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Influence of liquid enzymes supplementation on growth performance, blood parameters and muscle fatty acids in broilers

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Abstract

Objective: The present study aimed to investigate the effect of liquid enzyme supplementation to drinking water on growth performance, plasma lipids and muscle fatty acids profile in broilers.

Methods: One hundred and twenty one-day old broilers were divided into four groups (30 birds), each group included three replicates of 10 chicks. The first group was control without any additives supplementation in feed or water. The second, third and fourth groups were supplemented in drinking water with 2, 4, and 8 ml/ L water of Non-Starch Polysaccharides (NSP) liquid enzyme, respectively.

Results: Body weight and body weight gain were increased by 8 ml/ L water liquid enzyme supplementation. Feed intake was decreased and thus, feed conversion was significantly improved. The addition of NSP liquid enzymes in water did not affect the carcass, muscle relative weights, while abdominal fat relative weight was significantly decreased by the addition of 8ml/L NSP enzymes in drinking water. Spleen weight was increased. Plasma total cholesterol, LDL-cholesterol, glutamic oxalacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) were significantly decreased following the addition of 8ml/L NSP enzymes. Plasma HDL-cholesterol was significantly increased. Liver MDA and muscle palmitic acid concentration were decreased, however, alpha-linolenic acid was increased by liquid enzyme supplementation.

Conclusion: It could be concluded that liquid enzyme supplementation to drinking water improved growth performance, modified plasma lipids and muscle fatty acids profile in broilers.

Keywords: liquid enzymes, fatty acids, lipid peroxidation, cholesterol, broilers

1. Introduction

Broilers have great benefits as a source of human protein. Therefore, numerous studies focus on broiler's nutrition to maintaining sustainable broiler production to meet the human demand for protein. So that the balanced ration formulation is of great importance for poultry production (Ravindran, 2010; Field et al. 2000). In the poultry industry, the cost of energy-contributing ingredients constitutes about 65% of the dietary cost. Therefore, several trials were done for lowering the cost by decreasing the percentages of some energy ingredients along with stimulating the growth performance of broiler chickens (Donohue and Cunningham 2009). One strategy is the enzyme supplements to broiler which enhances the growth performance parameters such as feed intake, feed conversion ratio (FCR) or weight gain (Horvatovic et al. 2015). Enzymes are effective to be supplemented to cereals-based diets, wheat, barley and corn due to the high content of non-starch polysaccharides (NSP) for enhancing the performance of monogastric animals (Bedford 2000). FCR was improved by the dietary inclusion of exogenous enzymes in the grower phase due to enhanced digestibility and lessened viscosity of digesta (Horvatovic et al. 2015; Almirall et al. 1995). Besides, the stimulation of growth performance as a result of enzyme supplementation could be attributed to their role in decreasing the viscosity of intestinal contents, and modulation of gut microbiota (Abdel-Latif et al. 2017; Choct et al. 1996). The addition of xylanase (EC 3.2.1.8) to a wheat-based diet could significantly decrease heat loss, leading to greater net energy and a better FCR and modulating the development of intestinal microbes of broiler chickens (Nian et al. 2011). Olukosi et al. (2015) studied the effect of protease (EC 3.4.21.62) alone or in a mixture with xylanase and amylase (EC 3.2.1.1) on broiler chicken's diet and they concluded that protease, at the lower dose, improved nutrient utilization and increased solubilization of NSP components and also could improve the digestibility of protein and amino acid in broilers (Romero et al. 2014; Olukosi et al. 2015; Saleh et al. 2020). The combination of xylanase, amylase, and protease is better than protease alone. The enzyme β-mannanase in broiler chicken's diet could hydrolyze β-mannans, decreasing the viscosity of intestinal content and enhancing the nutrient digestibility, as well as improving the gut environment (Barros et al. 2015).

One of the major objectives of the current study was to evaluate the impacts of adding liquid enzymes in drinking water on the growth performance, lipid peroxidation and blood constituents in broilers.

From this point of view, the addition of exogenous enzymes to the broiler chicken's diets aids in proper digestibility that produces the building blocks (fatty acids, monosaccharides, and amino acids) of lipid, carbohydrates and protein. Therefore, the current study was carried out to assess the impact of various commercial multi-enzyme on growth performance

2. Materials and methods

The study was approved by the Ethics Committee of Local Experimental Animals Care Committee and conducted in accordance with the guidelines of Kaferelsheikh University, Egypt (Number 4/2016 EC).

2.2. Study design and diets formulation

Ross 308 broiler chicks (n= 120), one-day old were divided into four groups, each included three replicates of 30 chicks (floor pens; ten birds/m^2). Chicks were raised in a windowed experimental farm. The experiment was started during February and brooding heat was provided and the temperature inside the barn was maintained at around 32 to 34°C from day 1 to day 5 post-hatch, and gradually decreased to 24°C at 21d. The control diet was based on corn, soybean meal, and corn gluten meal (Table 1). The first group was control without any additives in feed or water. The second, third and fourth groups were supplemented with 2, 4 and 8 ml/ L water of NSP liquid enzyme, respectively. The NSP liquid enzymes used is commercial enzymes product (FRAZYME MSP) from FRAMELCO BV Company, Netherland. Each 1 ml NSP contained 1600 BXU Xylanase, 2400 BU 1,3 (4) Beta- glucan, 210 U Pectinase, 2100 IU Alpha- Amaylase, 3000MNU Mannanese, 0.7 mg Protease, 1000 FTU Phytase. Broilers were fed *ad-libitum* starter, grower and finisher mash diets (day 1 to 42 of age) and water was available freely. Mortality was registered daily through the experiment period.

2.3. Growth performance

The body weight (BW) and feed consumption were registered every week. Feed conversion ratio (FCR) was considered by dividing feed intake by the weight gain.

2.4. Blood and liver sampling

At day 42, broiler chickens (n= 24) based on average final BW were selected (6 birds/ group). They were weighed individually, slaughtered, and dissected to collect visceral organs. Weights of hot carcass, breast muscle, liver and abdominal fat were recorded. Blood samples were collected from the wing vein immediately before slaughtering, gathered into heparinized test tubes, and then rapidly centrifuged (3000 rpm for 20 min at 5 ˚C) to separate the plasma. Plasma was stored at -20 ˚C pending analysis.

2.5. Biochemical analysis

Total protein, albumin, total cholesterol (CHO), triglycerides (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, glucose, glutamic oxalacetic transaminase (GOT), and glutamate pyruvate transaminase (GPT) were measured using kits from (Diamond Diagnostics, Egypt) according to the procedure outlined by the manufacture

2.6. Fatty acids levels in Breast Muscle

Palmatice, oleic and linolenic acids contents in breast muscle were analysed according to (Ceylan and Aksu 2011). Briefly, 10g of breast muscle and 40 ml of 0.1 N HCl were homogenized for 45 s at 4 °C then were centrifuged at 15,000 × g for 50 min at 4 °C. The supernatants were filtered and were analyzed using (GC-4 CM-PFE, Shimadzu gas chromatograph, Tokyo, Japan) equipped with a flame ionization detector (FID).

Table 1 . Composition and calculated levels of the basal diets

	Starter (0-10 days)	Grower (11-24 days)	Finisher (25-32days)
Ingredients (g/kg)			
Yellow corn	575	615	650
Soybean meal, 44%	320	278	240
Corn gluten meal, 60%	58	50	40
Premix[1]	3	3	3
Plant Oil	7.5	17.5	30.5
Di-calcium phosphate	15	15	15
Limestone	13.9	13.2	12.5
NaCl	3	3	3
DL- Methionine	1.85	1.8	1.65
L- Lysine. Hcl	2.75	3.5	4.35
Total	1000	1000	1000
Nutrients Levels,%			
Crude Protein (Analyzed)	23	21	19
Digestible Lys	1.19	1.14	1.11
Digestible Meth + Cysteine	0.84	0.78	0.71
Digestible Thr	0.77	0.70	0.63
Calcium	1.0	0.94	0.91
Available phosphorus	0.46	0.45	0.44
ME, MJ/Kg	12.43	12.85	13.15

[1] Hero mix® (Hero pharm, Cairo, Egypt). Composition (per3 kg): vitamin A 12,000,000 IU, vitamin D3 2,500,000 IU, vitamin E 10,000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30,000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10,000 mg, manganese 60,000 mg, zinc 50,000 mg, iron 30,000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg

2.7. Assaying malondialdehyde

Lipid peroxidation was evaluated by assaying the level of malondialdehyde (MDA) in the liver using kits from Cell Biolabs Inc. (San Diego, CA, USA).

2.8. Statistical analysis

The differences between the experimental treatments and the control were analyzed with a General Liner model using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). Tukey's multiple comparison test was used to identify which treatment conditions were significantly different from each other at a significance level of $p < 0.05$.

3.Results and discussion

The data presented in Table 2 show that the addition of NSP liquid enzymes in water significantly ($p < 0.05$) increased body weight gain and improved FCR. On the other hand, the feed intake was decreased. The inclusion of liquid enzymes in the present study improved the growth performance in broilers, and this improvement might be related to the enhancement of nutrient digestibility by NSP enzyme supplementation. This is in harmony with Nortey et al. (2008), who suggested that dietary exogenous enzyme addition had a beneficial effect on nutrient digestibility in swine specimens. Moreover, Slominski et al. (2000) reported that the inclusion of a debranching

enzymes mixture improved the overall enzyme effectiveness and consequently enhanced the nutrient digestibility and alleviation of negative impacts of NSP Slominski et al. (2000). Recently, Ravn et al. (2018) stated that the addition of an enzyme combination (xylanase (Xyl) and arabinofuranosidase (Abf)) improved duodenum villi length, which was probably involved in enhancing the growth performance, including body weight and FCR, in broilers Saleh et al. (2020).

Growth performance was improved by 8 ml/ L water liquid enzyme supplementation. These findings are in correspondence with Cozannet et al., (2017) who demonstrated that a dietary combination of Xyl and Abf had a positive effect on the energy utilization and digestibility of protein, starch, fat, and insoluble and soluble fibers. Additionally, Cowieson and Ravindran (2008) reported improvements in crude protein and amino acid digestibility when a multiple enzyme mixture including protease, Xyl, and amylase was employed to supplement corn-soybean diets (Cozannet et al. 2017). Similarly, Rutherfurd et al. (2007) found enhancements in crude protein and amino acid digestibility in broilers fed commercial diets supplemented with multiple enzymes complex, including amylase, β-glucanase, and Xyl. Cowieson and Ravindran (2008) reported that the mechanisms that enhanced the amino acid utilization due to the addition of exogenous enzymes are connected with minimizing endogenous losses related to a decreased secretion of endogenous enzymes. Moreover, Meng et al. (2005) stated that dietary enzymes take off the nutrient encapsulating effect of NSP, thus enhancing the nutrient availability to endogenous enzymes and improving the overall nutrient digestibility and intestinal microbial environment (Cozannet et al. 2017).

Breast muscle relative weights were significantly increased by added NSP liquid enzymes in water, while abdominal fat relative weight was significantly decreased in both low-energy diets compared to the control. However, carcass, gizzard, heart relative weights were not affected by added NSP liquid enzymes in water. These findings are in agreement with previous reports (Francesch et al. 1994; Liu et al. 2007; Saleh et al. 2019) who reported that organ weights were increased by added NSP enzymes in broiler diets. However, Farran et al. (2010) found that breast muscle, pectoralis major, thigh, and drum yields were not affected by the inclusion of enzyme preparations. Garipoglu et al. (2006) reported that the dressing percentage was reduced, while abdominal fat weight was not influenced, by feeding diets supplemented with multienzymes. Similarly, Kocher et al. (2003) found that Contrarily, Garcia et al. (1997) reported that Xyl and β-glucanase supplementation of barley–wheat-based diets elevated the abdominal fat content in broilers. In the present study (Table 3), the lower abdominal fat relative weight noted in low-energy diets, with or without enzyme supplementation, might be attributed to the fact that the lower energy diets caused less fattening and were also connected with numerically reducing the feed intake.

Table 4 shows the effect of the addition of NSP liquid enzymes in the water on blood biochemical parameters. Plasma total protein, globulin, and HDL-cholesterol were significantly increased by adding 8 ml/L of liquid enzymes in drinking water. However, plasma total cholesterol was significantly reduced; however, plasma GOT, GPT, albumin, triglycerides, glucose and, LDL-cholesterol were not affected. Regarding the plasma lipid profile, the data tabulated in Table 4 indicated that the addition of NSP liquid enzymes in water decreased plasma total cholesterol (P = 0.016), triglycerides (P = 0.2), and LDL-cholesterol (P = 0.6), and increased HDL-cholesterol (P = 0.04). These modifications in plasma lipids might be related to a low numerical feed intake (P = 0.18). Liver function indicators (plasma GOT and GPT) were not significantly affected. These results are in harmony with Ahmad et al., (2013) who evaluated the effect of dietary Xyl addition on plasma biochemical constituents in broilers, and illustrated that Xyl might be safe in poultry rations without negative effects on vital organ functions. Additionally, Saleh et al. (2018) reported that the serum concentrations of GOT, GPT, and creatinine were not significantly affected by dietary enzyme supplementation.

The data presented in Table 5 illustrate the shows the effect of the addition of NSP liquid enzymes in the water on the muscle content of fatty acids, and liver MDA content. Liver MDA content was not affected, while muscle oleic and linolenic acid content were significantly increased by feeding. However, the muscle contents of palmatic acid were not significantly influenced by dietary treatments.

Regarding muscle lipid peroxidation index, the liver MDA content was significantly reduced. However, the muscle contents of oleic, and linolenic acids were significantly increased by dietary treatments. These findings are in agreement with Cowieson and Ravindran, (2008) who found enhancements in the digestibility of lysine, methionine, cysteine, and threonine when a multiple enzyme mixture possessing protease, Xyl, and amylase was used to supplement corn-based diets, but these improvements did not affect the amino acid contents in muscle. Furthermore, Head et al. (2019) reported that dietary α-linolenic acids in the form of linseed resulted in a significant increase of hepatic n-3PUFA; however, the inclusion of a multiple enzyme complex of Xyl and amylase in a linseed-based diet resulted in a reduction in the n-6PUFA-like linoleic acid, but oleic and linolenic acids were not affected. Muscle Thiobarbituric acid reactive substance (TBARS) concentration was decreased by reducing the energy in diets and this agreed with (Cho and Kim 2013; Haščík et al. 2015; Saleh et al. 2019; Saleh et al. 2020) who observed that muscle MDA concentration was decreased in low-energy density diets supplemented with or without β-mannanase and Xyl supplementation in pigs.

4. Conclusion

Based on the data presented above, it could be concluded that the addition of NSP liquid enzyme in drinking water by 8 ml/L water might be involved in growth performance, lipid peroxidation and modified plasma and muscle lipids profile in broilers.

Table 2. Influence of liquid enzymes supplementation on growth performance in broilers.

	Control	2ml/L	4ml/L	8ml/L
Initial body weight, gram	44.63±0.09	44.7±0.17	44.53±0.19	44.93±0.07
Final body weight, gram/42 day	2408±40.278^{b}	2417±46.6^{b}	2550±63.9^{ab}	2624±51.3^{a}
Body weight gain, gram/42 day	2363.7±40.4^{b}	2372.8±46.4^{b}	2505.8±63.7^{ab}	2579.7±51.4^{a}
Feed intake, gram/42 day	4008.6±23.1^{a}	3686.7±46.7^{b}	3695.2±84.3^{b}	3796.3±54.6^{b}
FCR	1.697±0.020^{a}	1.555±0.046^{b}	1.478±0.065^{b}	1.472±0.009^{b}

^{a,b} Mean values with different letters in the same column differ significantly at $p < 0.05$. Values are expressed as means ± standard error.

Table 3. Influence of liquid enzymes supplementation on organs weights in broilers.

	Control	2m/L	4ml/L	8ml/L
Carcass, g/100g bw	63.420±1.160	65.547±0.762	64.164±0.797	64.040±0.757
Heart weight, g/100g bw	0.430±0.027	0.422±0.028	0.423±0.019	0.447±0.013
Liver weight, g/100g bw	2.318±0.106	2.277±0.111	2.160±0.135	2.812±0.502
Spleen weight, g/100g bw	0.062±0.012^{c}	0.072±0.010^{b}	0.098±0.022^{a}	0.122±0.030^{a}
Gizzard weight, g/100g bw	1.165±0.139	1.128±0.058	1.177±0.073	1.175±0.053
Abdominal fat weight, g/100g bw	1.740±0.115^{a}	1.782±0.165^{a}	1.692±0.187^{ab}	1.580±0.074^{b}
Breast muscle weight, g/100g bw	23.327±0.423	23.120±0.792	23.033±0.469	23.225±0.885
Thigh muscle weight, g/100g bw	17.063±1.062	17.877±0.555	17.533±0.800	17.257±0.404

^{a,b,c} Mean values with different letters in the same row significantly differ at $P < 0.05$. Values are expressed as means ± standard error. bw, body weight.

Table 4. Influence of liquid enzymes supplementation on blood parameters in broilers.

	Control	2ml/L	4ml/L	8ml/L
GPT, I/U	8.307±0.191^{a}	6.265±0.192^{b}	6.603±0.298^{b}	6.207±0.169^{b}
GOT, I/U	269.33±13.52^{a}	272±9.74^{a}	223.83±12.52^{b}	222.67±7.98^{b}
Total cholesterol, mg/dL	154±8.33^{a}	158.67±8.67^{a}	149.33±9.36^{a}	137.33±4.78^{b}
Glucose, mg/dL	169.67±11.16	150.83±10.99	150.67±11.21	130.50±23.66
Total protein, g/dL	5.20±0.14	5±0.21	5.22±0.19	5.60±0.28
Albumin, g/dL	2.57±0.10^{b}	2.90±0.07^{a}	2.72±0.08^{ab}	2.67±0.14^{ab}
Globulin, g/dL	2.73±0.12^{a}	2.27±0.19^{ab}	2.17±0.10^{b}	2.43±0.18^{ab}
LDL-cholesterol, mg/dL	109.83±6.77^{a}	97.17±12.94^{ab}	97.67±4.20^{ab}	73±9.12^{b}
HDL-cholesterol, mg/dL	33.83±1.70^{c}	35.83±1.96^{bc}	41.83±2.46^{ab}	43.67±3.28^{a}

^{a,b,c} Mean values with different letters in the same row significantly differ at $P < 0.05$. Values are expressed as means ± standard error. Glutamic oxalacetic transaminase (GOT), and glutamate pyruvate transaminase (GPT); high-density lipoprotein (HDL); low-density lipoprotein (LDL). International Units (I/U).

Table 5. Influence of liquid enzymes supplementation on muscle fatty acids and liver MDA content in broilers.

	Control	2m/L	4m/L	8m/L
Palmatic, mg/100 g fat	0.618±0.025^{a}	0.605±0.022^{a}	0.496±0.018^{b}	0.462±0.070^{b}
Oleic Acid, mg/100 g fat	0.212±0.004	0.217±0.008	0.228±0.008	0.227±0.008
Linolenic Acid, mg /100 g fat	0.234±0.007^{b}	0.230±0.008^{b}	0.404±0.047^{a}	0.485±0.073^{a}
Liver MDA, nanomole/gram	15.333±0.491^{a}	15.170±0.393^{a}	15.300±0.751^{a}	14.037±0.581^{b}

^{a,b} Mean values with different letters in the same row significantly differ at $P < 0.05$. Values are expressed as means ± standard error. Thiobarbituric acid reactive substance (TBARS).

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