



EFFECT OF USING EFFECTIVE MICROORGANISMS (EM) AS A GROWTH PROMOTER ON BROILERS PERFORMANCE, THYROID HORMONES, LIPID PROFILE, HEPATOSOMATIC INDEX, IMMUNE RESPONSE, ENTERIC PATHOGENS, AND ANTIOXIDANT PARAMETERS

I. A. Abdelkader, A. S. Ramadan, M. S. Bahnas, R. A. S. Abdelrasoul

Poult. Prod. Dep., Fac. of Agric., Fayoum Uni., 63514 Fayoum, Egypt.

Corresponding author: I. A. Abdelkader Email: iaa03@fayoum.edu.eg

Received: 19/03/2023

Accepted: 05 /05/2023

ABSTRACT:The current study was carry out to assess the effects of effective microorganism(EM) as feed additive on performance, digestive enzymes, thyroid hormones, lipid profile, hepatosomatic index, immune response, enteric pathogens, and antioxidant parameters of broiler chicks. A total number of 180 broilers (Arbor Acres) one day-old were randomly into 4 groups, with three replicates of 15 chicks each. The experimental groups consist of control fed basal diet while the other three groups fed control diet supplied with 5.0, 7.5 and 10 ml EM/kg diet and labled as EM5, EM7.5, EM10, respectively. The results show that all groups received graded levels of effective microorganisms (EM), improved all the studied growth parameters during allover period as body weights, body weight gain, feed conversion ratio, growth rate and performance index, respectively. The best values for the previous growth parameters were recorded for group fed high dietary EM level. Chicks of EM5 improved their kidney functions, in term of decreasing uric acid. Also, creatinine level did not influenced by any tested diets. With regard to the liver function, the results showed a significant improvement in ALT and AST with each level of EM compared to the control group, and the best values of liver functions were for EM10. The use of EM in the feed improved serum cholesterol, HDL, LDL immune response, thyroid functions and gut microflora compared to the control group. Generally, supplementation with EM could enhance growth performance, blood biochemical and ameliorate the structure of the gut microflora.

Key words: effective-microorganism, broiler, growth promoter, serum parameters.

1. INTRODUCTION

Effective microorganisms (EM) are a group of useful regenerative microorganisms that live freely in nature and are not manipulated in any way. Preparations of Effective microorganisms are mostly formed from yeasts, lactic acid bacteria, actinomycetes, fungi and photosynthetic bacteria (EMRO, 2010). Effective microorganisms as a novel trend for probiotics in the production of poultry, have displayed some useful influences in the poultry industry like enhancement in growth rate, feed conversion ratio, nitrogen utilization, and intestinal infections prevention (Sugiharto *et al.*, 2018).

In the livestock sector, EM is extensively utilized to better and improve growth performance, health conditions, odor problems, disease control, and as a tool for dropping recycling in poultry production. When the birds that received EM in water and feed, useful microorganisms play a role to ameliorate the gut flora, thus boosting of efficient digestion and the immune system. Therefore, the growth rate, reproductive performance, egg production, and health condition of chickens will be improved (EMRO, 2010 and Jwher *et al.*, 2013).

Several studies reported that effective microorganisms improved intestinal microflora balance (Hamad *et al.*, 2020); immune response (Apata, 2008 and Ashraf and Shah 2014); decreasing triglycerides and total cholesterol in serum (Ignatova *et al.*, 2009 and Şahan *et al.*, 2021) also, lowering fat and cholesterol in chicken meat (Mansoup, 2011). Also, Awad *et al.*, (2009) and Sugiharto *et al.*, 2018 proved that effective microorganisms bettered broilers performance index and economic efficiency. Abd El-Hack *et al.*, (2020)

concluded that supplementation of effective microorganisms has been shown to benefit poultry performance; through modulation of immune, structural alteration and increased cytokine production, which positively affected the lining of intestinal mucosa against pathogen.

This study aimed mainly to assess the impact of effective microorganism (EM) as a feed additive on broilers growth performance, thyroid hormones, lipid profile, hepatosomatic index, immune response, enteric pathogens, and antioxidant parameters.

2. MATERIALS AND METHODS

The current study was carried out at the Poultry Research Center, Faculty of Agriculture, Fayoum University, Egypt. Research on live animals met the guidelines approved by Fayoum University Institutional Animal Care and Use Committee in Egypt (Code No. of the proposal: AEC 2218).

2.1. Effective microorganisms(EM):

The EM were purchased from the *Agricultural Research Center*, Ministry of Agriculture, Giza, Cairo, in the form of liquid containers and were prepared according to the processes of the Asia-Pacific natural agriculture Network (1995) and were mixed with the basal diets and left for seven days before use to form the soiled fermented feed (bokashi). According to EMRO (2010), EM culture is a high cocktail of live beneficial microorganisms including high level of Lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei* and *Streptococcus lactis*), yeast (*Saccharomyces cerevisiae* and *Candida utilis*) and fewer populations of photosynthetic bacteria (*Rhodospseudomonas palostris* and

effective-microorganism, broiler, growth promoter, serum parameters.

Rhodobactor Spaeroids) Ray fungi (Streptomyces albs and Streptomyces griseus) and fungi (Aspergillus oryza and Mucor hiemalis).

2.2. Experimental birds design and diets:

At one-day old, a total number of 180 broilers (Arbor Acres) were bought from Elwattania company, were randomly divided at equal body weights into 4 groups, with three replicates of 15 chicks each. The first group served as control and fed the basal diet, while, second, third and fourth groups fed the basal diet added with 5.0, 7.5 and 10 ml EM/kg diet, respectively and marked as EM5, EM7.5, EM10. Birds were housed in batteries consisting of three decks, two sections of cages with automatic watering and were fed and watered *ad libitum*. The ingredients and chemical composition of basal diets are displayed in Table1.

2.3. Growth performance and carcass traits measured:

Chicks' live body weights (LBW) and feed consumption (per pen) were recorded, then body weight gain (BWG) was calculated by the difference between the final live body weight (35 days of age) and initial live body weight (1 day old). Also, the feed conversion ratio (FCR) was calculated by BWG dividing to feed consumption and according to North (1981) the performance index (PI) was computed through $PI = BWkg/FCR$. the Growth rate was computed as follows: $GR = (LBW_{35} - LBW_1) / 0.5 (LBW_1 + LBW_{35})$ as reported by (Brody, 1945). At 35 old days of age, 24 chickens (4 treatment x 3 samples x 2 sex) were reweighed and slaughtered by cutting the Jugular vein, then de-feathered and eviscerated. Carcass yield and giblets weight was measured and the dressing% and giblets were computed. The blood

samples were taken, then the serum was separated (through centrifugation at 3000 rpm for 15 minutes) and stored till use for blood analyses. Hepatosomatic Index was calculated according to Nur-Azri *et al.*, (2018) using the following equation
Hepatosomatic Index (HSI) = $\frac{\text{weight of the liver (g)}}{\text{final body weight (g)}} \times 100$

2.4. Blood biochemical, anti-oxidant and immunity:

All following determinations were done in serum; total cholesterol (Chol), low density lipoproteins (LDL), high density lipoproteins (HDL) according to James (2001), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lipase and Amylase enzymes were measured (Friedman and Young (2005) and trypsin enzyme was determined (Bovine Trypsin ELISA Kit MBS706461). Triiodothyronine (T₃) and thyroxine (T₄) were quantitatively detected as an immunoassay by ELISA technique using the automated ELISA reader Expert Plus UV, Biochrom., G 020151. All blood biochemical parameters determined through utilization commercial diagnosing kits (manufactured by Spectrum Diagnostics Company, Egypt). Immunoglobulins Isotypes(IgA and IgG) were assayed in Sandwich ELISA (Erhard *et al.*, 1992).

2.5. Microbial analysis:

After the slaughtering process, in sterile glass bottles, the intestinal digesta was collected, emptied and mixed. The containers were stored at 4°C till the enumeration of the microbial population. The microbial population examined for E. coli, Salmonella spp and Lactobacilli spp according to (Abd El-Wahab *et al.*, 2022).

2.6. Economic Efficiency

The economical efficiency was calculated according to Kalia *et al.* (2018). The extra

price of the EM was added to the feed price, whereas other productive costs were disregarded since they were constant.

2.7. Statistical analysis

Statistical analysis was conducted using one-way ANOVA of SPSS software (SPSS, 2014). The model used was $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} = observed value in the i th treatment of the k th individual, μ = overall mean, T_i = Treatment effect ($i=1-4$), and e_{ij} is the error associated with Y_{ij} . As reported by Duncan, (1955) all tested means (treatment) were compared by Duncan's new multiple-range test. A probability of $P < 0.05$ was required for significance

3. RESULTS AND DISCUSSION

3.1. Growth performance:

Table 2 shows the effect of feeding broilers on graded levels of EM on different growth promoters (LBW, BWG, FCR, GR and PI) during overall period (1 to 35d of age). The findings showed that all levels of EM addition were significantly superior ($P < 0.01$) to the control group, and the results were of a linear trend, whether between levels and control or among levels, where the highest levels of addition (EM10) were the best values for the studied growth parameters LBW (2358.33g), BWG (2312.03 g), FCR (1.64), GR (192.25) and PI (145.72) while, and the lowest values were shown for the control group being 2194.87g, 2148.54g, 1.77, 191.62 and 127.68, for the same previous parameters respectively. It was found that all performance parameters were better in broilers fed in diets containing EM than those fed in basal diet ($P < 0.001$). This improvement in broiler performance may be related to EM could be caused by enhancing crude protein digestibility and crude fiber digestibility.

These results are agree with those of Wondmeneh *et al.*, (2011), Jwher *et al.*, (2013) and Hatab *et al.*, (2016) on broiler, Dorra *et al.*, (2016) on turkey, Nur-Azri *et al.*, (2018) on quail and Ye *et al.* (2021) on Partridge. Recently, Zhang *et al.*, (2021) revealed that microbial additives during the period from 0–42 days of age, markedly augmented LBW, average daily gain, and average daily consumption in female chickens and significantly ameliorated LBW and feed conversion ratio in male chickens. In contrast the previous authors, Nuengjamnong and Luangtongkum (2014) and Jayathilaka *et al.*, (2017) reported that broiler growth performance didn't affected significantly by feeding diets or drinking water added with microbial additives. Various studies point to the role and importance of EM in better feed utilization efficacy. Effective microorganisms supplementation improved digestibility, reduced the feed consumed quantity and enhanced animal growth performance (Jwher *et al.*, 2013; Wondmeneh *et al.*, 2011; Dorra *et al.*, 2016; Hatab *et al.*, 2016; Nur-Azri *et al.*, 2018; Ye *et al.*, 2021; Zhang *et al.*, 2021).

This enhancement in growth performance as a result of feeding diets enriched with EM may be related to decreasing the counts of pathogenic bacteria (*E. coli*) and increasing counts of beneficial bacteria (*Lactobacillus Spp.*) as will be shown later in table (6). The same conclusion was reported by Chen *et al.* (2013) who showed that supplementing broilers diets with EM affect the gut system by improving of digestive enzyme activity, intestinal bacteria composition and intestinal pH.

effective-microorganism, broiler, growth promoter, serum parameters.

3.2. Blood parameters:

Table 3 displays the effect of feeding broiler chicks different levels of EM on serum liver and kidney functions. The results showed that with regard to the kidney function, creatinine level was not significantly affected by any treatments studied, and the numerical improvement in creatinine values was achieved for group of EM5 which record 0.3 mg/dl comparing with the control group while the uric acid positively affected by EM supplementation only with EM7 (5.49 mg/dl) which superior control group comparing and the rest two levels EM 5.0 and EM 10 ml/kg diet (5.08, 4.14 and 4.64 mg/dl), respectively. With regard to the liver function, the findings displayed a significant improvement in ALT and AST with each level of EM compared to the control group, and the best values of liver functions were with the highest levels of EM. With regard to sex effect, there was no significant difference between males and females, whether in liver or kidney functions. With respecting to liver enzymes (in serum), the current results detected that there significantly was a reduction in liver enzymes ALT and AST compared with the control group which agreed with the findings of Abdel-Aziz *et al.*, (2020) and disagreed with the results reported by Abd (2014) who reported that EM feeding groups had a higher liver enzymes compared with the control group while Ladine *et al.*, (2014) indicated that EM had no effects on the serum ALT and AST concentrations. Hepatocytes have an important role in absorbing and metabolizing various poisonous chemicals, so they are susceptible to damage by many chemicals included in the food. Liver function and health can be detected by Aspartate aminotransferase

and Alanine aminotransferase activities in serum, so liver cellular damage may augment the AST and ALT level in serum. Alanine aminotransferase chiefly exists in the liver and is considered as being more specific than Aspartate aminotransferase for revealing injury of liver cells (Michalska *et al.*, 2021). The high levels of hepatic enzymes serum (ALT and AST) reflect the hepatic injury and enzymes leakage in the bloodstream and vice versa. Furthermore, Hatab *et al.*, (2016) found no significant effects of microbial additives to diets of chicken on serum creatinine concentrations, while, uric acid, serum AST, ALT, cholesterol and triglycerides concentrations in all tested groups were lower significant than in the control group.

Effect on hepatosomatic index (HSI):

Hepatosomatic (HSI) is an indicator utilized to test liver weight relative to final body weight. In this study, group of EM5 recorded the highest ($P>0.03$) HSI value also, the other groups (EM7.5 and EM10) recorded higher HIS values but without significant differences to control. Hepatosomatic index is a trusty parameter of hepatic growth and development related to age and the liver's physiochemical or physiological status. The hepatosomatic index is important because it describes the changes caused by the feed supplementation so, it is a good parameter for the activity of animal feeding. Also, the current study displays that the broilers had a higher dose of EM in the feed, which had a positive impact on health. This denotes that the liver maintains a normal size without any reverse influence on its function. This finding agreed with those of Nur-Azri *et al.*, (2018). On the other hand, Chen *et al.*, (2013) reported that the weight of the liver was not influenced by the

treatments. Also, Awad *et al.*, (2009) showed that there are no significant differences in liver weights caused by adding probiotics to feed of broiler chickens.

3.3. Lipid profile:

Table 4 represents the influence of feeding broiler chicks on different levels of effective microorganisms on total cholesterol, its fractions and abdominal fat percentage. Where it is clearer to note that total cholesterol and LDL had the highest significant value in the control and then took a decline by increasing the level of effective microorganisms. Group of EM5 recorded the highest HDL value without significant differences to control. While, the other groups of EM7.5 and EM10 recorded significantly lower HDL values than control. All tested groups' achieved significantly lower abdominal fat percentage rather than control.

Effective microorganisms are live microbial, when added to feed, affect serum triglycerides and total cholesterol. The present findings are in the same line with those of Sjojfan *et al.*, (2021), Şahan *et al.*, (2021) and Abd (2014) who point out that feed containing EM may have some beneficial impacts by decreasing the concentration of serum triglycerides and cholesterol in chickens. Also, Esatu *et al.*, (2011) showed that the levels of serum total cholesterol were decreased by adding EM to a bird's diet. The reduction in total cholesterol in serum may be due to synthesis of hepatocyte cholesterol which is regulated and converted to bile acids, the excretion of these acids is promoted by the adherence of Effective Microorganism. so, it may have a role in diminishing serum cholesterol level. Furthermore, probiotics could be inhibited hepatic 3-hydroxy-3-

methylglutaryl coenzyme A reductase activity and related to a lowered recycling of bile salts in the gut. Lactic acid bacteria, as probiotics, are capable to elicit the effect of hypocholesterolemic by interfering with the transport and absorption of intestinal bile acid, which causes an augment in the excretion of bile acid (Jamshidparvar *et al.*, 2017).

3.4. Thyroid hormones and immune response:

The data summarized in table 5 shows the impact of feeding broiler chicks diets supplemented with graded EM levels on blood thyroid hormones, immune globulin and spleen index (g/100g BW). It is clear from the data that there is no significant effect among the different treatments on thyroid(T3) hormone regarding to T4 hormone the highest level detected value was recorded for EM5 group followed by control group, then EM7.5 group without significant differences between them but the lowest value was recorded for EM10 group, to be 132.22, 130.58, 128.77 and 125.07 ng/dl respectively. In contrary to the results, Chotinsky and Mihaylov (2013) reported that broiler chickens that fed diets containing probiotics significantly increased their serum triiodothyronine level. Hatab *et al.* (2016) provided a potential causative relation between thyroid serum hormone and the EM effects as a growth-promoting of layer chickens.

As for the immunoglobulins (IgG and IgA), the control treatment showed the lowest level of immune response compared to the rest of the treatments, while the rest of the treatments added with microbes had a significantly high immune response and the highest were EM5 in IgG (1049.93 mg/dl) and EM7.5 in IgA (210.76 mg/dl). When tracking the

effective-microorganism, broiler, growth promoter, serum parameters.

percentage of spleen to LBW, it is clear that the lowest percentage appears with the control treatment, while the highest percentage is with the treatments of EM5 and EM7.5. EM can boost the secretion of immunoglobulins, and immune cells proliferation and promote nonspecific immunity stimulation such as the induction of the phagocytic activity of macrophages (Stefaniak *et al.*, 2020). Ahmed (2006) observed that birds fed with probiotics in their diets have increased serum antibodies production levels including IgM, interferon γ and IgG. Zhang *et al.* (2021) reported that probiotics and feeding with EM highly promoted the IgG and IgA concentration in female and male broilers which strongly agreed with the current study. Poultry lymphatic organs are the bursa of Fabricius, spleen, thymus and their weight directly explains the internal immune function strength (Slawinska *et al.*, 2014). Stefaniak *et al.* (2020) showed that the treatment of a single probiotic or in combination can improve spleen, bursa of Fabricius, and thymus weight in broilers. In our study, different EM expended the best effect on the broiler spleen index which agreed with the previous findings.

3.5. Digestive enzymes and enteric pathogens:

The data shown in Table 6 shows the impact of adding graded levels of effective microorganisms on the digestive enzymes in the blood serum (Amylase Lipase and Trypsin) and gut microbes (*E. coli* and *Lacto bacillus*), where the values of the three previous enzymes showed an upward trend starting from the control group, which included the lowest values of enzymes and the more the addition of microbes increased the values of the three enzymes to achieve the peak height with

treatment EM7.5 to be (520.50 U/L Amylase, 26.17 U/L Lipase and 48.17U/L Trypsin), then the enzyme values decreased with the increase of the addition of microbes in the EM10 treatment to be (Amylase 519.17, Lipase 24.50 and Trypsin 47 U/L). This results are in same line with those of Zhang *et al.*, (2021) who showed that probiotics supplementation for broiler chickens (male and female) influentially decreased the number of harmful *E. coli* and *Salmonella* bacteria in the gut and augmented the digestive enzymes. Also, according to recent investigations by Ye *et al.*, (2021) and Abd El-Hack *et al.*, (2020) who were conducted that broiler chickens that received probiotics had higher amylase and protease activity

Regarding to gut microorganisms, *E. coli* shows the highest values with the control coefficient 7.85 Log₁₀ cfug and it decreases with the augment in the EM level of in the diet until it reaches the lowest significant value with the treatment EM10 6.68 Log₁₀ cfug. As for *Lacto*, the lowest value appears with the control 6.89 the values increase with the addition of effective microorganisms in the diet to achieve the peak height with treatment EM10 7.87 Log₁₀ cfug. The present findings agreed with those of Hamad *et al.* (2020) who showed that 1% EM had the best impact in decreasing the pathogenic tested bacteria growth *E. coli*. and *S. aureus*.

Effective microorganism, specially *Lactobacillus* is much appropriate for poultry and animals, because its growth inhibits the activation of pathogenic microorganism and promoting nonpathogenic microorganism by producing different metabolites, which causes an improvement to the environment of intestinal microecological

(Attia *et al.*, 2018). The wide spread encountered pathogenic microorganism in poultry farming are *Salmonella*, *E. coli* enterica (Vieco *et al.*, 2019). In this study, Effective microorganism supplementation which consists of *Lactobacillus plantarum* and *Lactobacillus casei* decreased *E. coli* and improve *Lactobacillus* counts. The findings were consistent with the results obtained by Zhang *et al.* (2021) who noticed the same trend in the gut microbial population in broilers fed with probiotics. In general, supplementation of Effective microorganism in feed could improve competition in favor of beneficial bacteria in the gut's internal environment.

3.6. Economic efficiency:

Data presented in Table 7 shows the effect of adding different levels of EM to broiler diets on the economical efficiency indicating that all levels of EM had the highest economic efficiency than the control group, and the treatment EM10 was the highest value in economic efficiency and relative economic efficiency. This may be due to the highest final body weight of those treatments compared to the control group. Our findings are in the same trend as mentioned by Dorra *et al.*, (2016) on Turkey and El-Deep (2011) who reported that the supplementation of Effective Microorganisms improved economic efficiency as compared with the control.

CONCLUSION

In general, supplementation of effective microorganism in broiler diets could up to improve growth performance, blood biochemical and ameliorate the structure of the gut microflora...

effective-microorganism, broiler, growth promoter, serum parameters.

Table (1): Composition of the basal diet at different ages of broiler chicks (Arbor Acres).

Ingredient%	Pre –starter 1-7 day	Starter 8-20 day	Grower 21-28 day	Finisher 29-35 day
Yellow Corn	55.80	57.17	61.50	68.70
Soybean Meal (44%)	28.20	28.97	25.00	19.31
Corn Gluten Meal (60%)	10.17	7.78	7.46	5.82
Soyabean Oil	1.20	1.40	2.00	2.50
L-Lysine HCL	0.50	0.50	0.50	0.50
DL Methionine	0.13	0.18	0.14	0.16
Calcium Carbonate	1.60	1.60	1.33	1.22
Calcium Phosphate, Mono	1.73	1.73	1.40	1.12
Salt, NaCl	0.37	0.37	0.37	0.37
Vitamin and mineral mix*	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
Calculated Analysis**				
Dry Matter%	87.73	87.53	86.95	86.46
Crude Protein%	23.8	22.85	21.26	18.40
M.E. Kcal/Kg	3000	2990	3084	3181
Ether Extract%	2.77	2.78	2.88	3.03
Crude Fiber%	3.55	3.58	3.39	3.11
Lysine%	1.46	1.46	1.35	1.18
Methionine%	0.60	0.61	0.55	0.52
Methionine + Cysteine%	0.98	0.98	0.90	0.82
Calcium%	0.97	0.97	0.90	0.85
Available Phosphorus%	0.51	0.51	0.44	0.40
Determined				
Dry Matter%	88.5	88.7	86.8	85.6
Crude Protein%	23.89	22.77	20.85	18.29
Ether Extract %	3.00	3.00	3.00	2.95
Ash%	5.22	4.9	4.6	5.1

*Each 3.0 kg of mineral and vitamin mix contain: Vit. A, 12000000 I.U; Vit. E, 10 g; **Vit. D₃, 2500000 I.U; Vit. K₃, 2.5 g; Vit.B₁,1 g; Vit. B₂, 5 g; Vit.B₆,1.5 g; Vit.B₁₂,10g; Biotin 50 mg; Folic acid, 1 g; Nicotinic acid, 30 g; Pantothenic acid, 10 g; Choline 250000mg, Zn, 55 g; Cu, 10 g; Fe, 35 g; Co,250 mg; Se,150 mg; I, 1 g; Mn, 60 g; and antioxidant, 10 g. **According NRC, 1990.

Table (2): Effect of dietary treatments (effective microorganism (EM)) on broilers growth performance.

Item	LBW1d	LBW 35d	BWG 1-35d	FI 1-35d	FCR 1-35d	GR 1-35d	PI 1-35d
Treatment effect							
Control	46.33	2194.87 ^b	2148.54 ^b	3746.57 ^c	1.77 ^a	191.62 ^b	127.68 ^b
EM 5	46.48	2291.18 ^a	2244.69 ^a	3807.77 ^a	1.69 ^b	191.98 ^a	138.26 ^a
EM 7.5	46.25	2320.76 ^a	2274.50 ^a	3756.43 ^{bc}	1.68 ^b	192.14 ^a	139.64 ^a
EM 10	46.30	2358.33 ^a	2312.03 ^a	3803.98 ^a	1.64 ^b	192.25 ^a	145.72 ^a
SEM	0.26	29.07	29.39	5.04	0.02	0.11	3.52
P- value							
Treatment	0.9870	0.0013	0.0012	0.0001	0.0025	0.0006	0.0102

^{a..b} Means within the same column with different superscript differ significantly (P≤0.05)

SEM¹: Pooled standard error, LBW :Live body weight BWG: body weight gain, FCR: feed conversion ratio, PI: performance index GR: growth rate EM 5= basal diet + 5ml EM/kg, EM7.5 = basal diet + 7.5ml EM/kg EM10 = basal diet + 10ml EM/kg

Table (3): Effect of dietary treatments (effective microorganism (EM)) on serum kidney and liver functions of broilers.

Item	Kidney functions		Liver functions		Hepatosomatic index (HSI)
	Creatinine (mg/dl)	Uric acid (mg/dl)	ALT (U/L)	AST (U/L)	
Treatment effect					
Control	0.33	5.08 ^{a b}	6.57 ^a	264.17 ^a	1.33 ^b
EM 5	0.3	4.14 ^b	5.8 ^b	239.17 ^b	1.71 ^a
EM 7.5	0.32	5.49 ^a	5.72 ^b	221.67 ^b	1.48 ^{ab}
EM 10	0.32	4.64 ^{a b}	5.68 ^b	219.17 ^b	1.50 ^{ab}
SEM	0.02	0.97	0.21	8.82	0.08
P- value					
Treatment	0.89	0.045	0.026	0.008	0.03

ALT Alanine aminotransferase : AST Aspartate aminotransferase SEM: Pooled standard error Creat : Creatinine. ^{a,b and c}: Means within the same column with different superscript differ significantly (P≤0.05 EM 5= basal diet + 5ml EM/kg, EM7.5 = basal diet + 7.5ml EM/kg EM10 = basal diet + 10ml EM/kg

effective-microorganism, broiler, growth promoter, serum parameters.

Table (4): Effect of dietary treatments (effective microorganism (EM)) on serum cholesterol profile of broilers.

Item	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Abdominal fat % to LBW
Treatment effect				
Control	132.10 ^a	93.07 ^{ab}	39.03 ^a	1.00 ^a
EM5	127.07 ^{b3}	94.70 ^a	32.35 ^b	0.93 ^b
EM7.5	121.17 ^c	90.72 ^b	30.39 ^c	0.90 ^b
EM10	115.93 ^d	87.29 ^c	28.64 ^d	0.91 ^b
SEM	1.38	0.98	0.46	0.01
P- value				
Treatment	P< 0.001	P< 0.001	P< 0.001	P< 0.001

^{a,b and c}: Means within the same column with different superscript differ significantly (P≤0.05).
SEM: Pooled standard error HDL: High density lipoprotein , LDL: Low density lipoprotein
EM 5= basal diet + 5ml EM/kg, EM7.5 = basal diet + 7.5ml EM/kg EM10 = basal diet + 10ml EM/kg

Table (5): Effect of dietary treatments (effective microorganism (EM)) on serum thyroid functions and immune response kidney and liver fuctions of broilers.

Item	Thyroid hormones		Immune response		Spleen % to LBW
	T3 (ng/dl)	T4 (ng/dl)	IgG (mg/dl)	IgA (mg/dl)	
Treatment effect					
Control	2.64	130.58 ^a	936.15 ^c	175.53 ^c	0.12 ^c
EM5	2.46	132.22 ^a	1049.93 ^a	196.86 ^b	0.16 ^a
EM7.5	2.30	128.77 ^{ab}	1124.03 ^b	210.76 ^a	0.15 ^{ab}
EM10	2.48	125.07 ^b	1015.23 ^b	190.36 ^b	0.13 ^{bc}
SEM	0.16	1.79	15.96	2.99	0.01
P- value					
Treatment	0.53	0.064	P< 0.001	P< 0.001	P< 0.001

T₃: Triiodothyronine T₄: Thyroxine IgG , IgA Immunoglobulins Isotypes G and A , LBW : Live body weights of chicks ^{a...d}: Means within the same column with different superscript differ significantly (P≤0.05) SEM: Pooled standard error EM 5= basal diet + 5ml EM/kg, EM7.5 = basal diet + 7.5ml EM/kg EM10 = basal diet + 10ml EM/k

Table (6): Effect of dietary treatments (effective microorganism (EM)) on serum digestive enzymes and intestinal microbial count of broilers.

Item	Digestive Enzymes (U/L)			Microbial Count Log 10 CFU/g	
	Amylase	Lipase	Trypsin	<i>E. coli</i>	<i>Lacto.</i>
Treatment effect					
Control	341.00 ^b	14.00 ^c	34.33 ^b	7.85 ^a	6.89 ^b
EM5	512.33 ^a	21.17 ^b	43.67 ^a	7.29 ^{ab}	7.32 ^{ab}
EM7.5	520.50 ^a	26.17 ^a	48.17 ^a	6.86 ^b	7.75 ^a
EM10	519.17 ^a	24.50 ^a	47.00 ^a	6.68 ^b	7.87 ^a
SEM	13.56	0.69	2.56	0.3	0.29
P- value					
Treatment	P< 0.001	P< 0.001	0.006	0.08	0.10

E. coli: Escherichia coli cfug: logarithm of colony forming unit per gram of digesta *Lacto*: Lactobacillus spp. ^{a-b}: Means within the same column with different superscript differ significantly (P≤0.05 EM 5= basal diet + 5ml EM/kg, EM7.5 = basal diet + 7.5ml EM/kg EM10 = basal diet + 10ml EM/kg

Table (7): Effect of dietary treatments (effective microorganism (EM)) on broiler diets economic efficiency.

Item	Control	EM5	EM7.5	EM10
Av. Feed intake, Kg feed/ Kg meat a	1.77	1.69	1.68	1.64
Price Kg feed (L.E.) *b	8.30	8.35	8.38	8.40
Total feed cost C= (a×b)	14.691	14.112	14.078	13.776
Price / one Kg gain** d	24.00	24.00	24.00	24.00
Net revenue (L.E.) = d-c = e	9.309	9.889	9.922	10.224
Economic efficiency *** (e/c)	0.634	0.701	0.705	0.742
Relative efficiency ****	100.00	110.59	111.22	117.12
Viability rate (%)	100.00	100.00	100.00	100.00

*Price of Kg feed according local market December 2020 ** Price of Kg live body weight according to the local market December 2020. ***net revenue per unite cost. ****compared to the economic efficiency of the control group EM 5= basal diet + 5ml EM/kg, EM7.5 = basal diet + 7.5ml EM/kg EM10 = basal diet + 10ml EM/kg

REFERENCES

- Abd El-Hack, M. E., M. T. El-Saadony, M.E. Shafi, Y. A. Shaza, G. E. Qattan, Batiha, F. Asmaa Khafaga, A.E. Abdel-Moneim, and M. Alagawany 2020.** Probiotics in poultry feed: A comprehensive review. *J. Anim. Physiol. Anim. Nutr.*;104:1835–1850. DOI: 10.1111/jpn.13454
- Abd El-Wahab, A. E. W. A. E. A., M. M. Aly, M. S. Bahnas, , and R. A. S. Abdelrasol, 2022.** Influence of dietary supplementation of marigold flower powder and extract (*Calendula officinalis* L.) on performance, nutrient digestibility, serum biochemistry, antioxidant parameters and immune responses of growing Japanese quail. *Journal of Animal Physiology and Animal Nutrition*, 106(4), 742-751.
- Abd, S.K. 2014.** Effect of effective microorganisms on some biochemical parameters in broiler chicks. *Iraqi J. Vet. Sci.* 2014, 28, 1–4.
- Abdel-Aziz, M., M .Bessat, A. Fadel, and S. Elblehi, 2020).** Responses of dietary supplementation of probiotic effective microorganisms (EMs) in *Oreochromis niloticus* on growth, hematological, intestinal histopathological, and antiparasitic activities. *Aquac. Int.* , 28, 947–963.
- Ahmed A. 2006.** Effect of probiotics on broilers performance *Int. J. Poult. Sci.* 5, 593-597.
- Apata, D. F. 2008.** Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. *J. Sci. Food Agric.*, 88: 1253-1258.
- Ashraf, R., and N. P. Shah 2014.** Immune system stimulation by probiotic microorganisms. *Crit. Rev. Food Sci. Nutr.*, 54: 938–956. doi: 10.1080/10408398.2011.619671
- Asia-Pacific Natural Agriculture Network-APNAN, EM application manual, 1st edition, 1995, <https://www.agriton.nl/apnanman.html>
- Attia, Y.A., A.E.E. Hamid ., Ismaiel, A.M., Oliveira, M.C.D and G.A. Simon 2018.** Nitrate detoxification using antioxidants and probiotics in the water for rabbits. *Rev Colombiana de Cienc Pecuarias.*; 31:130–8.
- Awad, W. A., K .Ghareeb, Abdel-Raheem, S. and J.Bohm, 2009.** Effect of dietary inclusion of probiotic and symbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens . *Poult. Sci.*, 88, 1: 49-56.
- Brody, S. (1945):** Bioenergetics and Growth. New York: Reinhold Publishing Co.
- *Chen, W. , J.P. Wang and L. Yan 2013.** Evaluation of probiotics in diets with different nutrient densities on growth performance, blood characteristics, relative organ weight and breast meat characteristics in broilers. *Br. Poult. Sci.*; 54 (5): 635–641. PubMed Abstract | Publisher Full Text
- Chotinsky, D and R. Mihaylov 2013.** Effect of probiotics and avotan on the level of thyroid hormones in the blood plasma of broiler chickens *Bulg. J. of Agric. Sci.*, 19 (4) 817-821
- Dorra, T,I., Z. M. Kalaba, M.Y. Mostafa and Sh.M. Zayed 2016.** growth performance and hematological parameters of turkey poult as affected by effective

- microorganisms. *J. Anim. and Poult. Prod.*, Mansoura Univ., Vol. 7 (1): 15 - 22.
- Duncan, D. B. 1955.** Multiple range test and multiple F. test. *Biometrics*, 11, 1-42.
- El-Deep, M. M. H. 2011.** Effect of using effective microorganisms as an alternative antibiotics in local domestic fowls nutrition. Ph. D. Thesis, Faculty of Agriculture, Poultry Production Department, Kafr El-Sheikh University. Egypt.
- EM Research Organization (EMRO) 2010.** Working with EM Technology for Sustainable Environment. [<http://www.emsustains.co.uk>] site visited on 5 August 2010.
- Erhard, M. H., Von Quistorp, I., Schraner, I., Jüngling, A., Kaspers, B., Schmidt, P. and R. Kühlmann, 1992.** development of specific enzyme-linked immunosorbent antibody assay systems for the detection of chicken immunoglobulins g, m, and a using monoclonal antibodies. *Poult. Sci.* Volume 71, Issue 2, 1 February 1992, Pages 302-310. <https://doi.org/10.3382/ps.0710302>.
- Esatu .W, T.Dessie and S .Adey 2011.** Effect of effective microorganisms on growth parameters and serum cholesterol levels in broilers. *African J. of Agric. Res.* Vol. 6 (16), pp. 3841-3846, 18
- Friedman, R. B. and D. S. Young 2005.** Effects of disease on clinical laboratory tests. Columbia University press
- Hamad M.A., S.A. Hussein, E.N. Mahmmoud and A.M. Al-Aalim 2020.** The inhibitory role of effective microorganisms on the growth of pathogenic bacteria. *Iraq. J. of Vet. Sci.*, Vol. 34, No. 1, 2020 (153-158)
- Hatab, M.H., M.A. Elsayed and N.S. Ibrahim 2016).**Effect of some biological supplementation on productive performance, physiological and immunological response of layer chicks, *Journal of Radiation Research and Applied Sciences*, 9, 2, 185-192, ISSN 1687-8507, <https://doi.org/10.1016/j.jrras.2015.12.008>.
- Ignatova, M., V .Sredkova and V. Marasheva 2009.**Effect of dietary inclusion of probiotic on chicken performance and some blood indices.*Biotech.in Anim. Husbandry.*, 25, 1029-1085
- James, I. 2001.** Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA: the Journal of the American Medical Association*, 285(19), 2486-2497. <https://doi.org/10.1001/jama.285.19.2486>.
- Jamshidparvar, A., F. Javandel and A . Seidavi 2017.** Effects of golpar (*Heracleumpersicum* Desf.) and probiotics in drinking water on performance, carcass characteristics, organ weights, blood plasma constituents, and immunityof broilers. *Environ Sci. Poult. Res. Int.* 24 (30): 23571-23577. PubMed Abstract Publisher Full Text

effective-microorganism, broiler, growth promoter, serum parameters.

- Jayathilaka B.R, W.A.D. Nayananjalie, Adikari A.M.J.B and B.W.N.J .Samaraweera 2017.** influence of effective microorganisms on growth performances of broiler chicken .Int.l J. of Multidisciplinary Studies (IJMS) Volume 4, Issue 1, 2017 DOI: <http://doi.org/10.4038/10.4038/ijms.v4.i1.39>
- Jwher, D.M.T, S.K. Abd, and A. G.Mohammad 2013.** The study of using effective microorganisms(EM) on health and performance of broiler chicks. Iraq. J. of Vet. Sci., :27(2):73-78.))
- Kalia, S., V. K. Bharti, A. Giri, B. Kumar, A. Arora, and S. S. Balaje. 2018.** Hippophae rhamnoides as novel phytogetic feed additive for broiler chickens at high altitude cold desert. Sci. Rep. 8:5954.
- Ladine, C. , C. ustafa., Ugur,S. , Yusuf1 ,U., Sait ,P., Fatma ,Y., Mehmet , C., Osman,G. , and K, Rustu 2014.** Effects Of Supplemental Effective Microorganism On Performance, Some Histological And Blood Parameters Of Broiler. The 17th Asian-Australasian Association of Animal Production Societies Animal Science Congress.
- Mansoup, N. H. 2011.** Comparitive effect of butyric acid and probiotic on performance and serum composition of broiler chickens.Advances in Environ. Biolo., 5: 1188-1191.
- Michalska, K.; M. Gesek,; Sokól, R.; Murawska, D.; Mikiewicz, M.; and A. Chłodowska.2021.** Effective microorganisms (EM) improve internal organ morphology, intestinal morphometry and serum biochemical activity in japanese quails under clostridium perfringens challenge. molecules 2021, 26, 2786. <https://doi.org/10.3390/molecules26092786>
- North, M.O 1981.**Commercial chicken production manual, 2nd Edition. AVI Publishing Company Inc, USA.
- Nuengjammong, C and T. Luangtongkum 2014.** Effects of Effective microorganisms on growth performances, ammonia reduction, hematological changes and shedding of *Salmonella enterica* and *Campylobacter spp.* in broilers. Thai J. Vet. Med44 (1): 15-22.
- Nur-Azri, M A ., N. R. Wan., N, Ahmad Sanusi., N. Sabli., A, Kamarud and N. Ismail 2018.** The Effect of Em-1® (*Lactobacillus Spp.*) on growth performance of quails, Coturnix Japonica. J. Agrobiotech. Vol. 9 (1S), 2018, p. 283–293. © Universiti Sultan Zainal Abidin. ISSN 1985-5133 (Press)
- Şahan, Z., H. Kutay and L . Çelik, 2021.** Influence of effective microorganism supplementation to the drinking water on performance and some blood parameters of laying hens exposed to a high ambient temperature. Braz. J. Poult. Sci., 23 (01) • 2021 • <https://doi.org/10.1590/1806-9061-2020-1351>
- Sjofjan, O. D., N. Adli and R.P. Harahap 2021.** The effects of lactic acid bacteria and yeasts as probiotics on the growth performance, relative organ weight, blood parameters, and immune responses of broiler: A meta-analysis [version 1;peer review: 1 approved with reservations] F1000Research 2021, 10:183 <https://doi.org/10.12688/f1000research.51219.1> First published: 05 Mar 2021, 10:183 <https://doi.org/10.12688/f1000research.51219.1>

- Slawinska A. S. M, Zylinska J. Bardowski J. Brzezinska J. and K. A. Gulewicz 2014.** Influence of synbiotics delivered in ovo on immune organs development and structure. *Folia Biol. (Krakow)*. :62:277–85.
- SPSS 2007.** *User's Guide : statistics. Version 16.* SPSS Inc. Chicago, IL USA.
- Stefaniak .T, J.P .Madej, Graczyk .S, Siwek .M, Łukaszewicz. E and A. Kowalczyk 2020.** Impact of prebiotics and synbiotics administered in ovo on the immune response against experimental antigens in chicken broilers. *Animals (Basel)*.:10:643.
- Sugiharto .S., I . Isroli, Yudiarti .T and E. Widiastuti 2018.** The effect of supplementation of multistrain probiotic preparation in combination with vitamins and minerals to the basal diet on the growth performance, carcass traits, and physiological response of broilers. *Vet. World*11:240-247.
- Vieco-Saiz. N., Y. Belguesmia ., Raspoet . R., Auclair. E., Gancel . F and I . Kempf 2019.** Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Front Microbiol.* 2019;10:57.
- Wondmeneh E., M, Adey and D. Tadelles 2011.** Effect of effective microorganisms on growth parameters and serum cholesterol levels in broilers. *Afri. J. Agri. Res*6 (16):3841-3846.
- Ye .Y., Li. Z, Wang P, Zhu. B, Zhao. M, Huang .D, Ye .Y, Ding .Z, Li. L. Wan G. Wu .Q. Song .D and Y. Tang 2021.** Effects of probiotic supplements on growth performance and intestinal microbiota of partridge shank broiler chicks. *Peer J.* 9:e12538 <http://doi.org/10.7717/peerj.12538>
- Zhang , L., R. Zhang , Jia . H., Zhu. Z., Li. H. and Y. Ma 2021.** Supplementation of probiotics in water beneficial growth performance, carcass traits, immune function, and antioxidant capacity in broiler chickens. *Open Life Sciences* 2021; 16: 311–322 <https://doi.org/10.1515/biol-2021-0031>

الملخص العربي

تأثير استخدام الكائنات الحية الدقيقة الفعالة كمحفز للنمو على أداء دجاج التسمين ، وهرمونات الغدة الدرقية ، وصورة الدهون في الدم ، ومؤشر الكبد ، والاستجابة المناعية ، ومسببات الأمراض المعوية ، ومعايير مضادات الأكسدة

إبراهيم عبدالقادر ، محمد سعد بهنس ، علاء شعبان رمضان ، رمضان علام سيد عبدالرسول
قسم إنتاج الدواجن – كلية الزراعة – جامعة الفيوم.

أجريت الدراسة الحالية لتقييم تأثير الكائنات الحية الدقيقة الفعالة كمضاف غذائي على الأداء ، والإنزيمات الهضمية ، وهرمونات الغدة الدرقية ، و صورة الدهون في الدم ، ومؤشر الكبد ، والاستجابة المناعية ، ومسببات الأمراض المعوية ، ومعايير مضادات الأكسدة لكتاكيت التسمين. اجمالى 180 فرخا (اربور ايكرز) عمرها يوم واحد تم تقسيمها عشوائيا الى 4 مجموعات ، بثلاث مكررات كل منها 15 ككتوت. تتكون المجموعات التجريبية من مجموعة تناولت عليقة كمنترول كغذاء أساسى بينما تم تغذية المجموعات الثلاث الأخرى على عليقة كمنترول مزود بـ 5.0 و 7.5 و 10 مل من EM / كغم وسميت EM5 و EM7.5 و EM10 على التوالي. أظهرت النتائج أن جميع المجموعات تلقت مستويات متدرجة من الكائنات الحية الدقيقة الفعالة EM، مما أدى إلى تحسين جميع متغيرات النمو المدروسة خلال الفترة المدروسة مثل أوزان الجسم وزيادة وزن الجسم ونسبة التحويل الغذائي ومعدل النمو ومؤشر الأداء على التوالي. تم تسجيل أفضل القيم مقاييس النمو السابقة للمجموعة التي تم تغذيتها بمستوى EM الغذائي العالي. تحسنت وظائف الكلى للصيصان التي تناولت EM5 ، من حيث تقليل حمض البوليك. أيضا ، لم يتأثر مستوى الكرياتينين بأي نظام غذائي تم اختياره. فيما يتعلق بوظائف الكبد ، أظهرت النتائج تحسناً ملحوظاً في ALT و AST مع كل مستوى من EM مقارنة بمجموعة الكمنترول ، وكانت أفضل قيم وظائف الكبد لـ EM10 . أدى استخدام EM في العلف إلى تحسين مستوى الكوليسترول في الدم ، HDL ، LDL ، استجابة مناعية ووظائف الغدة الدرقية وميكروفلورا الأمعاء مقارنة بمجموعة الكمنترول. بشكل عام ، يمكن أن تعزز المكملات بـ EM أداء النمو والكيمياء الحيوية للدم وتحسن بنية الأمعاء الدقيقة.