Urea Treated Cocoa Pod as Barley Grain Substitution in Ruminant Ration on Microbial Metabolism and Feed Degradation (Rusitec Study)

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Abstract

Limited sustainable supply of high quality feedstuff is the major constrain in ruminant production in Indonesia. Aim to convert underutilized cocoa pod to quality feedstuff and its inclusion in ruminant ration have been done. Cocoa pod was treated with 20 g urea per kg fresh material. The effects of replacing barley grain by urea treated cocoa pod on methane release, SCFA production as well as the amount and efficiency of microbial-N fixation in Rusitec have been studied. The experiment included six different rations (T1 = 10 g/d hay; T2 = T1 + 4 g/d barley-soybean mixture (barley); T3 = T1 + 3 g barley + 1 g cocoa pod); T4 = T1 + 2 g barley + 2 g cocoa pod; T5 = T1 + 1 g barley + 3 g cocoa pod; T6 = T1 + 4 g cocoa pod) and three runs of Rusitec in a block random design. Substitution of barley by urea treated cocoa pod up to 100% in hay based rations did not decrease the rate of ration DM disappearance, and fiber degradation even tended to be increased. Microbial N-fixation decreased with the inclusion of cocoa pod in the rations, but the efficiency of microbial N-fixation was increased in line with the cocoa pod level. Methane release per unit NDF disappearance decreased inversely to the cocoa pod level. Although urea treated cocoa pod cannot replace barley/soybean meal-mixture equivalently in feed rations for ruminants, in fact, the inclusion of urea treated cocoa pod at the expense of higher fermentable feed components will reduce the fermentation intensity thereby yielding lower amounts of SCFA and microbial protein for the host animal. Nevertheless, the urea treated cocoa pod can be used as a feedstuff for low performance ruminants as a substitute for barley/grain meal-mixture or as a supplement to hay based rations without reducing the efficiency of microbial synthesis. Moreover, no toxicity effects were observed with the inclusion of urea treated cocoa pod in the ration as to a dramatically disturbed fermentation or a too high concentration of ammonia.

Keywords: Rusitec, urea treated cocoa pod, microbial protein, Methane, ruminant

Introduction

Background

Cocoa pod is a potential feed source for ruminants. Their availability increase inline with the escalation of world-wide cocoa demand. ICCO (2003) forecasted of 3 million tons world cocoa seed production which released about 6 million tons of cocoa pod meal (a 1 to 2 cocoa bean to cocoa pod meal ratio (DUKE, 1983)). Cocoa pods is quite palatable, however its utilisation by ruminant is limited according to low protein content and high cell wall constituents. As a late-maturing plant component, the pod contains high lignocellulosic and low non-polysaccharide substances. To be used as ruminant feedstuff, cocoa pod needs quality upgrading.

Based on in vitro study using gas test (Menke et al., 1997), Despal (2005) reported that cocoa pod treated with 14 kg urea per 100 kg DM produced higher gas in compare to 0, 7 and 21 kg urea/100 kg DM treatments. Relative to untreated cocoa pod, the urea treatment increased gas production (Gb) by 38%. Since the amount of urea used in this study were higher than that applied by CHENOST (2001) for rice straw equivalent to 5.3 kg urea per 100 kg DM or WILLIAMS et al. (1984) who applied 4 kg urea per 100 kg barley straw, it seems necessary to test the possible toxicity of urea treated cocoa pod in a more physiological rumen model such as Rusitec. A too high consumption of dietary urea would however be toxic for the animal (HELMER & BARTLEY, 1971; BARTLEY & DEYOE, 1981; VAN SOEST, 1982).

According to SMITH (1974), urea toxicity can be minimized by ensuring an adequate matched supply of available energy instead of replacing urea with more expensive NPN sources. In this study, the urea treated cocoa pods were mixed with different amounts of barley as an energy source and constant amounts of these mixtures were combined with constant amounts of hay in Rusitec.

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Objectives

The research aimed at investigating the effects of replacing barley/soybean meal-mixture by urea treated cocoa pod in ruminant feed rations on methane release, SCFA production and N-metabolism as well as the efficiency of protein synthesis of rumen microbes. Another objective of this research was to study the effect of the substitution level on rate of disappearance of DM and nutrients from the feed used as substrate in Rusitec.

Material and methods

Urea treated cocoa pod preparation

The cocoa pods (CPs) of AFR (Forastero) cultivar were collected after harvesting the beans. The cocoa trees at the Cikasungka Cocoa Plantation in Bogor-Indonesia were about 20 years old and ranged from 1.5 to 3 m height at the time of the harvest. The cocoa pods were treated with urea (20 g/kg fresh materials or equal to 140 g/kg DM (w/w)).

The treatment was processed manually. Firstly, cocoa pods were sliced into about 2 mm thickness and samples of 2 kg each were weighed. The pods were put into 40 x 50 cm and 0.12 cm thin poly-bags. Urea was added layer by layer in order to achieve homogeneous mixtures. The bag was then pressed to reduce the air contents and sealed with nylon tape. Finally, the bag was stored at room temperature. After 14 days the bag was opened and the contents sun dried (about 18 hours light intensity). The dried material was then ground with a laboratory bur mill to pass a 0.5 mm sieve for further analyses.

Experimental ration

The experiment was conducted in rumen simulation technique fermenters. The fermenters were supplied with 10 g of hay without or with 4 g of concentrate daily. Barley grain enriched with extracted soybean meal (to achieve the same crude protein (CP) content as urea treated cocoa pod) was used as standard concentrate. The standard concentrate was gradually replaced with increasing levels of treated cocoa pod.

The resultant six treatments were (T1 = 10 g/d hay; T2 = T1 + 4 g/d barley-soybean mixture (barley); T3 = T1 + 3 g barley + 1 g cocoa pod); T4 = T1 + 2 g barley + 2 g cocoa pod; T5 = T1 + 1 g barley + 3 g cocoa pod and T6 = T1 + 4 g cocoa pod. The nutritional composition of the feed components is shown in Table 1. The daily amounts of the different feed components supplied to six fermenters in Rusitec and their chemical composition are shown in Table 2.

Table 1: Nutritional composition of the feed components

Component	DM	ASH	CP	XL	CF	NDF	ADF
	%			% DM			
Hay	93.4	7.92	15.8	1.32	30.2	57.3	33.9
Barley	87.6	2.48	12.7	2.48	7.26	30.8	8.46
Soy bean meal extract.	89.2	7.12	52.2	1.82	4.66	10.6	5.86
Treated cocoa pod	90.5	8.58	15.9	0.74	51.9	76.9	59.4

Table 2: Composition of the feed rations

Ingredients	T1	T2	T3	T4	T5	T6
Hay (g)	10	10	10	10	10	10
Barley (g)	0	2.4	1.8	1.2	0.6	0
Soy bean meal extract.(g)	0	1.5	1.2	0.8	0.4	0
Treated cocoa pod (g)	0	0	1.0	2.0	3.0	4.0
Mineral mix (g)	0.15	0.15	0.15	0.15	0.15	0.15
Nutrient contents						
DM (%)	93.5	91.9	92.1	92.3	92.5	92.7
ASH (%DM)	9.73	7.64	8.02	8.40	8.78	9.15
CP (% DM)	15.6	15.9	15.8	15.8	15.7	15.7
XL (% DM)	1.30	1.60	1.49	1.37	1.26	1.14
CF (% DM)	29.7	23.6	26.7	29.8	32.8	35.9
NDF (% DM)	56.4	48.9	52.2	55.5	58.8	62.1
ADF (%DM)	33.3	26.5	30.0	33.6	37.0	40.5

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Rusitec procedure

The study was conducted in Rusitec fermenters following the procedure which has been developed by CZERKAWSKI & BRECKENRIDGE (1977). The trial was repeated three times (3 replications) with 6 fermenters for each run. Each run lasted 14 days including day 0 to day 9 as preliminary phase and day 10 to 14 as the collecting phase. The Rusitec procedure is explained below:

Water bath and fermenter. Water bath was set at 39°C using a thermostat heater. Water circulated through a circulating pump. The water bath contained 6 fermenters. Each fermenter had a volume of 1060 ml and was filled with 690 ml rumen fluid which was collected from two rumen fistulated steers. A nylon bag containing 80 g of rumen solid fraction was introduced into a covered bottle at day 0 together with two bags containing hay and concentrate respectively. The cover and the bottom of the bottle had several holes to allow free fluid movement when introduced into the cylindrical fermenter. The bottle cover was connected to a wheel allowing an automatic stroke frequency of the bottle of about 400 times per hour. The fermenter was filled with buffer and closed. Nitrogen gas was blown for about 2 minutes to pull oxygen out and to maintain anaerobic conditions. The overflow of the fermenter was connected to a 1000 ml Erlenmeyer containing 1 ml $HgCl_2$. The gas produced was collected in a gas bag.

Buffer (McDOUGAL, 1948) was pumped continuously using an automatic pump at a flow rate of 620 ml/d. The fermenter was maintained under anaerobic conditions by infusing N_2 gas each time it was opened. The arrangement of fermenters, pump and thermostat heater in the Rusitec system is shown in Figure 1.
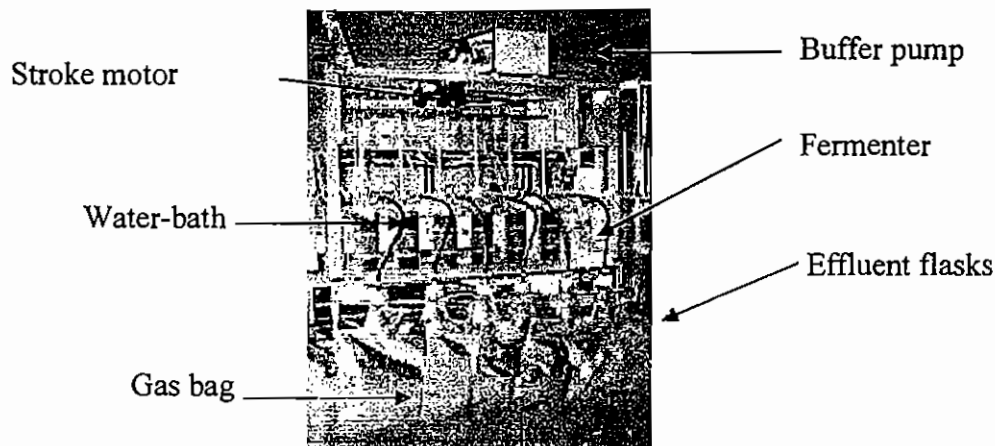

Figure 1: Rumen simulation technique instruments

Ration. The daily ration was put into two nylon bags. A 60 μm pore size bag was used for concentrate and a 100 μm pore size bag for hay. At day 0, the bag containing rumen solid fraction was introduced and removed after 24h. For the rest of experimental period, the fermenters were fed with feed ration only. Each bag containing feed ration was incubated in the fermenter for 48 h. The residual ration in the nylon bag served to calculate the rate of disappearance and was therefore washed with 60 ml buffer which was returned to the fermenter.

^{15}N infusion. ^{15}N-Urea with 95% enrichment was used as a tracer for the determination of microbial growth. The amount of 0.2259 g $(^{15}NH_2)_2CO$ was weighed and solved in 100 ml distilled water. Five ml of the solution was added to 5 liters of fresh buffer. The quasi-steady state condition in the fermenters with a nearly constant ^{15}N-concentration of the ammonia pool was achieved after 5 days.

Parameters. Overflow, gas production, pH, redox-potential and NH_3 concentration were measured daily. Protozoal counts and gas composition were analyzed every two days during the preliminary phase and daily during the collection phase. The SCFA and ^{15}N-NH_3 were analyzed in the overflow samples of the collection phase, while the disappearance rate of the feed ration was measured during the collection phase by analyzing the residual contained in the nylon bag after fermentation.

Rate of disappearance

The DM-disappearance rate of the feed ration was determined by subtracting the residual ration in nylon bags after 48 h of incubation from the amount of ration introduced into the fermenter. The disappearance of OM, CP, CF, NDF and ADF was determined by analyzing the residual solids for crude ash (XA), crude protein (CP) and crude fiber (CF) according to NAUMANN & BASSLER (1997) and for NDF and ADF according to VAN SOEST et al. (1991).

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Amount and composition of fermentation gas

The composition of produced fermentation gas was analyzed by gas chromatography (SimadzuGC-8A) in collected gas samples on a packed silica gel column, at 80°C injection port and detector temperature and 40°C column temperature (isothermal), TC-detector (Simadzu C-R1B) and argon as carrier gas (DA COSTA GOMEZ, 1999). Gas samples were taken from the gas bag using a 1 ml syringe.

A standard gas consisting of CO_2, N_2, CH_4 and H_2 in the proportions of 65, 30, 4 and 0.9% respectively was used. The peak of graph was achieved at the 2nd minute for H_2, after 2.85 minutes for N_2, 3.89 minutes for CH_4 and 20.95 minutes for CO_2.

Short chain fatty acid (SCFA)

A 10 ml sample of the effluent was centrifuged in a Martin Christ centrifuge (Type UJ IS) for 10 minutes at 3900 g. The supernatant was separated from the sediment. An internal standard solution consisting of 4% 2-methyl valeric acid in formic acid (w/w) was prepared. 250 μl of the internal standard solution was mixed with 5 ml supernatant using a vortex. About 1.5 ml of the mixture was transferred to a covered centrifuge cup and centrifuged at 15000 g (Biofuge A, Heraues Sepatech) for 10 minutes. The amount of 1.4 μl of the centrifuged sample was injected to GC using a 10 μl syringe.

SCFA was detected using a gas chromatograph (GC-14B, Simadzu) equipped with a packed column (10% Carbowax 20 MTPA SP 1000 with 1% H_3PO_4 on Cromosorb WAW 80/100) and flame ionization detector connected to a chromato-integrator (D-2000 Merck-Hitachi). H_2 at 120 kPa pressure served as carrier gas. The injection oven temperature was set at 170°C while the column and detector temperatures were set at 120°C and 220°C respectively.

The peak curve was measured after a retention time (min) of approximately 3.33 for C_2 (acetate), 4.90 for C_3 (propionate), 5.66 for iC_4 (iso-butyrate), 7.51 for nC4 (butyrate), 9.23 for iC5 (iso-valerate), 12.73 for nC5 (valerate) and 14.64 for the internal standard.

Additionally to the internal standard, an external standard was also used which consisted of (μmol/ml) 30 acetic acid, 20 propionic acid, 1 iso-butyric acid, 10 butyric acid, 2 iso-valeric acid and 3 valeric acid. To determine the concentration of SCFA, a combined internal/external standard procedure was applied.

Microbial protein synthesis (MPS)

Microbial protein synthesis (MPS) was determined by the continuous infusion technique applying ^{15}N-urea as a tracer (ABEL et al., 1990). Three drops of silicon oil (anti foam), 8 drops of Thymolphthalein (2% in ethanol solution) or methyl red, 10 ml boric acid buffer (in 1 N KCl solution adjusted with 1 N KOH to pH 9.5) and 4.5 ml 1N KOH were added to 100 ml effluent (pooled 10% collection phase overflow samples). The solution was distilled and the distillate collected in a beaker glass containing 0.1 N H_2SO_4. Basically 0.1 N H_2SO_4 would be enough to bind the NH_3 released. The amount of 0.1 N H_2SO_4 needed to bind NH_3 was calculated as the concentration of NH_4-N/100 ml sample divided by 1.401 plus 1 ml. The beaker volume was filled to 40 ml with distilled water. The distillation process was terminated when 200 – 250 ml of distillate were collected which occurred in approximately 12 minutes.

Part of the distillate volume (equal to approximately 0.5 mg nitrogen) was evaporated. The amount of distilled volume to be evaporated was calculated as the volume of distillate in the beaker divided by NH_4-N/100 ml rumen fluid and divided by 2. This amount was transferred into a 50 ml beaker glass and evaporated at a temperature of 60°C to give a volume of 3 – 4 ml. The temperature was then increased to 100°C to further reduce the volume to 750 μl. The remaining solution was transformed into a tin cup and further dried at 105°C. The cup was then formed to a small pellet and placed into a pallet. The pellets were analyzed for ^{15}N enrichment by means of a mass spectrometer (Finnigan MAT Delta C, Bremen, connected to an elemental analyzer, Fisons 1108 Rodano, Milano).

Assuming steady state conditions, microbial nitrogen was derived according to equation: Microbial N (mg/d) = r_i x ((s_i/s_p) – 1), where r = infusion rate (mg/d), s_i = ^{15}N-excess in infusion, s_p = ^{15}N-excess in NH_4-N pool. Microbial protein synthesis (MPS) in mg/d can be calculated as microbial-N divided by (8/100), assuming 8% N in microbial cells (CZERKAWSKI, 1986). The efficiency of microbial protein synthesis (EMPS) is calculated according to EMPS (mg/g OMAD) = MPS/OMAD, where OMAD is organic matter apparently degraded.

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Experimental design and statistical analysis

The trial had a randomized block design with 6 rations in 6 fermenters as treatments and 3 runs as block. The linear model used for the analysis was: $Y_{ij} = \mu + \mu_i \beta_j \mu_{ij}$, where, Y_{ij} is the observation value of ration i and run j, μ is the overall mean of observations, μ_i is the additive effect of ration i, β_j is the effect of run j and μ_{ij} is experimental error of ration i and run j.

Results

Fermentation characteristics

The effect of replacing the barley/soybean meal-mixture with urea treated cocoa pod on fermentation in Rusitec is shown in Table 3. There was a significant decrease in pH due to the addition of concentrate (T2 – T5). However, the substitution of barley/soybean meal-mixture by treated cocoa pod resulted in a gradual increase in pH at a rate that was nearly proportional to the increased cocoa pod proportion in the ration (T3 – T6). A 100% replacement of barley/soybean meal-mixture by urea treated cocoa pod resulted in a pH similar to that in T1 with hay as the sole substrate.

Table 3. Effect of replacing barley/soybean meal-mixture by urea treated cocoa pod on fermentation characteristics in Rusitec

Parameter	Treatment					
	T1	T2	T3	T4	T5	T6
PH	6.63[d]	6.51[a]	6.52[ab]	6.54[b]	6.59[c]	6.63 [d]
Protozoa (cts/ml)	17475	17868	16310	14267	13672	12436
CH_4 (mmol/d)	5.45[a]	7.51[de]	7.71[e]	6.95[cd]	6.38[bc]	6.02[ab]
NH_4^+ (mmol/l)	5.64[a]	8.31[d]	7.42[c]	7.28[c]	6.68[b]	6.48[b]
SCFA (mmol/d)	24.88[a]	35.61[b]	33.59[b]	31.94[ab]	30.94[ab]	28.78[ab]
acetate	14.46[a]	18.84[b]	18.47[b]	17.85[cb]	17.80[ab]	17.26[ab]
propionate	6.07[a]	9.20[b]	8.57[b]	7.76[ab]	7.52[ab]	6.59[a]
iso-butyrate	0.17	0.25	0.18	0.16	0.15	0.20
n-butyrate	3.02	5.14	4.58	4.45	3.94	3.41
iso-valerate	0.60[a]	1.12[d]	0.98[cd]	0.88[bc]	0.79[abc]	0.70[ab]
n-valerate	0.56	1.06	0.81	0.84	0.75	0.63
Microbial cells (mg DM/d)	700[a]	859[c]	821[b]	782[b]	793[b]	776[b]

Addition of concentrate (barley/soybean meal-mixture) to hay based ration (T2) tended to increase protozoal counts. Replacing the concentrate with urea treated cocoa pods, however, reduced the protozoal counts inversely to the cocoa pod level below the value of T1. Methane production was significantly increased by the addition of concentrate (T2 – T5). The increment could be reduced to the T1 level by replacing 100% concentrate with urea treated cocoa pods (T6).

Although ammonium production was decreased by the replacement of barley/soybean meal-mixture with urea treated cocoa pods (T3 – T6), the level was still higher than T1. Total and partial SCFA production showed the same trend (significantly or just by tendency). Addition of concentrate (T2 – T6) increased the values compared to the pure hay ration T1. Replacement of barley/soybean meal-mixture with urea treated cocoa pod (T3 – T6) decreased the production inversely to the cocoa pod level and approached the T1 level at the 100% substitution (T6).

Microbial cell production was also enhanced by the addition of concentrate (T2 – T6). However, urea treated cocoa pod (T3 – T6), resulted in lower increment compared to the barley/soybean meal-mixture, T2.

Development during Rusitec

The average pH, concentration of NH_4^+ and counts of protozoa during Rusitec are illustrated in Figure 2. Daily pH ranged from 6.4 to 6.8. The pH tended to fluctuate during the preliminary phase (day 0 – 9) and then stabilized during the collecting phase (day 10 – 14). Ammonium production also showed the same trend and ranged from 4.8 – 9.0 mmol/l. The number of protozoa decreased with increasing time. The average count of protozoa at day 0 was about 50000/ml and was reduced to a tenth in the collection phase.
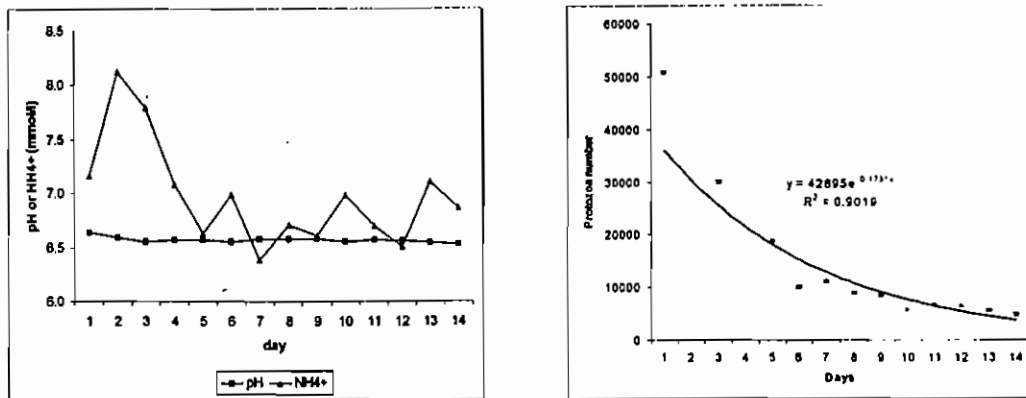
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Figure 2: The mean pH, NH_4^+ concentration and protozoa counts during Rusitec

Rate of disappearance

The rate of disappearance of DM and nutrients is shown in Table 4. There were no significant differences in hay disappearance between the treatments. The disappearance of DM of concentrate T3 (substitution of 25% barley/soybean meal-mixture by urea treated cocoa pod) was not significantly different from that in T2, however, increasing the proportion of urea treated cocoa pod (T4 – T6) resulted in a gradual decrease in DM disappearance. The OM disappearance of concentrate also tended to decrease with increasing amounts of cocoa pod, while the disappearance of CF decreased significantly when barley/soybean meal-mixture was partially or completely replaced by cocoa pod. The fiber fractions of concentrate disappeared in most cases in higher amounts with the cocoa pod containing rations.

Table 4: Disappearance of dry matter and nutrients of the feed ration in Rusitec (g/d)

Parameter	Treatment					
	T1	T2	T3	T4	T5	T6
Disappearance of hay						
DM	4.741	4.344	4.622	4.675	4.395	4.577
OM	4.216	3.818	4.057	4.129	3.879	4.001
CP	0.773	0.716	0.724	0.734	0.698	0.730
CF	1.168	1.008	1.131	1.074	1.002	1.066
ADF	1.202	1.029	1.174	1.153	1.053	1.108
NDF	1.908	1.699	1.961	1.958	1.755	1.868
Disappearance of concentrate						
DM		1.779[c]	1.566[bc]	1.393[ab]	1.270[a]	1.169[a]
OM		1.721	1.483	1.266	1.098	0.960
CP		0.272[b]	0.215[a]	0.183[a]	0.212[a]	0.223[a]
CF		0.016[a]	0.083[a]	0.146[ab]	0.266[b]	0.438[c]
ADF		0.002[a]	0.019[a]	0.042[a]	0.130[u]	0.306[b]
NDF		0.436[a]	0.400[a]	0.396[a]	0.466[a]	0.651[b]
Disappearance of ration						
DM	4.741[a]	6.123[b]	6.188[b]	6.068[b]	5.665[b]	5.747[b]
OM	4.216	5.539	5.540	5.395	4.978	4.962
CP	0.773[a]	0.988[b]	0.939[b]	0.917[b]	0.910[b]	0.953[b]
CF	1.168[ab]	1.024[a]	1.214[ab]	1.219[ab]	1.268[ab]	1.504[b]
ADF	1.202[ab]	1.031[a]	1.193[ab]	1.196[ab]	1.183[ab]	1.413[b]
NDF	1.908[a]	2.135[ab]	2.362[ab]	2.354[ab]	2.220[ab]	2.519[b]

The disappearance of DM and CP of the total feed ration increased compared to the pure hay ration (T1) when concentrate regardless of substitution level was added. There was no clear effect of the substitution level on fiber disappearance. The hay/cocoa pod concentrate 0-ration (T2) showed significantly lower crude fiber and ADF disappearance than the hay/cocoa pod concentrate 100-ration (T6), whereas, the lowest NDF disappearance was observed for the pure hay ration T1.

The proportions of the disappeared DM and nutrients (apparent degradability) are shown in Table 5. There were no significant differences in hay degradability between treatments. The inclusion of urea treated cocoa pod decreased the degradability of DM, OM, CP and NDF of the concentrate whereas it was increased for CF and ADF.

Table 5: Apparent degradability of the feed ration in Rusitec (%)

Parameter	Treatment					
	T1	T2	T3	T4	T5	T6
Degradability Hay						
DM	49.91	45.72	48.65	49.20	50.12	48.18
OM	48.99	44.36	47.14	47.97	49.39	46.48
CP	49.33	50.64	49.63	48.93	48.40	52.26
CF	41.38	35.69	40.04	38.02	38.64	37.74
ADF	37.99	32.52	37.10	36.44	36.99	35.01
NDF	35.61	31.71	36.61	36.54	36.37	34.86
Degradability Concentrate						
DM		50.59[d]	44.12[c]	38.99[bc]	35.65[ab]	32.28[a]
OM		50.43[d]	43.71[c]	37.60[b]	33.22[ab]	29.00[a]
CP		46.30[c]	36.66[ab]	31.29[a]	37.26[ab]	38.67[b]
CF		6.57[a]	12.72[ab]	13.70[ab]	19.68[ab]	23.31[b]
ADF		0.59	2.55	3.48	10.00	14.20
NDF		43.18[b]	27.48[a]	20.84[a]	21.73[a]	23.39[a]
Degradability Ration						
DM	49.91[b]	47.03[ab]	47.42[ab]	46.41[ab]	46.15[ab]	43.79[a]
OM	48.99[c]	46.08[bc]	46.17[bc]	45.06[abc]	44.87[ab]	41.63[a]
CP	52.26[b]	47.81[a]	45.45[a]	44.45[a]	46.87[a]	46.34[a]
CF	41.38	33.36	34.89	31.37	32.14	31.97
ADF	37.99[b]	29.85[a]	30.44[ab]	27.26[a]	27.60[a]	26.58[a]
NDF	35.61	33.53	34.66	32.43	31.92	30.94

The substitution of up to 75% barley/soybean meal-mixture by cocoa pod (T5) did not significantly decrease the degradability of DM and OM of the ration. The substitution of 100% barley/soybean meal-mixture did not affect the degradability for CP, CF, ADF and NDF of the feed ration. Compared to pure hay (T1), the addition of concentrate decreased the degradability of the total rations.

Discussion

The ammonium concentration in the liquid phase (4.8 - 9.0 mmol/l) and the pH (6.4 - 6.8) during the experiments showed that the conditions in the fermenters were within the physiological range for rumen microbes (SATTER & SLYTER, 1974; MCDONALD et al., 1995). However, the counts of protozoa in the rumen fluid (4×10^4 - 6×10^4 per ml) were less than is usual for the normal rumen physiology where up to 10×10^6 protozoa per ml may be expected (MCDONALD, et al., 1995). The protozoa were even decreased in Rusitec to about 5000 counts/ml during the collection phase. Apart from methodological reasons associated with the discrepancy between protozoal generation interval and substrate exchange, the survival of only one tenth of the initial introduced protozoa during the collection phase might also have been caused by the restricted substrate available for protozoa when high fiber diets are used (WELLER & PILGRIM, 1974; COLEMAN et al., 1980).

The amounts of microbial cell synthesis measured in Rusitec are comparable to theoretical expectation (Table 6). The amount of synthesized microbial cells can be calculated using the basic stoichiometry of SCFA production. According to DEMEYER & VAN NEVEL (1975) and DEMEYER et al. (1995) the amount of hexose fermented (HF) can be calculated as HF = hexose fermented (mol/d) = (A + P)/2 + B + V, where A is acetate, P is propionate, B is butyrate and V is valerate expressed in mol/d respectively. The amount of hexose fermented (g/d) is calculated as 162 x HF. The fermentation of 100 g carbohydrate (hexose) is assumed to result in the synthesis of 30 g microbial cells (MC; BERGNER & HOFMANN, 1996). MC synthesis measured for the different treatments was similar to those theoretically calculated on the basis of HF.

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Table 6: Stoichiometry of fermentation in Rusitec

Parameter	Treatment					
	T1	T2	T3	T4	T5	T6
Microbial cell (MC) measured in Rusitec (mg/d)	700[a]	859[c]	822[bc]	783[b]	793[b]	776[b]
Hexose Fermented (HF)						
HF (mmol/d)	13.9[a]	20.3[c]	19.1[de]	18.1[cd]	17.4[bc]	16.0[b]
HF (g/d)	2.25[a]	3.28[c]	3.09[de]	2.93[cd]	2.82[bc]	2.60[b]
MC calculated from HF(mg/d)	675[a]	985[c]	928[de]	881[cd]	845[bc]	779[b]

The efficiency of microbial metabolism depends on the amount of microbial cells as well as the amounts of methane per unit of fermented substrate. It can be expressed in different terms. Calculated values of microbial N in relation to organic matter apparently degraded (OMAD), to truly digested organic matter (TOMD = truly degraded organic matter), to hexose fermented, to SCFA and to ATP are shown in Table 7. The highest efficiencies for microbial growth can be stated for the pure hay ration (T1). Addition of barley (T2) led to a significant reduction in EMPS, whereas the replacement of increasing barley/soybean meal-mixture by urea treated cocoa pod caused a slight recovery of EMPS and reaching almost similar to T1 values at replacement of 100% barley with cocoa pod (T6).

Table 7: Efficiency of microbial protein synthesis expressed in different terms

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
TOMD (HF + MC) g/d	2.95[a]	4.14[c]	3.91[de]	3.72[cd]	3.61[bc]	3.37[b]
Microbial-N/OMAD (mg/g)	13.3[b]	12.4[ab]	11.9[ab]	11.6[a]	12.9[ab]	12.5[ab]
Microbial-N/TOMD (mg/g)	19.1[c]	16.6[a]	16.8[a]	16.9[a]	17.7[ab]	18.5[bc]
Microbial-N/HF (mg/g)	25.1[c]	21.0[a]	21.3[a]	21.5[a]	22.7[ab]	24.0[bc]
Microbial-N/SCFA (mg/mmol)	2.27[c]	1.93[a]	1.96[ab]	1.97[ab]	2.07[abc]	2.16[bc]
Microbial-N/ATP* (mg/mmol)	1.27[b]	1.09[a]	1.10[a]	1.10[a]	1.15[a]	1.20[ab]
CH_4/SCFA (mmol/mmol)	0.22	0.21	0.23	0.22	0.21	0.21
CH_4/OMAD (mmol/g)	1.29	1.37	1.40	1.29	1.28	1.23
CH_4/TOMD (mmol/g)	1.84	1.83	1.99	1.88	1.78	1.82
CH_4/NDF disappearance (mmol/g)	2.86[ab]	3.55[c]	3.29[bc]	2.97[abc]	2.89[ab]	2.42[a]

*) ATP = 2 Acetate + Propionate + 3 Butyrate (mmol/day)

With the exception of methane per unit NDF disappearance, the methane release remained unaffected by the different treatments. However, less methane was produced per unit NDF disappearance when 100% cocoa pod concentrate (T6) was supplied to the fermenters instead of 0% (T2), 25% (T3), and 50% (T4) cocoa pod concentrates. It may also be noted that the lower amounts of produced microbial protein observed when cocoa pod containing concentrates served as the substrate, were associated with a slightly but not significantly increase in EMPS.

Conclusions

It was found that the substitution of barley/soybean meal-mixture by urea treated cocoa pod up to 100% in hay based rations did not decrease the rate of ration DM disappearance, and fiber degradation even tended to be increased. Even though the microbial N-fixation decreased with the inclusion of cocoa pod in the rations, the efficiency of microbial N-fixation was increased in line with the cocoa pod level. Methane release per unit NDF disappearance decreased inversely to the cocoa pod level.

Although urea treated cocoa pod cannot replace barley/soybean meal-mixture equivalently in feed rations for ruminants, in fact, the inclusion of urea treated cocoa pod at the expense of higher fermentable feed components will reduce the fermentation intensity thereby yielding lower amounts of SCFA and microbial protein for the host animal. Nevertheless, the urea treated cocoa pod can be used as a feedstuff for low performance ruminants as a substitute for barley/grain meal-mixture or as a supplement to hay based rations without reducing the efficiency of microbial synthesis. No toxicity effects were observed with the inclusion of urea treated cocoa pod in the ration as to a dramatically disturbed fermentation or a too high concentration of ammonia.

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