Research Article

Effect of Adding Benefit Microorganisms and Enzymes in Diets on Growth Performance, Carcass Traits and Intestinal Health of Guinea Fowls (*Numidia meleagris*)

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Abstract | This study was conducted to assess effects of the benefit microorganisms and enzymes supplementation in diets. A total of 150 Guinea fowls at 5-week old was placed in a completely randomized design with 5 nutritional treatments and 3 replicates, including ten birds per experimental unit. The treatments were supplement levels of 0 (no supplement),0.2% and 0.4% benefit microorganism and 0.4 and 0.8%vitamin-enzyme (VE)to concentrate-basal diets corresponding to the S0, SP0.2, SP0.4, VE0.4 and VE0.8 treatments. The results indicate that the treatments supplemented SP and VE improved final live weight and daily weight gain ($p<0.05$) and the birds in the PS0.2 treatment had the highest daily weight gain (17.3 g/day), final live weight (1543 g/bird) and better feed conversion ratio ($p<0.05$). Similarly, the carcass, breast and thigh weights were significantly higher for the SP0.2treatment ($p<0.05$). The supplement of SP and VE products significantly decreased density of *E. coli* and *C. perfringens* in the chicken excreta ($p<0.05$). It was concluded that the Guinea fowls supplemented SP and VE in concentrate basal diets improved growth rate, carcass performance as well as had better intestinal health and the treatment offered at 0.2% SP gave the highest results.

Keywords | Guinea fowl, Growth rate, Meat performance, Probiotic, Intestinal health

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INTRODUCTION

Poultry production plays an important role in the Mekong Delta of Vietnam due to increasing consumption requirements. The Guinea fowls are originally come from African countries (Vignal et al., 2019), have successfully adapted to Vietnam's ecological and nutritional circumstances. Due to the high quality meat with higher price as compared to the native chicken (around 20%) of the Guinea fowls, Vietnamese farmers have reared them in an effort to increase economic returns and diversify poultry output. Traditionally, in Vietnam's chicken industry, antibiotics as growth promoter chemicals have been utilized to increase productivity and avoid disease outbreak (Agostini et al., 2012).

Guinea fowls are distinct from both imported commercial chicken breeds and native chicken breeds in that they have a significantly higher slaughter performance (Ebegbulem and Asuquo, 2018), good ratios of essential sections in the carcass, and satisfactory meat sensory attributes (Kyere et al., 2020). In terms of its nutritional value, the flesh of the Guinea fowl is distinguished from that of other chicken species by its higher protein level and lower fat content

(Ayorinde, 1991). As of the year 2020, the administration of antibiotics for the purpose of preventing animal diseases or adding them to animal feed as growth promoters was prohibited in Vietnam (USDA, 2021). The beneficial microorganism and enzymes which have been applied in animals as additives because of being not leave residues in meat products (Fluton et al., 2002; Muzaffer et al., 2003). They could improve feed digestion and nutrient absorption, growth performance, balance of intestinal microorganisium, inhibit harmfull bacteria, to increase body resistance and immune system for chicken (Gibson et al., 2017). The supplement of microorganisms such as *Saccharomyces cerevisiae* and *Pediococcusacidilactici* improved feed conversion ratio (Merati et al., 2021). The *Saccharomyces cerevisiae* absorbs oxygen in the digestive tracts supporting the development of beneficial bacteria, while limiting the growth of the harmful ones as a result to improve the health and performace of animals. As the same ways of using microorganisms, Lee et al. (2022) recorded that *Saccharomyces cerevisiae* and *Bacillus amyloliquefaciens*in-feed supplementation improve growth rate and modulated the cecal microbiota. The supplement of enzymes has also brought positive effects on growth performance and microbiota (Jabbar et al., 2021). Not only can exogenous enzymes increase feed efficiency, but they also decrease proteolytic fermentation, nutrient excretion in feces, and bacterial toxins (Mahmood et al., 2017).

However, the supplement of microorganisms and vitamin-enzymes was limited for applying in Guinea fowls. Thus, this study was implemented aiming to evaluate effects of benefit microbes and enzymes in diets on growth performances, carcass quality and pathogenic bacteria density in the excreta of Guinea fowls for an application.

MATERIALS AND METHODS

Local and time

The experiment took place between May and September. The research was carried out at the Experimental farm in An Binh village, Phong Dien district, Can Tho city. In the laboratory of the Department of Animal Sciences in the Faculty of Agriculture at Can Tho University, feeds were chemically analyzed.

Experimental animals

Guinea fowls originated in Hungary and were subsequently bred at the Experimental farm. Before entering the trial, all chickens were immunized against Gumboro, Newcastle, H5N1, and chicken pox. The experiment followed the ethical management process in experimental animals of Can Tho University of Vietnam, No. 3965/QĐ ĐHCT and Published in Oct 15-2021.

Feeds and supplements

All feed materials were purchased at once from a feed store for the duration of the experiment (Table 1). Prior, they were analyzed chemical composition, then the concentrate basal diet was formulated with contained 18% CP and 13 MJ ME/kg DM (Table 2).

Table 1: Composition of concentrate baseline diet ingredients in the Experiment (%DM)

Feed	Percentage (%)
Rice bran	15
Broken rice	48
Extracted soybean	19
Coconut meal	16
Bone meal	2.0
Total	100

The composition of benefit microorganisms contained per 1 kg including *Saccharomyces spp (Saccharomyces cerevisiae)*: 10^8CFU, *Pediococcuss spp (Pediococcus acidilactici)*: 10^8CFU and wheat flour (SP).

The composition of vitamin- enzymes contained per 1 kg including vitamin D3: 200.000 IU; Vitamin A: 1.000.000 IU; Protease: 10.000 IU; Amilase: 75.000 IU, Cellulase: 4.000 IU, Pectinase: 2.000 IU; Lipase: 3.000 IU, and calcium carbonate (VE).

Experimental design

One hundred fifty Guinea fowls at 5 weeks of age (330 ± 2.38 g/bird) were randomly assigned to one of five treatments and three replicates, with 10 birds per experimental unit (balanced sex). Different supplement doses of 0 (no supplement of SP or VE); 0.2, 0.4% SP and 0.4 and 0.8% VE were added to concentrate-based meals as the S0, SPO.2, SP0.4, VEO.4 and VEO.8 treatments, respectively. The duration of the experiment was 10 weeks. Table 3 presents the composition of feed ingredients in treatments.

Housing and management

Wood and steel wire net were used to construct a birdhouse, and a plastic sheet was placed under each cage floor to collect excreta. The birds were confined in each cage of 1.80 m2/10 birds.

In each of the pens, feeders and drinkers were installed, and they were cleaned each morning along with the chicken feces that were removed each day.

The birds were given food at 7.30, 13.00, and 17.00 hours each day, and the diets that were offered to the treatments were varied weekly depending on actual feed intakes. The birds also had full access to water throughout the experiment.

Table 2: The chemical composition of the feed items and concentrate used in the experiment (%DM)

Feed, %	DM	OM	CP	EE	CF	NDF	ADF	Ash	ME (MJ/kgDM)
Broken rice	88.9	99.4	7.85	2.86	0.71	7.85	4.87	0.60	14.0
Rice bran	89.7	91.4	11.7	9.89	10.4	20.6	14.2	8.60	10.6
Extracted soybean	87.9	93.8	42.1	3.89	9.25	22.9	18.7	6.20	13.1
Coconut meal	89.2	92.3	21.3	18.4	13.2	58.5	33.5	7.70	13.2
Bone meal	93.0	35.3	21.4	4.80	0.52	4.42	3.46	64.7	5.50
Cabbage residue	7.52	84.7	15.1	5.31	15.6	21.6	19.4	15.3	8.25
Concentrate	88.9	94.5	18.0	6.67	5.96	21.0	13.7	5.46	13.0

CP: crude protein, EE: ether extraction; DM: dry matter; OM: orgarnic matter, CF: crude fiber, ADF: acid detergent fiber, NDF: neutral detergent fiber, ME: metablolizable energy.

Table 3: Feed ingredient composition in treatments

Treatment	Concentrate (%)	*Saccharomyses- Pediococcus* (SP) (%)	Vitamin-enzymes (VE) (%)
S0	100	0	0
VE0.4	99.6	0	0.4
VE0.8	99.2	0	0.8
SP0.2	99.8	0.2	0
SP0.4	99.6	0.4	0

Measurements taken

Daily feed and nutrient intakes: The morning routine consisted of collecting and weighing the previous day's feed and refusals in order to calculate daily intakes of feed and nutrients.

The growth rate and feed conversion ratio were determined by weighing the birds weekly and at the conclusion of the experiment.

Carcass and meat quality: Three days following the conclusion of the trial, four birds (two males and two females) were slaughtered for evaluating of their carcasses and meat quality.

The colony counting method was used to quantify the number of *E. coli, Salmonella. spp*, and *Clostridium perfringens* in chicken feces at the age of 12 weeks. The samples were obtained directly from the cloaca of three males and three females per unit. Then, it was homogenized and transported to the Biology Laboratory of the Analysis Service Center in Can Tho City, where the chicken survival rate per experimental unit was monitored.

Chemical analyses

Following AOAC (1990) protocols, chemical compositions of CP, OM, DM, CF, EE and Ash in offered feeds were evaluated. While NDF and ADF analysis was conducted according to Van Soest et al. (1991), ME was determined using the formula proposed by Janssen (1989). Bacteria density in excreta were determined following specific methods: *Salmonella. spp* (Quantitative) by ISO-6579-1:2017; *E. coli* (Quantitative) was analyzed accoding to ISO-16649-2-2021 and *Clostridium perfringens* (Quantitative) by ISO 7937.

Statistical analysis

General Linear Model (GLM) of the Minitab 16.2.0 program was used to evaluate the data, and the Tukey method was used to compare the significant differences between two treatments.

RESULTS AND DISCUSSION

Growth performance of Guinea fowls

The daily feed and nutrient intakes were presented in Table 4. Within all items, nutrient intake of Guinea fowl did not change between treatment or daily intakes of DM and OM, CP, EE, CF and ME were resemble among the treatments ($p>0.05$).

Daily weight gain, final live weight and feed conversion ratio of Guinea fowls over treatments were presented in Table 5. The initial weight was not significant in five treatments ($p>0.05$) which can prove that the significant differences of final weight were not affected by initial weights. Besides, the results of daily weight gain were the lowest in the S0 treatment ($p<0.05$), the higher performance was for the treatments supplemented SP or VE, and the high est result (17.3 g/day) was showed in the SP0.2 treatment ($p<0.05$). Also, the final live weights were significantly highest (1.543g/con) in the SP0.2 treatment ($p<0.05$).

Table 4: Daily feed, nutrient and metabolizable energy (ME) intakes of Guinea fowls (g DM/bird)

Item (g/bird)	Treatment					SE	P
	S0	VE0.4	VE0.8	SP0.2	SP0.4		
DM	60.8	60.3	60.7	60.8	60.3	0.45	0.809
OM	57.3	56.8	57.3	57.3	56.9	0.43	0.827
CP	10.9	10.8	10.9	10.9	10.8	0.08	0.814
EE	4.04	4.00	4.03	4.04	4.00	0.03	0.806
CF	3.77	3.73	3.76	3.76	3.74	0.03	0.821
NDF	12.8	12.6	12.7	12.8	12.6	0.09	0.809
ADF	8.44	8.36	8.42	8.43	8.36	0.06	0.790
Ash	3.47	3.44	3.46	3.47	3.44	0.03	0.802
ME (MJ/bird)	0.894	0.886	0.892	0.893	0.887	0.01	0.784

Table 5: Daily weight gain, final live weight (g/bird) and FCR of Guinea fowls

Item	Treatment					SE	P
	S0	VE0.4	VE0.8	SP0.2	SP0.4		
Initial live weight	329	329	331	330	329	2.38	0.869
Final live weight	1465[c]	1518[ab]	1505[b]	1543[a]	1527[ab]	7.73	0.001
Daily weight gain	16.2[c]	16.9[ab]	16.8[b]	17.3[a]	17.1[ab]	0.09	0.001
FCR	3.75[a]	3.55[b]	3.62[ab]	3.51[b]	3.52[b]	0.03	0.004

[abc] Mean values in the same row with different superscripts are different $p<0.05$

Table 6: Carcass values and internal organs of Guinea fowls supplemented SP and VE

Item	Treatment					SE	p
	S0	VE0.4	VE0.8	SP0.2	SP0.4		
Slaughter weight, g	1455[c]	1528[ab]	1515[b]	1555[a]	1530[ab]	7.45	0.001
Carcass weight, g	1068[b]	1097[ab]	1086[ab]	1125[a]	1104[ab]	9.67	0.02
% Carcass	73.4	71.8	71.7	72.3	72.2	0.63	0.382
Breast meat weight, g	238[b]	257[ab]	254[ab]	278[a]	265[ab]	6.61	0.018
Breast meat, %	23.8	23.4	23.4	24.7	24.0	0.33	0.102
Thigh meat weight, g	238[b]	262[ab]	257[ab]	276[a]	268[a]	5.74	0.009
Thigh meat, %	22.3	23.9	23.7	24.5	24.3	0.61	0.167
Gizzard weight, g	23.8	24.0	24.8	22.9	24.0	1.27	0.729
Small intestine L., cm	94.8	94.3	97.8	98.0	96.6	1.53	0.358
Large intestine L., cm	12.9	13.8	13.4	11.9	11.2	0.86	0.256
Cecum L., cm	15.7	14.6	15.2	16.1	15.6	0.58	0.466

[a,b,c] Mean values in the same row with different superscripts are different $p<0.05$

The supplement of SP and VE improved growth performance of Guinea fowls including body weights and feed conversion ratio in this study. It was in line with the study of Merati et al. (2021), Jabbar et al. (2021) and Lee et al. (2022). This could be explained that the supplementation of SP or VE improving antibody resistance and stimulating feed digestion and nutrient absorption, resulted in increasing weight gain. Merati et al. (2021) also noted that it shows the improved efficiency of feed conversion, which may be associated with enhanced intestinal absorption. In addition, the positive effect of S. cerevisiae may be linked to a reduction in the stress responses of birds. This may occur due to a rise in vitamin absorption, enzyme production, and protein metabolism. This enhances digestion and nutrient absorption, resulting in enhanced growth performance (Merati et al., 2021). By producing numerous enzymes, including as lipase, protease, and amylase, the use of microbes may improve nutritional digestion and utilization by broilers. This also explained that the addition of vitamin-enzymes in the diets could be a potential approach to improve growth rate without antibiotics. The final weight of Guinea fowls of our experiment are lower than the

Table 7: The nutrient composition of Guinea fowl meat (% in fresh form)

Item	Treatment					SE	p
	S0	VE0.4	VE0.8	SP0.2	SP0.4		
DM	26.6	26.0	26.7	26.0	25.9	0.32	0.115
OM	98.4	98.2	97.7	98.4	97.6	0.28	0.211
CP	20.8	21.4	21.7	21.8	21.3	0.57	0.844
EE	1.73	1.99	1.58	1.84	1.79	0.24	0.352
Ash	1.58	1.79	2.27	1.59	2.38	0.26	0.223

Table 8: Bacteria density in excreta at 12 -week-old Guinea fowls

Item	Treatment					SE	P
	S0	VE0.4	VE0.8	SP0.2	SP0.4		
E. coli (10^6 CFU/g)	9.34[a]	6.72[ab]	7.23[ab]	4.38[b]	4.82[b]	0.314	0.013
Clostridium perfringens (10^5 CFU/g)	5.28[a]	4.33[ab]	4.28[ab]	3.24[b]	3.63[b]	0.262	0.022
Salmonella. spp/25 g (+/-)	N	N	N	N	N	-	-
Survivable rate (%)	93.3	100	96.7	100	100	1.65	0.061

[a,b] Mean values in the same row with different superscripts are different p<0.05
N: negative

study of Batkowska et al. (2021) and higher than the study of Bernacki et al. (2012) with 1884-1968 g/bird and 1268-1318 g/bird respectively. The values obtained in this study ranged being lightly higher from 1.250-1.470 g/bird, reported by Bernacki et al. (2012). The carcass percentages of Guinea fowls in a present trial were in a range from 69.9 -73.6%; 71.5-73.9% in Guinea fowl works reported by Singh et al. (2015) respectively. The breast meat and thigh meat percentages in a current study was in agreement with the values (22.2-24.7% and 22.2-24.6 %, respectively) from an experiment on Guinea fowls of Dong (2016), while being lightly higher than those (23.1% and 21.2%, respectively) in the Guinea fowl research of Bernacki et al. (2012).

Carcass characteristics and nutrient composition of Guinea fowl meat

The carcass values of Guinea fowls were shown significant differences among treatments (Table 6). The slaughter weight, carcass, breast meat and thigh meat weights were significantly different among treatments which had the higher values in the treatment of 0.2% microorganisms (p<0.05) than those in the others. For other criteria, there were no significant differences.

For meat composition of Guinea fowl (Table 7), the supplements did not improve any changes in DM, OM, CP, EE and Ash (p>0.05). However, from the results, treatments with supplementation were slightly higher than treatment without supplement microorganisms and vitamin-enzymes (p>0.05).

The carcass characteristics were improved by the addition of microorganisms/vitamin enzymes. The significant differences may be due to the increase in body weight and final weight gain. The synthesis of enzymes, and the metabolism of proteins was increased when added enzymes in the diets (Merati et al., 2021) or when added microorganisms (stimulating feed digestion and nutrient absorption) and protein is one of the most important substance to formulate muscles in animals. Thus, the increase in carcass weights could be associated to the increase levels of microorganisms/vitamin.

Intestine health status

Bacteria density in excreta was significant recorded in treatments (Table 8). The amount of *E. Coli, Clostridium perfringens* was different between treatments (p<0.05). The highest amount of *E. Coli, Clostridium perfringens* was detected in treatment without supplementation. In this study both *E. coli* and *C. perfringens* accounts were significantly higher (p<0.05) in chicken excreta of the treatment without supplement (S0), lower values for the VE0.4 and VE0.8 treatments and the lowest for the SP0.2 and SP0.4 treatments (p<0.05). However, *Salmonella spp*. were undetectable in excreta at 12-week-old Guinea fowls (Table 8).The surviral rate was lower (93.3%) for the birds in the S0 and VE0.8 treatments (96.7%), and got100% for the VE0.4 and SP0.2 treatments. *Salmonella spp., E. coli* and *C. Perfringens* are potentially pathogenic bacteria (Yesilbag and Coplan, 2006). The results obtained show that supplementation of SP at 0.2 and 0.4% in concentrate diet reduced *E. coli* and *C. perfringens density* in the intestinal tract of the birds. Therefore, addition of *Saccharomyses cerevisiae* and *Pediococcus acidilactici*has prevented the growth of pathogenic bacteria in the intestines, resulting in enhance nutrient digestibility and reduce mortality for chicken (Fatufe and Matanmi, 2011). Moreover, the supplement of vitamin-digestible enzymes improved feed digestion and nutrient absorption for the birds. Due to the ability of these probiotics to enhance immunity, promote the growth of intestinal microflora, and produce lactic acid, the intestinal environment becomes hostile to the formation of pathogens, hence decreasing the quantity of harmful bacteria in the intestine. Therefore, the growing Guinea fowls had good intestine health due to adding the supplements of benefitial microbes and digestible enzymes besides vaccination of major diseases prior introduce the trial and carefully management.

CONCLUSION

The supplementation of *Saccharomyses cerevisiae*, *Pediococcus acidilactici* and vitamin- enzymes in diets of Guinea fowls improved growth rate, carcass performance and health. At level of 0.2% *Saccharomyses cerevisiae*, *Pediococcus acidilactici* supplemented in concentrate diet gave the higher daily weight gain and carcass values, better FCR and decreased density of *E. coli* and *C. perfringens* in the chicken excreta leading to enhancing intestinal health.

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CONFLICT OF INTEREST

The authors declared that there were no conflicts of interest.

NOVELTY STATEMENT

The research outcomes ongrowth rate, carcass performance and intestinal health of Guinea fowls supplemented *Saccharomyses cerevisiae, Pediococcus acidilactici* and vitamin-enzymes

AUTHORS CONTRIBUTION

1-Nguyen Thi Kim Dong conducted the trial and wrote the article for publication

2- Nguyen Van Thu designed the experiment and analysed data of the experiment.

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