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## Effective Microorganisms (EM) as an Alternative to Antibiotics in Broiler Diets: Effect on Broiler Growth Performance, Feed Utilisation and Serum Cholesterol.

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**Abstract :** *An experiment was conducted to evaluate the effect of using Effective Microorganisms (EM) as an alternative to antibiotics (AB) on growth performance, feed utilisation and serum cholesterol of broilers. Dietary treatments consisted of supplementation with neither AB nor EM, AB only, EM only or AB plus EM. The EM was supplemented at either 15 g/kg or 30 g/kg while the AB (Zinc Bacitracin) was added at 500 mg/kg. At six weeks of age, birds fed diets with neither the EM nor AB had significantly ( $P < .05$ ) lower weight gains (2066 g) than the rest of the treatments. Birds fed the diet containing AB and EM at 0 g/kg had significantly ( $P < .05$ ) higher body weight gain (2096g) than the rest of the treatments. The improvements in BWG were associated with slight enhancement of feed efficiency while the EM effects were more pronounced at the higher dosage (30 g/kg). The poorest feed:gain ratio (1.82) was observed in birds fed diets containing neither EM nor AB. Apart from improving dressing percentage, EM supplementation also resulted in birds with low serum cholesterol levels. This study has shown that EM has growth promoting and hypocholesteremic effects and offers a potential alternative to antibiotics in broiler diets.*

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### Introduction

Antibiotics (AB) continue to be used in the poultry industry as growth stimulants and therapeutic agents. However, due to the fact that continued use of AB tends to stimulate development of resistance from harmful micro-organisms, there is currently an outcry from the consumer society and health sector to ban their (AB) use as feed additives in animal and poultry feeds. It is therefore urgent and imperative that an alternative to replace antibiotics should be found. However, such an alternative should elicit positive results similar to those of AB without compromising bird growth, feed utilisation and the quality of final product. According to this criteria, and based on the current available knowledge on feed additives, probiotics seem to be the best alternative.

Probiotics have been shown to have growth promoting (Cavazzoni et al, 1998; Jin et al, 1998; Yeo and Kim, 1997; Mohan et al, 1996) prophylactic (Cavazzoni et al, 1998; Yeo and Kim, 1997) and hypocholesteremic effects (Jin et al, 1998; Mohan et al, 1996). Jin et al (1998) attributed enhanced growth rates by probiotics to improved feed efficiency.

There are several probiotics on the market world wide. In South Africa, Effective Microorganisms (EM)<sup>1</sup> is a probiotic that has recently been introduced. Use of EM has been shown to improve animal health (Phillips and Phillips, 1996). However, there is paucity of information regarding the efficacy and beneficial effects of EM *vis a vis* AB. Without

any tangible evidence from empirically derived data, the adoption of this innovation in the poultry industry cannot be guaranteed.

This experiment was conducted to evaluate the effects of supplementing broiler diets with EM at two different inclusion rates as an alternative to AB (Zinc bacitracin) on body weight gain (BWG), feed efficiency (FCR), dressing percentage/ carcass yield (CARC) and serum cholesterol (CHOL) of broilers from 1-42 days of age.

## **Materials and Methods**

### **Birds**

Four hundred and fifty chicks were randomly selected and assigned to 6 treatments. The treatments (Trt.) involved addition of AB at 500 mg/kg (AB) and EM at either 15 g/kg (EM15) or 30 g/kg (EM30). Some diets had no EM (EM0) or AB (AB0) added. The 6 treatments were as follows: Trt.1= EM0, AB; Trt. 2= EM0, AB0; Trt. 3 = EM15, AB; Trt. 4 = EM15, AB0; Trt. 5 = EM30, AB; Trt. 6 = EM30, AB0.

Each treatment had five replicates of 15 birds each. Birds were housed in an environmentally controlled broiler house with a floor covered with wood shavings for the whole experimental period.

### **Feed**

Birds were fed a commercial starter mash (12.8 MJ/kg ME, 22.99 CP) from 1-28d followed by finisher mash (13.4 MJ/kg ME, 20.03 CP) from 29-42 d of age.

Liquid EM was initially mixed with a portion of the basal diet which contained maize meal, soybean meal, molasses and fish meal to make "Bokashi". The Bokashi was made as per the method of Phillips and Phillips (1996) before being mixed with the rest of the feed.

### **Measurements**

Bird weight gains and feed intake (FI) measurements were determined at weekly intervals. On day 42, five birds were randomly selected from each treatment for collection of blood through the brachial vein. The blood was drained into a polythene tube and centrifuged at 5,000 rpm for 10 minutes. Serum CHOL was then determined using the Synchron CX System (Beckman Instruments, Inc., 1995). The birds were then killed by cervical dislocation for determination of carcass. The carcass yield excluded feathers, feet, head and viscera and was expressed as percent of live weight and reported as dressing percentage.

### **Data Analysis**

Data were analysed using the General Linear Models procedures of SAS (SAS Institute, 1988) at  $P < .05$ .

**Table 1 Effects of EM and Antibiotic Supplementation on Final Body Weight Gain, Food Intake, Feed Conversion Ratio of Broilers at 42 Days of Age**

	Treatment					
	1	2	3	4	5	6
BW Gain (g, 0-42d)	2084.9629.3 <sup>b</sup>	2065.9928.1 <sup>a</sup>	2090.5929.6 <sup>b</sup>	2075.75+27.8 <sup>c</sup>	2095.6625.9 <sup>c</sup>	2091.7028.4 <sup>b</sup>
Food Intake, g	3737.3133.4 <sup>a</sup>	3755.1532.8 <sup>c</sup>	3733.3731.6 <sup>c</sup>	3739.13+30.7 <sup>d</sup>	3769.6129.4 <sup>c</sup>	3762.432.6 <sup>c</sup>
Feed: Gain, g:g	1.790.03	1.820.05	1.790.04	1.80+0.06	1.790.05	1.800.07
Dressing Percentage, % of BW	67.121.35 <sup>b</sup>	67.261.12 <sup>b</sup>	68.910.53 <sup>b</sup>	68.72+0.76 <sup>b</sup>	72.130.92 <sup>a</sup>	71.801.12 <sup>a</sup>
Serum Cholesterol, (mmol/l)	3.38 0.19 <sup>a</sup>	3.36 0.15 <sup>a</sup>	3.32 0.18 <sup>a</sup>	3.10+0.15 <sup>b</sup>	3.08 0.17 <sup>b</sup>	3.02 0.13 <sup>b</sup>

<sup>a,b,c,d</sup> Means with no common superscripts are significantly different

Treatment 1. = 0 EM, 500mg/Kg Zinc Bacitracin

Treatment 3. = 15 g/kg EM, 500mg/Kg Zinc Bacitracin

Treatment 5. = 30 g/kg EM, 500mg/Kg Zinc Bacitracin

Treatment 2. = 0 EM, 0mg/Kg Zinc Bacitracin

Treatment 4. = 15 g/kg EM, 0mg/Kg Zinc Bacitracin

Treatment 6. = 30 g/kg EM, 0mg/Kg Zinc Bacitracin

**Results**

The effect of EM and AB supplementation on BWG, FI and CARC are presented in Table 1.

Birds fed diets containing AB and EM0 (Trt.1) had significantly ( $P<.05$ ) higher BWG than those fed diets containing neither AB nor EM (Trt. 2) or the diet containing EM15 (Trt. 4). Diets supplemented with AB, EM15 (Trt. 3) produced BWG similar to diets that contained AB, EM30 and AB0, EM30. A combination of AB and EM30 produced the highest BWG. The high BWG was associated with increased FI. Though not significantly ( $P >. 05$ ) different, feed conversion ratio tended to improve with addition of AB, EM or both. Dressing percentage was significantly ( $P<.05$ ) higher for birds containing EM30 with or without AB supplementation (Trt.5 and 6).

Effects on serum CHOL are presented in the Fig. 1. Supplementing diets with EM resulted in birds with reduced serum CHOL. The CHOL reducing properties of EM occurred in a dose dependent manner. The lowest serum cholesterol content was observed in birds fed diets containing EM 30.

Two other notable results were observed in this study. A negligible mortality rate of only 0.22 per cent (1 out of 450 birds) occurred for the whole experimental period. The bird died in week 5 (Trt. 5) probably due to Sudden Death Syndrome. Another observation was that during the first few days (1-21d), there was a propensity for birds fed diets containing EM diet to have pasted vents.

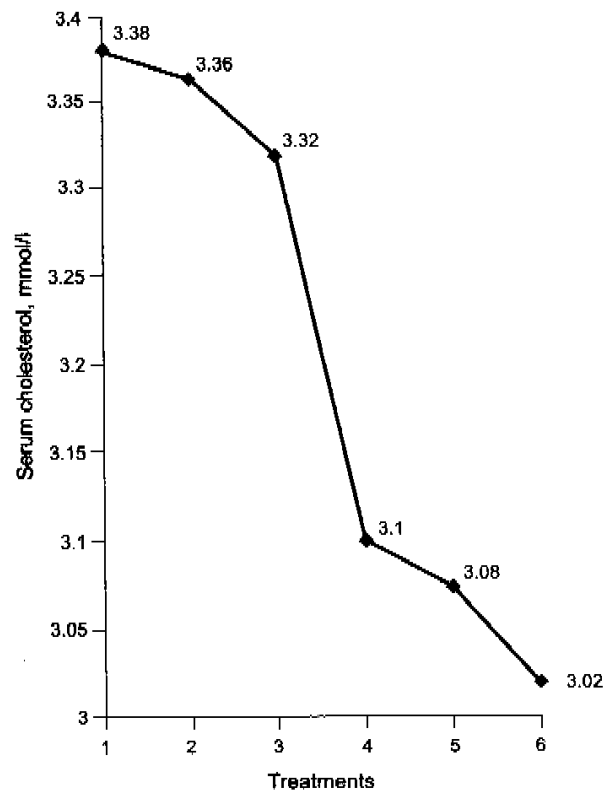
**Discussion  
and  
Conclusions**

Diets containing AB, EM0 had better BWG than those with AB0, EM0. This is a clear manifestation and demonstration of the growth stimulation effect of AB. Addition of EM at 15g/kg elicited no beneficial effects on BWG unless supplemented together with AB. However, an inclusion rate of 30g/kg of EM with or without AB resulted in improved BWG. These results suggests that a right dosage (30 g/kg of feed) of EM is required to exhibit growth stimulation effects in broilers. At this inclusion level, addition of AB produces a complimentary effect. There were no differences in the FCR reported in this study. However, the FCR tended to improve with addition of EM or AB which may be a reason for the improvements in BWG. Increased BWG was also associated with high FI. Mohan et al (1996), attributed the growth promotion effects of probiotics to improvements in utilisation of apparent metabolisable energy while enhanced feed utilisation was mentioned by Jin et al (1998).

The high dressing percentage for birds fed the 30 g/kg EM supplemented diet was concomitant with the high BWG observed in the respective treatments. Other studies have shown no differences in dressing percentage between probiotic and no-probiotic supplemented diets (Mohan et al, 1996).

In agreement with other studies involving broilers (Mohan et al, 1996; Jin et al, 1998), this study has demonstrated that probiotics such as EM have serum CHOL reducing properties. These effects are dose dependent and according to this study, the appropriate dosage is 30 g/kg. Eyssen (1973) reported that deconjugation of bile acids in the small intestine may be responsible for reduction of concentrations of serum cholesterol because deconjugated

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**Fig. 1. Effect of Probiotic and Antibiotic Supplementation on Serum Cholesterol of Broilers at 42 d of age**

bile acids do not function as well as conjugated bile acids in solubilisation and absorption of lipids. Chikai et al (1987) reported that adherence of deconjugated free bile acids to bacteria and dietary fibre enhances excretion of the bile acids. This mechanism has been implicated to trigger a feedback mechanism that regulates hepatic cholesterol synthesis and subsequent transformation into bile acids which may be responsible for lowering serum cholesterol levels.

A mortality rate of only 0.22 per cent was recorded for the whole trial. Although a prophylactic effect by probiotics has been reported in other studies, (Cavazzoni et al, 1998), it is difficult to draw a similar conclusion in the study presented here because there are no differences in the morbidity and mortality cases among the six treatments some of which had no EM added.

In conclusion, this study has demonstrated and substantiated earlier reports that probiotics such as Effective Microorganisms (EM) has growth promoting effects. Additionally, supplementation of broiler diets with EM has serum cholesterol reducing properties, an element important for the health conscious consumers. A dosage of 30 g/kg is required if the aforementioned benefits are to be attained.

Finally, although further research on beneficial effects of dietary EM is required, the results reported herein bear testimony to the fact that probiotics such as EM offer a potential alternative to antibiotics in broiler diets.

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## Effective Microorganisms (EM) for Animal Production

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*Abstract :Potential of EM in agriculturally dominated Nepal is high where livestock is an integral part the of agricultural system and plays an important role in the nutrition and economy of the people. The role of EM to enhance production, to control malodor and to induce immune response against diseases seems promising. To evaluate the effect of EM on production and health of broilers and the economical feasibility of its use in broiler farming, two demonstration trials were conducted in 1997 and 1999 at a village farm and Institute of Agriculture and Animal Science (IAAS) livestock farm respectively. Another trial was conducted at IAAS livestock farm to see the effect of EM on growth performance of goat in 1997. Further, a demonstration trial on use of EM in the pigsty was conducted at a village farm in 1998. The results of trials on broilers indicated that EM promoted growth, improved health, reduced cost of production and controlled malodor in the farms. Out of different methods of EM applications (EM solution through drinking water, EM Bokashi through feed and both of them) tested, EM solution through drinking water was found to be cost effective and labour saving. In goat, the body weight gain was highest with feed mixed with 10 % EM Bokashi as compared to 5 % and control. In pigsty, EM effectively controlled malodor resulting in a healthy and socially acceptable environment. The economic analysis of using EM in broilers indicated that EM was a cheaper product and could be used profitably in broiler farming.*

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### **Introduction**

Nepal has a farming based economy and livestock is an integral part of the agricultural system. Livestock has been reared principally for manure and provides almost all the draft power used in cultivation. This contribution together with production of food (meat, milk, egg), fibers, hides/skin and transportation amounts to 15 percent of GDP (LMP 1993). This sector, on the other hand, contributes about 1/3<sup>rd</sup> of agricultural GDP (NPC 1998). As the present status of the country indicates that the consumption of meat, milk and egg is very low by Asian standards this sector is facing pressure to commercialize itself in order to meet the nutritional demand of the growing population. However, the sustainability of the existing farming system has remained the issue of concern, because of many problems associated with the modern system moving towards chemical based farming.

Notwithstanding the potential of income generation for rural farmers, the poor management, unhygienic conditions, the low immune status and digestibility prevailing in the livestock and poultry farms are attributed to the reduced appetite, greater incidence of health problems, poor growth and consequently high mortality. All these lead to great economic loss to the farmers. Moreover the use of antibiotics and other drugs to treat the animals increases the cost of production as well as creates other problems like drug residues and drug resistance, which is of serious concern to public health. But the benefits of using EM in livestock farming were such that it eradicated odours, keeps the animals healthy, does away with the



need to use antibiotics and cuts down the unnecessary labour (Higa, 1994). Ahmed et al. (1996) has concluded in his report that EM is a safe product and this technology can be applied for promoting growth and for inducing immune response in broilers. Use of EM in layers showed increased resistance, reduced mortality, increased egg production and average egg weight rate whereas in pig rearing it showed not only even growth and quicker growth speed but also hog's skin and hair gradually turned to luster (Li Wei-Jiong et al. 1994). EM contains many naturally occurring beneficial microorganisms, which are both oxybiotic and anaerobic microbes. After entering the body of animals as foodstuffs these microbes may multiply rapidly and they not only check the growth of other pathogenic microbes but also form the normal microbial group within the host body to produce main vitamins for the host, provide nutrients and prevent attack of the pathogens (Li Wei-Jiong et al. 1994). So EM can be used in livestock farming especially more effectively in biological animal husbandry to address the above mentioned problems in Nepal. Thus, a few demonstration trials were conducted to observe the influence of EM on performance of broilers, goats and on pigsty.

#### **Materials and Methods**

##### **Effect of EM on Broiler Production**

Two demonstration trials were conducted using EM in broiler farming in 1997 at a village farm and in 1999 at the IAAS livestock farm. In both cases the broilers were divided into four groups namely G1, G2, G3 and G4. The owner of the village farm and the caretaker of poultry farm at IAAS were trained to prepare solid and liquid forms of EM for use. The liquid EM used was the secondary EM (or EM-2) which was prepared from primary EM or EM stock solution provided by the Community Welfare and Development Society (CWDS), a professional NGO involved in the extension of EM technology in Nepal. The solid form was the EM Bokashi prepared anaerobically from rice bran. Both forms were prepared by the methods as suggested by CWDS. The prepared liquid EM was given through drinking water at the rate of 1 ml. per litre of drinking water to G2, whereas G3 was given the solid form of EM at the rate of 50 gm. per kg. of feed and G4 was given both solid and liquid form of EM continuously after 10 days of age. G1 served as control. In case of village farm, chicken were fed *ad libitum* the commercial broiler ration throughout the study period but in the IAAS livestock farm equal quantity of ration was given to the broilers in all the treatments and control. The trials at village and IAAS livestock farm were conducted during summer and winter respectively. However, deep litter management system was practised in both cases.

#### **Results and Discussion**

Results from village farm showed that the live body weight of the broilers in all treatment groups were greater than the control. The average live body weight of the four groups i.e. G1, G2, G3 and G4 at 54 days of age were 1492, 1812, 1788 and 1860 g respectively (Table.1). The net weight gain of the broilers in G2 (EM solution), G3 (EM Bokashi) and G4 (both EM solution and Bokashi) over the control were 30.5, 24.6 and 30.5 per cent respectively (Table.2). In case of IAAS livestock farm the final average live body weight at 53 days of age in G1( control), G2 (EM solution), G3 (EM Bokashi) and G4(both EM solution and Bokashi) were 2276, 2380, 2395 and 2261 respectively (Table 3) whereas the percentage net weight gain of the broilers in G2(EM solution), G3 (EM Bokashi) and G4

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**Table 1. Effect of EM on Live Body Weight (g) of Broilers at Village Farm, Chitwan, 1997**

Age (Days)	G1 (Control)	G2(EM solution)	G3(EM Bokashi)	G4 (Solution + Bokashi)
18	307	266	311	314
27	590.5	563	584	645.5
36	957.5	1077	1066	1085
45	1120	1338	1320	1410
54	1492	1812	1788	1860

\*\* Each figure represents mean of 10 birds.

**Table 2. Percentage Net Weight Gain (g) over the Control at Village Farm, Chitwan, 1997**

Groups	Initial Weight(x)	Final Weight(y)	Net Weight Gain (y-x)	Percentage(%) Net Weight Gain
G1 (control)	307	1492	1185	---
G2 (EM solution)	266	1812	1546	30.5
G3 (EM Bokashi)	311	1788	1477	24.6
G4 (solution+ Bokashi)	314	1860	1546	30.5

\*\*Each figure represents mean of 10 birds

**Table 3. Effect of EM of Live Body Weight (g) of Broilers at IAAS Livestock Farm, 1999**

Age (Days)	G1(Control)	G2 (EM solution)	G3 (EM Bokashi)	G4 (Solution +Bokashi)
11	474	448	461	435
18	675	635	615	605
25	690	690	680	653
32	960	953	928	888
39	1480	1445	1425	1410
46	1760	1905	1954	1734
53	2276	2380	2395	2261

\*\* Each figure represents mean of 10 birds

**Table 4. Percentage Net Weight Gain over Control of Broilers at IAAS Farm, 1999**

Groups	Initial Weight(x)	Final Weight(y)	Net Weight Gain (y-x)	% Net Weight Gain
G1 (control)	474	2276	1802	---
G2 (EM solution)	448	2380	1932	7.2
G3 (EM Bokashi)	461	2395	1934	7.3
G4 (solution+ Bokashi)	435	2261	1826	1.3

(both EM solution and Bokashi) over the control (G1) were 7.2, 7.3 and 1.3 respectively (Table 4). The average feed consumption was 4.46, 4.49 and 4.61kg in G2 (EM solution), G3 (EM Bokashi) and G4 (both EM solution and Bokashi) respectively as compared to 4.27kg. in G1; and the feed conversion ratios were 2.46, 2.51 and 2.47 in G2 (EM solution), G3 (EM Bokashi) and G4 (both EM solution and Bokashi) respectively as compared to 2.86 in G1 in the broilers of village farm (Table 5). The average feed consumption was 6.78kg. in all groups of IAAS livestock farm (Table 6). The variation in the above results in two different trials could be due to the differences in season, breed, feed and the environment of the poultry house. The variation was clearly reflected in terms of feed intake, final average live body weight and mortality of the broilers, as there were more feed intake, greater live body weight and no mortality in case of IAAS livestock farm. However, the consistent results of both trials were as follows.

- The net weight gain in the treatment groups were greater indicating that EM enhanced production.
- There was more efficient utilization of feed in the treatment groups indicating that EM helped in digestion. In both cases the feed intake was observed to be more in treatment groups.
- There was low mortality in the treatment groups observed at the village farm which was located in the disease prone area. It indicated that EM improved the immune system and produced healthy birds, which was shown by earlier study in experimental broilers (Ahmed et al., personnel communication). However, the higher mortality in G4 (both EM solution and Bokashi) as compared to G2 (EM solution) and G3 (EM Bokashi) could be due to overcrowding of birds in summer stress as the space was not sufficient to rear that number of broilers ( Table. 7).
- The litter of the birds in the treatment groups were well decomposed and free from malodor indicating that EM controlled malodor and produced good quality manure for crop production.
- Economic analysis of using EM indicated that EM was a cheaper product and could be used profitably in broiler production. Moreover, it seemed that EM could decrease the cost of production by reducing the need to use antibiotics and other drugs which further could make it a more beneficial product to use in broiler production ( Annex 1).

**Table 5. Total Feed Consumption(TFC) in Average and Feed Conversion Ratio (FCR) at Village Farm, 1997**

	G1(Control)	G2(EM solution)	G3(EM Bokashi)	G4 (Solution+ Bokashi)
TFC	4270	4460	4492	4610
FCR	2.86	2.46	2.51	2.47

\*\* FCR = TFC/ Total weight gain

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**Table 6. Total Feed Consumption (TFC) and Feed Conversion Ratio (FCR) at IAAS Livestock Farm, 1999**

	G1 (Control)	G2 (EM solution)	G3 (EM Bokashi)	G4 (EM solution + Bokashi)
TFC	6780	6780	6780	6780
FCR	3.76	3.51	3.51	3.71

\*\* FCR = TFC/ Total weight gain

**Table 7. Mortality of Broilers at Village Farm, Chitwan, 1997**

Groups	Total No. of Broilers	No. of Broilers Dead	Mortality %
G1(control)	75	10	13.33
G2(EM solution)	75	5	6.66
G3(EM Bokashi)	75	4	5.33
G4(solution+Bokashi)	200	20	10

**Effect of EM on Goat Production****Materials  
and  
Methods**

A demonstration trial was conducted on use of EM in goat production at IAAS livestock farm during Aug.-Sept., 1997. Nine kids of local breed were selected and divided into three groups of three each namely G1, G2 and G3. Then G2 and G3 were given Bokashi at the rate of 5 percent and 10 percent of their feed respectively whereas G1 served as control. The Bokashi used in feeding the treatment groups was prepared anaerobically from rice bran by the same method as above. Initially the kids were dewormed and tagged. In the treatment groups Bokashi was mixed with wheat bran and commercial feed that is used by the farm to feed the goats. But the existing system of grazing was not disturbed. All kids were allowed to graze two times a day. The trial was conducted for two months and the observation was taken fortnightly.

**Results  
and  
Discussion**

Results showed that the net weight gain was more in both treatment groups. The net weight gains in G1 (control), G2 (Bokashi 5 percent) and G3 (Bokashi 10 percent) were 2.2, 3.64 and 5.27kg. respectively (Table.8).

**Table 8. Effect of EM on Performance of Goats, IAAS Livestock Farm, 1997**

S.N.	Tag No.	Trea- tments	Initial Wt.	Wt. After 15 days	Wt. after 30 days	Wt. after 45 days	Wt. After 60 days	Avg. Initial Wt.	Avg. Final Wt.	Net Wt. Gain
1	035	G1	12.5	13.1	13.65	14.1	14.5	9.8	12.0	2.2
2	09		8.0	8.7	9.3	9.8	10.25			
3	03		9.0	9.7	10.3	10.8	11.45			
4	07	G2	4.1	4.75	5.4	6.25	7.1	6.66	10.3	3.64
5	011		11.0	12.25	13.1	13.9	14.7			
6	097		4.9	6.25	7.5	8.4	9.1			
7	02	G3	4.8	6.5	8.1	9.6	11.0	5.93	11.2	5.27
8	022		3.75	5.0	6.3	7.5	8.8			
9	031		9.25	10.75	12.05	13.1	14.0			

The inclusion of Bokashi at the rate of 10 percent of feed showed better performance of goats. Thus, even in ruminants like goats, EM was shown to have positive impact on growth performance.

#### **Effect of EM in Pigsty**

#### **Materials and Methods**

A demonstration trial was conducted using EM in the pigsty in a village farm. Diluted EM (1:1000) was prepared and sprayed in the pigsty two or three times a week in the beginning. Later the frequency of EM application was reduced to once a week and then fortnightly as the malodor of the farm was found to be reduced to a minimum.

#### **Results and Discussion**

The result showed that EM can effectively control malodor and help keep the environment healthy. This result is in corroboration with the finding of Li et al., (1995). Although the result was observed qualitatively, EM was found to be effective in controlling malodor in the diversified ethnic community of the farmers in the village.

#### **Conclusions and Perspectives**

The trials on the use of EM in animal production, though not widely conducted, have demonstrated beneficial effects not only on health and production but also on environmental pollution. The results of the trials are in corroboration with the finding that the naturally occurring microorganisms contained in EM after they enter into the body create more effective intestinal microflora with a greater synthetic capability i.e. one that can synthesize vitamins, hormones and enzyme systems that improve digestion, enhance growth, provide disease resistance, suppress malodor, inhibit pathogen and improves product quality (Li et al., 1994). Though many commercial products containing yeast and lactic acid bacteria are costlier and their range of beneficial activities has not been claimed to be equal to that shown by EM they are readily available in the market and are widely used by the farmers in livestock and poultry farming. On the other hand, antibiotics are unscrupulously used by the farmers as growth promoters and prevent means for diseases which could lead to public health hazard. Thus, its highly desirable that this environment friendly economical and safe technology from a public health point of view should be incorporated in the traditional animal husbandry practice with necessary amendments, instead of so called modern animal husbandry which encourages use of chemicals and drugs. However, future activities need to be focussed on scientific and applied researches in line with the concept of Kyusei Nature Farming at the research institutes of home country. Then, massive extension programs along with the basic information and literature on EM technology should be carried out to familiarize it with the large as well as the small rural farmers, so that a sustainable and nature friendly livestock farming systems could be established.

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**Annex 1****Economical Analysis (for 1000 broilers)**

The use of EM either through feed or through water alone is profitable both from more production and labor saving point of view. Among them, using EM through feed is costlier than through drinking water. Economic analysis of using EM through both means is given out below.

**If EM given through feed**

Increase in live body weight per bird	= 100 gm. ( though the result shows more than 100 gm.).
Total increase in live body weight	= 100x 1000 = 100,000 gm. = 100 kg.
Average price of live body weight	= Rs.60/-
Total income through increase in live body weight (a)	= 100 x 60 = 6,000/-
Average feed consumption	=6780 gm.
Total feed consumption	= 6.78 x1000 kg. = 6780 kg.
Rice bran required to prepare bokashi	= 6780 x 5% = 339 kg.
Average price of rice bran	= Rs.10/ kg.
Total price of rice bran (x)	= 339 x10 = 3,390/-
Molasses required	= 5 kg. (maximum ).
Average price of molasses	= Rs.15/-
Total price of molasses (y)	= Rs.15 x 5= Rs. 75/-
Cost of EM	
Total cost of EM (z)	= Rs.20/litre x5= Rs. 100/-
Total labor cost to prepare bokashi (w)	= Rs.500 (maximum).
Total cost incurred in preparing EM(b)	= x + y+ z+ w = 3,390+75+100+500 =Rs. 4065/-
Net income = a - b	= 6000 - 4065 = Rs.1935/-
Net income per bird = 1935/1000	= Rs.1.935/-

**If EM given through water**

Total cost incurred in preparing EM (b)	= y+z+w = 75+100+500= Rs. 675/-
Net income = a-b	= 6000- 675= Rs.5325/-
Net income per bird	= 5325/1000= Rs. 5.325/-

## Use of Effective Microorganisms against Deg Nala Disease in Buffaloes in the Rice Growing Areas of Punjab Province (Pakistan)

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**Abstract :** *Deg Nala disease which causes necrosis and gangrene of the dependent part in buffaloes and cattle is known to exist in the rice grown areas of the Punjab, A study was undertaken to evaluate the efficacy of Effective Microorganisms (EM), Pentasulphate, Terramycin-LA, zinc sulphate and nitroglycerine ointment. EM gave the highest cure rate. Feeding of EM treated rice straw was an effective prevention for the control of disease than sodium hydroxide treated rice straw. In another study, field trials were carried out by using EM treated rice straw (Group A), dry (Group B) and mouldy rice straw (Group C) to observe the fungal growth, lesion of Deg Nala disease, body weight and blood picture of the animals. No fungal growth appeared in rice straw of groups A and B and no lesions of Deg Nala disease were observed in animals of group C and lesions of Deg Nala disease were also recorded in animals of this group. Buffalo calves fed on EM treated rice straw gave a significantly higher percent in body weight as compared with the calves fed on dry and mouldy rice straw. There were no significant changes in blood values but haemoglobin content, R.B.C counts were decreased and total leukocytic counts increased in animals fed on mouldy rice straw. However, values in groups A and B of animals were within the normal range.*

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### Introduction

Deg Nala disease which causes necrosis and gangrene of the dependent parts in buffaloes and cattle is known to exist in Indo-Pakistan since some cases around Muridke (District Sheikupura) a place close to Nala Deg - a monsoon rain water stream were recorded (Shirlaw, 1939). Widespread occurrence of the disease has been reported from rice grown areas of Indo-Pakistan (Irfan & Maqbool, 1986; Irfan, 1971; Kalra et al, 1972) and causes considerable economic losses. The present study was designed to evaluate the comparative efficacy of different drugs against Deg Nala disease. Comparative therapeutic trials with EM, Penta-sulphate, Terramycin-LA, zinc sulphate and nitroglycerine ointment were carried out and control measures were also carried out with EM and 4 percent sodium hydroxide. The study was also designed to see the effect of EM treated, dry and mouldy rice straw on fungal growth and on weight gain and haematology.

### Materials and Methods

A total of 110 randomly selected naturally affected animals were divided into 6 groups of 20 animals in group A, B, C, D and E, while, group F had 10 animals kept as the untreated control. The following therapeutic regimens were tried.



**Treatment A**

A Penta-sulphate mixture (Ferrous sulphate-166 g, Copper sulphate-24 g, Zinc sulphate-75 g, Cobalt sulphate 15 g, and Magnesium sulphate-100 g) was used at the rate of 60 g (first day) orally followed by 30 g daily for 15 days with sufficient quantity of linseed and molasses. The lesions were washed with lukewarm water and dressed with nitroglycerine 2 percent ointment.

**Treatment B**

Treatment of group B was carried out with EM i.e, *Lactobacillus* 1.1 percent  $10^4$  cfu/ml, Anaerobic bacteria  $1.9 \times 10^7$  cfu/ml, *Actinomycetes*  $<1 \times 10^6$  cfu/ml, Nitrogen fixing bacteria  $<1 \times 10^2$  cfu/ml and yeast and mould  $2.2 \times 10^3$  cfu/ml) at the rate of 80 ml daily for 15 days. Local treatment of lesions proceeded as in treatment A.

**Treatment C**

A single intramuscular injection of Terramycin-LA (Pfizer; 200 mg oxytetracycline/ml) @ 20 mg/kg b.wt. Local treatment of lesions proceeded as in treatments A and B.

**Treatment D**

Only local treatment of lesions with 2 percent nitroglycerine ointment as in treatments A, B and C.

**Treatment E**

Daily oral administration of 2 g of zinc sulphate for 15 days.

**Group F**

No treatment was given to the animals of this group and acted as the control.

**Prophylactic Trials**

A total of 40 animals were randomly divided into two groups of 20 animals each. The following chemoprophylaxis drugs were used.

**Group a) Experimental feeding on NaOH treated rice straw**

For this purpose 4 percent solution of NaOH (@ 1 g/400 ml of water for 20 kg rice straw) was sprinkled on rice straw containing multiple dark specks daily for 20 days starting from first week of December.

### Group b) Experimental feeding of EM treated rice straw

For this purpose 2 percent solution of EM (@ 2 ml / 100 ml of water for 20 kg rice straw) was sprinkled on rice straw containing multiple dark specks daily for 20 days starting from first week of December.

Each animal of both groups was daily fed on 8-10 kg treated rice straw and 10-15 kg of green fodder for 10 days depending upon the age and weight of animals. Animals were examined daily and were kept under observation for a period of 45 days.

For another study fresh rice straw was obtained from the field during harvesting season of paddy. The straw was divided into three portions. First portion was treated with 2 percent solution of EM (@ 2ml/100ml of water) for 20 kg rice straw. This solution was sprinkled onto straw daily for 15 days (Group A). Second portion of straw was kept in a room and no treatment was given (Group B), while the third portion of straw was kept in the open and no treatment was given (Group C).

Ninety animals varying in age from 3 to 11 were randomly divided into three groups A, B and C each having 30 animals. Animals in group A were fed on 6 kg of EM treated straw along with 6-10 kg green fodder (*Trifolium alexandrinum*) for 4 weeks depending upon the age and size of animals. Animals in group B were fed on 6 kg of straw along 6-10 kg green fodder, whereas animals in group C were fed 6 kg mouldy straw along with 6-10 kg green fodder depending upon the age and size of animals. These animals were kept under observations for a period of 45 days following the termination of experimental feeding trials.

EM treated, dry and mouldy rice straw were examined macroscopically and microscopically. Cultures were made on Sabouraud's agar (Irfan and Maqbool 1986) at different intervals for observing the growth of fungus. Observations recorded were skin lesions, increase in body weight, hematological values and general body conditions of animals.

### Results

Therapeutic trials with EM gave highest cure rate (95 percent) i.e. treatment B, followed by treatment A (90 percent). While, treatment C gave 70 percent cure rate. Treatment D and E, ointment alone and zinc sulphate both with a cure rate of 60 percent were tied for the last slot in terms of cure rate, whereas, animals in control group remained positive throughout the course of the treatment.

For another study, rice straw samples from groups A, B and C were taken weekly and examined macroscopically for the presence of multiple dark specks and microscopically after culturing it on Sabouraud's agar media. No fungal growth was observed in EM treated and dry rice straw throughout the course of treatment. In rice straw of group C multiple dark specks were seen and when this rice straw was cultured on Sabouraud's agar media, mixed fungal growth i.e. *Aspergillus niger*, *Alternaria alternate*, *Fusarium avenaceum*, *Mucor heimalis*, *Fusarium oxysporum*, *Fusarium fusarioides*, *Cladosporium cladosporoides*, *Aspergillus flavus* and *Penicillium notatum* were observed.

In animals of group C lesions of Deg Nala disease were also observed. The affected animals were invariably weak, ulcerative wounds and gangrene developed on the limbs and other dependent parts of the body. Almost all cases showed gangrene of the tail, which was shrivelled and cold to touch. Invariably one or both ears showed dry gangrene. In two cases muzzle and tips of the tongues became gangrenous.

In one or more feet lesions showed at different stages of development. In some cases the affected feet were swollen up to the knee, the hair was denuded and inflammatory changes set in. Later wounds appeared on the coronet, fetlock, pastern, knee and hock region. In very advanced cases the lower region of the feet became gangrenous, hooves were shed and bones were exposed in some cases. The gangrenous portions of the tail, tip of the ears, tongue and other affected parts of the body dropped off in long standing cases.

### Weight Gain

Overall percent increase in body weight in case of calves fed on EM treated straw, dry straw and mouldy rice straw were 39.2, 33.0 and 28.0 respectively (Table 1). Percent increase in case of EM treated straw in group A of calves was higher as compared to other groups of animals.

**Table 1. Effect of EM Treated, Dry and Mouldy Rice Straw on Body Weight of Buffalo Calves**

Group	No. of Animals	Rice Straw Fed	Zero Day Wt. kg	30 <sup>th</sup> day (% increase)	60 <sup>th</sup> day (% increase)
A	30	EM treated	44	58 kg (32.4)	70 kg (39.2)
B	30	Dry	45	52 kg (28.6)	60 kg (33.0)
C	30	Mouldy	47	50 kg (26.5)	53 kg (28.0)

**Table 2. Effect of EM Treated, Dry and Mouldy Rice Straw on Blood Picture of Buffalo Calves**

Group	RBC 10 <sup>6</sup> /mm <sup>3</sup>	Hb. Gm/Dl	PCV(%)	TLC(%)	Neu(%)	Lym(%)	Mon(%)	Eos(%)	Bas(%)	T. Protein	T. Albumin
A	7.03	12.8	44	8110	38.5	57	4.2	4.1	0.4	6.4	3.2
B	6.93	12.2	37	8755	38.4	65	3.4	3.6	0.6	4.9	4.2
C	5.81	10.0	35	9180	26.2	62	3.9	3.2	0.0	5.3	3.2

### Hematological Values

There were no significant changes in blood values. Haemoglobin contents, red blood cell counts decreased in group C fed on mouldy rice straw (Table 2). The results indicated that there was also a rise in total leukocytic count in animals of group C. However, values in other groups of animals were within the normal range.

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**Discussion**

Therapeutic trials with EM given orally and a vasodilator (nitroglycerine ointment) applied locally on the lesions affected the highest percentage (95 percent) cure rate.

This cure rate is in a broad agreement with the findings of earlier workers (Khan et al., 1992; Nahashan et al., 1994; Haddadin et al., 1996). They reported a high percent cure rate with EM chickens. Secondary bacterial infection of the lesions are at least partially responsible for the severity of disease. The precise modus operandi of nitroglycerine remain uncertain. The use of EM treated rice straw proved to be beneficial for the prevention of Deg Nala disease probably by virtue of its ability to check the growth of mycotoxin producing fungi. This agent not only controls the disease but also increases the growth rate, feed consumption and feed efficacy. Similar results were also recorded in broilers by Khan et al. (1992) and Haddadin et al. (1996). Although treatment of EM has been tried for fattening of cattle and buffaloes under the feed lot system, practical demonstration of the technique to farmers on a large scale would be more beneficial for the control of this disease in the rice growing areas of Pakistan.

The feeding of rice straw treated with EM proved beneficial for the prevention of Deg Nala disease probably by virtue of its ability to check the growth of mycotoxin and no lesions of Deg Nala disease occurred in animals of this group. This feeding trial was not associated with any ill effects on the weight and health of experimental animals. Similar results were also observed by Maqbool et al (1997). Similarly, lesions were not reported in animals of group B.

Under suitable conditions of humidity and temperature during winter months saprophytic fungi grow in the form of multiple dark specks on the rice straw kept in the open field. When this infested rice straw is fed in large quantities in the winter due to scarcity of green fodder, it produced lesions of Deg Nala disease. Fungi isolated from the infested rice straw in these studies are known to produce mycotoxin which cause vasoconstriction resulting in gangrene and necrosis of dependent parts as was also discussed by Irfan & Maqbool (1986).

Percent increase in the weight gain of animals in groups A is more than in group B and C. This could be due to the fact that rice straw become more palatable and nutritious after treatment with EM as was also recorded by Maqbool et al., (1997).

The results of blood examination of animals in group A and B in these studies indicated that values remained within the normal range as reported by Gillani (1984) for healthy buffaloes. Some increase in total leukocytic count with neutrophilia in group C as also reported by Bhatia & Kalra (1981) and Kalra et al. (1972) could be due to inflammatory reaction and secondary infection.

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