

IMPACT OF DIETARY SUPPLEMENTATION WITH MULTI-ENZYME AND/OR PROBIOTICS ON PRODUCTIVE PERFORMANCE AND NUTRIENTS DIGESTIBILITY OF BROILER CHICKENS.

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SUMMARY

One hundred and twenty unsexed one-day-old Cobb broiler chicks were randomly distributed into four groups (5 replicates per group) to study the effect of multienzymes and/or probiotics supplementation on productive performance and nutrients digestibility of broiler chickens. The first group was used as control and fed the basal diet without supplementation, while, chicks in the 2nd, 3rd and 4th groups were fed the basal diet supplemented with multienzymes (Avizyme at 0.20 g/kg diet), probiotics (Biacton at 0.50 g/kg diet) and multienzymes plus probiotics (0.20 g Avizyme /kg diet + 0.50g/kg Biacton), respectively. Body weight (BW), body weight gain (BWG), and feed intake (FI) were recorded. Feed conversion rate (FCR), production index and economical efficiency were calculated. At the end of the experiment a digestibility trial was carried out. Results showed that the chickens fed diet supplemented with multienzymes and probiotics during days 22-35 and 0-35 of age had significantly higher BWG and FI and better FCR followed by those fed diet supplemented with either multienzymes or probiotics compared to those fed control diet. The multienzymes and/or probiotics supplementation had significantly improved the digestibility of dry matter (DM), crude protein (CP) and ether extract (EE) compared to the control. In addition, dietary supplementation with multienzymes plus probiotics or multienzymes alone had significantly improved the digestibility of crude fiber (CF) compared to the control. Chicks fed diet supplemented with multienzymes plus probiotics had significantly better production index and economical efficiency followed by those fed diet supplemented with multienzymes or probiotics alone compared to those fed the control diet. In conclusion: multienzyme and /or probiotics are good growth stimulators to the broiler chicks and can be added together to the diet to improve productive performance and feed utilization.

Keywords: *Broiler, multienzymes, probiotics, growth performance, nutrients digestibility.*

INTRODUCTION

The consumption and trade of poultry products are increasing rapidly as the human population increases, making it the second largest source of meat (FAO, 2014). The animal feed industry as a whole has been worked toward solutions to lower costs up front and as a result have a lower cost at the end product for the consumer. Despite there are several ways to reduce feed cost, the enzymes supplementation (ES) has demonstrated to be an effective way to reduce it. Enzymes not only make the animals able to benefit from the parts of feedstuffs that were not previously utilized, but also allow the nutritionist to change the diet content. The multienzymes allow the producer to lower feed costs while maintaining optimum production rates (Hahn, 2010; Attia *et al.*, 2012, 2014). Also, Plant feedstuffs contain non-starch polysaccharides (NSP), which are indigestible and able to reduce use of nutrients in poultry by lowering their digestion and absorption (Kim *et al.*, 2005). Thus, the presence of NSP in the diet of poultry can also limit the efficiency of nutrient usage. Addition of carbohydrate enzymes in poultry diets may reduce the anti-nutritional effects for NSP. Thus, the ES in poultry diets could improve poultry performance (Peric *et al.*, 2008; Attia *et al.*, 2012, 2014).

Probiotics are live microorganisms (such as bacteria, fungi and yeast) that make a positive impact on the host physiological status. This is frequently accomplished by their capacity to modify the gut

microbial balance in a helpful way, which thus will enhance the health of animals and birds (Bengmark, 1998; Fuller, 2001).

Nowadays, probiotics have been utilized as a feed additive in diets of poultry to improve performance and immunity (Higgins *et al.*, 2008). Moreover, probiotics addition to poultry diets had positive impacts on growth rate, feed intake (Afsharmanesh and Sadaghi, 2014; Lei *et al.*, 2015), Feed conversion rate (FCR) (Zhang and Kim, 2014) and nutrient digestibility (Li *et al.*, 2008; Zhi-gang *et al.*, 2014; Zhang and Kim, 2014) in broiler chickens. This study aimed to study the effect of multienzymes and/or probiotics supplementation on productive performance and nutrients digestibility of broiler chickens.

MATERIALS AND METHOD

This study was carried out at the Poultry Farm, Faculty of Agriculture, Damanhour University, Egypt, from March to April, 2017.

One hundred and twenty unsexed one-day old Cobb broiler chicks, obtained from a commercial hatchery, were randomly distributed into four groups with 5 replicates and 6 birds per replicate (i.e., 30 birds per treatment) and reared on same managerial conditions. The first group was used as control and fed only a basal diet without supplementation (T1), while, chicks in the 2nd, 3rd and 4th groups were fed the basal diet supplemented with multienzymes (Avizyme at 0.20 g/kg diet; T2), probiotics (Biacton at 0.50 g/kg diet; T3) and multienzymes plus probiotics (0.20 g Avizyme /kg diet + 0.50g/kg Biacton; T4), respectively. The enzymes complex preparation Avizyme® 1505 is a product of Danisco Animal Nutrition, Marlborough, Wiltshire, UK., containing 1500 U/g endo-1, 4-β-xylanase, 2000 U/g α-amylase and 20000 U/g protease and its recommended dose of use in broiler and turkey diets is 0.20 g per kg diet. Biacton is a product of Chemvet DK A/S, Denmark. It consists of *Lactobacillus farciminis* CNCM MA67/4R at a concentration of 1×10^9 CFU g⁻¹, together with the carrier materials being waxy maize corn starch (19.8%), yeast extract (7.5%), oil-free soybean lecithin (2.4%), and corn starch (70.1%) and its recommended dose of use in broiler and turkey diets is 0.5 kg Biacton per ton feed. The experimental diets were formulated according to NRC (1994) as shown in Table (1). The chicks were housed in wire cages (60 × 50 × 40 cm) in semi-opened room. Chicks were fed the experimental diets *ad libitum* and given free access to water. A light schedule similar to commercial conditions was applied until the 7th day being 23 h light followed by 20 h light from 8-35 days of age. The brooding temperature (indoor) was 32, 30, 27 and 21-24 °C during 1-7, 8-14, 15-20 and 21-35 days of age (declined gradually). Chicks were vaccinated against Newcastle Disease and Infectious Bronchitis. Body weight and feed intake were recorded at 1, 21 and 35 days of age, and the body weight gain (BWG, g/chick) and FCR (g feed/g gain) were calculated. Production index value was calculated throughout the experimental period (1-35d of age) as reported by Attia *et al.* (2012).

PI= BW (kg) x SR / (PP x FCR) x 100 Where:

PI = Production Index BW = Body weight (kg)

SR = Survival rate (100% - mortality) PP = Production period (days)

FCR = Feed conversion ratio (kg feed / kg gain)

At 35 days of age the apparent digestibility of nutrients and ash retention were evaluated using five birds per treatment housed individually in metabolic cages by using total collection method as cited by (Abou-Raya and Galal, 1971). Dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content of the dried excreta as well as those of feed were determined according to AOAC (2004). Economical evaluation for all experimental treatments was made according to Zeweil, (1996).

Statistical analysis

Data were analyzed by the GLM procedure (Statistical Analysis System (SAS), 2002) using one-way ANOVA with the following model:

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

Where Y is the dependent variable; μ is the general mean; T_i is the effect of experimental treatments; e_{ik} is the experimental random error.

Before analysis, all percentages were subjected to arcsine transformation ($\log_{10} x + 1$) to normalize data distribution. The significance of the differences among means was determined using Duncan's new

multiple range test (Duncan, 1955) (at $P < 0.05$).

Table (1): Ingredients, determined and calculated analysis (%) of the basal diets

Ingredient	Diet (% as fed)	
	Starter (1-21 d of age)	Grower (22-35 d of age)
Corn, Grain	53.60	56.30
Soybean meal (44% CP)	31.00	20.00
Wheat Bran	0.00	5.00
Gluten Meal (60% CP)	5.00	5.00
Full fat soybean seed	4.00	7.00
Vegetable oil	2.75	3.00
Dicalcium phosphate	1.80	1.60
Vit+min premix*	0.30	0.30
Limestone	1.00	1.00
NaCl	0.30	0.45
DL-Methionine	0.15	0.20
L-Lysine HCl	0.10	0.15
Total	100.00	100.00
Determined ¹ and calculated ² composition (% as fed)		
Nutrient	Supplied	Supplied
Dry matter ¹	86.59	86.66
ME (kcal/kg) ²	3061	3107
Crude protein ¹	23.16	20.56
Ether extract ¹	5.88	6.83
Crude fiber ¹	3.68	3.64
Calcium ²	0.91	0.85
Ash ¹	6.09	5.97
Total Phos. ²	0.74	0.71
Available phosphorus ²	0.49	0.47
Lysine ²	1.22	1.08
Methionine ²	0.55	0.56

* Vitamins and minerals mixture provide per kilogram of diet: Vitamin A (as all-trans-retinyl acetate); 12000 IU; Vitamin E (all rac- α -tocopheryl acetate); 10 IU; k_3 3mg; Vit.D₃, 2200 ICU; riboflavin, 10 mg; Ca pantothenate, 10 mg; niacin, 20 mg; Choline chloride, 500 mg; Vitamin B₁₂, 10 μ g; Vitamin B₆, 1.5 mg; Thiamine (as thiamine mononitrate); 2.2 mg; Folic acid, 1 mg; D-biotin, 50 μ g. Trace mineral (milligrams per kilogram of diet) Mn, 55; Zn, 50; Fe, 30; Cu, 10; Se, 0.1 and Ethoxyquin 3mg.

RESULTS AND DISCUSSION:

Growth performance

The growth performance of broilers fed diet supplemented with multi-enzymes and/or probiotics during 1-35 days of age is shown in Table 2. The chicks in T4 group fed MEnz+ Pro had significantly heavier BWG during the period from 0-21 days of age than chicks in T1, T2 and T3 groups. Meanwhile, BWG of Chicks in T4 was significantly the highest during the periods 22-35 and 0-35 days of age followed by those in T2 and T3 compared to those in T1. Moreover, the chicks in T4 consumed significantly higher in feed intake during 22-35 and 0-35 days of age than chicks in T1, T2 and T3 without significant differences between T1, T2 and T3. However, feed intake during 0-21 days of age was insignificantly affected by treatments. In addition, the chicks in T3 and T4 have significantly better FCR during the period 0-21 days of age than chicks in T1 without any difference between T1 and T2 groups. Also, the chicks in T4 had significantly better in FCR during 22-35 days of age than chicks in T1 without significant differences between T1, T2 and T3. However, the chicks in T2, T3 and T4 had significantly better in FCR during the whole period than chicks in T1 without significant differences between T2, T3 and T4 (Table 2). The present results are in agreement with those reported by Persia *et al.* (2002); Al-Harthi (2006) and Attia *et al.* (2008, 2012, 2014). In this regard, Peric *et al.* (2008); Raza *et al.* (2009); Abdollahi *et al.* (2016) and Amerah *et al.* (2017) concluded that enzymes supplement (ES) increased BWG and improved FCR of broiler chicken. In addition, Sherif (2009a) observed a positive effect for

adding different types of enzyme preparations in the grower-finisher diets of broilers, on final BW, and BWG. In another study, Sherif (2009b) found that dietary supplementation with different ES improved FCR for broiler chicks fed plant protein diets. Moreover, Zhou *et al.* (2009) suggested that ES in broiler chicken diets could improve the efficiency of energy utilization, especially in low metabolizable energy diets. Sun *et al.* (2015) indicated that ES increased BWG and improved FCR from 0 to 14 d and from 0 to 21 days of age. In this respect, supplementation of exogenous enzymes in poultry diets increased BWG and FI and improved FCR as compared to poultry diets in which enzyme was not supplemented during day 1-21 and 1-35 (Mahmood *et al.*, 2017). In the literature, the improvements in growth rate, FCR and economic traits due to supplementation of multi-enzymes were attributed to the enhancement in the availability and absorption of nutrients through increasing the digestibility of the ingested diets (Abudabos, 2012; Nourmohammadi *et al.*, 2012; Attia 2014). In addition, multienzyme containing amylase, xylanase and protease was found to improve energy utilization in corn-SBM and sorghum-SBM diets due to the breakdown of starch, cereal cell walls, and endogenous proteins (Hong *et al.*, 2002; Attia *et al.*, 2008). Similarly, Cowieson *et al.* (2003) and Ghazalah *et al.* (2005) showed that the improvement in BWG obtained upon feeding enzyme mixtures may be attributed to the presence of amylase and NSPs degrading enzymes in enzymes mixture, rather than protease, that making the nutrients more available to the birds and improve chick's growth performance.

Supplementation of probiotics to poultry diets increased broiler growth (Shoeib and Madian, 2002; Willis *et al.*, 2007; Sherief *et al.*, 2012; Zhi-gang *et al.*, 2014; Zhang and Kim, 2014), feed intake (Landy and Kavyani, 2013; Afsharmanesh and Sadaghi, 2014) and improve FCR (Shim *et al.*, 2012; Zhang and Kim, 2014) as compared to poultry diets without supplementation. In addition, Timmerman *et al.* (2006) observed that broilers given drinking water supplemented with chicken specific probiotic had a productivity index, taking into account daily BWG, FCR and mortality, superior to that of the non-supplemented birds. Sherief *et al.* (2012) recorded that probiotic addition increased ($P < 0.05$) the final BW, BWG and FI and improved FCR of broiler chickens compared with those fed the control diet. In this regard, Dersjant-Li *et al.* (2015) suggested that multi-enzymes plus probiotics supplementation increased FI and improved BWG and production efficiency in broilers under commercial settings. The improvement in growth rate in the probiotic treated birds was associated with increased FI (Landy and Kavyani, 2013; Lei *et al.*, 2015) and improved FCR (Shim *et al.*, 2012; Zhang and Kim, 2014) compared with untreated birds. Therefore, increased digestibility of feed resulting in improved FCR could be one of mode of actions for improved growth rate. The improvement in performance and productivity of poultry due to the probiotics addition in diets has been attributed to increased FI and improved FCR (Shim *et al.*, 2012).

Table (2): Performance of broilers fed diet supplemented with multienzymes and/or probiotics.

Items	Control (T1)	MEenz (T2)	Prob (T3)	MEenz+Pro (T4)	SEM	Sig
Body weight gain (g):						
0-21d	716b	740b	737b	778a	2.05	0.001
22-35d	907c	973b	991b	1089a	45.02	0.008
0-35d	1623c	1713b	1728b	1867a	44.35	0.006
Feed intake (g):						
0-21d	1079	1076	1049	1089	21.39	0.617
22-35d	1702b	1717b	1745b	1850a	19.56	0.003
0-35d	2780b	2793b	2794b	2939a	29.52	0.005
Feed conversion ratio (g feed/g gain):						
0-21d	1.51a	1.42ab	1.39b	1.38b	0.032	0.041
22-35d	1.88a	1.81ab	1.80ab	1.72b	0.043	0.117
0-35d	1.71a	1.63b	1.62b	1.57b	0.025	0.009

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); MEenz = Multi-enzyme; Prob = Probiotics; MEenz+Pro = Multi-enzyme + Probiotics; SEM = Standard error of mean.

Nutrients digestibility (%)

Data presented in Table 3 showed the effect of supplementing multi-enzymes and/or probiotics to experimental diets on the nutrients digestibility and ash retention of broiler chicks. The digestibility of nutrients in groups T2, T3 and T4 were significantly better for crude protein, ether extract and dry matter digestibility than T1 without any significant differences between T2, T3 and T4. Moreover, chicks in T2 and T4 had significantly greater digestibility of crude fiber than chicks in T1. However, apparent ash retention was insignificantly affected by treatments. These results agree with the findings of Sherif (2009a), who found that adding different types of commercial enzyme preparations to broiler diets had a positive impact on the digestibility of CP and EE, and nitrogen retention as compared to those fed the control diets. Similarly, Sherif (2009b) reported that digestibility of DM of boiler chicks were improved in response to adding ES (Avian plus and Natuzyme) to their diets. In addition, Wang *et al.* (2005) demonstrated that apparent CP digestibility in broiler chicks increased in response to feeding diets supplemented with enzymes. Amerah *et al.* (2017) concluded that ES improved nutrient digestibility of broiler chicken. The improvement in starch digestibility was associated with the augmentation of endogenous α -amylase and improvement each of digestion resistant starches, access to cell resistant starches and access to cell contents via a reduction in cell wall integrity. In addition to the benefit effects with corn, amylase, xylanase and protease that have additional benefit effects with soybean meal. These benefits when apply to corn and soybean meal, as the protease component of the enzyme combination can have a positive effect on trypsin inhibitor (Cowieson and Ravindran, 2008). Supporting this, energy and amino acids digestibility of a corn-soy based diet could be improved by nearly 3% when supplemented with xylanase, amylase and protease (Cowieson and Ravindran, 2008). The combination of amylase, xylanase and protease can also improve ileal digestibility by 2% (Cowieson and Ravindran, 2008). Also, Cowieson and Adeola (2005) concluded that combination of phytase and xylanase, amylase and protease improved ileal digestibility by a factor greater than 100 kcal/kg diet. Similar results were also reported by Fuller (1997); Palliyaguru *et al.* (2004); Zhi-gang *et al.* (2014) who detected improvements of CP retention in broilers at the starter phase of growth when supplementing the diet with 0.02% probiotics. This effect may be mediated by an improvement in the main functions of digestion and absorption in the gut (Fioramonti *et al.*, 2003). These findings suggested that part of the beneficial impacts of probiotics on early phase of growth performance may be mediated via improvement in the apparent retention of CP through reducing nitrogen excretion. Probiotics can also enhance the synthesis of some vitamins, raised digestive enzymes and increasing the volatile fatty acids production that beneficial for the animals (Fuller, 2001). They may also raise the efficiency of nutrient usage through enhancing the gut health resulting in higher activities of gut enzymes and nutrient availability (Fuller, 2001) and increase the absorption of nutrients from gut through their indirect impact on its permeability (Higgins *et al.*, 2008).

Table (3): The apparent digestibility of the nutrients and ash retention (%) of broilers fed diets supplemented with multi-enzyme and/or probiotics.

Items	Control T1	MEenz T2	Prob T3	MEenz+Pro T4	SEM	Sig
Crude protein	63.9b	73.5a	73.5a	76.9a	1.121	0.006
Ether extract	66.2b	76.6a	76.7a	78.9a	1.038	0.001
Crude fiber	16.6b	19.0a	17.9ab	19.6a	0.582	0.008
Ash retention	29.0	33.5	33.8	35.1	1.621	0.072
Dry matter	67.2b	72.0a	72.4a	72.0a	0.882	0.001

^{a,b} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); MEenz =Multi-enzyme; Prob= Probiotics; MEenz+Pro =Multi-enzyme+ Probiotics ; SEM=Standard error of mean.

Economic efficiency and Production Index

The chicks in T4 had significantly higher feed cost, total costs, total revenue and net revenue during the whole period than chicks in T1, T2 and T3 without any differences in feed cost and total costs between T1, T2 and T3 (Table 4). Moreover, chicks in T2 and T3 had significantly higher in total revenue and net revenue during the whole period than chicks in T1 without any difference in total revenue and net revenue between T2 and T3. On the other hand, Chicks fed MEenz+Pro (T4) had significantly better economical efficiency followed by those in T3 then T2, compared to those in T1. Moreover, Chicks of T4 had significantly better production index followed by those of T2 and T3, compared to T1. These results are similar to those reported by Jahan *et al.* (2006) and Attia *et al.* (2014) who indicated that production

index and economical efficiency increased with supplementing multienzyme to the diets compared with control diets. Also, Osman (2003) and Zeweil *et al.* (2006) reported that, probiotic addition to diet improved economical efficiency as compared with control diet. This improvement could be due to improving BW and FCR. The same result was confirmed by Mostafa *et al.* (2015) who indicated that economical efficiency increased with supplemented ES (Bio-Mos) diets compared with their control. **In conclusion:** Multienzyme and /or probiotics are good growth stimulators to the broiler chicks and can be added together to the diet to improve productive performance and feed utilization.

Table (4): Economical efficiency and production index of broilers fed diets supplemented with multienzymes and/or probiotics

Item	Control T1	MEenz T2	Prob T3	MEenz+Pro T4	SEM	Sig
Feed cost	11.12b	11.45b	11.46b	12.34a	0.123	0.001
Total cost	22.12b	22.45b	22.46b	23.34a	0.123	0.002
Total revenue	26.75c	28.21b	28.45b	30.65a	0.341	0.006
Net revenue	4.628c	5.761b	5.989b	7.313a	0.349	0.001
Economic efficiency	20.94c	25.67cb	26.67ab	31.34a	1.585	0.003
Production Index	279c	309b	314b	348a	7.757	0.001

^{a,b} Means in the same row followed by different letters are significantly different at ($p \leq 0.05$); MEenz =Multienzymes; Prob= Probiotics; MEenz+Pro =Multienzymes+ Probiotics
SEM=Standard error of mean.

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تأثير إضافات مخلوط الإنزيمات و/ أو البروبيوتك على الأداء الإنتاجي وهضم المركبات الغذائية في دجاج اللحم.

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استخدم عدد مائة وعشرون كتكوت (كب) عمر يوم غير مجنس قسمت بالتساوي عشوائيا الى أربع مجموعات تجريبية (5 مكررات لكل مجموعة) لدراسة تأثير إضافة مخلوط الإنزيمات و/ أو البروبيوتك على الأداء الإنتاجي وهضم المركبات الغذائية لدجاج اللحم. غذيت المجموعة الأولى على العليقة الأساسية بدون أي إضافات (كنترول)، بينما تغذت الكتاكيت في المجموعات الثانية والثالثة والرابعة على العليقة الأساسية مضاف إليها مخلوط الإنزيمات (أفيزيم 0,20 جم / كجم من العليقة)، البروبيوتك (بياكوتون 0,50 جم / كجم من العليقة) ومخلوط الإنزيمات مع البروبيوتك (أفيزيم 0,20 جم / كجم من العليقة + بياكوتون 0,50 جم / كجم من العليقة) على التوالي. تم تسجيل وزن الجسم، الزيادة في وزن الجسم، الغذاء المأكول وحساب كل من معدل التحويل الغذائي ودليل الإنتاج والكفاءة الاقتصادية. وفي نهاية التجربة أجريت تجربة الهضم. أوضحت النتائج أن الكتاكيت المغذاه على عليقة مضاف لها مخلوط الإنزيمات مع البروبيوتك خلال 22-35 و35-0 يوم من العمر كانت أعلى معنويا في الزيادة في وزن الجسم والعلف المأكول وأفضل معامل تحويل غذائي تليها المغذاه على عليقة مضاف لها مخلوط الإنزيمات أو البروبيوتك منفردا بالمقارنة مع تلك المغذاه على العليقة الكنترول. أدت إضافة مخلوط الإنزيمات و/ أو البروبيوتك إلى تحسن معنوي في هضم المادة الجافة والبروتين الخام والمستخلص الأثيري مقارنة بالكنترول. بالإضافة إلى ذلك، فإن الإضافات الغذائية من مخلوط الإنزيمات مع البروبيوتك أو مخلوط الأنزيمات منفردا قد أدى إلى تحسن معنوي في هضم الألياف الخام مقارنة بالكنترول. أظهرت الكتاكيت التي تم تغذيتها على عليقة مضاف لها مخلوط الإنزيمات مع البروبيوتك أفضل دليل إنتاج وكفاءة اقتصادية تليها تلك التي تغذت على عليقة مضاف لها مخلوط الإنزيمات أو البروبيوتك منفردا مقارنة بتلك التي تغذت على العليقة الكنترول. و تشير النتائج إلى أن مخلوط الإنزيمات و/ أو البروبيوتك يمكن استخدامها كمنشطات نمو جيدة من أجل تحسين الأداء الإنتاجي والاستفادة من الغذاء في علائق دجاج اللحم.