	1.99
5	Ash	%	6.06
6	Crude fiber	%	3.36

During the experiment, chickens were fed with the rations (consisting of corn, rice bran, anchovy meal, soybean meal, shellfish, vitamin premix, mineral premix, $CaCO_3$, L-lysine, and DL-methionine) that fully meet the needs of broiler chickens according to Vietnamese Standards (TCVN 2265:2007) with nutritional components in Table 2. Feed and water for chickens were provided *ad libitum* during the experiment. Experimental chickens were vaccinated against Marek, Newcastle, Fowl pox, and Infectious bursal (Gumboro) disease.

Experimental design

The experiments were arranged in a completely randomized design with 180 chicks in 5 treatments, designated as CE1, CE2, GE1, GE2, and CG, in which the rations to chickens were supplemented correspondingly with 0.5 and 1% chive extract preparation, 0.5 and 1% ginger extract preparation, and combined 0.5% chive and 0.5% ginger preparations, in parallel with a control group (CT), in which neither chive nor ginger preparation was added (Table 3).

Performance parameters

- Bodyweight: All one-to-five-week-old chicks were weekly weighed in groups (10 birds/pen) with an electronic scale (1 kg ± 2 g); from five-week age, the birds' weights were determined individually with a 5-kg scale.
- Feed intake: Feed intake in each group was determined weekly via the difference between the amount of daily delivered feed and its left-over amount (g/chicken/day) and feed conversion ratio (FCR), or the feed amount expended for each kilogram of weight gain, was calculated.

Health indicators

- Daily record of dead and eliminated chickens.
- Percentage of diarrhoea syndrome: number of chickens with diarrhoea/total number of chickens observed daily. Signs of chicken with diarrhoea syndrome are diluted stool, moodiness, lim eyes, inactiveness, saggy wings, thirstiness, usually with stool stuck to the anus.
- The ratio of chickens infected with respiratory syndrome is the number of chickens showing respiratory/total number of chickens observed daily. Signs of chickens with

Table 3. Experiment design

Items	CT	CE1	CE2	GE1	GE2	CG
Chive extract (%)	–	0.5	1.0	–	–	0.5
Ginger extract (%)	–	–	–	0.5	1.0	0.5

respiratory syndrome are moodiness, ruffled feathers, runny nose, coughing in the evening, face swelling due to sinusitis.

The performance efficiency index is calculated according to the following formula:

PEI = The final body weight (g) × Survival rate (%)/ ((total experiment days) × FCR) [4].

Small intestinal morphology

The morphology of the small intestine was assessed with the method reported in our previous study [8]. Three chicks per treatment were sacrificed at the end of the experiment with the cervical dislocation method. Collected jejunum was fixed in 10% formalin for 24 h. Sections of about 3–5 mm from the middle part of the jejunum were cut out and embedded in paraffin. Cross-sections of 6-μm thickness were made by perpendicularly slicing the gut specimens with a microtome and stained with hematoxylin and eosin (HE). After fixing the specimen to a slide with ethanol, the diameter and length of the villus and the depth of the intestinal gland layer (crypt, located at between the two intestinal villi) were measured with an image analysis program (Leica QWin Standard, Version 2.8, Germany). Five cross-sections per chick were then examined by using a light microscop.

Statistical analysis

Statistical analysis for all data was performed by using the ANOVA procedure in SPSS software (26.0), and significance among treatments ($p < 0.05$) was determined with the Generalized Linear Models (GLM) test. The statistical model for data analysis is outlined as follows:

$$Y_{ij} = \mu + C_i + e_{ij}.$$

where Y_{ij} is the measured value for each observation (data); μ is the parameter common to all treatments (overall mean); C_i is the treatment effect; e_{ij} represents experimental error.

3 Results and discussion

Broiler performance

The data in Table 4 show that a non-significant difference was observed in treatment groups compared with CT from one- to six-week-old chickens. However, bodyweight gain (g) of experimental chickens supplemented with 1% CE (CE2) show significantly ($p < 0.05$) higher values than with CT and other experimental groups from seven-week-old chickens. At the end of the experiment (ten-week-old), the weights of chickens in the CT (1470.21 g/head), GE1 (1488.01 g/head), and GE2 (1462.81 g/head) are lower than those in the CE1 (1504.44 g/head), CE2 (1528.74 g/head), and CG (1528.75 g/head) treatments. However, the difference is

statistically significant ($p < 0.05$) only in treatments supplemented with C1% compared with the other treatments. The improvement in weight gain of the experimental chickens fed with C1% may be due to the activity of compounds like allicin and organosulfur, responsible for inhibiting pathogenic bacteria and fungi in the gut environment. Our results are consistent with those of Aji et al. [3], who stated that garlic (*Allium sativum*, which is closely related to chive) in the diet had a positive effect on the broiler body weight gain.

Table 4. Average body weight (Mean ± SD, g/chick)

Age (week)	CT		CE1 (C0.5%)		CE2 (C1%)		GE1 (G0.5%)		GE2 (G1%)		CG (C0.5%+G0.5%)		*p*-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
1	64.17	5	64.83	6.4	64.86	6.9	63.17	4	63.67	6.4	67.83	6.5	0.766
2	150.33	10.5	149.17	11.6	150.33	9.3	144.17	9.6	145.33	11.6	149.17	10.5	0.362
3	246.17	20.9	233.67	10.6	242.33	16	240.5	14.1	245.83	19.5	239.33	21.0	0.273
4	362.67	21.4	358.5	27.4	365.15	33.1	365.5	19.1	366.83	25.7	358.67	25.4	0.447
5	510.5	21.6	521.33	28.2	521.83	22.9	520.33	20.8	518.83	26.3	514.67	28.2	0.342
6	684.83	16.7	688.33	26.9	715.05	24.6	701.50	27.7	703.50	37.1	706.67	31.6	0.116
7	871.70^{a}	32.1	890.83^{ab}	32.0	910.18^{a}	38.5	904.70^{ab}	29.3	899.00^{ab}	32.5	907.85^{a}	33.3	0.042
8	1072.40^{b}	44.5	1099.68^{b}	41.1	1127.13^{a}	29.7	1092.30^{b}	35.6	1107.30^{ab}	29.8	1110.82^{ab}	24.6	0.035
9	1268.31^{b}	39.8	1302.64^{ab}	39.8	1308.33^{a}	36.5	1292.30^{ab}	27.9	1289.70^{ab}	34.6	1288.50^{ab}	42.4	0.028
10	1470.21^{b}	44.2	1504.44^{ab}	51.3	1528.74^{a}	44.8	1488.01^{b}	38.9	1462.81^{b}	47.5	1506.95^{a}	38.7	0.011

* Different letter (a, b) indicates significant difference within row ($p < 0.05$)

Table 5. Performance and economic evaluation

Items	CT	CE1	CE2	GE1	GE2	CG	*p*-value
Feed intake (g/chick/day)	58.02	57.80	61.25	58.60	58.10	56.20	0.243
FCR	2.83^{a}	2.75^{ab}	2.87^{a}	2.87^{a}	2.80^{a}	2.67^{b}	0.036
PEI	69.26	72.84	76.08	71.52	69.67	75.17	0.398
Survival rate (%)	93.3	93.3	100.0	96.7	93.3	93.3	0.687
Respiratory syndrome (%)							
1–4w-old	1.8	2.7	3.6	3.1	2.3	3.4	0.127
5–10w-old	6.7	4.3	3.1	3.3	3.0	2.1	0.087
Diarrhoea syndrome (%)							
1–4w-old	1.6	–	–	2.1	2.7	–	–
5–10w-old	3.2	2.8	4.1	2.2	1.6	1.2	0.122

* Different letter (a, b) indicates significant difference within row ($p < 0.05$)

The use of ginger in this study did not improve the chicken weight like what was reported by Zhang et al. [25] when using ginger powder (5 g/kg) in broiler diets. However, Ademola et al. [1], Mohamed et al. [14], and Sadeghi et al. [19] reported that ginger in the diets stimulated lactic acid bacteria and reduced the number of pathogenic bacteria such as mesophilic bacteria, aerobic bacteria, and coliform. This diet improved the absorption of nutrients, leading to a better weight gain of the birds [24].

In this experiment, unlike with the CT, the improvement of chicken weight gain in combination group CG shows possible synergistic effects between the active principles of the herb. Chung et al. [6] claimed a positive effect on growth in the Rilai broiler in Thua Thien Hue province, with the supplement of ginger mixed with other herbs. However, the combination ratio and dosage should be considered to control the balance between synergistic and antagonistic effects on the health of animals and the toxicity of herbal additives [13].

Table 5 shows that the addition of chive and ginger to chicken's diets did not affect feed intake compared with the CT (56.20–58.60 g/head/day). Thus, there is no adverse effect of smell and/or taste of chive and ginger on the palatability of the feed in the chick's diets.

Experimental chickens in the CG trial group show significantly ($p < 0.05$) lower average FCR than in the other groups (except CE1). These results are consistent with the findings of Mohamed et al. [14], who reported that the chickens fed with 0.2% ginger had a higher FCR. The addition of ginger and chive alone in chicken diets did not significantly affect the FCR compared with the CT. These results are similar to the findings of Aji et al. [3], who reported a non-significant effect of chive on the FCR. These results also agree with those of Thayalini et al. [23], who did not observe any significant improvements in the feed conversion ratio of the broilers fed on a diet containing ginger powder compared with the CT.

The productive efficiency index (PEI) is a general indicator of economic and technical efficiency; the higher PEI is, the more significant the economic effect. Usually, the PEI in chickens is greater than 70 [4]. In this experiment, PEI is lowest (69.26) in the CT group and highest in CE2 (76.08); however, a non-significant difference is observed among all treatment groups and the CT.

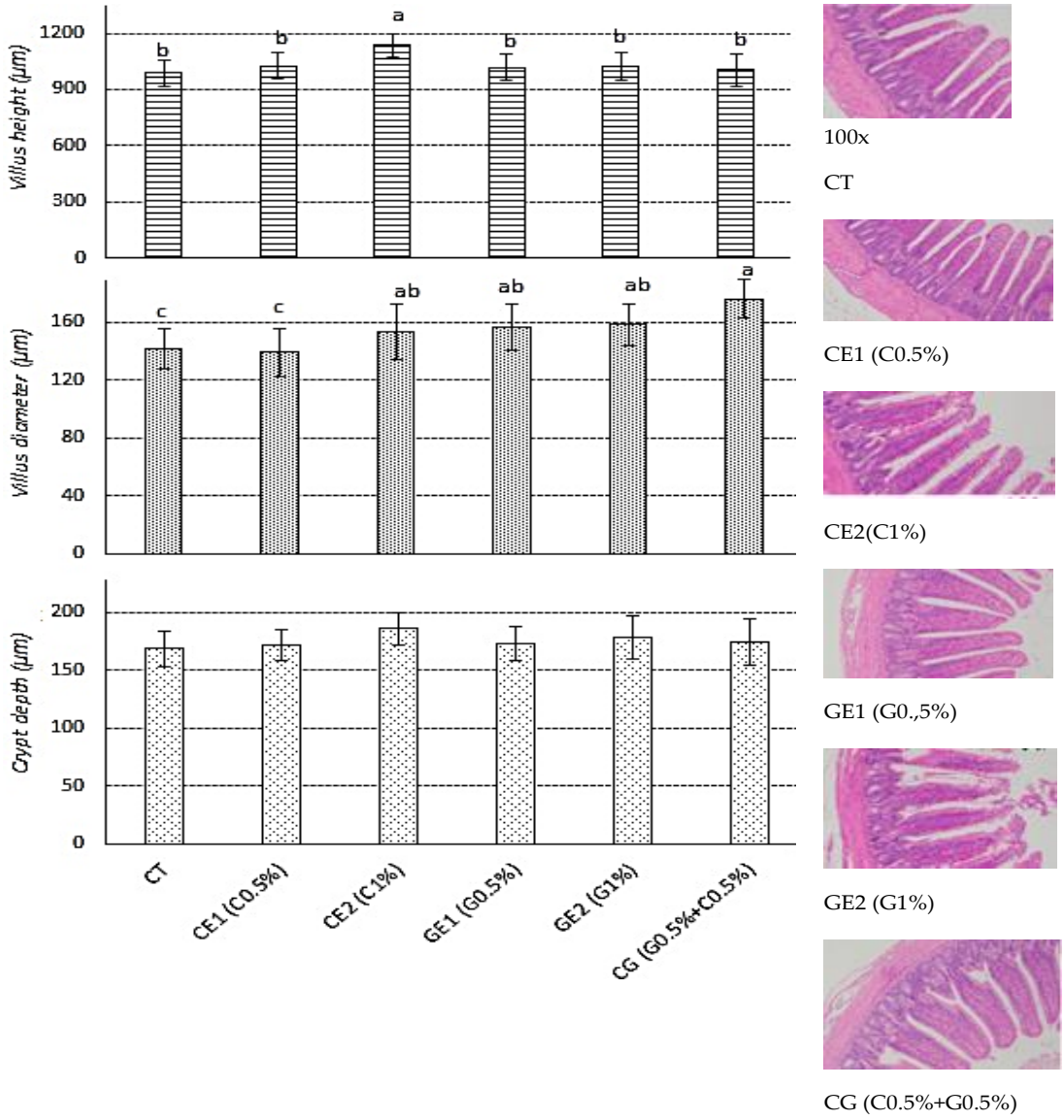
Health performance

The two most common diseases in poultry production are diarrhoea and respiratory disorders. The prevalence of diarrhoea did not differ in treatment groups compared with the CT at one- to four-week-old birds. The chickens fed with chives and ginger show a lower rate of respiratory symptoms (2.1–4.3%) than with the CT (6.7%) at five- to ten-week-old age, but no statistical significance ($p > 0.05$) was found. The chickens in the chive and ginger supplemented groups show a high survival rate (93.3–100%), though insignificantly different from the CT (93.3%). It

was reported that the chemical components in chive and ginger, such as alkaloid, saponin, flavonoid, and tannin (Table 2), have the therapeutic potential to improve the health of the chickens. Concerning the digestive and respiratory diseases caused by bacteria, both ethanol extracts of ginger and chive bulbs have bacteriostatic/bactericidal effects on *E. coli* and *Salmonella* spp. [11]. The antibacterial ability of ginger extract may be due to compounds such as sesquiterpenoid, zingiberene, bisabolene, farnesene, and monoterpenoid [17]; meanwhile, diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide are found in chive bulbs [18].

Gut morphometry

The values of villus heights are significantly larger ($p < 0.05$) in the CE2 chicks than in the other groups. Meanwhile, the villus width in the chicks of the CG group is greater than that of the CT group. This shows promising effects of chive prescribed separately and in combination with ginger on chicken's gut health. Murugasan et al. [15] reported that stem cells at the base of crypts are known to be the source of all the cells that line the crypts and the villi. Because a larger cryptal depth causes a faster mucosal proliferation activity, more efficient digestibility and absorption of ingested feed may occur in group CE2. This shows that chive can increase the digestive and absorptive capacity of the small intestine of chicken by increasing the crypt depth and the absorptive surface area of the intestine. This finding agrees with that of Oladele et al. [16]. They reported that an increase in the absorptive capacity of the intestine might be due to the increased absorptive surface area, which results in a higher body weight gain and a lower FCR of the broilers.



*Different letter (a, b, c) indicates significant difference among groups ($p < 0.05$)

Figure 1. Schematic illustration of villus height and diameter, and crypts' depth of small intestine of broilers treated with different doses of chive and ginger and their combination

5 Conclusion

The supplementation of ethanol extract preparations of chive (1%) and its mixture (0.5%) with ginger (0.5%) to chickens' diets improves the growth performance and economically-related indices of chickens. Chive can be a potential alternative to antibiotics as a growth promoter in feeding broiler chicken.

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