Agriculture **2015**, *5*, 427-440; doi:10.3390/agriculture5030427

OPEN ACCESS

agriculture

ISSN 2077-0472

www.mdpi.com/journal/agriculture

Review

The Occurrence and Toxicity of Indospicine to Grazing Animals

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Academic Editor: Wayne Bryden

Received: 17 May 2015 / Accepted: 6 July 2015 / Published: 13 July 2015

Abstract: Indospicine is a non-proteinogenic amino acid which occurs in *Indigofera* species with widespread prevalence in grazing pastures across tropical Africa, Asia, Australia, and the Americas. It accumulates in the tissues of grazing livestock after ingestion of *Indigofera*. It is a competitive inhibitor of arginase and causes both liver degeneration and abortion. Indospicine hepatoxicity occurs universally across animal species but the degree varies considerably between species, with dogs being particularly sensitive. The magnitude of canine sensitivity is such that ingestion of naturally indospicine-contaminated horse and camel meat has caused secondary poisoning of dogs, raising significant industry concern. Indospicine impacts on the health and production of grazing animals *per se* has been less widely documented. Livestock grazing *Indigofera* have a chronic and cumulative exposure to this toxin, with such exposure experimentally shown to induce both hepatotoxicity and embryo-lethal effects in cattle and sheep. In extensive pasture systems, where animals are not closely monitored, the resultant toxicosis may well occur after prolonged exposure but either be undetected, or even if detected not be attributable to a particular cause. Indospicine should be considered as a possible cause of animal poor performance, particularly reduced weight gain or reproductive losses, in pastures where *Indigofera* are prevalent.

Keywords: indospicine; indigofera; non-protein; hepatotoxic

1. Introduction

Indospicine (L-6-amidino-2-amino-hexanoic acid) is a non-proteinogenic amino acid found in *Indigofera* plant spp. which in recent decades has gained notoriety for its ability to form persistent and sometimes toxic residues in tissues of grazing animals. Ingestion of indospicine-contaminated horse meat [1] and more recently camel meat [2] has resulted in the secondary poisoning of dogs which are particularly sensitive to indospicine hepatotoxicosis. The impact of this plant toxin on the health and production of grazing animals *per se* has been less widely documented and is the focus of this review.

As an arginine analogue, indospicine (Figure 1) has the potential to interfere with a range of mammalian arginine metabolic pathways, as arginine is a precursor for the synthesis not only of proteins but also of nitric oxide, urea, polyamines, proline, glutamate, creatine and agmatine. Indospicine has been shown experimentally to cause both liver degeneration and also teratogenic and embryo-lethal effects [3,4], but the mechanism of this toxicity has never been fully elucidated.

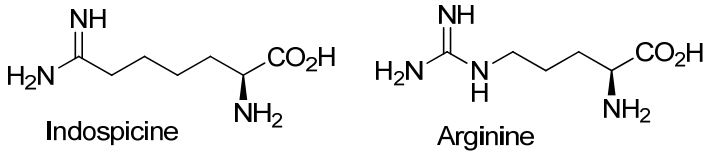

Figure 1. Indospicine an analogue of arginine.

2. Plant Sources of Indospicine

Indospicine has been found only in *Indigofera* plant species, and then primarily restricted to a small number of species within this genus. As a cumulative, non-acute toxin it is possible that this toxin may be as yet unidentified in additional members of this genus of some 700 species which are distributed geographically in tropical Africa, Asia, Australia and North and South America. *Indigofera* plant species, like other Leguminosae, are high in protein, and are considered agronomically very desirable due to their ability to tolerate drought, floods and salinity [5]. Many *Indigofera* species in Africa and Asia have been utilized as forage, green manure in coffee, tea and rubber plantations, and as cover crops. Several species (notably *Indigofera spicata*) were initially introduced as pasture legumes into mainland USA [6] and into Australia [7], before their potential toxicity was recognised. Members of this genus range from prostrate or ascending perennial herbs (such as *I. spicata*) to shrubby species. These perennial shrubby species are deep-rooted and can represent valuable forage plants, due to their palatability, insect resistance and ability to respond with growth to small rainfall events [8], however indospicine has also been determined within some such species [9].

2.1. Indospicine Content of Indigofera Species

This toxin was isolated as the first naturally occurring hepatotoxic amino acid in 1970 by Hegarty and Pound from *Indigofera spicata* hence the name indospicine [4]. Plant classification within this species has been confused over the years and the *I. spicata* complex now comprises both *I. spicata* (Forsk.) and *I. hendecaphylla* (Jacq.) (or *I. endecaphylla* auctt., orth. var.). These species names appear interchangeably in early literature and it was not until 1993 that these were shown to be 2 separate

species [10]. It is thus difficult to ascertain with any certainty which of these species was actually used in early studies unless herbarium voucher specimens are examined [11]. Retrospective inspection of herbarium data has however established that the study by Hegarty and Pound utilized specimens of both species, and that indospicine was determined in both *I. spicata* and *I. hendecaphylla* in their work [11].

Since its discovery, indospicine content has been investigated in more than 30 different *Indigofera* species of both shrubby and herbaceous types with widely different results depending on both species and locality. Species in which high levels of indospicine have been determined in foliage (in excess of 500 mg/kg DM) include *I. hendecaphylla* [4,11], *I. linnaei* [12,13], *I. lespedezioides* [7,14,15], *I. spicata* [4,11,13–16], *I. vicioides* [9] and *I. volkensii* [7]. Indeed levels up to an exceptional 1.2% (DM basis) have been reported in *I. spicata* [7]. Species with low to moderate levels of indospicine (typically less than 100 mg/kg DM and sometimes up to some 300 mg/kg DM) include *I. amorphoides* [9], *I. arrecta* [9], *I. brevicalyx* [9], *I. circinella* [15], *I. coerulea* [9], *I. colutea* [13], *I. costata* [9], *I. cryptantha* [9], *I. heteroticha* [15], *I. hirsuta* [15], *I. linifolia* [13], *I. nigritana* [7] and *I. trita* [15].

It is perhaps worth noting that in a large proportion of the *Indigofera* species that have been analysed, indospicine has not been detected [7,13,15,17]. *Indigofera* species in this non-indospicine containing category include *I. adesmiifolia* [13], *I. alternans* [7], *I. antunesiana* [7], *I. astragalina* [7], *I. georgei* [13], *I. glandulosa* [7], *I. leucotricha* [13], *I. mucronata* [7], *I. oblongifolia* [13], *I. parviflora* [7,13], *I. praticola* [14], *I. schimperi* [7], *I. suffruticosa* [7], *I. tinctoria* [7,14], and *I. vohemarensis* [7]. Likewise Miller and Smith [17] measured indospicine in the defatted seed meals of "*I. endecaphylla*" (*I. spicata*/*I. hendecaphylla*) but did not detect indospicine in seed meal of 16 other *Indigofera* species namely *I. arrecta*, *I. brachystachya*, *I. densiflora*, *I. frutescens*, *I. hirsuta*, *I. jaliscensis*, *I. kirilowii*, *I. linifolia*, *I. oblongifolia*, *I. pilosa*, *I. schimperi*, *I. sphaerocarpa*, *I. suffruticosa*, *I. sulcata*, *I. tinctoria*, and *I. zoilingeriana*.

Wide variations in indospicine content have been reported within individual *Indigofera* species with some suggestion that levels are influenced by the locale in which the taxa is growing, and particularly wide ranges of concentration (more than 10 fold variations between individual samples) have been reported for plant samples of *I. linnaei* [13] and *I. lespedezioides* [14]. Growth stage also effects indospicine levels [4], with highest levels of indospicine found in harvested seed of *I. spicata* [4] and *I. linnaei* [12] and lesser concentrations found in other parts of the plant including tips, leaves and the stem.

In other examples of intra-species variation, indospicine was detected in *I. hirsuta* and *I. trita* by Charlwood [15] but not by other researchers [7,13], and in *I. arrecta* and *I. amorphoides* by Hassen [9] but not by Aylward [7]. Aylward reported the "universality" of indospicine in *I. spicata* [7], whereas Charlwood [15] reported the absence of indospicine in 75% of seed samples of this species, and most remarkably detected only trace indospicine levels in the same seed accession [CPI 16110] that had contained 1240 mg/kg fresh weight when analysed by Hegarty and Pound [4]. The seed analysed by Hegarty and Pound in 1970 was green seed from fresh field-grown plants [4], while that analysed by Charlwood in 1984 was from a seed collection "collected from sites throughout Australia" and of unreported age [15]. This difference in seed source may well be a major contributor to the difference in reported results. Unsuccessful attempts were made to select for low-indospicine varieties of species with high agronomic potential such as *I. spicata*, and populations of this species were treated with

a chemical mutagen in non-entirely successful attempts to develop indospicine-free cultivars of *I. spicata* [18].

2.2. Prevalence of Indospicine-Containing Indigofera Species

The 6 species with highest indospicine content *I. hendecaphylla*, *I. linnaei*, *I. lespedezioides*, *I. spicata*, *I. vicioides* and *I. volkensii*, are all prostrate or semi-prostrate herbs that occur in pasture and are potentially grazed by livestock. *I. hendecaphylla*, *I. linnaei*, *I. lespedezioides*, *I. spicata*, and *I. volkensii* are relatively closely phylogenetically aligned within the Tethyan Clade, whereas *I. vicioides* is distinct within the Pantropical clade [19].

I. spicata and *I. hendecaphylla* are widely spread across tropical regions. *I. hendecaphylla* has a native range in Africa, Comoros, Madagascar, Reunion, Asia to Papua New Guinea and the Philippines, but it has been introduced and is now naturalized extensively in Florida (USA), French Polynesia and also Australia [20]. Similarly, *I. spicata* has a native range in Africa, Madagascar, Mauritius and Yemen, and has been naturalized extensively in Australia, Hawaiian islands, Japan, New Caledonia, Micronesia, Cook Islands among other places [21]. *I. hendecaphylla* and *I spicata* are often confused in literature, and it was only recently established that the species of *Indigofera* found in Hawaii was *I. spicata* [22]. Even though their toxicity has been recognized for more than 70 years [6], these species are still utilized as forage in their native range [23]. *I. linnaei* has a less extensive global distribution and is native across northern Australia, India, Indochina through Malesia, Melanesia to New Guinea [24]. This species has a seasonally variable prevalence in pasture, and can at times be a predominant plant in some arid regions of central and northern Australia. With a strong tap root the perennial *I. linnaei* is able to withstand dry conditions and responds quickly after rain [25]. It is most abundant at the start of the summer wet season when first rains stimulate the growth of this plant but are not sufficient to cause the germination of annual grasses and herbage. Horses in this region suffer from the neurological syndrome Birdsville horse disease following consumption of this plant [25], but cattle and sheep are seemingly unaffected by *I. linnaei* under normal grazing circumstances [26]. *I. lespedezioides* is restricted to the Americas and is native from Mexico and throughout South America [27] where the plant, particularly the root, is used medicinally as a febrifuge and stomachic (Venezuela); to halt diarrhea (Honduras); and as a treatment for gonorrhea, leucorrhea, jaundice, and epilepsy (Brazil) [28]. In Brazil, the roots and leaves are employed in the Mato Grosso to poison fish [28]. This species is responsible for a neurological syndrome in horses in Brazil [29] and is reported to be poisonous to livestock in Bolivia [30] and cattle in Brazil [29].

I. vicioides and *I. volkensii* have less extensive distributions and there are no literature reports of toxicity in livestock consuming these two species. *I. vicioides* is a spreading annual or perennial herb with native range in Africa and India [31] and *I. volkensii* has a more limited native range in east and north east Africa [32].

3. Etiology of Indospicine Toxicosis

Indospicine toxicosis manifests itself in two ways, acting as both a hepatotoxin with associated fat accumulation in liver and secondly as a teratogen and abortifacient. These effects were first observed

in livestock consuming *Indigofera* plant material, with indospicine subsequently identified as the responsible toxin through *in vivo* rodent toxicity studies [3,4].

I. spicata plant extracts were shown to induce a site-specific cleft of the secondary palate, generalized somatic dwarfism, and intrauterine embryolethality [33,34]. The abortive effect was also reported in pregnant rabbit does where feeding as little as 5% *I. spicata* in daily rations resulted in 100% stillborn offspring when fed in the last 15 days of pregnancy [35]. Pure indospicine administered orally at day 13 of gestation in pregnant rats caused cleft palate in more than 80% of viable fetuses exposed with histological features identical to those produced by the crude *I. spicata* extract, and also high embryolethality [3]. No other physical defects were observed in the embryopathic syndrome. Rat pups born with cleft palate induced by *Indigofera* did not live for more than a few hours after birth, with this observation attributed to either inhalation of milk into the lungs or a secondary metabolic toxic effect [33].

Indospicine also interferes with hepatic protein metabolism. Subcutaneous injection of indospicine into mice produced fat accumulation and cytological changes in the liver, which was inhibited by simultaneous injection of arginine [4]. In *in vivo* rat studies, oral dosing caused a pronounced inhibition of incorporation of ^{14}C-leucine into hepatic and serum protein, with this inhibition of protein synthesis then followed by a marked rise in the level of liver triglycerides [36]. Incorporation of ^{14}C-arginine into protein was depressed in cell-free fractions from the livers of rats treated with indospicine and also on addition of indospicine *in vitro* [37]. Increasing the level of arginine in the mixture decreased the magnitude of this effect. It was shown also that the formation of ^{14}C-arginyl-tRNA was inhibited by indospicine, but not the formation of ^{14}C-leucinyl-tRNA. Indospicine was concluded to be a competitive antagonist of arginine preventing arginine being incorporated into tRNA as a prelude to ribosomal polypeptide synthesis. Indospicine was also shown to be a competitive inhibitor of rat liver homogenate arginase with the affinity of arginase for indospicine being similar to that of the enzyme for arginine [38]. Inhibition of arginase negatively impacts aminoacylation of arginine and adversely leads to dysfunction of urea cycle. It was considered that other enzymes involved in arginine metabolism may be similarly affected by indospicine. The effect of indospicine on multiplying cells was tested by measuring the incorporation of ^{3}H-thymidine into newly-formed DNA using cultures of human lymphocytes stimulated with phytohaemagglutinin. Indospicine inhibited DNA synthesis particularly at early stages of incubation, and the inhibition was reversible by arginine proportional to the relative concentrations of the two substances [39]. This inhibition was attributed to blocking of protein synthesis which in turn is essential for DNA synthesis. Inhibition of DNA synthesis was also demonstrated by addition of indospicine to mouse bone marrow cell cultures, and this inhibition could also be prevented by supplementation by arginine [40].

In addition to indospicine, some *Indigofera* species contain a further non-proteinogenic amino acid, canavanine, also related to arginine. Canavanine can function in all enzymatic reactions for which arginine is a substrate and does not induce the same toxicosis as seen with indospicine [4]. Additionally some *Indigofera* species contain 3-nitropropanoic acid (3-NPA), present in the plant material as glucose esters. Independent rat trials demonstrated that 3-NPA is a neurotoxin and does not cause the hepatotoxic effects linked with indospicine [41]. Horses (but not ruminants) develop a neurological syndrome thought to be related to 3-NPA effects as discussed below.

4. Persistence of Indospicine in Animal Tissues

Indospicine is an unusual amino acid in that it is not incorporated into protein but exists in both plant and animal tissues as the free amino acid [42]. It is seemingly not readily metabolized by mammalian systems and accumulates as persistent residues in all tissues of animals consuming a diet containing the amino acid. Indospicine has been shown to accumulate in the tissues of horses [1,42,43], cattle [44] and goats [45] consuming *I. linnaei*, and in rabbits fed *I. spicata* seed [12]. The persistence of these residues has been demonstrated in experimental studies and tissue indospicine levels were still detectable several months after the cessation of feeding *Indigofera* plant material in horses [43], cattle [44], goats [45] and rabbits [12]. The cumulative and persistent nature of indospicine may well contribute to the long term toxic effects seen in field studies.

5. Indospicine Effects on Animals Consuming *Indigofera* Plants

The toxicity of *Indigofera* plants to livestock and other animals has been recorded over many decades with differing manifestations of indospicine toxicosis depending on animal species.

5.1. Cattle

Cattle consuming *Indigofera* have been reported to experience both hepatotoxic effects with associated liver lesions, and also teratogenic and embryo-lethal effects [35]. Cattle can, however, seemingly tolerate relatively small proportions of *Indigofera* with no observable ill effects. In Australia, cattle are reported to graze *I. linnaei* with no ill effect under normal grazing circumstances [26]. In Kenya, researchers, in 1949, reported various *Indigofera* including "*I. endecaphylla*" (*I. spicata* or *I. hendecaphylla*) were palatable to cattle with no toxicity reported [46]. In Hawaii, studies in the 1940s showed no adverse effects on young cattle in 10 years of short-interval pasture trials with relatively small proportions of the *I. spicata* [35]. However as reported by Nordfeldt *et al.*, in 1952, when the concentration of the legume in the diet was increased and the trials were continued for longer periods of time, signs of toxicity began to appear. Cattle fed a diet containing increasing percentages from 30% to 100% fresh *I. spicata* for 15 days gradually became inappetent and lost weight. The central nervous system was reportedly affected in some animals that appeared sluggish and drowsy, walking in circles with low head carriage, although these signs usually disappeared within one or two days. Animals fed for longer time periods showed depressed weight gain and liver lesions [35]. Loss in bodyweight and liver lesions were also reported in calves fed the fresh "*I. endecaphylla*" as a third of their diet for 2 weeks in Ceylon (Sri Lanka) in 1953 [47]. These authors recorded elevated levels of albumin in the urine and swollen, congested livers and pale kidneys on necropsy. Histological examination of the livers revealed periacinar necrosis, haemorrhage, and distention of the capillaries and sinusoids.

In addition to weight loss and hepatoxicity, cattle consuming *Indigofera* have also been reported to suffer reproductive loses, with "*I. endecaphylla*" (*I. spicata* or *I. hendecaphylla*) recognised as causing abortion in cattle in Africa as early as 1937 [48]. In trials in Hawaii in 1949, Nordfeldt reported *I. spicata* caused abortion or stillbirths in pregnant cows and heifers fed *I. spicata* as 16% to 52% of the diet for up to 44 days [35,49]. Dead or stillborn calves were born prematurely by as much as

100 days after heifers were fed green *I. spicata* for 25 days as a pasture supplement. In Fiji, abortions and placental retention were reported in all 4 cows fed between 25% and 100% *I. spicata* for one to three months in 1959 [50]. No autopsy information was provided for dead or stillborn calves and it is not known whether the cleft palate seen in rats fed indospicine was present. We are unable to locate any primary literature that describes cleft palate in calves as a result of maternal consumption of *Indigofera* or indospicine, however the Merck Veterinary Manual states that *I. spicata* is suspected of causing cleft palate in calves [51]. It has been further suggested that the widespread occurrence and utilization of *Indigofera* by livestock in Australian rangelands may also be an unattributed cause of malformations in domestic livestock [52]. *I. linnaei* has a widespread distribution in Australian rangelands [53], and given the extensive nature of livestock production systems in arid regions of central and northern Australia, pre-natal and peri-natal losses due to maternal consumption of indospicine may well occur and contribute to observed reproduction losses in these regions without any specific attribution. Causes of a large proportion of both pre- and peri-natal losses in these extensive herds are unknown [54]. In a recent study of factors affecting calf mortality in this region, 67% of calves that died did so within a week of their birth, with cause of death most frequently recorded as unknown [55].

5.2. Sheep

Fewer *Indigofera* feeding experiments have been conducted with sheep than cattle, but it would seem that sheep also experience both the hepatotoxic effects of indospicine with associated liver lesions, and embryo-lethality. The clinical signs of toxicoses in sheep fed *I. spicata* are largely the same as those seen in cattle [35,56]. Nordfeldt reported a more pronounced toxic effect on sheep than on cattle in feeding trials in Hawaii in 1952. In addition to inappetence, weight loss and drowsiness, sheep also exhibited an ocular discharge, a discharge from the nostrils and, in later stages, corneal opacity. Anorexia developed in sheep after two weeks on an *I. spicata* diet with deaths occurring after 28 days. Most of the sheep exhibited general weakness and an unsteady gait, while one animal developed severe diarrhea and one pregnant ewe aborted its foetus. Post-mortem examination revealed fatty degeneration of the liver. In later Australian trials, sheep fed *I. spicata ad lib* were reported to suffer inappetence, weight loss and drowsiness, with liver lesions seen post-mortem [56].

Like cattle, sheep can however, seemingly tolerate relatively small proportions of *Indigofera* with no observable ill effects. In Australia, sheep are reported to graze *I. linnaei* with no apparent ill effect [26]. In Kenya researchers in 1949 reported that various *Indigofera* spp. including "*I. endecaphylla*" (*I. spicata* or *I. hendecaphylla*) were palatable to sheep with no toxicity reported [46]. Feeding trials in India suggested *I. cordifolia* and *I. linnaei* (then called *I. enneaphylla*) were highly palatable feeds for sheep [57], and these plants are still considered valuable pasture constituents in India [58].

In contrast, sheep fed unrestricted amounts of *I. teysmannii* (*I. zoilingeriana*) leaves for a period of 24 days in India suffered hepatotoxic and nephrotoxic changes [59]. Rams were jaundiced with significant serum concentrations of albumin, glucose, bile salts and bilirubin. Fat deposits were gelatinized in livers at necropsy and histological examinations revealed fatty changes in the liver and mononuclear infiltrates with areas of necrosis and fibrosis, and regenerative foci. Lesions were also present in the

gastrointestinal tract, heart, spleen, lungs and kidney. However the indospicine content of this species is not known, indeed Miller reported the absence of indospicine in seeds of this species [17]. It is thus not clear whether the reported toxicity of *I. teysmannii* can be attributed to indospicine.

5.3. Horses

Horses consuming *I. linnaei* and *I. spicata* appear resistant to the hepatotoxic effect of indospicine [1,11]. Microscopic examination reveals only mild liver lesions consistent with descriptions of indospicine-related hepatopathy, including periacinar and periportal lymphocytic infiltrations, hydropic degeneration of mid-zonal hepatocytes and multifocal periacinar necrosis [11]. Horses however don't escape embryolethality indospicine effects, and abortion is commonly observed in mares consuming *I. lespedeziodes* in Brazil [29].

Equine neurological syndromes known as "Birdsville horse disease" in Australia [11] and "Grove disease" in USA [60] are attributed to consumption of *Indigofera* plant species but do not relate to indospicine toxicosis. *Indigofera* species particularly *I. hendecaphylla*, *I. linnaei* and *I. spicata* have been shown to contain glucose esters of 3-nitropropanoic acid (3-NPA) co-occurring with indospicine, and 3-NPA is believed to be the toxin responsible for the neurological syndrome seen in horses (but not ruminants) consuming these plants [11,61–63]. In non-ruminants, esters of 3-NPA are hydrolysed by mammalian esterase activity in the upper gastrointestinal tract but rapid absorption of the nitroacid precludes its metabolism by microbes in the hindgut [64]. In ruminants 3-NPA can be metabolised and detoxified by ruminal bacteria before absorption from the intestine, and hence the lesser toxicity of 3-NPA in these species. *I. lespedeziodes* also contains both indospicine and 3-NPA [14,29], but it has been queried whether detected levels of 3-NPA (up to 2.5 mg/g) were sufficient to cause the neurological syndrome seen in Brazil and these authors suggested that indospicine could be responsible for the neurological syndrome [29]. Notably however none of the tested plant samples were directly related to poisoning incidents.

There is no evidence that indospicine causes these neurological syndromes in horses, but we propose that is possible that indospicine acting as an arginine antagonist could attenuate the 3-NPA effects in horses. Creatine has been demonstrated to offer a protection against the neurological effects of 3-NPA [65], and it is thus conceivable that an indospicine-induced arginine (and resultant creatine) deficiency could exacerbate the neurological effects of 3-NPA.

Interestingly, even though unaffected by the hepatotoxic effects of indospicine, horses consuming *Indigofera* plants are known to accumulate indospicine in their tissues [1,11]. Indospicine accumulation occurs in all tissues including muscle and is residual. Fatal hepatotoxicity has occurred in dogs fed indospicine-contaminated meat from horses that had grazed *I. linnaei* in central Australia [1]. As a consequence, the Northern Territory of Australia *Meat Industries Act* [66] bans the slaughter of horses, donkeys, mules or hinnies for human consumption if the animals are exhibiting signs of Birdsville horse disease (*I. linnaei* poisoning), or for pet food if these animals originate from an area in which Birdsville disease occurs.

5.4. Camels

I. linnaei is reportedly a preferred food plant for feral camels present in arid Australian rangelands [67]. There are no field reports of impacts on camel health, but canine deaths have resulted from the consumption of indospicine-contaminated camel meat [2]. Camels fed *I. spicata* for 35 days accumulated indospicine in their tissues but showed no clinical signs [68]. At autopsy only very mild liver degeneration was observed.

5.5. Goats

I. hochstetteri has been demonstrated to be toxic to goats in Sudan, with animals developing inappetence, weight loss, anaemia and hind limb weakness followed by incoordination, recumbency and death [69]. Fat accumulation and congestion of the liver were reported at necropsy as well as damage to other tissues, especially the kidneys, intestine and central nervous system. Histological lesions included fatty degeneration of the liver. This is suggestive of indospicine toxicity, even though indospicine content of the plant had not been established.

An Angora goat fed dried *I. linnaei* at an average dose rate of 0.2 mg indospicine/kg BW/day for 52 days accumulated indospicine in plasma and liver but did not develop liver lesions [45]. A second goat was fed at dose rates of 0.2 mg to 0.6 mg indospicine/kg BW/day over a 30 week period and demonstrated the persistence of plasma indospicine levels with a half-life of some 2 weeks after cessation of *Indigofera* consumption.

5.6. Poultry/Birds

Following the reports of *I. spicata* toxicity in grazing livestock by Nordfeldt [35], feeding trials with 11 different *Indigofera* species were undertaken with poultry chicks [70]. Of the species tested only one induced toxicity. Consumption of 5% "*I. endecaphylla*" (*I. spicata* or *I. hendecaphylla*) meal from various global sources resulted in decreased growth rate and paralysis of the neck, legs and wings, commonly followed by death [70]. Subsequent investigation established that the toxin responsible for producing these neurological signs in chicks was 3-NPA and that the severity of the toxic signs was related to the 3-NPA content of the plant material [63,71–73]. Chickens were intoxicated by pure 3-NPA and by the leaf and stem of *I. spicata* containing 3-NPA as well as indospicine, but were unaffected by *I. spicata* seed, which contains indospicine, but no 3-NPA [74]. This evidence suggests that chickens are not susceptible to the hepatotoxic effects of indospicine.

6. Conclusions

Indospicine is not an acute toxin, but it is present in a number of *Indigofera* species which have a somewhat ubiquitous presence in grazing pastures across tropical regions from Africa, Asia, Australia, the Americas and islands of both Pacific and Indian Oceans. Livestock can thus have a chronic and cumulative exposure to this toxin, with such exposure experimentally shown to induce both hepatotoxicity and embryonic losses in cattle and sheep. In extensive pasture systems, where animals are not closely and individually monitored, the resultant toxicosis may well occur after prolonged exposure but either not be detected/identified or even if detected then be attributed to an unknown

cause. The literature would suggest that for cattle or sheep grazing pastures where *Indigofera* (particularly *I. spicata*, *I. hendecaphylla*, and *I. linnaei*) are prevalent, indospicine should be considered as a possible cause of poor performance, particularly where reduced weight gain or reproductive losses are involved.

Author Contributions

Mary T. Fletcher reviewed the literature and wrote the initial draft of the manuscript with assistance from A. Judith Cawdell-Smith and Rafat Al Jassim. All three authors reviewed and edited the final submission.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Hegarty, M.P.; Kelly, W.R.; McEwan, D.; Williams, O.J.; Cameron, R. Hepatotoxicity to dogs of horse meat contaminated with indospicine. *Aust. Vet. J.* **1988**, *65*, 337–340.
2. FitzGerald, L.M.; Fletcher, M.T.; Paul, A.E.H.; Mansfield, C.S.; O'Hara, A.J. Hepatotoxicosis in dogs consuming a diet of camel meat contaminated with indospicine. *Aust. Vet. J.* **2011**, *89*, 95–100.
3. Pearn, J.H.; Hegarty, M.P. Indospicine—The teratogenic factor from *Indigofera spicata* extract causing cleft palate. *Br. J. Exp. Pathol.* **1970**, *51*, 34–36.
4. Hegarty, M.P.; Pound, A.W. Indospicine, a hepatotoxic amino acid from *Indigofera spicata*: Isolation, structure, and biological studies. *Aust. J. Biol. Sci.* **1970**, *23*, 831–842.
5. Skerman, P.J.; Cameron, D.G.; Riveros, F. *Tropical Forage Legumes*, 2nd ed.; Food and Agricultural Organisation of the United Nations: Rome, Italy, 1988.
6. Emmel, M.W.; Ritchey, G.E. The toxicity of *Indigofera endecaphylla* Jacq. for rabbits. *J. Am. Soc. Agron.* **1941**, *33*, 675–677.
7. Aylward, J.H.; Court, R.D.; Haydock, K.P.; Strickland, R.W.; Hegarty, M.P. *Indigofera* species with agronomic potential in the tropics. Rat toxicity studies. *Aust. J. Agric. Res.* **1987**, *38*, 177–186.
8. Hassen, A.; Rethman, N.F.G.; van Niekerk, W.A.; Tjelele, T.J. Influence of season/year and species on chemical composition and *in vitro* digestibility of five *Indigofera* accessions. *Anim. Feed Sci. Technol.* **2007**, *136*, 312–322.
9. Hassen, A.; Rethman, N.F.G.; Apostolides, Z.; van Niekerk, W.A. Forage production and potential nutritive value of 24 shrubby *Indigofera* accessions under field conditions in South Africa. *Trop. Grasslands* **2008**, *42*, 96–103.
10. Du Puy, D.J.; Labat, J.N.; Schrire, B.D. The separation of two previously confused species in the *Indigofera spicata* complex (Leguminosae: Papilionoideae). *Kew Bull.* **1993**, *48*, 727–733.
11. Ossedryver, S.M.; Baldwin, G.I.; Stone, B.M.; McKenzie, R.A.; van Eps, A.W.; Murray, S.; Fletcher, M.T. *Indigofera spicata* (creeping indigo) poisoning of three ponies. *Aust. Vet. J.* **2013**, *91*, 143–149.

12. Pollitt, S. Residue Implications of Indospicine, a Toxic, Non-Protein Amino Acid. Ph.D. Thesis, Department of Physiology and Pharmacology, The University of Queensland, Brisbane, Australia, 2001.
13. Tan, E.T.T.; Silcock, R.G.; Al Jassim, R.; D'Arcy, B.R.; Fletcher, M.T. The University of Queensland, Brisbane, Queensland, Australia. Unpublished work, 2015.
14. Gardner, D.; Riet-Correa, F. Analysis of the toxic amino acid indospicine by liquid chromatography-tandem mass spectrometry. *Int. J. Poisonous Plant Res.* **2011**, *1*, 20–27.
15. Charlwood, B.V.; Morris, G.S.; Grenham, M.J. A chemical database for the Leguminosae. In *Database in Systematics*; Alkin, R., Bisby, F.A., Eds.; Academic Press: London, UK, 1984; pp. 201–208.
16. Aylward, J.H. Screening legumes for toxicity. In *CSIRO Division of Tropcal Crops and Pastures Annual Report 1981–1982*; CSIRO: New South Wales, Australia, 1982; pp. 80–81.
17. Miller, R.W.; Smith, C.R., Jr. Seeds of *Indigofera* species. Their content of amino acids that may be deleterious. *J. Agric. Food Chem.* **1973**, *21*, 909–912.
18. Duke, J.A. *Handbook of Legumes of World Economic Importance*; Plenum Press: New York, NY, USA, 1981.
19. Schrire, B.D.; Lavin, M.; Barker, N.P.; Forest, F. Phylogeny of the tribe Indigofereae (Leguminosae–Papilionoideae): Geographically structured more in succulent-rich and temperate settings than in grass-rich environments. *Am. J. Bot.* **2009**, *96*, 816–852.
20. PIER (Pacific Islands Ecosystems at Risk). *Indigofera Hendecaphylla* Jacq., Fabaceae. Available online: http://www.hear.org/pier/species/indigofera_hendecaphylla.htm (accessed on 9 July 2015).
21. PIER (Pacific Islands Ecosystems at Risk). *Indigofera spicata* Forssk., Fabaceae. Available online: http://www.hear.org/pier/species/indigofera_spicata.htm (accessed on 9 July 2015).
22. Wagner, W.L.; Herbst, D.R.; Lorence, D.H. Flora of the Hawaiian Islands website. Available online: http://botany.si.edu/pacificislandbiodiversity/hawaiianflora/index.htm (accessed on 9 July 2015).
23. Bezabih, M.; Pellikaan, W.F.; Tolera, A.; Khan, N.A.; Hendriks, W.H. Chemical composition and *in vitro* total gas and methane production of forage species from the Mid Rift Valley grasslands of Ethiopia. *Grass Forage Sci.* **2014**, *69*, 635–643.
24. GRIN (USDA Germplasm Resources Information Network). Taxon: *Indigofera linnaei* Ali. Available online: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?70382 (accessed on 9 July 2015).
25. Gracie, A.; Brown, A.; Saville, P. AgNote: Birdsville Disease (Northern Territory Government). Available online: http://www.nt.gov.au/d/Content/File/p/Anim_Dis/633.pdf (accessed on 9 July 2015).
26. Dowling, R.M.; McKenzie, R.A. *Poisonous Plants: A Field Guide*; Department of Primary Industries: Brisbane, Australia, 1993.
27. GRIN (USDA Germplasm Resources Information Network). Taxon: *Indigofera lespedezioide*s Kunth. Available online: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?20025 (accessed on 9 July 2015).
28. Hastings, R.B. Medicinal legumes of Mexico: Fabaceae, Papilionoideae, Part One. *Econ. Bot.* **1990**, *44*, 336–348.

29. Lima, E.F.; Riet-Correa, F.; Gardner, D.R.; Barros, S.S.; Medeiros, R.M.T.; Soares, M.P.; Riet-Correa, G. Poisoning by *Indigofera lespedezioides* in horses. *Toxicon* **2012**, *60*, 324–328.
30. Altschul, S.V.R. Drugs and Foods from Little-Known Plants: Notes in Harvard University Herbaria; Harvard University Press: Cambridge, MA, USA, 1973.
31. GRIN (USDA Germplasm Resources Information Network). Taxon: *Indigofera vicioides* Jaub. & Spach. Available online: http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl (accessed on 9 July 2015).
32. GRIN (USDA Germplasm Resources Information Network). Taxon: *Indigofera volkensii* Taub. Available online: http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl (accessed on 9 July 2015).
33. Pearn, J.H. Report of a new site-specific cleft palate teratogen. *Nature* **1967**, *215*, 980–981.
34. Pearn, J.H. Studies on a site-specific cleft palate teratogen. The toxic extract from *Indigofera spicata* Forssk. *Br. J. Exp. Pathol.* **1967**, *48*, 620–626.
35. Nordfeldt, S.; Henke, L.A.; Morita, K.; Matsumoto, H.; Takahash, M.; Younge, O.R.; Willers, E.H.; Cross, R.F. *Feeding Tests with Indigofera Endecaphylla Jacq. (Creeping Indigo) and Some Observations on its Poisonous Effects on Domestic Animals*; Technical Bulletin No. 15; Hawaii Agriculture Experiment Station, University of Hawaii: Honolulu, HI, USA, 1952; pp. 5–23.
36. Christie, G.S.; Madsen, N.P.; Hegarty, M.P. Acute biochemical changes in rat liver induced by the naturally-occurring amino acid indospicine. *Biochem. Pharmacol.* **1969**, *18*, 693–700.
37. Madsen, N.P.; Christie, G.S.; Hegarty, M.P. Effect of indospicine on incorporation of L-arginine-^{14}C into protein and transfer ribonucleic acid by cell-free systems from rat liver. *Biochem. Pharmacol.* **1970**, *19*, 853–857.
38. Madsen, N.P.; Hegarty, M.P. Inhibition of rat liver homogenate arginase activity *in vitro* by the hepatotoxic amino acid indospicine. *Biochem. Pharmacol.* **1970**, *19*, 2391–2393.
39. Christie, G.S.; de Munk, F.G.; Madsen, N.P.; Hegarty, M.P. Effects of an arginine antagonist on stimulated human lymphocytes in culture. *Pathology* **1971**, *3*, 139–144.
40. De Munk, F.G.; Christie, G.S.; Hegarty, M.P. The effects of indospicine on bone marrow cells in liquid culture. *Pathology* **1972**, *4*, 133–137.
41. Hutton, E.M.; Windrum, G.M.; Kratzing, C.C. Studies on the toxicity of *Indigofera endecaphylla*: II. Toxicity for mice. *J. Nutr.* **1958**, *65*, 429–440.
42. Pollitt, S.; Hegarty, M.P.; Pass, M.A. Analysis of the amino acid indospicine in biological samples by high performance liquid chromatography. *Nat. Toxins* **1999**, *7*, 233–240.
43. Hegarty, M.P. Non-metallic chemical residues in toxic plants with potential importance to animal and human health. In *Vet Update 92*; Osborne, H.G., Ed.; Continuing Professional Education, University of Queensland: Brisbane, Australia, 1992; pp. 323–332.
44. Arid Zone Research Institute. Indospicine in beef. In *Northern Territory Department of Primary Industries and Fisheries Technical Annual Report 1987–1988*; Technical Bulletin No. 150; NTDPIF: Alice Springs, Australia, 1989; p. 39.
45. Young, M.P. Investigation of the Toxicity of Horsemeat due to Contamination by Indospicine. Ph.D. Thesis, School of Veterinary Science, University of Queensland, Brisbane, Australia, 1992.

46. Bogdan, A.V. Observations on palatability of some leguminous plants of Kenya. *East Afr. Agric. J.* **1949**, *15*, 38–40.
47. Jeganathan, P. Toxic effects of feeding *Indigofera endecaphylla* (Jacq.) to calves. *Ceylon Vet. J.* **1953**, *1*, 83–85.
48. Dalziel, J.M. *The Useful Plants of West Tropical Africa*; The Crown Agents for the Colonies: London, UK, 1937; pp. 243–247.
49. Nordfeldt, S.; Young, O.R. Toxicity of creeping indigo to livestock. *Hawaii Agric. Expt. Station Univ. Hawaii Prog. Notes* **1949**, *55*, 1–2.
50. Yelf, J.D. The toxicity of creeping indigo in Fiji. *Agric. J.* **1959**, *29*, 9–10.
51. Maxwell, H.S.; Nettleton, P.; Kirkland, P.D. Overview of congenital and inherited anomalies. In *Merck Veterinary Manual*. Available online: http://www.merckvetmanual.com/mvm/ (accessed on 9 July 2015).
52. Keeler, R.F. Plant metabolites that are teratogenic in offspring and toxic in the dam. *Toxicon* **1983**, *21*, 221–225.
53. Australia's Virtual Herbarium. Available online: http://avh.chah.org.au/ (accessed on 9 July 2015).
54. Burns, B.M.; Fordyce, G.; Holroyd, R.G. A review of factors that impact on the capacity of beef cattle females to conceive, maintain a pregnancy and wean a calf—Implications for reproductive efficiency in northern Australia. *Anim. Reprod. Sci.* **2010**, *122*, 1–22.
55. Bunter, K.L.; Johnston, D.J.; Wolcott, M.L.; Fordyce, G. Factors associated with calf mortality in tropically adapted beef breeds managed in extensive Australian production systems. *Anim. Prod. Sci.* **2014**, *54*, 25–36.
56. Maskasame, C. Toxicity and Nutritional Value of Some Promising Pasture Legumes in Rats and Sheep. Master's Thesis, Department of Physiology and Pharmacology, The University of Queensland, Brisbane, Australia, 1984.
57. Nath, K.; Malik, N.; Singh, O. Chemical composition and nutritive value of *Indigofera enneaphylla* and *I. cordifolia* as sheep feeds. *Aust. J. Exp. Agric.* **1971**, *11*, 178–185.
58. Srinivas, B.; Swain, N. Seasonal dynamics in vegetation and rumen microbial nitrogen production and nutritional status of grazing sheep in a semiarid rangeland in eastern Rajasthan, India. *Grassland Sci.* **2011**, *57*, 219–224.
59. Krishna, L.; Vaid, J.; Singh, B. Pathological study on *Indigofera teysmanni* toxicity in sheep. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **1986**, *7*, 14–17.
60. Morton, J.F. Creeping indigo (*Indigofera spicata* Forsk.) (Fabaceae): A hazard to herbivores in Florida. *Econ. Bot.* **1989**, *43*, 314–327.
61. Majak, W.; Benn, M.; McEwan, D.; Pass, M.A. Three nitropropanoyl esters of glucose from *Indigofera linnaei*. *Phytochemistry* **1992**, *31*, 2393–2395.
62. Williams, M.C. Nitro compounds in *Indigofera* species. *Agron. J.* **1981**, *73*, 434–436.
63. Cooke, A.R. The toxic constituent of *Indigofera endecaphylla*. *Arch. Biochem. Biophys.* **1955**, *55*, 114–120.
64. Majak, W.; Pass, M.A. Aliphatic nitrocompounds. In *Toxicants of Plant Origin, Volume II, Glycosides*; Cheeke, P.R., Ed.; CRC Press: Boca Raton, FL, USA, 1989; pp. 143–159.

65. Shear, D.A.; Haik, K.L.; Dunbar, G.L. Creatine reduces 3-nitropropionic-acid-induced cognitive and motor abnormalities in rats. *NeuroReport* **2000**, *11*, 1833–1837.
66. Northern Territory Dept Primary Industries Fisheries & Mines. Northern Territory of Australia Meat Industries Act. Available online: http://www.austlii.edu.au/au/legis/nt/consol_act/mia184.txt/cgi-bin/download.cgi/download/au/legis/nt/consol_act/mia184.rtf#_Toc263691324 (accessed on 9 July 2015).
67. Dörges, B.; Heucke, J.; Dance, R. *The Palatability of Central Australian Plant Species to Camels*; Technote No. 116; Department of Primary Industry, Fisheries and Resources, Northern Territory Government: Alice Springs, Australia, 2003. Available online: www.nt.gov.au/d/Content/File/p/Technote/TN116.pdf. 2003 (accessed on 9 July 2015).
68. Tan, E.T.T.; Al Jassim, R.A.M.; Cawdell-Smith, J.A.; Ossedryver, S.M.; Fletcher, M.T. The University of Queensland, Brisbane, Queensland, Australia. Unpublished work, 2015.
69. Suliman, H.B.; Wasfi, I.A.; Tartour, G.; Adam, S.E.I. The effects of *Indigofera hochstetteri* on goats. *Rev. Elev. Med. Vet. Pays Trop.* **1983**, *36*, 393–402.
70. Rosenberg, M.M.; Zoebisch, O.C. A chick test for toxicity in forage legumes. *Agron. J.* **1952**, *44*, 315–318.
71. Morris, M.P.; Pagan, C.; Warmke, H.E. Hiptagenic acid, a toxic component of *Indigofera endecaphylla*. *Science* **1954**, *119*, 322–323.
72. Britten, E.J.; Matsumoto, H.; Palafox, A.L. Comparative toxic effects of 3-nitropropionic acid, sodium nitrite and *Indigofera endecaphylla* on chicks. *Agron. J.* **1959**, *51*, 462–464.
73. Britten, E.J.; Palafox, A.L.; Matsumoto, H. Selection for toxicity in single plants of *Indigofera endecaphylla* by biological assay. *Agron. J.* **1959**, *51*, 651–654.
74. Britten, E.J.; Palafox, A.L.; Frodyma, M.M.; Lynd, F.T. Level of 3-nitropropanoic acid in relation to toxicity of *Indigofera spicata* in chicks. *Crop Sci.* **1963**, *3*, 415–416.