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An update on leucaena toxicity: Is inoculation with *Synergistes jonesii* necessary?

*Una actualización sobre la toxicidad de leucaena: ¿Es necesaria la inoculación con *Synergistes jonesii*?*

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Abstract

Concern about mimosine toxicity and its management has contributed to the restricted adoption of leucaena as a forage for ruminants. The toxicity is a function of the antimetabolic effects of mimosine, which is rapidly converted to isomers of hydroxypyridone (DHP), also toxic compounds, by plant and microbial enzymes. Work by R.J. Jones and colleagues (1960–1994) identified a rumen bacterium (*Synergistes jonesii*) capable of degrading DHP, and rumen fluid containing this bacterium was subsequently made available in Australia as a commercial inoculum for cattle producers.

Research by University of Queensland and CSIRO over 15 years, commencing in 2003, found evidence for another pathway of toxin management in Indonesia, where hundreds of Balinese farmers had fed uninoculated Bali bulls (*Bos javanicus*) up to 100% leucaena without experiencing toxicity symptoms, apart from an initial 1–2 week period while their cattle became adapted to the new diet. Tests showed that the Indonesian cattle were not degrading all DHP, as it appeared in high concentrations in urine samples, predominantly as 2,3-DHP and almost all (>97%) in a conjugated form. The conjugating compounds (glucuronic acid and sulfate compounds), produced in the liver, appeared to be the major pathway for neutralizing the toxicity of DHP. Other work revealed that *S. jonesii* was a ubiquitous organism in the rumen fluid of animals in all countries but always as a minor population, just detectable using new PCR-based assays, and sometimes not detected in all animals studied.

Since the Indonesian cattle fed leucaena suffered symptoms of mimosine toxicity for only a short time before quickly recovering, we hypothesize that conjugation of DHP by the liver was the major detoxification pathway for these animals. This detoxification pathway is also operative in Australia and other countries but further studies are needed to determine its significance.

Keywords: Conjugation, ‘leucaena bug’, microbial detoxification, ruminants, tree legumes.

Resumen

La preocupación sobre la toxicidad de la mimosina y su manejo ha contribuido a que la adopción de leucaena como forraje para los rumiantes estuviera restringida a nivel mundial. La toxicidad se debe a los efectos antimetabólicos de la mimosina, la cual mediante enzimas microbianas y de la planta se convierte rápidamente en compuestos también tóxicos, isómeros de la hidroxipiridona (DHP). Los trabajos de R.J. Jones y sus colegas (1960–1994) identificaron una bacteria ruminal (*Synergistes jonesii*) que es capaz de degradar el DHP. Posteriormente el líquido ruminal conteniendo esta bacteria se convirtió en Australia en un inoculante comercial para los productores ganaderos.

En investigaciones realizadas por la Universidad de Queensland y CSIRO durante los últimos 15 años se encontró evidencia de otra vía de manejo de toxinas en Indonesia, donde cientos de productores balineses habían alimentado toretes no inoculados de ganado Bali (*Bos javanicus*) con hasta 100% de leucaena sin experimentar síntomas de toxicidad, aparte de un período inicial de 1–2 semanas durante el cual los animales se adaptaron a la nueva dieta. Las pruebas mostraron que el ganado

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indonesio no estaba degradando todo el DHP ya que aparecía en altas concentraciones en muestras de orina, predominantemente como 2,3-DHP y casi todo (>97%) en forma conjugada. Los compuestos de conjugación (ácido glucurónico y compuestos de sulfato), producidos en el hígado, parecieron ser la principal vía para neutralizar la toxicidad del DHP. Otro trabajo reveló que *S. jonesii* es un organismo ubicuo que se puede detectar en el líquido ruminal de animales en todos los países, pero siempre en poblaciones bajas, muchas veces solo detectables usando nuevos métodos basados en PCR y a veces no detectadas en todos los animales examinados.

En vista de que el ganado indonesio alimentado con leucaena mostró síntomas de toxicidad por mimosina solo por poco tiempo y se recuperó rápidamente, nuestra hipótesis es que la conjugación del DHP por el hígado es la principal vía de detoxificación en estos animales. Esta vía de detoxificación también se presenta en Australia y otros países pero se necesitan estudios para determinar su significancia.

Palabras clave: Conjugación, detoxificación microbiana, leguminosas arbóreas, ‘leucaena bug’, rumiantes.

Background

Concern about mimosine toxicity and its management has contributed to the restricted adoption of leucaena as a forage for ruminants. Along with other factors [establishment and management limitations, the psyllid insect (*Heteropsylla cubana*) and weediness concerns (Buck et al. 2019; Dahlanuddin et al. 2019)], toxicity concerns have prevented the realization of the huge potential of leucaena pastures as the most productive, sustainable and profitable improved pasture option for northern Australia (Shelton and Dalzell 2007), and for many other tropical regions worldwide (Aung 2019; Chará et al. 2019; Nimbkar 2019; Pachas et al. 2019; Ramírez-Avilés et al. 2019; Zapata Cadavid et al. 2019). The toxicity of leucaena results from the presence of a non-protein free amino acid, mimosine, which occurs in high concentrations in its foliage (Honda and Borthakur 2019) and can severely affect animal health and performance (Jones and Lowry 1984).

The mode of toxicity is initially due to the antimetabolic effects of mimosine, which are most pronounced on rapidly dividing cells, causing hair loss, salivation, oesophageal lesions, low bull fertility, foetal abortion and occasionally death (Hegarty et al. 1964; Jones et al. 1978; Holmes 1980; Holmes et al. 1981). However, after an initial adaptation period in ruminants (1–2 weeks), mimosine is rapidly and effectively converted to less acutely, but still toxic compounds, stepwise through isomers of hydroxypyridone (3,4-DHP and then 2,3-DHP). Plant enzymes are involved in the initial conversion of mimosine to 3,4-DHP (Lowry et al. 1983). Thereafter, mimosine does not appear in urine samples (O'Regain et al. 2014). However, DHP is chronically toxic and was reported to be a goitrogen inhibiting thyroid hormone synthesis, plus reducing feed intake and animal performance (Jones and Lowry 1984). Both compounds have toxic effects as strong ligands and chelate with essential metal ions leading to mineral deficiencies (Tsai and Ling 1971).

R.J. Jones and colleagues conducted the pioneering research into leucaena toxicity between 1960 and 1994 (Hegarty et al. 1964; Allison et al. 1992; Jones 1994) and published widely on the symptoms, chemistry, microbiology and management of toxicity (Jones 1994).

They identified a rumen bacterium (*Synergistes jonesii*) capable of completely degrading DHP in vitro, and rumen fluid containing this organism was subsequently made available as a commercial inoculum for cattle producers in Queensland by Queensland Department of Agriculture and Fisheries (DAF) (Klieve et al. 2002). While this resolved the problem within Australia, an equivalent service was not available in other tropical countries. Despite this, while fear of toxicity has limited the expanded use of the legume in some countries, e.g. Paraguay (Glatzle et al. 2019), many farmers in Asia (Phaikaew et al. 2012) and Latin America (Ramírez-Avilés et al. 2019) have a long history of feeding leucaena to ruminant animals without inoculation, and appear to experience no long-term effects of leucaena toxicity.

Research workers from University of Queensland and CSIRO began studying leucaena toxicity in 2003 and immediately found anomalies and discrepancies with earlier reports, that indicated *S. jonesii* was not as effective as reported (Dalzell et al. 2012; Halliday et al. 2013, 2018) and that there were other mechanisms for neutralizing the toxins in ruminants consuming leucaena (Halliday et al. 2018). The many studies conducted during 2003–2016 are briefly reviewed and a new hypothesis provided to explain how cattle adapt to diets containing high percentages of leucaena. The implications of the hypothesis for future R&D on leucaena toxicity are also discussed.

The evidence

Australia (2003–2011)

Following a report of mortality of hungry cattle when introduced to lush leucaena during a drought in January

2003 (Dr Bevan Peters pers. comm.), a survey of the urine chemistry of a sample of Australian cattle herds grazing leucaena was conducted in 2004 (Dalzell et al. 2012). It showed that half of all herds studied (all grazing leucaena) had high levels of DHP in urine, indicating that it was not being completely degraded despite previous inoculation with rumen fluid by graziers or use of alternative strategies for introducing *S. jonesii* to their herds (Dalzell et al. 2006). Testing for effectiveness of toxin degradation involved detection of the amount of undegraded DHP in urine samples. Jones (1994) employed a simple crush-side colorimetric test in which acidified FeCl₃ was added to the urine samples leading to color changes (red color for mimosine and 3,4-DHP and blue color for 2,3-DHP). It was later discovered that incomplete hydrolysis of conjugated DHP was occurring in the colorimetric test, leading to the underestimation of the concentration of DHP in the urine samples (Halliday 2018). A modified colorimetric urine test protocol was developed to provide a more robust and reliable routine test (Graham et al. 2014). This involved collecting and storing urine samples in HCl, and heating them for 1 hour at 80 °C, prior to conducting the FeCl₃ colorimetric test and high performance liquid chromatography (HPLC). However, HPLC analysis revealed that incomplete hydrolysis of conjugated DHP continued to occur, resulting in ongoing underestimation of the amount of undegraded DHP in urine samples (Halliday 2018).

Pen-feeding studies found that the commercially available inoculum from DAF was not fully effective in degrading all DHP in steers fed leucaena rations (Halliday et al. 2018). It was originally postulated that, while ruminants were inherently capable of degrading mimosine to the isomer 3,4-DHP via plant and microbial enzymes, the isomerization of 3,4-DHP to 2,3-DHP (Allison et al. 1994) required *S. jonesii*. Therefore, presence of the isomer 2,3-DHP in urine samples was regarded as an indication that bacterial degradation had begun and that 2,3-DHP was transitory. However, several studies showed that this was not the case and that 2,3-DHP was frequently the dominant isomer found in urine samples from cattle on long-term high leucaena diets (Dalzell et al. 2012; Halliday et al. 2014a).

Mexico and Thailand (2005–2009)

A survey of the toxicity status of goat herds in Mexico in 2005 (H.M. Shelton unpublished data) and in Thailand in 2009 (Phaikaew et al. 2012) showed that many herds fed diets of predominantly leucaena (often 100% leucaena diets) were excreting very high levels of DHP. In Thailand, herd averages for total urinary DHP concen-

trations ranged from 375 to 3,357 µg/mL with most herds excreting >1,000 µg/mL, the majority as 2,3 DHP, indicating that the toxin was not being fully degraded and confirming the Australian findings. Despite this, the goats appeared healthy and productive (Phaikaew et al. 2012).

Indonesia (2011–2016)

The main evidence for an additional pathway of toxin management was discovered in Indonesia. An ACIAR-funded project (LPS/2008/054) (2011–2016) (Shelton 2017) found that, for more than a decade, hundreds of Balinese farmers on the island of Sumbawa had been feeding up to 100% leucaena to Bali bulls (*Bos javanicus*) in profitable fattening enterprises (Panjaitan et al. 2014; Dahlanuddin et al. 2019). Similar practices were observed in West Timor (Kana Hau and Nulik 2019) (Figures 1 and 2).



Figure 1. Bali bulls consuming 100% leucaena diets on Sumbawa Island, Indonesia.



Figure 2. Bali bull fattened on 100% leucaena diet on Sumbawa Island, Indonesia.

The Indonesian animals had not been inoculated with *S. jonesii* and liveweight gains and other measurements showed that they were free from toxicity symptoms and were growing at rates similar to their genetic potential (Panjaitan et al. 2014). When questioned, Indonesian farmers reported that newly purchased cattle, naïve to leucaena, initially showed toxicity symptoms, such as hair loss, salivation and reduced appetite, but recovered within 2–3 weeks and subsequently showed excellent growth performance.

Subsequent tests showed that the cattle were not degrading all DHP as it appeared in high concentrations in urine samples, predominantly as 2,3-DHP (Halliday et al. 2014a; 2014b). However, HPLC analysis revealed almost all (>97%) DHP present in urine was in a conjugated form, detected using a PDA detector (HPLC diode array detector) and by analysis of the UV absorption spectra of the chromatographs (Halliday 2018). The conjugating compounds (glucuronic acid and sulfate compounds) are produced in the liver and are especially effective in conjugating hydroxy compounds, such as DHP (Hegarty et al. 1979). They act by bonding with DHP, neutralizing its toxic activity and increasing its solubility, enabling rapid excretion in urine.

In concurrent microbiological investigations, analysis of rumen fluid from the Indonesian cattle revealed the presence of different strains of *S. jonesii*, including the ATCC type strain (78-1) (Padmanabha et al. 2014; Halliday 2018), albeit at low population levels (<10⁶ cells/mL rumen fluid), and always accompanied by high levels of DHP in urine. We concluded that *S. jonesii*, while present, was incapable of degrading all the DHP generated from high leucaena diets and that conjugation played a key role in preventing DHP toxicity.

Other evidence

The work of Padmanabha et al. (2014) and McSweeney et al. (2019) showed that *S. jonesii* was not specific to regions where leucaena was being fed, but was an ubiquitous organism detectable in many ruminants, in all countries tested including cold climates (e.g. yaks in Tibet), and in a variety of non-ruminants, but always at low population numbers (<10⁶ cells/mL rumen fluid) using new PCR-based assays, and sometimes not detectable in all animals studied. They further observed that strains of *S. jonesii* that differed from the type strain 78-1 occurred within animals and at different geographical locations. However, these studies were unable to determine whether there is variation in the DHP-degrading ability of the different strains, which may influence their contribution to the overall

detoxification process in the animal. It has also been observed that the main substrates for *S. jonesii* and related genera in the *Synergistetes* phylum are amino acids and their survival does not appear to depend on the presence of DHP, i.e. it is not specifically a ‘leucaena bug’ as often reported.

In re-examining HPLC chromatographs from earlier studies in Australia (Dalzell et al. 2012; Graham et al. 2013; Halliday 2018), it was evident that additional conjugated DHP was also present in urine samples taken from Australian cattle consuming leucaena. This indicated that the toxic effects of DHP were also reduced by conjugation in Australian cattle consuming leucaena and that the amount of DHP present in those samples had been underestimated. However, since the samples had been immediately acidified at the point of collection, it was not possible to re-estimate the level of conjugated DHP present in the urine.

Discussion, conclusions and future research

We propose that hepatic conjugation was the major pathway for control of DHP toxicity in Indonesian cattle consuming high leucaena diets. Since indigenous strains of *S. jonesii* were already present, albeit at low population density, and almost all excreted DHP was conjugated, presumably negating its toxic effects, and since the animals were gaining weight at a rate close to their genetic capacity, we conclude that inoculation with *S. jonesii* was not necessary in these ruminants.

The process of conjugation of DHP in ruminants has long been recognized, as hydrolysis of the conjugate was a necessary step in the method for measurement of DHP (Hegarty et al. 1964), although it was not historically considered a protective mechanism (Hegarty et al. 1979). The initial focus of DHP toxicity was on its inhibition of thyroid hormones (Jones and Hegarty 1984), even though the conjugated form of DHP had less negative effect on thyroid function than unconjugated DHP (Christie et al. 1979), since conjugation reduces the biological activity of the toxin (Galanello 2007; Crisponi and Remelli 2008). Conjugation also increases the water solubility of the compound, increasing the speed of its clearance in urine (Galanello 2007; Sooriyaarachchi and Gailer 2010).

Contrary to much of the original work on the goiterogenic nature of DHP (Jones et al. 1978; Jones and Hegarty 1984), goitre is rarely observed in leucaena-fed ruminants worldwide. Reduced thyroxin levels were not encountered following the feeding of high leucaena diets to steers in the work of Halliday et al. (2018), suggesting that conjugation of DHP may diminish any direct toxic

activity of the compound on tissues such as the thyroid gland. Conjugation would also reduce the potential for DHP to bind with divalent transition metals such as Zn, Mg and Cu, which are essential for regular cellular function ([Berdoukas et al. 1993](#); [Hoffbrand and Wonke 1997](#)). Jones et al. (1978) reported that supplementation of steers on a sole diet of leucaena with minerals (Fe, Cu, Zn) significantly increased mean daily intake and daily liveweight gain, and decreased hair loss and skin lesions although it did not alleviate the low serum T4 levels.

While mimosine can initially induce severe toxicity symptoms such as hair loss, salivation, foetal abortion and even death, it is rapidly and effectively converted to DHP by plant and microbial enzymes. Thus naïve animals, when first fed high leucaena diets, can show symptoms of toxicity, but recover within 2–3 weeks and acute toxicity resulting in death rarely occurs. The Indonesian cattle required a short period of adaptation to firstly degrade mimosine to DHP and then become fully capable of conjugating DHP, thereby preventing negative effects on health and productive performance.

The practical implications of our findings were that feeding of diets containing up to 100% leucaena by Indonesian smallholders was successful in providing a low-cost, low-labor feed source for the productive fattening of bulls ([Halliday 2018](#)).

Our current hypothesis after 1.5 decades of research into leucaena toxicity, arising principally from our Indonesian studies, is: when naïve ruminants are introduced to leucaena the mimosine consumed causes immediate symptoms (hair loss, salivation and reduced appetite), from which animals quickly recover as mimosine is converted to DHP. Our understanding of mimosine degradation remains unchanged – plant and microbial enzymes have the capacity to deal effectively with high concentrations of mimosine in the diet within 2–3 weeks in naïve ruminants. Thereafter, conjugation of hydroxypyridone (DHP) plays the major role in protecting animals from residual leucaena toxicity when they consume high leucaena diets. Microbial detoxification by low populations of *S. jonesii*, or by other organisms ([Aung 2019](#)), may also play a role but their relative contributions need to be quantified in terms of the amount of DHP degraded.

This finding, if confirmed as applicable to other countries in the tropical world, has great significance for the adoption and use of leucaena for feeding ruminant livestock.

There are several possible explanations for the differences between our current hypothesis and that previously

reported, with regard to the need for inoculation with *S. jonesii*, namely:

- There have been advances in methodologies, particularly in rumen molecular techniques, enabling detection of *S. jonesii* when present in the rumen at low populations; and
- We have new understanding of the sample preparation necessary (acid strength and heating requirements) to achieve complete hydrolysis of conjugated DHP prior to measurement by colorimetric or improved HPLC techniques. All previous measurements of concentrations of DHP in urine were almost certainly substantial underestimates.

Future research needs

Confirmation that inoculation of ruminants with *S. jonesii* may not be necessary removes a major world-wide barrier to adoption of leucaena for feeding ruminants. Nevertheless, while there is evidence of similar hepatic conjugation in ruminants consuming leucaena in Australia and other countries where leucaena is being fed, our hypothesis needs to be confirmed by additional studies in those countries.

A number of issues still need clarification, namely:

- More work is required to understand the relative significance of chelation versus effects on thyroid hormones as the principal mode of toxicity of DHP;
- What alternative pathways exist for the isomerization of 3,4-DHP to 2,3-DHP?;
- What rumen organisms can degrade DHP other than *S. jonesii*? An audit of total mimosine ingested and total DHP voided in urine and feces might indicate the possible contribution of other micro-organisms in the detoxification of DHP.
- Are there differences in ability to adapt to leucaena toxicity among species of cattle and between other ruminant species?

Further study is also needed to clarify the effects on the reproductive performance of ruminants of feeding high leucaena diets. Infertility in cattle grazing leucaena was reported by Holmes (1980) and Holmes et al. (1981) in Papua New Guinea. Recent anecdotal evidence ([O'Neill and O'Neill 2019](#)) from Australia indicates that foetal abortion can occur when females in the first trimester of pregnancy, naïve to leucaena, are fed high-leucaena diets, leading to lowered calving percentages. In contrast, high calving percentages are achieved in breeding herds where females have adapted to high leucaena diets (J. Schmidt and P. Larsen pers. comm.). Thus, it may

be possible to avoid negative effects on herd reproduction by appropriate herd management.

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