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Effect of treatment of cocoa-pods with *Aspergillus niger* on liveweight gain and cocoa-pod intake of Bali (*Bos sondaicus*) cattle in South-East Sulawesi

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Abstract. Cocoa-pods, a by-product of the cocoa industry, could potentially be used as a feed resource for ruminants in eastern Indonesia. However, little is known regarding the optimal amount to be included in the diet or the effect of treatment with *Aspergillus niger* on cocoa-pod quality. In this experiment the effect of rate of inclusion (0 or 10 g DM/kg liveweight.day or *ad libitum*) of *A. niger*-treated or untreated cocoa-pods in the diet on intake and liveweight gain of Bali cattle (*Bos sondaicus*) was investigated. *Ad libitum* intake of cocoa-pods was greater when they were treated with *A. niger* (17.1 \pm 0.07 g DM/kg liveweight.day; mean \pm s.e.m.) compared with untreated cocoa-pods (13.9 \pm 0.19 g DM/kg liveweight.day) when offered as the sole component of the diet. The digestibility of *A. niger*-treated cocoa-pods (448.9 \pm 23.7 g/kg) was not different to untreated cocoa-pods (422.9 \pm 13.9 g/kg) when fed *ad libitum*, which was lower than native grass (527.2 \pm 10.7 g/kg). Animals offered *A. niger*-treated cocoa-pods lost less liveweight than animals offered untreated cocoa-pods when offered *ad libitum* (-0.104 \pm 0.02 and -0.280 \pm 0.02 kg/day, respectively), and grew faster when included in the diet at 10 g DM/kg liveweight.day (0.233 \pm 0.02 and 0.129 \pm 0.02 kg/day, respectively). In conclusion, in areas where cocoa plantations exist, cocoa-pods may be a useful feed resource for ruminants when fed at low levels of inclusion in the diet. The treatment of cocoa-pods with *A. niger* will result in increased liveweight gain. However, it is unlikely such treatments will be adopted by small-holder farmers due to the increased requirements for inputs, such as time, labour, funds, equipment, and technical skills.

Introduction

In South-East Sulawesi (Sulawesi Tenggara) small-holder beef cattle production, is restricted by both the quality and quantity of feed available. This occurs during both the dry season, when forage availability is low, and the wet season, when large areas of land are allocated to rice production and plantation crops, such as cocoa (*Theobroma cacao*). The area of land under cocoa plantations is increasing rapidly in South-East Sulawesi due to good financial returns to small-holders. Cocoa trees have a dense canopy, limiting the potential for the establishment of forages within plantations and the continued expansion of the cocoa industry is placing increasing pressure on the availability of land for forage production. The reduction in forage-producing land area has resulted in increased labour inputs of small-holder farmers to cut and carry sufficient feedstuffs to meet the requirements of their cattle.

Recently, cocoa yields in South-East Sulawesi have declined due to several pests and diseases, the most threatening of which is the cocoa-pod borer (*Conopomorpha cramerella*). One strategy to control the cocoa-pod borer is to remove trash that remains within the plantation, including previously harvested cocoa-pods, thereby limiting the opportunity for infestation of the subsequent cocoa crop. Cocoa-pods could potentially be used as a ruminant feed resource, thereby removing the reservoir for the cocoa-pod borer and addressing the issues of seasonal feed shortages and increased labour inputs.

The use of cocoa-pods as a feed resource for ruminants is often dismissed due to a perception that they are low in crude protein (CP) and high in fibre, although this is not supported in the literature where moderate values of 63-85 g CP/kg DM (Bateman and Fresnillo 1967; Ashun 1975; Wong et al. 1987) and 240-360 g crude fibre/kg DM (Bateman and Fresnillo 1967; Ashun 1975; Wong et al. 1987) are reported. Earlier workers (Bateman and Fresnillo 1967; Olubajo et al. 1976; Smith and Adegbola 1985) indicated that the DM digestibility of cocoa-pods was low and was within the range 45-52%. Cocoa products also contain theobromine (3,7-dihydro-3,7dimethyl-H-purine-2,6-dione), which can be toxic to animals at high intakes. The European Food Safety Authority (European Food Safety Authority 2008) reported decreased milk yield and altered milk composition in cows, hyperactivity and increased respiration rates in calves and decreased feed intake and liveweight gain in sheep, when chocolate, cocoa byproducts or theobromine were included in diets. It is therefore important to confirm that the theobromine content of cocoa-pods used in the present study is within the safe feeding range recommended by the European Food Safety Authority (2008).

Some Indonesian agencies have advocated the treatment of cocoa-pods with Aspergillus niger, as a means of improving cocoa-pod quality, however, there is little available data to support this. A. niger is an aerobic filamentous fungi widely used in industrial fermentation processes. A. niger has been used as a source of phytase to improve utilisation of phytin-bound phosphorus (P) in pig diets (Sands et al. 2009), with the aim of reducing P excretion and increased liveweight gain, gain : feed and bone strength, length and ash content (Veum and Ellersieck 2008) and as a source of ferulic acid esterase in multi-enzyme mixtures to improve the in vitro rumen fluid degradability of oat hulls (Yu et al. 2005). A. niger decreased total soluble phenolics and tannins of Shea nut meal and increased growth rates of broilers (Dei et al. 2008), increased DM, organic matter (OM) and CP and decreased crude fat and crude fibre of vegetable wastes used in aquaculture diets (Rajesh et al. 2010), while a comparable increase in CP content resulted when cassava byproducts were incubated with A. niger (Aderemi and Nworgu 2007). The addition of A. niger cellulase to a grass/concentrate (70:30% DM basis) substrate resulted in increased DM and neutral detergent fibre (NDF) disappearance after in vitro incubation for 6 and 24 h and increased total volatile fatty acid production (Giraldo et al. 2007). A preliminary in vitro study suggested that treatment of cocoa-pods with A. niger may increase the nitrogen (N) content and decrease the NDF content of cocoapods (Marsetyo et al. 2008); however, the effect of A. niger treatment of cocoa-pods on liveweight gain and intake of Bali cattle (Bos sondaicus) has not been reported.

This experiment investigated the response of Bali cattle to increasing amounts of cocoa-pods, with or without *A. niger* treatment, in the diet.

Materials and methods

All procedures were conducted in accordance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were reviewed and approved by the University of Queensland Animal Ethics Committee.

Location, animals and experimental design

The experiment was conducted at the University of Haluoleo cattle research facility, Kendari, South-East Sulawesi, Indonesia $(3^{\circ}58'17S, 122^{\circ}34'40E)$. Male Bali cattle (n = 25), ~12 months of age and 118 ± 3 kg (s.e.m.) liveweight were weighed and allocated to individual pens and to one of five treatments (n = 5 per treatment) in a randomised block design. The animals remained in the same individual pens throughout the experiment. The five treatments were native grass *ad libitum* (C), untreated cocoa-pods (10 g DM/kg liveweight.day) plus native grass *ad libitum* (UTNG), treated cocoa-pods (10 g DM/kg liveweight.day) plus native grass *ad libitum* (TNG), untreated cocoa-pods *ad libitum* (TAL). The animals were adjusted to their treatments over a

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1-week preliminary period, which was followed by an 8-week experimental period.

Feeds and feeding

Cocoa-pods (~20 t) were collected from cocoa plantations in South-East Sulawesi, within 2 days of harvest. These cocoa-pods were obtained from plantations in the same region and were sufficient for the conduct of the entire experiment. The cocoapods were chopped into 1 by 5-cm-dimension pieces, mixed thoroughly and divided into two equal lots. The first lot of cocoapods were dried in the sun for 3 days before grinding through a 2-mm screen and storing in sealed plastic bags. The remaining cocoa-pods were treated with an A. niger solution (Balai Pengkajian Teknologi Pertanian, Bali). The treatment solution was prepared by adding 10 g urea, 10 g sugar, 2.5 g SP36 (36% total P2O5 fertiliser), 2.5 g KCl and 5 mL A. niger (starter culture containing 1×10^6 spores/mL) to 1 L of water, which was diluted in an additional 10 L of water. The diluted solution was then applied to cocoa-pods at a rate of 1 L/kg fresh cocoa-pods. This was the equivalent of 4.55×10^5 spores of A. niger and 1 g of urea/kg fresh cocoa-pod. Treatment occurred for 6 days under aerobic conditions, followed by sun drying for 3 days before grinding through a 2-mm screen and storing in sealed plastic bags. Ground, dried cocoa-pods were stored in sealed plastic bags for 6 months after harvest and treatment before the commencement of the feeding study. Native grass is the local term used to describe feeds that are representative of locally available cut-and-carry forages typically used by small-holder farmers in the region. Native grass used in the present experiment was cut fresh each day from areas around the University of Haluoleo campus and consisted of a 9:1 mixture of various grasses (Axonopus compressus, Setaria sphacelata, Cvnodon dactylon, Brachiaria decumbens and Paspalum dilatatum) and legumes (Macroptilium artopurpureum and Calopogonium muconoides).

Animals offered native grass (C) or cocoa-pods (UTAL and TAL) *ad libitum* were offered the previous day's feed intake plus 20%, divided across three separate feedings at 0800, 1200 and 1600 hours each day. Animals offered a fixed allowance of cocoa-pods (UTNG and TNG) were offered their allocation of cocoa-pods, based on their most recent liveweight measurement, at 0800 hours and were then provided with native grass *ad libitum* at 1400 hours. Cocoa-pods were offered to animals as a 40% DM slurry, prepared with water.

Measurements

Liveweight was measured once each week and feed intake was measured daily. Feed intake was determined by measuring total feed offered and refused over a 24-h period. Digestibility was measured by total faecal collection over 7 consecutive days on three separate occasions, during Weeks 2, 4 and 6 of the experimental period.

Sample collection, analyses and calculations

Subsamples of feed offered were collected daily, dried to a constant weight at 60°C in a fan-forced oven, bulked over 1 week, mixed thoroughly and stored for analysis [i.e. there was one bulk sample for each feed for each week (n = 8) of

the experiment]. Feed residues were weighed daily and bulked over 1 week, mixed thoroughly and subsamples were dried to a constant weight at 60°C in a fan-forced oven. Faeces were collected and weighed daily during the digestibility period, mixed thoroughly and 5% subsamples were stored at -20° C. After 7 days daily faecal subsamples were combined, mixed thoroughly and dried to a constant weight at 60°C in a fanforced oven. All feed, residues and faeces were ground through a 1-mm screen before further analysis. The OM content of subsamples of feed offered, feed residues and faeces was determined by combustion in a muffle furnace at 550°C for ~4.5 h. Subsamples of feed offered were analysed for N content by the Kjeldahl method (AOAC 2000) and ash-free NDF by digestion in a neutral detergent solution (Goering and Van Soest 1970), followed by washes in boiling water and acetone before combustion at 550°C for ~4.5 h. All analyses were done in duplicate.

Metabolisable energy (ME) intake was estimated from Freer *et al.* (2007) where ME (MJ/kg DM) = $0.0157 \times \text{digestible organic}$ matter in dry matter (DOMD; g/kg), and DOMD (g/kg) = [(feed OM (kg/day) – faeces OM (kg/day))/feed DM (kg/day)] × 1000.

Theobromine content was determined in duplicate on lipid-extracted cocoa-pods by high performance liquid chromatography (HPLC) based on the method of del Rosario Brunetto *et al.* (2007). Sample clean-up was achieved by passing 5 mL of centrifuged extract through a 45- μ m filter followed by a Sep-Pak C18 cartridge (Waters, Milford, MA, USA). Theobromine was washed off the cartridge using 20% methanol. Chromatographic analysis was performed on a Shimadzu 10A series HPLC (Shimadzu, Tokyo, Japan) using a Luna 5- μ C18 (2) 100 Å 250 × 46-mm column (Phenomenex, Torrance, CA, USA), with 20% methanol at 1 mL/min as the mobile phase and detection was conducted at 274 nm.

Statistical analyses

All data were analysed using the generalised linear model procedure in Statistical Analysis Software (SAS 1999).

Liveweight gain over the experiment included initial liveweight as a covariate. Models included treatment, week of experiment and their interaction; the interaction term and week of experiment were sequentially removed from the model if not significant (P > 0.05). Differences in least square means between treatments were accepted as significantly different at P < 0.05. In addition, orthogonal contrasts were used to compare (i) the diets of cocoa-pods *ad libitum* with diets of cocoa-pods at 10 g DM/kg liveweight.day and (ii) cocoa-pods treated with *A. niger* with cocoa-pods not treated with *A. niger*.

Results

Treatment of cocoa-pods with *A. niger* resulted in lower DM, OM and ash-free NDF content and a higher N and theobromine content compared with untreated cocoa-pods (Table 1). Untreated cocoa-pods were similar in N content to native grass but contained less OM and ash-free NDF.

Bali cattle offered treated and untreated cocoa-pods only *ad libitum* lost liveweight, while cattle offered cocoa-pods with native grass or native grass alone gained liveweight, over

Table 1. Chemical composition of native grass (NG) and untreated (UTCP) and treated (TCP) cocoa-pods offered to 12-month-old Bali cattle at South-East Sulawesi

NM, not measured. Values are least square means and standard error of the mean. Each value represents the mean of duplicate assays conducted on bulked weekly feed offered samples over an 8-week experimental period (n=8 per feedstuff). Within a row, means without a common lowercase letter are different (P < 0.05)

Parameter		s.e.m.		
	NG	UTCP	TCP	
Dry matter (g/kg)	204a	829c	782b	2
Organic matter (g/kg DM)	918c	894b	878a	3
Nitrogen (g/kg DM)	12.1a	12.5a	21.7b	0.4
Neutral detergent fibre (g/kg DM)	658c	598b	539a	5
Theobromine (mg/kg DM)	NM	56.3a	67.5b	2.6

Table 2. Average daily liveweight gain, feed intake and digestibility of 12-month-old Bali cattle fed native grass *ad libitum* and increasing levels of untreated and *Aspergillus niger*-treated cocoa-pods

Treatments were native grass *ad libitum* (C), untreated cocoa-pods with native grass (UTNG), treated cocoa-pods with native grass (TNG), untreated cocoa-pods *ad libitum* (TAL) and treated cocoa-pods *ad libitum* (TAL). DOMI, digestible organic matter intake; OM, organic matter; MEI, metabolisable energy intake; NT, not offered in treatment diet. Values are least square means and s.e.m. is standard error of the means over an 8-week experimental period (liveweight gain and intake) or over three digestibility periods (digestibility, DOMI and estimated MEI). Within a row, means without a common lowercase letter are different (*P* < 0.05)

Parameter	С	UTNG	TNG	UTAL	TAL	s.e.m.
Liveweight gain (kg/day)	0.164cd	0.129c	0.233d	-0.280a	-0.116b	0.02
Native grass intake (g DM/kg liveweight.day)	22.5b	20.1a	20.9a	NT	NT	0.5
Cocoa-pod intake (g DM/kg liveweight.day)	NT	5.9a	7.0a	13.9b	17.1c	0.3
Total intake (g DM/kg liveweight.day)	22.5c	26.0d	27.9e	13.9a	17.1b	0.5
Total N intake (g/kg liveweight.day)	0.27b	0.32c	0.41e	0.17a	0.37d	0.01
Total NDF intake (g/kg liveweight.day)	14.8c	16.8d	17.5d	8.3a	9.2b	0.3
DM digestibility (g/kg)	527b	523b	536b	423a	449a	15
OM digestibility (g/kg)	539b	532b	556b	439a	474a	13
DOMI (g/kg liveweight.day)	10.9b	12.7bc	14.0c	5.6a	7.5a	0.5
Estimated MEI ^A (MJ/kg liveweight ^{0.75} .day)	0.57b	0.66bc	0.73c	0.28a	0.38a	0.03

^ARefer to Materials and methods for calculation.

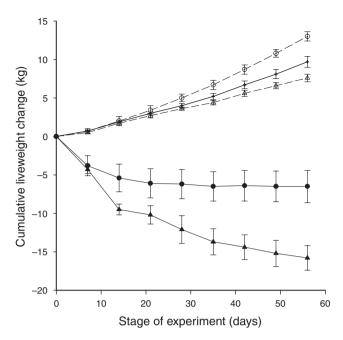


Fig. 1. Cumulative liveweight change of male Bali cattle (n = 5 per treatment) offered native grass *ad libitum* (- + -), native grass *ad libitum* plus 10 g untreated cocoa-pods DM/kg liveweight.day ($-\triangle$ -), native grass *ad libitum* plus 10 g treated cocoa-pods DM/kg liveweight.day ($-\bigcirc$ -), untreated cocoa-pods *ad libitum* ($-\triangle$ -) and treated cocoa-pods *ad libitum* ($-\triangle$ -). Error bars indicate standard error of the mean.

the 8-week experimental period (Table 2). The daily liveweight loss was less for cattle offered the TAL compared with UTAL treatments. Similarly, liveweight gain was greater in cattle offered the TAL compared with UTAL treatments (Fig. 1). Orthogonal contrast analysis confirmed that liveweight gain was reduced for animals fed cocoa-pods ad libitum compared with 10 g DM/kg liveweight.day (P < 0.001) and for untreated compared with treated cocoa-pods (P < 0.001). Ad libitum intake of cocoa-pods was greater when cocoa-pods were treated with A. niger compared with untreated cocoa-pods, however, treatment had no effect on cocoa-pod intake when offered with native grass. Intake of N was greater for animals fed treated cocoa-pods, compared with animals fed the control or untreated cocoa-pod diets, at both ad libitum and restricted cocoa-pod intakes. NDF intake was lower in cattle fed cocoa-pods ad libitum than those fed the control diet or restricted intakes of cocoa-pods. There was no difference in NDF intake of treated or untreated cocoa-pods, at ad libitum intake. DM digestibility, OM digestibility, digestible OM intake and estimated ME intake did not differ between the three digestibility periods and were all lower when cocoa-pods were offered ad libitum as the sole component of the diet. Orthogonal contrast analysis confirmed that DM and OM digestibility, digestible OM intake and estimated ME intake were all reduced for diets consisting solely of cocoa-pods compared with diets containing cocoa-pods with native grass (P < 0.001). The orthogonal contrast analysis showed DM digestibility of A. niger-treated cocoa-pods was not different to untreated cocoa-pods (P = 0.18); however, OM digestibility, digestible OM intake and estimated ME intake were all greater when the diets contained *A. niger*-treated cocoa-pods compared with untreated cocoa-pods (P < 0.05).

Discussion

The utilisation of cocoa-pods as a feed source for cattle in Indonesia addresses the issues of inadequate seasonal feed supply for cattle and decreasing cocoa yield and quality. Bali cattle, ~12 months of age, offered cocoa-pods treated with *A. niger* had higher average daily liveweight gain than those animals offered untreated cocoa-pods at a similar level of feeding. Cattle offered cocoa-pods *ad libitum* either treated with *A. niger* or untreated, as the sole component of the diet, grew slower than cattle offered cocoa-pods at a fixed level (10 g DM/kg liveweight. day) with native grass *ad libitum*.

Cocoa-pods used in the present study had a similar N content to locally available grasses, before treatment with the A. niger solution. Treatment with the A. niger solution resulted in an increase in N content and a decrease in ash-free NDF content of cocoa-pods. The secretion of cellulases from A. niger may be responsible for the breakdown of polysaccharides in the cocoapods and the apparent decrease in DM and NDF, which is supported by the increased disappearance of DM and NDF in response to A. niger treatment reported to occur in vitro (Giraldo et al. 2007). Similarly, Trichoderma longibrachiatum cellulose treatment of substrates, under the same conditions as A. niger, resulted in increases in DM and NDF disappearance and volatile fatty acid production but in addition increased daily ammonia-N and microbial-N flow were also reported (Giraldo et al. 2007). While total DM production, as a result of A. niger treatment, was not measured in the present study it is unlikely that the decrease in NDF content was due to a proportional increase in A. niger relative to cocoa-pod on a DM basis alone. In contrast, the increased N content of the cocoa-pods is presumably a result of the growth of the mycelia of A. niger and the inclusion of urea in the A. niger media. In the present experiment, the inclusion of urea in the A. niger treatment solution contributed only slightly to the increased N content of cocoa-pods (0.47 g N/kg fresh cocoa-pod) suggesting that the growth of A. niger itself accounted for the majority of the increase in N content. Other studies have reported a similar increase in N content of cocoa-pods treated with A. niger when no urea was included in the media (Marsetyo et al. 2008) or when vegetable wastes (Rajesh et al. 2010) and cassava byproducts (Aderemi and Nworgu 2007) were treated with A. niger. The source of this additional N used for growth of A. niger remains unknown at this stage.

Young Bali cattle offered cocoa-pods *ad libitum* lost liveweight over the experimental period, while cattle offered 10 g DM/kg liveweight.day cocoa-pods with native grass grew at a similar rate to those fed native grass *ad libitum*. Bali cattle have a low growth potential and growth rates in the order of 0.1-0.2 kg/day are typical of this class of cattle offered native grass (Quigley and Poppi 2009). A negative response in liveweight gain and feed conversion efficiency to increasing levels of cocoa-pods in the diet of cattle was also reported by Smith and Adegbola (1982), although this was only significant when cocoa-pods comprised ~60% of the diet. Bali cattle fed cocoa-pods treated with *A. niger* had higher average daily liveweight gain over the experimental period than those cattle offered a similar amount of untreated cocoa-pods. Goats offered a diet containing Shea butter cake treated with A. niger also had greater liveweight gain than those offered untreated Shea butter cake, at two fixed intake allowances (Belewu and Yahaya 2008). Direct application of a commercial enzyme (a mixture of A. niger and T. longibrachiatum) into the rumen of sheep fed a grass/concentrate diet resulted in increased DM and NDF disappearance of the feed from the rumen, a decrease in acetate : propionate with no differences observed for DM, OM and NDF digestibility or urinary purine excretion, however, change in liveweight in response to treatment was not reported (Giraldo et al. 2008). Responses in liveweight gain, digestibility and feed conversion ratio to Trichoderma species-derived cellulases have been reported in beef cattle (Beauchemin et al. 1995, 1997) but the responses appear to be dependent on the basal feed offered. Therefore, the response of ruminants to treatment of feeds with fibrolytic enzymes is variable and appears to be dependent on the enzyme used, the basal feed offered and the physiological state of the animal.

Total daily DM intake was lower in animals offered cocoapods ad libitum, compared with those animals offered cocoa-pods as a proportion of the diet. The response was similar for treated and untreated cocoa-pods, suggesting the decreased feed intake was due to palatability or anti-nutritional features of the cocoapods which could not be overcome by treatment with A. niger. There were cocoa-pod residues when 10 g DM/kg liveweight.day cocoa-pods were offered to animals, with intakes ranging from 5.9 to 7 g DM/kg liveweight.day, suggesting palatability of cocoa-pods may be an issue. Cocoa-pod intake did not vary significantly between weeks throughout the experiment, suggesting that the decreased intake was not simply due to the time required for adaptation to the diet. The form of the diet (40%)DM slurry of 2-mm ground particles) may also have contributed to the reduced intake of animals offered cocoa-pods ad libitum, as they were not offered any additional roughage. The higher intake of treated compared with untreated cocoa-pods by cattle, at both levels of inclusion in the diet, may also be the result of the increased N content in treated cocoa-pods. Responses in ad libitum intake to higher N diets (Panjaitan et al. 2010), supplements of non-protein N into the rumen (Hennessy and Williamson 1990) and post-ruminal protein infusions (Egan 1965) are related to increased rumen microbial activity, increased digestibility and decreased retention time of material within the rumen and metabolic status, all of which will stimulate intake of low N forages.

Theobromine has been implicated with liveweight loss and decreased feed intake in sheep fed cocoa-bean shell (10 g theobromine/kg cocoa-bean shell on an as-fed basis) at greater than 10% of the diet and theobromine at greater than 1 g/kg DM of a concentrate ration, equivalent to \sim 30–40 mg theobromine/kg liveweight.day (Tarka *et al.* 1978), over a 14-week period. Theobromine levels in cocoa-pods are much lower than those in other components of the cocoa fruit (Wong *et al.* 1987). In the present experiment, Bali cattle offered cocoa-pods *ad libitum* consumed \sim 1 mg theobromine/kg liveweight.day (based on approximate values of 60 mg theobromine/kg liveweight.day), which was markedly lower than levels expected to have a negative impact on animals (Tarka *et al.* 1978; European Food Safety

Authority 2008). This suggests the theobromine content was not likely to have contributed to the low feed intake and low liveweight gain observed for animals fed cocoa-pods *ad libitum*, in the present study.

In conclusion, growing Bali cattle in eastern Indonesia can successfully be offered a diet containing cocoa-pods at 10 g DM/ kg liveweight.day but not *ad libitum*. Treatment of cocoa-pods with *A. niger* will result in a modest increase in average daily liveweight gain and feed intake above untreated cocoa-pods for this class of cattle. However, within the context of the smallholder crop-livestock system in eastern Indonesia it is unlikely that such a strategy would be adopted and sustained due to the additional labour requirements, costs involved and technology and training required. The use of *A. niger* to improve the feed value of low quality feed resources available in Australia warrants investigation.

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