OPEN ACCESS Journal of Animal Health and Production

Research Article

Effect of Natural Multi-Enzyme Supplementation on Growth Performance, Gut Microflora, Carcass Characteristics, and Meat Quality of Broilers Reared in Tropical Climates

Keatisak Soisuwan[1], Hatairat Plaimast[2], Atichat Thongnum[3], Soranot Chotnipat[4], Manatsanun Nopparatmaitree[4*]

[1]Department of Animal Science, Faculty of Agriculture, Rajamagala University of Technology Srivijaya, Thungsong, Nakhon Si Thammarat, 80110, Thailand; [2]Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand; [3]Department of Animal Science and Fisheries, Faculty of Sciences and Agricultural Technology, Rajamangala University of Technology Lanna, Phitsanulok 65000, Thailand; [4]Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT Campus, Cha-am, Phetchaburi 76120, Thailand.

Abstract | This study aimed to investigate the effect of natural multiple-enzyme supplementation on growth performance, gut microflora, carcass characteristics, and the meat quality of broilers reared in tropical climates. This study employed a completely randomized design (CRD). One thousand Ross 308® one-day-old chicks were divided into two treatments, each with 10 replicates and 50 chicks (25 females and 25 males) per experimental unit. Dietary treatment consisted of commercial broilers diets (corn-soybean based diet) supplemented with 0 or 100 g/ton of natural multi-enzymes feed supplementation (top-up feeding). Birds were provided *ad libitum* access to water and experimental diets. The results showed that natural multi-enzyme supplementation had no significant effect on the growth performances of broilers as compared to the control ($P > 0.05$). However, supplementation of natural multi-enzymes increased the viability rate of broilers during the starter period ($P < 0.05$). In addition, natural multi-enzyme supplementation exhibited positive effects on the productivity index, salable bird return, net profits return per bird, return on investment, and feed cost per gain compared to the control group ($P < 0.05$). Likewise, supplementation of natural multiple enzyme had no effect on the viscosity of the duodenal content and cecal microbial population, of broilers ($P > 0.05$). In addition, dietary treatments had no significant changes on carcass composition and meat quality ($P > 0.05$). Inclusion of 100 g/ton feed of natural multi-enzymes supplementation (top up feeding) of broiler chicken significantly affected the economic return of broiler production.

Keywords | Natural multi-enzymes, Performances, Gut microflora, Carcass and meat quality, Broilers

Received | October 25, 2022; **Accepted** | February 25, 2023; **Published** | April 10, 2023
***Correspondence** | Manatsanun Nopparatmaitree, Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT Campus, Cha-am, Phetchaburi 76120, Thailand; **Email:** Nopparatmaitree_m@silpakorn.edu
Citation | Soisuwan K, Plaimast H, Thongnum A, Chotnipat S, Nopparatmaitree M (2023). Effect of natural multi-enzyme supplementation on growth performance, gut microflora, carcass characteristics, and meat quality of broilers reared in tropical climates. J. Anim. Health Prod. 11(2): 129-138.
DOI | http://dx.doi.org/10.17582/journal.jahp/2023/11.2.129.138
ISSN | 2308-2801

INTRODUCTION

Broiler production industry places a strong emphasis on the production in order to achieve goals such as high productivity management, effective nutrient utilization, and high production efficiency (Dersjant-Li et al., 2015). Approximately 60–70% of the cost of producing broilers in this context is attributed to the cost of animal feed (Alqaisi et al., 2017). The corn-soybean diet is a typical feed formulator used in the industrial production of broiler feed. However, the scope for improving the nutritive values of maize and soybean meal-based diets with carbohydrates is limited, since the nutrients in maize and soybean are considered to be highly digestible (Mohiti-Asli et al., 2020). A considerable quantity of non-starch polysaccharides (NSP) are present in maize (9 g/Kg soluble NSP and 6 g/Kg insoluble NSP). Shirmohammad and Mehri (2011) reported that β-glucan, cellulose, and arabinoxylans are all found in the corn cell wall. In addition, Soybean contains 60 g/Kg soluble NSP and 180 – 210 g/kg insoluble NSP, and these may affect energy utilization of diets containing these ingredients (Pluske et al., 2001). Currently, many alternative grains such as wheat, barley, sorghum, canola, or rapeseed are used in feed formulas to lower feed cost, but these ingredients have different nutrient digestibility (Mohammed et al., 2018; Babatunde et al., 2021). In addition, agro-industrial byproducts can be utilized as alternative feedstuffs to reduce the cost of broiler diets. However, feed sources made from agro-industrial byproducts have a high concentration of plant cells, especially non-starch polysaccharides and lignin, which make up 20% of plant-based poultry diets and cannot be readily broken down by broiler's endogenous enzymes (Mohammed et al., 2018). Hamaker and Tuncil, (2014) reported that because NSP differ structurally in terms of linkage patterns, monomer composition, degree of branching, and side chain compositions, it has a range of digestibility in each animal's digestive system. Modern broiler diet formulations frequently include enzymes to reduce anti-nutritive effects impacts of NSP, improve and balance feed resource utilization, decrease the discharge of unused nutrients, and create a more environmentally friendly broiler production process (Musigwa et al., 2021). This enhances NSP utilization and lessens its anti-nutritive effects (Kim et al., 2022). Variations in feed ingredient quality are caused by a variety of factors.

The endogenous enzymes of broiler chickens cannot adequately digest the dietary non-starch polysaccharides (NSP), and a high level of ingestion of these NSP by carbohydrases may exert anti-nutritional effects in broilers by increasing the digesta viscosity and decreasing the nutrient digestibility (Zeng et al., 2015). Exogenous enzymes are supplemented into corn-soya based poultry diets to reduce the effects of differing raw material quality, improve homogeneity and enhance nutrient digestibility (Yaqoob et al., 2022; Kutlu et al., 2019).

Previous research has shown that adding exogenous carbohydrases in poultry diets, including cellulase, glucanase, xylanase and amylase, reportedly increased the nutrient digestibility (Cozannet et al., 2017) and improve energy and protein utilization, and growth rates (Saleh et al., 2019). Indeed, several research groups have reported beneficial responses to pure xylanase and a mixture of carbohydrates in maize-soybean based diets for ducks and chicks (Olukosi et al., 2007). Even so, it is suggested that, since carbohydrases may improve energy digestibility, their beneficial effects are more likely to be observed when applied to nutritionally marginal diets (Kocher et al., 2003). The main mechanism involved includes the exogenous enzymes' ability to improve nutrient availability by reducing the intestinal digesta viscosity through the degradation of non-starch polysaccharides (Musigwa et al., 2021). Cellulase is class of enzymes that breaks down cellulose by hydrolysis. Amylase breaks down starch into sugar, and β-xylanase degrades the linear polysaccharide β-1,4-xylan into xylose, thus digesting the hemicellulose, the major components of plant cell walls. The digesta viscosity is reduced primarily by β-glucanase which catalyzes the digestion of plant's α-glucosan into glucose. Most of the proteases, which breaks down proteins by hydrolyzing the protein's peptide bond, work best in acidic conditions, except for alkaline proteases (Rahman et al., 2014). Several previous experiments have demonstrated fermentation of wheat bran using *Trichoderma* sp. to produce exogenous enzymes in animal feed production. Cavalcante et al. (2007) reported that wheat bran was the best substrate for producing *Trichoderma* spores for all strains tested, especially *T. harzianum* sp. (28.301 spores/gds) and *T. viride* sp. (24.101 spores/gds). Furthermore, Chua et al. (2017) reported that cellulase and xylananse activity of fermented wheat bran peaked at day 4, and solid-state fermentation (SSF) increased the reducing sugar content. Furthermore, they proposed that replacing a basal diet with fermented wheat bran could not only improve growth performance, but also cause optimal gut morphometry in broilers.

Therefore, the objectives of this study were to evaluate the effects of natural multi-enzyme from *Trichoderma* fermented wheat bran on the growth performances, gut microflora, carcass characteristics, and meat quality of broilers reared in tropical climates.

MATERIAL AND METHODS

Ethical Approval

This study was conducted in accordance with the protocol approved by The Animal Care Protocol Management and Review Committee of the Faculty of Animal Science and Agricultural Technology, Silpakorn University, Thailand (Record no. ASAT SU005/2562).

Animals, Experimental Design And Treatment Diets

In this study, one thousand one-day-old mixed-sex Ross 308® broiler chicks were randomly allocated to two dietary treatments in a completely randomized design (CRD).

Each treatment was broken down into 10 replicates with 50 chicks (25 females and 25 males) each. Chicks were vaccinated for Newcastle disease, Marek's disease, and Infectious Bronchitis disease at the hatchery, and for the Gumboro vaccine at 12 days of age. For the 42-day period of the experiment, all birds were raised in an open-sided house system in floor pens with fresh rice hulls. Dietary treatments were composed of group 1 (T1): normal commercial Thai diet (control) and group 2 (T2): T1 + natural multi-enzymes in the levels of 100 g/ton feed (top-up feeding). The natural multi-enzymes used in this study were a micro granular product made of endo-1, 4 xylanase (EC 3.2.1.8), endo-1, 4 glucanase, endo-1, 3(4) glucanase, 2-amylase, and protease, which were fermented on wheat bran by *Trichoderma* sp. All birds received drinking water provided by 4 water cups with 2-gallon capacity per replicate and received the feed in feeding tubs at 4 feeding tubs per replicate. All birds were provided *ad libitum* access to feed and water. The starter (from day 1 to day 21), grower (from day 21 to day 35), and finisher diets (from day 35 to day 42) were used. The nutrient content of the diets used in this study corresponded to the needs of broilers raised in tropical climates according to the National Research Council (1994) as presented in Table 1.

Measurement Of Productive Performance And Economic Benefit Return

Broiler chickens were raised for 42 days and observed daily. The feed intake, body weight, and mortality of the birds were recorded on days 21 (end of the starter phase), day 35 (i.e., the end of the grower stage), and day 42 (i.e., the end of the finisher phase). Feed intake and body weight by replicate were recorded at the beginning and the end of each week, and dead birds were recorded daily. Average daily feed intake (ADFI) (g/bird/day) = cumulative feed intake / (number of birds x number of days); Average daily gain (ADG) (g/bird/day) = final weight gain-initial weight/ number of days; Feed conversion ratio (FCR: Feed/Gain) = cumulative feed intake (kg)/total weight gain (kg), were calculated as indicators of production performance following the formulas described by Marcu et al. (2013). Moreover, Mortality rate = (total birds died / total birds at time of housing) x 100 following the formulas described by Awobajo et al. (2017), viability rate = 100 - mortality rate, and productive index (PI) = [(Viability x BWG x 100) / (FCR x rearing day)] was calculated following the formula of Barbosa et al. (2017). In addition, economic benefit return indicators, such as feed cost per gain (FCG), net profits return per bird (NPR), salable bird return (SBR), and return of investment (ROI), were enumerated using the following formula: FCG = (FCR x feed cost x BWG), NPR = (SBR – FCG), SBR = (price of live chicken × BW), and ROI = (NPR/FCG) × 100 calculated after the experimental period according to the formulas of Ojediran et al. (2017).

Microbial Population In Cecal Content And Determination Of Viscosity In The Duodenal Content

At 42 days of age, a microbial assay was carried out by the method proposed by Torrallardona et al., (2003). One gram of mixed cecal contents from 4 birds (2 females and 2 males) from each replicate were diluted with 9 ml of Buffer-field phosphate buffer solution, followed by further serial dilution in Buffer-field phosphate buffer solution. Duplicated plates were incubated with a 0.1 ml sample and incubated. The microbial groups analyzed were anaerobic bacteria: *Lactobacillus* spp. and *Bifidobacterium* spp., which were grown on MRS ager at 35°C for 72 h under the anaerobic condition in an anaerobic jar (Byrd et al., 2022). Coliform bacteria and *Escherichia coli* were grown on violet red bile agar and MacConkey agar, respectively, at 24 h under aerobic conditions (Keeratipibula et al., 2011). Colonies on each plate were counted on a colony counter, and colony-forming units (CFU) were defined as distinct colonies measured more than 1 mm in diameter (Brugger et al., 2012). The bacterial population was then transformed using the base 10 log method suggested by Feng et al. (2014). The digestive tract was then removed in its entirety, and digesta was manually extracted into a 50-mL centrifuge tube. The method used to prepare the digesta sampling was carried out according to Ayres et al. (2018). The determination of digesta viscosity methodology used was carried out by the procedure suggested by Lamp et al. (2015).

Carcass Characteristics And Meat Quality Measurements

On day 42, four representative birds (2 females and 2 males) from each replicate nearest to the average pen weight were sampled for processing after fasting for 12 hours. The birds selected for processing were weighted for calculation of carcass and cutting percentages. Birds were electrically stunned, bled, scalded, mechanically picked, and manually eviscerated according to Sanchai (2000). The dressed weight (live weight - (weight of blood + feathers + shanks + head)), carcass weight (dressed weight - weight of viscera), and viscera weight (heart, liver, and gizzard) were recorded according to Singh et al. (2010). The carcass was placed on ice for 24 hour before the musculature from the breast area (breast fillet (*Pectoralis major* muscle), tender (*Pectoralis minor* muscle)), thigh, drum-stick, and wing (outer wing and inner wing) were recorded. Dressed percentage ((dressed weight / live weight) × 100), Eviscerate percentage ((carcass weight / live weight) × 100), and cutting percentage ((cutting part weight / live weight) × 100) were calculated using the method described by Singh et al. (2010).

The quality of meat was assessed using the breast meat

Table 1: Feed formula and analyzed composition of the experimental diets (dry matter basis)

Experimental diets	Starter (1–21 days)	Grower (21–35 days)	Finisher (35–42 days)
Ingredients composition (% as fed basis)			
Maize	51.00	53.00	55.00
Rice bran	10.00	10.00	10.00
Soyabean meal, (48% CP)	26.46	24.36	21.50
Fish meal, (58% CP)	6.00	6.00	6.00
Rice bran oil	3.63	4.16	5.00
Dicalcium phosphate (DCP)	0.95	0.69	0.69
Calcium carbonate	0.93	0.81	0.81
Sodium chloride	0.35	0.35	0.35
DL-Methionine	0.18	0.14	0.15
Vitamin–mineral premix[1]	0.50	0.50	0.50
Total	100.00	100.00	100.00
Laboratory analyzed composition (%)[2]			
Dry matter	88.34	90.21	88.19
Organic matter	94.83	95.17	95.83
Crude protein	21.61	19.03	17.10
Ether extract	4.89	5.52	7.17
Crude fiber	2.38	3.03	3.48
Total calcium	0.89	1.03	0.92
Total phosphorus	0.59	0.57	0.58
Salt (NaCl)	0.42	0.43	0.47

[1]Each one kilogram of vitamin–mineral premix contained 20.02 MIU of retinal palmitate, 9.10 MIU of cholecalciferol, 136.50 g of DL-3-tocophyryl acetate, 5.46 g of phylloquinone, 5.46 g of thiamine, 14.56 g of riboflavin, 27.30 g of Ca-D-pantothenate, 7.28 g of pyridoxine, 109.20 g of niacin, 3.64 g of folic acid, 29.12 mg of cobalamin, 237.00 mg of D-biotin, 120 g of manganese, 3.00 g of selenium, 1,000 mg of zinc, 160.00 mg of copper, 400.00 mg of ferrous, 12.50 g of iodine

[2]The analyzed composition of the basal diets used in this experiment which a commercial broiler chicken diets from Mittraphap Feed Mill Co., Ltd.

samples. For pH score at 24 hour, 3 g samples of chicken breasts (at 24 h after slaughter) were homogenized with 27 mL of distilled water in a homogenizer at 6,000 rpm for 30 sec. The pH value was then measured three times using an electronic pH meter according to Tuell et al., (2020). Color of breast meat (at 24 h after slaughter) measured including lightness (L*), redness (a*), and yellowness (b*) using a Minota 410 Chromameter as described by Pathare et al. (2013). Drip loss and cooking loss were measured as an indication of the water holding capacity, following the procedure according to the previously established method described by Yaqoob et al. (2022). To measure the shear force of the meat using the Warner-Bratzier Meat Shear apparatus, the cooked places of the breast meat were cut to obtain six cores (20 x 13 x 13 mm) on each breast sample 6-8 (carrot) according to Zakaria et al. (2010).

Statistical Analysis

Data were analyzed using ANOVA and the difference between dietary treatment means was compared by T-test according to the recommendation of Steel and Torrie (1980) with the R Core Team's description of R program version 3.5.1 (2018). Treatment effects were declared significant at $P < 0.05$, and tendencies for treatment effects were declared at $0.05 < P \leq 0.01$. The following statistical model for the experiment was used:

$Y_{ij} = \mu + T_i + e_{ij}$

Where;

Y_{ij} = Measured response,

μ = Overall mean,

T_i = effect of treatment (I = supplementation of natural multi-enzymes in the levels of 0 and 100 g/ton feed (top-up feeding)),

e_{ij} = random error associated with Y_{ij} observation.

RESULTS AND DISCUSSION

The effects of natural multi-enzymes supplementation on the growth performances of broilers reared in tropical climate are shown in Table 2. The dietary treatments had no effects (P > 0.05) on the feed intake, body weight, body weight gain, feed conversion ratio, and feed cost per kilogram body weight of broilers during 1-21 days of age. However, it was found that the viability rate of the treatment that was supplemented with natural multiple-enzymes at the level of 100 g/ton feed by top-up feeding was significantly (P < 0.05) higher when compared to the control group not supplemented with natural multiple-enzymes. Additionally, feed intake, body weight, body weight gain, feed conversion ratio, feed cost per kilogram bodyweight, and viability rate were unaffected by the dietary treatments between 22 and 35 days of age. During 35-42 days of age, the dietary treatments had no effect (P > 0.05) on the feed intake, body weight, body weight gain, feed conversion ratio, feed cost per kilogram body weight, and viability rate. The dietary treatments during the 35–42 day period did not affect feed intake, body weight, body weight gain, feed conversion ratio, feed cost per kilogram bodyweight, and viability rate (P > 0.05). The dietary treatments had no effects on the total productivity between 1 and 42 days (P > 0.05) with respect to feed intake, body weight, body weight gain, feed conversion ratio, and viability rate. This result was supported by the report of Dalólio et al. (2016) who reported that supplementation of exogenous enzyme complex consisting of phytase, protease, xylanase, β-glucanase, cellulase, amylase, and pectinase in broiler diets did not significantly affect the feed intake, body weight, body weight gain and feed to gain ratio of broilers. Mohammed et al. (2018) reported that multi-enzyme supplementation in broiler chicken diets had no effect on the performance parameters, with the exception of feed intake, body weight, body weight gain, and feed conversion ratio on days 22–35 (P > 0.05).

Although the performance parameters were not clearly affected by the addition of natural multi-enzyme to the diet in this trial. However, the current study found that adding natural multi-enzyme to the diet increased the productive index of broiler significantly over the control group during 0–21 (P < 0.05), 35–42 (P < 0.01), and 0–42 days (P < 0.01). Furthermore, adding natural multi-enzyme to the diet decreased feed cost per gain in comparison to the control group (P < 0.05). In addition, the natural multi-enzyme supplementation in the diet increased salable bird return, net profits return per bird and return on investment as compared to the control group (P < 0.05). Because the broiler industry prioritizes high production rates, the efficient use of external enzyme would serve as an important factor to increase the economic returns (Ravindran, 2013). These findings can be explained by the following scientific principles as follows. In the gastrointestinal tract of broilers, there is no endogenous enzyme for NSP digestion. As a result, the inability of NSP to be digested in the gut resulted in a loss of energy in broiler feed. The use of exogenous enzymes can assist in converting the NSP into short-chain carbohydrates that the broilers can utilize (Rahman et al., 2014). This enzyme preparation contains β-glucanase, protease, and cellulose activity, which may improve the digestion of NSP and protein because it improves the partially broken-down cell wall matrix, allowing encapsulated intracellular nutrients to be released, (Shirmohammad and Mehri, 2011) as well as facilitating contact between endogenous digestive enzymes and their substrates, thus improving the overall nutrient utilization in corn-soy base diets (Zeng et al., 2015). The addition of glucanase counteracted these effects by significantly increasing villi compared to the control group, resulting in changes in nutrient absorption and enhanced productivity performance (Balamurugan et al., 2011).

The duodenal viscosity, which is generally the results of the use of viscous grains and microbial population in the cecal content of broilers, can be improved by natural multi-enzyme supplementation. At 42 days of age (Table 3), the dietary treatments had no effect (P > 0.05) on duodenal viscosity, *Escherichia coli*, Coliform bacteria, *Bifidobacterium* spp., and *Lactobacillus* spp. population in cecal. The authors had suggested that if the formula composed of the regular ingredients such as corn or soybean meal, which had very low non-starch polysaccharide, it has no effect on the gut viscosity when supplemented with exogenous enzyme. These results are consistent with the report of Engberg et al. (2004) who reported that the counts of *Loctobicllus* spp. tend to be higher and the counts of Coliform bacteria tend to be lower in caecum content of broilers fed diets with exogenous xylanase supplementation than that the control group. In addition, these results are in agreement with the report of Gao et al. (2008) who reported that supplementation of exogenous xylanase in broiler diets did not significantly affect the viscosity in duodenum and also did not significantly affect the cecal microbial population.

The effect of natural multiple–enzyme supplementation on carcass characteristics and meat quality of broilers reared in tropical climates are shown in Table 4. The dietary treatments exhibited no significant different effect (P > 0.05) on carcass composition such as dressed percentage, eviscerate percentage, cutting percentage, and viscera percentage as compared to the control. In addition, the pH, meat color in terms of lightness, redness, and yellowness as well as water holding capacity in terms of cooking loss and drip loss were unaffected by the dietary treatments. Moreover, it was also found that supplementation of natural multiple-enzyme not significantly improved the breast meat tenderness (P>0.05). This result is in agreement with previous reports of Dalólio et al. (2016) who reported that exogenous enzyme supplementation had no effect on the relative weight of carcass composition, meat quality and

Table 2: Effect of natural multiple-enzyme supplementation on growth performances and economic benefit return of broilers

Growth performances and economic benefit return[1/]	Level of natural multi-enzyme supplementation in diets		*P*-value
	Control	**100 g/ton**	
Growth performances			
1-21 days of age			
Feed intake (g/bird)	1,195.90±9.22	1,188.60±7.17	NS
Body weight (g/bird)	846.81±12.11	852.16±14.65	NS
Body weight gain (g/bird)	801.92±11.83	808.51±14.74	NS
Feed conversion ratio	1.46±0.014	1.45±0.017	NS
Viability rate (%)	99.40±0.43	99.80±0.20	*
Productive index	259.98±1.86	264.99±2.54	*
22-35 days of age			
Feed intake (g/bird)	1,738.40±20.46	1,704.10±12.38	NS
Body weight (g/bird)	1,766.80±24.93	1,751.30±16.64	NS
Body weight gain (g/bird)	906.95±20.32	899.36±17.37	NS
Feed conversion ratio	1.92±0.02	1.89±0.02	NS
Viability rate (%)	97.82±1.06	99.00±1.34	NS
Productive index	331.78±3.45	336.36±2.91	NS
35-42 days of age			
Feed intake (g/bird)	1,113.70±24.61	1,109.90±40.94	NS
Body weight (g/bird)	2,285.50±19.98	2,305.90±32.11	NS
Body weight gain (g/bird)	539.46±18.27	553.68±23.18	NS
Feed conversion ratio	2.06±0.07	2.02±0.08	NS
Viability rate (%)	94.75±2.10	95.90±1.56	NS
Productive index	354.16±3.69	375.05±4.27	**
1-42 days of age			
Feed intake (g/bird)	4,047.90±35.47	4,002.60±42.54	NS
Body weight (g/bird)	2,285.50±19.98	2,305.90±32.11	NS
Body weight gain (g/bird)	2,248.30±24.32	2,261.50±32.21	NS
Feed conversion ratio	1.80±0.07	1.76±0.08	NS
Viability rate (%)	92.18±2.70	94.90±1.70	NS
Productive index	274.10±1.67	290.27±2.08	**
Economic benefit return[#](USD/bird)			
Feed cost per gain	2.38+0.01	2.34+0.01	*
Salable bird return	2.68±0.02	2.71±0.03	*
Net profits return	0.30±0.01	0.37±0.01	*
Return of investment (%)	12.60±0.20	15.81±0.31	*

[1/] Values are means of ten replicates of 50 chicks each, presented as means ± SEM
NS: not significant, *: P < 0.05, **: P < 0.01.
1 USD = 38.325 THB,
[#] Feed cost per gain (FCR x feed cost (0.587 USD/Kg) x BWG), Salable bird return = (Price of live chicken (1.175 USD) x Body weight), Net profits return per bird = (SBR – FCG), and Return of investment = (NPR /FCG) x 100

pH of broilers. In addition, these findings were consistent with previous reports of Mohammed et al. (2018) who found that adding multi-enzymes to broiler diets had no impact on carcass and meat quality parameters as well as Yaqoob et al. (2018) who discovered that the addition of multi-enzymes to broiler diets had no effect on the cutting weight, internal organs weight, shear force and color of the breast meat.

Table 3: Effect of natural multiple-enzyme supplementation on viscosity of duodenal content and cecal microbial population of broilers at 42 days of age

Duodenal content viscosity and cecal microbial population[1/]	Level of natural multi-enzyme supplementation in diets		*P*-value
	Control	**100 g/ton**	
Viscosity of duodenal content (CPS)	14.36±1.405	14.58±2.08	NS
Cecal microbial population (Log $_{10}$ CFU/ml)			
Bifidobacterium spp.	5.79±0.25	5.96±0.28	NS
Lactobacillus spp.	5.87±0.23	5.88±0.26	NS
Escherichia coli	5.46±0.27	5.72±0.29	NS
Coliform bacteria	5.35±0.23	5.30±0.23	NS

[1/] Values are means of ten replicates of 50 chicks each, presented as means ± SEM
NS: not significant

Table 4: Effect of natural multiple-enzyme supplementation on carcass characteristic and meat quality of broilers 42 days of age

Carcass characteristic and meat quality[1/]	Level of natural multi-enzyme supplementation in diets		*P*-value
	Control	**100 g/ton**	
Carcass characteristic			
Live weight (g/b)	2,292.00±27.85	2,302.00±35.18	NS
Carcass percentage (%)			
Dressed percentage	91.97±0.20	92.51±1.30	NS
Eviscerate percentage	88.47±0.18	88.09±0.22	NS
Cutting percentage (%)			
Pectoralis major muscle	19.40±0.37	18.96±0.36	NS
Pectoralis minor muscle	3.71±0.04	3.61±0.08	NS
Thigh	12.46±0.22	12.48±0.31	NS
Drumstick	10.21±0.16	9.92±0.13	NS
Outer wing	3.74±0.33	3.75±0.04	NS
Inter wing	4.17±0.09	4.15±0.07	NS
Viscera organ percentage (%)			
Liver	2.22±0.05	2.24±0.07	NS
Gizzard	1.49±0.05	1.53±0.03	NS
Heart	0.52±0.02	0.49±0.01	NS
Meat quality			
Breast meat pH (score)	5.87±0.03	5.84±0.03	NS
Breast meat color			
L* (Lightness)	57.50±3.44	58.50±2.11	NS
a* (Redness)	3.04±0.15	3.21±0.17	NS
b* (Yellowness)	13.51±0.31	12.82±0.28	NS
Water holding capacity (%)			
Cooking loss	21.32±1.37	22.58±1.40	NS
Drip loss	3.40±0.38	3.24±0.29	NS
Shear force (kg/cm^2)	1.34±0.10	1.32±0.05	NS

[1/] Values are means of ten replicates of 50 chicks each, presented as means ± SEM
NS: not significant

CONCLUSION

Natural multi-enzyme supplementation, when fed with commercial broiler diets, had no effect on the growth performance of broilers reared in tropical climates. However, supplementation of natural multi-enzyme significantly increased viability rate of broilers during the starter period. In addition, natural multi-enzyme supplementation showed positive effects on the productivity index as well as salable bird return, net profits return per bird, and return of investment of broiler chicken production. It was also found that supplementation of natural multi-enzyme had no effect on duodenal content viscosity, cecal microbial population, carcass composition and meat quality of broilers.

ACKNOWLEDGEMENT

The authors would like to express a great appreciation to the Department of Animal Science, Faculty of Agriculture, Rajamagala University of Technology Srivijaya for the facility and technical support for this trial. The authors are very grateful to the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University for the laboratory and technical support for this experiment.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

NOVELTY STATEMENT

This research investigated a novel feed additive from natural source with high safety for broiler production in Tropical climates. The research also provides evidence of improved economic benefit return from the experimenented natural feed additive.

AUTHORS' CONTRIBUTION

KS: Conceptualized, husbandry work, laboratory work, data collected and analysis. HP and AT: laboratory work. SC: Editing and revising the manuscript. MN: Conceptualized, and wrote the manuscript. The final manuscript was read and approved by all authors.

REFERENCES

Alqaisi O, Ndambi OA, Williams RB (2017). Time series livestock diet optimization: cost-effective broiler feed substitution using the commodity price spread approach. Agric. Food Econ. 5:25. https://doi.org/10.1186/s40100-017-0094-9

Awobajo SAK, Akintan YM, Igbosanu AO, Mako AA, Olatokunbo OT (2007). The mortality rate in the two breeds of broiler on brooding. World Appl. Sci. J. 2(4): 304-308.

Ayres V, Baldwin HL, Li X, Xu H, Raab RM, Boney JW, Broomhead JN, Moritz JS (2018). The effect of corn-expressed carbohydrase on performance and digesta viscosity of broilers fed a high non-starch polysaccharide diet. J. Appl. Poult. Res. 27:499–506. https://doi.org/10.3382/japr/pfy049

Babatunde OO, Park CS, Adeola O (2021). Nutritional Potentials of Atypical Feed Ingredients for Broiler Chickens and Pigs. Animals (Basel). 11(5): 1196. https://doi.org/10.3390/ani11051196

Balamurugan R, Chandrasekaran D, Kirubakaran A (2011). Effects of multi-enzyme supplementation on gut morphology and histomorphology in broilers. Indian J. Sci. Technol. 4(1): 15-18. https://doi.org/10.17485/ijst/2011/v4i1/29924

Barbosa FJA., Almeida M, Shimokomaki M, Pinheiro JW, Silva CA, Michelan FT, Bueno FR, Oba A (2017). Growth performance, carcass characteristics and meat quality of griller-type broilers of four genetic lines. Braz. J. Poult. Sci. 19(1):109-114. https://doi.org/10.1590/1806-9061-2016-0261

Brugger SD, Baumberger C, Jost M, Jenni W, Brugger U, Mühlemann K (2012). Automated counting of bacterial colony forming units on agar plates. PLoS One. 7(3): e33695. https://doi.org/10.1371/journal.pone.0033695

Byrd PM, Fallico V, Tang P, Wong C (2022). Novel microaerobic agar plate method delivers highly selective and accurate enumeration of probiotic lactobacilli in freeze-dried blends containing bifidobacteria. J. Microbiol. Methods. 195(2022): 106451. https://doi.org/10.1016/j.mimet.2022.106451

Cavalcante RS, Lima HLS, Pinto GAS, Gava CAT, Rodrigues S (2007). Effect of moisture on *Trichoderma Conidia* production on corn and wheat bran by solid state fermentation. Food Bioprocess Technol (2008) 1:100–104. https://doi.org/10.1007/s11947-007-0034-x

Chua TY, Lob CT, Changc SC, Lee TT (2017). Effects of *Trichoderma* fermented wheat bran on growth performance,intestinal morphology and histological findings in broiler chickens. Italian J. Anim. Sci. 16(1): 82–92. http://dx.doi.org/10.1080/1828051X.2016.1241133

Cozannet P, Kidd MT, Neto RM, Geraert PA (2017). Next-generation non-starch polysaccharide-degrading, multi-carbohydrase complex rich in xylanase and arabinofuranosidase to enhance broiler feed digestibility. Poult. Sci. 96(8): 2743-2750. https://doi.org/10.3382/ps/pex084

Dalólio FS, Moreira J, Vaz DP, Albino LFT, Valadares LR, Pires AV, Pinheiro SRF (2016). Exogenous enzymes in diets for broilers. Rev. Bras. Saúde Prod. Anim. Salvador. 17(2):149-161. https://doi.org/10.1590/S1519-99402016000200003

Dersjant-Li IY, van de Belt K, van der Klis JD, Kettunen H, Rinttilä T, Awati A (2015). Effect of multi-enzymes in combination with a direct-fed microbial on performance and welfare parameters in broilers under commercial production settings. J. Appl. Poult. Res. 24(1): 80-90. https://doi.org/10.3382/japr/pfv003

Engberg RM, Hedemann MS, Steenfeldt S, Jensen BB (2004). Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83(6): 925-938. https://doi.org/10.1093/ps/83.6.925

Feng C, Wang H, Lu N, Chen T, He H, Lu Y, Tu XM (2014). Log-transformation and its implications for data analysis. Shanghai Arch. Psychiatry. 26(2): 105–109. https://doi.org/10.3969/j.issn.1002-0829.2014.02.009

Gao F, Jiang Y, Zhou GH, Han ZK (2008). The effects of xylanase supplementation on performance, characteristics of the gastrointestinal tract, blood parameters and gut microflora in broilers fed on wheat-based diets. Anim. Feed Sci. Technol. 142(1-2): 173-184. https://doi.org/10.1016/j.anifeedsci.2007.07.008

Hamaker BR, Tuncil YE (2014). A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. J. Mol. Biol. 426(23): 3838-3850. https://doi.org/10.1016/j.jmb.2014.07.028

Keeratipibula S, Phewpana A, Lursinsapb C (2011). Prediction of coliforms and *Escherichia coli* on tomato fruits and lettuce leaves after sanitizing by using Artificial Neural Networks. LWT - Food Sci. Technol. 44(1): 130-138. https://doi.org/10.1016/j.lwt.2010.05.015

Kim E, Morgan NK, Moss AF, Li L, Ader P, Choct M (2022). The flow of non-starch polysaccharides along the gastrointestinal tract of broiler chickens fed either a wheat- or maize-based diet. Anim. Nutr. 9 (2022): 138-142. https://doi.org/10.1016/j.aninu.2021.11.004

Kocher A, Choct M, Ross G, Broz J, Chung TK (2003). Effect of enzyme combinations on apparent metabalizable energy of corn-soybean meal-based diets in broilers. J. Appl. Poult. Res. 12(3): 275-283. https://doi.org/10.1093/japr/12.3.275

Kutlu HR, Saber SN, Kutay H, Celik L, Uzu Y, Toy N, Kutlu M, Yucelt O, Burgut A, Thiery P, Yavuz B (2019). Effect of multi-enzyme produced by a single fungus on growth performance and some carcass parameters of broiler chicks fed on maize-soya based diets. Kafkas Univ. Vet. Fak Derg. 25 (2): 221-230, https://doi.org/10.9775/kvfd.2018.20765

Lamp AE, Evans AM, Moritz JS. (2015). The effects of pelleting and glucanase supplementation in hulled barley based diets on feed manufacture, broiler performance, and digesta viscosity. J. Appl. Poult. Res. 24(3): 295-303. https://doi.org/10.3382/japr/pfv028

Marcu A, Opriş IV, Dumitrescu G, Ciochină LP, Marcu A, Nicula M, Peţ I, Dronca D, Kelciov B and Mariş C (2013). The influence of genetics on economic efficiency of broiler chickens growth. J. Anim. Sci. Biotechnol. 46(2): 339-346.

Mohammed AA, Habib AB, Eltrefi AM, Shulukh ESA and Abubaker AA (2018). Effect of different levels of multi-enzymes (Natuzyme Plus®) on growth performance, carcass traits and meat quality of broiler chicken. Asian J. Anim. Vet. Adv. 13(1): 61-66. https://doi.org/10.3923/ajava.2018.61.66

Mohiti-Asli M, Ghanaatparast-Rashti M, Akbarian P Mousavi SN (2020). Effects of a combination of phytase and multi-carbohydrase enzymes in low-density corn–soybean meal based diets on growth performance and ileal nutrients digestibility of male broilers. Ital. J. Anim. Sci. 19(1): 1533–1541 https://doi.org/10.1080/1828051X.2020.1857311

Musigwa S, Morgan N, Swick RA, Cozannet P, Kheravii SK, Wu SB (2021). Multi-carbohydrase enzymes improve feed energy in broiler diets containing standard or low crude protein. Anim. Nutr. 7(2021): 496-505. https://doi.org/10.1016/j.aninu.2020.08.008

National Research Council. (1994). Nutrient requirement of poultry. 9th Edn. National Academy Press, Washington, DC.

Ojediran TK, Fasola MO, Oladele TO, Onipede TL, Emiola IA (2017). Growth performance, flock uniformity and economic indices of broiler chickens fed low crude protein diets supplemented with lysine. Arch. Zootec., 66 (256):543-550.

OluKosi OA, Bedford MR, Adeola O (2007). Xylanase in diets for growing pigs and broiler chickens. Can. J. Anim. Sci. 87: 227-235. https://doi.org/10.4141/CJAS06005

Pathare PB, Oparaand UL, Al-Said FA (2013). Colour measurement and analysis in fresh and processed foods: a review. Food Bioproc. Tech. 6: 36–60; https://doi.org/10.1007/s11947-012-0867-9

Pluske JR, Kim JC, McDonald DE, Pethick DW, Hampson DJ (2001). Non-starch polysaccharides in the diets of young weaned piglets. In: Varley, M.A. and Wiseman, J., (eds.) the weaner pig: nutrition and management. CABI Publishing, Wallingford, UK, pp. 81-112.

R Core Team (2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Austria. URLhttp:/www.R-project.org/

Rahman A, Saima, Pasha TN, Younus M, AbbasY, Ditta YA. (2014). Effect of multi-enzymes supplementation on growth performance of broiler. Pakistan J. Zool. 46(2): 417-422.

Ravindran V (2013). Feed enzymes: The science, practice, and metabolic realities. Journal Applied of Poultry Research. 22(3): 628-636. https://doi.org/10.3382/japr.2013-00739

Saleh AA, Kirrella AA, Abdo SE, Mousa MM, Badwi NA, Ebeid TA, Nada AL, Mohamed MA (2019). Effects of dietary xylanase and arabinofuranosidase combination on the growth performance, lipid peroxidation, blood constituents, and immune response of broilers fed low-energy diets. Animals. 9(7): 467. https://doi.org/10.3390/ani9070467

Sanchai C (2000). Meat science Technology. Department of Animal Science Faculty of Agriculture, Chiangmai University. Thailand.

Shirmohammad F, Mehri M (2011). Effects of dietary supplementation of multi-enzyme complex on the energy utilization in rooster and performance of broiler chicks. Afri. J. Biotechnol. 10(38): 7541-7547. https://doi.org/10.5897/AJB10.2260

Singh J, Verma CP, Sharma RK (2010). Effect of period of supplementation of multi-enzyme on carcass yield and serum minerals in broilers during hot humid weather. Haryana Vet. 49(December, 2010): 59-61.

Steel R, Torrie JH. (1980). Principle and procedures of Statistics: A Biometrical Approach 2th ed.: McGraw Hill Book Co. New York. USA.

Torrallardoma D, Conde MR, Badiola I, Polo J, Brufau J (2003). Effect of fish meal replacement with spray dried animal plasma and colistin on intestinal structure, intestinal microbiology and performance of weaning pigs, challenged with *Escherichia coli* K99. J. Anim. Sci. 81(5): 1220-1226. https://doi.org/ 10.2527/2003.8151220x

Tuell JR, Park JY, Wang W,Cheng HW, BradKim YH (2020). Functional/physicochemical properties and oxidative stability of ground meat from broilers reared under different photoperiods. Poult. Sci. 90(7): 3761-3768. https://doi.org/10.1016/j.psj.2020.04.021

Yaqoob MU, Yousaf M, Iftikhar M, Hassan S, Wang G, Imran S, Zahid MU, Iqbal W, Wang M (2022). Effect of multi-enzymes supplementation on growth performance, meat quality, ileal digestibility, digestive enzyme activity and caecal microbiota in broilers fed low-metabolizable energy diet. Anim. Biosci. 35(7): 1059-1068. https://doi.org/10.5713/ab.21.0402

Zakaria HAH, Jalal MAR, Ishmais MAA (2010). The influence of supplemental multi-enzyme feed additive on the performance, carcass characteristics and meat quality traits of broiler chickens. Int. J. Poult. Sci. 9(2): 126-133.

Zeng Q, Huang X, Luo Y, Ding X, Bai S, Wang J, Xuan Y, SuZ, Liu Y, Zhang K (2015). Effects of a multi-enzyme complex on growth performance, nutrient utilization and bone mineralization of meat duck. J. Anim. Sci. Biotechnol. 2015(6):12. https://doi.org/10.1186/s40104-015-0013-4