

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.*

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.

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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 1.9×10^7 cfu/ml, *Actinomyces* $<1 \times 10^4$ cfu/ml, Nitrogen fixing bacteria $<1 \times 10^2$ cfu/ml and yeast and mould 2.2×10^3 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

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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 alexandrium*) 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 fusariodes*, *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 th day (% increase)	60 th 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/mm ³	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.

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 at 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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