Spin-off technologies from 2nd generation biofuel: potential to transform fodder quality of crop residues

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Abstract

The work on 2nd generation biofuels (biofuels based on lignocellulosic biomass rather than on grains as in 1st generation biofuel) has attracted US multi-billion dollars of investment during the last two decades. It may be feasible to utilize spin-offs from 2nd generation biofuel technologies to upgrade lignocellulosic biomass for animal feeding thereby increasing the accessibility of sugars in plant cell walls. Key processes in 2nd generation biofuel that matter for livestock feed resources are: 1) post-harvest collection and mechanical pre-treatment of lignocellulosic biomass; 2) physical-chemical-biological pre-treatment to disrupt lignin-hemicellulose-cellulose matrices, partially hydrolyze weaker linkages of pentoses in hemicellulose structures and make hexoses in cellulose more susceptible to enzymatic hydrolysis; and 3) design and application of targeted and tailored enzyme cocktails. For animal nutritionists pre-treatment technologies up to the generation of glucose (or equivalents) are interesting, from here on rumen microbes and mammalian enzymes can take over.

Three 2nd generation biofuel technologies – 1. Steam treatment, 2. Ammonia Fiber Expansion (AFEX) and 3. Two Chemical Combination Treatment (2CCT) were applied to a wide range of cereal straws and stovers. Increases in *in vitro* gas production and true *in vitro* organic matter digestibility (TIVOMD) were greatest upon 2CCT, followed by AFEX and finally steam treatment. Two Chemical Combination Treatment on average increased TIVOMD by 38.2 percentage units from 55.9 in untreated straws and

stovers to 94.1% after treatment. When fed to sheep the 2CCT had the greatest effect on livestock productivity promoting an accumulated live weight gain (LWG) after 10 weeks of 6.12 kg which was 3.7 times that of a total mixed ration containing untreated rice straw. Steam explosion treatment was less effective in increasing *in vitro* digestibility but had a dramatic positive effect on voluntary feed intake, resulting in an organic matter intake of 4% of live weight in male sheep. Applying spin-off technologies from 2nd generation biofuel to upgrading the feeding values of crop residues can transform feed resourcing and feeding. It will not be a farmers' technology but should be embedded into small and medium business enterprises.

1. Introduction

Lignocellulosic biomass from forest, agricultural wastes and crop residues is the most abundant renewable biomass on earth with a total annual production of about 10 - 50 billion metric tons (Sanchez and Cardena, 2008). About 3.8 billion metric tons are contributed by crop residues with cereals contributing 74%, sugar crops 10%, legumes 8%, tubers 5% and oil crops 3% (Lal, 2005) and crop residues are the single most important livestock feed resources in most low and middle income countries (Blümmel et al., 2014a; Duncan et al., 2016). Cellulose is the major constituent in lignocellulosic biomass ranging from about 300 to 550 g/kg followed by hemicellulose which constitutes about 150 to 350 g/kg and lignin which constitutes about 60 to 300 g/kg (Ivetic and Antov, 2013). Cellulose is a linear polymer of cellobiose which itself is made up of a glucose to glucose dimer in the β 1-4 glucan configuration. This β 1-4 glucan configuration conveys molecular stability to cellulose when compared to starch, a glucose to glucose dimer in the α 1-4 glucan configuration (Van Soest, 1994). Thus, lignocellulosic biomass is, in its essence, not that different from the primary products of cereals, the starch in grains, even though their respective accessibility to mammalian digestive enzymes is very different (Van Soest, 1994). Considering the huge quantities of lignocellulosic biomass available and the high nutritive quality of their hexose and pentose sugars, it comes as no surprise that attempts to upgrade lignocellulosic biomass for livestock fodder reach back to the beginning of the 20th century (Fingerling and Schmidt, 1919; Beckmann, 1921).

The work on 2nd generation biofuels (biofuels derived from lignocellulosic biomass) was motivated by reasons very similar to those of the early animal nutritionists: the abundance of lignocellulosic biomass and its content of basic sugars. The work on 2nd generation biofuels has attracted US multi-billion dollars of investment during the last two decades (Blümmel et al., 2014b). It may be feasible to utilize spin-offs from 2nd generation biofuel technologies to upgrade lignocellulosic biomass for animal feeding thereby increasing the accessibility of sugars in plant cell walls. The current paper explores the impact of three 2nd generation biofuel technologies on the fodder quality of a wide range of cereal straws and stovers: 1) Steam explosion treatment; 2) Ammonia Fiber Expansion (AFEX); and 3) Two Chemical Combination Treatment (2CCT).

2 Materials and Methods

2.1. Steam explosion treatment

For laboratory analysis maize stover from a superior dual-purpose hybrid, a superior dual purpose sorghum variety and two sorghum stovers purchased from the fodder market were steam-treated using intermittent live steam injection to heat stovers to 160°C for 10 minutes. After 10 min the stovers were exploded into a receiver tank and dried (Dhanalakshmi et al., 2015). For sheep feeding trials rice straw from variety MTU 1010 was purchased and steam explosion treated in an analogous fashion.

2.2. Ammonia Fiber Expansion (AFEX)

This technique was developed by Dale and Weaver (2000). During AFEX treatment, ammonia vapor is added to the biomass under moderate pressure (100 to 400 psi) and temperature (70 to 200°C) before rapidly releasing the pressure and recovering more than 95% of the ammonia used in the process. For laboratory analysis ten cereal straws and stovers from India consisting of two rice straws, three sorghum stovers, one wheat straw, two pearl millet stovers and two maize stovers were treated. (AFEX is a Trademark and no larger amounts of rice straw could be treated for feeding trials)

2.3. Indian Institute for Chemical Technology (IICT) 2 Chemical Combination Treatment (2CCT)

This technology has been developed by the Indian Institute for Chemical Technology (IICT) for biofuel production and is currently being prepared as a joint CSR-IICT and ILRI patent application. The approach is therefore only described as 2 Chemical Combination Treatment (2CCT). For laboratory analysis four pearl millet stover, three sorghum stover, two maize stover and two were treated. For sheep feeding trials rice straw from variety MTU 1010 was purchased and steam explosion treated in an analogous fashion.

2.4 Laboratory analysis

Nitrogen was analyzed by the Kjeldahl method. Neutral (NDF) and acid (ADF) detergent fibre and acid detergent lignin (ADL) were analyzed according to Van Soest et al. (1991). *In vitro* apparent digestibility and ME content were calculated from *in vitro* gas production after 24 h using the equations of Menke and Steingass (1988) but with the *in vitro* incubation procedure modified according to Blümmel and Ørskov (1993). True *In vitro* organic matter digestibilities (TIVOMD) after 24 and 48 h were analysed according to Goering and Van Soest (1970) by refluxing of incubation residues from the gas syringes as described by Blümmel and Ørskov (1993) with neutral detergent solution.

2.5 Sheep feeding trials

Three isonitrogenous total mixed rations (TMR) each consisting of about 70% untreated, 2CCT and steam explosion treated rice straw were designed and each fed to six sheep housed in metabolic cages.

The TMR were fed *ad libitum* allowing for about 10 to 15% of refusals. Measured were daily intake and liveweight changes.

3. Results

3.1 Comparison of effectiveness of steam explosion, AFEX and 2CCT treatment on in vitro measurements

Increases in *in vitro* gas production (GP) and true IVOMD measured after 48 h of incubation were greatest upon 2CCT followed by AFEX and finally steam treatment (Table 1). The increases in *in vitro* GP were 10, 20 and 68% upon steam explosion, AFEX and 2CCT treatment, respectively. TIVOMD increased by 14, 30 and 68% respectively upon steam explosion, AFEX and 2CCT treatment, respectively. In absolute terms after 48 hr of incubation AFEX and 2CCT on average increased true IVOMD by 19.3 (65.1 to 84.4%) and 38.2 (55.9 to 94.1%) percentage units, respectively.

Table 1 about here

3.2 Effectiveness of steam explosion and 2CCT treatment on rice straw in total mixed rations (TMR) on laboratory fodder quality traits

Organic matter, CP, NDF, ADF, ADL, *in vitro* GP and TIVOMD measured after 24 and 48 hr in the TMRs consisting of about 70% of untreated and two chemical combination (2CCT) and steam explosion (SE) treated rice straw are reported in Table 2. The TMRs were close to isonitrogenous. NDF, ADF and ADL seem unsuitable laboratory analytical techniques for testing effects of 2CCT and steam explosion treatments. For example, 2CCT treatment could increase NDF and ADF content over that of untreated TMR while steam explosion treatment increased the recovery of ADF over NDF and almost doubled the ADL content relative to untreated TMR.

The *in vitro* GP and TIVOMD responded to treatments in that GP more than doubled when compared after 24 hr of incubation and was 84% higher when compared after 48 hr in 2CCT TMR relative to control

TMR (Table 2). TIVOMDs also increased by 50 and 40% after 24 and 48 hr over control TMR, respectively reaching more than 90% of TIVOMD. Steam explosion treatment resulted in 23 and 12% higher GP after 24 and 48 hr, respectively, relative to control TMR while TIVOMD was 28 and 21% higher after 24 and 48 hr, respectively.

Table 2 about here

3.3 Effectiveness of steam explosion and 2CCT treatment on rice straw in total mixed rations (TMR) fed to sheep

The responses of sheep fed TMR consisting of about 70% untreated, steam explosion and 2CCT treated rice straw are reported in Figure 1. The 2CCT treatment had the greatest effect on livestock productivity promoting an accumulated live weight gain (LWG) of 7.85 kg, which is 3.4 times that of the TMR containing untreated rice straw. While steam treatment was less effective in increasing *in vitro* digestibility than 2CCT (Table 1) its positive effect on voluntary feed intake was found to be dramatic, resulting in an organic matter intake of about 4% of live weight in male sheep! This very high intake promoted accumulating LWG of 6.28 kg., which is 2.7 times that of sheep fed TMR with untreated rice straw.

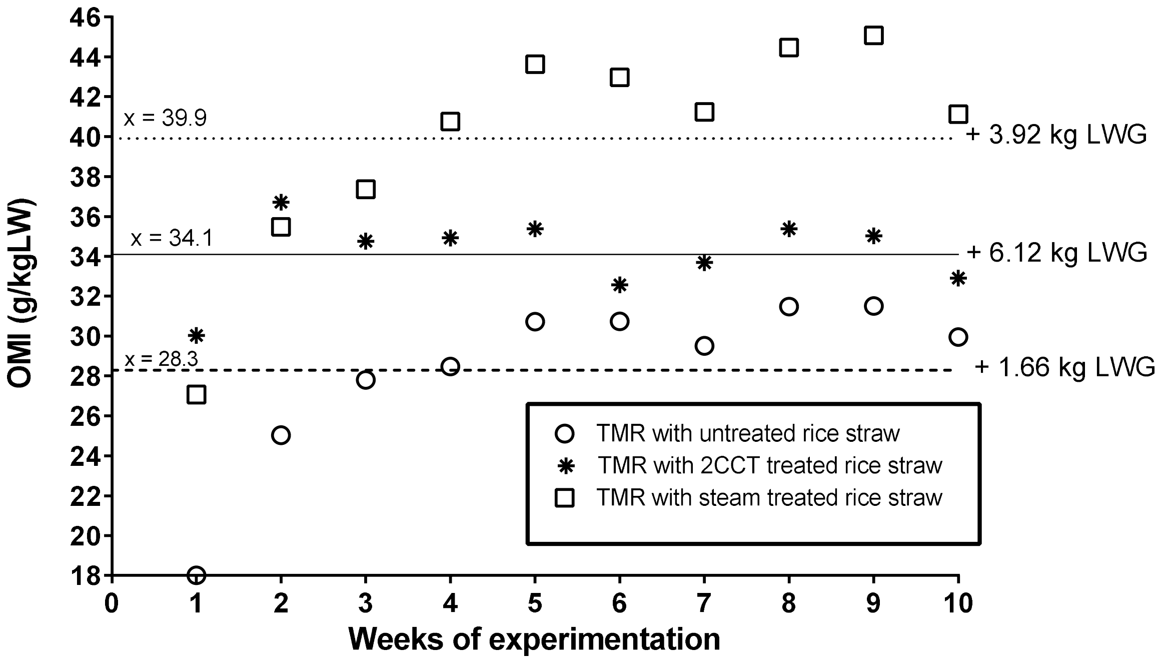

Figure 1: Response of sheep fed total mixed rations containing 70% of untreated, 2CCT treated and steam explosion treated rice straw

4. Discussion

4.1 Urgency of increasing fodder quality of crop residues such as straws and stover

Feed, both shortage and costs, acts as a major constraint to higher livestock yields; this feed constraint will become more binding with the increasing demand for animal sourced food (ASF). Opportunities for improving feed resources are constrained by shortages of arable land and increasingly water; and these constraints are likely to become aggravated by climate change (Blümmel et al., 2015). Feed supply-demand scenarios for South Asia and East and West Africa have shown that crop residues such as straws, stover and haulms are generally the single most important feed resources often providing between 50 and 70% of the feed resources in small holder systems (Blümmel et al., 2014a; Duncan et al. 2016). Crop residues are considered to be of low fodder quality, though this is essentially only true for cereal crop residues since leguminous residues can have excellent fodder quality. Even the poor fodder quality of cereal crop residues is not because of an inherent low nutrient content but due to the molecular formation of its basic sugars. In ligno-cellulose, glucose to glucose dimers are in the β 1-4 glucan configuration and this β 1-4 glucan configuration conveys molecular stability to ligno-cellulose when compared to starch, a glucose to glucose dimer in the α 1-4 glucan configuration (Van Soest, 1994). Thus, lignocellulosic biomass is, in its essence, not that different from the primary products of cereals, the starch in grains, even though their respective accessibility to mammalian digestive enzymes is very different (Van Soest, 1994). The high content of basic sugars of ligno-cellulose biomass of course prompted the 2nd generation biofuel work with the aim of converting ligno-cellulose biomass into Ethanol. Blümmel et al. (2014b) argued that technologies developed for converting ligno-cellulose biomass into ethanol could be useful in animal nutrition to make basic sugars in lignified plant cell walls more accessible to rumen microbes and even mammalian enzymes.

4.2 Effectiveness of steam explosion, AFEX and 2CCT treatment on laboratory fodder quality traits

The current work investigated three 2nd generation biofuel technologies, steam explosion, AFEX and 2CCT. These three technologies are in order of increasing complexity in that steam explosion treatment is based only on water and pressure (Dhanalakshmi et al., 2015), AFEX on one chemical (ammonia) and mild pressure (Dale and Weaver, 2000) and 2CCT (Blümmel et al., 2018) on two chemicals, one pH changing and the other one oxidative. Increases in *in vitro* GP and TIVOMD measured after 48 h of incubation were greatest upon 2CCT, followed by AFEX and finally steam explosion treatment (Table 1). The increases in *in vitro* GP were 10, 20 and 68% upon steam explosion, AFEX and 2CCT treatment, respectively, while TIVOMD increased by 14, 30 and 68% upon steam explosion, AFEX and 2CCT treatments, respectively (Table 1). In absolute terms increments of TIVOMD upon steam, AFEX and 2CCT treatments were 8.9, 19.3 and 38.2 percentage units, respectively (Table 1). Particularly AFEX and 2CCT treatments will have potentially very significant impacts on fodder quality of cereal straws and stovers if these TIVOMDs can be translated into animal performance, in effect turning cereal crop residues into concentrates (Table 1).

The TIVOMD measurement is gravimetric in nature and calculated from the truly undegraded residue; all substrate not recovered is supposed to have been fermented. This might not always be the case particularly in treated feed stuffs where some unfermentable substrate might have been solubilized and so not recovered in the incubation residue (Blümmel et al., 2005). There is some indication that this might have happened upon steam explosion and AFEX treatments where percentage increases in *in vitro* GP were less than increases in TIVOMD. In 2CCT, however, the increase in TIVOMD of 68% agreed with the average increase *in vitro* GP of 66% (66.7 vs 39.7 ml) and *in vitro* GP reflects generation of fermentation products and so is not gravimetric in nature. Put differently, the tremendous increases in straws and stover quality upon 2CCT treatment seem real.

4.3 Effectiveness of steam explosion and 2CCT treatment on sheep performance

The effectiveness of steam explosion and 2CCT treatments were not restricted to laboratory fodder quality traits, as shown by feeding of TMRs with untreated and treated rice straw to sheep. The responses of sheep fed TMR consisting of about 70% untreated, steam explosion and 2CCT treated rice straw are reported in Figure 1. The 2CCT treatment had the greatest effect on livestock productivity promoting an accumulated live weight gain (LWG) 7.85 kg which is 3.4 times that of the TMR containing untreated rice straw. While steam treatment was less effective in increasing *in vitro* digestibility than 2CCT (Table 2) its positive effect on voluntary feed intake was found to be dramatic, resulting in an organic matter intake of about 4% of live weight in male sheep. This very high intake promoted accumulating LWG of 6.28 kg, which is 2.7 times that of sheep fed TMR with untreated rice straw.

5 Conclusions

Spin- off technologies from 2nd generation biofuels have considerable potential for transforming the fodder quality of crop residues. Animal nutritionists can, and should, leverage from these multi-billion investments into 2nd generation biofuel in collaboration with the private sector. Small and medium enterprises applying these spin-off technologies would: 1) very significantly increase fodder quality of crop residues thereby increasing livestock productivity at decreased feed costs; and 2) generate employment and income opportunities for rural population; and 3) decrease environmental hazards from localized burning of crop residues (mainly rice straw). We also want to stress that the three pre-treatments explored are not exhaustive and other potentially useful spin-off technology from 2nd generation biofuel are out there and need to be investigated.

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Table 1: Summary of effects of steam, ammonia fiber expansion and 2CC treatment on *in vitro* gas production (GP) and true *in vitro* organic matter digestibility[-1] after 48 h of incubation. U = untreated; T = Treated

Spin-off technology	n	*In vitro* GP after 48 h (ml/200 mg)		True IVOMD after 48 h (%)	
		U	T	U	T
Steam Treatment	4	48.6	53.6	62.9	71.8
AFEX Treatment	10	42.9	51.5	65.1	84.4
2CC Treatment	11	39.7	66.7	55.9	94.1

[-1] The average difference between true and apparent IVOMD is about 12.9 percentage units (van Soest, 94).

Table 2: Organic matter (OM), crude protein (CP), neutral (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL) *in vitro* gas production (GP) and true *in vitro* organic matter digestibility (TIVOMD) measured after 24 and 48 hr in total mixed rations (TMR) consisting of about 70% of untreated and two chemical combination (2CCT) and steam explosion (SE) treated rice straw

Treatment	**OM**	**CP**	**NDF**	**ADF**	**ADL**	**GP 24**	**GP 48**	**TIVOMD 24**	**TIVOMD 48**
			%			ml		%	
Untreated	81.7	12.7	56.6	43.7	3.7	27.9	37.7	60.7	68.0
2CCT	85.8	12.4	63.6	53.2	2.1	58.4	69.3	90.8	94.7
SE	80.1	12.7	41.1	47.4	6.1	34.4	42.3	77.9	82.5
P > F	<0.0001	0.5	<0.0001	0.002	0.0001	<0.0001	<0.0001	<0.0001	0.0001
LSD	0.80		2.3	3.5	0.91	6.4	5.7	2.4	5.9

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