UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

INFLUÊNCIA DA PALHA E TEMPO DE PREPARO DA CALDA ANTES DA HIDRÓLISE DA CANA-DE-AÇÚCAR EM DIETAS DE VACAS LEITEIRAS

Viviane Endo
Zootecnista

2016

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Orientador: Prof. Dr. Mauro Dal Secco de Oliveira

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para obtenção do título de Doutor em Zootecnia.

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EPIGRAPH

"You may never know what results come of your actions, but if you do nothing, there will be no results."
Mahatma Gandhi

"Education is the most powerful weapon which you can use to change the world."
Nelson Mandela

"The great thing in this world is not so much where we stand, as in what direction we are moving."
Oliver Wendell Holmes

DEDICATION

To my parents, Antônio Toshihar Endo and Marilúcia Emico Endo.

To my fiancé, André Marubayashi Hidalgo.

To my brothers, Wagner Endo and Evandro Endo.

To my sisters-in-law, Thaís C.C. Guimarães Endo and Thaís M. Zequim Endo.

I love you guys so much!

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INFLUÊNCIA DA PALHA E DO TEMPO DE PREPARO DA CALDA ANTES DA HIDRÓLISE DA CANA-DE-AÇÚCAR EM DIETAS DE VACAS LEITEIRAS

RESUMO – A cana-de-açúcar tem papel importante no agronegócio brasileiro para a produção de etanol e açúcar. A palha da cana-de-açúcar é um resíduo orgânico pobre em sacarose para produção de etanol e de baixo valor nutritivo para a alimentação animal. Devido a isso, o processo de queima dos canaviais ocorre em larga escala com intuito de facilitar a colheita da cana-de-açúcar, além de limpar o canavial para a próxima safra. Entretanto, a queima causa danos à saúde e impacto ambiental. No estudo foram avaliados dois fatores da cana-de-açúcar: a influência da palha e a influência dos tempos de preparo da calda de hidróxido de cálcio ($Ca(OH)_2$) antes da hidrólise da cana-de-açúcar. Os parâmetros avaliados neste estudo foram: composição bromatológica, temperatura e pH da cana-de-açúcar, digestibilidade de nutrientes, desempenho de vacas leiteiras e uso de imagens de microscopia eletrônica de varredura (MEV) da cana-de-açúcar antes e após a digestão *in vitro*. O primeiro estudo foi um arranjo fatorial 2 x 5 [cana-de-açúcar com ou sem palha e cinco tempos de preparo da calda de $Ca(OH)_2$ antes da hidrólise da cana-de-açúcar (72, 48, 24, 0,5 horas e sem preparo)]. No segundo estudo foi avaliado a digestibilidade *in vitro* dos nutrientes em um delineamento inteiramente casualizado, com cinco tratamentos: cana-de-açúcar fresca sem palha, HPT0,5 (cana-de-açúcar hidrolisada com palha e a calda preparada 0,5 horas antes da hidrólise), HPT72 (cana-de-açúcar hidrolisada com palha e a calda preparada 72 horas antes da hidrólise), HT0,5 e HT72, cana-de-açúcar hidrolisada sem palha usando os mesmos procedimentos de preparo da calda na cana-de-açúcar com palha. Posteriormente, utilizou-se um delineamento em quadrado latino 5 x 5 (cinco vacas em lactação e cinco tratamentos) para estudo de desempenho. Imagens de MEV foram feitas como complemento para visualização da cana-de-açúcar com e sem digestão *in vitro*. Como resultados, a cana-de-açúcar fresca sem palha e hidrolisada com palha e a calda preparada 72 horas antes apresentou menor teor de fibra em detergente neutro (FDN). O pH da cana-de-açúcar hidrolisada, devido à presença do agente alcalinizante foi maior em comparação à cana-de-açúcar fresca independente da palha. A temperatura foi influenciada pela palha, apresentando-se maior quando esta estava presente. Em relação à digestibilidade dos nutrientes, a cana-de-açúcar hidrolisada com palha, quando comparada à hidrolisada sem palha, apresentou menor digestibilidade *in vitro* de matéria seca (MS) e maior digestibilidade *in vitro* da FDN. Para o desempenho das vacas, houve menor consumo de MS para a cana-de-açúcar com palha em comparação à cana-de-açúcar sem palha. A produção de leite manteve-se constante entre os tratamentos, assim como a sua composição. Nas imagens da MEV, algumas pequenas partículas de cana-de-açúcar que foram observadas nas amostras não digerida, após a digestão *in vitro* parecem ter sofrido alguma digestão. O que permaneceu foram partículas maiores, ou seja, partículas indigestíveis. A utilização da cana-de-açúcar com palha e a calda de $Ca(OH)_2$ preparada 72 horas antes da hidrólise é recomendada devido à melhor eficiência alimentar das vacas, além de facilitar a mão-de-obra.

Palavras-chave: eficiência alimentar, hemicelulose, pH, temperatura

INFLUENCE OF STRAW AND TIME OF LIME PREPARATION BEFORE THE HYDROLYSIS OF SUGARCANE IN DAIRY COW DIET

ABSTRACT – Sugarcane has an important role in agribusiness because of ethanol and sugar production. Straw of sugarcane is an organic residue, it has low nutritional value to animal feed and a poor sucrose content which is used to produce ethanol. Because of this, burning of the sugarcane is performed on a large scale aiming to facilitate the sugarcane harvest, besides cleaning the sugarcane fields for the next crop. Burning, however, is not safe for health and causes harmful environmental impact. The study evaluated two sugarcane factors: the influence of straw and the time of lime preparation on the sugarcane hydrolysis. The variables analyzed on this study were: chemical composition, temperature and pH of sugarcane, nutrient digestibility, performance of dairy cows and the use of scanning electron microscopy (SEM) images of sugarcane before and after *in vitro* digestion. First study was arranged as a factorial 2 x 5 [sugarcane with or without straw and five times of the lime preparation before hydrolysis of sugarcane (72, 48, 24, 0.5 hours and no lime used)]. The lime used was calcium hydroxide ($Ca(OH)_2$. In the second study the *in vitro digestibility* of nutrients was evaluated and arranged as a completely randomized design with five treatments: fresh sugarcane without straw, HST0.5 (hydrolyzed sugarcane with straw and the lime prepared 0.5 hours before the hydrolysis), HST72 (hydrolyzed sugarcane with straw and the lime prepared 72 hours before the hydrolysis), HT0.5 (hydrolyzed sugarcane without straw and the lime prepared 0.5 hours before the hydrolysis) and HT72 (hydrolyzed sugarcane without straw and the lime prepared 72 hours before the hydrolysis). Posteriorly, a Latin square design 5 x 5 five lactating cows and five treatments was arranged for performance of dairy cows. As complement, SEM images of sugarcane before and after the *in vitro* digestion were done. As results, fresh sugarcane without straw and hydrolyzed with straw and the lime prepared 72 hours in advance presented lower neutral detergent fiber (NDF) content. The pH of hydrolyzed sugarcane was higher compared to fresh sugarcane, due to the presence of the alkalizing agent, regardless of straw. Temperature was higher when the straw was present. Regarding the digestibility of nutrients, sugarcane with straw showed lower *in vitro* digestibility of dry matter (DM) and higher *in vitro* digestibility of NDF compared with hydrolyzed sugarcane. For the cow's performance, a lower DM intake was observed for sugarcane with straw compared with sugarcane without straw. Milk production was similar among treatments, as well as its composition. Providing sugarcane with straw and the lime of $Ca(OH)_2$ prepared 72 hours before hydrolysis of sugarcane is recommended because it provided better feed efficiency to the cows. Additionally, it facilitates the labor.

Keywords: feed efficiency, hemicellulose, pH, temperature

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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 019284/13 do trabalho de pesquisa intitulado **"Cana-de-açúcar hidrolisada em duas formas de utilização na alimentação de vacas leiteiras"**, sob a responsabilidade do Prof. Dr. Mauro Dal Secco de Oliveira está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 06 de setembro de 2013.

Jaboticabal, 06 de setembro de 2013.

Prof. Dr. Andrigo Barboza De Nardi
Coordenador - CEUA

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CAPÍTULO 1 – Considerações gerais

1. Introdução

A cana-de-açúcar tem importante impacto no agronegócio brasileiro e com isso, altas tecnologias são empregadas anualmente para o desenvolvimento da produção. A sustentabilidade é tema atual e vem ganhando espaço tanto no mercado, quanto na comunidade científica. Com isso, a busca do aproveitamento de todo e qualquer tipo de resíduo gerado pelas culturas é pertinente. A palha da cana-de-açúcar é um resíduo agrícola, produzido em larga escala nos canaviais, e tem gerado preocupação de produtores por ser um resíduo pobre em carboidratos solúveis para produção de etanol (primeira geração) e pobre em nutrientes para produção animal. A prática de queima da palha da cana-de-açúcar vem sendo proibida nos últimos anos em âmbito regional, estadual e nacional devido, principalmente, aos malefícios que esta prática provoca à saúde humana, animal, bem como ao meio ambiente.

Há constante esforço na redução das queimadas da palha da cana-de-açúcar devido à busca da sustentabilidade nos sistemas de produção. Com a diminuição e proibição das queimadas, surge um problema, a excessiva quantidade de palha que permanece nos canaviais sem destino aparente. Alguma palha é utilizada para manutenção do solo para a safra seguinte de cana-de-açúcar, mesmo assim, a quantidade que permanece sem destino ainda é grande. Para se ter uma ideia, a produção de palha nos canaviais é de aproximadamente 12 toneladas de palha por hectare em revisão feita por Santos e colaboradores sobre o opotencial da palha da cana-de-açúcar para produção de etanol.

O uso da palha para a produção de energia dentro da própria usina é um destino interessante do ponto de vista econômico e de sustentabilidade. Além da produção de etanol de segunda geração e a produção de papel a baixo custo a partir da palha da cana-de-açúcar. O fornecimento da cana-de-açúcar como volumoso para os animais geralmente é recomendada para pequenos e médios produtores. Nestes casos, normalmente o corte da cana-de-açúcar é manual com uso de facão ou mecânico com uso trator para os pequenos e médios produtores, respectivamente. Economicamente, não se justifica o transporte da palha que permanece nestes canaviais para produção de energia ou etanol de segunda geração. Diante disso, o setor de produção animal sente-se estimulado a desenvolver estudos que utilize este resíduo agrícola na alimentação animal para produção de leite. É uma tarefa de todas as áreas pesquisar mais sobre os resíduos agrícolas e encontrar destinos economicamente e logisticamente aplicáveis.

2. Revisão de literatura

2.1 Cana-de-açúcar na alimentação de ruminantes

A cana-de-açúcar é uma forrageira de clima tropical, e vem ganhando interesse pelos produtores por apresentar custo de produção reduzido, boa disponibilidade nos períodos de escassez de forragem, além de possuir altos teores de matéria seca e energia (Oliveira, 2010).

Para a escolha da variedade de cana-de-açúcar na alimentação animal, Siqueira et al. (2012) recomendam utilizar variedades que apresentem alta digestibilidade da fração fibrosa e boa produção de massa. Como exemplo temos as variedades de cana-de-açúcar IAC 86-2480 e a IACSP 93-3046. A maior produção de matéria seca digestível por hectare é uma característica importante na escolha da variedade de cana-de-açúcar, pois leva em conta aspectos quantitativos e qualitativos (Siqueira et al., 2012).

Além do baixo teor de proteína bruta (PB) da cana-de-açúcar, o seu modo de fornecimento limita o uso na alimentação animal pela necessidade de cortes diários. Esta prática se torna inviável por não haver mão-de-obra disponível e/ou equipamentos adequados para a realização desta tarefa. Como alternativa para os produtores, a hidrólise da cana-de-açúcar com cal virgem (CaO) ou hidratada ($Ca(OH_2)$) prolonga o tempo de armazenamento do volumoso. Este procedimento atua na melhoria da digestibilidade da cana-de-açúcar ao agir na porção fibrosa da parede celular (Oliveira, 2010). A ação do agente alcalinizante eleva a taxa de digestão da fibra presente na cana-de-açúcar, provavelmente devido as quebras nas ligações das frações do complexo celulose-hemicelulose-lignina, tornando-as mais disponíveis para os microrganismos ruminais.

Segundo Jackson (1977) e Klopfenstein (1980), a celulose quando tratada com agentes alcalinizantes, sofre expansão e quebra das ligações intermoleculares das pontes de hidrogênio, que ligam essas moléculas. Coombre (1979) relatou que os produtos alcalinos provocaram redução na fibra em detergente neutro (FDN) devido à solubilização de parte da hemicelulose (HEM). Segundo este mesmo autor, a celulose se torna mais degradável no ambiente ruminal também em função da sua deslignificação. A ação hidrolisante dos agentes alcalinos no sentido de reduzir os teores de FDN, HEM e de fibra em detergente ácido (FDA) da cana-de-açúcar já é bastante antiga e está relacionada com a melhoria na digestibilidade e no aumento do consumo, o que possibilita melhor desempenho animal.

2.1.1 Desempenho de vacas leiteiras alimentadas com cana-de-açúcar hidrolisada

O desempenho animal está relacionado com o seu consumo de matéria seca e a produção. O consumo de alimentos determina o nível de nutrientes ingeridos, com efeito sobre a resposta animal (Van Soest, 1994). Entender os mecanismos que controlam o consumo de alimentos é importante, pois dependerá da quantidade total de nutrientes que o animal deverá receber para o crescimento, a saúde e a produção (Berchielli et al., 2011). A quantidade de nutrientes absorvidos está sujeita a variações tanto da digestibilidade dos nutrientes quanto no seu consumo. O consumo é regulado pelo alimento quanto a sua densidade energética, teor de nutrientes, necessidade de mastigação, capacidade de enchimento e regulado pelo animal quanto ao peso corporal, ao estado fisiológico e ao nível de produção, além das condições de alimentação, como o espaço no comedouro, a disponibilidade de alimento, o tempo de acesso aos alimentos e frequência de alimentação (Mertens, 1992).

Corrêa et al. (2003) avaliaram o desempenho de vacas holandesas alimentadas com cana-de-açúcar ou silagem de milho de diferentes texturas de grão. Como resultados, obtiveram redução no consumo, na digestibilidade da FDN e na produção de leite em vacas alimentadas com cana-de-açúcar em comparação às vacas que receberam silagem de milho, porém a digestibilidade da matéria orgânica foram semelhantes entre os tratamentos. Os autores concluíram que a cana-de-açúcar, como volumoso, na dieta aparenta ser boa alternativa para vacas em lactação nas fases de menor demanda nutricional.

Ao avaliarem o desempenho produtivo de vacas mestiças Girolanda, com média de 508 kg de peso corporal, alimentadas com cana-de-açúcar fresca e hidrolisada, Alves et al. (2010) não encontraram diferença no consumo de matéria seca (MS) entre os tratamentos. Porém, vacas alimentadas com cana-de-açúcar hidrolisada apresentaram maior produção de leite (16,5 litros/dia) quando comparadas às vacas alimentadas com cana-de-açúcar fresca (14,8 litros/dia), ou seja, aumento de 10,4% na produção de leite. Estes resultados demonstram melhor aproveitamento dos nutrientes quando a cana-de-açúcar foi hidrolisada.

A avaliação de diferentes volumosos na dieta de vacas mestiças (Holandesa x Gir) com 14,4% de PB e produção média de leite de 13,60 kg/dia, Martins et al. (2011) encontraram maior consumo de MS para a cana-de-açúcar (17,8 kg/dia) em comparação à silagem de girassol (13,3 kg/dia), maior consumo de carboidratos não fibrosos (7,3 kg/dia) em relação à silagem de girassol (3,6 kg/dia), à silagem de sorgo (1,8 kg/dia) e ao capim-tanzânia (1,9 kg/dia). Estes mesmos autores encontraram menor consumo de FDN da cana-de-açúcar (5,8 kg/dia) em comparação à silagem de sorgo (10,6 kg/dia) e ao capim-tanzânia (11,6 kg/dia). No desempenho de vacas leiteiras mestiças (Holandesa x Gir) alimentadas com cana-de-açúcar associada à ureia e tratada com cal virgem e 13,1% de PB, Silva Junior et al. (2015) encontraram 11,9 kg/dia para consumo de MS, 4,9 kg/dia de consumo de FDN e 13,5 kg de produção de leite.

O aumento na produção de leite em si não significa muito se a sua qualidade for prejudicada com o uso do alimento testado. O leite é constituído de aproximadamente 87% de água e 13% de sólidos totais (todos os elementos do leite, menos a água). Dos sólidos totais, aproximadamente 3,5% são de proteínas, 3,6% de gordura, 4,9% de lactose e 0,7% de cinzas (minerais) (PRATA, 2001). O leite é levemente ácido, com pH variando de 6,5 a 6,7. Trabalhos têm demonstrado semelhante produção e composição do leite de vacas alimentadas com cana-de-açúcar hidrolisada em comparação com a dieta padrão com silagem de milho e outros volumosos (CORRÊA et al., 2003, MARTINS et al., 2011).

2.1.2 Digestibilidade da cana-de-açúcar hidrolisada

O tratamento químico de volumosos é relativamente antigo e a utilização de agentes alcalinizantes é realizada com o intuito de melhorar a digestibilidade e o consumo de alimentos fibrosos (Ezequiel et al., 2005). Outro propósito é aumentar o tempo de armazenamento da cana-de-açúcar, dispensando necessidade de cortes diários (Oliveira, 2010). O hidróxido de cálcio é um agente alcalinizante de fácil aplicação e de custo baixo quando comparado ao hidróxido de sódio, mas a digestibilidade da cana-de-açúcar é menor quando esta é hidrolisada com o $Ca(OH)_2$ (Rodrigues e Souza, 2005; Oliveira 2010). As variedades de cana-de-açúcar distinguem-se com a época de maturação, que pode ser precoce, média ou tardia.

A época de colheita da cana-de-açúcar para o seu fornecimento aos animais é importante, pois quando colhida antes da maturação irá apresentar baixo teor de carboidratos solúveis e alto teor de fibras (Siqueira et al., 2012). A cana-de-açúcar apresenta baixa porcentagem de FDN (40-50%); porém a fração fibrosa indegradável da cana-de-açúcar é elevada (Siqueira et al., 2012). Como consequência, é observado baixo consumo devido ao enchimento ruminal. Sendo assim, o estudo da digestibilidade dos nutrientes dos alimentos fornecidos é importante e está relacionado com a cinética e a taxa de passagem da digesta pelo trato digestivo. Porém, a avaliação do seu valor nutritivo e as formas de aproveitamento pelo trato gastrintestinal ainda permanecem como um desafio para os pesquisadores da área de nutrição animal.

A digestão é o processo de quebra das macromoléculas de nutrientes em compostos mais simples (Van Soest, 1994). Estes compostos mais simples são absorvidos pelo trato gastrintestinal dos animais. As medidas de digestibilidade qualificam os alimentos quanto ao seu valor nutritivo. É expressa pelo coeficiente de digestibilidade e indica a quantidade percentual de cada nutriente que o animal pode aproveitar.

Alguns trabalhos têm analisado o efeito da cal virgem ou hidratada na estabilidade, na composição bromatológica da cana-de-açúcar e na digestibilidade dos nutrientes (Silva et al., 2006; Oliveira et al., 2007; Oliveira et al., 2008; Domingues, 2009; Oliveira, 2010). Após a aplicação da cal, observaram melhora tanto na estabilidade, quanto na DIVMS e na composição bromatológica (redução nos teores de FDN, FDA e de HEM). Apesar dos benefícios citados, há necessidade de estudos visando o efeito do tempo de preparo da calda para uso na hidrólise da cana-de-açúcar, assim como o efeito da palha para a hidrólise.

Segundo Oliveira (2010) vários fatores interferem na hidrólise, no entanto, não foram relatadas informações bibliográficas sobre a relação tanto do tempo do preparo da suspensão da calda quanto da influencia da palha na eficiência da hidrólise da cana-de-açúcar. Assim, torna-se oportuna a realização de estudos para entender a ação de tais fatores sobre a hidrólise da cana-de-açúcar. Este aspecto é importante, pois muitos produtores preparam a calda com antecedência para facilitar as operações relacionadas com a hidrólise e alguns utilizam picadeiras móveis (cana-de-açúcar integral picada). Todavia, os produtores rurais, principalmente produtores de leite, utilizam picadeiras fixas (normalmente despalham a cana-de-açúcar antes de picá-la). Portanto, é importante o conhecimento sobre até que ponto esses procedimentos podem interferir na hidrólise da cana-de-açúcar.

2.1.2.1 Microscopia eletrônica de varredura (MEV)

Além de análises em laboratório que quantificam os teores de fibras do volumoso, existe a técnica de microscopia eletrônica de varredura (MEV) que tem sido utilizada, segundo Lempp (2007), para estabelecer uma base entre a parede celular e a digestibilidade de forragens, pois proporciona profundidades de campo muito maiores, possibilitando a observação e registro de imagem tridimensional (SANTOS, 1995). A MEV permite esclarecer o processo de quebra das fibras, possibilitando a identificação e até mesmo a quantificação de substratos provenientes da degradação da forragem. Lempp (2007) em sua revisão sobre a MEV na avaliação de alimentos para ruminantes, estudou forragens tropicais, milho para ensilagem, tratamento de resíduos agrícolas (bagaço de cana-de-açúcar), leguminosas (alfafa), forragens com tanino e milho grão.

Este tipo de estudo em forrageiras visa apurar o efeito da anatomia na digestibilidade e fornece informações sobre os fatores que interferem na qualidade das forragens. Lempp (1997) verificou, com a MEV, que a parede celular da gramínea *Paniccum maximum*, cv. Vencedor apresentou-se mais rígida em relação à cv. Aruana, sem verificar diferenças na composição bromatológica entre estas cultivares. Jung e Casler (2006) estudaram a degradabilidade *in vitro* e a micrografia do resíduo da incubação *in vitro* (24 e 96h) do entrenó do milho em diferentes fases de desenvolvimento, e observaram que o aumento da parede celular no entrenó do milho foi associado ao espessamento da parede celular secundária na região do córtex e identificaram também as regiões não degradadas (córtex) e regiões parcialmente degradadas (medular) pelas imagens obtidas a partir da fotomicrografia.

Ao observarem o efeito do tratamento com hidróxido de sódio (NaOH) e bicarbonato de amônia (NH_4HCO_3) no colmo de palha de arroz, Wang et al. (2006) perceberam que com o tratamento com NH_4HCO_3, houve aumento da ruptura da camada de cutícula, mas a epiderme permaneceu inalterada. Já com o NaOH, a camada da cutícula se dissolveu, resultando em degradação da epiderme. A avaliação com uso da microscopia é importante, pois nem sempre a análise química e a digestibilidade são capazes de elucidar todas as variações no consumo das forrageiras pelos ruminantes (LEMPP, 2007).

Técnicas de microscopia eletrônica de varredura possibilita maiores esclarecimentos das relações de causa e efeito na degradabilidade das forragens, assim como sua escolha em relação ao potencial qualitativo (FERRARI, 2013). Há escassez de trabalhos com a técnica da MEV para melhor compreender a ação de agentes alcalinizantes na cana-de-açúcar, o que dificulta a discussão e interpretação das imagens (FERRARI, 2013).

2.2 Palha da cana-de-açúcar como forragem

A nomenclatura do resíduo agrícola da cana-de-açúcar apresenta falta de padronização do que seria a palha, a palhada ou o palhiço (Ripoli e Gamero, 2007). O palhiço da cana-de-açúcar é o material remanescente no canavial após a colheita. É constituído de folha verde, folha seca, ponteiro e pedaços de colmo industrializáveis ou não (Ripoli e Gamero, 2007). A palha (folha seca) constitui de uma parte do palhiço. Do ponto de vista industrial, a palha é caracterizada como toda a parte aérea da planta (ponteiro), constituído por folha verde e folha seca; porém, do ponto de vista de produção animal, a palha é caracterizada como sendo a folha seca. A palha da cana-de-açúcar é constituída principalmente de 40-44% de celulose, 30-32% de hemicelulose e 22-25% de lignina (Santos et al., 2012).

A oferta de forragem para a produção animal sofre variações ao longo do ano. No inverno é o período de maior escassez de forragem devido à baixa temperatura e, principalmente, por caracterizar um período seco. Com isso, o custo de produção se eleva devido à necessidade de obtenção de concentrados e silagens de alto custo. Uma interessante alternativa para evitar, ou então minimizar estas variações de oferta de forragem é o uso de alimentos alternativos de baixo custo. O uso de resíduos agrícolas, como a palha, na alimentação de ruminantes deve ser levado em consideração. O animal ruminante é capaz de aproveitar o material fibroso presente em forragens devido à fermentação microbiana que ocorre no rúmen. O produto desta fermentação são os ácidos graxos voláteis (ácido acético, propiônico e butírico) que serão utilizados pelo animal como fonte de energia para produção.

Apesar da escassez de trabalhos bibliográficos que abordam a palha da cana-de-açúcar na alimentação de ruminantes, Rodrigues e Souza (2005) descreveram bem este resíduo agrícola. É um volumoso de baixo valor nutricional, portanto, não pode ser fornecido como alimento único na dieta. Nas épocas de escassez de forragem, o uso da palha é interessante por diminuir o custo da dieta. A palha, que é um resíduo lignocelusósico, apresenta alto teor de parede celular (celulose, hemicelulose e lignina), baixo teor de proteína bruta e minerais, principalmente fósforo e enxofre. Para melhor aproveitamento das palhadas pelos ruminantes, pesquisadores sugerem, primeiramente, a correção nutricional da palhada antes de qualquer tipo de tratamento da forragem, seja ele biológico, químico ou físico.

Para a correção nuticional da palha da cana-de-açúcar, deve-se levar em consideração o suprimento de energia e proteína na dieta de forma balanceada. O balanceamento entre energia e proteína é exigido pelos microrganismos do rúmen para a síntese de proteína microbiana. O tratamento do resíduo agrícola tem como principal objetivo aumentar a digestibilidade das fibras, além de aumentar o consumo de matéria seca. O baixo valor nutritivo da palha é devido ao incrustamento da parede celular pela lignina (RODRIGUES e SOUZA, 2005). Tratamentos químicos da palha com uso de hidróxido de sódio, de cálcio, de potássio e de amônio, por serem produtos alcalinos, acabam hidrolisando a lignina, causando expansão das fibras. O resutaldo é aumento da digestibilidade da celulose e hemicelulose (RODRIGUES e SOUZA, 2005).

Carvalho et al. (2010) ao estudarem a digestibilidade *in vitro* da fibra em detergente neutro (DIVFDN), encontraram 11,9% para digestibilidade dos colmos e 23,9% para digestibilidade das folhas. Entretanto, o teor de FDN do colmo foi de 38,2% e das folhas de 68,7%. Os valores de digestibilidade da palha não foi considerado, mas provavelmente, segundo Siqueira et al. (2012), possuía teores de FDN superiores aos da folha.

Os ruminantes, com seu sistema digestivo compartimentado, são capazes de ulitizar as palhadas quando corretamente suplementados com fontes de nitrogênio e minerais. Souza e Cardoso (2003) afirmaram que a identificação de alternativas no uso da palhada no Brasil representa considerável desafio econômico, ambiental e, sobretudo, técnico-científico.

3. Objetivos gerais

O objetivo do primeiro estudo foi avaliar a influência da palha e dos tempos de preparo da calda de hidróxido de cálcio antes da hidrólise da cana-de-açúcar na composição bromatológica e nos valores de pH e temperatura.

O segundo estudo objetivou avaliar a digestibilidade dos nutrientes, o desempenho produtivo a composição do leite de vacas leiteiras alimentadas com os tratamentos obtidos a partir dos melhores resultados bromatológicos no primeiro estudo.

Como estudo complementar, imagens de microscopia eletrônica de varredura foram feitas e avaliou-se a ação da digestão *in vitro* e do hidróxido de cálcio na cana-de-açúcar com ou sem palha.

4. Referências

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CHAPTER 2 – Evaluating the influence of straw and the time of lime preparation before the hydrolysis of sugarcane

ABSTRACT – This study aimed to evaluate the influence of straw and the time of lime preparation before the hydrolysis of sugarcane on the chemical composition, pH and temperature of sugarcane. A completely randomized 2 x 5 factorial design with four replications for each of the ten combinations of the two factors (n=40) was used. Treatments were two ways of use chopped sugarcane (with or without straw), and five times of lime preparation before the hydrolysis of sugarcane (72h, 48h, 24h, 0.5h and no lime preparation for fresh sugarcane). Calcium hydroxide [$Ca(OH)_2$] was the lime used. Chemical composition of sugarcane was analyzed nine hours after the hydrolysis process. Internal and external temperatures of the stacks of sugarcane as well as pH were measured 0.5h, 3h, 6h and 9h after hydrolysis. In general, the highest neutral detergent fiber ash-free values were obtained for sugarcane with straw treatments (450 g/kg) compared to sugarcane without straw (412 g/kg). Hence higher residual of $Ca(OH)_2$ remained in the container used to prepare the suspension. Fresh sugarcane had lower ash (36.2 g/kg) due to non-use of additive $Ca(OH)_2$ with mineral properties. The pH of hydrolyzed sugarcane decreased as time increased (0.5h, 3h, 6h and 9h), from 11.3 to 9.1 and fresh sugarcane was similar as time increased with average of 5.3. Sugarcane with straw did not have different pH values compared to sugarcane without straw. After 9 hours of hydrolysis, sugarcane with straw had higher internal (18.6^{o}C) and external (21.1^{o}C) temperatures than without straw (14.3 and 15.4^{o}C). From the chemical composition point of view, fresh sugarcane without straw and hydrolyzed sugarcane with straw using the lime prepared 72 hours before the hydrolysis can be used to feed cows because, in general, provided lower neutral detergent fiber. Hydrolyzed sugarcane has some advantages such as the possiblility to store it, facilitating the labor. Additionally, it provides lower temperatures of the stacks of sugarcane and higher pH which is interesting for conservation.

Keywords: chemical composition, pH, temperature

Influência da palha e do tempo de preparo da calda antes da hidrólise da cana-de-açúcar

RESUMO – O estudo teve como objetivo avaliar a influência da palha da cana-de-açúcar e do tempo de preparo da calda antes da hidrólise da cana-de-açúcar a fim de verificar seus efeitos na composição bromatológica, pH e temperatura da canad-de-açúcar. Foi utilizado um delineamento inteiramente casualizado em arranjo fatorial 2 x 5, com quatro repetições para cada uma das 10 combinações dos dois fatores (n=40). Os tratamentos foram, duas formas de utilização da cana-de-açúcar picada (com e sem palha) e cinco tempos de preparo da calda antes da hidrólise (72h, 48h, 24h, 0,5h e sem preparo para cana-de-açúcar fresca). A cal utilizada foi o hidróxido de cálcio ($Ca(OH)_2$). Após o processo de hidrólise da cana-de-açúcar, foram feitas as análises bromatológicas. Foram mensuradas as temperaturas interna e externa, e o pH dos amontoados de cana-de-açúcar durante os tempos 0,5h, 3h, 6h e 9h após a hidrólise. Em geral, os valores de fibra em detergente neutro livre de cinzas foram maiores para a cana-de-açúcar com palha (450 g/kg) em comparação à cana-de-açúcar sem palha (412 g/kg). Sendo assim, maior $Ca(OH)_2$ residual permaneceu no recipiente utilizado para preparar a calda. A cana-de-açúcar fresca apresentou menor teor de cinzas (36,2 g/kg) devido à ausência do aditivo $Ca(OH)_2$ com propriedades minerais. O valor de pH da cana-de-açúcar hidrolisada diminuiu de 11,3 para 9,1 com o passar das horas (0,5h, 3h, 6h e 9h) e para a cana-de-açúcar fresca isto não aconteceu, sendo o pH médio 5,3. A cana-de-açúcar com palha não teve seus valores de pH alterados em comparação com a cana-de-açúcar sem palha. Após 9 h de hidrólise, a cana-de-açúcar com palha teve maior temperatura interna (18,6^{o}C) e externa (21,1^{o}C) que a cana-de-açúcar sem palha (14,3 e 15,4^{o}C). Do ponto de vista de composição bromatológica, a cana-de-açúcar fresca sem palha e a cana-de-açúcar hidrolisada com palha e a calda preparada 72 horas antes da hidrólise pode ser usada na alimentação de vacas, pois proporciona menor fibra em detergente neutro. A cana-de-açúcar tem alguma vantagem, como a possibilidade de estocá-la, o que facilita a mão-de-obra. Além disso, possibilita menor temperatura dos amontoados de cana e maior pH, que é interessante para sua conservação.

Palavras-chave: composição química, pH, temperatura

1. Introduction

Sugarcane is a tropical roughage widely used in cattle production feed in Brazil. It has up to 60% of total digestible nutrient on a dry matter basis, which is interesting from the animal nutritional point of view (OLIVEIRA, 2010). Another positive aspect of sugarcane is the high production per hectare, 74.8 ton fresh matter (FM)/ha (CONAB, 2014), especially when forage is scarce (winter season). It has, however, few drawbacks: low crude protein content (up to 4.0% in DM basis) that requires protein supplementation and a need for daily harvest due to the high concentration of soluble carbohydrates. Due to this high concentration of soluble carbohydrates, the environment becomes favorable for the development of microorganisms that deteriorate the chopped sugarcane. The amount of neutral detergent fiber (57% in DM basis) is not a problem, but because of its low digestibility of the fibers, it becomes a drawback (SIQUEIRA et al., 2012).

Previous studies have evaluated the effect of some additives such as calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$) on sugarcane stability and its chemical composition (RIBEIRO et al., 2009; MOTA et al., 2010; OLIVEIRA, 2010). They observed an improvement on sugarcane stability and chemical composition after using additives, such as lower amount of neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicelluloses (HEM).

Several factors affect the hydrolysis process, e.g. particle size of chopped sugarcane and the lime type used for hydrolysis (OLIVEIRA, 2010). However, the influence of straw and lime preparation time on the efficiency of sugarcane hydrolysis has not been reported. Lime is prepared (by mixing the $Ca(OH)_2$ with water) every day by producers to hydrolyze the sugarcane. If a large amount of lime is prepared and no loss is observed, then this procedure can be interesting for producers to facilitate the labor.

It is already known, that the amount of straw in the sugarcane fields is considerably high and for long time was considered useless. Most of times, the straw is burned to eliminate it and also to facilitate the harvesting. This practice is common for small and medium producers. Burning, however, is not safe for the environment, human and animal health. Burning plants residues causes emissions of greenhouse gases (GHG) such as carbon dioxide (CaO_2), nitrous oxide (N_2O) and methane (CH_4) (CANÇADO et al., 2006). According to Bordonal et al. (2013), preliminary burning (burned harvest) of sugarcane fields is performed when the harvest is done manually. Sugarcane straw biomass accounts for 1/3 of the gross energy potential of sugarcane (SANTOS et al., 2012). São Paulo State Law n^o 11.241/2002, aims to eliminate the burned harvest until 2021.The Green Ethanol Protocol (Protocolo Etanol Verde) was an initiative of São Paulo state to anticipate the pre harvest burning of sugarcane to 2014, but in March, 2015 Federal Supreme Tribunal considered this practice unconstitutional.

Some studies evaluated the potential of straw from sugarcane for ethanol production, and also the use of residual straw in ruminant feed (RODRIGUES; SOUZA, 2005 and SANTOS et al., 2012). This study aimed to evaluate the influence of straw and the time of lime preparation before the hydrolysis of sugarcane on chemical composition, pH and temperature.

2. Material and methods

The IAC 86-2480 was the variety of sugarcane used, harvested at 18 months of growing, used with or without straw, and chopped in particle size of 1.0 cm average. A completely randomized 2 x 5 factorial design with four replications for each of the ten combinations of the two factors (n=40) was used. Treatments consisted in two ways of use chopped sugarcane (with or without straw), and five times of lime preparation before the hydrolysis of sugarcane, i.e., different times of mixing the $Ca(OH)_2$ with water before hydrolysis process (72h, 48h, 24h, 0.5h and no lime was used for fresh sugarcane). The final product obtained after this mixing was named $Ca(OH)_2$ suspension. Fresh sugarcane with or without straw was evaluated as control.

Sugarcane with straw was harvested and immediately chopped using a tractor with a chopper of knives coupled. Sugarcane without straw was harvested manually using a machete, and the straw was removed around the sugarcane’ stems before to chopping with a fixed chopper of knives. The entire plant (stem, leaves and dry leaves, i.e. straw) was defined as sugarcane with straw, and sugarcane without straw was defined as just stem and leaves. Sugarcane was hydrolyzed using 0.5% of $Ca(OH)_2$, as fresh basis, i.e., for each 100 kg of fresh sugarcane, 0.5 kg of $Ca(OH)_2$ diluted in 2 liters of water was used (OLIVEIRA, 2010). Chemical composition of the hydrolyser additive consisted of 94.1% of $Ca(OH)_2$ and 1.5% of magnesium oxide.

After harvesting of sugarcane, all the material obtained was then stored in a shed. The hydrolyzed sugarcane was obtained by spreading it on a tarp, forming a stack. After that, $Ca(OH)_2$ suspension was poured over the sugarcane and the stack was revolved for homogenization. Fresh sugarcane did not receive any type of hydrolyser additive. All the stacks were identified with tags and each one was constituted by 30 kg of sugarcane.

According to Oliveira (2010), eight hours of hydrolysis is enough so that the fibers breaking occur and the intake is not affected by the high pH values of hydrolyzed sugarcane. On the times 0.5h, 3h, 6h and 9h after the hydrolysis process, measurements of pH, internal and external temperature (IT and ET) were obtained. Infrared thermometer and digital pH meter were used according to Silva and Queiroz (2002). Samples of sugarcane from all treatments were collected after 9 hours of hydrolysis and were pre-dried at 55ºC forced-ventilation during 72h and posteriorly milled with 1.0 mm grinding plates. The milled samples were used to determine the contents of dry matter (DM), organic matter (OM) and ash following AOAC (1990) methodologies. The content of neutral detergent fiber ash-free (NDFa) was determined according to Van Soest et al. (1991) whereas acid detergent fiber (ADF) was determined according to Silva and Queiroz (2002). Total carbohydrates (TC) values and non-fiber carbohydrates (NFC) were determined according to Mertens (1997), by TC = 100 – (%CP + %EE + %ash); NFC = 100 – (%CP + %EE + %ash + %NDFa).

The statistical model was as follows:

Model for the chemical composition,

$$Yijk = \mu + si + tj + si*tj + eij,$$

where *Yijk* is the measured variable; *μ* is the overall mean; *si* is the effect of the straw of the i^{th} condition (with or without straw); *tj* is the effect of the lime preparation before the hydrolysis of the j^{th} time; *si*tj* is the interaction between straw and lime preparation before the hydrolysis; *eij* is the residual error.

Regression model for the pH and temperature along the time,

$$Y_{ijk} = b0 + b1x_{i1} + b2x_{i2} + b3x_{i3} + b4x_{i4} + \alpha_j + e_{ij},$$

where *Yij* is the measured of dependent variable; b's are the coefficients of the regression; x*i* is the independent variable; α*j* is the deviation of j^{th} regression; *eij* is the residual error.

Data were subjected to normality and homogeneity analysis and adjusted when necessary. For chemical analysis of sugarcane data were subjected to analysis of variance by PROC GLM using SAS (2001). When significant differences were detected, means were tested by Tukey HSD range test. For the pH and temperature, a polynomial regression analysis with split plots was performed.

An additional study using scanning electron microscopy (SEM) was done. Samples from fresh and hydrolyzed sugarcane with and without straw were taken to be observed before and after *in vitro* digestion. The digestion from sugarcane samples was determined using a Fermenter machine (Rumen Ankom®, "Daisy Fermenter II"). Sugarcane was analyzed from 12 to 24 hours after the hydrolysis process.

An amount of 0.5 g of sample for *in vitro* digestion was weighed in the bag F57® digestion. These were then sealed and placed in digestion jars (up to 25 bags per jar) containing the previously prepared solution. In each jar, 1600 mL of pre-warmed buffer (at 39°C) was added, consisting of the mixture in a 5:1 ratio of two solutions called, 'A' and 'B' respectively. The 'A' solution consisted of, 10g/L of KH_2PO_4; 0.5 g/L of $MgSO_4.7H_2O$; 0.5g/L of NaCl; 0.1g/L $CaCl_2$, and 0.5g/L of reactive urea-grade. The 'B' solution consisted of, 15g/L Na_2CO_3, and 1.0g/L of $Na_2S.9H_2O$.

After that, the rumen inoculums liquid was added into each jar (400 mL per jar). The inoculum of rumen fluid was obtained from a donor animal. The animal was subjected to a 20-day adaptation period to the diet. A diet consisting of 50:50% fresh and hydrolyzed sugarcane, concentrate, mineral mixture and *ad libitum* water was supplied. Samples were incubated during 48 hours. After that period, a second stage was carried out with the addition of 8 g of pepsin and 40mL of 6N HCl in each jar, keeping the system heated at 39^oC for more 24 hours. After the incubation period, the bags with samples inside were dried at 55^oC.

Samples were fixed on a metalic specimen holder of cylindrical shape using double-sided tape of conductive material (copper) (Figure 1). After fixed the samples, the metallization was made. Metallization is essential for the material to become electrically conductive and comprises in the deposition of a thin layer (about 35 nm) of palladium gold (JEOL JFC1100, Figure 2). Evaluations by observation in a SEM were made using the JEOL brand JSM5410 model machine (Figure 2). This analysis aimed to make possible the microscopic understanding of the role of calcium hydroxide on the sugarcane fibers before and after the *in vitro* digestion.

Figure 1. Specimen holder with the double-sided copper tape and sugarcane samples (1.0 mm particle size)

Figure 2. Machine (JEOL JFC 1100) on the left and scanning electron microscope on the right (JSM5410).

3. Results and discussion

Evaluating the influence of supplying sugarcane with straw for the animals as well as the time of lime preparation before the hydrolysis of sugarcane and its chemical composition is important for the choice on how this roughage should be provided to the ruminants.

Regarding the straw aspect (Figure 3), a higher NDFa (450 g/kg) and ADF (231 g/kg) were observed for sugarcane with straw than for sugarcane without straw

(NDFa, 412 g/kg and ADF 204 g/kg). Straw, according to Santos et al. (2012) is composed basically by 420 g/kg of cellulose, 315 g/kg of hemicellulose and 235 g/kg of lignin. It is possible to confirm the higher NDFa and ADF of sugarcane with straw. NDF is constituted by cellulose, hemicellulose and lignin, while ADF is composed by cellulose and lignin. Lower DM (218 g/kg) and NFC (461 g/kg) were observed for sugarcane with straw compared to sugarcane without straw (DM, 224 g/kg and 496 g/kg). Sugarcane has an important amount of NFC, mainly because sucrose is a main compound of this roughage. This can collaborate to increase DM of sugarcane.

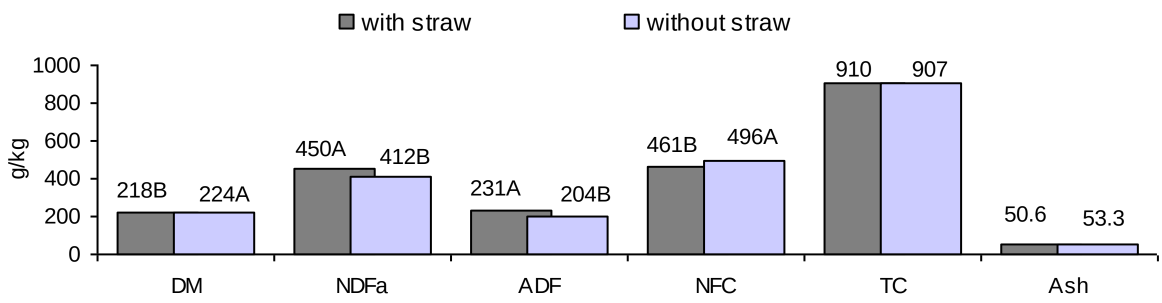

Figure 3. Chemical composition of sugarcane with or without straw. Dry matter (DM), neutral detergent fiber ash-free (NDFa), acid detergent fiber (ADF), non-fiber carbohydrates (NFC), total carbohydrates (TC) and ash (g/kg in DM basis). Tukey test ($p<0.05$)

Time of lime preparation interfered in the amount of NDFa (Figure 4a), NFC, TC and ash (Figure 4c), but did not interfere in the amount of DM and ADF (Figure 4a and 4b). $Ca(OH)_2$ suspension prepared 48 hours before the hydrolysis of sugarcane provided higher NDFa (458 g/kg) compared to fresh sugarcane (402 g/kg). Other treatments did not differ ($p>0.05$).

The obtained NFC values (Figure 2c) acted oppositely compared to those of NDFa, i.e. NFC of sugarcane hydrolyzed using $Ca(OH)_2$ suspension prepared 24 hours (465 g/kg) and 48 hours (446 g/kg) before its application was lower than fresh sugarcane (522 g/kg). Value of TC for fresh sugarcane (924 g/kg) was higher compared to hydrolyzed sugarcane, regardless the time of lime preparation before the hydrolysis. Regarding the ash content, $Ca(OH)_2$ suspension prepared 0.5 hour before the hydrolysis was lower (51.9 g/kg) compared to $Ca(OH)_2$ suspension prepared 24 hours before (59.4 g/kg).

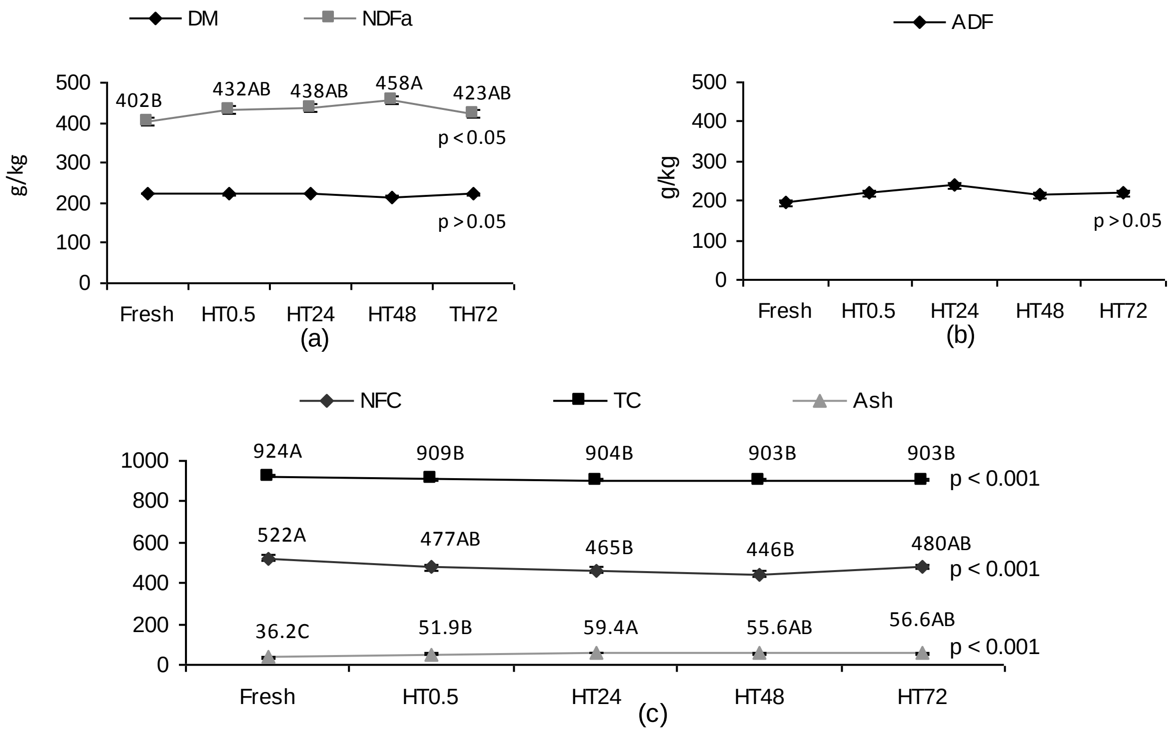

Figure 4. Chemical composition of fresh sugarcane or hydrolyzed sugarcane (mean ± SEM) using the lime prepared 72h, 48h, 24h and 0.5h before the hydrolysis: (a) dry matter (DM) and neutral detergent fiber ash-free (NDFa), (b) acid detergent fiber (ADF), (c) non-fiber carbohydrates (NFC), total carbohydrates (TC) and ash (g/kg in DM basis)

Oliveira (2010) recommendation of 0.5 kg of $Ca(OH)_2$ diluted in 2 liters of water is not concerned to obtain a solution, but a facilitator vehicle to hydrolyze the sugarcane. Only 1.73 g of $Ca(OH)_2$ can be dissolved per 1 liter of water (OMRI, 2002). That means, 0.5 kg of $Ca(OH)_2$ diluted in 2 liters of water, only 3.46 g was dissolved. The rest should have remained in a suspension form and exactly 467 g was sinking to the bottom. Even though the $Ca(OH)_2$ suspension was mixed by agitation before the hydrolysis process, time 0.5 hour of the lime preparation could have been not enough to saturate the 3.46 g of mixture. Hence higher residual of $Ca(OH)_2$ remained in the container used to prepare the suspension. Fresh sugarcane had lower ash (36.2 g/kg), due to non-use of additive $Ca(OH)_2$ with mineral properties.

Mota et al. (2010) evaluated the hydrolysis of sugarcane using calcium oxide and calcium hydroxide, and found 250 g/kg of DM, 401 g/kg of NDF, 187 g/kg of ADF and 33.0 g/kg of ash. In the current study, it was found similar averages for hydrolyzed sugarcane without straw 224 g/kg of DM, 412 g/kg of NDFa, 204 g/kg of ADF and 53 g/kg of ash.

Interaction between straw and time of lime preparation was observed for NDFa (Table 1, Figure 5), NFC (Table 2, Figure 6), TC (Table 3, Figure 7) and ash (Table 4, Figure 8). In general, the highest NDFa values were obtained for sugarcane with straw treatments. It, however, did not affect the NDFa value when $Ca(OH)_2$ suspension was prepared 0.5 and 24 hours before the sugarcane hydrolysis (Table 1, Figure 5).

Table 1. Unfolding the interaction, straw and time of lime preparation of neutral detergent fiber ash-free (NDFa) of sugarcane (g/kg in DM basis)

	NDFa					
Straw	Fresh	HT0.5	HT24	HT48	HT72	P-value
With	432^{Aab}	442^{ab}	424^{b}	488^{Aa}	461^{Aab}	0.038*
Without	371^{Bb}	422^{ab}	453^{a}	428^{Bab}	384^{Bb}	0.003**
P-value	0.007**	0.355ns	0.187ns	0.008**	0.001**	

Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis. Columns with different capital letters are significantly different, Tukey test. Line with different small letters are significantly different, Tukey test. *(p<0.05), **(p<0.01), ns = non-significant.

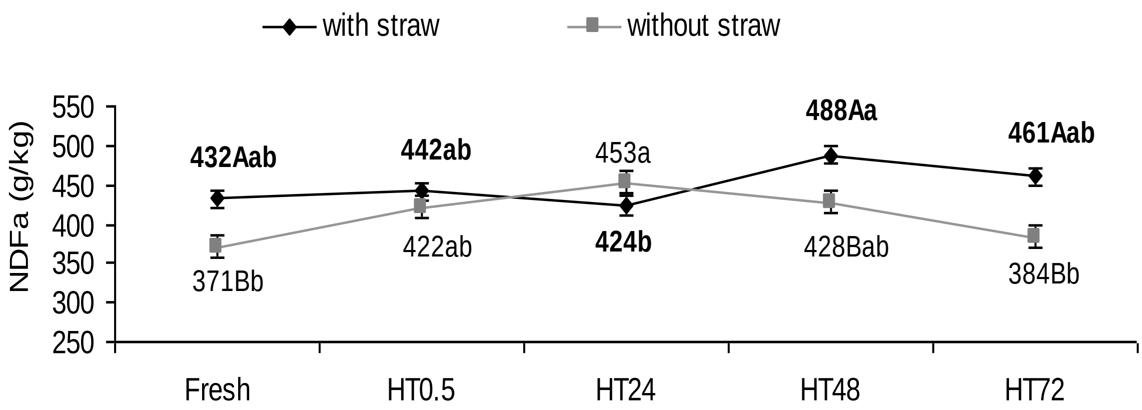

Figure 5. Interaction of straw and time of lime preparation of neutral detergent fiber ash-free (NDFa) of sugarcane (g/kg in DM basis) (mean ± SEM). Treatments: Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis. Different capital letters are significantly different between straw. Different small letters are significantly different between the times of lime preparation before the hydrolysis, Tukey test

The lowest values of NDFa were observed for fresh sugarcane without straw (371 g/kg) and hydrolyzed sugarcane without straw using $Ca(OH)_2$ suspension prepared 72 hours before the hydrolysis (HT72, 384 g/kg). This is a nutritionally important factor because it is related to intake regulation in ruminants (Berchielli et al., 2011). This is confirmed because the straw is basically composed of cellulose (444 g/kg), hemicellulose (307 g/kg) and lignin (198 g/kg) (Santos et al., 2014), which are three known components of NDF.

For NFC (Table 2, Figure 6), the results were opposite to NDFa values, i.e. the lowest NFC values were obtained in sugarcane with straw treatments. It, however, did not affect (p>0.05) the NFC value when $Ca(OH)_2$ suspension was prepared 0.5 and 24 hours before the hydrolysis of sugarcane. The lowest NFC values were observed for sugarcane with straw HT48 (419 g/kg).

Table 2. Unfolding the interaction, straw and time of lime preparation of non-fiber carbohydrates (NFC) of sugarcane (g/kg in DM basis)

	NFC					
Straw	Fresh	HT0	HT24	HT48	HT72	P-value
With	493^{Ba}	468^{ab}	486^{ab}	419^{Bb}	438^{Bab}	0.014*
Without	552^{Aa}	486^{abc}	444^{c}	474^{Abc}	523^{Aab}	<0.001**
P-value	0.016*	0.442ns	0.084ns	0.023*	0.001**	

Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis. Columns with different capital letters are significantly different, Tukey test. Line with different small letters are significantly different, Tukey test. *(p<0.05), **(p<0.01), ns = non-significant.

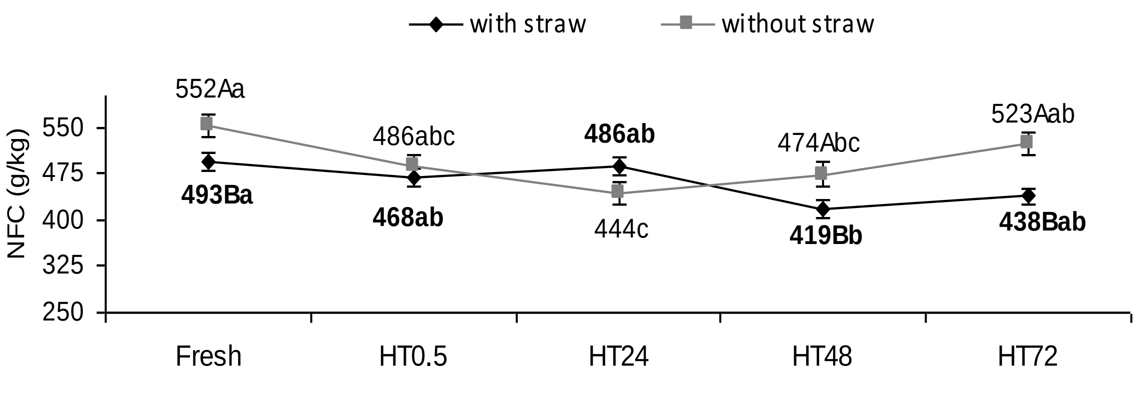

Figure 6. Interaction of straw and time of lime preparation of non fiber-carbohydrates (NFC) of sugarcane (g/kg in DM basis) (mean ± SEM). Treatments: Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis. Different capital letters are significantly different between straw. Different small letters are significantly different between the times of lime preparation before the hydrolysis, Tukey test

Regardless of the presence or absence of straw in sugarcane, the TC was higher for fresh sugarcane ($p<0.05$) compared to hydrolyzed sugarcane mainly due to the presence of ash from $Ca(OH)_2$ additive (Table 3, Figure 7). As it is known, TC is obtained by TC = 100 - (%CP + %EE + %ash) (Mertens, 1997). Hydrolyzed sugarcane with straw, using $Ca(OH)_2$ suspension prepared 24 hours before the hydrolysis had higher TC value (910 g/kg) compared to hydrolyzed sugarcane without straw in the same time of lime preparation (897 g/kg).

Table 3. Unfolding the interaction, straw and time of lime preparation of total carbohydrates (TC) of sugarcane (g/kg in DM basis)

	TC					
Straw	Fresh	HT0	HT24	HT48	HT72	P-value
With	925^{a}	910^{b}	910^{Ab}	907^{b}	899^{b}	<0.001**
Without	923^{a}	908^{b}	897^{Bb}	902^{b}	907^{b}	<0.001**
P-value	0.734ns	0.682ns	0.005**	0.280ns	0.835ns	

Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis. Columns with different capital letters are significantly different, Tukey test. Line with different small letters are significantly different, Tukey test. *($p<0.05$), **($p<0.01$), ns = non-significant.

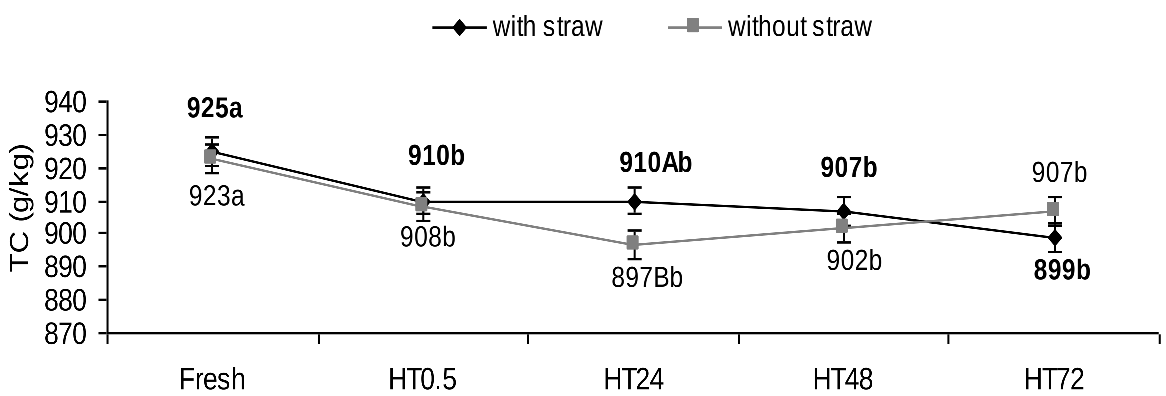

Figure 7. Interaction of straw and time of lime preparation of totan carbohydrates (TC) of sugarcane (g/kg in DM basis) (mean ± SEM). Treatments: Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis. Different capital letters are significantly different between straw. Different small letters are significantly different between the times of lime preparation before the hydrolysis, Tukey test

Sugarcane with straw differed ($p<0.05$) in ash content, when the $Ca(OH)_2$ suspension was prepared 24 and 48 hours before the hydrolysis of sugarcane (Table 4, Figure 8). Lower ash for hydrolyzed sugarcane with straw HT24 (5.5 g/kg) and HT48 (5.2 g/kg) compared to hydrolyzed sugarcane without straw (HT24: 6.3 g/kg and HT48: 6.0 g/kg). Higher values ($p<0.05$) of ash were found for treatments with hydrolysis. This behavior was expected due to the presence of minerals such as calcium and magnesium on the additive used, $Ca(OH)_2$.

Table 4. Unfolding the interaction, straw and time of lime preparation of ash of sugarcane (g/kg in DM basis)

	Ash					
Straw	Fresh	T0	T24	T48	T72	P-value
With	3.4^{b}	5.2^{a}	5.5^{Ba}	5.2^{Ba}	5.9^{a}	<0.001**
Without	3.8^{c}	5.1^{b}	6.3^{Aa}	6.0^{Aab}	5.4^{b}	<0.001**
P-value	0.242ns	0.754ns	0.017*	0.015*	1.107ns	

Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis. Columns with different capital letters are significantly different, Tukey test. Line with different small letters are significantly different, Tukey test. *($p<0.05$), **($p<0.01$), ns = non-significant.

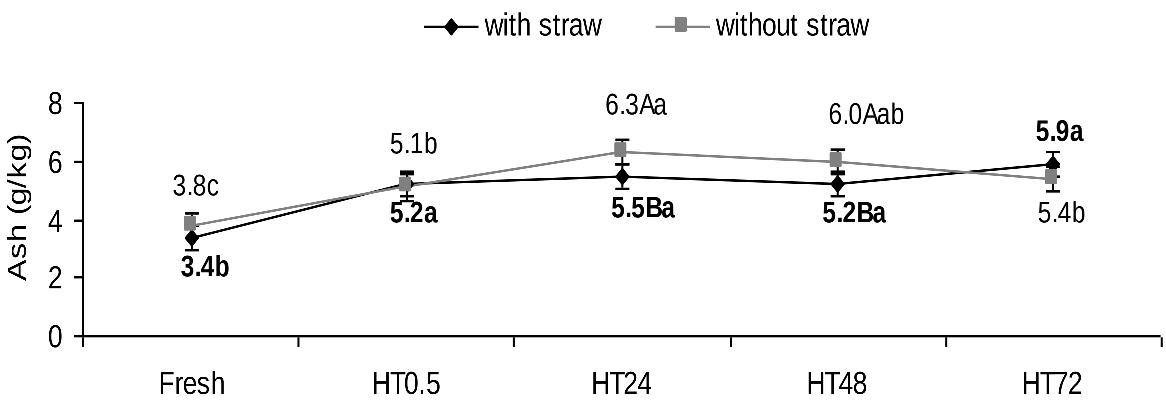

Figure 8. Interaction of straw and time of lime preparation of ash of sugarcane (g/kg in DM basis) (mean ± SEM). Treatments: Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis. Different capital letters are significantly different between straw. Different small letters are significantly different between the times of lime preparation before the hydrolysis, Tukey test

From the chemical composition of sugarcane point of view, lower NDFa was found for fresh sugarcane without straw and hydrolyzed sugarcane with straw using the lime prepared 72 hours before the hydrolysis. Higher NFC was found for fresh sugarcane without straw and hydrolyzed sugarcane without straw using the lime prepared 72 hours before the hydrolysis. Those were the criteria used to choose the treatments that were provided to the cows to analyze performance, milk composition and *in vitro* digestibility of nutrients (Chapter 3).

In general, pH of sugarcane stacks decreased ($p<0.05$) as time increased from 0.5 to 9 hours (Figure 9). Just on time 3 and 6 hours, pH was similar ($p >0.05$). Remarkable decrease of pH was observed during the first three hours and at the end of the hydrolysis process. Domingues et al. (2011) evaluated the aerobic stability of sugarcane hydrolyzed using CaO, and they found 9.8 of initial pH and 7.1 of final pH, after 24 hours of hydrolysis.

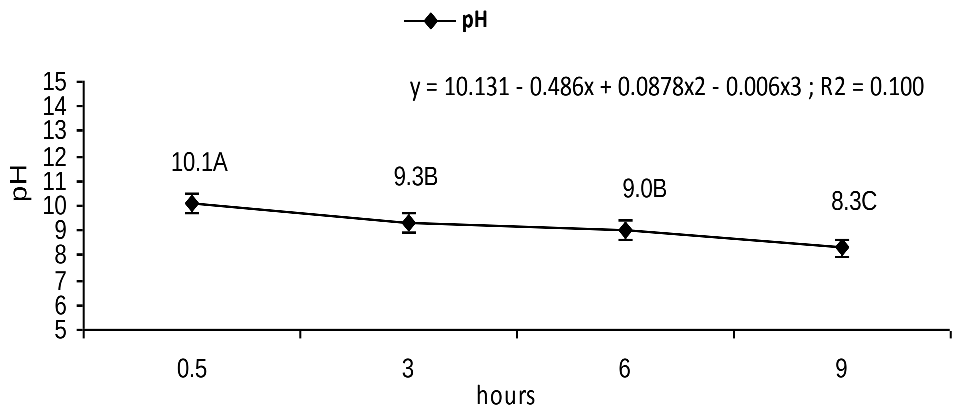

Figure 9. pH values (mean ± SEM) according to the treatments over time

Fresh sugarcane with or without straw had lower pH values compared to hydrolyzed sugarcane with or without straw. This fact demonstrates that the alkalizing action of the lime ($Ca[OH]_2$) suspension on hydrolyzed sugarcane occurred (Figure 10). Additionally, straw did not affect the pH values of sugarcane among treatments.

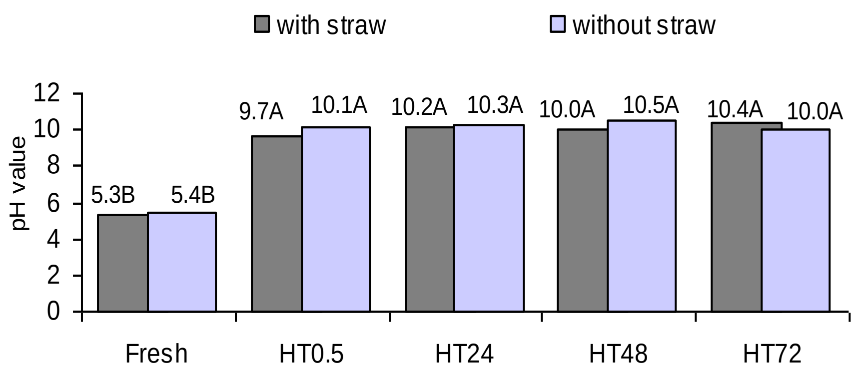

Figure 10. pH values (mean ± SEM) according to the treatments. Treatments: Fresh sugarcane i.e. no lime used, HT0.5, HT24, HT48, HT72, i.e. hydrolyzed sugarcane and time 0.5, 24, 48 and 72 hours of lime preparation before the hydrolysis

Fresh sugarcane with (Figure 11a) or without (Figure 11b) straw had constant behavior of pH, around 5.4. pH of hydrolyzed sugarcane decreased as time increased from 0.5 to 9 hours, showing quadratic and cubic behaviors. However, it seems that hydrolyzed sugarcane with straw using the lime prepared 24 and 48 hours before the hydrolysis causes an unstandardized pH decrease, i.e., cubic behavior.

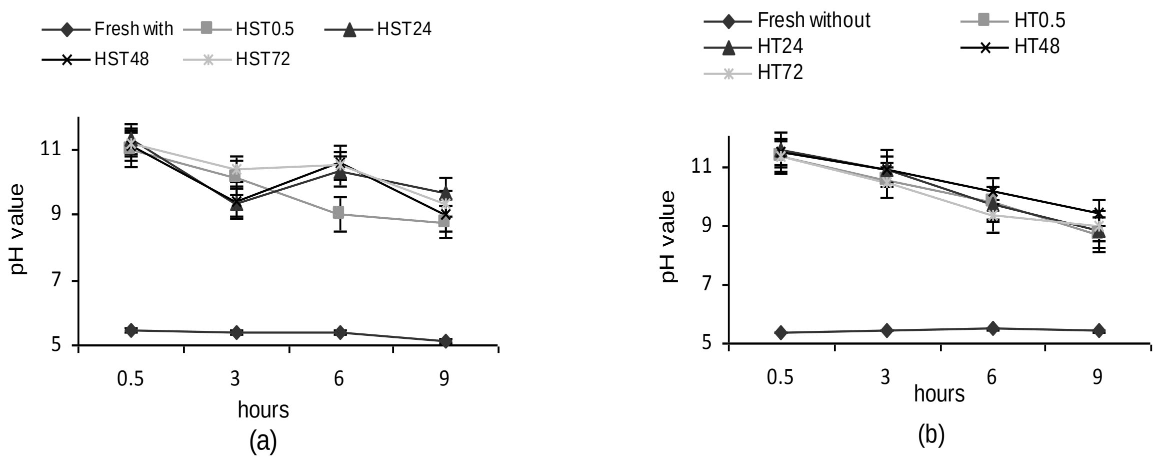

Figure 11. pH values of sugarcane (mean ± SEM) (a) with or (b) without straw, using the lime prepared 72h, 48h, 24h and 0.5h before the hydrolysis

Lower pH for fresh sugarcane (5.3) was observed compared to hydrolyzed sugarcane (10.2), regardless of the presence or not of straw. Use of $Ca(OH)_2$ naturally increases the pH of hydrolyzed material due to presence of alkaline property in their composition. The pH during the hydrolysis time (0.5, 3, 6 and 9 hours) is shown in Table 5.

Immediately after the application of $Ca(OH)_2$, i.e., $Ca(OH)_2$ suspension prepared 0.5 hour before the hydrolysis, pH had an average of 11.3, and after 9 hours of hydrolysis, pH had an average of 9.1. There was a decrease in pH as time increased for treatments submitted to hydrolysis; but this decrease did not achieve the average pH obtained for fresh sugarcane (5.3). Oliveira (2010) evaluated sugarcane hydrolyzed using 0.5% $Ca(OH)_2$, and found initial pH of 10.9, whereas after 6 hours of hydrolysis the pH was 9.7.

Table 5. Unfolding the interaction, time of lime preparation within treatment for pH.

Sugarcane	0.5 hours	3 hours	6 hours	9 hours	P-value
Fresh, with straw	5.4	5.4	5.4	5.2	0.94ns
Fresh, without straw	5.4	5.4	5.4	5.4	0.99ns
HST0.5	11.0^{A}	10.2^{AB}	9.0^{BC}	8.8^{C}	<0.0001**
HT0.5	11.4^{A}	10.5^{AB}	9.8^{BC}	8.7^{C}	<0.0001**
HST24	11.3^{A}	9.4^{B}	10.4^{AB}	9.7^{B}	0.0008**
HT24	11.6^{A}	10.9^{AB}	9.7^{BC}	8.9^{C}	<0.0001**
HS48	11.2^{A}	9.4^{BC}	10.6^{AB}	9.0^{C}	<0.0001**
HT48	11.5^{A}	10.9^{AB}	10.1^{BC}	9.4^{C}	0.0006**
HST72	11.2^{A}	10.4^{AB}	10.6^{AB}	9.3^{B}	0.0038**
HT72	11.3^{A}	10.4^{AB}	9.3^{BC}	9.0^{C}	<0.0001**
P-value	ns	ns	ns	ns	
SEM	0.075	0.325	0.284	0.580	

HST0.5 and HT0.5 = with and without straw, time 0.5 hour of lime preparation before the hydrolysis. HST24 and HT24 = with and without straw, time 24 hours of lime preparation before the hydrolysis. HST48 and HT48 = with and without straw, time 48 hours of lime preparation before the hydrolysis. HST72 and HT72 = with and without straw, time 72 hours of lime preparation before the hydrolysis. Line with different letters are significantly different, Tukey test. ** ($p<0.01$), ns = non-significant.

Internal temperature of sugarcane stacks had cubic behavior as time increased, but no temperature increase was observed (Figure 12). External temperature of sugarcane stacks had quadratic behavior as time increased, and temperature increase was observed just during the first three hours of hydrolysis (Figure 12). This behavior indicates that during the beginning of hydrolysis process there is an increase of temperature, probably due to chemical reactions that occurred with aerobic exposure. External temperature (17.5^{o}C) was lower than internal temperature (18.9 ^{o}C) after 9 hours of hydrolysis. This is because the external area of the stacks was under wind action.

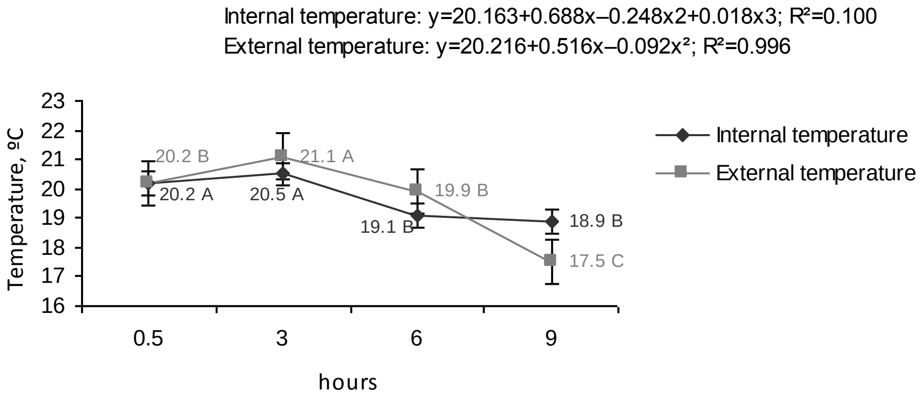

Figure 12. Internal and external temperatures (mean ± SEM) according to the treatments over time

Hydrolyzed sugarcane without straw, had lower ($p < 0.05$) internal (16.5 ºC) and external (15.6ºC) temperatures of stacks after 9 hours. Whereas, the temperatures of hydrolyzed sugarcane with straw were 21.2 and 19.1°C, respectively (Figure 13).

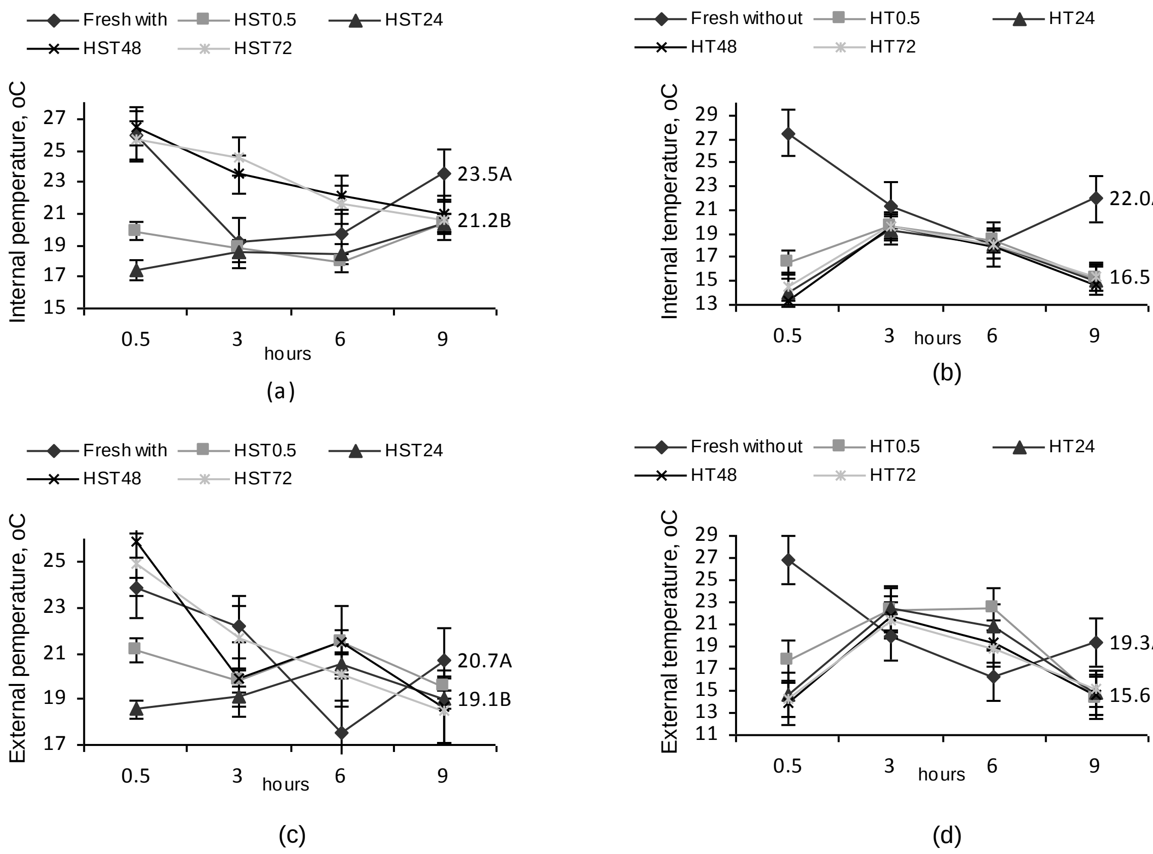

Figure 13. Internal temperature of sugarcane (mean ± SEM) with (a) or without (b) straw, using the lime prepared 72h, 48h, 24h and 0.5h before the hydrolysis. External temperature of sugarcane with (c) or without (d) straw, using the lime prepared 72h, 48h, 24h and 0.5h before the hydrolysis.

Fresh sugarcane, temperatures ranged from 19.3ºC (without straw) and 23.5ºC (with straw). Oliveira et al. (2008) evaluated the internal and external temperature of hydrolyzed sugarcane stacks using 0.5% of $Ca(OH)_2$ and found 23.0°C of internal and 24.6°C of external temperatures after 9 hours of hydrolysis. Straw of sugarcane consists of approximately 95% of DM, i.e. 5% water. It is known that water is a thermal insulator, therefore the highest temperature observed in stacks of sugarcane with straw can be explained by the lower level of water acting as a thermal insulator. Internal and external temperatures of fresh sugarcane were lower compared to hydrolyzed sugarcane because the uses of high levels of lime reduces the water activity and the development of microorganisms (SANTOS et al., 2008).

It is possible to observe the SEM images of sugarcane before and after the *in vitro* digestion in Figure 14. Small particles are observed in the images of sugarcane before the *in vitro* digestion (Figure 14 a, c and e). These small particles suffered the *in vitro* digestion (Figure 14 b, d and f). More particles remained undigested in hydrolyzed sugarcane without straw samples compared with fresh and hydrolyzed sugarcane with straw samples, after *in vitro* digestion. As shown in Figure 3, NDF content was higher for sugarcane with straw (450 g/kg) than sugarcane without straw (412 g/kg). Using the SEM images, it is possible to say that the straw suffered digestion. Higher digestion of hydrolyzed sugarcane with straw occurred probably because of the straw hydrolysis.

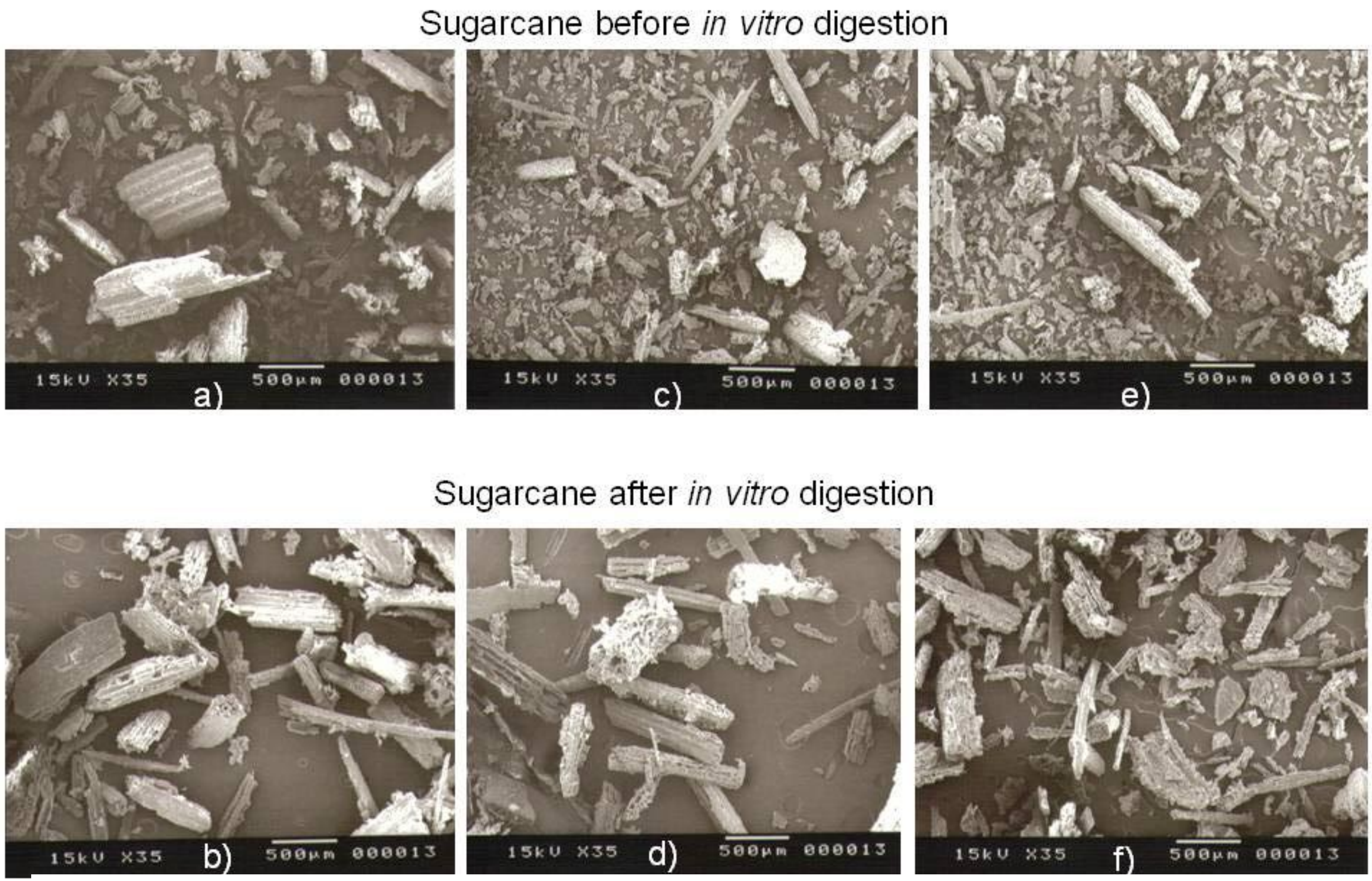

Figure 14. Fresh sugarcane (a) and (b), hydrolyzed sugarcane with straw (c) and (d), hydrolyzed sugarcane without straw (e) and (f).

4. Conclusions

From the chemical composition point of view, fresh sugarcane without straw and hydrolyzed sugarcane with straw using the lime prepared 72 hours before the hydrolysis can be used to feed cows because, in general, provided lower neutral detergent fiber. Hydrolyzed sugarcane has some advantages such as the possiblility to store it, facilitating the labor. Additionally, it provides lower temperatures of the stacks of sugarcane and higher pH that is interesting for conservation.

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CHAPTER 3 – EVALUATING THE INFLUENCE OF STRAW AND TIME OF LIME PREPARATION BEFORE THE HYDROLYSIS OF SUGARCANE ON NUTRIENT DIGESTIBILITY AND PERFORMANCE OF DAIRY COWS

ABSTRACT – This study aimed to evaluate the influence of straw and the time of lime preparation before the hydrolysis of sugarcane on *in vitro* digestibility of nutrients, nutrient intake, milk production and composition in cows fed hydrolyzed sugarcane. Five treatments were used: fresh sugarcane without straw, HST0.5 (hydrolyzed sugarcane with straw, using the lime prepared 0.5 hour before the hydrolysis), HST72 (hydrolyzed sugarcane with straw, using the lime prepared 72 hours before the hydrolysis, HT0.5 and HT72, hydrolyzed sugarcane without straw using the same procedures for sugarcane with straw. Calcium hydroxide ($Ca(OH)_2$) was the lime used. Experiment 1 was arranged as a completely randomized design to evaluate the *in vitro* dry matter (IVDMD), neutral detergent fiber (IVNDFD) and acid detergent fiber (IVADFD) digestibilities of sugarcane. Experiment 2 was arranged as a Latin square 5 x 5 to evaluate the performance of the dairy cows fed sugarcane as roughage. Hydrolyzed sugarcane with straw had lower values of IVDMD (HST0, 57.3% and HST72, 55.2%) and higher values of IVNDFD (HST0, 45.5% and HST72, 47.0%) compared to sugarcane without straw (fresh or hydrolyzed). The highest IVADFD was obtained for HST72 (29.9%), followed by HST0 (26.1%), then HT72 (21.9%) and the lowest values were for sugarcane without straw for fresh (17.6%) and HT0 (18.3%). Regardless of the time of lime preparation, sugarcane with straw provided lower DM intake (HST0, 13.5 kg/d and HST72, 12,0 kg/d) compared to the fresh (15.4 kg/d) or hydrolyzed sugarcane without straw (HT0, 15.5 kg/d and HT71,16.0 kg/d). The lowest intake of OM, TC and TDN was observed for sugarcane with straw regardless of the time of lime preparation. For sugarcane hydrolyzed with the lime prepared 72 hours before the hydrolysis process, a lower intake of CP, NDF, ADF and ash compared with fresh or hydrolyzed sugarcane was found. Milk production was 12.6 kg/d, whereas composition was 3.6% of fat, 3.5% of protein, 4.9% of lactose, 8.9% of solids-non-fat and 12.9% of total solids. Milk production and composition were similar among the treatments. As the feed efficiency of dairy cows was been improved and the digestibility of fibers increased, sugarcane with straw can be used in animal nutrition. In addition, the $Ca(OH)_2$ suspension may be prepared three days before the hydrolysis of sugarcane to facilitate the labor.

Keywords: feed efficiency, intake, milk production, neutral detergent fiber

Influência da palha e do tempo de preparo da calda antes da hidrólise da cana-de-açúcar na digestibilidade dos nutrientes e no desempenho de vacas

RESUMO – Este estudo teve como objetivo avaliar a influência da palha e do tempo de preparo da suspensão de hidróxido de cálcio na produção e na composição do leite, na digestibilidade *in vitro* de nutrientes, e no consumo pelas vacas alimentadas com cana-de-açúcar hidrolisada. Cinco tratamentos foram utilizados: cana-de-açúcar fresca sem palha, HPT0,5 (cana-de-açúcar hidrolisada com palha e a calda preparada 0,5 hora antes da hidrólise), HPT72 (cana-de-açúcar hidrolisada com palha e a calda preparada 72 horas antes da hidrólise), HT0,5 e HT72 que foram a cana-de-açúcar hidrolisada sem palha submetidas aos mesmos procedimetos da cana-de-açúcar com palha. A cal utilizada foi o hidróxido de cálcio $Ca(OH)_2$. O experimento 1 foi um delineamento inteiramente casualizado para avaliar a digestibilidade *in vitro* da matéria seca (DIVMS), fibra em detergente neutro (DIVFDN) e fibra em detergente ácido (DIVFDA). O experimento 2 foi distribuído em quadrado latino 5 x 5 para avaliar o desempenho de vacas leiteiras. Em comparação com cana-de-açúcar sem palha (fresca ou hidrolisada), a cana-de-açúcar hidrolisada com palha obteve menores valores de DIVMS (HST0, 57,3% e HST72, 55,2%) e valores mais elevados de DIVFDN (HST0, 45,5% e HST72, 47,0%). A maior DIVFDA foi obtida para HPT72 (29,9%), seguido por HPT0 (26,1%), em seguida, HT72 (21,9%) e os valores mais baixos foram para a cana de açúcar sem palha fresca (17,6%) e HT0 (18,3%). Independentemente do tempo de preparo do $Ca(OH)_2$, a cana-de-açúcar hidrolisada com palha diminuiu a ingestão de MS (HPT0, 13,4 kg/dia e HPT72, 11,9 kg/dia), em comparação a cana fresca (15,4 kg/dia) ou cana-de-açúcar hidrolisada sem palha (HT0, 15,5 kg/dia e HT72 16,0 kg/dia). O menor consumo de MO, CT e NDT foi observado para cana-de-açúcar com palha. Para cana-de-açúcar hidrolisada com $Ca(OH)_2$ preparado 72 horas antes da hidrólise, encontramos menor consumo de PB, FDN, FDA e cinzas em comparação com cana-de-açúcar fresca ou hidrolisada. A produção de leite foi de 12,6 kg/d, enquanto que a composição de gordura foi de 3,6%, proteína de 3,5% lactose de 4,9%, extrato seco desengordurado de 8,9% e de sólidos totais de 12,9%. A produção e a composição do leite foram semelhantes entre os tratamentos. Como a eficiência alimentar das vacas leiteiras foi melhor, bem como a digestibilidade das fibras também melhorou, a cana-de-açúcar com palha pode ser utilizada na alimentação animal. Além disso, a suspensão de $Ca(OH)_2$ pode ser preparada três dias antes da hidrólise da cana-de-açúcar a fim de facilitar a mão-de-obra.

Palavras-chave: consumo, eficiência alimentar, fibra em detergente neutro, produção de leite

1. Introduction

Sugarcane has been used by farmers because it has positive aspects, such as high energy content and high potential production of dry matter, especially when the forage is scarce (Oliveira, 2010). However, sugarcane has some drawbacks such as low crude protein content requiring supplementation. In addition, there is a need of daily cuts due to the high level of soluble carbohydrates, which makes the environment favorable for the development of microorganisms that deteriorate the sugarcane. Hydrolysis of sugarcane has been used to improve its nutritional value and minimize these problems (Carvalho et al., 2011). Another benefit of hydrolysis is the possibility to store the sugarcane for up to three days, which eliminates the need of daily cuts (Oliveira, 2010).

Particle size of chopped sugarcane and the lime type used for hydrolysis are factors that affect the hydrolysis of sugarcane (Oliveira, 2010). However, factors such as the straw of sugarcane and the time of lime preparation of calcium hydroxide before the hydrolysis process have not been reported. Usually lime is prepared (by mixing the $Ca(OH)_2$ with water) every day by producers to hydrolyze the sugarcane. It is relevant therefore to lead experiments to understand the action of such factors on hydrolyzed sugarcane.

Frequently, burning of the sugarcane to remove the straw and the pointer is performed on a large scale. This burning practice is justified by the sugarcane's harvest style, which is usually manually done using a machete. The mechanized harvesting system has advanced, but the manual harvest, still prevails in medium and small producers. Pre-harvest burning of the sugarcane fields rises the temperature of sugarcane, and causes sucrose losses by exudation (Georges, 2011). According to the same author, burning of the sugarcane fields is made in a controlled way and is characterized by being an efficient and economical cleaning process. Also, it reduces the hand labor and the incidence of poisonous animals in the sugarcane field.

The necessity to decrease the burning process in the sugarcane fields, however, has been discussed in the Federal and State Laws aiming to a sustainable production and a reduction of environmental impacts. By reducing the burning, a pertinent question about what to do with all the straw produced in the fields emerges. According to Macedo and Nogueira (2004), a ton of sugarcane produces on average 140 kg of straw on DM basis. The current challenge is to find alternatives for the efficient use of sugarcane straw (Georges, 2011). The use of sugarcane straw in animal feed has been taken considered, but special attention in balancing diets containing straw is required because of its low nutritional value (Rodrigues and Souza, 2005).

Evaluating crossbred Gyr and Holstein cows (508 kg body weight), between 60-80 lactation days, Alves et al. (2010) observed an increase in milk production of 1.7 kg (10.4%) when cows were fed diets containing sugarcane hydrolyzed using 1% of $Ca(OH)_2$. The technique of hydrolysis provides, for small and medium milk producers good economic return since the cost per kilogram to produce milk is lower compared with corn silage diets (Oliveira, 2010). This study aimed to evaluate the influence of straw and the time of lime preparation before the hydrolysis of sugarcane on *in vitro* digestibility of nutrients, nutrient intake, milk production and composition in Holstein cows.

2. Material and methods

In the digestibility study (experiment 1), the effect of straw and time of lime preparation before the hydrolysis on sugarcane was analyzed and studied. Then, in experiment 2, the effect of feed on cows' performance was evaluated.

2.1 Location and management

All procedures involving animals were approved by the Ethical Committee of Experimental Animals of Brazil. The study was carried out on Sao Paulo State University, campus of Jaboticabal, Brazil.

The IAC 86-2480 was the variety of sugarcane used, harvested at 18 months of age, used with or without straw, and chopped in particle size of 1.0 cm. Part of sugarcane harvested was manually obtained using a machete, and other part was obtained using a tractor with chopper of knives coupled. Sugarcane with straw was characterized as the entire plant (stem, leaves and dry leaves, i.e. straw), and sugarcane without straw was defined as stem and leaves.

For the hydrolysis process from 100 kg of fresh sugarcane, 0.5 kg of $Ca(OH)_2$ diluted in 2 liters of water was used (Oliveira, 2010). Chemical composition of the additive was 94.1% of $Ca(OH)_2$ and 1.5% of magnesium oxide.

Harvest of sugarcane was made every other day, as well as its hydrolysis. After harvest, sugarcane was transported to be stored in a shed for later uses in treatments. Hydrolyzed sugarcane was obtained by cutting and spreading it on a tarp, forming a stack. Calcium hydroxide suspension was poured over the sugarcane, and the stack was revolved for homogenization. This is an important process for proper hydrolysis of the fibers.

2.2 Experiment 1 – *in vitro digestibility*

2.2.1 Treatments

A completely randomized design with eight replicates per treatment was applied. Five treatments were used: fresh sugarcane without straw, HST0.5 (hydrolyzed sugarcane with straw, using the lime prepared 0.5 hour before the hydrolysis), HST72 (hydrolyzed sugarcane with straw, using the lime prepared 72 hours before the hydrolysis, HT0.5 (hydrolyzed sugarcane withouth straw, using the lime prepared 0.5 hour before the hydrolysis), HT72 (hydrolyzed sugarcane without straw, using the lime prepared 72 hours before the hydrolysis).

2.2.2 Procedures and data collection

In vitro dry matter (IVDMD), neutral detergent fiber (IVNDFD), and acid detergent fiber (IVADFD) digestibilities from sugarcane samples of treatments was determined using a Fermenter machine (Rumen Ankom®, "Daisy Fermenter II"). Eight replicates per treatment for the analysis were performed. Sugarcane was analyzed from 12 to 24 hours after the hydrolysis process.

An amount of 0.5 g of sample for *in vitro* digestibility of DM, NDF and ADF was weighed in the bag F57® digestion. These were then sealed and placed in digestion jars (up to 25 bags per jar) containing the previously prepared solution. In each jar, 1600 mL of pre-warmed buffer (at 39°C) was added, consisting of the mixture in a 5:1 ratio of two solutions called, 'A' and 'B' respectively. The 'A' solution consisted of, 10g/L of KH_2PO_4; 0.5 g/L of $MgSO_4.7H_2O$; 0.5g/L of NaCl; 0.1g/L $CaCl_2$, and 0.5g/L of reactive urea-grade. The 'B' solution consisted of, 15g/L Na_2CO_3, and 1.0g/L of $Na_2S.9H_2O$.

After that, the rumen inoculums liquid was added into each jar (400 mL per jar). Samples were incubated during 48 hours. After that period, a second stage was carried out with the addition of 8 g of pepsin and 40mL of 6N HCl in each jar, keeping the system heated at 39^{o}C for more 24 hours. After the incubation period, the bags with samples inside were dried at 55^{o}C and then DM content of the samples was determined in an oven at 105^{o}C. *In vitro* digestibility of NDF and ADF were determined in a sequential method, the bags were dried at 55^{o}C and weighed, and were analyzed by the Ankom® Fiber Analyzer.

The inoculum of rumen fluid was obtained from a donor animal. The animal was subjected to a 20-day adaptation period to the diet. A diet consisting of 50:50% fresh and hydrolyzed sugarcane, concentrate, mineral mixture and *ad libitum* water was supplied.

2.2.3 Statistical analysis

The experimental design was completely randomized. Data were subjected to analysis of variance by PROC GLM using SAS (2001). When significant differences were detected, means were tested by Tukey HSD.

The statistical model was as follows:

$$Yi = \mu + ti + ei,$$

where *Yi* is the measured variable; *µ* is the overall mean; *ti* is the fixed effect of the ith treatment; and *ei* is the residual error.

2.3 Experiment 2 – Performance

2.3.1 Diet and animals

Chemical composition of concentrate and sugarcane is presented in Table 1 and the diet composition is showed in Table 2.

Table 1. Chemical composition of concentrate and sugarcane (g/kg in DM basis)

Components	Conc.[2]	Sugarcane[1]				
		Fresh	HST0.5	HST72	HT0.5	HT72
Dry matter	885	250	248	248	251	252
Crude protein	192	44.0	45.0	45.0	41.0	39.0
Ash	78.0	53.0	51.0	50.0	53.0	51.0
NDF[3]	282	532	541	507	541	492
ADF[4]	72.0	200	222	204	214	215
Hemicellulose	209	331	318	302	327	277
Cellulose	183	153	165	165	172	163
Lignin	26.0	47.0	57.0	39.0	41.0	51.0
Ether extract	23.0	5.0	3.0	3.0	7.0	4.0
Organic matter	921	946	949	949	949	948
TC[5]	705	896	900	904	897	898
NFC[6]	423	363	358	397	356	412
TDN, %[7]	55.6	63.1	61.9	62.9	62.4	62.3

[1]Fresh, sugarcane without straw; HST0.5 and HST72, sugarcane with straw hydrolyzed using 0.5% of $Ca(OH)_2$ prepared 0.5 and 72 hours before the hydrolysis (respectively); HT0.5 and HT72, sugarcane without straw hydrolyzed using 0.5% of $Ca(OH)_2$ prepared 0.5 and 72 hours before the hydrolysis (respectively). [2]Conc. = concentrate. [3]NDF= neutral detergent fiber. [4]ADF= acid detergent fiber. [5]TC=total carbohydrates. [6]NFC= non-fiber carbohydrates. [7]TDN = total digestible nutrient, determined according to NRC (2001).

Table 2. Chemical composition of the diets (ratio roughage concentrate, 50:50) (g/kg in DM basis).

Components	Treatments[1]				
	Fresh	HST0.5	HST72	HT0.5	HT72
Dry matter	504	503	503	504	506
Crude protein	103	104	104	101	100
Ash	63.0	62.0	61.0	63.0	62.0
NDF[2]	432	437	417	437	408
ADF[3]	149	162	151	157	158
Hemicellulose	283	275	265	280	250
Cellulose	165	173	172	176	171
Lignin	39.0	44.0	34.0	35.0	41.0
Ether extract	12.0	11.0	11.0	13.0	11.0
Organic matter	936	938	938	938	937
Total carbohydrates	819	822	824	820	821
Non-fiber carbohydrates	387	384	407	382	416
TDN, %[4]	66.3	66.0	67.1	66.0	67.6

[1]Fresh, sugarcane without straw; HST0.5 and HST72, sugarcane with straw hydrolyzed using 0.5% of $Ca(OH)_2$ prepared 0.5 and 72 hours before the hydrolysis (respectively); HT0.5 and HT72, sugarcane without straw hydrolyzed using 0.5% of $Ca(OH)_2$ prepared 0.5 and 72 hours before the hydrolysis (respectively). [2]NDF= neutral detergent fiber. [3]ADF= acid detergent fiber. [4]TDN = total digestible nutrient, determined according to NRC (2001).

Diets were formulated to be isonitrogenous (100 g/kg of CP in DM basis). Roughage concentrate ratio was 50:50, and concentrate consisted of ground corn, soybean meal, cottonseed meal, wheat bran, urea, and mineral mixture, adding up to 19.3% of CP.

Five Holstein cows, at a mean body weight of 518.8 ± 11.6 kg were used. Cows were selected according to milk production (12 kg daily production), parturition order (second and third calving cows) and just after lactation peak (100 lactation days). Cows were identified and randomly distributed in each treatment.

2.3.2 Treatments

A Latin square with five cows and five treatments (5 x 5) was arranged, totaling five experimental periods. Treatments were the same of Experiment 1.

2.3.3 Procedures and data collection

Cows were kept in a tie stall system, provided with mixed diet at the individual feeder and fed were provided twice a day, at 7 and 15 hours, allowing up to 10% refusals of what was supplied. Sugarcane was provided to the cows from 12 to 48 hours after the hydrolysis process. The trial lasted 20 days, which 15 days were used for adaptation and five days to the data collection for each experimental period. Refusals of supplied feed were weighed daily to determine the dry matter intake (DMI); daily milk production was measured as well. Cows were weighed each experimental period. During the experimental period, cows were milked mechanically twice a day, at 6:00 and 14:00 hours.

Samples of the refusals and supplied feed were collected during the last five days of each experimental period and stored in a freezer at -18°C. After the field experiment, samples were pre-dried in a forced-ventilation at 55^{o}C during 72 hours and milled with 1 mm grinding plates. From milled samples, the contents of dry matter (DM), organic matter (OM), ash, crude protein (CP), and ether extract (EE) were determined according to AOAC (1990). Lignin (LIG), neutral detergent fiber ash-free (NDFa) according to Van Soest et al. (1991) and acid detergent fiber (ADF) according to Silva and Queiroz (2002). Total carbohydrates (TC) were calculated by the equation: TC = 100 – (%CP + %EE + %ash), while the non-fiber carbohydrates (NFC) were calculated by the equation: NFC = 100 – (%NDFa + %CP + %EE + %ash) (MERTENS, 1997). Nutrient intake was calculated by the difference between the amount of nutrient in the feed supplied and in the refusals.

Daily milk production was corrected to 4.0% fat according to Sklan et al. (1992). Sampling of milk was done on the 18th day of each experimental period from the two daily milkings (morning and afternoon), according to production. Analyses of composition of fat, protein, lactose and solids-non-fat (SNF) were performed using the ultrasonic analyser milk, Ekomilk Total®.

Blood sample were collected aiming to verify the metabolic parameters of the cows. The blood sampling was done on the 19th day of each experimental period from the tail vein after previous disinfection of the tail. Vacum tubes without anticoagulant were used to obtain the blood serum. After the blood sampling, the material collected was centrifuged at 3000 rpm during 15 minutes. The serum was obtained and stored in 1.5 mL eppendorffs and stored in a freezer at -18^{o}C. The analysis was done in the Laboratory of analysis from University of São Paulo, Pirassununga. Blood metabolites analyzed were total protein determined by the Biuret method (Labtest Diagnostica SA, Brazil); albumin, according to the Green Bromocresol method (Labtest Diagnostica S.A., Brazil); urea by UV enzymatic method (Labtest Diagnostica SA, Brazil); β- hydroxybutyrate (BHB) using the RANBUT kit.

2.3.4 Statistical analysis

The experimental design was completely randomized in a Latin square 5 x 5. Data were subjected to analysis of variance by PROC GLM using SAS (2001). When significant differences were detected, means were tested by Tukey HSD.

The statistical model was as follows:

$$Yijk = \mu + \alpha i + \beta j + tk + eijk,$$

where *Yijk* is the measured variable; *μ* is the overall mean; *αi* is the fixed effect of the i^{th} treatment; *βj* is the random effect of the j^{th} animal; *tk* is the random effect of the k^{th} period; and *eijk* is the residual error.

3. Results and discussion

The study of nutrient digestibility and performance of dairy cows fed sugarcane with or without straw and using the lime prepared in different times before the hydrolysis process was important to determine whether any decrease in nutrient digestibility or performance occurred in lactating cows.

3.1 Experiment 1 – *In vitro digestibility*

Hydrolyzed sugarcane with straw (Table 3) had lower values of IVDMD (HST0.5, 57.3% and HST72, 55.2%) than fresh and hydrolyzed sugarcane without straw (fresh, 67.4% HT0.5, 69.8%, and HT72, 71.3%). For IVNDFD, however, higher values for sugarcane hydrolyzed with straw (HST0.5, 45.5% and HST72, 47.0%) were found compared with sugarcane without straw (fresh, 29.8%, HT0.5, 32.8%, and HT72, 35.9%).

Fresh sugarcane without straw, HT0.5 and HT72 were not different ($p < 0.05$) from each other for IVNDFD. The highest IVADFD value was obtained for HST72 (29.9%), followed by HST0.5 (26.1%), then HT72 (21.9%) and lower digestibility was obtained for fresh sugarcane (17.6%) and HT0.5 (18.3%). Due to the higher IVNDFD in the treatment in which the straw was present, we concluded that, the hydrolysis of the straw was effective enough to improve the digestibility of NDF.

Daniel et al. (2013) found apparent digestibility of DM, 65.9% and NDF, 38.6% in a meta-analysis of the nutritional value of the hydrolyzed sugarcane in Brazil. Silva Junior et al. (2015) evaluated the digestibility of Gyr cows fed hydrolyzed sugarcane and found 56.5% of DM, 27.3% of NDF and ADF 27.7% digestibilities. Assessing fresh sugarcane fed dairy cows, Martins et al. (2011) found DM digestibility of 60.3% and NDF digestibility of 27.9%.

Table 3. Percentages of *in vitro* dry matter (IVDMD), neutral detergent fiber (IVNDFD) and acid detergent fiber (IVADFD) digestibilities of sugarcane under treatments

Treatments[1]	IVDMD	IVNDFD	IVADFD
Fresh	67.4A	29.8D	17.6D
HST0.5	57.3B	45.5A	26.1B
HST72	55.2B	47.0A	29.9A
HT0.5	69.8A	32.8C	18.3D
HT72	71.3A	35.9B	21.9C
CV (%)	2.21	2.63	4.87
SEM[2]	1.82	1.84	1.27
P-value	<0.0001**	<0.0001**	<0.0001**

[1]Fresh, sugarcane without straw; HST0.5 and HST72, sugarcane hydrolyzed with straw using 0.5% of $Ca(OH)_2$ suspension prepared 0.5 and 72 hours before hydrolysis (respectively); HT0.5 and HT72, sugarcane hydrolyzed without straw using 0.5% of $Ca(OH)_2$ suspension prepared 0.5 and 72 hours before hydrolysis (respectively). [2]standard-error mean. Columns with different letters are significantly different, **p< 0.001 (Tukey test).

Sugarcane with straw showed lower DM (24.8%) and higher NDF (52.4%) than the sugarcane without straw (25.1% of DM and 51.6% of NDF) (Table 1). These values possibly affected the IVDMD and IVNDFD of treatments. Sugarcane with straw expressed lower IVDMD and higher IVNDFD compared with sugarcane without straw (Table 3). The digestibility of sugarcane fibers is considered low, approximately 35% (Oliveira, 2010). Daniel et al. (2013) concluded that the hydrolysis of sugarcane improves the digestibility of NDF, but not enough to offset the losses of water soluble carbohydrates due to aerobic exposure.

3.2 Experiment 2 – Performance

Intake of EE (0.2 kg/day), NFC (6.5 kg/day) and LIG (0.5 kg/day) from the sugarcane by dairy cows was not influenced (p> 0.05) by treatments (Table 4). Regardless of the time preparation of $Ca(OH)_2$ suspension, hydrolyzed sugarcane with straw provided lower DM intake compared with the fresh or hydrolyzed sugarcane without straw.

The lower intake of CP, NDFa, ADF and ash in sugarcane with straw was observed when it was hydrolyzed with $Ca(OH)_2$ suspension prepared 72 hours before the hydrolysis process. Despite no large variation was found for nutritional value of the diets (Table 2), in general, sugarcane with straw provided lower intake of some nutrients, such as DM, OM, TC and TDN. It is already known that straw has a low palatability and its intake is limited by the lower rate of passage through the digestive tract due its slow ruminal degradation (Rodrigues and Souza, 2005).

Table 4. Nutrient intake (kg/day) of cows fed sugarcane according to treatments

Intake[2]	Treatments[1]					SEM[3]	P-value	CV (%)
	Fresh	HST0.5	HST72	HT0.5	HT72			
DM	15.4[A]	13.4[B]	11.9[B]	15.5[A]	16,0[A]	0,39	<0.0001**	5.35
CP	1.5A[B]	1.5[AB]	1.3[B]	1.6[A]	1,7[A]	0,11	0.007**	7.04
EE	0.1	0.1	0.1	0.2	0,2	0,04	0.318ns	33.60
OM	14.4[A]	12.6[B]	11.2[B]	13.5[A]	15,1[A]	0,37	0.0001**	5.42
NDF	6.3[A]	4.7[AB]	3.3[B]	6.1[A]	6,1[A]	0,32	0.0005**	13.92
ADF	1.8[AB]	1.5[AB]	0.7[B]	1.9A[B]	2,2[A]	0,17	0,05*	35.94
TC	12.7[A]	10.9[B]	9.7[B]	12.6[A]	13,1[A]	0,33	<0.0001**	5.44
NFC	6.4	6.1	6.4	6.4	6,9	0,18	0,09ns	5.57
Ash	0.9[A]	0.8[AB]	0.7[B]	0.9[A]	0,9[A]	0,04	0.0019**	9.31
LIG	0.5	0.4	0.1	0.5	0,7	0,06	0.18ns	56.63
TDN	9.4[A]	8.3[B]	7.7[B]	9.4[A]	9,6[A]	0,20	0.0001**	4.48

[1]Fresh, sugarcane without straw; HST0.5 and HST72, sugarcane hydrolyzed with straw using 0.5% of $Ca(OH)_2$ suspension prepared 0.5 and 72 hours before hydrolysis (respectively); HT0.5 and HT72, sugarcane hydrolyzed without straw using 0.5% of $Ca(OH)_2$ suspension prepared 0.5 and 72 hours before hydrolysis (respectively). [2]DM: dry matter; CP: crude protein; EE: ether extract; OM: organic matter; NDF: neutral detergent fiver; ADF: acid detergent fiber; TC: total carbohydrates; NFC: non-fiber carbohydrates; LIG: lignin; TDN = total digestible nutrient. [3]standard-error mean. Line with different capital letters are significantly different, Tukey test. **(p<0.01), ns = non-significant.

When evaluating the productive performance of dairy cows fed sugarcane corrected with 1% urea mixture over ammonium sulfate (9:1) in the proportion 50% roughage, Costa et al. (2005) obtained: 17.5 kg/day of DM intake, 2.6 kg/day of CP, 0.3 kg/day of EE, 16.7 kg/day of OM, 5.0 kg/day of NDF, 13.8 kg/day of CT, 8.8 kg/day of NFC, and 11.7 kg/day of TDN. Silva Junior et al. (2015) evaluated the nutrient intake of Gyr cows fed hydrolyzed sugarcane. They found 12.4 kg/day of DM intake, 2.1 kg/day of CP, 5.2 kg/day of NDF, and 2.4 kg/day of ADF. Assessing fresh sugarcane in dairy cows diet, Martins et al. (2011) found 17.8 kg/day of DM intake, 2.9 kg/day of CP, 1,0 kg/day of EE, 5.8 kg/day of NDF and 7.3 kg/day of NFC. According to NRC (2001), the optimal concentration of NFC in diets for lactating cows is not well defined, but to avoid acidosis and other metabolic problems, the maximum concentration should be around 32 to 43% of the ration DM. According to Valadares et al. (1999), 35% of non-fiber carbohydrates (NFC) in the diet correspond to the optimum level for using non-protein nitrogen (NPN) in the diets of dairy cattle that contain only alfalfa silage as forage. The NFC contents of the experimental diets were according to the references.

Milk production (MP) and energy corrected milk (ECM) to 4.0% fat remained constant among the treatments (Table 5). Milk composition was not affected (P> 0.05) by the straw on the sugarcane, as well as the time of lime preparation. MP was 12.4 kg/day, ECM (4.0%) 11.8 kg/day. For the composition of milk, fat content was 3.6%, protein 3.4%, lactose 4.9%, total solids (TS) 8.8%, and solids-non-fat (SNF) 12.9%.

Table 5. Milk production and composition from dairy cows fed the experimental diets

	Treatments[1]							
Variable	Fresh	HST0.5	HST72	HT0.5	HT72	SEM[7]	P-value	CV(%)
BW[2]	529.9	525.2	506.0	513.8	522.0	11.61	0.09ns	2.39
FE[3]	0.9^B	0.9^B	1.1^A	0.8^B	0.8^B	0.04	0.003*	8.70
	Milk daily production, kg							
Milk	12.7	12.8	12.4	11.4	13.1	0.65	0.79ns	14.73
ECM[4]	12.0	11.6	12.2	10.7	12.7	0.63	0.46ns	10.97
Fat	0.4	0.4	0.4	0.4	0.5	0.02	0.30ns	12.24
Protein	0.4	0.4	0.4	0.4	0.4	0.02	0.84ns	13.67
Lactose	0.6	0.6	0.6	0.5	0.6	0.03	0.78ns	12.96
	Milk composition, %							
Fat	3.6	3.4	3.8	3.5	3.8	0.11	0.74ns	15.48
Protein	3.4	3.4	3.4	3.5	3.4	0.01	0.70ns	2.15
Lactose	4.9	4.9	4.9	4.9	4.9	0.02	0.78ns	2.48
SNF[5]	8.8	8.8	8.9	8.9	8.8	0.04	0.73ns	2.20
TS[6]	12.8	12.6	13.1	12.8	13.0	0.15	0.76ns	5.36

[1]Fresh, sugarcane without straw; HST0.5 and HST72, sugarcane hydrolyzed with straw using 0.5% of $Ca(OH)_2$ suspension prepared 0.5 and 72 hours before hydrolysis (respectively); HT0.5 and HT72, sugarcane hydrolyzed without straw using 0.5% of $Ca(OH)_2$ suspension prepared 0.5 and 72 hours before hydrolysis (respectively). [2]Body weight. [3]Feed efficiency. [4]Energy corrected milk (4% fat). [5]Solids-non-fat. [6]Total solids. [7]Standard-error mean. Tukey test at 5% of significance (ns = non-significant).

Feed efficiency (FE) is the relation between milk production 3.5% fat-corrected milk and DM intake. HST72 (1.1) had the better FE, i.e. cows fed hydrolyzed sugarcane with straw and the lime prepared 72 hours before the hydrolysis needed to eat less to produce the same amount of milk compare to the other treatments. Body weight (BW) of the cows ranged from 506.0 to 529.9 kg and no difference was found among the treatments. That means the better FE is attributed to the diet and not to the mobilization of body reserve to maintain the milk production by the cows.

When evaluating the productive performance of cows fed sugarcane, Costa et al. (2005) found milk production of 16.9 kg/day and ECM (3.5%) of 16.7 kg/day. Whereas, for milk composition, they found 3.4% of fat, 3.6% of protein, 4.1% of lactose and 12.1% of TS. In crossbred cows Holstein x Gyr, fed hydrolyzed or fresh sugarcane with sunflower seed, Alves et al. (2010) observed 14.7 kg/day of milk production, 3.2% of fat, 3,4% of protein, 4,3% of lactose and 8,7% of SNF. Silva Junior et al. (2015) obtained 13.4 kg/day of milk production, protein 3.4%, fat 4.1%, SNF 9.8%, and lactose 5.6%.

Values considered acceptable to commercial market of milk, according to Normative Instruction n^{o}62/2011, are a minimum of 3.0% of fat, 2.9% of protein, 4.3% of lactose, 8.4% of SNF and 11.4% of TS. All the values on this study are according to the Brazilian recommendations. Quality Payment Program rewards milk that has more than 3.4% of fat and 3.05% of protein with a bonus of 6% for each percentage point (Bandeira, 2004).

The blood parameters are shown in Table 6. The average of the observed parameters are: 59.0 mg/dL of glucose, 25.1 mg/dL of urea, 2.9 g/dL of serum albumin, 7.6 g/dL of total protein, 4.7 g/dL of globulin, 0.6 of A/G ratio and 0.2 mmol/L of β- hydroxybutirate (BHB) (ketone bodies).

Table 6. Blood parameters according to treatments

	Treatments[1]							
	Fresh	HST0.5	HST72	HT0.5	HT72	SEM[5]	P-value	CV(%)
Glucose, mg/dL	59.9	59.5	58.1	59.9	57.6	1.09	0.60ns	5.94
Urea, mg/dL	20.4^{B}	27.6^{A}	27.7^{A}	24.7^{AB}	26.4^{A}	1.28	0.02*	14.87
TP[2], g/dL	7.7	7.5	7.9	7.2	7.7	0.17	0.33ns	7.89
Albumin, g/dL	3.0	2.9	2.9	2.7	3.1	0.09	0.55ns	9.63
Globulin, g/dL	4,7	4,6	5,0	4,5	4,6	0.14	0.31ns	9.53
A/G[3] ratio	0.6	0.6	0.5	0.6	0.6	0.02	0.63ns	9.66
BHB[4], mmol/L	0.2	0.1	0.1	0.1	0.1	0.01	0.86ns	23.77

[1]Fresh, sugarcane without straw; HST0.5 and HST72, sugarcane hydrolyzed with straw using 0.5% of $Ca(OH)_2$ suspension prepared 0.5 and 72 hours before hydrolysis (respectively); HT0.5 and HT72, sugarcane hydrolyzed without straw using 0.5% of $Ca(OH)_2$ suspension prepared 0.5 and 72 hours before hydrolysis (respectively). [2]Total protein. [3]Albumin/Globulin. [4]β- hydroxybutyrate. [5]Standard-error. Line with different capital letters are significantly different, Tukey test. *($p<0.05$), ns = non-significant.

The reference range for blood glucose in cows are 45.0 to 75 mg/dL, urea ranges from 20.0 to 40.0 mg/dL, total protein from 6.7 to 7.4 g/dL, albumin from 3.0 to 3.5 g/dL, globulin from 3.0 to 3.4 g/dL, BHB of 0.4 ± 0.03 (KANEKO, HARVEY and BRUSS, 2008; REECE et al., 2004). Values of glucose, urea and total protein are normal; globulin level is slightly high, but albumin and BHB are slightly below the standardized values.

Glucose is important on milk production, because of the total requirement of glucose for lactating cows around 60% is for the production of milk (KANEKO, HARVEY and BRUSS, 2008). Total protein gives us the real nutritional and healthy status of the animal.

Albumin is responsible to maintain intravascular osmotic pressure and for transportation of substances. The most common cause of decreased plasma albumin levels is related to inflammatory processes. Globulin has importance on transport of proteins, serves as substrates to other substances and has a vital role in natural and acquired immunity to infection. High levels of globulin can indicate inflammatory disease or some liver disease. The relative proportions of Albumin and globulin are nearly equal (REECE et al. 2004). A/G ratio is relatively constant in healthy cattle, ranging from 0.84 to 0.94 (KANEKO, HARVEY and BRUSS, 2008). A/G ratio lower than the reference values usually occurs mainly because of an increase in the globulin level, followed by a small decrease in the albumin level.

The β- hydroxybutyrate (BHB) is an important parameter to control blood nutritional status of the animal (CHUNG et al, 2008; ENJALBERT et al., 2001). Level above 1.4 mmol/L of BHB is an indicative of subclinical ketosis (GEISHOUSER et al., 2000). The BHB accumulation occurs when the liver is overwhelmed and beyond its ability to oxidize fatty acids that the body is mobilizing. Mobilization of body fat occurs to meet the energy deficit during the early lactation (ENJALBERT et al., 2001). Frigotto et al. (2009) evaluated the blood metabolic parameters of high-producing dairy cows during the transition period, they found BHB values on days 1, 5 and 10 postpartum of 0.548, 0.665 and 0.506 mmol/L, respectively.

Silva Junior et al. (2015) evaluating the blood parameters from cows (Holstein x Gyr) fed hydrolyzed sugarcane, found 59.7 mg/dL of glucose and 28.6 mg/dL of urea. Martins et al. (2011) evaluated forage (sugarcane, sorghum silage, sunflower and Tanzania grass) in the diet of dairy cows. These authors found 57.7 mg/dL of glucose content and 39.2 mg/dL of urea for dairy cows fed sugarcane as roughage. Sforcini (2014) assessing levels of inclusion of sugarcane in the cow's diets containing corn silage as roughage, found 5.5 g/dL of total protein, and 2.0 g/dL of albumin.

Nery et al. (2010) evaluated the blood metabolites of cows from different genetic groups. They found 6.7 g/dL of total protein and 21.6 mg/dL of urea. According to Broderick (1995), low blood level of TP and urea may indicate low intake of rumen degradable protein (RDP), as well as low efficiency in using RDP due to deficiency in energy:protein ratio in the diet. In general, the blood concentration of a specific metabolite, when is outside the normal range, may represent a nutritional disturb or incidence of a disease in the animal.

4. Conclusions

Providing sugarcane with straw and the calcium hydroxide suspension prepared 72 hours before the hydrolysis of sugarcane is recommended because it provides better feed efficiency to the cows. Hydrolyzed sugarcane with straw despite decreasing IVDMD, increases the IVNDFD. Additionally, calcium hydroxide suspension prepared 72 hours in advance of its use facilitates the labor-work.

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CHAPTER 4 – Final considerations

Supplying hydrolyzed sugarcane to the dairy cows (low and medium milk production) is already established as a viable alternative of roughage. However, some studies suggest that the sugarcane supply can be offered up to 40% as part of roughage for high producing cows without affecting production. The current study suggested the use of hydrolyzed sugarcane with straw to feed dairy cows in order to use this residue (straw) which is produced in large scale in the sugarcane crops. Further research on the botanical composition, i.e, separation, quantification and chemical analysis of sugarcane for stem, leaves and straw is recommended. Having this information, the discussion of the results can be better explored in relation to composition of component of the sugarcane.

It is suggested for future studies the inclusion of straw levels of sugarcane in the diet of dairy cows (medium and high milk production). By using pre-determined inclusion of straw, it will be possible to consider the actual effects on animal performance. Furthermore, the preparation time of the lime of calcium hydroxide, in future studies, might involve longer intervals as 7, 14, 21, and 28 days. The agricultural residue (straw) of sugarcane is a relevant topic that can be studied more in depth on issues involving chemical composition, aerobic stability of straw in the hydrolysis process, scanning electron microscopy of the cell wall and animal performance. On top of that, sustainability and environmental issues caused by the pre-harvest burning of sugarcane and economic evaluation should be studied.

APPENDIX

Internship report at Swedish University of Agricultural Sciences

ABSTRACT – This report aimed to present all activities developed during the internship period from October 2014 to September 2015. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provides scholarship to PhD students encouraging them to learn, improve and share knowledge with other Universities in several countries. Swedish University of Agricultural Science (SLU), located in Uppsala, was chosen as the training camp for Viviane Endo (PhD student at São Paulo State University, Unesp, Brazil). Rolf Spörndly was Viviane's internship supervisor and is the head of a three-year project entitled, "Feeding grass and cereals only to high producing dairy cows at organic farms". For the first year of experiment, 25 cows were followed through the complete lactation (44 weeks) where two feeding regimes were tested. Group 1 (G1) was fed only grass silage and a mixture of rolled barley, wheat and oats grain and no additional protein concentrate. Group 2 (G2) was fed a traditional ration consisting also of protein concentrate such as soy bean meal and rape seed meal. Protein content in experimental diet (G1) was around 14 % of CP per kg of DM compared with 18 % of CP in the control diet (G2). The study aimed to evaluate individual differences of dairy cows in the potential to produce milk at low protein levels. Preliminary results showed no differences on milk production on early and mid lactation between feeding regimes. So far, feeding cows with cereal grains only has been viable. In addition to the project, other activities as conferences, courses, seminars and lectures were attended by the PhD student. Two abstracts were published and other two were accepted to be presented during the internship period in different Conferences, 66th Annual Meeting of the European Federation of Animal Science (EAAP) and XXIV Congreso de la Asociación Latinoamericana de Producción Animal (ALPA). One of the abstracts was from the SLU project, and the other three were from the main PhD thesis.

Keywords: Brazil, CNPq, dairy cows, PhD, Sweden, Unesp.

Relatório de estágio na Swedish University of Agricultural Sciences

RESUMO – O relatório teve como objetivo apresentar as atividades desenvolvidas durante o periodo de estágio, de outubro de 2014 a setembro de 2015. O Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) oferece bolsas para estudantes de Doutorado encorajando-os a melhorar e compartilhar conhecimentos com outras universidades de diversos países. A Swedish University of Agricultural Science (SLU), câmpus em Uppsala, foi o local escolhido pela Viviane (estudante de Doutorado da Universidade Estadual Paulista, Unesp, Brasil). Rolf Spörndly foi o orientador de estágio da Viviane e o líder de um projeto que compreenderá três anos intitulado, "Vacas de alta produção em sistema orgânico alimentadas com gramínea e grãos de cereais". No primeiro ano de experimento, foram utilizadas 25 vacas por toda a lactação (44 semanas) e dois sistemas de alimentação foram testados. No grupo 1, as vacas foram alimentadas com silagem de gramínea, uma mistura de cevada, trigo e aveia, sem adição de concentrado proteico. No grupo 2, as vacas foram alimentadas com uma dieta tradicional constituída de concentrado proteico (grãos de soja e canola). O teor proteico na dieta do grupo 1 foi de aproximadamente 14% de preteína bruta (PB) por kg de MS comparado ao grupo 2 (controle), com 18% de PB. O estudo objetivou avaliar diferenças individuais das vacas leiteiras no potencial de produção de leite com baixo nível de proteína. Resultados preliminares não mostraram diferença na produção de leite no início e meio da lactação em ambas as dietas. Até o momento, a alimentação de vacas apenas com grãos de cereais tem sido viável. Adicionalmente ao projeto, outras atividades como conferências, cursos, seminários e palestras foram atendidas pela estudante. Durante o período do estágio, dois resumos foram publicados e outros dois foram aceitos para serem apresentados em diferentes conferências, 66th Annual Meeting of the European Federation of Animal Science (EAAP) e XXIV Congreso de la Asociación Latinoamericana de Producción Animal (ALPA). Um dos resumos é referente aos dados obtidos no projeto desenvolvido na SLU e outros três com resultados da tese de Doutorado.

Palavras-chave: Brasil, CNPq, vacas leiteiras, Doutorado, Suécia, Unesp.

1. Introduction

Swedish University of Agricultural Sciences (SLU) was formed in 1977 through a merge of the College of Agricultural, College of Forestry and Veterinary College. SLU has operations mainly in Alnarp, Skara, Uppsala and Umeå. It has 36 departments and units that are spread across four faculties (Faculty of Landscape Architecture, Horticulture and Crop Production Science, Faculty of Forest Sciences, Faculty of Natural Resources and Agricultural Sciences, and Faculty of Veterinary Medicine and Animal Science).

The Department of Animal Nutrition and Management has undergraduate and graduate levels. The department's topics covers knowledge about animal behavior to nutrition, food science and management issues and it embraces food-producing animals, sport animals and pets. The department conducts research and teaches on issues affecting ruminant nutrition, feeding, lactation, growth, behavior and housing. The focus is on how these factors affect animal production and welfare, and quality of animal products.

Internship is important for the professional development of the student's career, to establish contact between universities from the same or different countries, as well as to exchange knowledge, experience and culture. The internship allows interaction with internationally recognized professionals in the academic area, also to experience the activities of everyday life in a different workplace. Internship also enables the comparison of the planning and execution of tasks, procedures and conferences. Professional and personal qualification is expected when the internship is done by the student. From a professional point of view, the exchange student is able to learn new methodologies, discuss the thesis' data with the foreign supervisor, network with other professionals, or even establish a partnership in the research field. In all these points, the student must have a social relationship with other people, whether professor, researcher, other student or janitor.

This report aims to describe the activities developed by Viviane Endo, and her involvement with SLU from October 2014 to September 2015.

2. Background of the project

A feeding ration with only forage and cereal grain would be possible for on-farm production at most organic farms in Sweden. The model has been tested in short-term experiments covering the initial part of the lactation and results indicate lower milk production but also lower feed costs resulting in better or unchanged net. Before the model can be recommended, it has to be verified over a full lactation. In this project, cow health, fertility and feed metabolism are monitored by a range of metabolic markers. Differences between individuals in their ability to utilize low-protein rations are also investigated. The project will be concluded in an evaluation of low-protein diets for dairy cows regarding economy, production and health consequences.

The cost of organically-produced protein feeds is high and the supply is limited. It is difficult to cultivate at the farm, therefore most farmers buy it from the feed industry, often based on organically-grown soy beans. This forms a limitation for organic-milk farmers. It is therefore important to investigate if current high-quality forages in combination with only cereal grains can be a competitive alternative to protein supplements that have to be imported to the farm. Forage and cereal grain is produced at the farm and provide a stable production economy since the dairy farmer will be independent of fluctuations of commodity prices.

One could expect a lower milk production from such a feeding regime due to lower protein content in the total ration. Recent experiments, however, have shown that dairy cows have an optimum production at lower protein content in the feed than earlier assumed. Rations with forage and cereal grains only have proved to be economically attractive in experiments. Before recommendation of such rations in practice, it is important to study the effect on animal health and fertility. In the planned project we will particularly study whether there are individual differences in the potential to produce milk at low protein levels.

3. Presentation of the project

At the department of Animal Nutrition and Management, a three year project entitled “Feeding grass and cereals only to high producing dairy cows at organic farms”, started up during 2014, October. This project is in accordance with the Ethical committee, protocol number C 199/14, dated August 1st, 2014. Project leaders are Rolf Spörndly, Torsten Eriksson, Eva Spörndly, and Kjell Holtenius. Viviane Endo (Brazilian PhD student internship) actively worked with the project during the first year.

For the first year of experiment, June, 2014, the grass was harvested and ensiled at the research farm Lövsta, situated 14 km from the university campus. Fifty dairy cows will be part of the entire experiment, which will run for two years. For the first year, 25 cows will be followed through the complete lactation. Second year 25 more cows will be also followed through the complete lactation to obtain different forage qualities available to the animals. Two feeding regimes were tested. One group was fed only grass silage and a mixture of rolled barley, wheat and oats grain and no additional protein concentrate. The control group was fed a traditional ration consisting also of protein concentrate such as soy bean meal and rape seed meal. Protein content in experimental diet was around 14 % of CP per kg of DM compared with 18 % of CP in the control diet.

4. Material and methods

4.1 Animals

Twenty-five Swedish Holstein and Swedish Red and White (SRB) breed dairy cows were used. After calving, cows were randomly assigned to one of the two treatments and marked using a collar to be identified among the other cows in the same group. First-calving cows represented 1/3 of total cows on year 2014/2015. The automatic milking rotary (AMR) system was used in the experiment for the whole lactation until cows were dried off, i.e., 305 days lactation (44 weeks). Milk yield and intake were daily registered automatically.

4.2 Handling of animals

Blood and urine samples were collected three times in total from all the experimental cows. First time in lactation week (lw) 2-3. Second time in lw 5-6 and third time in lw 11-12. Cows were weighed daily during the lactation, once a month all the cows were subjected to body condition scoring (BCS). Milk production was recorded daily, and milk analysis was made twice a month. One time by official milk recording system and the other time performed by the department laboratory. Cows were milked in AMR system twice a day.

From October to May, 2014-2015, cows were kept in a free stall barn system, provided concentrate and grass silage, separately in individual feeders. Concentrate was fed restricted according to yield and grass silage was fed *ad libitum* where the consumption was pleasured individually.

During the spring and summer seasons, cows were grazing on timothy, ryegrass, white and red clover pasture (described later on 4.5). Concentrate was supplied inside the barns, according to the treatments. In addition, 5.0 kg of DM of silage was supplied to the cows, when necessary.

4.3 Diet and treatments

Diets were formulated according to the rules of European organic milk production. Cows were divided in two groups. Group 1 (G1), the experimental diet, cows were fed only grass silage and one concentrate consisting of cereal grains. Group 2 (G2), the control, cows were fed two concentrate types of which one consisted of cereal grains and one consisted of protein feeds.

Cereal grain pellets contained 36% of barley, 34% of wheat, 25% of oats, 2% of molasses and 3% of vitamin and mineral premix. Protein pellets contained 47% of soybean meal, 16% of rapeseed expeller, 15% of oats, 12% of rapeseed, 4% of soybean, 3% of wheat, 1% of molasses and 2% of vitamin and mineral premix.

Protein content in the experimental diet was around 14% of CP per kg DM compared with 18% of CP in the control diet. High energy content (>10.6 MJ/kg DM) and normal protein content (130-160 g CP/kg DM) of forage was supplied to cows.

The forage was fed *ad libitum* and silage, of first harvest with high digestibility, was available in the feeders.

During the first three months of lactation, a maximum of 50% of total DM was concentrate. After that, a maximum of 40% concentrate was allowed. Individual intake of concentrate and silage were registered daily using the Basreg software program. Sampling of concentrate and silage was made once a week, and pasture was taken daily, according to the routine management.

4.4 Sampling protocol

The nitrogen metabolism, metabolic status and health status was monitored with physiological markers. As mentioned before, blood and urine samples were collected three times from all the experimental cows. First time in lactation week (lw) 2-3, second time in lw 5-6 and third time in lw 11-12.

Blood samples were used to determine the histidine level. Histidine is believed to be the first limiting amino acid in protein low rations based on grass silage. Urine spot sampling was used to analyze the allantoine (microbial protein marker), urea (marker of urine nitrogen loss) and creatinine (marker of urine quantity in order to convert concentration of allantoine and urea to daily quantity) levels. Milk samples were collected twice a month to control the milk composition of fat, protein lactose and milk cell count.

4.5 Pasture management and measures (grazing season)

During the spring and summer seasons, all the experimental cows started the grazing season. First week on the pasture (6^{th}-13^{th} May), cows were submitted to day and night supplemented silage indoors *ad libitum*. From 13^{th} May onwards, no silage indoors were supplied, only pasture as roughage was provided to the cows, with day and night grazing. A maximum of 5 kg DM silage was provided to cows when necessary. According to Swedish recommendation, during early season, 4-6 cows/ha with full time grazing is suggested, mid season 2-3 cows/ha with full time grazing, and later season 4-6 cows/ha, but half the day cows are grazing and supplied silage indoors.

Rotational grazing system was used and the paddocks were consisted mainly of timothy, ryegrass, red and white clover. A regression equation (Figure 1) was used to assure the cows had access to at least 25 kg DM pasture/cow/day. Utilization of the pasture was 70%, i.e., cows had available 17.5 kg DM of grass to eat. The equation was made during the last summer as a guide of herbage mass. A minimum of 100 measures of the sward height was made using a pasture meter EC-09. The sward height value (cm) was included on the equation, $Y_{(kg\ DM/ha)} = 233.92 * x_{(cm\ sward\ height)} - 55.899$ ($R^2 = 0.85$), to estimate the herbage mass.

Pasture sample was taken on weekdays (Monday-Friday only) by ripping off pasture manually to imitate the cows grazing. Samples were dried at 60°C overnight. Later on, ash, crude protein (CP), amylase neutral detergent fiber ash-free (aNDFom), and *in vitro* organic matter digestibility (IVOMD) were determined on the laboratory to control the pasture quality.

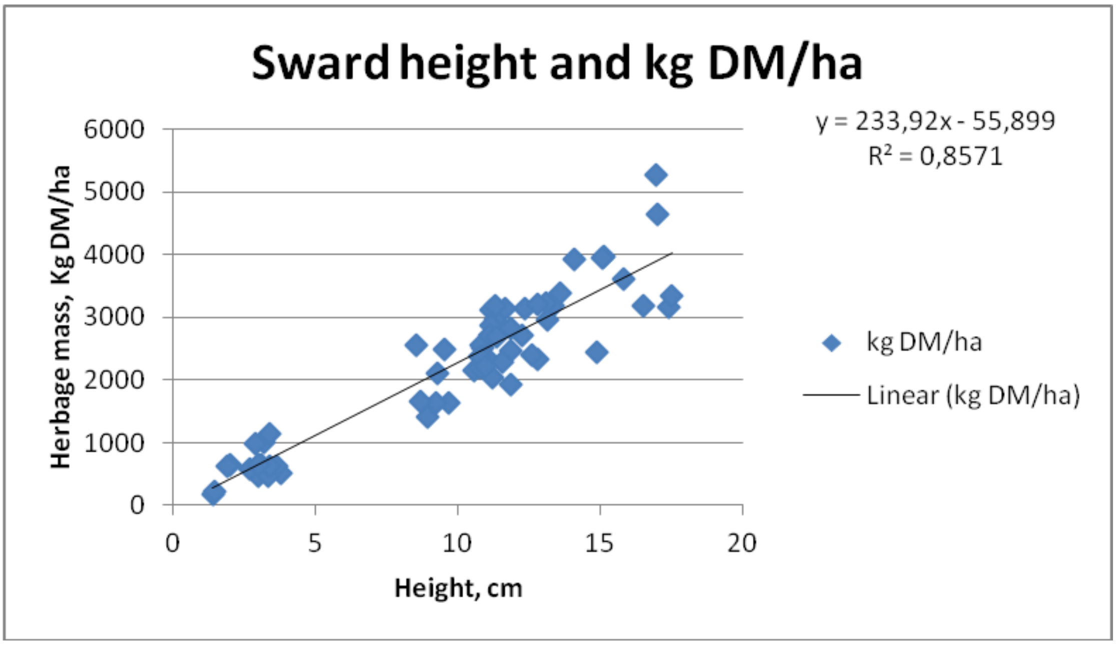

Figure 1. Regression equation made during summer season at the experimental farm belonging to SLU, 2013.

4.6 Experimental design

Cows were divided in two groups. One group was fed only one concentrate, consisting of cereal grains. The other group was fed two concentrate types of which one consists of cereal grains and the other one consists of protein feeds. A completely randomized design for the first year of experiment was used. Data were subjected to analysis of variance by PROC GLM of SAS.

5. Preliminary results

The blood and urine analyses were not included in this report because until the end of Viviane's internship, those analyses were not being analyzed yet. Also no other health parameters have been compiled.

5.1 Early lactation

Results from the first three lactation months of a major project with the aim to evaluate the potential to produce milk with protein supplied only from forage and cereals. Silage intake and milk production on early-lactation dairy cows fed cereal grains are showed in Tables 1 to 4.

Group 1 (G1) was supplied with 9.9 kg cereal grains/d (rolled barley, wheat and oats; 10.3% CP of DM). Group 2 (G2) was supplied with 7.6 kg cereal grains/d and 4.5 kg protein feeds/d (mainly soy beans and rapeseed meal; 32.3% CP of DM) according to Swedish feeding standards. In the first month of lactation, silage intake was not affected by treatment (12.5 kg DM/d). In the second and third months of lactation, however, G1 cows consumed more ($p < 0.05$) silage (15.5 and 17.4 kg DM/d) than G2 cows (13.3 and 15.0 kg DM/d). Diets did not differ ($p > 0.05$) regarding production of raw milk (36.5 kg/d for G1 and 38.8 kg/d for G1 and G2) or energy corrected milk (37.3 kg/d for G1 and 37.8 kg/d for G1 and G2). In early lactation, feeding cereal grains and high quality silage to cows was sufficient to maintain milk production.

Table 1. Intake and milk production average of early lactation (1-3 months)

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	% milk fat	% milk protein	kg BW
Grain	9.9	-	15.4	36.5	4.06	3.35	679
Grain+protein feed	7.6	4.49	13.3	38.8	3.86	3.28	646
P<	-		0.0784	0.4422	0.2818	0.4542	0.373

Table 2. Intake and milk production average of first lactation month

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	% milk fat	% milk protein	kg BW
Grain	9.6	-	13.2	36.3	4.1	3.5	683
Grain+protein feed	7.4	4.49	11.7	38.1	4.1	3.5	646
P<	-		0.3027	0.5914	0.9615	0.9424	0.3515

Table 3. Intake and milk production average of second lactation month

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	% milk fat	% milk protein	kg BW
Grain	10.0	-	15.5	37.2	3.7	3.3	675
Grain+protein feed	7.7	4.49	13.3	40.1	4.0	3.1	642
P<	-		0.0451	0.3752	0.4326	0.127	0.3701

Table 4. Intake and milk production average of third lactation month

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	% milk fat	% milk protein	kg BW
Grain	10.1	-	17.4	35.9	4.3	3.2	680
Grain+protein feed	7.7	4.49	15.0	38.3	3.6	3.2	651
P<	-		0.0445	0.4183	0.0342	0.9779	0.429

5.2 Mid lactation

Results of silage intake and milk production on mid lactation (4^{th} to 7^{th} months) dairy cows fed cereal grains are showed on Tables 5 to 9. Mid lactation, Group 1 (G1) was supplied with an average of 7.3 kg cereal grains/d (rolled barley, wheat and oats; 10.3% CP of DM). Group 2 (G2) was supplied with an average of 6.6 kg cereal grains/d and 2.2 kg protein feeds/d (mainly soy beans and rapeseed meal; 32.3% CP of DM). Milk production (31.9 kg/cow/day) and DMI (22.1 kg DM/cow/day) were not different between groups. In the fourth and seventh lactation month, G1 cows consumed more ($p < 0.05$) silage (18.3 and 19.8 kg DM/cow/day) than G2 cows (15.9 and 15.6 kg DM/cow/day). In mid lactation, feeding cereal grains and high-quality silage to cows was also sufficient to maintain milk production.

Table 5. Intake and milk production average of mid lactation (4-7 months)

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	DMI
Grain	7.3	-	19.3	31.5	23.0
Grain+protein feed	6.6	2.2	16.0	32.4	21.2
P>	-	-	0.0916	0.0731	0.7824
Mean	7.0	2.2	17.8	31.9	22.1

DMI, dry matter intake

Table 6. Intake and milk production average of fourth lactation month

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	DMI
Grain	9.6	-	18.3	33.6	28.0
Grain+protein feed	8.1	3.6	15.9	36.7	27.7
P<	-	-	0.04	0.1780	0.2377
Mean	8.9	3.6	17.2	35.1	27.8

DMI, dry matter intake

Table 7. Intake and milk production average of fifth lactation month

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	DMI
Grain	8.2	-	19.4	32.3	27.6
Grain+protein feed	7.3	2.4	16.3	34.6	25.9
P<			0.1535	0.5409	0.4875
Mean	7.7	2.4	17.9	33.4	26.8

DMI, dry matter intake

Table 8. Intake and milk production average of sixth lactation month

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	DMI
Grain	6.4	-	19.9	30.6	24.6
Grain+protein feed	6.2	1.5	16.0	31.0	20.8
P<			0.4433	0.6448	0.7072
Mean	6.3	1.5	18.2	30.8	22.8

DMI, dry matter intake

Table 9. Intake and milk production average of seventh lactation month

Treatment	kg Grain/day	kg Protein feed/day	kg DM silage/day	kg milk/day	DMI
Grain	5.0	-	19.8	29.3	11.6
Grain+protein feed	5.0	1.2	15.6	27.3	10.5
P<	-	-	0.0336	0.8019	0.5548
Mean	5.0	1.2	18.0	28.4	11.1

DMI, dry matter intake

6. Activity report

6.1 Field experiments

The main project followed was to assess milk from forage and cereal grain only. The objective will be concluded in an evaluation of low-protein diets for dairy cows regarding economy, production and health consequences.

One parallel experiment was partially followed, entitled: “Voluntary milking system (VMS) - Production pasture compared with exercise pasture in the morning and evening grazing system”. The objective was to compare production, feed intake and behavior of cows on production pasture compared with exercise pasture in part-time grazing system.

6.2 Laboratory

For the laboratory analysis, Viviane Endo learned new methodologies of rumen *in vitro* organic matter digestibility (IVOMD), using the Swedish method (VOS), to determine metabolizable energy. Amylase neutral detergent fiber (aNDFom) method, developed by SLU laboratory as well. Crude protein (CP), water soluble carbohydrates (WSC) and pH were other analysis made.

6.3 Conferences, courses, seminars, lectures and other

Conferences:

The 6th Nordic Feed Science Conference, no abstracts were presented.

The 66th EAAP Conference, two abstracts were presented. One was entitled: "Evaluation of milk production and composition of cows fed hydrolyzed sugarcane" from the PhD thesis at São Paulo State University. The other was entitled: "Silage intake and milk production on early lactation dairy cows fed cereal grains" from the internship PhD at SLU.

The XXIV Congreso de la Asociación Latinoamericana de Producción Animal (ALPA), two abstracts were accepted to be presented. One was entitled: "*In vitro* digestibility of nutrients of hydrolyzed sugarcane with straw". One was entitled: "Nutrient intake and energy corrected milk production in dairy cows fed hydrolyzed sugarcane with straw", both of them from the PhD thesis.

Courses completed as student:

1.

"Information retrieval and methods for scientific communication" aimed to develop the participant's knowledge of factors affecting search process, scientific communication, source criticism and critical evaluation of publications. It also aimed to develop the skills needed for effective and methodological work with information retrieval, reference management and scientific material. Finally it aimed to illustrate and problematize research information management, as well as the conditions and practices of scientific communication and publication.

Content:

- search process, search strategies
- international reference databases
- e-journals, e-books, other resources
- searching the web, web tools for communication
- reading skills/critical and analytical reading
- reference management
- bibliometrics and bibliometric tools
- scientific communication, open access, strategic publishing
- management and preservation of research data
- copyright

Examination:

Oral presentation exam.

2.

"Swedish Legislation, Ethics and Animal Use" aimed to comply with Swedish and EU Legislation in important issues related to research involving laboratory animals. The course consisted of five modules: Ethics and animal use, Swedish Legislation, animal records, identification methods, and humane endpoints. In this course the student learned to distinguish between legal and ethical norms, to distinguish between scientific content and value based arguments, to know the 3Rs (replacement, reduction and refinement) and their intention and potential regarding human-animal relationships. The students learned how to translate legal and ethical requirements into practical design of an animal experiment, to identify the legal requirements for record keeping in a laboratory animal facility, to know the responsibilities associated with record keeping, the requirements for group records, and list what must be in the animal records. Further, to know which methods are allowed and which are forbidden for identification of laboratory animals. An important issue is to be able to explain the significance of a humane endpoint, to describe how to know whether a humane endpoint has reached, and use the concept of humane endpoint.

Examination:

Multiple-choice exam answering forty questions.

3.

"Forage evaluation in ruminant nutrition" aimed to increased knowledge of cell-wall factors limiting digestibility and intake in ruminants, deeper understanding about different techniques for evaluation of forages, the effects of antinutritional factors in plants on animal performance, the use of stable isotopes in forage science, and to understand the importance of forages in ruminant nutrition for the production of greenhouse gases (methane).

Content:

- the course focused on how forage cell walls limit digestion in ruminants.
- new and improved analytical methods for estimation of the quantity and quality of cell walls.
- technology to measure degradation and passage through the gastrointestinal tract.
- laboratory exercises with analytical techniques *in vitro* for a better understanding of the theoretical presentations.
- group work and oral presentations.
- scientific articles, presenting and discussing to highlight important scientific progress in the area.

Examination:

Written exam, answering questions given by each teacher responsible for the different topics in the course.

Laboratory report, including introduction, material & methods, results and a brief discussion on the results, in relation to the focus of the course.

Presentation of a poster about the own PhD research.

Courses as listener:

1.

“Cattle Production course” focused on Sweden and Northern Europe cattle production (milk and beef), but international views and comparisons were taken into account. The emphasis on efficiency, product quality, animal health and welfare, including fertility and the impacts on the environment were also other issue of the course.
https://student.slu.se/en/studies/courses/?anmkod=30030.1415

2.

“Advanced Feed Science” aimed to propose appropriate processing techniques, harvesting and storage methods and evaluate its impact on the

nutritional and hygienic quality of feed under various conditions. Propose and practice the relevant analysis to determine the nutritional content / value of the feed. Describe the nutritional and antinutritional properties in various animal feeds. Plan, conduct and analyze nutrient utilization experiments with animals. Understand the principles of optimization of a diet or feed mixture and critically evaluate various methods. Discuss the ethical considerations in the selection of feeds and feeding strategy.
https://student.slu.se/en/studies/courses/?anmkod=30026.1415

3.

“Active learning classrooms” a pedagogical complement course on positive changes made to improve student engagement and achievement. During the workshop, Dr. Mark Decker from the University of Minnesota demonstrated how active learning principles can be implemented within the teaching spaces. This course was geared toward individuals who will work as university teachers or research advisers at SLU.
http://spectare.ucl.slu.se/adm/sus/2015/upc_workshop_mars_2015/Upc_M_decker.html

Seminars:

1.

“Welfare and sleep” arranged by Future Animal Health and Welfare and Department of Animal Nutrition and Management in collaboration with Welfare and sleep is about the importance of sleep.

Content:

- individual differences in time budgets – a matter of social strategy (Susanne Waiblinger, Institute of Animal Husbandry and Animal Welfare, University of Veterinary Medicine, Wien, Austria)
- sleep in sheep (Michael Cockram, Sir James Dunn Animal Welfare Centre, University of Prince Edward Island, Canada)

- sleep in dairy calves (Laura Hänninen, Research Centre for Animal Welfare, University of Helsinki, Finland)
- sleep in humans (Jan-Erik Broman, Department of Neuroscience, Uppsala University)

2.

"Techniques for writing a scientific paper" by Professor Michael Grossman, University of Illinois, USA, aimed to enable the participants to increase and improve the scientific paper production by more effective construction of a good text. The workshop includes lectures and group activities about techniques for scientific writing (title, abstract, introduction, material and methods, and conclusions), as well as how to design effective tables and figures, and ten ways to make your writing easier to read.

Lectures:

"A forage-only diet and reduced high intensity training distance in Standardbred horses" (Sara Ringmark PhD Thesis defence) – October 31th 2014, 14:00.

"Sleep in dairy cows" (Emma Ternman PhD Thesis defence) – December 12th 2014, 14:00.

"Transition from nomadic pastorialism to livestock based agro-pastorialism" The case of animal husbandry in West Pokot, Kenya (Antonia Grönvall - Master Thesis defence) – February 25th 2015, 08:45.

"Addicted to the Icelandic horse – our own horse breeding and trekking tours" (Guðrún Stefánsdóttir and Víkingur Gunnarsson – Researchers at SLU), March 4th 2015, 12:15.

"Long-term effect of daily herbage allowance restriction on pasture sustainability of grazing systems in Southern Chile" (Verônica Merino – PhD student at Ciencias Agrarias Universidad Austral de Chile). Seminar lunch, March 11th 2015, 12:15.

"Shorter dry period in dairy cows –so far, and variation in IgG and total protein content in plasma in young dairy calves" (Lisa Andrée O'Hara – PhD student at SLU). March 18th 2015, 12:15.

"Milk intake and risk of mortality and fractures in women and men: cohort studies" (Professor Karl Michaëlsson – Uppsala Clinical Research Centre; Department of Surgical Science, Orthopaedics). May 25th 2015, 11:30.

"Seasonal and interannual variability in habitat selection in wolves: an example from Yellowstone National Park" (AlessiaUboni – Post Doctoral Research at SLU, Umeå), May 11th 2015, 12:15.

"Using genomics and transcriptomics to study host-pathogen interactions: a tale of two mycos" (Ana Marcia de Sá Guimarães – PhD at Universidade de São Paulo), May 27th 2015, 12:15.

Other:

Presented lecture entitled: "Evaluation of hydrolyzed sugarcane with straw in the diet of dairy cows" (Viviane Endo – PhD at São Paulo State University), March 25th 2015, 12:15.

Visit at Andersta Gård Farm. As a brief description of the property, is a dairy cow farm, located 55.8 km to the Southwest from Uppsala. Address, Sparrsätra Andersta, 2, SE-74595, Enköping, Sweden. The farm has a total of 338 ha (142 own land, 88 ha leased land and 108 ha of forest). The farm has three DeLaval Robotic milking system, in a voluntary milking system (VMS), because is the test farm of DeLaval, which means that new technologies and applications are tested on the farm. Swedish Red Breed (SRB) represent around 80% of the total herd and 20% is Holstein cows. The farm consists of 170 cows, from which 156 are lactating. Cows are milked around 2.3 times per day and total production is 10,500 kg energy-corrected milk/cow/year. Calving interval average is 12.4 months with an age at first calving at 29 months. Milking cows are supplied with 12-13 kg DM silage/day and pasture during spring and summer season. Silage consists of Valletta crop, whole crop silage (spring wheat and peas). Forage from pasture consists of Timmoty, alfalfa and two types of red clover.

7. Conclusions

Internship is a high-impact complement in the professional life of a PhD student. It provides good interaction with internationally recognized professors and researchers. It allows the acquisition of new skills and knowledge. Comparison of methodologies, procedures and execution of tasks is also a valuable aspect of the internship. Expanding and sharing knowledge, besides improvement of the foreign language are relevant as well.