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

Phan Vu Hai*, Pham Hoang Son Hung, Ho Thi Dung, Le Minh Đuc, Đinh Thi Thuy Khuong, Tran Ngoc Liem

University of Agriculture and Forestry, Hue University, 102 Phung Hung St., Hue, Vietnam

Abstract: This study was to evaluate the effects of supplementation of ethanol extracts of chive (CE) and ginger (GE), and their combination (CG) in diets of broiler chickens on their growth, health performance and economic efficiency. Totally 180 male Egyptian broilers from one-day age were randomly assigned to 6 dietary treatments consisting of 3 replicates of 10 chicks each, of which in the diet of the control group (CT) neither ginger nor chive preparation were introduced, meanwhile the diets of birds in the 5 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 plus 0.5% GE preparations, respectively. In general, supplementation of CE and GE did not significantly affect feed consumption, mortality, performance efficiency index (PEI), and respiratory syndrome in chickens. However, the herb extract preparations could reduce the incidence of diarrhea in broiler chickens during five to-ten-week age periods. Microscopic examination of small intestinal mucosa showed that the heights of villi and the depths of crypts of chicks in the CE2 treatment as well as the diameters of villi of chicks in the CG treatment were significantly ($p < 0.05$) higher than in the others. In general, supplementation of 1% CE and the combination of 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 in feeding chicken.

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

1 Introduction

Feed is the major component of total costs of poultry venture as 80% of the total expenditure is on procurement of feed [9]. Feed additives are a group of nutrient and non-nutrient compounds which helps in improving the efficiency of feed utilization and thus reducing the cost of feed. The introduction of feed-additive antibiotics as a growth promoter is actually used for some decades. However, nowadays the use of antibiotics is banned in many countries due to many 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 as a result of inappropriate antibiotic use [2]. In order to replace antibiotic, natural growth promoters such as prebiotics, probiotics, synbiotics, enzymes, plant extracts, etc., can be used to feed the broilers [15], [24].

Corresponding: hai.phanvu@huaf.edu.vn
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Among potential candidates for this purpose we focused on ginger and chives, which are popular 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. A lot of bioactive substances such as allicin, diallyl disulfide, ajoene, organosulfur, polyphenols, saponins, fructans and fructo-oligosaccharides produced in chives [22], have antibacterial, antioxidant activities and stimulate immunity of animal [20]. Ginger (*Zingiber officinale*), commonly used in many 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 are often practically used in the form of raw powder or freshly squeezed juice in broiler production. However, it is difficult to quantify and qualify substances in the herbs and determine the dosage, and so, to control the pharmacological effect. Besides, for chive and ginger bulbs, the biggest drawback when used directly is the pungent and unpleasant taste, affecting the palatability of animals. On the other hand, hot and humid monsoon climate, and poor storage conditions lead to the deterioration of herb officinal quality. To solve that problem for wider use of them we have tested chive and ginger ethanol extract preparations that were tested *in vitro* previously on growth and health performance of broilers.

2 Materials and methods

Time and location

The experiment was conducted in Thuy An Laboratory Practice Center, Faculty of Animal Science and Veterinary Medicine, Hue College of Agriculture and Forestry, Hue University from November 2019 to February 2020 with temperature ranging from 15 to 31°C and an average rainfall of 3210 mm.

Solidified herbal extract

Fresh bulbs of ginger (12–14 months old, in A Luoi, TT-Hue) and fresh chives (4–5 months old, in 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 were prepared by the cool extraction method with ethanol solvent as described in our previous studies [10], [11]. The yield of solid extraction (moisture content of ~15%) obtained from fresh material was 10.55% for chives and 9.60% for ginger. The extracts were quantitatively analyzed by 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 a such mount 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.

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

Experimental animals

One-day-old Egyptian breed male chickens purchased from National Institute of Animal Science (Thuy Phuong, Tu Liem, Hanoi) with the average body weight of 35.21 ± 0.12 grams were raised on floor (10 chickens/m2) for 12 weeks. From the first day of age to the end of the experiment at 12–weeks of age, chickens were fed with the rations (consisting of corn, rice bran, anchovy meal, soybean meal, shellfish, vitamin premix, mineral premix, $CaCO_3$, L-lysine, 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 was provided *ad libitum* during the experiment. Experimental chickens were vaccinated against Marek, Newcastle, Fowl pox and Infectiuos bursal (Gumboro) disease.

Table 2. Nutrient composition of feed used on the experiment

No.	Composition, as fed	%	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

Experimental design

The experiment was arranged in a completely randomized method (CRD) 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, with 0,5% and 1% ginger extract preparation, with combined 0.5% chive and 0.5% ginger preparations, in parallel with a control group (CT), in which neither the chive nor ginger preparation was added (Table 3).

Table 3. Experiment design

Items	CT	CE1 (C0.5%)	CE2 (C1%)	GE1 (G0.5%)	GE2 (G1%)	CG (C0.5% + G0.5%)
Chive extract (%)	-	0,5	1,0	-	-	0,5
Ginger extract (%)	-	-	-	0,5	1,0	0,5

Performance parameters

+ Body weight: From one to five-week age all chicks were weekly weighted in group (10 birds/pen) with an electronic scale of 1 kg (±2g), 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 by subtraction of the amount of delivered feed by its left-over amount, from which daily feed consumption (g/chicken/day) and feed conversion ratio (FCR), or the feed amount expended for each kilogram of weight gain, were calculated.

Indicators of chicken health

+ Daily record of dead and eliminated chickens.

+ Percentage of diarrhea syndrome: number of chickens with diarrhea/total number of chickens observed daily.

Signs of chicken with diarrhea syndrome in chickens: the chickens with diluted stools, moodiness, lim eyes, inactiveness, saggy wings, thirstiness; usually with stool stick to the anus.

+ The ratio of chickens infected with respiratory syndrome: number of chickens showing respiratory/total number of chickens observed daily.

Signs of chickens with respiratory syndrome: moodiness, ruffled feathers, runny nose, coughing in the evening, face swelling due to sinusitis.

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

Small intestinal morphology

The morphology of small intestine was accessed by following our previous study [8]. Three chicks per treatment were sacrificed at the end of experiment by cervical dislocation method. Collected jejunum was fixed for 24 hours in 10% formalin. Sections of about 3–5 mm from the middle part of the jejunum were cut out and embedded in parafin. 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 specimen to a slide with ethanol, the diameter and length of the villus, depth of the intestinal gland layer (crypt, located at between the two intestinal villi) were measured by image analysis program (Leica QWin Standard, Version 2.8, Germany). Five cross sections per chick then were examined using a light microscopy.

Statistical analysis

Statistical analysis for all data was performed by using the ANOVA procedure of SPSS software (26.0) and significance among treatments ($p < 0.05$) determined by the Generalized Linear Models (GLM) test. The statistical model for data analysis is outlined as following: $Yij = \mu + Ci + eij$

Where Yij is measured value for each observation (data), μ is a parameter common to all treatments (overall mean), Ci is the treatment effect; eij represents experimental error.

3 Results and discussion

Broiler performance

The results in Table 4 showed that non-significant difference was observed in treatment groups compared to CT from one to six-week old. However, body weight gain (g) of experimental chickens supplemented with 1% CE (T2) showed significantly ($p < 0.05$) higher values as compared to CT and other experimental groups from seven-week old age. At the end of the experiment (ten-week old), the weights of chickens in the CT (1470.21 g/head), GE1 (G0.5%, 1488.01 g/head) and GE2 (G1%, 1462.81 g/head) were lower than CE1 (C0.5%, 1504.44 g/head), CE2 (C1%, 1528.74 g/head) and CG (C0.5% + G0.5%, 1528.74 8 g/head). However, the difference was statistically significant ($p < 0.05$) only in treatments supplemented with C1% compared to the other treatments. The improvement in weight gain of experimental chickens fed with C1% may be due to the activity of compounds like allicin and organosulfur responsible for inhibition of pathogenic bacteria and fungi in gut environment. Our results are consistent to those of Aji et al. [3], who stated that the use of garlic (*Allium sativum*, which are closely related to chive) in the diet had a positive effect on broiler body weight gain.

Table 4. Average body weigh (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 (C0.5%)	CE2 (C1%)	GE1 (G0.5%)	GE2 (G1%)	CG (C0.5%+G0.5%)	*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
Diarrhea 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 is like that 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], Sadeghi et al. [19] reported that ginger in the diets stimulated lactic acid bacteria and decreased the number of pathogenic bacteria such as mesophilic bacteria, aerobic bacteria, coliform and thus improved absorption of nutrients led to better weight gain of the birds [24].

In this experiment, in comparison to the CT, the improvement of chicken weight gain in combination group of C0.5% and G0.5% dietary supplementation shows possible synergistic effects between the active principles of the herb. Research of Chung et al. [6] on the supplement of ginger mixed with other herbs also showed positive effect on growth in Rilai broiler in Thua Thien Hue province. However, combination ratio and dosage should be considered to control the balance between synergistic and antagonistic effects on the health of animal and 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 to the CT (56.20–58.60g/head/day). Thus, there is no adverse effect of smell and/or taste of chive and ginger on the palatability of feed in the diets of chickens.

Experimental chickens in the CG trial group showed significantly ($p < 0.05$) lower average FCR as compared to the other groups (except CE1). These results are in accordance with the findings of Mohamed et al. [14], who reported that chickens fed with 0.2% ginger had better FCR. The addition of ginger and chive alone in chicken diets did not have any significant effect on FCR

compared to CT. These results are in accordance with the findings of Aji et al. [3] who has reported non-significant effect of chive on FCR. These results are also in agreement with Thayalini *et al*. [23] who did not observe any significant improvements in the feed conversion ratio of broilers fed on a diet containing ginger powder as compared to CT.

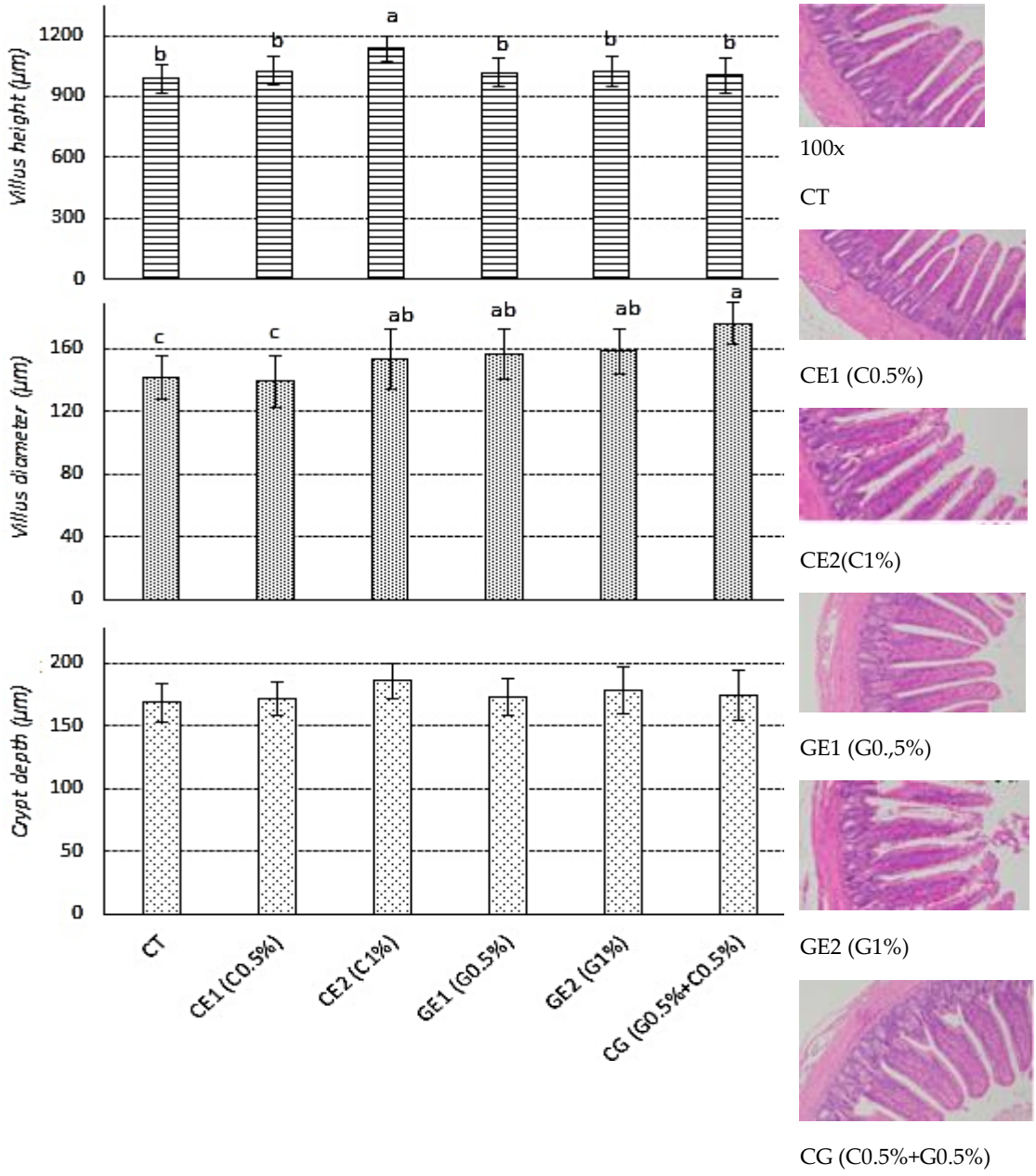
Productive efficiency index (PEI) is a general indicator of economic and technical efficiency; The higher PEI is, the greater economic effect. Normally, the PEI in chickens is greater than 70 [4]. In this experiment, PEI was lowest (69.26) in the CT group and highest in CE2 (76.08), however non-significant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

Health performance

There are two most common diseases in poultry production are diarrhea and respiratory problem. The prevalence of diarrhea was not differ in treatment groups compared to the CT at one to four-week old. Chickens fed with chives and ginger showed lower rate of respiratory symptom (2.1–4.3%) compared to the CT (6.7%) at five to ten-week old age, but no statistical significance ($p > 0.05$) was found. Chickens in the chive and ginger supplemented groups showed high survival rate (93.3–100%), though there were no difference with 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. In relation to digestive and respiratory diseases caused by bacteria, both ethanol extracts of ginger and chive bulbs had 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 were found in chive bulbs [18].

Gut morphometry

The values of villus heights were significantly higher ($p < 0.05$) in the CE2 chicks compared to the other groups. Meanwhile, the villus width in the chicks of the CG group was greater than that of the CT group. This shows the promising effects of chive separately prescribed as well as in the 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 which lines the crypts and the villi. That a higher cryptal depth appears the faster mucosal proliferation activity is for more efficient digestibility and absorption of ingested feed may take play group CE2 . This shows that chive has ability to increase the digestive and absorptive capacity of the small intestine of chicken by increasing the crypt depth as well as the absorptive surface area of the intestine. This finding agrees with results of Oladele *et al.* [16] who reported that an increase in the absorptive capacity of intestine might be due to increase absorptive surface area which results in higher body weight gain and lower FCR of broiler.



*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 the small intestine of broilers treated with different doses of chive and ginger and their combination.

5 Conclusion

Supplementation of ethanol extract preparations of chive (1%) and mixture of it (0.5%) with ginger (0.5%) to chickens' diets improves the growth performance and economically-related indices of chickens. This may be due to the chemical components in the bulb of the herbs that enhance health status of the flock through improving the intestinal villi, which helps the chicken's digestion. Chive can be considered as potential alternatives to antibiotics as growth promoters in feeding of broiler chicken.

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