Biochar reduces enteric methane and improves growth and feed conversion in local "Yellow" cattle fed cassava root chips and fresh cassava foliage

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Abstract

Twelve local "Yellow" cattle with initial live weight ranging from 80 to 100 kg were assigned in a completely randomized block design to a 2*2 factorial arrangement of four treatments with three replications. The factors were: biochar at 0.6% of diet DM or none; and potassium nitrate at 6% of diet DM or urea at 1.83% of diet DM. The basal diet was cassava root chips fed ad libitum and fresh cassava foliage at 1% of LW (DM basis). Sodium sulphate and sodium chloride were added to the diet at the rate of 0.4% and 0.5% in the DM. The trial lasted 98 days following a 21 day adaptation to the diets.

Live weight gain was increased 25% by adding biochar to the diet DM and tended to be decreased when nitrate replaced urea as the source of NPN. DM feed conversion was improved by biochar and by urea replacing nitrate. DM feed intake was not affected by supplementation with biochar nor by the NPN source. Both biochar and nitrate reduced methane production by 22 and 29%, respectively, the effects being additive (41% reduction) for the combination of biochar and nitrate.

Key words: Biofilm, climate change, consortia, global warming, greenhouse gases, methanogens, methanotrophs, micro-organisms, protein:energy ratio,

Introduction

Modification of rumen fermentation to minimize enteric methane production is a high priority research area because of the large contribution herbivorous animals make to this greenhouse gas. In recent times (UNEP 2011) a greater emphasis has been assigned to methane as, together with abatement of carbon black emissions, this appears to be the only means of regulating global warming in the short term. Without this short term amelioration of methane and carbon black emissions it is estimated that 3.1 million people are at risk of reduced life expectancy because of the reactions of methane with oxygen in the troposphere releasing the more deadly ozone (UNEP 2011). Ruminant methane production is a targeted area for mitigation of methane release since it produces a large proportion of world methane production. .

In an earlier report from our laboratory we showed that biochar derived from rice husks reduced methane production in an *in vitro* incubation with rumen fluid and a substrate of cassava root meal and cassava leaf meal supplemented with urea or potassium nitrate as the major fermentable N source (Leng et al 2012).

In an earlier report from our laboratory we showed that biochar derived from rice husks reduced methane production in an *in vitro* incubation with rumen fluid and a substrate of

cassava root meal and cassava leaf meal supplemented with urea or potassium nitrate (Leng et al 2012).

In this paper we show that the same biochar reduces enteric methane and also improves growth and feed conversion in growing cattle.

The hypotheses tested were that:

- In cattle fed a basal diet of fresh cassava root chips supplemented with fresh cassava leaves, supplementation with biochar will improve the growth rate and reduce the production of methane.
- There will be an additive effect on reduction of methane emissions from adding both biochar and nitrate to the diet of cattle fed a basal diet of fresh cassava root chips supplemented with fresh cassava leaves.

Photo 1: Chopping the cassava root by machine

Photo 2: Biochar from updraft gasifier stove

Photo 3: Cassava foliage from farmer areas

Photo 4: Cattle experiment facility

Materials and methods

Location and duration

The experiment was conducted in the farm of Souphanouvong University, 7 km from Luang Prabang city, Luang prabang province, Lao PDR.

Treatments and experimental design

The experiment was carried out for 98 days, with an extra 21 days at the beginning for adaptation to the pens and diets. Twelve local “Yellow” cattle were assigned in a completely randomized block design (CRBD) within a 2*2 factorial design with 3 replications. The treatments were:

NPN source

- Potassium nitrate at 6% of diet DM
- Urea at 1.83% of diet DM

Biochar:

- Biochar at 0.6% of diet DM
- No biochar

The basal diet was composed of cassava root chips fed ad libitum and fresh cassava foliage at 1% of LW (DM basis). Sodium sulphate and sodium chloride were added to the diet at the rate of 0.4% and 0.5% in the DM.

Animals and housing

Twelve young local “Yellow” male and female cattle were used with initial live weight ranging from 80 to 100 kg. The animals were confined in separate pens. Vaccination against epidemic diseases and treatment against internal parasites were done before the commencement of the experiment.

Feeding and management

Animals were were slowly brought on to the experimental feeds over three weeks to allow adaptation to the NPN source and the cassava foliage. The cassava roots and cassava foliage were obtained from a private farm near the University. The roots were obtained at 3 weekly intervals and the cassava foliage daily. The roots were sliced by machine prior to feeding fresh during the first 6 weeks and then after sun-drying until the end of the experiment. The biochar was produced by combusting rice husks in a “Top-lit updraft (TLUD)” gasifier stove in which the temperature of carbonization exceeds 400 °C (Olivier 2010). The biochar was suspended in water, in which urea or potassium nitrate had previously been dissolved, and the suspension sprinkled on the surface of the cassava chips. The feeds were offered in individual wooden troughs, two times a day at 7.00 am and 4.30 pm. The offer level of the cassava roots was set at 120% of the recorded intake during the previous week. Cassava foliage was given in the fresh state at the rate of 10 g/kg live weight (DM basis). Water was supplied during the whole period.

Data collection and measurements

The cattle were weighed before feeding in the morning at the beginning of the experiment and at 14 day intervals. Feeds offered and residues were recorded daily.

Samples of rumen fluid were taken by a stomach tube, two hours post feeding in the morning at the end of the experiment for determining ammonia and pH. At the end of the experiment, a sample of mixed eructated and respired gas from each animal was analysed for methane and carbon dioxide using the Gasmet equipment (GASMET 4030; Gasmet Technologies Oy, Pulttitie 8A, FI-00880 Helsinki, Finland), based on the approach suggested by Madsen et al (2008). Individual animals were held for 5 minutes in a wooden crate covered with polyethylene film before taking the measurements, so that the gases emitted from the animal could equilibrate with the air in the box (Photo 5). Samples of air in the animal house were also analysed.

Photo 5: Wooden crates enclosed in plastic used to house the cattle during the 5 minute period of adaptation/measurement using the GASMET infra-red analyser.

Chemical analysis

Samples of feeds offered and residues were collected every day to determine dry matter (DM), OM, crude protein (CP) and protein solubility following the procedure of Ly and Nguyen Van Lai (1997).

Statistical analysis

The data were analyzed by the general linear model option of the ANOVA program in the Minitab (2000) software (version 13.31). In the model the sources of variation were: blocks, level of biochar, NPN source, interaction biochar*NPN and error. Weight gains were measured by the linear regression of live weight (Y) on days in the experiment (X).

Results

The composition of the cassava root and foliage was in accordance with most published values for these feed ingredients (Göhl 1975).

Table 1. Chemical composition of the feeds

		% in DM	
	DM, %	**CP**	**OM**
Fresh cassava root chips	31.3	3.1	91.3
Dried cassava root chips	65.1	2.9	89.6

Fresh cassava foliage			
Leaves	30.9	23.3	93.3
Stem	27.2	17.2	93.7
Biochar	71.4	*NA*	12.5

NA Not analysed

DM feed intake was not affected by supplementation with biochar nor by the NPN source (Table 2). Concentrations of crude protein in the DM of the 4 diets (12.7 to 13.0%) and of the total N as NPN (24.9 to 25.7%) were similar on all diets.

Table 2. Mean values of feed intake for local "Yellow" cattle fed cassava root chips, fresh cassava foliage supplemented with biochar and NPN source

Item	Biochar		Prob.	NPN source		SEM	Prob.
	BIO	NOBIO		Urea	Nitrate		
Fresh feed intake, g/day							
Cassava root chips	3242	3242	1.00	3242	3242	30	1.00
Cassava foliage	2046	2030	0.79	2037	2039	42.6	0.96
Biochar	20.0			11.2	10.8	0.40	0.49
Total	5308	5272		5290	5291		
DM intake, g/day							
Cassava root chips	1377	1373	0.91	1378	1373	26.6	0.89
Cassava foliage	819	810	0.75	816	813	21.2	0.92
Biochar	14			8.0	7.7	0.29	0.50
Na_2SO_4	5.8	5.8	1.00	5.8	5.8	0.12	1.00
NaCl	6.7	6.7	1.00	6.7	6.7	0.18	1.00
Urea	14.3	12.5		25.8		0.42	
K-nitrate	40.8	46.8			84.7	1.39	
Total	2278	2255	0.75	2240	2290	48.5	0.48
CP in DM, %	12.9	12.9		13.0	12.7		
NPN as % of total N	25.5	25.7		24.9	25.0		
Biochar, % of diet DM	0.62	0.00		0.30	0.29		
DM intake, g/ kg LW	25.6	25.3	0.71	25.4	25.5	0.49	0.86

Live weight gain was increased (P=0.056) by biochar and tended to be decreased (P=0.11) by nitrate replacing urea as the source of NPN (Table 3; Figures 1 and 2). DM feed conversion was improved by biochar and by urea replacing nitrate.

Table 3. Mean values for change in live weight, feed intake and DM feed conversion of local “Yellow” cattle fed cassava root and cassava foliage supplemented or not with biochar and with urea or potassium nitrate as NPN source

	Biochar		Prob.	NPN		**Prob.**	**SEM**
	BIO	NOBIO		Nitrate	Urea		
Live weight, kg							
Initial	83.5	82.7		83.8	83.5		11.8
Final	96.6	94.3	0.69	94.5	96.4	0.987	3.7
LW gain, g/day	129	103	0.056	105	127	0.11	7.0
DM intake, g/day	2252	2304	0.90	2234	2279	0.989	252
DM conversion	19.1	23.2	0.031	22.8	19.5	0.009	2.77

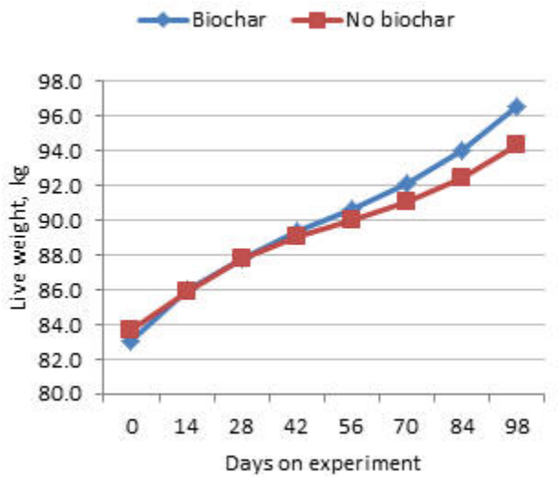

Figure 1. Growth curves of "Yellow"cattle fed cassava root and cassava foliage supplemented or not with biochar and with urea or potassium nitrate as NPN

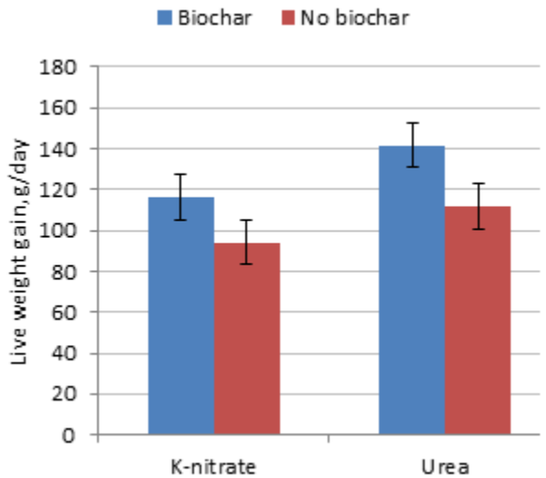

Figure 2. Effect of biochar and source of NPN on growth rate of "Yellow" cattle fed cassava root and cassava foliage

Both biochar and nitrate reduced methane production, the effects being additive for the combination of biochar and nitrate (Table 4 and Figures 3 and 4).

Table 4. Mean values for: concentrations of methane and carbon dioxide in mixed air and eructated gases and in background air and in in air; and for ratios of methane to carbon dioxide in mixed air and eructed gases in local "Yellow" cattle fed cassava root and cassava foliage supplemented or not with biochar and with urea or potassium nitrate as NPN source

	Biochar			NPN			
	BIO	NOBIO	Prob.	Nitrate	Urea	Prob	SEM
Mixed gas, ppm							
CO_2	2647	2351	<0.001	2454	2545	0.026	57.0
CH_4	76.2	85.7	0.066	68.0	93.9	<0.001	3.56
CO_2#	2234	1938	<0.001	2041	2132	0.26	57.0
CH_4#	64.0	84.5	0.066	68.1	99.3	<0.001	
Ratio: CO_2:CH4	0.0332	0.0418	<0.001	0.0433		<0.001	0.0012

 # Corrected for concentrations of CO_2 and CH_4 in background air which were 413 and 1.97 ppm, respectively

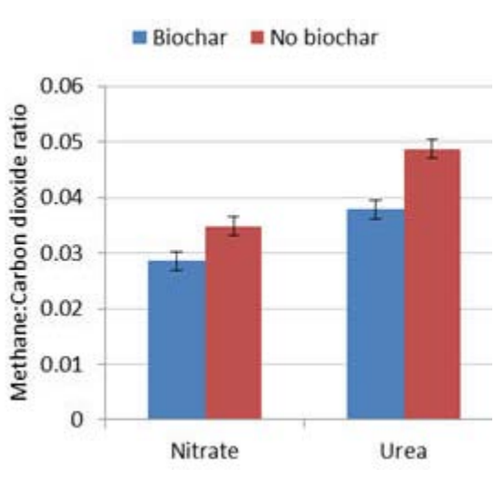

Figure 3. Effect of biochar and NPN source on ratio of methane to carbon dioxide in mixed air and eructed rumen gases for "Yellow" cattle fed cassava root and cassava foliage

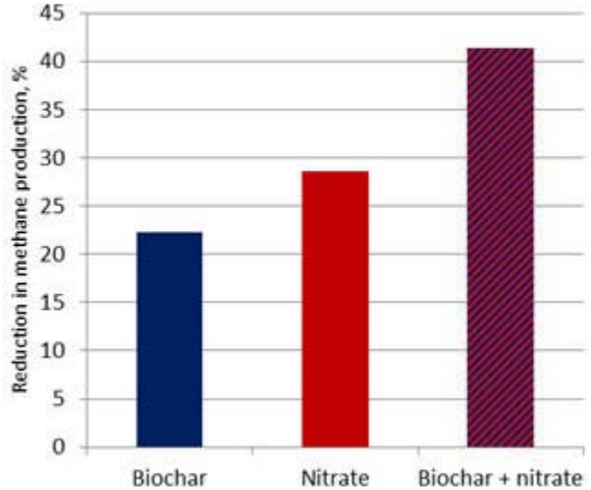

Figure 4. Reduction in methane due to biochar and nitrate in local "Yellow" cattle fed cassava root and cassava foliage supplemented or not with biochar and with urea or potassium nitrate as NPN source

Rumen pH and ammonia were increased by biochar and were greater with urea than with nitrate as NPN source (Table 5).

Table 5. Mean values for pH and ammonia in rumen fluid from local "Yellow" cattle fed cassava root chips, and fresh cassava foliage with or without biochar and nitrate or urea as NPN source

	Biochar		Prob.	NPN source		SEM	Prob.
	BIO	NOBIO		Urea	Nitrate		
Rumen pH	7.2	7.0	<0.001	7.13	7.06	0.02	0.030
NH_3, mg/litre	207	187	<0.001	205	190	2.92	0.005

Discussion

Charcoal produced by traditional carbonization of bamboo was reported by Do Thi Van et al (2006) to increase growth rates in goats fed foliage of *Acacia mangium.* However, as far as we are aware, this is the first report showing beneficial effects on growth and feed conversion, and on reduction of enteric methane emissions, from adding biochar to the diet of growing cattle. The reduction in enteric methane due to the biochar corroborates earlier findings in an *in vitro* incubation with rumen fluid and the same substrates (Leng et al 2012).

The reduction in methane production when nitrate replaced urea is in line with almost all other reports in the literature (see review by Cottle et al 2011). In contrast, the indication of poorer growth and feed conversion with nitrate, relative to urea, adds to the uncertainty relating to the effect of nitrate on production parameters in ruminant animals. Most of the reports on this topic show no effect on growth and feed conversion in goats (Anh Nguyen Ngoc et al 2011; Sophea and Preston 2011), in sheep (Thanh et al 2011) and in cattle (Phuong et al 2012). Similarly, milk production was not affected by nitrate supplementation of sheep (Sabri Yurtseven et al 2009) and dairy cows (Van Zijderveld et al 2011). There appears to be only one report that nitrate supplementation improved better growth rate and feed conversion when the basal diet was lime-treated rice straw fed to local Yellow cattle (Sangkhom et al 2012).

Many research scientists have emphasized the critical need for methanogenesis in maintaining a low partial pressure of hydrogen in the rumen which allows both simple and

complex structural carbohydrates to be fermented to short chain VFA with the coupling of ATP production with growth of microbial cells. In the rumen and most other anaerobic processes (waste water treatment, biodigestors and sediments) microbes carry out the processes of fermentation and mineralization through organized consortia arranged within a self-produced polymeric substance (EPS) (see Vu et al 2009), which forms the basis for biofilm matrix (Costerton 2007). Hydrogenotrophic organisms are necessarily in close contact with both the hydrolytic and fermentative consortia that break down relatively inert plant materials to end products that provide nutrients to the animal at sufficient rates to support productive processes.

Until recent years the microbial ecology of the rumen has been a neglected area. In the early 1960's the rumen was viewed as a mixed milieu of planktonic bacteria and protozoa. The involvement of anaerobic fungi were reported in the 70's (Orpin 1974) and the need for microbes to adhere to particulate materials to facilitate digestion of structural carbohydrates was recognized sometime after this (see Cheng et al 1995).The concept of the biofilm mode of digestion in the rumen started to be unraveled in key laboratories in the early 1900's (see Costerton 2007). However surprisingly few established authorities in the field of ruminant nutrition appear to have grasped the concept of the biofilm mode of digestion and its importance to achieving significantly high rates of fermentative digestion (see Wang and Chen 2009), and even recent publications from sources such as FAO (Background paper No 61 see McSweeney and Mackie 2012) make no mention of the biofilm research that has revealed the importance of this mode of digestion in ruminant nutrition (see McAllister et al 1994). However, considerable emphasis has been placed on the importance of microbes adhering to feed particle surfaces for the initial hydrolysis of the structural carbohydrate components exposed on the surface of feed particles (see Wang and McAllister 2002).

Leng (2011) has recently discussed the mechanism of fermentation in the rumen emphasizing the important roles of the biofilm consortia on (or in) feed particles in the breakdown of feed particles.

Methanogens and other hydrogenotrophs have been found to reside in the biofilm adhering to solid substrate surfaces or inside the feed particles in pockets where fermentable substrate can be readily accessed by hydrolytic and fermentative microbes (Cheng et al 1981; McAllister et al 1994) They are positioned on the outer layers of the biofilm where they access the hydrogen diffusing from the site where fermentation of carbohydrates is occurring (Song et al 2005). Biofilms with a high level of digestion ability are always composed of complex multi-species in layers (Stoodly et al 2002) where the distance between one group's metabolic end products are close and therefore readily available as substrate to the next group. Feedback inhibition by end product build-up from one colony of organisms, affects all colonising organisms, so the removal of end products by capture of these by other organisms in this way results in enhanced breakdown of feed particles allowing a many fold increase in cellulose breakdown as compared to that in microbial communities that are planktonic and not in organised consortia (see Wang and Chen 2009). This particularly applies to the removal of hydrogen that has a negative feedback on the oxidation of reduced cofactors produced in fermentation. In the" normal rumen", methanogens maintain a suitably low hydrogen tension in the biofilm to allow fermentation to progress at a rate that optimises the breakdown of feed particles.

Methane emissions from anaerobic biological sources are a balance between production by methanogenic *Archae* and oxidation by an - as yet to be characterized - methanotrophic consortia (Knittel and Boetius 2009). Methane oxidation has been reported in both aerobic and anaerobic environments (Hanson and Hanson 1996). Stocks and McCleskey (1964) isolated methane-oxidising bacteria/Archae from the rumen of steers that were similar to methanotrophic anaerobes isolated from soil and water and Mitsumori et al (2002) demonstrated that methanotrophs were present in both rumen fluid and in biofilm attached to the rumen wall although it appears that an insignificant amount of the methane was anaerobically oxidized (Kajikawa and Newbold 2000; Kajikawa et al 2003).

Biochar amendement greatly increased the ratios of methanotrophic to methanogenic abundances in paddy soils (Feng et al 2012) which led us to test a hypothesis that increasing the potential microbial habit (inert surfacs) in the rumen by adding biochar would lower the net yield of methane. In an *in vitro* incubation of rumen fluid, that had not been adapted to the presence of biochar, a 15% net reduction in methane release (Leng et al 2012) resulted when biochar was present. The question raised by this research is "does a biochar with its relatively large surface area (see Photo 6) (published with permission at http://en.wikipedia.org/wiki/BET_theory) and highly porous structure provide a favourable habitat for the organisms involved in a methanogenic- methanotrophic interaction, increasing the potential for anaerobic methane oxidation. We hypothesised here that methanotrophic consortia form on inert surfaces in the presence of methane which may also be associated with the same surfaces such as are available in biochar. The results of the *in vitro* studies have been repeated here showing that biochar reduces the net methane production. From the above discussion it is proposed that this is at least partially a result of increased surface area of inert material allowing a larger and better habitat for methane oxidation and microbial growth efficiency in general.

The BET surface area is a measure of the ability of a material to absorb gases (Brunauer et al 1938) and therefore its accessible surface for microbial attachment. Biochars often

have BET surface areas of 2-40 m^2 /g biochar but much greater surface areas maybe produced by particular production technologies (Day et al 2005). As shown in the electron micrograph (Photo 6) the potential to create surfaces that become habitats for biofilm residing microbes is substantial. One explanation now is that the inert material is providing a habitat for microbes within the biofilm increasing the efficiency and rapidity of the interactions leading to a higher efficiency of microbial growth. Biochar has a large surface area to weight depending on how it is produced (largely the temperature and starting material for the production of biochar). In our studies rice hull biochar was used which was produced at a temperature greater then 400^{o}C which should result in a large surface area to weight (see Chen et al (2011). Day et al (2005) showed that process temperature greatly affects the surface area of biochars; in one study the surface area increased from 120 m^3/g at a production temperature of 400^o C to 460 m^3/g at 900^{o}C (Day et al 2005). · Recent research has shown that where biochar has been added to biodigesters the rate and efficiency of methane production has been increased (Sangkom et 2012); and this research together with the reported effect of 1% biochar in the *in vitro* study of methane production by un-adapted rumen fluid from cassava root meal, which indicated a 15% decrease in net methane release (Leng et al 2012), raises questions: (i) is the additional surface area for microbial establishment in close association with soluble substrate responsible?; or (ii) is it possible that anaerobic methanotrophs are supported within a biofilm associated with the biochar surface in population densities sufficient to increase methane oxidation?. A further possibility is that the biochar improves the efficiency of microbial growth through closer association of microbial colonies, increasing the efficiency of ATP production and utilisation. Such an increased microbial cell production (microbial cells are more reduced than the substrate) may be responsible for the lowered methane production. If the latter is correct then increased efficiency of feed utilisation for growth and other productive indices should be improved particularly on low true protein diets where the N source is mainly nitrate or urea (see Leng 2004, 2005).

The concept may also explain why 25g of sodium bentonite in the diet of sheep improved wool growth (Fenn and Leng 2000). Bentonite is a montmorilanite clay that has been shown to improve protein nutrition in ruminants (Fenn and Leng 1989). Bentonite like biochar has no known nutritional attributes but has a large surface area to weight ratio due to its porosity. It is again possible that bentonite's beneficial effects are associated with improved microbial habitat where microbial consortia can come together for mutual benefits and efficient use of each of their metabolic end products.

Results from incubating rumen fluid from cattle fed diets with or without biochar (Inthapanya et al 2012) indicate that added biochar gave the greatest reduction in methane production in rumen fluid from animals adapted to biochar. This is possibly due to a larger population of methanotrophs in the system allowing greater oxidation but it could also indicate a population of organisms producing more reduced end products of feed break down such as propionate and a higher growth efficiency of the digesting microbial colonies.

In these studies we have not only demonstrated the effect of biochar for mitigation of methane production from ruminant animals but also that biochar increases the growth rates of young cattle and increases the efficiency of feed conversion. We hypothesize that these benefits are imposed by increasing microbial habitat in the rumen which indirectly increases microbial growth efficiency in the rumen (Y-ATP) but also increases the efficiency of animal production because of an improved essential amino acid to energy in the substrates (microbial cells and VFA) absorbed (see Preston and Leng 1987) . In addition methane production is reduced perhaps through stimulation of microbial growth (microbial cells are more reduced then the substrate they use and are therefore a hydrogen sink) and perhaps by stimulating an increased biomass of the usually small biomass of microbial consortia that oxidise methane. Research is ongoing to examine the various biochars with different attributes.

These appear to be the first results reported in the literature where a biochar has been demonstrated to reduce enteric methane production and considerably more research is needed before this becomes an "economic" application. Our objective is to make these concepts available at the earliest in order to stimulate research in an area with obvious implications for amelioration of global atmospheric contamination We are adamant that this information should be in the public domain as soon as possible and, freely available to scholars world wide. We are concerned that so much of the research on the use of biochar appears to be motivated by commercial interests aimed at patenting processes and thus restricting the free flow of information. With the health of future generations already compromised by the potential adverse effects of global warming everyone has the obligation to promote as rapidly and as widely as possible technologies that are environmentally friendly, such as the widespread application of bichar where it enhances environmental quality involving the active participation of miccrobes.

Conclusions

Live weight gain was increased 25% by adding 0.62% biochar to the diet DM and tended to be decreased when nitrate replaced urea as the source of NPN. DM feed conversion was improved by biochar and by urea replacing nitrate. DM feed intake was not affected by supplementation with biochar nor by the NPN source.

- Biochar and nitrate reduced methane production by 22 and 29%, respectively, the effects being additive (41% reduction) for the combination of the two additives. .

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Go to top