Nutritional Properties of Cocoa Pods as Ruminant Feedstuff

Despal[1] and H. Abel[2]

Dept. Animal Nutrition & Feed Technology, Faculty of Animal Science, Bogor Agriculture.
Jl. Agatis Kampus IPB Darmaga, 16680 Bogor. Email: despal@ipb.ac.id
2) Institute for Animal Nutrition and Physiology, Faculty of Agriculture, Georg-August University, Goettingen- Germany

Abstract

An attempt to increase cocoa pods quality feed for ruminant have been done using fermentation techniques with or without urea addition. Five treatments (Fresh cocoa pod = C; fermentation with addition urea 0 g/kg = U0 or ensilage; 10 g/kg = U1; 20 g/kg = U2 and 30 g/kg fresh substances of cocoa pods) have been examined for their effect on pH, proximate composition, N-fractions, amino acid compositions, cell wall constituents, anti nutritive compounds, *in vitro* gas production (Gb), estimate organic matter digestibility (OMD) and metabolizable energy (ME) of cocoa pods. Ensilage (U0) improve feed values of cocoa pods by weakening the lingo-cellulose or –hemicellulose bonds, resulting in higher in vitro gas production as well as higher OMD and ME contents compare to fresh cocoa pods. The protein value of cocoa pods, expressed as true protein (TP) or non ammonia nitrogen (NAN) minus urea nitrogen (UR) was also improved. Addition of urea affected the composition of the fibre fraction as to the proportion of NDF, ADF and ADL and the content of TP was slightly increased. Urea treatment increased Gb, OMD and ME compared to ensiled or fresh cocoa pods. The optimum level of urea for maximum Gb was 6.7 g/100 g DM cocoa pods. The optimum level to reach maximum OMD and ME were also reached at urea level 6.5 – 6.7 g/100 g DM cocoa pods.

Keywords: cocoa pod, ensilage, feed quality, ruminant, urea

Backgrounds

Based on FAO main findings about world agriculture 2030, patterns of food consumption in developing countries are shifting towards higher-quality and more expensive foods such as meat and dairy products. It is reported that meat consumption in developing countries has risen from only 10 kg per person annually in 1964-66 to 26 kg in 1997-99. It is projected to rise to 37 kg per person per year in 2030. Milk and dairy products have also seen rapid growth, from 28 kg

per person per year in 1964-66, to 45 kg now, and could rise to 66 kg in 2030. Although Indonesia currently are among the least milk consumer (7 liter/capita/year), almost 75% of the milk consumed are still imported. Meat productions are slightly better than milk production figure. About 72% of the demand could be supplied by domestic production although their sustainability is still a question mark. It is hard to imagine how milk and meat domestic production could fulfill the increasing demand in 2030 if FAO projection is become reality.

In the past five years, cattle population for meat and milk production remained at the same numbers. There are several problems in increasing ruminant population in Indonesia including feeding, breeding, disease and socio-economic problems. Feeding problem such as lack of good quality feed resources and suboptimum of byproduct utilizations are among the problems that have been noticed for long time period but still lack of action to solve them. Naturally, Indonesian grass like almost all tropical grasses has very low quality in compare to temperate grass. It contained 8 – 12% crude protein and high fibre component in contrast to temperate grasses which have prime to 1st grade grasses quality (17 – 20% CP and low fibre component). The low quality of grasses are not the only problem in providing nutrition for ruminant in Indonesia, their availability are also highly depended on season and very few planning on their conservation have been made. In dry season, farmers are frequently forced to use low quality but available by product such as cocoa pods, rice straw, palm press fibre, sugarcane-bagasse which could only provide limited nutrient for animal.

Cocoa pods are among the prospective feed resources for ruminant because of their high availability (2 to 1 of cocoa pods meal to cocoa seed ratio (Duke, 1983)) and concentrate in an area so that easily to handle and process. Cocoa pods contained 9% crude ash, 10% crude protein, 2 – 3% fat, 35% crude fibre (Barnes and Amega, 1984). It may resembles that of king grass quality (Sutardi, 1988) and superior than palm press fibre and rice straw (Toharmat et al., 1997). However, as a late mature plant component, the pods contained high cell walls (35% cellulose, 11% hemicelluloses, 6% pectin and 15% lignin (Sobamiwa and Longe, 1994)) which then restrict their utilization (Reynolds, 1995). Theobromine, a toxic alkaloid present in cocoa plants, may also limit its use as ruminant feedstuff (ICCO, 200). To increase utilization of cocoa pods in ruminant ration, therefore their quality upgrading should be done.

Flachowsky *et al*. (1990) have been reviewed several treatments to improve cellulosic material by using physical, chemical and biological treatments. Alkali source like urea have been

reported to be effective in improving fibrous feed quality (Schiere and Ibrahim, 1989; Sunstol et al, 1993; Chenost and Kayouli, 1997). The urea did not only improve fibre utilization but may also supply N for rumen microbial growth.

Since addition of urea increase amount of nitrogen in the treated feed, therefore their evaluation could not be assayed based on crude protein only (N x 6.25). There should be others N fractions that could describe true protein quality improvement on urea treated feed.

Objectives

The objectives of this study were to find the amount of urea level for cocoa pods quality improvement and evaluating their improvement using parameters that describe their improvement at best.

Methods

1. Urea treatments

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 randomly subdivided into five treatments, each of three replications which were fresh cocoa pods (C) and four levels of urea applications: 0 (U0), 10 (U1), 20 (U2) and 30 (U3) g/kg fresh materials respectively (w/w).

All treatments were processed manually. Firstly, cocoa pods were sliced into about 2 mm thickness and samples of 2 kg each were weighed. The pods in the control treatment (C) were sun dried immediately, while the other samples were put into 40 x 50 cm and 0.12 cm thin polybags. Urea was added layer by layer in order to achieve homogeneous mixtures. The bags were then pressed to reduce the air contents and sealed with nylon tape. Finally, the bags were stored at room temperature. After 14 days, the bags were 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.

2. Chemical analysis

The pH and DM were determined directly in fresh cocoa pods and in the ensiled, while all other parameters were measured in sun-dried samples. The pH was measured according to Naumann & Bassler (1997). Ten grams fresh samples were mixed with 100 ml distilled water and stirred with a laboratory blender for 3 min. The pH was measured in the filtrate with an electrode (Mettler Toledo In lab 417).

Contents of dry matter (DM), crude ash (ASH), crude protein (CP), crude lipid (XL), and crude fibre (CF) were analysed according to the conventional Weende procedure (Naumann & Bassler, 1997).

Non protein nitrogen (NPN) was determined by precipitation of true protein (TP) with tungstic acid, filtration and determination of the insoluble nitrogen in the residue. NPN was calculated as difference between total crude protein nitrogen and true protein nitrogen (Licitra *et al*., 1996).

Residual urea (UR) was measured according to Naumann & Bassler (1997). The sample was cleared with Carrez-solution I and II and agitated using an automatic shaker (Co Köttermann type 5627) for 30 minutes after the addition of water. The suspension was then filtrated. After the addition of 4-Dimethylamino-benzaldehyde, the absorbance in the filtrate was measured at 436 nm wavelength (Co. Eppendorf 1101 M) and the amount (concentration) calculated by plotting the absorbance value in a urea standard curve.

Non ammonia nitrogen (NAN) was determined using the same method as used by Carro & Miller (1999). The sample was wetted with distilled water, adjusted with 1 M NaOH to pH above 10, and dried at 90^{o}C for 16 h to remove NH_3-N. The N-content of the dried residues accounting for NAN content was determined by micro Kjeldhal (Naumann & Bassler, 1997).

The amino acid (AA) composition was measured first by ion exchange chromatograph. The cocoa pod samples (250 mg) were hydrolysed with 50 ml phenolic hydrochloric acid at 110^{o}C for 24 hours. The hydrolysed sample was transferred into a volumetric flask and adjusted to pH 2.2 with 7.5 M NaOH under cooling temperature at which condition all amino acids existed as cations. The volume was filled up to 250 ml after adding an internal standard (norleucine). The sample was centrifuged for 10 min at 15 000 g. The supernatant was analysed

using an amino acid analyser LC 3000 (Co Eppendorf Biotroniks). The sulphur containing amino acids (methionine and cysteine) must be oxidised before hydrolysis with 5 ml performic acid (0.5 ml H_2O_2) and 4.5 ml 88% phenolic formic acid.

The determination of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were carried out according to Van Soest *et al.* (1991). The contents of hemicellulose, cellulose, and crude lignin were calculated by difference. Hemicellulose were calculated as the difference between NDF and ADF, cellulose as the difference between ADF and ADL and crude lignin was calculated by subtracting ash residue (muffle-oven at 550^{o}C) from ADL.

Theobromine content was analysed according to Naumann & Bassler (1997). Theobromine was extracted with chloroform and the extract was dried and then resolved in water and treated with silver-nitrate solution. The free saltpetre acid was titrated with sodium hydroxide. Theobromine content was calculated according to the formula:

Theobromine (% DM) = (ml NaOH x 18)/(mg sample weight x %DM)

Where 18 is the coefficient of conversion for each ml 0.1 N NaOH used in titration.

3. In vitro gas test

In vitro gas production was measured applying the Hohenheim Gas Test (Menke *et al*., 1979). Approximately 200 mg sample (of about 90% DM) was weighed and incubated with 30 ml rumen fluid-buffer solution (1:2) in a 100 ml glass syringe. The syringes were put in a double wheel plat and rotated using a rotor in a 39^{o}C water bath. The fermentation process was stopped after 24 hours of incubation. The amounts of gas produced (Gb) in the syringes were read. The gas production was calculated for 200 mg DM.

The experiment was completely random designed with fresh cocoa pods as control and 4 levels of urea applications. Each treatment was repeated 3 times. Significant differences between treatments were analysed using analysis of variance (ANOVA) and continued with Tukey's test. Analyses were done using SPSS statistical software version 10.0.

Results

1. Chemical compositions

The pH and the results of proximate analyses of cocoa pods (CPs) are shown in Table 1. Ensilage (U0) reduced pH of CPs from 5.35 in the control (C) to 4.72. Addition of urea significantly increased pH (up to 8.38). However, there was no significant difference between U2 and U3. Dry matter (DM) contents tended to be reduced by ensiling with or without urea.

Table 1: PH and crude nutrient contents of cocoa pods

Parameter	Treatment				
	C	U0	U1	U2	U3
PH	5.35^{b}	4.72^{a}	7.93^{c}	8.20^{d}	8.38^{d}
DM (%)	18.98	18.06	18.06	18.42	18.24
ASH (% DM)	6.55^{a}	8.43^{b}	6.32^{a}	5.81^{a}	5.49^{a}
XL (%DM)	0.50	0.55	0.40	0.40	0.46
CF (%DM)	52.30^{b}	42.09^{a}	47.18^{ab}	49.98^{b}	46.83^{ab}

Different superscripts in the same line indicate significant differences ($p< 0.05$)

Crude ash content of cocoa pods increased by ensilage compared to the control. However, addition of urea (U1 to U3) resulted in the same crude ash value as in the control. No significant difference was found in crude lipid content (XL). Inversely to crude ash, crude fibre content (CF) was decreased by ensilage. Urea addition tended to increase CF but still to a lower level than the control.

The results for N-fractions are shown in Table 2. Total N and N-fractions were not significantly influenced by the ensilage process, whereas urea treatment increased these parameters in a dose dependent manner. However, NAN and UR were not significantly increased from U1 to U2, whereas further addition of urea to U3 level increased NAN and UR contents almost two and threefold respectively. NAN minus UR (NAN – UR) tended to decrease from U0 to U3 in contrast to TP values which increased with the addition of urea.

Table 2: N-Fractions of cocoa pods (% N in DM)

Fraction	Treatments				
	C	U0	U1	U2	U3
Total N	1.35[a]	1.69[a]	2.90[b]	3.98[c]	6.77[d]
NAN	1.22[a]	1.57[a]	2.16[b]	2.73[b]	5.21[c]
NH_3	0.13[a]	0.12[a]	0.74[b]	1.25[c]	1.56[d]
UR	0.07[a]	0.03[a]	0.74[ab]	1.35[b]	3.86[c]
NAN – UR	1.15	1.54	1.42	1.38	1.36
TP	1.28[a]	1.21[a]	1.73[b]	1.93[c]	2.04[d]
AA	0.78	1.11	nd	0.83	nd

NPN = non protein nitrogen; TP = true protein; UR = residual urea; NAN = non ammonia nitrogen; AA = amino acids; nd = not determined. Different superscripts in the same line indicate significant differences ($p < 0.05$).

AA (calculation based on N-contents of measured amino acid) found in U0 was higher than in the control and in U2. No analysis has been made for U1 and U3. The amino acid composition changed as a result of ensilage (Table 3). This effect was more pronounced for proline, aspartic acid, and glutamine. Amino acids of U2 treated CPs was similar or slightly higher than for the control except for tyrosine, phenylalanine and lysine which were reduced.

Table 3: Amino acid contents of cocoa pods (mg/g DM)

Amino Acid	Treatment		
	F	U0	U2
Cysteine	1.10	1.65	1.26
Aspartic acid	5.64	8.47	6.01
Methionine	1.24	1.74	1.21
Threonine	2.90	4.23	3.23
Serine	3.33	4.81	3.39
Glutamine	7.11	10.89	7.76
Proline	4.18	9.37	4.88
Glycine	2.90	4.76	3.54
Alanine	3.80	5.46	4.36
Valine	3.65	5.49	4.22
Isoleucine	2.84	3.81	2.59
Leucine	4.64	6.38	4.49
Tyrosine	3.54	4.11	2.82
Phenylalanine	4.62	5.12	3.44
Histidine	1.89	2.48	2.17
Lysine	3.89	3.88	3.26
Arginine	2.71	3.89	3.42
Total amino acids	60.00	86.56	62.02

The analysed cell wall contents are shown in Table 4. There was no significant effect of ensilage and urea on NDF. ADF was decreased by urea treatment whereas ADL was increased by ensilage.

Table 4: Cell wall constituents of treated CPs (% DM)

Parameter	Treatment				
	C	U0	U1	U2	U3
NDF	79.48	82.04	82.59	81.92	80.07
ADF	63.62^{bc}	65.57^{c}	60.51^{ab}	60.66^{ab}	57.35^{a}
ADL	29.08^{a}	34.82^{b}	29.33^{a}	29.87^{a}	27.73^{a}
Hemicellulose	15.85	16.47	22.08	21.26	22.72
Cellulose	34.54^{b}	30.76^{a}	31.18^{ab}	30.79^{a}	29.62^{a}
Crude lignin	28.64	33.13	29.17	29.66	27.40

Different superscripts in the same line indicate significant differences ($p < 0.05$).

Hemicelluloses tended to increase by urea additions while cellulose was reduced in all urea treatments (U0 – U3). There were no significant effects of the treatment on crude lignin content. The contents of theobromine are shown in Figure 1. There was also no significant effect of the treatments on theobromine content of cocoa pods. Treatment U1 resulted in the lowest and the control in the highest theobromine contents (0.32 vs. 0.37 g/kg).

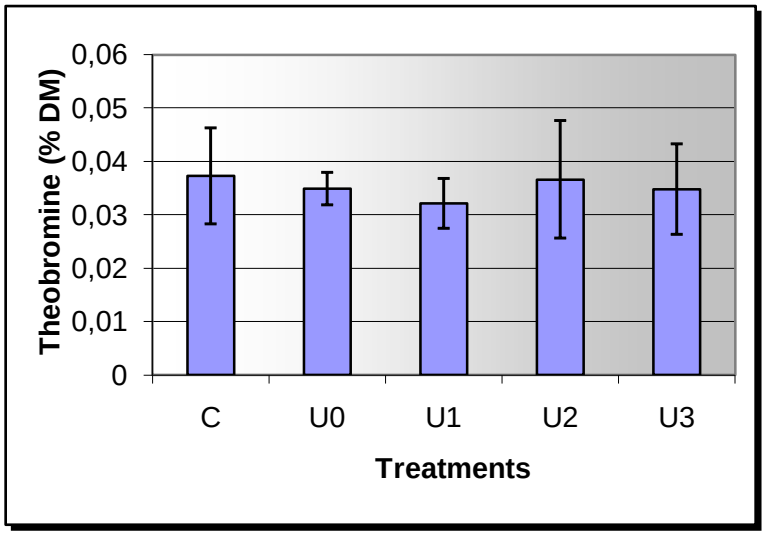

Figure 1: Contents of theobromine in cocoa pods
($n = 15$; $\xi \pm s$)

2. In vitro digestibility

In vitro gas production (Gb) of CPs is shown in Figure 2. Ensilage with or without urea treatment increased Gb significantly, treatment U3 excepted which decreased Gb sharply.

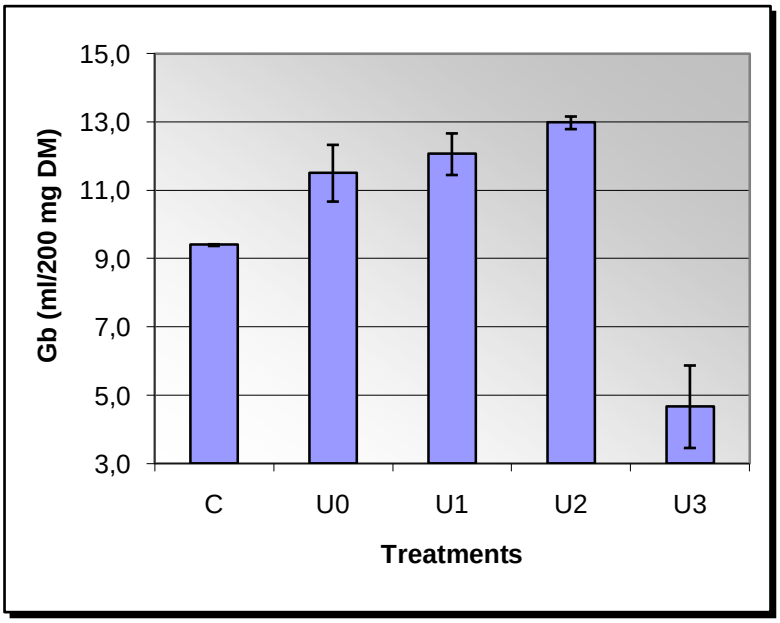

Figure 2: Gas productions of cocoa pods
(n = 15; ξ ± s)

Discussions

The proximate analyses of the cocoa pods indicated lower contents of CP (Total-N x 6.25), ash and crude lipid but higher CF than those observed by Barnes & Amega (1984). Theobromine content was also lower (0.032 vs. 0.32%) than reported by Barnes & Amega (1984). This may have been caused by the inclusion of pod husks in the samples. Although there was no effect of treatment, theobromine content of samples used in this study was very low and could be considered as being unaffective in ruminant nutrition. At present, there are no reports on theobromine toxicity in animals due to cocoa pods (ICCO, 2000).

The amount of N being lost from the time of addition to that of analysis increased with increasing levels of urea. While 10 g, 20 g and 30 g of urea had been added per kg fresh cocoa pods corresponding to 3.2 g, 6.4 g and 9.7 g urea-N per kg DM, only 1.7 g, 2.8 g and 5.6 g total-N (feed plus urea) were found in U1, U2 and U3 treatments respectively. Percentages of N losses as the ratio of urea-N added were 47%, 56% and 42% for U1, U2 and U3 respectively. It indicates an increased ureolytic activity (assuming N-losses as NH_3). This is confirmed by the similar trend in ammonia values. According to Chenost (2001), two-third of ammonia released from ureolysis is volatile and lost, and only one third of the ammonia binds on the forage cell wall.

The amount of N lost is also related to pH. Increasing urea level from U2 to U3 did not increase pH significantly. Possibly the time of incubation (14 days) was too short. Ureolytic activities are maximal at pH 7.0. The higher the pH the higher the ammonia-N lost. At an extremely high temperature of 90^{o}C and pH > 10, all ammonia is lost. According to this feature ammonia is used to determine NAN (Carro & Miller, 1999). According to Rexen & Knudsen (1984) the alkali process on feed depends on temperature, pressure, alkali concentration and reaction time.

The evaluation of TP as tungstic acid precipitable protein seems not to be valid for urea treated fibrous feed. TP may be overestimated and NPN underestimated since not all of the NPN might be separated from TP (Licitra et al., 1996).

Total N in U0 was slightly higher than in the control. It may partly be the result of indirect enrichment of N due to organic matter degradation during ensilage. Both, the decreased pH and the low ammonia concentration are indicators for an acid fermentation of ensiled cocoa pods (U0). Possibly the substrate for fermentation was too low for a stronger acid production which would have led to an even lower pH.

The decrease in ADF for treatment U1 – U3 in comparison to control is associated with lower cellulose and partially compensatory higher hemicellulose contents. The quality of the fibre fraction of urea treated cocoa pods was therefore modified and should be higher and/or faster degradable in the microbial rumen environment.

This is confirmed by in vitro gas production which was increased from treatments U0 to U2. The sharp decrease of Gb with U3 must be explained with a too high and therefore toxic

ammonia effect on the microbes in the in vitro test. According to Smith (1989), at pH above 7 urea splitting in the rumen results predominantly in NH_3 which is toxic to the rumen if present in large amounts. However, at pH below 7 a high concentration of non toxic NH_4^+ predominates.

For calculation of OMD and ME from in vitro gas production, cocoa pods are grouped to dry fodder or hay and the following formulas are applied:

OMD (%) = 16.49 + 0.9042 Gb + 0.0492 (CP or TP or (NAN – UR) x 6.25) + 0.0387 XA

ME (MJ/kg DM) = 2.43 + 0.1206 Gb (ml) + 0.0069 (CP or TP or (NAN – UR) x 6.25) + 0.0187 XL

Where Gb is in ml, while TP, ASH and XL are in g/kg DM (Menke & Steingass (1987). The results of these calculations are shown in Table 5.

Table 5: OMD (%) and ME (MJ/kg DM) of cocoa pods

Parameter	Treatment				
	C	U0	U1	U2	U3
OMD					
CP (TN x 6.25)	31.79^{a}	35.35^{b}			
TP (TP-N x 6.25)	31.48^{b}	33.90^{c}	35.20^{cd}	36.48^{d}	29.10^{a}
NAN – UR (NAN – UR x 6.25)	31.06^{ab}	34.90^{b}	34.00^{b}	34.72^{b}	27.02^{a}
ME					
CP (TN x 6.25)	4.25^{a}	4.65^{b}			
TP (TP-N x 6.25)	4.21^{b}	4.44^{b}	4.71^{c}	4.91^{c}	3.96^{a}
NAN – UR (NAN – UR x 6.25)	4.15^{a}	4.58^{b}	4.54^{b}	4.66^{b}	3.67^{a}

Different superscripts in the same line indicate significant differences ($p < 0.05$)

For C, the formula renders almost the same OMD- and ME-values with either CP or TP as dependent variables whereas (NAN-UR) delivers somewhat lower values. The lower values calculated for U0 when TP is used instead of CP can be explained with the relatively big difference between CP and TP.

CP cannot be used in the formula for the urea treated samples. TP and (NAN-UR) give the same trend as the gas production values, and can therefore be used to express the protein value of urea treated cocoa pods. Except U0, applying TP in the formulas leads to higher OMD and ME contents than (NAN–UR). Presumably some NPN is precipitated with tungstic acid

resulting in higher TP than (NAN-UR) values. The values calculated by applying (NAN-UR) are therefore suggested to be the nearest evaluations of the real feeding values of the samples. However, the OMD and ME values may be underestimated due to considering TP instead of CP.

The response of Gb, OMD and ME on urea applications followed the equations shown in Table 6. The maximum Gb was reached at approximately 67 g urea per kg DM cocoa pods. About the same urea level was estimated for OMD and ME maximum with (NAN–UR) as dependent variable.

Table 6: Response of OMD and ME on urea applications

No	Formula	Peak	n	r^2	F
	Based on Gb	U (g/kg DM)			
1	$Gb = 11.0306 + 0.067U – 0.0005U^2$	67	12	0.86	0.000
	Based on %TP (TP-N x 6.25)				
2	$OMD = 33.4659 + 0.0743\ U - 0.0004\ U^2$	93	12	0.84	0.000
3	$ME = 4.3889 + 0.0113\ U - 0.00006\ U^2$	94	12	0.84	0.000
	Based on (NAN – UR-N) x 6.25				
4	$OMD = 34.3873 + 0.0401\ U - 0.0003\ U^2$	67	12	0.75	0.000
5	$ME = 4.5191 + 0.0065\ U - 0.00005\ U^2$	65	12	0.72	0.000

The use of TP as dependent variable resulted in maximal OMD and ME at approximately 93 g urea per kg DM cocoa pods (Figure 3). The optimal level of urea found was higher than that for treated straw reported by Williams et al. (1984) and Chenost (2001) (40 and 53 g/kg DM respectively). It may have been caused by the higher lignocellulosic material in cocoa pods compared to rice straw.

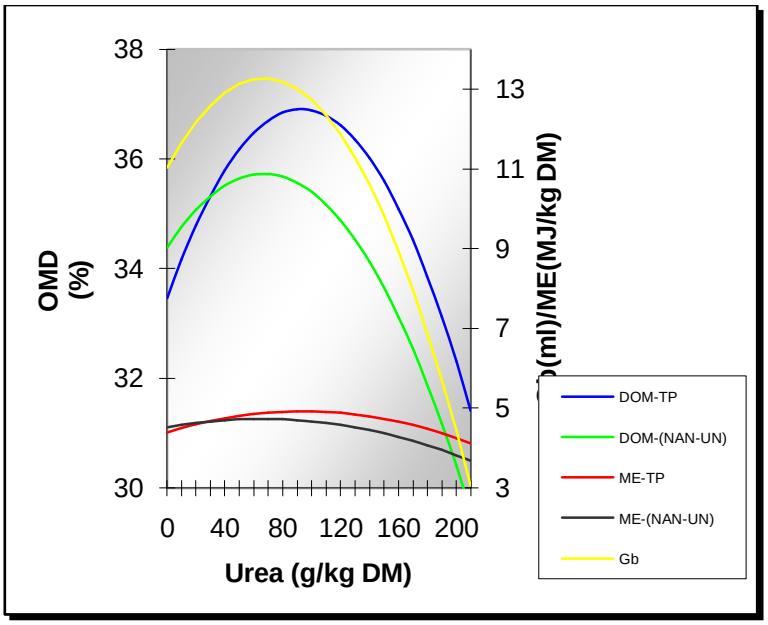

Figure 3: OMD and ME of CPs as response to urea treatment

Conclusions

The treatments (ensilage with or without urea) improve the nutritive value of cocoa pods by weakening the ligno-cellulose or –hemicellulose bonds which then can easier be penetrated by rumen microbes, resulting in higher in vitro gas production, as well as higher calculated OMD and ME values compared to the control. The protein value of cocoa pods, expressed as TP or (NAN-UR) was also increased. The (NAN-UR) value is suggested to be the nearest evaluation of the real protein value of urea treated cocoa pods.

The optimum level of urea to reach Gb maximum was 67 g/kg DM cocoa pods. The optimum level to reach maximal OMD (%) and ME (MJ/kg DM) based on TP as protein value was 93 – 94 g urea per kg DM, while using (NAN-UR) was reached at an urea level of 66 g/kg DM cocoa pods.

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