Use of DHP-degrading rumen bacteria to overcome toxicity in cattle grazing irrigated leucaena pasture

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Abstract

Leucaena toxicity in cattle in the Ord River Irrigation Area has been severe due to high mimosine concentrations over most of the year. Control of mimosine toxicity was achieved by dosing heifers with rumen fluid containing bacteria which are capable of degrading 3-hydroxy-4(1H)-pyridone (DHP).

Two groups of yearling Shorthorn heifers were grazed on irrigated leucaena-pangola pastures. Heifers in one group were each given an oral dose of DHP-degrading bacteria. Heifers in the other group were used as controls. Groups were separated by 4 ha of ungrazed verano-pangola pasture.

Within 4 weeks of dosing, excretion of DHP in the urine of treated heifers almost ceased, serum thyroxine levels returned to normal and clinical signs of toxicity were absent. Dosed heifers were 15 kg heavier ($P<0.01$) than the controls after 12 weeks.

By the 12th week after dosing, the bacteria had spread to the control group and those heifers then recovered from the toxicity and overall weight gains 20 weeks after dosing were similar to the dosed group. Four calves born to heifers in the control group were found dead, while 3 calves born in the treated group were normal.

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Resumen

La toxicidad de la leucaena para el ganado ha sido severa en el Area de Irrigación del Río Ord debido a la alta concentración de mimosina durante la mayor parte del año. El control de la toxicidad de la mimosina ha sido lograda mediante la dosificación ruminal a vaquillas con un liquido que contiene bacterias las cuales son capases de degradar 3-hydroxy-4(1H)-pyridone (DHP).

Pasturas de leucaena-pangola irrigadas fueron pastoreadas con dos grupos de vaquillas Shorthorn de año. Cada una de las vaquillas de uno de los grupos fueron suministradas con una dosis oral de bacterias degradadoras de DHP. Las vaquillas en el otro grupo fueron usadas como control. Los grupos fueron separados con una pastura de verano-pangola no utilizada.

Durante un período de 4 semanas, la eliminación de DHP en la orina de las vaquillas tratadas casi cesó, los niveles de thyroxina en suero retornaron a su normalidad y los signos clínicos de toxicidad estuvieron ausentes. Después de 12 semanas las vaquillas dosificadas fueron 15 kg más pesadas ($P<0.01$) que el control.

En la semana 12 después de la dosificación, las bacterias degradadoras del DHP se habían esparcido al grupo control el cual se recuperó entonces de la toxicidad y su ganancia de peso global 20 semanas después de la dosificación fue similar a la del grupo tratado. Cuatro becerros nacidos de las vaquillas del grupo control murieron, mientras que 3 becerros nacidos del grupo tratado fueron normales.

Introduction

The leguminous tree *Leucaena leucocephela* has been grown experimentally on the Ord River Irrigation Area (ORIA) in the Kimberley region of Western Australia for nearly 20 years. The high mimosine content of leucaena grown under irrigation in this region has been a severe constraint to its commercial exploitation (Blunt 1976, Blunt and Jones 1977, Jones and Hegarty 1984). The ingested mimosine is metabolized in the rumen to 3-hydroxy-4(1H)-pyridone (DHP) which is a potent goitrogen (Hegarty *et al.* 1979) and which reduces feed intake (Jones and Lowry 1984). Cattle and goats that have been either ruminally or orally dosed with cultures of DHP-degrading bacteria isolated from a goat in Hawaii have acquired the ability to degrade DHP (Jones and Megarrity 1986) and are therefore not adversely affected by diets containing high levels of mimosine.

In south-east Queensland where leucaena toxicity is rare in grazing cattle, the use of DHP-degrading bacteria led to an improvement in liveweight gain (Quirk *et al.* 1988). The effects of leucaena toxicity are greater in far northern Australia than in S.E. Queensland (Jones *et al.* 1976), and especially so in the ORIA. It was therefore important to evaluate the DHP-degrading bacteria in this area of severe toxicity.

This paper reports the effects of dosing Shorthorn heifers with DHP-degrading bacteria on the expression of leucaena toxicity and on their liveweight gain when grazing irrigated leucaena pastures known to contain high levels of mimosine.

Materials and methods

Site and climate

The experiment was conducted at the Kimberley Research Station (15°39′S and 128°43′E), now known as the Frank Wise Institute of Tropical Agricultural Research, situated in the ORIA, Western Australia. The ORIA has a tropical monsoon climate with an average annual rainfall of 787 mm. The wet season extends from early December until the end of March. In November, the hottest month, mean daily maximum is 39 °C and minimum 25 °C, while the coolest month is June with a mean maximum of 30 °C and mean minimum of 14 °C.

Treatments and design

The experiment consisted of two main treatments, one in which 20 heifers grazing a 4 ha leucaena pasture were dosed with DHP-degrading bacteria and the other in which 20 heifers grazing a separate 4 ha leucaena paddock were not dosed.

Half of each of these groups were given an intra-muscular injection of iodine as earlier work had shown that iodine reduced toxicity in leucaena-pangola pasture (R.J. Jones and C.J. Blunt, unpublished data).

The liveweight of the two groups of heifers was compared using the Student "t" test. Urinary DHP and serum T_4 levels were analysed by analysis of variance using animals as replicates. The pangola grass and leucaena on offer at each sampling date are presented as means with their standard deviation.

Procedure

Pasture management

Sixteen hectares of Cununurra clay (pH 8) were ploughed in August 1982. *Leucaena leucocephala* cv. Cunningham was sown in rows 4.5 m apart on half the area with pangola grass (*Digitaria eriantha* ssp. *pentzii*, previously *D. decumbens*) planted by runners between the rows. The remaining 8 hectares were planted to pangola grass and Caribbean stylo (*Stylosanthes hamata* cv Verano). Prior to planting, 150 kg/ha of zinc enriched double superphosphate (17.5% P, and 0.6% Zn) was applied and a similar amount applied aerially each year.

The leucaena-pangola pastures were in two blocks of 4 ha separated by a 4 ha block of verano-pangola that was not grazed during the experiment. This area served as a buffer between the two blocks of leucaena to prevent animal contact and the possibility of spread of introduced bacteria. The possibility was further reduced by weighing the two groups of cattle a day apart. The controls were weighed on the first day and the dosed group on the second day. Thus there was a 4 week period between use of the yards by dosed cattle and the control cattle.

Each block of leucaena was divided into four 1 ha paddocks. Each paddock was grazed for 1 week and rested for 3 weeks. The paddocks were stocked at 5 animals/ha for the first 8 weeks and subsequently at 3.5 animals/ha.

Water was applied fortnightly to field capacity using flood irrigation through syphons. Irrigation ceased in any week which received 25 mm or more of rainfall.

Cattle management

Forty yearling Shorthorn heifers, weighing approximately 180 kg, were allocated to the 2 treatments by stratified randomization based on unfasted liveweight on December 8 1983. Prior to allocation the heifers had all grazed together for one week on a leucaena-pangola pasture to assure a supply of substrate in the rumen for the DHP-degrading bacteria.

The DHP-degrading bacteria, which to date remain un-named, are anaerobic gram negative short rods (1.2 x 0.6μ). They convert 3-hydroxy-4(1H)-pyridone to 2-hydroxy-3(1H)-pyridone before ring cleavage as proposed by Jones (1985). However, the identity of the final breakdown products and whether they are metabolised or directly excreted has yet to be resolved.

Heifers in the treated group were orally infused with 600 ml of rumen fluid containing approximately 10^5 DHP-degrading bacteria/ml on December 12, 1983. This fluid was collected on December 11, 1983 from cattle that had been infused with DHP-degrading bacteria earlier in 1983 and were grazing leucaena pastures at the CSIRO Lansdown pasture Research Station, near Townsville, Queensland (Jones and Megarrity 1986). The excess rumen fluid returned to Townsville after transit to and from KRS, degraded DHP in 19 days indicating that active DHP-degrading bacteria were infused into the cattle.

Prior to grazing the paddocks the heifers were dosed with Nilverm (active ingredient 60g/l Levamisole) at the recommended rate of 5 ml/50 kg of liveweight for the control of intestinal worms, particularly *Ascarid* spp. and *Haemonchus contortus* and dipped in Grenade (active ingredient 200g/l Cyhalothrine), at the rate of 350 ml of Grenade to 1000 litres of water, to control cattle tick, *Boophilus microplus*.

Measurements

Every 4 weeks cattle were weighed unfasted and samples of urine and blood collected. Urine samples were acidified to pH 3-4 with HCl and stored at 4 °C. DHP concentrations in urine were measured after acid hydrolysis of any DHP conjugates (Megarrity 1981) by an automated colorimetric method (Megarrity 1978). Blood was collected by jugular puncture and serum stored at −20 °C prior to determination of total serum thyroxine (T_4) concentrations by radioimmunoassay.

Normal levels of serum thyroxine range from 60–80 n mol/l. This chemical is important in controlling the overall metabolism of the animal and is essential for proper growth and reproduction. Deficiency leads to goitre, low metabolic rate, loss of hair, lethargy and poor reproduction.

Samples of rumen fluid were collected by stomach tube from all heifers prior to, and 4 and 8 weeks after dosing with DHP-degrading bacteria. These samples were stored anaerobically in CO_2-enriched containers at 4 °C prior to measurement of *in vitro* DHP-degradation using 0.5 ml of rumen fluid in 9 ml of medium containing 50 mg of DHP. (Jones and Megarrity 1986).

Faeces were collected from the rectum at the start of the trial and every 28 days for $\delta^{13}C$ determinations (LeFeuvre and Jones 1988), to estimate the percentage of leucaena in the diet (Jones *et al.* 1979). Samples for the plus and minus iodine treatments were bulked prior to analysis for each group, i.e. there were four $\delta^{13}C$ determinations made at each sampling.

Pasture on offer to the heifers was measured on paddock 1 of the rotation every 28 days immediately prior to access. Leucaena yield was measured by harvesting leaf and edible stem (<6 mm diameter) from 10 m x 1 m lengths of row per paddock. The yield of inter-row pangola grass was measured by cutting to near ground level ten 0.5 m² quadrats per paddock.

Results

Urinary DHP concentrations

After 1 week of grazing leucaena, both groups had equal mean levels of urinary DHP (0.36%). By week 4 the mean DHP% had dropped to 0.04% in the dosed group and then to 0.01% at weeks 8, 12, 16 and 20. Levels for the control heifers increased to 0.78% by week 6 and then declined to values similar to those of the dosed group by week 20 (Figure 1).

Serum thyroxine (T_4) levels

The mean T_4 levels were identical for both groups at the start of the experiment (55 n mol/l). Dosing resulted in an increase in T_4 levels which persisted

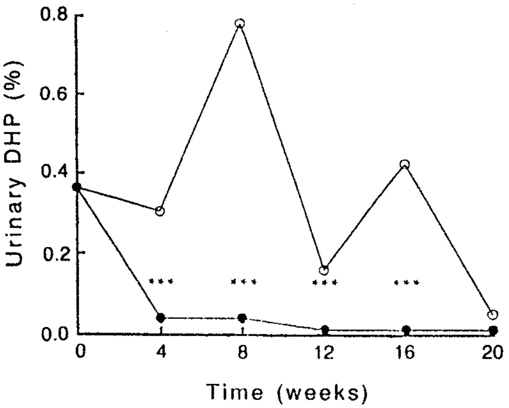

Figure 1. Changes in mean urinary DHP concentration of heifers grazing Leucaena-pangola pastures following infusion with DHP-degrading bacteria (—●—) or not infused (—o—).

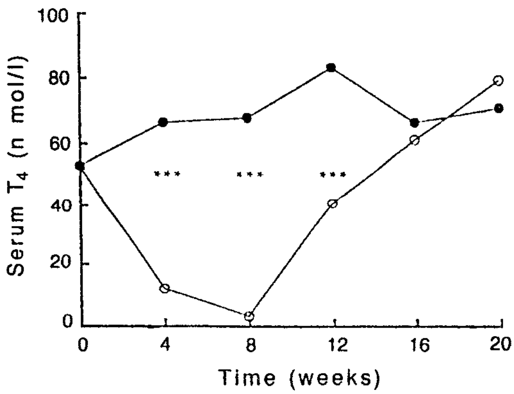

Figure 2. Changes in the mean serum T_4 levels of heifers grazing leucaena-pangola pastures following infusion with DHP-degrading bacteria (—●—) or not infused (—o—).

for the remainder of the experiment. In the control group, T_4 levels dropped to 2.7 n mol/l by week 8 then increased progressively so that by weeks 16 and 20 the levels for the two groups were similar (Figure 2).

DHP – degrading activity of rumen fluid samples

All rumen fluid samples taken from the experimental cattle at the commencement of the experiment failed to degrade DHP *in vitro* after 2 months of incubation. Four weeks after dosing, rumen fluid from all infused heifers degraded the DHP within 3 days of incubation whereas there was no degradation of DHP with rumen fluid from the controls, even after incubation for 20 days. At 8 weeks, rumen fluid from all infused heifers degraded DHP but, in addition, rumen fluid from 3 heifers in the control group also showed an ability to degrade DHP. The absence of DHP in the urine of the 20 control animals at the end of the experiment indicated that all these heifers had acquired the bacteria and so no rumen fluid samples were taken at this time to measure DHP-degrading activity.

Leucaena in the diet

The percentage leucaena in the diet was similar for the dosed and the control cattle at week 0 (December 1983) and week 4 (January 1984). Thereafter the percentage leucaena in the diet of the control heifers was significantly higher than in the diets of dosed heifers. There was no difference in the percentage leucaena in the diet due to the iodine treatment, and the mean values across the iodine treatment for the control and dosed heifers are presented in Figure 3.

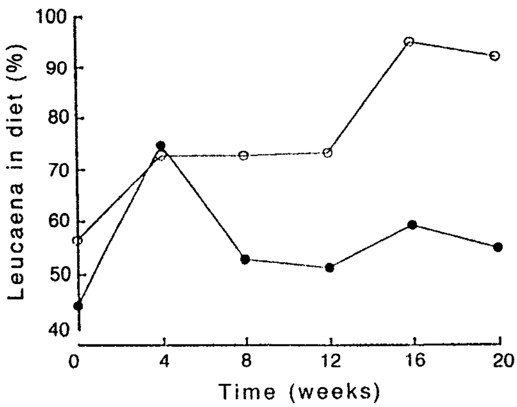

Figure 3. Seasonal changes in the percentage leucaena in the diet of infused (—●—) or not infused heifers (—o—).

Yields of leucaena and pangola

Pasture presentation yield was always higher on the control paddocks and the difference in favour of the control paddocks increased with time (Table 1). At the end of the experiment, yield on the control paddocks was almost double that on the paddocks grazed by dosed cattle. Leucaena availabilty ranged from 27-60 kg/heifer/week.

Table 1. Presentation yield of pangola grass and leucaena (mean ± s.d.) and mimosine concentration of shoots of leucaena during the experiment

Treatment	Pangola	Leucaena	Mimosine
	(kg/ha)		(% DM)
Week 0[1]			
Dosed	950±90	680±60	7.6
Control	1270±120	540±50	7.8
Week 4			
Dosed	870±90	470±40	9.3
Control	1440±100	390±30	7.6
Week 8			
Dosed	920±100	670±60	9.5
Control	1280±120	540±40	9.6
Week 12			
Dosed	800±70	510±50	8.2
Control	1020±130	710±70	7.3
Week 16			
Dosed	740±80	460±50	6.4
Control	1260±140	810±70	6.8
Week 20			
Dosed	730±70	470±40	6.8
Control	1390±110	850±70	5.1

[1]December 12, 1983

Mimosine concentration

The mimosine concentration in the shoots of leucaena as measured in the tip to first expanded leaf and expressed as a percentage of the dry matter is shown in Table 1. The levels ranged from 5.1% to 9.6% There was no significant difference in mimosine content between treatments at any date although concentrations were significantly higher in January (week 4) and February (week 8) compared with April (week 16).

Liveweight change

There was no effect of iodine on liveweight gain, so the liveweights of the iodine and non-iodine treated cattle were averaged. As 3 heifers in the dosed group and 4 in the control group proved to be pregnant, these animals were excluded from the liveweight gain calculations (Table 2). The mean of the two groups liveweight did not differ ($P>0.5$) 4 weeks after dosing, but dosed heifers had gained 8 kg more than controls after 8 weeks ($P<0.5$) and 15 kg after 12 weeks ($P<0.1$) Thereafter the liveweight gain of the control heifers improved more rapidly than the dosed heifers so that after 20 weeks there was only a 6 kg advantage from dosing which was not significant ($P>0.05$). The exclusion of the 3 heifers that were shown to have picked up the DHP-degrading bacteria at 8 weeks had no effect on the mean

Table 2. Mean liveweight of heifers with and without infusion with DHP-degrading bacteria, grazing leucaena-pangola pastures

	Week 0	4	8	12	16	20
	(kg/hd)					
Infused	180	191	208	212	227	242
Control	180	193	200	197	220	236
Difference		2	8	15	7	6
		NS	**	***	**	NS

weight of that group and so they have been included.

The physical signs of DHP toxicity exhibited by the control group after 6 weeks of grazing leucaena included loss of hair at the brush and around the feet. In some animals severe hair loss occurred over the whole leg and the brisket. There was excessive salivation from the majority of this group of heifers. In addition any small abrasions or cuts failed to heal and became festerous. These symptoms had started to abate by week 12 and were completely absent by week 20.

The 3 calves born to heifers in the control group were found dead while the 4 from the dosed heifers were normal and survived.

Discussion

The introduction of DHP–degrading bacteria into cattle on the ORIA was clearly effective in establishing a permanent bacterial rumen population which overcame the severe toxicity problems associated with leucaena at this site. The results confirm the experience with these bacteria in North Queensland (Jones and Megarrity 1986) and in S.E. Queensland (Quirk *et al.* 1988). In this experiment, the clinical signs of toxicity that occurred in the control group and the death of calves, was a recurrence of earlier experience with breeding cattle at this site (Jones *et al.* 1976).

The toxicity at this site, expressed as high urinary DHP concentrations, very low serum T_4 levels and hair loss on head, legs and body, was more severe than that experienced in the S.E. Queensland experiment where no outward signs of toxicity occurred (Quirk *et al.* 1988). This difference may be explained by the very high mimosine concentrations recorded on the ORIA.

The concentrations of 9.6% recorded in the height of summer on the ORIA are far higher than levels ever recorded at sub-tropical sites. Despite the high intakes of mimosine, the urine of dosed cattle was free of DHP, indicating that ruminal degradation of DHP was occurring. As a result, dosed cattle maintained normal concentrations of serum thyroxine throughout the experiment and did not exhibit hair loss or excessive salivation.

The changes in liveweight in this experiment, due to infusion with DHP-degrading bacteria, were not as clear cut as recorded in S.E. Queensland (Quirk *et al.* 1988). Low feed availability (which was lower than in the experiment of Quirk *et al.* 1988), on the paddocks grazed by treated cattle may have had some influence on liveweight gain throughout the period of the experiment. The lower feed-on-offer in paddocks grazed by dosed cattle was probably associated with higher intake of animals infused with the DHP-degrading bacteria (Jones and Lowry 1984). In addition, the control heifers apparently acquired the DHP-degrading bacteria after week 8 and they were then able to respond rapidly in terms of liveweight gain.

However, it has been shown that liveweight gains of only 0.5 kg/hd/d can be achieved by Shorthorn cattle while Brahman and Brahman cross cattle regularly gain at 0.75 kg/hd/d (D. Pratchett and T. Triglone, unpublished data). The average liveweight gain of the dosed cattle in this experiment was 0.45 kg/hd/d. Reducing the stocking from 5 to 3.5 animals/ha did increase liveweight gain marginally (Table 2).

The cross infection of the control group also occurred in an earlier experiment (Quirk *et al.* 1988), despite attempts to physically keep the groups of cattle apart, and to allow one month between the use of the yards by the control and treated cattle. It is now known (R.J. Jones, unpublished data), that although the bacteria are highly anaerobic in the growth phase, the faeces collected in the field do contain bacteria which, when incubated anaerobically, are capable of degrading DHP. It seems highly likely therefore, that infection of the first of the control cattle occurred when passing through the yards for weighing. Once a single animal in a group had been infected then spread within the control herd would be rapid.

The large difference in dietary leucaena between the two groups in April (week 16) and May (week 20) is difficult to explain, especially the very high percentage of leucaena in the diet of control heifers. However, by this time it is assumed they had acquired the DHP-degrading bacteria; also leucaena availability in the control paddocks was higher than on the treated paddocks, and their stocking rate was reduced so giving higher availability of leucaena.

It is significant that even with diets of 90% leucaena no toxicity occurred once the heifers had acquired the specific rumen bacteria. Since the completion of this experiment no further cases of mimosine toxicity have occurred on the ORIA and there are now 600 hectares being grazed with a total of 5,000 animals (protected by the bacteria) finished on leucaena in the past five years. Steer liveweight gains of 1200 kg/ha/year have been obtained at a stocking rate of 6.5 animals/ha (Pratchett et al. 1989) and as high as 1500 kg/ha/year at 7.5 animals/ha (D. Pratchett, unpublished data).

We therefore have confidence that these bacteria, which are unique and as yet un-named, can fully protect cattle from mimosine toxicity when grazing leucaena on the ORIA and elsewhere.

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References

Blunt, C.G. (1976) Preliminary cattle grazing trials on irrigated *Leucaena leucocephala* and pangola grass in the Ord Valley of north-western Australia. *Proceedings of the Australian Society for Animal Production*, 11:10p.

Blunt, C.G. and Jones, R.J. (1977) Steer liveweight gains in relation to the proportion of time on *Leucaena leucocephala* pastures. *Tropical Grasslands*, **11**, 159-164.

Hegarty, M.P., Lee, C.P., Christie, G.S., Court, R.D. and Haydock, K.P. (1979) The goitrogen 3-hydroxy-4(1H)-pyridone, a ruminal metabolite from *Leucaena leucocephala*: effects on mice and rats. *Australian Journal of Biological Sciences*, **32**, 27-40.

Jones, R.J. (1985) Leucaena toxicity and the ruminal degradation of mimosine. *In*: Seawright A.A., Hegarty, M.P., James, L.F., Kelley, R.F. (eds) *Plant Toxicology* pp. 111-119. (Queensland Department of Primary Industries: Brisbane.)

Jones, R.J., Blunt, C.G. and Holmes, J.H.G. (1976) Enlarged thyroid glands in cattle grazing leucaena pastures. *Tropical Grasslands*, **10**, 113-116.

JONES, R.J. and HEGARTY, M.P. (1984) The effect of different proportions of *Leucaena leucocephala* in the diet of cattle on growth, feed intake, thyroid function, and urinary excretion of 3-hydroxy-4(1H)-pyridone. *Australian Journal of Agricultural Research*, **35**, 317-325.

JONES, R.J. and LOWRY, J.B. (1984) Australian goats detoxify the goitrogen 3-hydroxy-4(1H)-pyridone (DHP) after rumen infusion from an Indonesian goat. *Experientia*, **40**, 1435-1436.

JONES, R.J. and MEGARRITY, R.G. (1986) Succesful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of leucaena. *Australian Veterinary Journal*, **63**, 259-262.

JONES, R.J., LUDLOW, M.M., TROUGHTON, J.H. and BLUNT, C.G. (1979) Estimation of the proportion of C_3 and C_4 plant species in the diet of animals from the ratio of natural ^{12}C and ^{13}C isotopes in the faeces. *Journal of Agricultural Science*, Cambridge, **92**, 91-100.

LEFEUVRE, R.P. and JONES, R.J. (1988) The static combustion of biological samples sealed in glass tubes as a preparation for ^{13}C determination. *Analyst*, **113**, 817-823.

MEGARRITY, R.G. (1978) An automated colorimetric method for the determination of mimosine in leucaena leaves. *Journal of the Science of Food and Agriculture*, **29**, 182-186.

MEGARRITY, R.G. (1981) Rapid estimation of DHP in urine. *Leucaena Research Reports*, **2**, 16.

PRATCHETT, D. and TRIGLONE, T. (1989) Prospects for leucaena on the Ord. *Journal of Agriculture of the Western Australian Department of Agriculture*, **2**, 62-66.

QUIRK, M.F., BUSHELL, J.J., JONES, R.J., MEGARRITY, R.G., and BUTLER, K.L. (1988) Liveweight gains on leucaena and native grass pastures after dosing cattle with rumen bacteria capable of degrading DHP, a ruminal metabolite from Leucaena. *Journal of Agricultural Science*, Cambridge, **111**, 165-170.

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