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*More meat, milk and eggs by and for the poor*

# Leverage spin-off effect from 2<sup>nd</sup> generation biofuels to turning Indian cereal straws and stover into concentrate feeds

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## Rational

Small holder livestock production is the main stay of large parts of India's rural population but benefits from it are constrained by availability of affordable feeds. Feed costs relative to farm gate prices of produce increase, reducing farmers' income and resulting in high cost of animal sourced food (ASF) for consumers. Improvement of feed resources is severely constrained by bio-physical factors such availability of arable land and water but increasingly also by on-farm labor competition and shortages. The way forward needs, therefore, to be two-pronged: First, increase the availability of affordable off-farm produced feed. Second; use feed resources that do not compete with land and water allocation for direct food production.

Indian feed resources have been categorized and inventorized by the National Institute for Animal Nutrition and Physiology (NIANP) and are summarized in Table I. Crop residues, that is straws, stover and haulms by-product from grain production of ligno-cellulosic nature, contribute the overwhelming (>70%) bulk to feed resources for livestock production in India. At the same time there is a substantial nutrient deficit which will need to be addressed to close yield gaps in livestock production. This nutrient deficit is due to the generally poor fodder quality of crop residues. On the other hand, a significant improvement of fodder quality of crop residues will result in a transformative event in the quality of Indian feed resources.

Table I: Feed resource supply and demand in India and their quantitative and qualitative balance (from NIANP 201; Blümmel et al., 2014)

Feed Resource	Contribution to Overall Feed Resources (%)
Greens from CPR, forests, grazing, fallows	8.0
Planted forages	15.1
Crop residues	70.6
Concentrates	6.3
Balance: feed availability versus feed requirement	
Dry matter (i.e. quantity)	-6.0
Digestible crude protein	-61.0
Total digestible nutrients	-50.0

## Current status of research

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 4 billion tons of this cellulosic biomass consist of crop residues, the direct and widely available byproduct of crop production (Lal 2005). Cellulose, the major constituent in lignocellulosic biomass, is a linear polymer of cellobiose which itself is made up of a glucose to glucose dimer in the  $\beta$  1–4 glucan configuration. This  $\beta$  1–4 glucan configuration conveys molecular stability to cellulose when compared to starch, a glucose to glucose dimer in the  $\alpha$  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. This was the rationale for work on 2<sup>nd</sup> generation bio-fuels (bio-fuels 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 2<sup>nd</sup> generation bio-fuels (bio-fuels based on lignocellulosic biomass rather than on grains as in 1<sup>st</sup> generation biofuel) has attracted US multi-billion dollars of investment during the last two decades from the public and private sector. This level of funding was, and is, entirely out of the reach of animal nutritionist but our hypothesis was that it will be feasible to leverage spin-offs from 2<sup>nd</sup> generation bio-fuel technologies to upgrade lignocellulosic biomass for animal feeding by increasing the accessibility of polymerized sugars in plant cell walls (Blümmel et al., 2014). Figure 1 outlines key processes in 2<sup>nd</sup> generation biofuel value chains. Process steps that matter for livestock feed resources are: 1) post harvest collection and mechanical pretreatment of lignocellulosic biomass; 2) physical-chemical-biological pre-treatment to disrupt lignin-hemicelluloses-cellulose matrices, partially hydrolyze weaker linkages of pentoses in hemicelluloses structure and make hexoses in cellulose more susceptible to enzymatic hydrolysis; and 3) design and application of targeted and tailored enzyme cocktails. For animal nutritionist pre-treatment technologies up to the generation of glucose (or equivalents) are interesting, from here on rumen microbes and mammalian enzymes can take over. That is 2<sup>nd</sup> generation investments into conversion of glucose into ethanol are of less interest to animal nutrition, though opportunities for some novel protein sources might reside in some of the processes.

Crop residues are of high bulk and low density making transport expensive and lessons from 2<sup>nd</sup> generation biofuel are helpful in optimizing the relations between harvest and collection of biomass, central versus decentralized approach and physical form and transport value to pre-treatment to voluntary feed intake (Figure 1). Of major interest to ruminant nutrition are process that swell and disrupt hemicellulose-cellulose-lignin matrix, partially hydrolyze xylan structure and increase surface and porosity of fiber structure. These are usually temperature, pressure and pH mediated applied either as single or combined interventions (Blümmel et al., 2014). Application of enzymes and enzyme cocktails can further convert pre-treated biomass into glucose suitable for monogastric nutrition.

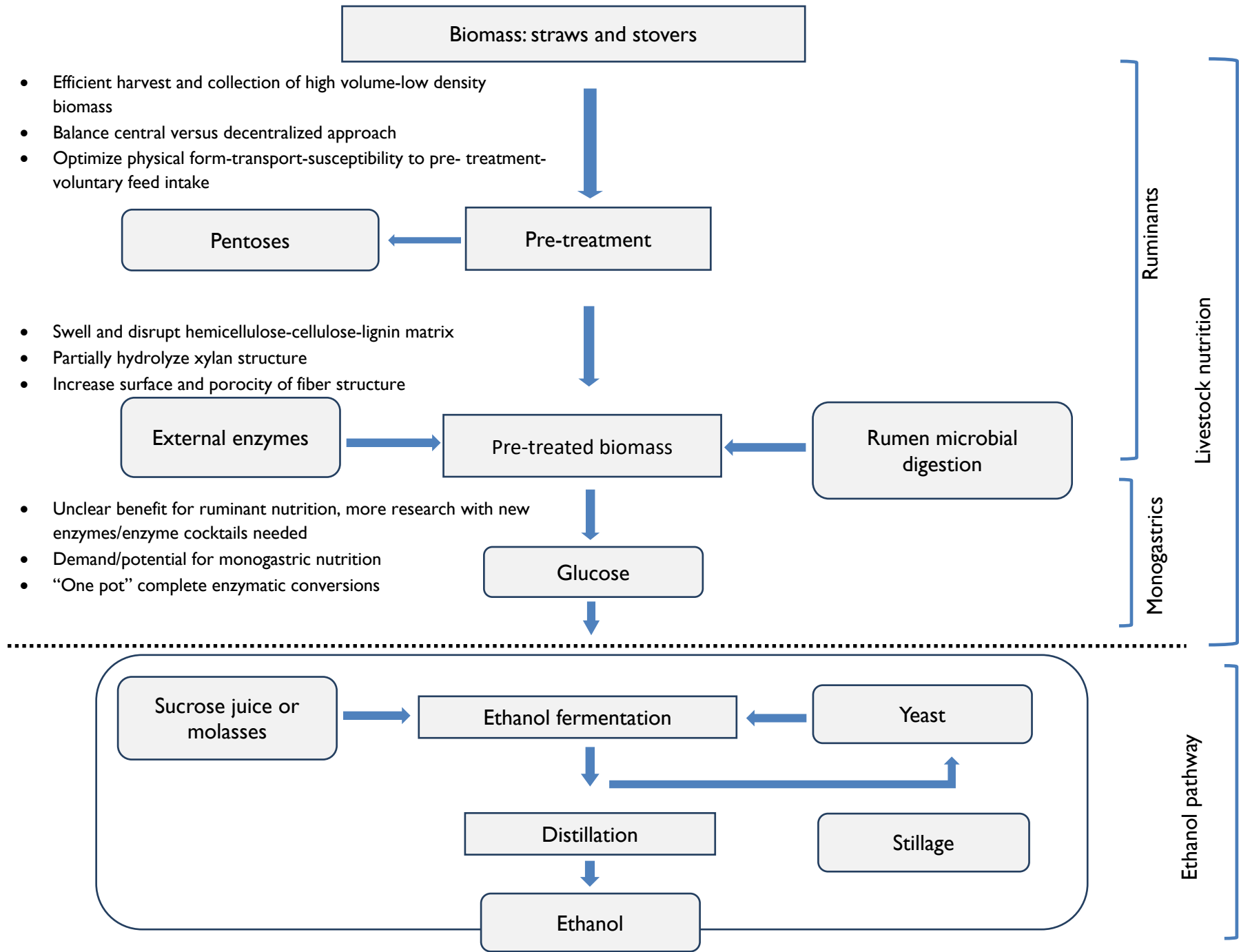


Figure 1: Process steps in second generation bio-fuel technology of interest to livestock nutrition (Blümmel et al.,2014)

## The preliminary work done so far

So far ILRI and partners have explored the impact of three 2<sup>nd</sup> generation biofuel technologies on the fodder quality of a wide range of Indian cereal straws and stovers: 1) Steam treatment, 2) Ammonia Fiber Expansion (AFEX); and 3) Two Chemical Combination Treatment (2-CCT). All three technologies were developed for converting ligno-cellulosic biomass into ethanol.

*Steam explosion:* Steam explosion was developed by Nargajuna Fertilizer and Chemicals Research and Development Center (R&D) in India as pre-treatment potentially effective without pH interventions. In a collaboration between ILRI and Nargajuna R&D maize stover from a superior dual purpose hybrid, a superior dual purpose sorghum variety and two sorghum stovers purchased from 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. Details of this work have been reported by Dhanalakshmi et al. (2015).

*Ammonia Fiber Expansion (AFEX):* This technique was developed by Dale and Weaver (2000) at the Michigan Biotechnology Institute (MBI), and is a TRADEMARK product: 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. In a collaboration between ILRI and MBI 10 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 investigated. Details of this work have been reported by Blümmel et al. (2018a).

*Indian Institute for Chemical Technology (IICT) 2 Chemical Combination Treatment (2CCT):* This technology has been developed by IICT a CSIR institute in Hyderabad. In a collaboration between ILRI and IICT four pearl millets stovers, three sorghum stover, two maize stover and two wheat straws were treated. Details of this work have been reported by Blümmel et al. (2018b).

## Comparison of effectiveness of steam, AFEX and 2-CCT treatment on *in vitro* measurements

Increases in *in vitro* gas production (GP) and true *in vitro* organic matter digestibility (IVOMD) measured after 48 h of incubation were greatest upon 2-CCT followed by AFEX and finally steam treatment (Table 2). After 48 h of incubation, steam treatment on average increased *in vitro* gas production significantly by 10% and *in vitro* true organic matter degradability by 14% or 8.9 percentage units (71.8 vs 62.9%), respectively. AFEX treatment increased *in vitro* gas production from 42.9 to 51.5 ml and *in vitro* true IVOMD from 65.1 to 84.4% that is by 19.3 % units. Two Chemical Combination Treatment on average increased true IVOMD by 38.2 percentage units from 55.9 in untreated straws and stovers to 94.1% after treatment. Such an increase is amazing and converts the straws and stovers in essence into concentrate feeds. The true IVOMD measurement is gravimetric in nature and calculated from the truly undegraded residue; all substrate not recovered is supposed to have been digested. This might not always be the case particularly in treated feed stuffs where some undigestible substrate might have been solubilized and so not recovered in the incubation

residue (Blümmel et al., 2005). However, the increases in true IVOMD of agreed with average increases in in vitro GP and GP reflects generation of fermentation products and is so not gravimetric in nature. Put differently, the increases in straws and stover quality are real.

Table 2: Summary of effects of steam, ammonia fiber expansion and 2CC treatment on in vitro gas production (GP) and true in vitro digestibility-I 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

-I The average difference between true and apparent IVOMD is about 12.9 percentage units (van Soest, 94). Increments in digestibility are similar independent of expression as apparent or true digestibility (Blümmel et al., 2018).

Preliminary in vivo observations using suggest that steam and 2-CCT treatments also increase voluntary feed intake that is treatments do not affect palatability, to the contrary they increase feed intake. That is treatments have additive effects: much higher quality per unit straw and stover plus higher feed intake. Preliminary

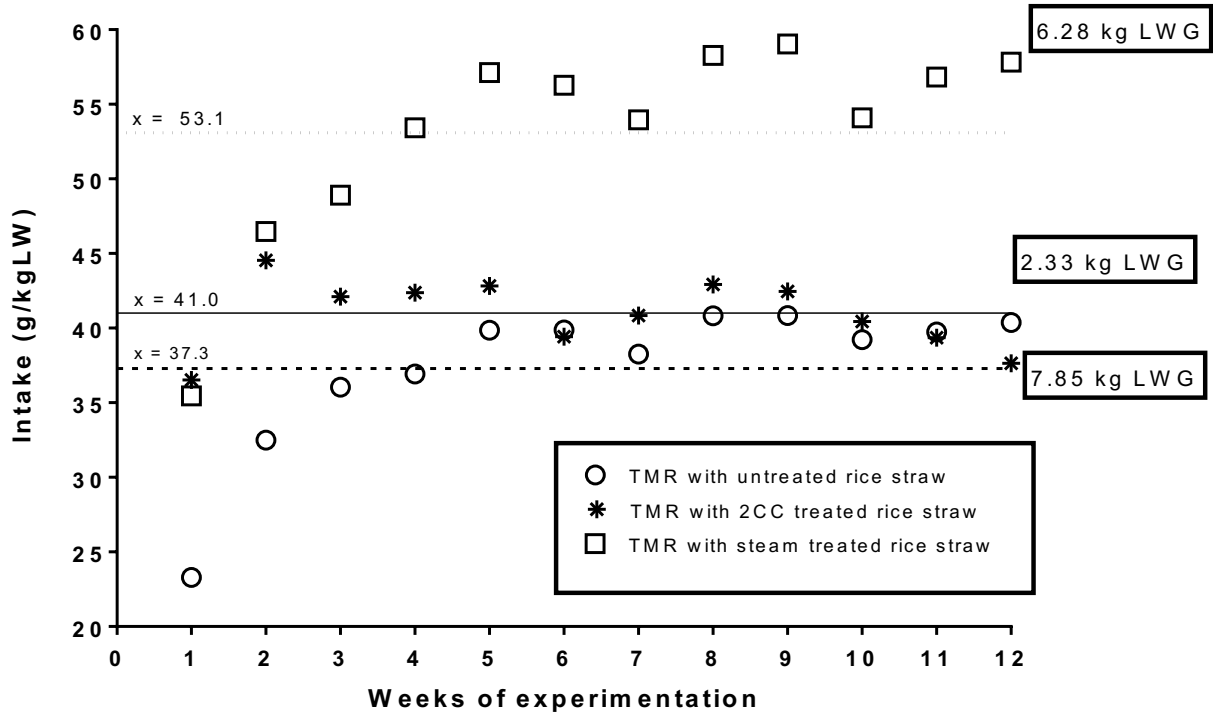


Figure 2: Comparison of untreated and steam and IICT treated rice straw when contributing about 70% to an iso-nitrogenous (12% CP) total mixed ration. Experimental animals were sheep (Blümmel et al., unpublished)



The 2CCT treatment had the greatest effect on livestock productivity promoting an accumulated live weight gain (LWG) 7.85 kg which is 3.7 times that of the TMR containing untreated rice straw. These findings give collateral to the statement derived from Table 2 that crop residues can be turned into concentrates by 2CCT treatment. Interestingly 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 intake of 5.3% of live weight in male sheep! This very high intake promoted accumulating LWG of 6.28 kg. digestibility. Steam treatment should, therefore be also be further explored together with 2CCT.