DETOXIFICATION OF MIMOSINE AND DIHYDROXYPYRIDONE: A REVIEW

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ABSTRACT

Subabul (Leucaena leucocephala) is considered as a miracle tree due to its protein rich foliage (20-30% CP), fast growing and drought tolerant habits. Use of its foliage as animal feed is being limited by presence of a toxic amino acid-mimosine and its metabolites 3,4 DHP and 2, 3 DHP. Inclusion of subabul leaves on ration of farm animals results in varied response to severe adverse effects. Treatment of subabul leaf meal with mimosine binding agents reduce mimosine toxicity significantly and this seems to be the most effective detoxification method for nonruminants. But for ruminants a new hope has arisen from the discovery of presence of DHP degrading rumen microbes in some regions of the world including Karnal area of India. Such microbes were identified as Clostridium strain 162 in Venezuela; Synergistes jonsii in USA and a strain of Streptococcus bovis in India. This paper reviews the topic and provides an update information about detoxification of mimosine and DHP.

Subabul (Leucaena leucocephala) is considered as a miracle tree for its ever-green protein rich foliage (20-30% CP), fast growing habit, drought tolerance, good high energy fuel, organic nitrogen fertilizer and its charcoal, gum etc. (NAS,1977). It also prevents soil erosion and fixes large amount of nitrogen in the soil. Its pest resistance and durability under grazing, cutting, fire and drought have become legendary. Its leaves are also rich in β-carotene and minerals (Akbar and Gupta, 1985). It produces more than 3t/ha crude protein with amino acid composition similar to lucerne (Jones, 1979; NAS, 1981). It is also considered suitable for alley cropping. Despite so many good qualities, its use as livestock feed is being limited by presence of a toxic amino acid-mimosine and its metabolites 3, 4 DHP and 2, 3 DHP. Through researchers have been trying to detoxify mimosine for more than 50 years, some major break-through have been achieved only very recently. This paper reviews upto date progress on detoxification of mimosine and DHP.

NUTRIENT COMPOSITION OF SUBABUL LEAF MEAL AND SEED:

The range of various nutrients in leucaena leaf meal reported by several workers is CP 15.22 to 31.43; EE 2.50 to 7.10 ; NEE 38.62 to 57; NDF 27.3 to 46.30; ADF 14.4 to 29.79; cellulose 7.10 to 16.7; Lignin 4.4 to 12.81; total ash 6.8 to 12.5; Ca 1.80 to 2.70; P 0.17 to 0.23 per cent, GE 19.0 to 20.1 MJ/kg, β-carotene 227 to 546.66 ppm. Upadhyay et al., 1974 : NAS, 1977; Kharat et al. 1980; Pal et al., 1979; D’Mello and Fraser, 1981; Jones and Megarity, 1989; Kurar et al., 1984; Akbar and Gupta, 1985; Gupta et al., 1986; Sunaria and Vidyasagar, 1989; Samanta et al., 1994 : Onwudike, 1995; Gupta, 1995). The range of various nutrients in subabul seed is CP 21 to 30.4; CF 6 to 11; EE 4.9 to 8; NEE 41.1 to 55.98; total ash 4.3 to 10.8 and P 0.3 to 0.38 per cent (Shukla et al., 1987; Gupta et al., 1986; Bhaskar et al., 1987 ; Chakraborty and Chhabra, 1988 ; Dharamsare et al., 1991).

INCRIMINATING FACTORS IN SUBABUL:

The use of subabul as livestock feed is being prevented by the presence of mimosine and its degradation products 3,4 DHP (Ross and Springhall 1963) and 2,3 DHP (D’Mello,1992). The mimosine content is different in different parts of the plant. In leaf meal and hay it varies from 1 to 4.40 per cent of DM (Hegarty et al., 1964; Sobale et al., 1978; D’Mello and Taplin, 1978; Meuline et al., 1984; Gupta et al., 1988; Sunaria and Vidyasagar, 1889; Samanta 1991; Ram 1992; Gupta 1995). The concentration in seed varies from 3.6 to 5.0 per cent of DM (Jones, 1979;Gupta and Raheja, 1988; Chakraborty and Chhabra, 1988). D’Mello and Acamovic (1982) observed upto 14.5 per cent mimosine in subabul seed. Mimosine is degraded to 3,4 DHP and further to 2,3 DHP by endogenous enzymes of leucaena plant. 3,4 DHP and 2,3 DHP are equally toxic and
both are potent goitrogen (D'Mello, 1992). Other less important toxic constituents of subabul are tannin, protease inhibitor and galactomannan (D'Mello, 1992; D'Mello and Acamovic, 1982).

**METABOLIC AND TOXIC EFFECT OF MIMOSINE AND ITS METABOLITES:** Mimosine being structurally very similar to tyrosine may inhibit tyrosine decarboxylase or tyrosinase by acting as tyrosine analogue (Crounse et al., 1962). It may complex with pyridoxal phosphate and various metal ions such as zinc, copper etc. (Hegarty, 1978; Stunzi et al., 1979). Jones et al., (1978) have reported that mineral supplement containing Zinc reduce the toxicity of Leucaena in cattle and suggested that some of the toxic signs (e.g., skin lesions) resembles zinc deficiency. In mice study it was observed that gross damage in hair follicle and alopecia may be due to inhibitory action of mimosine on the proliferative phase of hair growth i.e. mitotic phase rather than on keratinization phase (Montagna and Yun, 1963 Hegarty et al., 1964). Mimosine is an inhibitor of cystathionine synthetase and cystathionase enzyme involved in the conversion of methionine to cystine. This inhibition could also be an important factor in mimosine induced alopecia (Cheek and Shull, 1995). The metabolic product of mimosine, 3, 4 DHP prevents iodinisation of tyrosine-an amino acid responsible for synthesis of thyroxine. 3,4 DHP reduces level of peroxidase which is necessary for conversion of iodine molecule to iodide radical or nascent iodine, which is important for its incorporation into tyrosine, hence affecting thyroxine synthesis (Hegarty et al., 1976) which results in compensatory enlargement of thyroid gland (goitre). Mimosine and DHP has hepatotoxic effect and increase plasma SGOT and SGPT in rabbit (Gupta, 1995). 3, 4 DHP is comparatively less toxic than mimosine (Lowry, 1982). 3, 4 DHP and 2, 3 DHP are equally toxic and both are potent goitrogen (D'Mello, 1992). Mimosine, 3, 4DHP and 2, 3 DHP produce a variety of toxic effects in Livestock such as alopecia, depressed growth, excessive salivation, ulcer in mouth (Owen, 1958; NAS, 1981; Ram, 1994).

**METABOLISM OF MIMOSINE:** Hegarty et al., (1964) observed that mimosine was extensively degraded to 3,4 DHP and other unidentified products in sheep rumen but there was no degradation of mimosine during or after absorption in the body. There is no evidence of detoxification of mimosine or DHP on the animal body. Mimosine gets converted to 3, 4 DHP by Ruminal microbes e.g. Synergistes jonsii, Streptococcus etc. (Smith and Fowden, 1966) and other ruminal endogenous enzymes (pH & temperature). 3,4 DHP (Hegarty et al., 1964; Shiroma & Akeshi, 1976; Atreja et al., 1990; Allison et al., 1992 Paul et al., 1997) is then degraded by ruminal microbes e.g. Synergistes jonsii, Streptococcus etc. (Smith and Fowden, 1966). 2, 3 DHP (D'Mello, 1992; Gupta and Atreja, 1994; Paul et al., 1999) is then degraded by ruminal microbes e.g. Synergistes jonsii, Streptococcus etc. (Smith and Fowden, 1966). 3, 4 DHP prevents iodinisation of tyrosine-an amino acid responsible for synthesis of thyroxine. 3,4 DHP reduces level of peroxidase which is necessary for conversion of iodine molecule to iodide radical or nascent iodine, which is important for its incorporation into tyrosine, hence affecting thyroxine synthesis (Hegarty et al., 1976) which results in compensatory enlargement of thyroid gland (goitre). Mimosine and DHP has hepatotoxic effect and increase plasma SGOT and SGPT in rabbit (Gupta, 1995). 3, 4 DHP is comparatively less toxic than mimosine (Lowry, 1982). 3, 4 DHP and 2, 3 DHP are equally toxic and both are potent goitrogen (D'Mello, 1992). Mimosine, 3, 4DHP and 2, 3 DHP produce a variety of toxic effects in Livestock such as alopecia, depressed growth, excessive salivation, ulcer in mouth (Owen, 1958; NAS, 1981; Ram, 1994).

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**FIG. 1 : PATHWAYS OF MIMOSINE DEGRADATION.**
endogenous enzymes of Subabul plant (Lowry, 1982; Tangendjaja et al., 1984) by rumen microbes (Hegarty et al., 1997), by chemical reaction of mimosine under appropriate pH and temperature (Wills and Tangendjaja, 1981). 3, 4 DHP can be further converted to 2, 3 DHP by endogenous plant enzymes or rumen microbes (D'Mello, 1992; Ram et al., 1994; Gupta 1995; Paul et al., 1995). The pathway of biodegradation of mimosine and its metabolites are presented in Fig. 1. The products of ruminal mimosine degradation are DHP, pyruvic acid and ammonia (Smith and Fowden, 1966). There is considerable variation on ruminal metabolism of DHP in different part of the world. Rumen microflora from certain geographical regions can degrade 3, 4 DHP or 2, 3 DHP to unidentified nontoxic compounds (Allison et al., 1992; Hammond, 1995; Gupta and Atreja, 1994; Paul et al., 1999) whereas rumen microflora from certain region fail to do so (Kudo et al., 1989; Wang, 1992). Though the end products of DHP degradation are not clearly defined, probably pyridone ring is broken down.

**DETOXIFICATION OF MIMOSINE AND DHP:** In the following text, a review of the research findings on detoxification of mimosine and DHP is being made under two subheads.

1. **Physicochemical methods:** Ross and Springhall (1963) observed that application of ferrous sulphate to leucaena leaf meal (LLM) reduces its toxicity in poultry by increasing faecal mimosine excretion. They speculated that mineral form chelate with mimosine which prevent absorption of mimosine from GI tract. Lin et al., (1964) showed that L-phenylalanine and L-tyrosine supplementation could partially reduce mimosine toxicity in rats. But Labadan (1960) found tyrosine to be completely ineffective. Tsai and Ling (1973) showed that aluminium formed stronger complexes with mimosine than ferrous sulphate for inducing faecal excretion of mimosine. D’Mello and Acamovic (1982) observed that ferrous sulphate at 0.5% level and aluminium at 1.17% level were effective in increasing mimosine excretion through faeces. They suggested that ferric sulphate is more effective than ferrous sulphate. They obtained best result when both PEG and ferrous sulphate were used. Lowry (1982) reported that maceration helps in converting very toxic mimosine to less toxic 3, 4 DHP. Akbar and Gupta (1985) observed that spraying 6.7 per cent ferrous sulphate reduced mimosine content from 3.5 to 2.37 per cent. Sunaria and Vidyasagar (1989) reported 1.26 per cent ferrous sulphate to be optimum for detoxifying 4.04 per cent mimosine in LLM. Mali et al., 1994; reported 17 to 19 per cent reduction on dry heating at 100°C and 19 to 23 per cent reduction in mimosine content of seed in auto-claving but Atreja et al., (1990) did not observe any beneficial effect of dry heat treatment. Akbar and Gupta (1985) observed that moist heat at 70 to 100°C caused 50% reduction in mimosine content. But it is not clear whether mimosine was converted to DHP by moist heat treatment in these studies hence reduction in mimosine level might have resulted in increased DHP level which may be of no practical benefit. Supplementation of molasses to the leucaena diet increased excretion of mimosine and 3, 4 DHP via faeces and thus prevented lowering of thyroxine level (Elliot et al., 1985). Probably high level of minerals especially zinc, ferrous and copper present in molasses biids with mimosine and DHP and increase their excretion via faeces. Tawata et al., (1986) reported that leaching of leucaena leaves with 0.05 N sodium acetate removed 95 per cent mimosine without loss of any important nutrients. Shyam et al., (1990) observed 42.65% reduction in mimosine content of LLM when it was ensiled along with other roughages. Sharma et al., (1990) did not observe any beneficial effect of dry heat treatment. They also observed that iron supplementation at a level of 4g/kg or above level and copper supplementation at level of 10mg/kg and above reduced in vitro rumen microbial degradation of mimosine. This effect might possible be due to binding effect of copper and iron on mimosine which inhibited its conversion to 3,4 DHP by rumen microbes. Ram et al., (1994) reported improved gain when calves were given sole diet of LLM treated with Cu(10ppm). Through, at present, the nature of complex of mimosine with metalions is not known, it is clear that these metal ions are
quite effective in reducing mimosine toxicity and they prevent absorption of mimosine and DHP from GI tract.

(ii) **Biological Methods**: Recently, the most promising method of detoxification of mimosine and DHP in ruminant is manipulation of rumen function by incorporating microbes capable of degrading mimosine to unidentified residues (D’Mello, 1994). There is a considerable variation in ruminal metabolism of mimosine and DHP in different parts of the world and hence ruminants in some parts of the world consume sole Leucaena diet without any toxicity whereas in other parts they suffer even when fed at very low level. The goats were able to eat Leucaena diets without adverse effects in Hawaii but not in Australia (Henke, 1958; Jones et al., 1973). Subsequently, Jones and Megarrity (1983) observed that 71 per cent of added 3,4 DHP was degraded by Hawaiian goat rumen fluid and none by rumen fluid from Australian goats and thus substantiated the hypothesis that there was different metabolism of mimosine in Hawaiian goats as a result of which there was no toxicity in Hawaii. Jones and megarrity (1986) transferred inoculum from Hawaiian goat to the rumen of a steer and a goat fed 100 per cent Leucaena diet in Australia and reported cessation of DHP excretion in urine. They also reported that only bacteria and not protozoa were responsible for DHP degradation in rumen. Quirck et al., (1988) observed that dosing of cattle with rumen bacteria capable of degrading 3,4 DHP resulted in improved weight gain in cattle from 0.52 kg/d to 1.03 kg/d on only Leucaena pasture. The introduced bacteria spread naturally to untreated cattle after 19 weeks of dosing. Rumen bacteria which degrade 2,3 DHP but not 3, 4 DHP was isolated in cattle in US Virgin Island (Hammond et al., 1989). Kudo et al., (1989) reported that DHP degrading rumen microbes were absent in Malaysian cattle, buffaloes and sheep. Allison et al., (1990) observed that ruminal bacteria from animals in Virgin Island and Haiti degraded DHP. Dominguez-Bello and Stewart (1991) described a gram positive spore forming bacteria isolated from sheep in Venezuela. This isolate was named as Clostridium strain 162 which degraded products of mimosine metabolism 3, 4 DHP and 2, 3 DHP to normal rumen metabolites. Allison et al., (1992) isolated a bacteria that degraded both 3, 4 DHP and 2, 3 DHP and named it as *Synergistes jonsii*. They isolated 4 strains of this microbe all of which degraded both 3, 4 DHP and 2, 3 DHP to unidentified normal nontoxic metabolites. Wang et al., (1992) reported that rumen microbes in Weizong Island of China can degrade 3, 4 DHP but rumen microbes from Chinese mainland cannot degrade. Later on rumen microbes were successfully transferred from Weizong island to Chinese mainland and mimosine toxicity was alleviated. In India, Feng and Atreja(1994) observed that on 3 to 4 weeks adaptation cattle in Karnal area acquired rumen microbes which could degrade DHP to unidentified nontoxic product. This DHP degrading ability could be transferred from cattle to cattle (Gupta and Atreja, 1994). Buffaloes failed to develop 2,3 DHP degrading ability even on 42 days adaptation though rumen microbes of buffaloes was also able to convert mimosine to 3, 4 DHP and further to 2, 3 DHP (Gupta and Atreja, 1994). Hammond (1995) observed that susceptible animals could be imparted the ability to degrade 3,4 DHP and 2, 3 DHP within a weeks time of inoculum transfer either from the animal already possessing the ability or with cultures of S. Jonsii. Puniya et al., (1996) reported that 78.75 and 85 per cent of 3, 4 DHP was degraded after 20 days of incubation with ruminal fluid from cattle, buffalo and goat respectively. This provided evidence that the animals were colonized with 3, 4 DHP degrading bacteria, but the rate of degradation was very slow. Recently goats were adapted to Leucaena diet for 4 months and they did not show any toxicity symptoms even though they were consuming 70% of DM as leucaena. In *vitro* and in *vivo* studies on these goats showed presence of DHP degrading microflora in rumen that degraded 84.92 to 92.88 per cent of DHP in 24 hours. Urinary excretion of DHP was very low. Mimosine was rapidly degraded in rumen and was not detectable in faeces and urine. Later on rumen liquor from these goats were transferred to cattle and buffaloes and the cattle and buffaloes were switched over to 60% LLM diet immediately after inoculation. In subsequent weeks LLM level were increased up to 89 to 90% of DMI but there was no mimosine/DHP toxicity symptoms. The animals gained in body weight 500g/d. On in *vitro* study, it was proved that they attained 50-60% DHP degradability within 5 to 6 days of transfer of
rumen liquor inoculum (Paul, 1996). Scientist working at NDRI, Karnal, India has isolated DHP degrading microbes which was identified as Streptococcus bovis strain. Pure culture of these microbes (300 ml) when transferred to cattle and buffaloes, they also acquired 60% degradation of 3,4 DHP and complete degradation of 2,3 DHP and the animals did not show any toxicity symptoms (Anonymous, 1997).

**CONCLUSION**

From the above discussion it is amply clear that Leucaena leucocephala is an excellent fodder tree but presence of mimosine and DHP limits its utilization. Among various methods of physico-chemical detoxification, use of mimosine binding agents seems to be most effective in reducing mimosine toxicity. But for ruminants a new hope has arisen from the discovery of presence of extensive mimosine and DHP degrading rumen microbes in some regions of the world including Karnal area of India which can be commercially cultured, freeze dried and transferred to ruminants in those regions where they suffer from mimosine toxicity. Some long term studies should also be undertaken to find out maximum level of inclusion of subabul in different species of ruminants inoculated with DHP degrading rumen microbes.

**REFERENCES**