# Influence of Effective Microorganisms on Health and Immune System of Broilers under Experimental Conditions

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## **Abstract**

Eighty, day-old, commercial broilers were divided in four equal groups and were fed standard broiler ration. Effective microorganisms (EM) were administered in solution form through drinking water to 1st group, in solid form (Biofeed) through feed to 2nd group and both to 3rd group from 10 days of age to 56 days of age. The 4th group served as control. Birds were vaccinated with Newcastle disease (ND) vaccine at 7 days and 28 days of age.

After 45 days of EM treatment live body weight was  $2004=F1\ 45.1\ g$ ,  $1978=F1\ 61.5\ g$  and  $2022=F1\ 45.4\ g$ , respectively, in broilers given EM solution alone, biofeed alone and both simultaneously compared with  $1690=F1\ 37.1\ g$  in the control broilers (P<0.001).

Of the lymphoid organs bursa and thymus indices were significantly greater in broilers given EM solution alone and both EM solution and biofeed compared with the control broilers (P<0.05) at 45 days post-treatment. Spleen weight was not influenced with EM treatment.

Of the visceral organs liver index was significantly lesser in broilers given biofeed alone compared with the control broilers (P<0.01). Gizzard index was significantly lesser in birds given EM solution (P<0.05), biofeed (P<0.001) and both (P<0.01) than the control broilers. Heart index was significantly lesser in birds given EM solution (P<0.01), biofeed (P<0.01) and both (P<0.05) than the control broilers.

Proventriculus, intestine, kidneys, pancreas and thyroid Indices did not differ significantly between EM treated and control broilers at 45 days post-treatment.

Antibody geometric mean titre (GMT) value against ND vaccine virus during primary response was 13 in broilers given EM solution alone, 9.8 in broilers given biofeed alone and 9.8 in broilers given both simultaneously compared with 3.2 in the control broilers. The counterpart GMT values during secondary response were 445.7, 264, 256 and 68.6.

In conclusion, EM is a safe product. This technology can be applied for promoting growth and for potentiation of immune response in the chicken.

## Introduction

Effective microorganisms (EM) is a newer technology from Japan recently introduced in Pakistan by Hussain et al., 1996. EM is available in the form of solution and solid for commercial use. Until now the products are being used mainly in Agriculture and the technology has shown promising results in plant health and production. There were reports from abroad on EM use in livestock and poultry. This study was envisaged to investigate the effect of EM on health and immune system of poultry under local conditions.

#### Materials and Methods

Experimental birds Eighty day-old, commercial broiler chicks of certified health status were

procured from a breeder company (Mughal Chicks Sargodha). The chicks were divided into four equal groups and kept in cages under standard management conditions. The chicks were fed adlibitum commercial broiler ration (Punjab Feeds Ltd.) containing 23 per cent crude protein and 3000 ME/kg throughout the study period of eight weeks.

# Effective microorganisms

EM was supplied by Hussain et al. (1996) in solution and solid (biofeed) form. EM primarily contains Lactic acid bacteria and other useful bacteria. EM was given as solution at the dose rate of 1 ml/L drinking water to first group, as biofeed at the dose rate of 30 g/Kg in feed to second group and both simultaneously to third group continuously from 10 days of age to 56 days of age. The fourth group served as control.

## **Parameters**

The following parameters were observed.

- 1. Weekly live body weight.
- 2. Feed consumption and feed conversion ratio.
- 3. Organ weights at four weeks and eight weeks of age.
- 4. Antibody titre after primary and booster vaccination with Newcastle disease vaccine virus (LaSota strain, Bioteke Italy).

### **Results**

# Live body weight

Live body weight in experimental broilers is given in Table 1. It was significantly greater in broilers given EM solution alone or biofeed alone than the control from 4th week post-treatment onward (P<0.001). Whereas live body weight was significantly greater in broilers given both EM solution and biofeed simultaneously than the control broilers from 3rd week post-treatment onward (P<0.001). At the end of 45 days of treatment live body weight was 18.6 percent, 17 percent and 19.6 percent greater, respectively, in broilers given EM solution alone, biofeed alone and both simultaneously than the control broilers. Net weight gain in the three respective treatments was 1912 g, 1884 g and 1919 g compared with 1596 g in the control broilers.

## Feed conversion ratio

Feed consumption was 5.11 kg in broilers given EM solution, 5.12 kg in broilers given biofeed and 5.18 kg in broilers given both simultaneously compared with 4.93 kg in the control. Feed conversion ratio was 2.55 in broilers given EM solution, 2.59 in broilers given biofeed and 2.56 in broilers given both simultaneously compared with 2.92 in the control.

## Lymphoid organs

Actual weight of bursa and thymus and their indices were significantly greater in EM treated broilers compared with the control (Table 2, P<0.05).

Actual weight of spleen was significantly greater in EM treated broilers compared with the control (P<0.05) but its index did not differ significantly between EM treated broilers and the control (Table 2).

## Visceral organs

Table 3 shows that actual weight of liver was significantly greater in EM treated broilers

compared with the control but liver index was significantly lesser in EM treated broilers compared with the control (P<0.05). Actual weight of proventriculus was significantly greater in EM treated broilers compared with the control (P<0.05) but its index did not differ significantly between EM treated broilers and the control. Actual weight of gizzard did not differ significantly between EM treated broilers and the control but gizzard index was significantly lesser in EM treated broilers compared with the control (P<0.05). Actual weight of intestine and its Index did not differ significantly between EM treated broilers and the control. Actual length of intestine was significantly lesser in EM treated broilers compared with the control (P<0.01). Actual weight of pancreas was significantly greater in EM treated broilers compared with the control (P<0.05). Pancreas index did not differ significantly between EM treated broilers and the control. Actual weight of kidney and its index did not differ significantly between EM treated broilers and the comrol. Actual weight of heart did not differ significantly between EM treated broilers and the control. Heart index was significantly lesser in birds given EM solution (P<0.01), biofeed (P<0.01) and both (P<0.05) than the control broilers. Actual weight of thyroid and its index did not differ significantly between EM treated and control broilers at 45 days post-treatment.

# Antibody titre

Primary and secondary immune responses, in terms of antibody geometric mean titre (GMT) were determined against Newcastle Disease (ND) vaccine virus. GMT values during primary response were 13 in broilers given EM solution alone, 9.8 in broilers given biofeed alone and 9.8 in broilers given both simultaneously compared with 3.2 in the control broilers. The counterpart GMT values during secondary response were 445.7, 264, 256 and 68.6.

#### Discussion

Effective micro-organisms were administered to broiler chicks in the form of "EM solution" "biofeed" and "both simultaneously" to study their effect on health and immune response of broiler chicks. Live body weight was significantly greater in all EM treated groups compared with the control (Table 1, P<0.001). It confirms growth promoting activity of EM as reported by other workers (Hussien and El-Ashry, I991; Ahmad, 1996; Hussain 1996). The better weight gain in EM-treated broilers could be related to better digestibility of crude protein and crude fiber (Hussain, 1996). Interestingly, the increase in live body weight was also accompanied with a significant decrease in offal weight i.e., of liver index (P<0.05), gizzard index (P<0.01), intestinal weight index, intestinal length index (P<0.05). kidneys index (P<0.05) and heart index (P<0.01) (Table 3). In Pakistan there are numerous antibiotics widely used as growth promoters but all have associated risks of drug residues and drug resistance. The EM appears to be a safe growth promoter without any associated risks.

Of the visceral organs the decrease in heart index is of particular interest and needs further consideration. This has also been observed in a previous experiment (Ahmad, 1996). Heart enlargement is a common disease in humans. If constituents of the EM responsible for heart enlargement can be determined and purified, further research may indicate if this can help in treatment of heart enlargement.

The greater bursa and thymus index in birds supplemented EM as compared to the control

(Table 2, P<0.05) suggests that EM supported these lymphoid organs. The two lymphoid organs are responsible for recruiting B and T lymphocytes and make up the vital and basic components of humoral and cellular immunity. The gross support of lymphoid organs was also accompanied with better antibody production in EM treated broilers. In the present study GMT against Newcastle disease vaccine virus was 6.5 times in broilers given EM solution, 3.85 times in broilers given biofeed and 3.73 times in broilers given both EM solution and biofeed simultaneously than the control value.

The present study showed that EM potentiated immune response in the experimental broilers. Previous studies demonstrated that Lactic acid bacteria administered orally or intraperitoneally enhanced activity of the mononuclear phagocytic system (Kato et al., 1983) and increased the production of circulating antibodies for certain antigens in mouse (Saito et al., 1983; Perdigon and Alvare, 1996). However, further investigation is required for the elucidation of the mechanism through which EM produced systemic increase in the immune response.

## References

Ahmad, A. 1996. Personal communication

Hussain, I., 1996. Effect of microbial culture (EM4) on the performance of male broiler chicks. M.Sc. thesis, Department of Poultry Husbandry, UAF, Pakistan.

Hussain, T., R. Ahmad, G. Gilani and T. Higa, 1996. Applied EM Technology, Nature Farming Research Centre, University of Agriculture Faisalabad, Pakistan.

Hussein, H.H. and M.A. EI-Ashry, 1991. Some studies on the beneficial effect of Lactobacillus concentrate supplementation on broiler performance. Egyptian Journal of Animal Reproduction 28(1):85-91.

Kato, I., T. Yokokura and M. Mutai, 1983. Macrophage activation by Lactobacillus casei in mice. Microbial. Immunol., 27(7):611-618.

Perdigon, G. and S. Alvarez, 1995. Probiotics and the immune state. In: Probiolics. Ed. R. Fuller. pp.1-25.

Saito I. K. Sato and Y. Horikawa 1983. Enhanced humoral antibody production and delayed type hypersensitivity response in mice by Lactobacillus casei. Hiroshima J. Med. Sci., 32:223-225.

Table 1. Effect of effective microorganisms on live body weight (grams) of experimental broilers.

Age (wk)	Control	EM solution	Biofeed	EM solution + Biofeed
1	94.3=F1 4.0	91.8=F1 1.9	94=F1 2.4	103=F1 4.1
2	251=F1 7.9	233=F1 13.1	236=F1 8.1	243.1=F1 8.3
3	443=F1 25.8	459=F1 21.4	478=F1 12.2	524=F1 18.6***
4	556=F1 29.0	689=F1 39.6***	665=F1 25.2***	807=F1 32.9***
5	864=F1 35.5	1217=F1 35.8***	1207=F1 32.5***	1361=F1 20.1***
6	1052=F1 30.1	1267=F1 38.4***	1258=F1 23.8***	1352=F1 33.7***
7	1367=F1 39.2	1740=F1 40.7***	1663=F1 48.9***	1752=F1 50.9***
8	1690=F1 37.1	2004=F1 45.1***	1978=F1 61.5***	2022=F1 45.4***

Each figure represents mean (=F1 standard error of the mean) of 10 birds.

Table 2. Actual weight and index of lymphoid organs in experimental broilers at 45 days post EM treatment.

Control	EM solution	Biofeed	EM solution + Biofeed		
Bursa Actual weight (g)					
2.10=F1 0.15	3.52=F1 0.32**	2.94=F1 0.34*	4.02=F1 0.34***		
Index					
0.125=F1 0.009	0.177=F1 0.017*	0.147=F1 0.013	0.199=F1 0.017**		
Thymus Actual weight (g)					
6.56=F1 0.36	10.24=F1 0.79***	9.30=F1 0.78**	10.71=F1 1.02**		
Index					
0.39=F1 0.031	0.51=F1 0.041*	0.47=F1 0.033	0.53=F1 0.050*		
Spleen Actual weight (g)					
1.73=F1 0.14	2.05=F1 0.11	1.81=F1 0.15	2.32=F1 0.24*		
Index					
0.103=F1 0.009	0.103=F1 0.006	0.09=F1 0.008	0.114=F1 0.010		
	*	•	•		

Each figure represents mean (=F1 standard error of the mean) of 10 birds.

<sup>\*\*\*</sup>P<0.001 compared with the control.

<sup>\*</sup>P<0.05, \*\*P<0.01, \*\*\*P<0.001 compared with the control.

Table 3. Actual weight and index of visceral organs in experimental broilers.

Age	Control	EM solution	Biofeed	EM solution +		
(week)				Biofeed		
Liver Actual weight (g)						
4	26.8=F1 1.06	30.6=F1 1.50*	30.7=F1 1.33*	36.3=F1 0.72***		
8	34.0=F1 1.76	39.5=F1 0.83*	33.1=F1 1.11	39.9=F1 2.2*		
		Index				
4	3.13=F1 0.141	2.54=F1 0.078**	2.55=F1 0.110**	2.67=F1 0.055*		
8	2.01=F1 0.096	1.97=F1 0.038	1.68=F1 0.041**	1.97=F1 0.082		
		Proventriculus Ac	tual weight (g)			
4	5.67=F1 0.23	6.41=F1 0.37	5.99=F1 0.13	7.22=F1 0.21		
8	6.78=F1 0.16	8.35=F1 0.64*	7.36=F1 0.35	7.64=F1 0.27*		
		Index				
4	0.40=F1 0.013	0.53=F1 0.023	0.49=F1 0.015	0.53=F1 0.017		
8	0.40=F1 0.013	0.42=F1 0.036	0.37=F1 0.013	0.37=F1 0.013		
	(	Gizzard Actual weight (	g)			
4	18.7=F1 1.06	25.6=F1 1.38**	26.2=F1 0.89***	29.9=F1 0.77***		
8	37.8=F1 1.32	39.8=F1 1.46	35.3=F1 1.05	35.9=F1 2.15		
		Index				
4	2.17=F1 0.094	2.13=F1 0.076	2.18=F1 0.079	2.21=F1 0.057		
8	2.25=F1 0.086	1.99=F1 0.081*	1.79=F1 0.052***	1.77=F1 0.094**		
	It	ntestine Actual weight (	(g)			
4	30.9=F1 1.63	36.9=F1 1.72*	33.2=F1 2.02	37.6=F1 1.23**		
8	45.8=F1 2.05	51.9=F1 1.49	49.8=F1 2.01	51.4=F1 3.1		
		Index				
4	3.59=F1 0.15	3.09=F1 0.13*	2.73=F1 0.11***	2.76=F1		
0	2.72 - 51 0 1 4	2 (0 F1 0 07	0.54 E1.0.14	0.073***		
8	2.72=F1 0.14	2.60=F1 0.07	2.54=F1 0.14	2.53=F1 0.11		
4		testine Actual length (c		160 0 151 4 4**		
4	149.8=F1 3.31	165.4=F1 5.1*	153.7=F1 3.6	168.8=F1 4.4**		
8	184.2=F1 2.38	188.2=F1 4.7	169.9=F1 10.7	171.9=F1 4.5*		
4	17.5 F1.0.65	Index	12.0 E1.0.26***	10 4 E1 0 27***		
8	17.5=F1 0.65 10.9=F1 0.31	13.9=F1 0.39*** 9.4=F1 0.25**	12.8=F1 0.36*** 8.6=F1 0.53**	12.4=F1 0.37*** 8.51=F1 0.19***		
0		ancreas Actual weight (		8.31=F1 0.19****		
4	4.3=F1 0.24	3.77=F1 0.53	2.82=F1 0.08***	4.28=F1 0.14		
8	3.33=F1 0.24	4.22=F1 0.33*	4.12=F1 0.28*	4.42=F1 0.14 4.42=F1 0.42*		
0	J.JJ-11 U.24	4.22=F1 0.33** Index	4.12-11 0.20	4.42-11 U.42		
4	0.51=F1 0.035	0.32=F1 0.053*	0.23=F1 0.008***	0.32=F1		
+	0.31-11 0.033	0.32-11 0.033	0.23-11 0.000	0.52-61		
8	0.19=F1 0.014	0.21=F1 0.012	0.21=F1 0.013	0.22=F1 0.019		
Kidneys Actual weight (g)						

4	8.8=F1 0.25	10.1=F1 0.45*	9.5=F1 0.39	11.9=F1 0.48***		
8	15.9=F1 0.88	17.4=F1 0.8	16.2=F1 0.66	17.1=F1 0.95		
	Index					
4	1.042=F1 0.063	0.84=F1 0.027*	0.79=F1 0.029**	0.88=F1 0.036*		
8	0.94=F1 0.042	0.87=F1 0.032	0.82=F1 0.025*	0.84=F1 0.038		
	Heart Actual weight (g)					
4	4.13=F1 0.14	5.51=F1 0.19***	5.41=F1 0.27**	6.31=F1 0.24***		
8	7.71=F1 0.32	7.26=F1 0.39	7.57=F1 0.27	7.90=F1 0.39		
	Index					
4	0.49=F1 0.017	0.46=F1 0.018	0.45=F1 0.018*	0.46=F1 0.015*		
8	0.46=F1 0.019	0.36=F1 0.014**	0.38=F1 0.015**	0.39=F1 0.017*		
Thyroid Actual weight (g)						
4	0.13=F1 0.013	0.17=F1 0.013*	0.12=F1 0.014	0.15=F1 0.020		
8	0.16=F1 0.006	0.17=F1 0.012	0.17=F1 0.014	0.19=F1 0.024		
Index						
4	0.015=F1 0.001	0.015=F1 0.002	0.010=F1 0.001	0.011=F1 0.001		
8	0.010=F1 0.0005	0.009=F1 0.0006	0.009=F1 0.0007	0.010=F1 0.001		

Each figure represents mean (=F1 standard error of the mean) of 10 birds.

<sup>\*</sup>P<0.05, \*\*P<0.01, \*\*\*P<0.001 compared with the control group.