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Control of Leucaena Toxicosis in Myanmar Sheep Using IBT-Goettinger Bioreactor Grown Mimosine Degrading Ruminal Klebsiella spp.

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Abstract

Rumen juice of German steer was taken and then treated with mimosine using a fermenter for 16 days to develop mimosine degrading bacteria. After this treatment, mimosine degrading bacteria (Klebsiella spp) were selected, then isolated and multiplied by using IBT-Goettinger Bioreactor. For the use of field experiment, they were incorporated in sodium alginate.12 local sheep from Pyawbwe area, Myanmar, were allocated in 4 groups. The experiment was conducted with complete randomised design. Group I was fed with normal ration and used for control. Group II was used as treated control group fed with 40% leucaena of total ration and without inoculating with ruminal Klebsiella. Animals from group III and IV were fed with the same ration to group II. The microbes were inoculated to animals from group III once at the beginning of feeding trial and 14 days to animals from group IV continuously. Clinical sings, feed intake and body temperature were recorded daily. Experimental period was 14 days for feeding trial and 5 days for collection of faecal and urine samples. Clinical signs of leucaena toxicosis such as loss of hair and dullness were found in group II, but not in other treated groups. Daily intakes of animals from group II gradually decreased although it was increased in other groups. Body temperatures of treated control animals were also higher than others and ranged from 39.7 to 40.6 ºC while the others at the range of 38 to 38.9 ºC. The mean value of TDN intake (g/ d/ kg BW0.75) of group II (0.5) is significantly lower than those of group I (0.89), III (0.79) and IV (0.8) respectively. According to these findings, IBT-Göttinger Bioreactor grown ruminal Klebsiella shows in vivo degradation of mimosine in Myanmar sheep.

Introduction

Leucaena leucocephala is a leguminous plant and is potentially excellent source of crude protein (Blom, 1981). Its use increased the liveweight gain of cattle in south-east Queensland (Allison et al. 1990). However its use is limited because of the presence of a toxic amino acid, mimosine, to an extend of 2-10% in the dry matter of leucaena leaves and seed (D'Mello and Acamovic 1982; Dominiguez-Bello and Stewart 1990). Both mimosine and its primary degradation product 3-hydroxy, 4 (1H) pyridine (DHP) are toxic to animals (Hegarty et al. 1964). This toxicity prevents intensive use of leucaena as a forage. Henke (1958) noticed that there was tolerance of ruminant to leucaena in Hawaii. This tolerance might be related to the presence of microbes that degrade mimosine and 3,4-DHP (Jones, 1981). Jones and Megarrity (1984) inoculated ruminal juice of Hawaiian goats to Australian goats for degradation of 3,4-DHP ruminally. In this study, we selected and isolated mimosine degrading ruminal Klebsiella, multiplied by using IBT-Goettinger Bioreactor and entrapped those bacteria in alginate beads and fed to Myanmar sheep to control leucaena toxicosis.

Materials and method

Mimosine degrading ruminal bacteria were selected and isolated from German steers and multiplied by using IBT-Goettinger Bioreactor (Aung et al. 2006). These were identified by amplifying PCR and DNA sequencing as were Klebsiella spp. Those microbes were entrapped in alginate beads to maintain long life without their substrate mimosine outside the freezer.

Twelve local sheep from Pyawbwe area, Myanmar, were allocated in 4 groups. The experiment was conducted with complete randomised design. As untreatment control, the animals from group I were fed with normal ration without leucaena feed. Group II was used as treated control group fed with 40% leucaena of total ration and without inoculating with ruminal Klebsiella. Animals from group III and IV were fed with the same ration to group II. The microbes were (1.0 x 1011/ ml) inoculated to animals from group III once at the beginning of feeding trial and 14 days to animals from group IV continuously. All dietary treatments were adjusted to be isonitrogenous at the feeding level. Dietary treatments were weekly adjusted by the supplements at the level of crude protein not less than 17%. Clinical sings, feed intake and body temperature were recorded daily. Digestion trial was carried out to determine digestibility by conventional collection method. It consisted of 7 days for preliminary experiment, 14 days for feeding trial and 5 consecutive days for collection of faecal and urine samples. Daily samples of rice straw feed, sesame meal, rice bran and leucaena leaves were taken and rice straw residues were removed, weighed and sampled before next morning feeding. During collection period, faeces samples were taken (5% of the total mass) weighed, and put into the plastic bottles. Two to three drops of

formaldehyde solution was put into the bottles to prevent the putrefaction. For the collection of urine, sulphuric acid was added into the urine to become pH 4. Chemical analysis of feedstuffs, faeces and urine were done according to the methods of AOAC (1984).

Results and discussion

The control sheep inoculated with no microbes showed loss of hair and declined feed intake after 7 days of experiment. This finding is similar to earlier reports (Hegarty et al., 1964). They also reported that sheep shed the hairs after 6-7 days of feeding leucaena. Moreover, the toxic effects of leucaena feeding observed in this experiment are similar to those reported for cattle in earlier studies (Jones and Hegarty, 1984). In the experiment of Ram et al. (1994) calves on a sole diet of showed the same findings in this experiment. The body temperature of the sheep associated with toxic symptoms was 39.3-40.6 °C while animals inoculated with rumen Klebsiella and normal control groups was 38 to 38.9 ºC. Hegarty et al. (1964) also found that body temperature of sheep was 105 °F (40.56 °C) after infusion of mimosine. The cause of increase in temperature is not clear.

The mean value of TDN intake (g/ d/ kg $BW^{0.75}$) of group II (0.5) was significantly lower than those of group I (0.89), III (0.79) and IV (0.8) respectively ($p<0.05$). This may be due to decreased digestibilities of nutrients of animals from group II. Ruskin (1977) reported that mimosine reduced the activity of cellulolytic bacteria in the rumen.

As in treatment groups inoculated with mimosine degrading bacteria showed no clinical signs, and better TDN intake, it could be concluded, that IBT-Goettinger Bioreactor grown ruminal Klebsiella can detoxify *in vivo* in Myanmar sheep.

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