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Looking for Opportunities from Second Generation Bio-fuel Technologies for Upgrading Lignocellulosic Biomass for Livestock Feed

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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 billion metric tons (Sanchez and Cardena, 2008). About 4 billion tons consist of crop residues; the direct and widely available byproduct of crop production (Lal, 2005). Cellulose is the major constituent in lignocellulosic biomass ranging from about 30 to 55% followed by hemicellulose which constitutes about 15 to 35% and lignin which constitutes about 6 to 30%. 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 α 1-4 glucan configuration (Van Soest, 1994). Thus lignocellulosic biomass, say from crop residues 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). Mammals that utilize lignocellulosic biomass, notably ruminants, can do so because they host microbial populations in their fore-stomach that secrete enzymes that hydrolyze and break down β 1-4 glucan linkages and make pentoses and hexoses more accessible. However, even ruminants can digest lignocellulosic biomass only partially, and important contributors to livestock feed resources particularly in developing and transition countries such as the crop residues are conventionally considered to be of poor fodder quality. Nevertheless the widespread availability of crop residues and their importance as feed resource in crop livestock systems marks them as a strategic natural resource of high order (Blümmel et al., 2012). Furthermore, it is important to realize that crop residues are feed resources that do not need allocation of water and land, because the crops are grown for the production of the primary products of grains and pods (Dale et al., 2010; Blümmel et al., 2012).

Past efforts of upgrading lignocellulosic biomass, impact on livestock productivity and adoption of interventions

Considering the huge quantities of lignocellulosic biomass available and the high nutritive quality of their basic constituents, the hexose and pentose sugars, it comes as no surprise that attempts on upgrading lignocellulosic biomass for livestock fodder reach back to the beginning of the 20th century (Fingerling and Schmidt, 1919; Beckmann 1921). These and later attempts included chemical, physical and biological treatments but chemical treatments received maximum attention of researchers, particularly the use of hydrolytic agents such as NaOH and NH_4 (for review see Jackson 1977 and Owen and Jayasuriya, 1989). However, comparatively little uptake of these technologies was observed, even though considerable efforts were expanded by the international research community and development practitioners (Owen and Jayasuriya, 1989). For example, Owen and Jayasuriya (1989) listed and reviewed 12 major international conferences addressing the improved use of lignocellulosic biomass for livestock feed from 1981 to 1988 and concluded that large scale adoption of treatment interventions was very rare and un-sustained once project activities ceased this, despite the many efforts expended on simplifying treatment technologies and use of locally available materials and inputs.

There exist several explanations for the low adoption of these technologies for the upgrading of lignocellulosic biomass. Despite the simplification of technologies and use of locally available material, labor and costs and availability of inputs might still have posed major constraints. On the other hand, at the time of peak research and development activities in the 70s and 80s, too little attention was given to the socio-economic conditions of farm households and communities, particularly with regards to market orientation and opportunity, respectively the lack thereof. Where famers benefit little from higher livestock production and productivity, incentives for technology were absent. Occasionally doubts were also raised about the biological significance of achievable increases in lignocellulosic biomass digestibility, suggesting that the increases in the 5 to 10% percent unit range commonly observed would not raise livestock productivity in a significant enough way. However, these reservations have been generally rejected for several reasons. First, empirically farmer and fodder traders were well aware of differences in fodder quality in crop residues even within a species. This would hardly have been the case if crop residue fodder quality differences did not matter for livestock performance, after all these differences were large enough to effect adoption of new crop cultivars in mixed crop livestock systems (Kelley et al., 1996). Surveying commercial sorghum stover fodder traders in India, Blummel and Rao (2006), observed that a mean difference of 5% unit in *in vitro* stover digestibility was associated with price premiums of 25% and higher. These findings agreed well with ex-ante assessments by Kristjanson and Zerbini (1999), who estimated that a one-percentage unit increase in digestibility in sorghum and pearl millet stover would result in increases in milk, meat or draught power outputs ranging from 6 to 8%. Second, experimental evidence from livestock productivity trials showed that intuitively small differences in fodder quality in forages and roughages can have large effects. As shown by Vogel and Sleper (1994), differences of 3 to 5% units in forage digestibility were associated with 17 to 24% differences in livestock productivity. It is often not appreciated that increases in fodder quality through increased digestibility (or metabolizable energy content) upon treatments (or crop breeding and selection for that matter) result in: 1) more available nutrients per unit fodder; and 2) higher voluntary feed intake. This can be demonstrated by revisiting an influential key publication about NH_4 treatment of cereal straws and the prediction of their voluntary feed intake (Ørskov et al., 1988; Blummel and Orskov, 1993). Ammonia treatment increased straw digestibility on average by 34% and voluntary feed intake by 23% resulting in a doubling of daily weight gain in steers from 213 to 483 grams per day. Thus an average increase in digestibility of 11.2 percent units was associated with an increase in livestock output of 127%. In other words, a one unit increase in digestibility was associated with an increase in livestock output of about 11%. These interpolations agree generally well with the gist of above described work by Vogel and Sleper, (1994); Kristjanson and Zerbini, (1999); Blummel and Rao, (2006) proving that small differences in digestibility can matter for livestock performance. Table 1 compares the In Vitro digestibility, dry matter intake and weight gain in both untreated and ammonia treated barley, wheat and oat straw.

Relevant spin-off technologies from second generation biofuel for upgrading lignocellulosic biomass for livestock feed

As mentioned above, attempts to break β-glucosidic linkages to release glucose from plant cell walls through change of pH (sulphuric acid, ammonia, urea and ammonium hydroxide), steam, temperature and pressure stretch back more than 100 years but never matured into technologies that could be routinely used and that were widely adopted in agriculture and food industry. The recent global interests in 2nd generation bio-fuel technologies with billions of US $ investments from private and public sector can change this, and the development of cost-effective technologies for the conversion of lignocellulosic biomass to sugars could herald a livestock feeding revolution (Dixon et al., 2010; Dale et al., 2010). For 2nd generation bio-fuel technologies to succeed, economically efficient and environmentally acceptable technologies were/are required for hydrolysis of plant cell walls and release of glucose and other sugars from the lignified matrix. Animal nutritionists have the same objective. In addition, implementation of 2nd generation bio-fuel technologies is faced with optimizing collection and transport of low density widely dispersed biomass, processing the biomass in fermenter and distributing the produce (Sims et al., 2010). Animal feed processors and producers wanting to use cellulolytic biomass batter are facing very similar logistic and engineering problems. Second generation bio-fuel technology developments engaged a wide range of experts encompassing plant breeder and molecular geneticists, plant chemists, microbiologists/enzymologists, economists and manufacturing and process engineers. The present paper argues that from these research investments potential, spin-offs can be harvested for upgrading of ligno-cellulose

ble 1: Comparisons of untreated (U) and ammonia treated barley (A), wheat and oat straw in *in vitro* digestibility (IVOMD) and dry matter intake (DMI) and weight gain (g/d Gain) of steers

Cultivar	Treatment	IVOMD (%)	DMI (kg/d)	Gain (g/d)
Gerbel	U	27.6	3.43	106
Gerbel	A	37.8	4.70	359
Igri	U	29.5	3.56	126
Igri	A	37.5	4.82	332
Corgi	U	39.0	5.16	400
Corgi	A	54.1	5.86	608
Golden Promise	U	36.4	4.43	198
Golden Promise	A	45.6	4.93	602
Norman	U	31.7	4.57	237
Norman	A	44.8	5.81	516
Mean	U	32.8	4.23	213
Mean	A	44.0	5.22	483
Δ A/U		34%	23%	227%

Calculated from Ørskov et al. (1988)

biomass for ruminants and for making the boundaries between feed resources for ruminants and monogastrics permeable, thereby increasing the choice of feed material for these species. If ruminants achieve higher productivity on lignocellulosic biomass, they will require less concentrates which will reduce competition with monogastric animals, including humans.

Processes in second generation biofuel technologies relevant to livestock nutrition

Key processes in 2nd generation biofuel that matter for livestock feed resources are: 1) post-harvest collection and mechanical pretreatment of lignocellulosic biomass; and 2) physical-chemical-biological pretreatment to disrupt lignin-hemicelluloses-cellulose matrices, partially hydrolyze weaker linkages of pentoses in hemi-cellulose structure and make hexoses in cellulose more susceptible to enzymatic hydrolysis. The postharvest technology and mechanical pretreatment will reduce particle size which in turn will: a) affect transport and storage cost of biomass, b) partially collapse crystalline cellulose structures, c) increase surfaces and pore size of substrate thus facilitating enzymatic (microbial or otherwise) attachment and invasion; and d) influence later voluntary feed intake by livestock. Physical, chemical and biological pretreatments and their single and combined effect on 2nd generation biofuel outcomes have been discussed in detail by Sun and Cheng (2002), Hendriks and Zeeman (2009) and Agbor et al. (2011). A summary of these pretreatment methods is presented in Table 2. While increasing accessible surface area and alteration of lignin structure will have positive effect on fodder quality, toxic by-product formations (mostly furfural) might have negative implication for rumen microbes and host animal alike. Application of acids can result in removing considerable parts of the hemi-cellulose complex. The effect of solubilization of hemicellulose on fodder quality will be related to generations and ease of recovery/storage/drying of soluble fractions from the pre-treatment operation, for example, pre-treatment methods that use free water will result in separate liquid and solid streams and 10 to 40% of carbohydrates could be removed from the solid stream (Wyman et al., 2005).

Table 2: Options for pre-treatments of ligno-cellulosic biomass and effects on surface area, solubilization of hemi-cellulose, lignin (L) structure and toxic by product formations

Pre-treatment	Surface area	Solubilization HC	Structure L	Toxic BP
Mechanical	+	-	-	-
Steam treatment/stem explosion	+	+	+	+
Liquid hot water	+	+	-	-
Acid	+	+	-	+
Alkaline	+	-	+	-
Oxidative	+		+	-
Thermal + acid	+	-	+	+
Thermal + alkaline	+	-	+	-
Thermal + oxidative	+	-	+	-
Thermal + alkaline + oxidative	+	-	+	-
Ammonia fiber expansion	+	-	+	-
Carbon dioxide	+	+	+	-

Modified from Hendriks and Zeeman (2009)

In 2nd generation bio-fuel processes, pre-treatment are followed by enzymatic hydrolysis using an array of cellulases such as endoglucanase to create free cellulose chain ends, exoglucanases that generate cellobiose and β glucosidases that generate glucose residues. Cellulase enzymes can be combined with hemicellulases such as xylanase, acetylesterase β xylosidase, galactomannase and glucomannase in so called enzyme cocktails (Verardi et al., 2011). Enzymes have become more affordable, more stable and more recoverable with the investments in 2nd generation bio-fuel technologies and thus more attractive for use in animal nutrition too (see for example pertinent Feedinfo.com alerts). Enzymatic incubation methods will be very relevant for upgrading lignocellulosic biomass for use in feed for monogastrics but might add little improvement to roughage feed for ruminants that have already undergone pre-treatment processes (see also below). In untreated roughages, application of exogenous fibrolytic enzymes modified cell wall matrixes and increased *in vitro* digestibility by 2 to 3 percent units by facilitating rumen microbes easier access to cell wall structures (Van de Vyver and Cruywagen, 2013). However, many of the proposed pre-treatments (Table 2) will have this effect too.

Processes in second generation biofuel technologies applied to livestock nutrition

From the pre-treatments approaches summarized in Table 2, only the Ammonia Fiber Expansion (AFEX) treatments (Dale and Moreira, 1982) and its newer version FIBEX (Dale and Weaver, 2000) were systematically investigated for application to livestock feed improvement (Weimer et al., 2003; Bals et al., 2010). The AFEX system operates at 80 to 150°C and 200 to 400 psi with a treatment time of 5 to 30 minutes (Bals et al., 2010). It can be calculated from the data of Bals et al. (2010), that in 11 AFEX treated roughages, the average cell wall (estimated as neutral detergent fiber, NDF) degradability (g NDF/kg Forage DM) increased from 264 gram to 475 gram upon AFEX treatment and incubation in rumen fluid. In the same work, rumen microbes removed about twice as much substrate as an enzyme cocktail (Bals et al., 2010). These findings cast doubts on the use of exogenous enzymes to further increase hydrolysis of pre-treated roughages by rumen microbes. The average cell soluble content (NDS) of the forages in (Bals et al., 2010) can be calculated as 263 g NDS/ kg Forage DM and the total mean true digestibility in the untreated forages will then be 527 g/ kg Forage DM while the AFEX treated forages will have a mean true digestibility of 738 g/Forage DM. This is equivalent to an increase in digestibility of 21 percent units. When relating these increases to above described considerations by Vogel and Sleper (1994), Kristjanson and Zerbini (1999) and Blummel and Rao (2006), it appears evident that 2nd generation biofuel pre-treatment technologies can have substantial positive implication for livestock feeding. However, it needs to be pointed out that *in vitro* substrate digestibility measurements applied by Bals et al. (2010), were gravimetric techniques that relied on quantifications of undegraded residues, and substrate not recovered is supposed to have been fermented. This was found not to be the case in some NaOH and NH_4 treated straw (Blummel et al., 2005) and the gravimetric methods might provide an overestimation of true fermentability and there might, therefore, be an overestimation of the beneficial effects AFEX treatments on the nutritive value of roughages. On the other hand, Weimer et al. (2003), replaced alfalfa hay by FIBEX treated rice straw (which is on the lower side in terms of digestibility among cereal straws, Teufel el al. (2010) and observed daily milk yields of about 40 kg on rations containing 35% FIBEX treated rice straw.

Conclusion and Outlook

Exploring spin-off technologies from 2nd generation biofuel technologies for modification and adoption for livestock feeding in a systematic way should be a high priority for animal nutritionists given the incredible high investments and the interdisciplinary research efforts of some of the best contemporary minds in life science that have been already devoted to this research. Areas for systematic research should be around: 1) Assessment of lignocellulolosic biomass kind and availability, competition, utilization and costs and in potential value chains for meat, milk and fish for targeted interventions, 2) Design and pilot-testing of appropriate biomass processors and fermenters as intermediary step in the development of up-scalable technologies; and 3) Up-scale technologies that can be used along feed value chains with partners from farmers organization, cooperatives, NGO's and private sector.

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