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EFFECTS OF FEEDING EFFECTIVE MICROORGANISMS ON BLOOD LEVELS OF ANTIBODIES TO NEWCASTLE DISEASE VIRUS VACCINE AND TRACE ELEMENT IN BROILER CHICKS

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ABSTRACT

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A total of 60 unsexed day old broiler chicks of (Ross 308) were divided into 2 groups, each of 30 chicks were housed in a separate individual pens, during the period 1st/10 till 5th/11 2012, for 5 weeks. Effective Microorganisms (EM) was administered at a rate of 10 ml/liter of drinking water. Chicks were vaccinated against Newcastle disease (ND) (Lasota strain). Blood samples were collected and divided into two parts, one for estimation of trace elements (copper, zinc, cobalt and manganese) using Atomic absorption, while the other part for estimation of antibody titers against Newcastle disease virus. Results showed that there was a significant ($p<0.05$) increase in the antibody titers against Newcastle disease virus vaccine of 5347.07±117.03, in chicks amended with EM when compared with the control group of 2949.24±314.51. There was also a significant ($p<0.05$) increase in the mean of trace elements of Zn, Co, Cu and Mn which are (1.171 µg/ml, 6.128 µg/ml, 0.376 µg/ml and 0.146 µg/ml)in treated chicks with EM, in comparison with (0.587 µg/ml, 4.151 µg/ml, 0.232 µg/ml and 0.000) respectively in the control group.

Key word: *Effective Microorganisms, Trace elements, Antibody titers*

INTRODUCTION

Newcastle disease is a deadly viral contagious disease of poultry among chicken and other birds (Alexander and Senne, 2008). Newcastle disease is being controlled using prophylactic measures such as routine vaccination along with strict biosecurity measurements of many developed countries of the world (Liljebjelke *et al.*, 2008). Different vaccination programs against ND are being practiced as a means of protecting birds from the frequent outbreak of the disease in broilers have been applied in Mosul Governorate Newcastle disease (ND) is being considered as one of the most important constrains for the development of profitable poultry farming including broiler both in the urban and rural area of Iraq.

A survey of maternal immunity of broiler chicks reared in Mosul broiler farms showed that they were influencing the optimum age of birds for ND vaccination (Mohammadamin and Qubih 2010), in addition to other factors of seasonal variation and prevalence of certain disease (Allan *et al.*, 1978). As regard to the boosting effect of effective microorganisms on the immune system of birds when they are added to their diet or water, (Anjum *et al.*, 1996) and (Jwher *et al.*, 2013), reported greater bursa and thymus index which are the vital components of humoral and cellular immunity in birds supplemented with EM through drinking water and fermented feed. Effective microorganisms are formed at high pressures by the interaction of a diverse group of naturally occurring, aerobic and facultative anaerobic microorganisms. EM include high populations of lactic acid producing bacteria (*Lactobacillus* and *Pedicoccus*) at 1 x 10^5 CFU/ml suspension, yeast (*Sacharomyces*) at 2 x 106 CFU/ml suspension and fewer amounts of photosynthetic bacteria, actinomyces and other organisms (Gum *et al.*, 1998), Effective microorganisms are regarded in Japan to be effective as a probiotic, (Kitazato Environmental Science Center 1994). These flora could play an important role in the digestion process in intestinal tract of the birds, since their enzymes promote the digestion of protein, lipids and carbohydrates and bacteria also synthesize vitamins that contribute to the nutrition of the bird (Larbier and Leclerco 1994). Many authors were referred to the beneficial effects of effective microorganisms in improving growth parameters: feed intake, weight gain, feed conversion ratio in broilers (Jwher *et al.*, 2013; Safalaoh, 2006; Ergun *et al.*, 2000; Silva *et al.*, 2000; Kumprechtova *et al.*, 2000; Chantsawang and Watcharangkul 1999; ZuAnon *et al.*, 1998; Alvarez *et al.*, 1994; Jagdish and Sen 1993). This paper was therefore conducted to study the effect of Effective Microorganisms on the

Newcastle antibody titers and trace elements in blood of broiler chicks.

MATERIALS and METHODS

Management of Experimental Birds:
A total of 60 unsexed day old broiler chicks of (Ross 308) were divided into 2 groups each of 30 chicks were housed in a separate individual pens thoroughly cleaned and well prepared at the farm of the College of Veterinary Medicine/ Mosul University, during the period 1st/10 till 5th/11 2012. Each group was randomly assigned to 2 replicates for a study period of 5 weeks (table 1). All the treatment groups were fed to appetite with commercial starters, grower and finisher rations and clean water were made available all the times (table 2).

Table 1: Treatment Allocation to the Experimental Chicks.

Treatments	Rep/Treat.	Chicks/Rep.	Total
0 ml EM-1/Liter of drinking water, control (T1)	2	15	30
10 ml EM-1/ Liter of drinking water (T2)	2	15	30

Table 2: Feed composition (g/kg).

Ingredients	Starter	Grower	Finisher
Corn	26.8	54.3	50.6
Wheat	20.0	17.4	27.0
Barley	20.0	-	-
Soybean Meal (48%)	27.0	23.0	17.0
Fat	1.7	1.0	1.0
Ground Limestone	1.6	1.5	1.5
Calcium Phosphate (20% P)	1.5	1.5	1.5
Iodized Salt	0.3	0.22	0.3
Vitamin: mineral[1] premix	1.0	1.0	1.0
Methionine	0.10	0.08	0.10
Calculated analysis			
Crude Protein	22.0	18.0	16.1
Digestible protein (%)	17.7	14.4	12.9
Crude Fat (%)	5.9	3.4	3.4
Metabolized Energy (kcal/kg)	3060	3022	3050
Calcium (%)	1.00	0.95	0.96
Av. Phosphorus (%)	0.42	0.41	0.41
Sodium (%)	0.17	0.17	0.18
Methionine (%)	0.48	0.38	0.37
Methionine &Cysteine (%)	0.82	0.65	0.61
Tryptophan (%)	0.31	0.25	0.22
Lysine (%)	1.25	0.93	0.78
Threonine (%)	0.94	0.75	0.65

*(Leeson and Summers 1997)

Liquid form of EM product (Alannam company for natural agriculture, Tortuous–Syria under the supervision of EMRO Japanese institute - Okinawa-Japan) was used. EM stock solution is formed from Lactic acid bacilli: *Lactobacillus plantarum; L. casei Streptococcus Lactis.*; Photosynthetic bacteria *Rhodopseudomonas palustris*; *Rhodobacter sphaeroides*, Yeast; *Saccharomyces cerevisiae*; *Candida utilis torula, Pichia jadinii*; Actinomycetes; *Streptomyces albus*; *S. griseus* and Fermenting fungi; *Aspergillus oryzae*; *Mucor hiemalis*.as described by the manufacturer. EM was administered at a rate of 10 ml/liter of drinking water as shown in T2. Standard bio-security protocol was employed throughout the experimental period. Chicks were

vaccinated against Newcastle disease (Lasota strain) at 8 and18 days of age.

Blood collection

Blood samples were collected from each bird at the end of the experiment (35) days by wing vein puncture and then were divided into two parts, one part in test tubes with EDTA for estimation of trace elements (copper, zinc, cobalt and manganese) using Atomic absorption (GBC Scientific equipment PTY LTD Australia). The other part was allowed to clot in plastic tubes at room temperature (Alexander, 1988) for three hours. After that the clot was loosened and the tubes were stored at $4C^0$ overnight. Serum was removed in the second day and stored in -20 °C. The serum was then tested for antibody titers against Newcastle disease virus.

ELISA:

Sixteen sera samples were used for analyzing ND antibody titers using ELISA kits as prescribed by the manufacture (Symbiotic, Co., San Diego, USA).

Trace elements:

Trace elements (copper, zinc, cobalt and manganese) were estimated using Atomic absorption (GBC Scientific equipment PTY LTD Australia) with the methods represented in table 3.

Table 3: Instrument parameters for trace elements estimation in blood samples of broiler chicks.

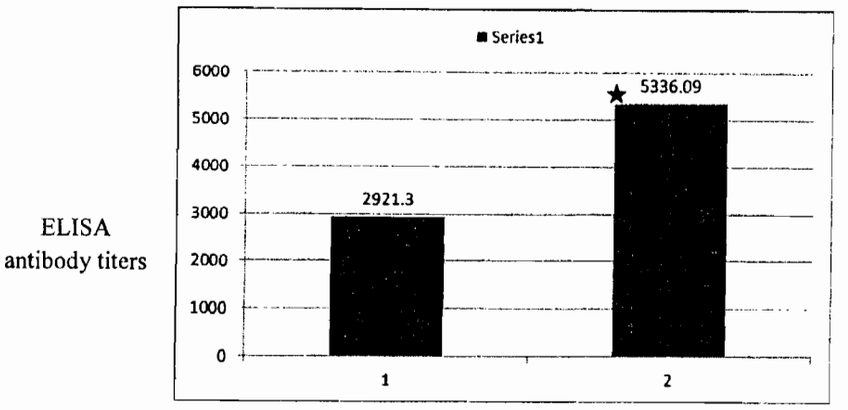
	Instrument parameters			
Elements	Zn	Cu	Co	Mn
System type	Flame	Flame	Flame	Flame
Lamp current	5.0 Am	3.0 mA	6.0 mA	5.0 Am
Wavelength	213.9 nm	324.7 nm	240.7 nm	279.8nm
Slit width	0.5 nm	0.5 nm	0.2nm	0.2nm
Measurement mode	Integration	Integration	Integration	Integration
Sample introduction	Manual	Manual	Manual	Manual
Read time	3.0	3.0	3.0	3.0
Calibration mode	Linear LS through Zero	Linear LS through Zero	Linear LS through Zero	Linear LS through Zero
Conc. unit	µg/ml	µg/ml	µg/ml	µg/ml
Conc. Decimal places	5	5	3	5
Check sample lower range	1.000 µg/ml	1.000 µg/ml	1.000 µg/ml	1.000 µg/ml
Flame type	Air- Acetylene	Air- Acetylene	Air- Acetylene	Air- Acetylene
Fuel flow	2.00 l/m	2.00 l/m	2.00 l/m	2.00 l/m
Oxidant flow	10.0 l/m	10.0 l/m	10.0 l/m	10.0 l/m

Statistical Analysis:

Data were presented as means ± S.E. and were analyzed using two way analysis of variance (ANOVA) using significant level of ($P<0.05$). Specific group differences were determined using Duncan's multiple range test as described by (Duncan, 1995).

RESULTS

ELISA antibody titers:
Addition of 10 ml of effective microorganisms /l drinking water has a significant ($p<0.05$) positive effect on the antibody titers against Newcastle disease virus vaccine of 5336.09±122.08, in comparison with the control group of 2921.51±275.40 (Fig.1).



★significant at ($p<0.05$)

Figure 1: Effect of Effective microorganisms on the ELISA antibody titers against Newcastle disease virus vaccine in broiler chicks.

Trace elements:
Addition of 10 ml of effective microorganisms /l drinking water has a significant ($p<0.05$) positive effect on the mean trace elements of Zn, Co, Cu and Mn of (1.171 µg/ml, 6.128 µg/ml, 0.376 µg/ml and 0.146 µg/ml) in comparison with (0.587 µg/ml, 4.151 µg/ml, 0.232 µg/ml and 0.000) respectively in the control group (Table 4).

Table 4: Effect of Effective microorganisms on the blood concentrations of trace elements in broiler chicks.

Elements	Mean µg/ml		Range µg/ml	
	T1	T2	T1	T2
Zn	0.587±0.0789	1.171±0.0831*	0.130 - 0.820	0.820 - 1.620
Co	4.151±0.507	6.128±0.549*	1.190 - 5.530	5.140 - 7.230
Cu	0.232±0.0204	0.376±0.0134*	0.281- 0.312	0.754 - 1.434
Mn	0.124±0.0428	0.284±0.134*	0.0598 - 0.550	0.128 - 1,620

*significant at ($p<0.05$)

DISCUSSION

Effective micro-organisms were administered to broiler chicks in the form of "EM solution" to study its effect on immune response and some of the blood trace elements of broiler chicks. Our results relating to Antibody titers against (ND) virus vaccine confirmed the previous work of (Monoura *et al.*, 2008). (Anjum *et al.*, 1996) who also reported that Antibody geometric mean titre (GMT) against Newcastle disease vaccine virus was 6.5 times in broilers given EM in drinking water, 3.85 times in broilers given EM fermented feed and 3.73 times in broilers given both EM in drinking water and fermented feed. Our results were also in the line of (Monoura *et al.*, 2008) on the ELISA antibody titre which was also higher in treated broilers with EM (4675.12±485.72) compared to (2169.38±724.45) in control group.

EM was reported to support immune system through significant increase in the relative weights of immune system organs (Bursa, thymus and spleen) (Liljebjelke *et al.*, 2008; Safalaoh, 2006; Anjum *et al.*, 1996). (Shoeib *et al.*, 1997), reported that the bursa of probiotic treated chickens showed an

increase in the number of follicles with high plasma cell reaction in the medulla. Since the mid-1980's, livestock researchers and producers in Japan began to test EM to be effective as a probiotic, (Kitazato Environmental Science Center 1994), and probiotics were reported in broiler chicks to have positive effects on immune organs (Willis *et al.,* 2007), by increase lymphocyte (Abdollahi *et al.,* 2003), on the increase of the phagocytic activity of leukocytes and the phagocytic index in broilers (Shareef and Al-Dabbagh 2009). The positive effect of feeding diet containing probiotics on the immune response could be due to their direct effect which may be related to stimulate the lymphatic tissue (Kabir *et al.,* 2004), or indirect via changing the normal microbial population flora of the lumen of gastrointestinal tract. Additionally, (Christensen *et al.,* 2002) suggested that some of these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Notably, It has been concluded that EM is immune modulator in broilers since treated birds had significantly more serum antibodies than those served as control birds (Haghighi *et al.,* 2005; Ahmed *et al.,* 1996).

The higher levels of all trace elements tested were shown in blood of broiler group amended with 10 ml of EM in the drinking water compared to those of the control. These findings may be related to the inoculation of the gastro-intestinal tract with beneficial microorganisms. The gastro-intestinal tract of birds is host to approximately 40 species of microorganisms with 3 or more different types of each one. The flora plays an important role in the digestion process. Bacterial enzymes promote the digestion of protein, lipids and carbohydrates and bacteria also synthesize vitamins that contribute to the nutrition of the bird (Larbier and Leclerco 1994). According to (Yongzhen and Waijiong 1994) EM Probiotic improves the coefficient of nitrogen absorption in the animal. After 45 days of EM treatment in day-old commercial broilers; live body weight was approximately 2004 grams for broilers given EM in drinking water, approximately 1978 grams for broilers given EM fermented feed and approximately 2022 grams for broilers given EM in both ways, compared to approximately 1690 g of the control broilers. (Yongzhen and Waijiong 1994) also found that the concentration of amino acids in the feed was improved 28% after the fermentation process with EM, indicating that EM improves the quality of the feed. A study that took place in the Aichi Prefecture in Japan with 70,000- 80,000 Arbor Acre broilers using EM for two years, showed an improvement in the feed conversion rate and an increase in the weight increase per day. The average broiler weight at shipment went from 2.68 Kg to 2.9 Kg. EM was given in the drinking water once a week and it was also sprayed inside and outside the chicken house before the birds were brought in (Kitazato Environmental Science Center 1994). In the same line were the results of (Jwher *et al.,* 2013), who found a significant improvement in body weight gain of broilers amended with EM in their drinking water (2019.8 ± 63.7 g of treated group vs. 1758 ± 34.52 in control group at 35 days of age. Moreover the intestinal mucosal architecture can reveal useful information on the intestinal function. The histological changes found in small intestines of the treated group probably had increased the intestinal surface area, facilitating the nutrient absorption to a greater extent and, thus boosted the promoting growth effect of certain probiotic (EM) supplementation. (Samanya, and Yamauchi 2002) found that longer villi in the ileum of adult male birds with slight improvement in feed efficiency after dietary addition of *Bacillus subtilis var. natto* and in broilers after addition *Enteroccocus faecium*. In addition to that, the increase releasing of mucin by goblet cells inhibit the reproduction of harmful bacteria in the intestine (Rahimi *et al.,* 2009) Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients. The intestinal mucosal architecture and its relation to nutrient absorption and performance enhancement was found by (Jwher *et al.,* 2013). The trace minerals which are of primary concern in poultry diets having recommended levels of supplementation in the ration formulated and given in this study which is in the same time as recommended by the (NRC 1984) Nutrient Requirements of Poultry include Zinc (Zn), Manganese (Mn), Copper (Cu), Iron (Fe), Selenium (Se) and Iodine (Leeson, 2005). The higher levels of Zn, Co, Cu and Mn in treated broilers with 10 ml of EM in their drinking water than those of control have a profound beneficial effect on the antibody titers against (ND) virus vaccine reported here, since Zn, Co, CU and Mn play a crucial role in human and animal immunity (Arthington and Havenga 2011; Sajadifar *et al.,* 2011; Larry and Berger 2006; Chaturvedi *et al.,* 2004; Patidar *et al.,*1999). Zinc as an example and its metalloenzymes in the poultry including carboxypeptidases and DNA polymerases enzymes, play important roles in the birds immune response, and their deficiency in poultry could include a suppressed immune system, both humoral and cellular responses (Sajadifar *et al.,* 2011; Sheila, 2008). In conclusion, and under the conditions of this experiment it is found that EM has a significant positive effect on the immunity of broiler chicks against vaccination to ND vaccine and to improve the trace element status of broiler chicks.

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تاثير اضافة الاحياء المجهرية الفعالة (EM) على مستوى الاجسام المضادة لفيروس مرض النيوكاسل ومستوى المعادن في افراخ فورج اللحم

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استخدمت افراخ فروج اللحم من نوع روص ٣٠٨ غير مجنسة حيث قسمت الى مجموعتين من ٣٠ فرخة لكل مجموعة ووضعت في اقفاص منفصلة، للفترة مابين ١٠/١ ولغاية ٢٠١٢/١١/٥ لمدة خمسة اسابيع. تم اضافة المنتج الحيوي الطبيعي EM بنسبة ١٠ مل /لتر من ماء الشرب، كما لقحت الافراخ بلقاح النوكاسل (بعترة لاسوتا). جمعت عينات الدم وقسمت الى قسمين، الاول استخدم لقياس المعادن (النحاس والزنك والكوبلت والمنغنيز) باستخدام جهاز الامتصاص الضوئي، والجزء الثاني لقياس مستوى الاجسام المناعية لفيروس النيوكاسل. اظهرت النتائج وجود اختلاف معنوي عند ($p<0.05$) زيادة في مستوى الاجسام المضادة عند 5347.07±117.03 للمجموعة المدعومة بالاي ام بالمقارنة مع مجموعة السيطرة التي بلغت 2949.24±314.51. وهناك فرق منوي عند ($p<0.05$) في معدل الاملاح للزنك والكوبلت والنحاس والمنغنيز للمجموعة المتناولة للاي ام بالمعازنة مع مجموعة السيطرة.