UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

CORN SILAGE INOCULATED WITH MICROBIAL ADDITIVES

Fernanda Carvalho Basso

Zootecnista

2013

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

2013

Basso, Fernanda Carvalho
B322c Corn silage inoculated with microbial additives/ Fernanda Carvalho Basso – – Jaboticabal, 2013
xix, 81 p. ; 28 cm

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2013
Orientador: Ricardo Andrade Reis
Banca examinadora: Clóves Cabreira Jobim, Carla Luiza da Silva Ávila, Izabelle Auxiliadora M. de Almeida Teixeira, Flávio Dutra de Resende
Bibliografia

1. Balanço de nitrogênio. 2. Eficiência alimentar. 3. Lactobacillus buchneri. 4. Lactobacillus plantarum. 5. Propionato. 6. Rendimento de carcaça I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 636.085.5:633.15

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação – Serviço Técnico de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.

DADOS CURRICULARES DO AUTOR

FERNANDA CARVALHO BASSO – filha de Verginia Teresa Carvalho Basso e Carlos Alberto Araújo Basso, nasceu em São Paulo – SP, em 8 de outubro de 1984. Ingressou na primeira turma do curso de Zootecnia da Faculdade de Engenharia da Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Campus de Ilha Solteira em agosto de 2003, onde foi vice-presidente do Centro Acadêmico de Zootecnia (2003-2004), discente membro titular do conselho de departamento de Biologia e Zootecnia (2004 a 2006) e bolsista da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) no período de outubro de 2005 a setembro de 2007, obtendo o título de Zootecnista em janeiro de 2008. Em março de 2008 ingressou no curso de pós-graduação em Zootecnia, da Faculdade de Ciências Agrárias e Veterinárias (FCAV) da UNESP, Campus de Jaboticabal – SP, obtendo o título de Mestre em Zootecnia, em fevereiro de 2010. Em março deste mesmo ano iniciou o curso de doutorado em Zootecnia também na FCAV/UNESP, com bolsa da FAPESP. De março a junho de 2012 realizou estágio de pesquisa no exterior, na Universidade da Flórida, na cidade de Gainesville, sob orientação do Dr. Adegbola T. Adesogan. Em agosto de 2013 obteve o título de doutora.

"In any moment of decision, the best thing you can do is the right thing. The worst thing you can do is nothing"

Theodore Roosevelt

ACKNOWLEDGEMENTS

I am very thankful for Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista for providing facilities during my Ph.D. work and opportunity to study in this excellent University.

I would like to acknowledge to São Paulo Research Foundation – FAPESP for their financial support to this research and my scholarship.

I express my sincere gratitude to my supervisor Prof. Dr. Ricardo Andrade Reis for his excellent guidance, support and encouragement throughout my research work; and I am extremely flattered for his trust in my abilities.

I would like to thank supervisory committee members Profª Drª Telma T. Berchielli, Profª Drª Izabelle A. M. A. Teixeira, Prof. Dr. Flávio D. de Resende, Profª Drª Ana Claudia Ruggieri, Prof. Dr. Clóves C. Jobim and Drª Carla L. S. Ávila for their valuable suggestions and dedication with this research work.

My sincere thanks are to my co-supervisor Dr. Gustavo R. Siqueira for his help, support and suggestions.

I express acknowledge to Prof. Dr. Américo G. S. Sobrinho for his support in these trials.

I would also like to thank Prof. Dr. Gilberto Macedo at Universidade Federal de Uberlândia for his valuable suggestions.

My special thanks to my supervisor at University of Florida Dr. Adegbola T. Adesogan for the opportunity to work with his research team and for his valuable suggestions in these manuscripts. I would like to thank all the research team from Dr. Adesogan for opportunity to work with them, Oscar Queiroz, Kathy Arriola, Miguel Zarate, Juan Romero, Rodolfo Daetz and Armando Schlaefli.

I would like to thank all the staff from Animal Science Department at FCAV/UNESP in special Ana Paula Sader, João Luiz Guariz and José Fernando Pereira for their assistance necessary during the experiments.

I am extremely thankful to friends and students Erika Lara, Flávia Assis, Carlos Rabelo, Pedro Salvo, João Yamaguchi, Fábio Kamada, Gustavo Gonçalves, Marcela Morelli, Lais Grigoletto, Virgilio Naves, Daniel Furlan, Heloísa de Godoy, Milena Marzocchi, Luana Jorge, Fernando Meneguello and Luis

Fernando Denadai for their help for me through endless hours in the field and laboratory.

I also want to thank my friend Andre Oliveira for valuable discussions.

I am grateful to all my friends and colleagues of graduate Fabiana Almeida, Bruna Rosa, Gabriela de Mello, Sheila Tavares, Max Ternero, João Paulo Ramos, Bruna Cabral, Leandro Galzerano, Eliane Morgado, Wilton Ladeira, André Valente, Carlos Stefenson, Kelen Basso, Patricia França, Karla Magalhães and Darcilene Figueiredo.

I thank my husband Bruno Lodo, for his love, support, patience and understanding.

I would also like to thank my parents (Teresa and Carlos) for their love, continuous support and patience.

I wish to express my sincere gratitude to all people around me, who have given me their support and time during these years.

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SILAGEM DE MILHO INOCULADA COM ADITIVOS MICROBIANOS

RESUMO – Objetivou-se determinar a qualidade da silagem de milho inoculada com aditivos microbianos. Para tanto, foram realizados três experimentos. No primeiro ano, em silos experimentais, foram testados quatro inoculantes que compuseram os seguintes tratamentos: forragem inoculada com *Lactobacillus buchneri* (LB), *Propionibacterium acidipropionici* (PA), *Bacillus subtilis* (BS), *Lactobacillus plantarum* (LP) e as combinações LBLP, BSLP e PALP, permanecendo uma silagem sem inoculante (Controle). Após 96 dias da ensilagem, a composição químico-bromatologica e a ocorrência de leveduras e fungos filamentosos foram avaliadas. A estabilidade aeróbia foi realizada durante 12 dias. Nos dias 4, 8 e 12 após a abertura, os valores de pH e a dinâmica de fungos foram determinados. No segundo e terceiro ano experimental avaliou-se a qualidade de silagens escolhidas a partir do primeiro ano. No segundo ano, as silagens estudadas foram: controle, LB e LBLP, confeccionadas em silos superfície. Na avaliação de desempenho, digestibilidade dos nutrientes e síntese de proteína microbiana, se utilizou 30 cordeiros mestiços. O estudo de fermentação ruminal foi realizado em seis cordeiros mestiços com cânula ruminal. No terceiro ano, avaliou-se a associação de silagem de milho inoculada a dois níveis de concentrado. Foram confeccionados dois silos trincheiras, sendo um a silagem controle e outro a LB. As dietas foram compostas das respectivas silagens associadas a 40 e 60% de concentrado, totalizando quatro tratamentos. As avaliações nos animais foram semelhantes às realizadas no segundo ano experimental. Os inoculantes afetaram positivamente as características fermentativas e reduziram a parede celular das silagens. A inoculação promoveu alteração na fermentação ruminal, maior fluxo de proteína microbiana e melhor desempenho dos cordeiros alimentados com dietas compostas pelas silagens inoculadas. A qualidade da silagem de milho foi melhorada com a inoculação de aditivo microbiano.

Palavras-chave: balanço de nitrogênio, eficiência alimentar, *Lactobacillus buchneri*, *Lactobacillus plantarum*, propionato, rendimento de carcaça

CORN SILAGE INOCULATED WITH MICROBIAL ADDITIVES

ABSTRACT - This study aimed to determine the quality of corn silage inoculated with microbial additives. Therefore, three experiments were carried out. In the first year, in experimental silos, four inoculants that comprised the following treatments were tested: forage inoculated with *Lactobacillus buchneri* (LB), *Propionibacterium acidipropionici* (PA), *Bacillus subtilis* (BS), *Lactobacillus plantarum* (LP) and combinations LBLP, BSLP and PALP, remaining silage without inoculant (control). After 96 days of ensiling, the chemical composition and the occurrence of yeasts and molds were evaluated. Aerobic stability was performed by 12 days. On days 4, 8 and 12 after opening the pH values and dynamics of yeasts and molds were determined. In the second and third experimental year the quality of silage chosen from the first year were evaluated. In the second year, the silages studied were: control, LB and LBLP, made in stack silos. In the study of performance, nutrient digestibility and microbial protein synthesis, 30 crossbred lambs were used. The trial of rumen fermentation was performed with six crossbred lambs with ruminal cannula. In the third year, we evaluated the association of corn silage inoculated with two levels of concentrate. Two bunker silos were made (control silage and LB). The diets were composed of the respective silages combined with 40 and 60 % concentrate, a total of four treatments. Assessments in animals were similar to those obtained in the second experimental year. The inoculants positively affected fermentation characteristics and reduced cell wall of silages. The inoculation changed ruminal fermentation and, the flow of microbial protein and performance of lambs fed diets containing the inoculated silages were increased. The quality of corn silage was improved by inoculation with microbial additive.

Keywords: carcass yield, feed efficiency, *Lactobacillus buchneri*, *Lactobacillus plantarum*, nitrogen balance, propionate

CHAPTER 1 – LITERATURE REVIEW

1. Introduction

Aerobic spoilage by yeasts and molds is a major cause of reduction in the nutritional value of silage, mainly corn silage that is susceptible to aerobic deterioration, especially in warm weather (ASHBELL et al., 2002). In this condition, yeasts utilise soluble carbohydrates, and lactic acid, produced by lactic acid bacteria (LAB), as energy source. Thus, silages become a favourable environment, for the growth of molds and aerobic bacteria, resulting in lower quality silages. Silos oversize and slow feedout rate are a problem, because beyond of surface, the face of silage also is exposed to air for a long time.

Microbial inoculants have been used in attempts of mitigate this problem, mainly because are biological, easy to use, not corrosive and not pollute the environment (CONTRERAS-GOUVEIA; MUCK, 2006). An obligatory heterofermentative LAB (^{he}LAB), *Lactobacillus buchneri*, has been suggested as an additive to improve the aerobic stability of silages (DRIEHUIS; OUDE ELFERINK; SPOELSTRA, 1999; RANJIT; TAYLOR; KUNG JR. JR, 2002; KLEINSCHMIT; KUNG JR., 2006; TABACCO et al., 2009; NKOSI et al., 2011; BASSO et al., 2012). This bacteria converts glucose and fructose to lactic acid, acetic acid and other end products (McDONALD; HENDERSON; HERON, 1991). *Lactobacillus buchneri* can also convert lactic acid to acetic acid, 1, 2-propanediol and small amounts of ethanol (OUDE ELFERINK et al., 2001). The presence of acetic acid protects the silage against spoilage by aerobic microorganisms, as yeasts and molds (MOON, 1983).

It has been suggested that other types of inoculants, such as propionic acid bacteria (PAB) and *Bacillus* species, can be used as microbiological additives to overcome the problem of silage aerobic spoilage (PHILLIP; FELLNER, 1992; FILYA; SUCU; KARABULUT, 2004; FILYA; SUCU; KARABULUT, 2006, ROWGHANI et al. 2008, BASSO et al. 2012b). *Propionibacterium acidipropionici* are used to improve aerobic stability by producing propionic and acetic acids, which have antifungal effects (MOON, 1983), and the antimycotic effects of *Bacillus* species may be due to the production of bacteriocins, such as zymocin, which is produced by some *Bacillus subtilis* strains (PAHLOW et al. 2003).

However, inoculation with ^{he}LAB promotes higher DM losses during fermentation (McDONALD; HENDERSON; HERON, 1991). Likewise, some concerns about the use of PAB are the loss of DM and the proteolytic activity that *Propionibacteria* possess (KUNG JR. JR., 2009). *Bacillus* species can produce lactic and acetic acids, and their growth is not suppressed by these fermentation products or by low pH. However, these organisms are generally less efficient than LAB in producing lactic acid (PAHLOW et al. 2003). Thus, combining ^{he}LAB, PAB or *Bacillus* with a facultative heterofermentative LAB, as *Lactobacillus plantarum*, may be an alternative strategy to decrease fermentation losses and protein degradation by producing greater quantities of lactate, thereby enhancing the aerobic stability of silages, as found by Driehuis et al. (2001); Filya (2003) and Rowghani et al. (2008).

Moreover, some strains of LAB and *Bacillus* ssp. can produce ferulate esterase enzyme, which may increase the susceptibility of plant cell walls to enzymatic hydrolysis because ferulic acid is released from cell wall arabinoxylans (DONAGHY; KELLY; McKAY, 1998; NSEREKO et al. 2008). Ferulic acid esterase breaks the ester linkage between ferulic acid and the attached carbohydrate, releasing ferulic acid from the cell walls of the plant (BARTOLOMÉ et al. 1997; WILLIAMSON; KROON; FAULDS, 1998), which leaves the remainder of the polysaccharide chain open for further hydrolysis by other cell wall degrading enzymes (YU; McKINNON; CHRISTENSEN, 2005).

Furthermore, these LAB used in inoculant for silage could survive in rumen fluid, to interact with rumen microorganisms, enhance rumen functionality and animal performance, providing a probiotic effect (WEINBERG et al. 2004 a, b).

Thus this study aimed to evaluate the quality of corn silage inoculated with microbial additives.

2. Literature Review

2.1. Microbial inoculant at level of silage

Microbial silage inoculants containing facultative heterofermentative LAB have long been used to improve silage fermentation, because they are fast and efficient producers of lactic acid (WEINBERG; MUCK, 1996). The main objective in using these facultative heterofermentative LAB inoculants is to reduce the risk of clostridial

fermentations and maintain the nutritional value (DRIEHUIS; OUDE ELFERINK; VAN WIKSELAAR, 2001). However, these types of inoculants are not always advantageous, because they sometimes impair the aerobic stability of silages (WEINBERG; MUCK, 1996; FILYA, 2003; DRIEHUIS; OUDE ELFERINK; VAN WIKSELAAR, 2001).

Thus, a heterofermentative LAB inoculant species, *Lactobacillus buchneri*, has become available commercially and produces high concentrations of acetic acid in silage that inhibit yeasts and molds preserving the silages to spoilage upon exposure to air (FILYA, 2003; WEINBERG et al., 2002). Nevertheless, heterolactic fermentation is deemed as undesirable compared with homolactic fermentation because the loss of dry matter is greater (McDONALD; HENDERSON; HERON, 1991). Heterolactic fermentation results in variable DM losses of 24 to 5% for fermentation of glucose and fructose respectively (KUNG JR., 2009). However, a facultative heterofermentative LAB, as *L. plantarum*, can be combining with *L. buchneri* in the attempt of decrease the fermentation losses and also protein degradation by greater production of lactate and, enhance the aerobic stability of silages (DRIEHUIS; OUDE ELFERINK; VAN WIKSELAAR, 2001; FILYA, 2003).

Some results were found in the literature last years. On laboratory studies, Weinberg et al. (2002), Filya (2003) and Hu et al. (2009) found increases in the lactate content from corn silage inoculated with *L. buchneri* combined with *L. plantarum* and increases in the acetic acid content from corn silage inoculated with *L. buchneri* alone or combining with *L. plantarum*. Reich and Kung Jr. Jr. (2010) observed greater concentrations of acetic acid in corn silage inoculated with *L. buchneri* and *L. plantarum* than uninoculated silage. Nishino et al. (2003) reported that inoculation of *L. buchneri* reduced the lactic acid content and increases acetic acid content from corn silage inoculated compared to untreated.

Ranjit, Taylor and Kung Jr. (2002) reported higher concentrations of acetic and propionic acid in the corn silage inoculated with *L. buchneri* than untreated silage; however the authors did not found differences on the lactic acid content on farm silos. Queiroz et al. (2012) also found higher concentrations of acetic acid and were not detected differences in the concentration of lactic acid in corn silage

inoculated with *L. buchneri* and *Pediococcus pentosaceus* compared to uninoculated on farm silos.

As in laboratory as in field, all authors verified that the growth of yeasts was inhibited and the aerobic stability was improved when they combined *L. buchneri* with a facultative heterofermentative LAB.

Further, Gollop, Zakin and Weinberg (2005) reported that treating silages with LAB could have advantages of imparting bacteriocins, as buchnericin produced by some strain of *L. buchneri* that inhibited the growth of some bacteria undesirable as *Listeria monocytogenes* and *Bacillus cereus* (YILDIRIM; AVŞAR; YILDIRIM, 2002).

With respect the efficiency of *P. acidipropionici and B. subtilis* strains in the yeasts control and improved aerobic stability of silages Filya, Sucu and Karabulut (2004) found that inoculation of *P. acidipropionici* can improve the aerobic stability of wheat, sorghum and corn silages, but inoculation with both *P. acidipropionici* and *L. plantarum* was not efficient. The authors reported the *P. acidipropionici*-inoculated silages had significantly higher levels of acetic and propionic acid than *P. acidipropionici* plus *L. plantarum*-inoculated silages.

Basso et al. (2012b) observed a lower occurrence of yeast and improved aerobic stability in corn silage inoculated with *B. subtilis*. Phillip and Fellner (1992) found that inoculation of high-moisture ear corn with *B. subtilis* and *L. plantarum* could improve its aerobic stability. Katz and Demain (1977) reported that 66 different peptide antibiotics are elaborated by strains of *Bacillus subtilis,* and the most of the peptide antibiotics produced by bacilli are active against gram-positive bacteria, whereas bacillomycin, mycobacillin, and fungistatin are effective agents against molds and yeasts.

Moreover, recently, Nsereko et al. (2008) reported that LAB can release the ferulate esterase enzyme that might increase susceptibility of plant cell wall to enzymatic hydrolysis, because the ferulic acid is released from cell wall arabinoxylans enhancing the fiber digestion of silages. Donaghy, Kelly and McKey (1998) also detected that *Bacillus subtilis* strains exhibit ferulate esterase activity. According Adaah et al. (2011) this property has become the target for development of a third generation of silage inoculants.

2.2. Microbial inoculant at level of rumen

Many researchs suggesting that the LAB might survive in the rumen, and these shifts in the ruminal fermentation may be an indication of a change in the rumen microbial population, but it is not totally clear, mainly when there are a effect associated at levels of concentrate (KEADY; STEEN, 1994; WEINBERG; MUCK, 1996; FELLNER et al., 2001; WEINBERG; MUCK; WEIMER, 2003; WEINBERG et al., 2004; WEINBERG; CHEN; GAMBURG, 2004; WEINBERG et al., 2007).

In vitro studies highlights the survive of LAB in the rumen, Weinberg, Muck and Weimer (2003) found that LAB as *L. buchneri* and *L. plantarum* can survive in the rumen fluid by 72 h. Weinberg et al. (2004) observed that LAB counts in the rumen fluid after 96 h of incubation. Contreras-Govea et al. (2011) found more microbial biomass in silages inoculated with *L. buchneri* or *Lactococcus lactis* after 9 and 48 h of *in vitro* incubation. With respect to *in vivo* study, Mohammed et al. (2012) reported greater relative population size of *L. plantarum* MTD/1 in rumen fluid of cows fed inoculated alfalfa silage compared to those fed untreated silage.

Weinberg et al. (2007) reported that lactic acid bacteria, mainly in presence of starch, may compete with other microorganisms in the rumen (lactate-producing such as *Ruminobacter amylophilus* and *Streptococcus bovis*) for readily fermentable substrate resulting in less lactic acid causing a higher ruminal pH, which in turn could be favorable for fibrolytic bacteria in the rumen.

About the effect of microbial inoculant on ruminal fermentation, Keles and Demirci (2011) did not found differences in the ruminal parameters of lambs fed of triticale–Hungarian vetch herbage inoculated with *L. plantarum* or *L. buchneri*. On the other hand, Mohammed et al. (2012) found that total volatile fat acid (VFA) tended to be greater in rumen fluid of cows fed alfalfa silage inoculated with *L. plantarum* MTD/1 than in the rumen fluid of animals fed uninoculated. Fellner et al. (2001) observed an higher concentration of acetate in the rumen fluid of steers fed high-moisture corn inoculated with *L. plantarum* and *Enterococcus faecium* than in the rumen fluid of animals fed untreated silage. Keady and Steen (1994) found lower acetate content and higher propionate content in the rumen fluid of steers fed grass silage inoculated with *L. plantarum* than in the rumen fluid of animals that consumed untreated silage.

2.3. Effects on animal performance

In a review between 1990 to 1995, Kung Jr. and Muck (1997) reported positive responses to microbial inoculants on intake, daily gain and milk production. The average increase in daily gain was 5%, whereas in milk production was 3% (KUNG JR.; MUCK, 1997).

Recents results are found about animal performance fed silage inoculated with microbial inoculant. Kung Jr. et al. (2003) found no effect on the dry matter intake (DMI) of lactating cows fed alfalfa silage inoculated with *L. buchneri* 40788, however they observed an increase of 0.8 kg (2.0%) more on milk production. In contrast, Taylor et al. (2002) did not verified increase on milk production of lactating cows fed barley silage inoculated with *L. buchneri* 40788. Kristensen et al. (2010) and Arriola et al. (2011) also found no effect of microbial inoculation in corn silage on intake and milk production of dairy cows. Bayatkouhsar et al. (2011) found greatest intake of corn silage inoculated with microbial additive in lactating dairy cows, but the milk production was not affected.

Keady and Steen (1994) observed improvements in weight gain of 120 g/d (13.3%) in steers fed grass silage inoculated LAB. McAllister et al. (1998) reported enhanced of 8.0% in DM intake and 124 g/d (13.9%) in the gain of steers fed alfafa silage inoculated with *L. plantarum* combined with *Enterococcus faecium*. Fellner et al. (2001) also found improvements in the daily gain of steers (160 g/d; 10.8%) fed high moisture corn silage inoculated with LAB. In recent study, Acosta Aragon et al. (2012) reported increase of 6.14% in DM intake and 100 g/d in average gain of young beef cattle fed corn silage inoculated with microbial additive.

Ranjit et al. (2002) observed no effect on the DMI of lambs fed corn silage inoculated with *L. buchneri* 40788, but sheep fed this diet gained 57 g (68.6%) more weight day than those fed untreated silage. On the other hand, Nkosi et al. (2009, 2011) found higher DM intake in the lambs fed inoculated silage than animals fed untreated silage. Nkosi et al. (2009) reported an increase on daily weight gain of 21.8 (14.1%) and 35.0 per day (22.7%) in lambs fed with corn silage inoculated *Pediococcus pentosaceus*, *L.* plantarum and *L. buchneri* and *L. buchneri* alone compared to animals fed untreated silage respectively.

Some assumptions are speculated by several authors about improvements in animal performance when they are fed silage inoculated with microbial additives. These improvements can be in response of increase in digestibility as it was found by McAllister et al. (1998) that verified increase in the DM and OM digestibility in lambs fed alfalfa silage inoculated with LAB. Aksu, Baytok and Bolat (2004) and Kamarloiy and Yansari (2008) also observed enhances in the DM and NDF digestibility in lambs and steers, respectively, fed corn silage inoculated with LAB.

Kamarloiy and Yansari (2008) reported that the increase in digestibility was result of increased surface area available for microbial attack, resulting in a more rapid rate of ruminal fermentation and increased intake, because the partial digestion of the fibrous components of silage during ensiling may alter ruminal digestibility. These changes could be due to removal the ferulic acid from cell wall arabinoxylans (NSEREKO et al., 2008). Futhermore, the effects of inoculants on digestibility may be a consequence of improved nutrient preservation during the fermentation process and conservation of a greater proportion of digestible nutrients (McDONALD; HENDERSON; HERON, 1991).

Moreover, an increase in N retention could result in improve in animal performance, as it was found by Nkosi et al. (2010; 2011) that reported the greatest N retention in the lambs fed silage inoculated with LAB and attributed to better digestibility of CP which resulted in increase N absorption, showing more efficient N use, or due a decrease of proteolysis in silage.

In general, inoculated silages have a greater concentration of non-fibrous carbohydrates and/or higher lactate content. Fermentation of non-fibrous carbohydrates in silage has a direct effect on the pattern of VFA production in the rumen, even as the lactic acid from silage is metabolized primarily to propionate, this shifts the balance of fermentation end products from lipogenic to glucogenic precursors (CHARMLEY, 2001). Thus, the enhanced on animal performance by feeding inoculated silage might be related to improved efficiency of energy utilization due to higher levels of propionate in the rumen caused by an inoculant, as reported Keady and Steen (1994).

However, sometimes with use of microbial inoculants the chemical composition and fermentation characteristics of silage are not affected and

nevertheless the animal performance is improved. A possible explains is that LAB inoculated in silage could survive in ruminal conditions; enhance rumen function and improved digestibility of fiber (WEINBERG et al., 2004a, 2004b). Moreover, treating silages with LAB could have advantages of imparting bacteriocins to the silages which might inhibit detrimental microrganisms in the silage or even in the rumen and thus, provide a probiotic effect what would result in a improve of animal health and performance (GOLLOP; ZAKIN; WEINBERG, 2005).

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CHAPTER 2 - CORN SILAGE INOCULATED WITH MICROBIAL ADDITIVES: NUTRITIVE VALUE AND AEROBIC STABILITY

Abstract

This study aimed to evaluate the effects of *Lactobacillus buchneri*, *Propionibacterium acidipropionici* or *Bacillus subtilis*, as well as their combinations with *L. plantarum* on the chemical composition, *in vitro* gas production, *in vitro* apparent digestibility and aerobic stability of corn silage. The inoculants, *Lactobacillus buchneri* (LB), *Bacillus subtilis* (BS), *Propionibacterium acidipropionici* (PA), *Lactobacillus plantarum* (LP), as well as combinations *L. buchneri* and *L. plantarum* (LBLP), *B. subtilis* and *L. plantarum* (BSLP) and *P. acidicipropionici* and *L. plantarum* (PALP) were applied to corn plant and ensiled in laboratory silos. After the ensiling period, the chemical composition, the *in vitro* gas production and the *in vitro* apparent DM and organic matter (OM) digestibility was determined. To evaluate the aerobic stability, the silages were subjected to aerobic exposure for 12 days. Silages containing *L. plantarum* had greater lactic acid content. The acetic acid content was higher in the LB and LBLP silages. The microbial inoculation promoted silages with lower NDF and hemicellulose content and higher NFC contents, which resulted in greater gas production and higher *in vitro* apparent DM and OM digestibility. Under aerobic conditions, the inoculated silages had lower yeast and mold counts and improved aerobic stability.

Keywords - acid lactic bacteria, inoculant, *in vitro* gas production, propionic acid bacteria

1. Introduction

Microbial additives containing facultative heterofermentative lactic acid bacteria $^{(HeFac)}$LAB such as *Lactobacillus plantarum* are used to decrease rapidly the pH of forage during ensilage; these microbes are used because the end product of the silage fermentation process is lactic acid, thereby avoiding undesired fermentation by Clostridia and Enterobacteria (Kung Jr. 2009). However, corn plants have a high content of soluble carbohydrates, which are fermented by the natural LAB, resulting

in lactic acid (McDonald et al. 1991), thus would be not necessary to use inoculants containing *L. plantarum*. Soluble carbohydrates and lactic acid are consumed by lactate-assimilating-yeast in the post-opening of silos, raising the silage pH and resulting in aerobic deterioration, which reduces the quality and digestibility of the silage (Lindgren et al. 1985).

Inoculants containing heterofermentative LAB (^{He}LAB), such as *Lactobacillus buchneri* are used to improve the aerobic stability of the silage by producing high levels of acetic acid (Nkosi et al. 2011, Basso et al. 2012a). It has been suggested that other types of inoculants, such as propionic acid bacteria (PAB) and *Bacillus* species, can be used as microbiological additives to overcome the problem of silage aerobic spoilage (Phillip and Fellner 1992, Filya et al. 2004, Filya et al. 2006, Rowghani et al. 2008, Basso et al. 2012b). *Propionibacterium acidipropionici* are used to improve aerobic stability by producing propionic and acetic acids, which have antifungal effects (Moon 1983), and the antimycotic effects of *Bacillus* species may be due to the production of bacteriocins, such as zymocin, which is produced by some *Bacillus subtilis* strains (Pahlow et al. 2003).

However, inoculation with ^{he}LAB can promote higher DM losses during fermentation (McDonald et al. 1991). Likewise, some concerns about the use of PAB are the loss of DM and the proteolytic activity that *Propionibacteria* possess (Kung Jr. Jr. 2009). *Bacillus* species can produce lactic and acetic acids, and their growth is not suppressed by these fermentation products or by low pH. However, these organisms are generally less efficient than LAB in producing lactic acid (Pahlow et al. 2003). Thus, combining ^{He}LAB, PAB or *Bacillus* with a ^{HeFac}LAB may be an alternative strategy to decrease fermentation losses and protein degradation by producing greater quantities of lactate, thereby enhancing the aerobic stability of silages, as found by Driehuis et al. (2001); Filya (2003) and Rowghani et al. (2008).

Moreover, this *Bacillus subtilis* strain has been few studied in the deterioration aerobic control of silages. Hence, this study aimed to evaluate the effects of *Lactobacillus buchneri*, *Propionibacterium acidipropionici* or *Bacillus subtilis*, as well as their combinations with *L. plantarum* on the chemical composition, *in vitro* gas production, *in vitro* apparent digestibility and aerobic stability of corn silage.

2. Material and methods

The trial was conducted at Faculdade de Ciências Agrárias e Veterinárias, Univ Estadual Paulista (UNESP), located at 21°14'14.04'' S and 48°17'27.92'' W. According to the Köppen classification the climate is AW type, which is characterized as a tropical wet and dry climate that is rainy in the summer and dry in the winter season.

An AG1051 corn hybrid (Monsanto, Barretos, SP, Brazil) was sown on January 10, 2009 and was harvested on April 21, 2009. The crop was manually cut at a height of 20 cm from the soil using a machete. Forages were chopped to achieve a theoretical cut length of 10 mm in the stationary machine (Penha, Ribeirao Preto, SP, Brazil).

The following treatments were applied to the fresh forages: untreated (**control**), *Lactobacillus buchneri* NCIMB 40788 (**LB** - 1 x 10^5 cfu g^{-1} of fresh forage), *Bacillus subtilis* AY553098 (**BS** - 1 x 10^5 cfu g^{-1}), *Propionibacterium acidipropionici* MA26/4U (**PA** - 1 x 10^5 cfu g^{-1}) and *L. plantarum* MA18/5U (**LP** - 1 x 10^5 cfu g^{-1}). The combination treatments were *L. buchneri* combined with *L. plantarum* (**LBLP,** 2 x 10^5 cfu g^{-1}), *B. subtilis* combined with *L. plantarum* (**BSLP,** 2 x 10^5 cfu g^{-1}) and *P. acidicipropionici* combined with *L. plantarum* (**PALP,** 2 x 10^5 cfu g^{-1}).

The application rate of the inoculants (concentration of microorganism per g of product) was determined in accordance with the manufacturer's instructions. The correct amount of inoculant for each treatment was weighed to achieve the desired application rates. Inoculants were diluted in water at rate of 5 mL kg^{-1} of fresh forage and were then applied in a uniform manner by spraying the fresh forage in a constant mixing by hand. The control silage received a similar amount of distilled water.

Immediately following inoculation, samples of the fresh forage from all the treatments were obtained to determine the DM, ash, crude protein (CP), neutral-detergent fiber (NDF), acid-detergent fiber (ADF), hemicellulose, ammonia N content in relation to total nitrogen contents (NH_3 TN^{-1}), pH values and microorganism' counts (yeasts and molds), thus characterizing the corn plant before ensiling. The average DM content was 354 g kg^{-1}. The concentrations of ash, CP, NDF, ADF and hemicellulose were 38 g kg^{-1}; 103 g kg^{-1}; 514 g kg^{-1}; 314 g kg^{-1} and 200 g kg^{-1} of the DM, respectively. The average pH value was 6.02 and the ammonia N content was

2.15% of total nitrogen. The yeast and mold counts were 7.23 and 5.63 (log_{10}) cfu g^{-1} of forage, respectively.

A sample of chopped forage (3.6 kg) from each treatment was packed into 7L plastic bucket silos in quadruplicate; these silos were sealed with a lid and adhesive tape, stored at room temperature (average 25°C) and remained closed for 96 days. Experimental silos were weighed after filling and at the end of the ensiling period to determine the gas and DM losses. The empty plastic buckets were also weighed. The gas losses (GL) were calculated by subtracting the final weight from the initial weight of the silos and dividing the resulting weight difference by the dry mass of the ensiled material (Jobim et al. 2007). The DM losses (DML) were obtained by subtracting the silage dry mass from the ensiled dry mass and dividing the resulting weight difference by the dry mass of the ensiled material (Jobim et al. 2007).

After the ensiling period, the silos were opened, and the silage was homogenized and sampled to determine the pH values, the NH_3/TN content, the concentrations of lactic acid (LA) and volatile fatty acids (VFA) and the yeast and mold counts. The DM, ash, OM, CP, NDF, ADF, N residuals (NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen), hemicellulose, cellulose and lignin, total carbohydrates (CHO) and non-fibrous carbohydrate (NFC) content was determined, in addition to the *in vitro* gas production and the *in vitro* apparent DM (IVDMD) and OM digestibility (IVOMD).

To determine the aerobic stability, 3 kg of silage were placed in plastic buckets and were kept closed at room temperature (average 26 °C). The temperature of the silage was measured every half hour for 12 days by a data logger placed in the silage during the aerobic exposure. The ambient temperature was measured by data loggers distributed near the experimental silos. The aerobic stability was defined as the number of hours that the temperature of the silage remained stable before rising more than 2°C above room temperature (Taylor and Kung Jr. 2002). During the aerobic exposure (4, 8 and 12 days), the silages were sampled to determine the pH values and the yeast and mold counts.

Samples to determine the fermentation characteristics (pH value, NH_3 TN^{-1} content, and concentrations of lactic acid and VFA) were stored at -20 °C until water

extraction was performed. Samples to determine the chemical composition of the silage were stored dried. Microbiological analyses were performed immediately.

A water extract was made from the wet silage according to the protocol established by Kung Jr. Jr. et al. (1984). The pH of the water extract was determined using a pH meter (MA522 model, Marconi Laboratory Equipment, Piracicaba, SP, Brazil). The VFA were measured in a Shimadzu GC2014 (SHIMADZU Corporation, Kyoto, Japan) gas chromatograph using a HP-INNOwax capillary column (30 m x 0.32 mm; Agilent Technologies, Colorado, USA) at an initial temperature of 80 °C and a final temperature of 240 °C. LA was determined using a colorimetric method (Barker and Summerson 1941). The NH_3 TN^{-1} was measured through distillation (AOAC 1996 – ID 941.04).

For the microbiological analyses, 25 grams of either the fresh forage or silage sample from each replicate were homogenized in 225 mL peptone sterile water (1 mg mL^{-1}). The yeast and mold counts were performed on a spread-plate of potato dextrose agar acidified with lactic acid (85%), and the plates were grown at 28 °C for 3 and 5 days, respectively. All the microbiological data were log-transformed.

Samples were oven dried at 55 °C for 72 h, processed in a knife Wiley mill (A. H. Thomas, Philadelphia, PA, USA) in order to pass through 1 mm screen sieves, and analyzed for DM following incubation at 105 °C for 12 h and ash following incubation at 500 °C for 5 h. The OM was calculated using the following equation:

$$OM\ (g\ kg^{-1}\ DM) = 100 - Ash \quad (1)$$

The NDF was analyzed using a neutral detergent solution and a heat-stable α-amylase without sodium sulphite, as according to Mertens (2002). The ADF was analyzed using an acid detergent solution, as described by Van Soest and Robertson (1985). Both the NDF and ADF samples were incubated in an autoclave at 110 °C for 40 min (Senger et al. 2008). Residual N (NDIN and ADIN) was analyzed. The ash present in the NDF residue was also determined. Lignin present in the ADF residue was measured following the hydrolysis of cellulose in H_2SO_4 (72%) (Van Soest and Robertson 1985). The extract ether (EE) was measured according to the procedures of the AOAC (1996 - ID 920.39). The N in the NDF and ADF residues and the total N (TN) were determined using the Kjeldahl method (AOAC 1996 – ID 954.01). The crude protein (CP) was calculated as TN x 6.25.

The total carbohydrate (CHO) (Eq. (1)) and the non-fibrous carbohydrate (NFC) (Eq. (2)) contents were estimated according to Sniffen et al. (1992) and Detmann and Valadares Filho (2010), respectively.

CHO (g kg^{-1} DM) = 100 – (CP + EE + Ash) (2)

NFC (g kg^{-1} DM) = 100 – ($NDFap$ +CP + EE + Ash] (3)

where NDFap = NDF corrected for ash and protein.

The *in vitro* apparent DM digestibility (IVDMD) was determined using filter bags (ANKOM F57) in a DAISY II incubator (ANKOM Technologies, Macedon, NY, USA). The samples were incubated with rumen inoculum and buffer solution, in a ratio of 4:1, for 48 h, followed by acid digestion with pepsin for another 24 h (Marten and Barnes 1979). The Kansas State buffer “synthetic saliva” solution, described by Marten and Barnes (1979), was used.

The *in vitro* apparent OM digestibility (IVDOM) was estimated from the gas production, as described by Menke et al. (1979) and Mauricio et al. (1999). The samples (200 mg) were incubated in a water bath at 39°C in serum bottles (115 mL) (Mauricio et al. 1999) with 30 mL buffered rumen fluid (Menke et al. 1979). Rumen fluid was collected from 2 rumen-cannulated steers in the morning before feeding; the rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermos flasks, homogenized and mixed with solution medium. The steers were fed 60% corn silage without inoculant and 40% concentrate, on DM basis.

The accumulated headspace gas pressure measurements were made using a needle attached to a pressure transducer connected to a visual display (Datalogger pressure - pressDATA 800, MPL, Piracicaba, SP, Brazil). Readings were taken at regular intervals throughout the incubation period and at an increased frequency during the initial lag and rapid fermentation phases (e.g. 2, 4, 6, 8, 10, 12, 24 and 48 and 72 h post-inoculation). Flasks containing buffered rumen fluid without samples were used as blanks. However, the blank correction was omitted because according to Cone et al. (1997) microbial turnover in blank begins after 1 h and that about 30% of the maximum blank reading can be attributed to this turnover, and in the presence of substrate, turnover is delayed so that the blank does not reflect accurately what happens in the sample.

The relative gas production for each *in vitro* bottle was calculated by dividing the gas production at a given time by the gas production for that bottle at 72 h to determine whether the relative rate of gas production was affected by the treatment.

The IVDOM was estimated (Eq. (3)) as described by Menke et al. (1979):

$$IVDOM\ (g\ kg^{-1}) = 14.88 + ((0.889*gas24)+(0.045*CP)+(0.065*Ash)) \quad (4)$$

where gas24 equals the gas production in 24 h (mL 0.2 g^{-1} DM) and the CP and ash contents are expressed in g kg^{-1} DM.

Data were analyzed using a completely randomized design with 4 replicates using the MIXED procedure of SAS (v. 9.0 SAS Institute Inc., Cary, NC). Aerobiosis study and *in vitro* gas production data were analyzed using a mixed model with repeated measures. Unstructured and Toeplitz were the best covariance structures chosen by the minimum Akaike information criterion. Differences between the means were determined using DIFF, which differentiates means based on Fisher's *F*-protected least significant difference test. Significance was declared at $p < 0.05$.

3. Results

The gas and DM losses were lowest in the control silage (GL: 2.7% DM and DML: 3.6% DM), whereas higher losses were observed in the LBLP silage (GL: 6.5% DM and DML: 7.8% DM; $p < 0.05$). The other silages had no significant differences among them. The gas and DM losses ranged from 3.6 to 4.7% DM and from 4.4 to 5.4% DM, respectively.

The control and LB silages had the lowest lactic acid contents, whereas the silages incubated with *L. plantarum*, either alone or in combination, had greater concentrations of lactic acid, particularly the LP and LBLP silages. The acetic acid content was lower in the control and PALP silages; otherwise the acetic acid content was higher in the silages inoculated with *L. buchneri* alone or in combination with *L. plantarum*. The highest lactic acid to acetic acid ratio was observed in the PALP silage, followed by the control, LP, and BSLP silages (Table 1). The highest butyric acid concentration was found in the LP and LBLP silages. Propionic acid was not detected in any of the silages.

Table 1. Fermentation characteristics, chemical composition and apparent digestibility of the corn silages inoculated with microbial additives.

	Control	LB	BS	PA	LP	LBLP	BSLP	PALP	SEM[6]	*p* value
Fermentation characteristics										
LA[1]	42^{c}	46^{c}	53^{bc}	56^{bc}	73^{a}	72^{a}	60^{b}	62^{b}	3.820	0.001
AA[1]	10^{e}	20^{b}	17^{c}	16^{c}	17^{c}	23^{a}	13^{d}	11^{e}	0.544	0.001
LA:AA[2]	5^{ab}	3^{c}	3^{c}	4^{bc}	5^{ab}	3^{c}	5^{ab}	6^{a}	0.368	0.001
BA[1]	0.5^{c}	1.4^{b}	1.5^{b}	1.1^{bc}	3.0^{a}	2.3^{a}	1.2^{b}	1.0^{bc}	0.282	0.001
pH	3.81^{c}	3.87^{a}	3.85^{ab}	3.81^{c}	3.85^{ab}	3.86^{a}	3.84^{b}	3.83^{bc}	0.008	0.001
NH_3/TN[3]	4.68^{d}	4.68^{d}	4.85^{bc}	4.84^{bc}	5.78^{a}	5.28^{ab}	4.73^{cd}	4.18^{e}	0.175	0.001
Chemical composition (g kg^{-1} DM)[4]										
DM	349	341	343	339	341	345	350	356	3.760	0.054
OM	968	963	971	967	967	967	969	966	1.672	0.154
Ash	32	37	30	36	33	33	31	34	1.672	0.154
CP	83^{c}	92^{a}	92^{a}	89^{a}	86^{bc}	90^{a}	88^{ab}	91^{a}	1.179	0.001
EE	22^{d}	26^{c}	28^{bc}	29^{bc}	33^{ab}	33^{ab}	32^{ab}	34^{a}	1.382	0.001
NDF	499^{a}	447^{bc}	435^{c}	447^{bc}	443^{c}	466^{b}	393^{d}	428^{c}	7.715	0.001
NDFap	489^{a}	423^{cd}	423^{cd}	434^{cd}	439^{bc}	455^{b}	383^{e}	417^{d}	6.938	0.001
ADF	261^{ab}	244^{bc}	247^{bc}	249^{bc}	263^{ab}	270^{a}	226^{d}	235^{cd}	6.148	0.001
Hem	238^{a}	203^{b}	188^{cd}	198^{bc}	180^{d}	197^{bc}	168^{d}	193^{bcd}	4.790	0.001
Cell	207^{b}	224^{a}	199^{bc}	220^{ab}	208^{bc}	217^{ab}	193^{c}	203^{c}	4.705	0.001
Lignin	41^{ab}	46^{a}	29^{c}	30^{c}	36^{bc}	36^{bc}	27^{c}	31^{c}	3.101	0.004
NDIN[5]	164	147	160	161	142	165	141	144	9.881	0.400
ADIN[5]	61^{b}	59^{b}	62^{b}	69^{b}	88^{a}	85^{a}	63^{b}	72^{b}	4.632	0.001
CHO	861^{a}	845^{bc}	851^{b}	848^{bc}	849^{bc}	845^{bc}	849^{bc}	841^{c}	2.806	0.002
NFC	373^{d}	422^{b}	427^{b}	414^{b}	410^{bc}	390^{c}	466^{a}	424^{b}	7.172	0.001
In vitro *apparent DM and OM digestibility (g kg^{-1})*										
IVDDM	560^{e}	638^{c}	683^{a}	640^{c}	658^{b}	610^{d}	693^{a}	669^{b}	5.615	0.001
IVDOM	659^{c}	708^{ab}	714^{ab}	702^{b}	724^{a}	696^{b}	710^{ab}	712^{ab}	6.694	0.001

[*]Means follows of the same letter did not differ to 5% of significance. Silages - Control: without inoculant; LB: *L. buchneri*; BS: *B.subtilis*; PA: *P. acidipropionici*; LP: *L. plantarum*; LBLP: *L. buchneri* and *L. plantarum*; BSLP: *B. subtilis* and *L. plantarum*, PALP: *P. acidipropionici* and *L. plantarum*.[1]LA: lactic acid; AA: acetic acid; BA: butyric acid – g kg^{-1} DM; [2]LA:AA: Lactic acid to acetic acid ratio; [3]TN: total nitrogen. [4]DM: Dry matter; OM: organic matter; CP: crude protein; EE: extract ether; NDF: neutral detergent fiber; NDFap: neutral detergent fiber corrected for ash and protein; ADF: acid detergent fiber; Hem: hemicellulose; Cell: cellulose; NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen; CHO: total carbohydrates; NFC: non-fibrous carbohydrates.[5]NDIN and ADIN – g kg^{-1} TN. [6]SEM: Standard error of the mean.

We found higher pH values in the silages inoculated with *L. buchneri* or *B. subtilis* alone or in combination with *L. plantarum* and in the silages inoculated with *L. plantarum* alone compared to the control and PA silages. The highest concentrations of NH_3 TN^{-1} were found in the LP and LBLP silages, whereas the PALP silage had the lowest NH_3 TN^{-1} concentration (Table 1).

The DM, OM, ash and NDIN contents were similar among the silages. The inoculation of microorganisms alone or in combination with *L. plantarum* resulted in silages with higher CP, EE and NFC contents than the control silage. In contrast, the concentrations of NDF, hemicellulose and CHO were lower in the inoculated silages (Table 1). The reduction in the NDF from the whole corn plant to corn silage in the control silage was 15 g kg^{-1}, whereas the lowest NDF decrease in the inoculated silage was 48 g kg^{-1}in the LBLP silage, and the highest decrease was 121 g kg^{-1}in the BSLP silage.

The ADF content was higher in the LBLP, LP and control silages. Silages inoculated with *L. buchneri* alone or in combination with *L. plantarum* and inoculated with *P. acidipropionici* alone had the highest cellulose concentrations and the BSLP and PALP silages showed lower cellulose contents. The concentrations of lignin were higher in the LB and control silages and PALP silages showed lower lignin contents. The highest ADIN content was found in the LP and LBLP silages. We observed a greater *in vitro* apparent DM and OM digestibility in the silages inoculated with the microbial additives than the control silage (Table 1).

The gas production was significantly affected by microbial inoculation for the first 24 h of the incubation time. Silages inoculated with *L. plantarum* alone or in combination with other microorganisms had higher gas production volumes than the control silage throughout this time (until 24 h). During the first hours of incubation (at 2 and 4 h), the LB and BS silages had similar gas production to the control silage. At 8, 10 and 12 h of incubation, the PA silage presented a gas production volume similar to the control silage (Table 2).

The silages inoculated with *L. plantarum* alone or in combination with ^{he}LAB, PAB or *Bacillus subtilis* had faster relative rates of gas production than the control silage until 12 h of incubation. After 24 h of incubation, only the BSLP and PALP silages had faster relative rates of gas production than the control silage. In the LP, LBLP, BSLP and PALP silages, we observed that 50% of the total of gas production (72-h) occurred between 10 and 12 h (Table 2).

Table 2. *In vitro* gas production (mL g^{-1} DM) and relative *in vitro* gas production (fraction of the 72 h production) of the corn silages inoculated with microbial additives for various incubation times (IT).

IT (h)	*In vitro* gas production									
	Control	LB	BS	PA	LP	LBLP	BSLP	PALP	*p* value	SEM[1]
2	67.9^{c}	75.5^{bc}	73.6^{cb}	76.5^{ab}	86.2^{a}	78.7^{ab}	80.1^{ab}	78.8^{ab}	0.006	2.702
4	97.7^{c}	106.6^{bc}	106.6^{bc}	107.7^{b}	117.6^{a}	109.3^{ab}	115.1^{ab}	111.5^{ab}	0.008	3.183
6	125.9^{c}	140.4^{b}	140.4^{b}	138.4^{b}	154.2^{a}	151.4^{a}	152.3^{a}	145.5^{b}	0.003	3.753
8	146.7^{d}	158.0^{bcd}	160.3^{bc}	156.8^{cd}	170.5^{a}	166.6^{ab}	169.9^{ab}	163.1^{abc}	0.008	4.162
10	162.3^{c}	177.2^{ab}	179.8^{ab}	173.7^{cb}	188.9^{a}	182.9^{ab}	189.5^{a}	180.8^{ab}	0.005	4.414
12	178.9^{c}	194.7^{ab}	195.4^{ab}	190.0^{bc}	205.2^{a}	192.3^{b}	204.9^{a}	197.4^{ab}	0.008	4.401
24	254.9^{c}	277.8^{ab}	277.9^{ab}	272.3^{b}	284.6^{ab}	274.6^{ab}	290.1^{a}	277.1^{ab}	0.013	5.648
48	336.4	352.5	359.6	349.2	347.1	346.3	351.1	329.3	0.846	3.456
72	380.7	396.0	411.5	396.5	409.7	388.5	398.3	377.9	0.852	7.540
	Relative *in vitro* gas production									
2	0.178^{c}	0.191^{bc}	0.180^{c}	0.193^{bc}	0.211^{a}	0.203^{ab}	0.201^{ab}	0.209^{a}	0.001	0.005
4	0.257^{c}	0.269^{bc}	0.260^{c}	0.272^{bc}	0.287^{ab}	0.282^{ab}	0.289^{ab}	0.295^{a}	0.006	0.007
6	0.331^{c}	0.354^{bc}	0.342^{c}	0.349^{c}	0.377^{ab}	0.390^{a}	0.382^{a}	0.384^{a}	0.001	0.008
8	0.385^{c}	0.399^{bc}	0.391^{bc}	0.396^{bc}	0.416^{ab}	0.428^{a}	0.426^{a}	0.432^{a}	0.004	0.009
10	0.426^{d}	0.447^{bcd}	0.439^{cd}	0.439^{cd}	0.461^{abc}	0.471^{ab}	0.476^{ab}	0.478^{a}	0.005	0.010
12	0.470^{d}	0.492^{bcd}	0.477^{cd}	0.480^{bcd}	0.501^{abc}	0.495^{bcd}	0.514^{ab}	0.522^{a}	0.014	0.010
24	0.700^{b}	0.702^{ab}	0.679^{b}	0.687^{b}	0.695^{ab}	0.707^{ab}	0.728^{a}	0.734^{a}	0.034	0.014
48	0.883	0.890	0.879	0.881	0.848	0.892	0.883	0.872^{a}	0.707	0.017

[*]Means follows of the same letter did not differ to 5% of significance. Silages - Control: without inoculant; LB: *L. buchneri*; BS: *B.subtilis*; PA: *P. acidipropionici*; LP: *L. plantarum*; LBLP: *L. buchneri* and *L. plantarum*; BSLP: *B. subtilis* and *L. plantarum*, PALP: *P. acidipropionici* and *L. plantarum*. [1]SEM: Standard error of the mean for the incubation time.

We observed an interaction between silages and the days of aerobic exposure in the occurrence of yeasts and molds. Until the eighth day of aerobic exposure, the microbial inoculation affected the yeast count. Yeast occurrence was lower in the silages inoculated with microorganisms alone than in the control silage on the opening day of the silos (day 0). After four days of aerobic exposure, all the inoculated silages had a lower yeast count, except the PALP silage. On the eighth day of aerobic exposure, only the LBLP silage had a lower yeast occurrence (Table 3).

On the opening day of the silos, the mold occurrence was similar among all the silages. However, on the fourth day of aerobic exposure, the LB, BS, PA, LP and LBLP silages had lower counts of mold than the control, BSLP and PALP silages. The silage inoculated with *L. buchneri* and *L. plantarum* maintained the lowest mold count until the twelfth day of aerobic exposure (Table 3).

Table 3. Occurrence of yeast and mold in the corn silages inoculated with microbial additives, during aerobic exposure (days).

Aerobic exposure	Control	LB	BS	PA	LP	LBLP	BSLP	PALP
	Yeast[1] (*p* value - Silage: 0.001; Days: 0.001; SxD[2]: 0.034; SEM[3]: 0.337)							
0	3.9^{aB}	1.2^{bC}	1.6^{bB}	2.4^{bC}	3.9^{aB}	2.6^{abC}	3.5^{aB}	3.9^{aB}
4	8.9^{aA}	6.2^{cB}	7.8^{bA}	7.9^{bB}	8.0^{bA}	6.8^{cB}	8.0^{bA}	8.2^{abA}
8	9.3^{aA}	8.5^{abA}	8.5^{abA}	8.8^{abA}	8.6^{abA}	8.3^{bA}	8.5^{abA}	8.8^{abA}
12	8.8^{aA}	7.8^{aA}	8.1^{aA}	8.2^{aA}	8.5^{aA}	7.8^{aA}	7.9^{aA}	8.1^{aA}
	Molds[1] (*p* value - Silage: 0.002; Days: 0.001; SxD[2]: 0.001; SEM[3]: 0.351)							
0	3.9^{aB}	3.9^{aB}	4.0^{aB}	3.6^{aC}	3.8^{aC}	3.6^{aB}	4.2^{aB}	3.6^{aC}
4	6.6^{aA}	3.8^{dB}	4.0^{cdB}	4.8^{cB}	5.6^{bB}	4.2^{cdB}	6.5^{aA}	6.4^{aB}
8	7.1^{aA}	7.2^{aA}	7.3^{aA}	7.2^{aA}	7.3^{aA}	5.3^{bA}	7.5^{aA}	7.2^{aAB}
12	7.5^{aA}	7.3^{aA}	7.5^{aA}	7.4^{aA}	7.3^{aA}	5.3^{bA}	7.6^{aA}	7.5^{aA}

*Means follows of the same letter (lowercase in the row and uppercase in the column) did not differ to 5% of significance. Silages - Control: without inoculant; LB: *L. buchneri*; BS: *B.subtilis*; PA: *P. acidipropionici*; LP: *L. plantarum*; LBLP: *L. buchneri* and *L. plantarum*; BSLP: *B. subtilis* and *L. plantarum*, PALP: *P. acidipropionici* and *L. plantarum*. [1]Yeast and Molds - $\log_{10}$ cfu/ g of silage. [2]SxD – Interaction between the silages and the days of aerobic exposure. [3]SEM: Standard error of the mean.

There was an interaction between the silages and the days of aerobic exposure in the pH values. On the fourth day of aerobic exposure, all of the inoculated silages presented lower pH values than the control silage, except the BSLP silage. On the eighth day of aerobic exposure, only the LBLP silage showed a lower pH value. On the twelfth day of aerobic exposure, the LB, PA and BSLP silages had lower pH values than the control, BS and PALP silages (Table 4).

Table 4. Values of pH in corn silage inoculated with microbial additives, during aerobic exposure (days).

Aerobic exposure	Control	LB	BS	PA	LP	LBLP	BSLP	PALP
	pH values (*p* value - Silage: 0.034; Days: 0.001; SxD[1]: 0.001; SEM[2]: 0.090)							
0	3.81^{aC}	3.87^{aD}	3.84^{aD}	3.81^{aD}	3.85^{aD}	3.86^{aD}	3.83^{aD}	3.83^{aC}
4	4.74^{aB}	4.17^{dC}	4.35^{cdC}	4.48^{bcC}	4.45^{cdC}	4.31^{cdC}	4.65^{abC}	4.70^{bB}
8	4.78^{abB}	4.70^{abB}	4.84^{abB}	4.86^{abB}	4.92^{aB}	4.64^{bB}	4.90^{aB}	4.81^{abB}
12	6.11^{aA}	5.70^{bA}	6.36^{aA}	5.72^{bA}	5.86^{abA}	5.90^{abA}	5.49^{bA}	6.05^{aA}

*Means follows of the same letter (lowercase in the row and uppercase in the column) did not differ to 5% of significance. Silages - Control: without inoculant; LB: *L. buchneri*; BS: *B.subtilis*; PA: *P. acidipropionici*; LP: *L. plantarum*; LBLP: *L. buchneri* and *L. plantarum*; BSLP: *B. subtilis* and *L. plantarum*, PALP: *P. acidipropionici* and *L. plantarum*. [1]SxD – Interaction between silages and days of aerobic exposure. [2]SEM: Standard error of the mean.

The silages inoculated with *L. buchneri* alone or in combination with *L. plantarum* had the highest aerobic stability, followed by the silages inoculated with *B. subtilis* and *P. acidipropionici*, respectively. In the silages inoculated with *L.*

plantarum alone or in combination with other microorganisms, except *L. buchneri*, the aerobic stability was reduced; nevertheless these silages inoculated with *L. plantarum* had aerobic stability higher than the control silage (P<0.0001 and SEM: 4.730; Figure 1 and Figure 2). The temperature peaked in the control, BSLP, PALP and LP silages at approximately 75, 78, 79 and 79 h, respectively (Figure 2), whereas the silages inoculated with single microorganisms, except LBLP silages, exhibited a delay in the rise and peak of the temperature (PA: 95 h; BS: 98 h; LB: 127 h and LBLP: 123 h; Figure 2).

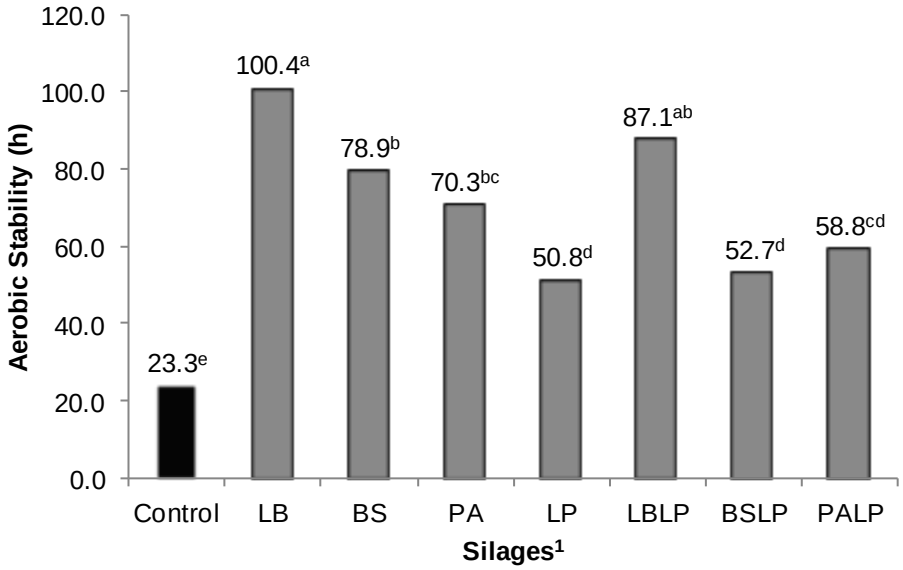

Figure 1. Aerobic stability of the corn silages inoculated with microbial additives.
*Means follows of the same letter did not differ to 5% of significance.
[1]Silages: Control – without inoculant; LB: *L. buchneri*; BS: *B. subtilis*; PA: *P. acidipropionici*; LP: *Lactobacillus plantarum*; LBLP: *L. buchneri* and *L. plantarum*; BSLP: *B. subtilis* and *L. plantarum*, PALP: *P. acidipropionici* and *L. plantarum*.

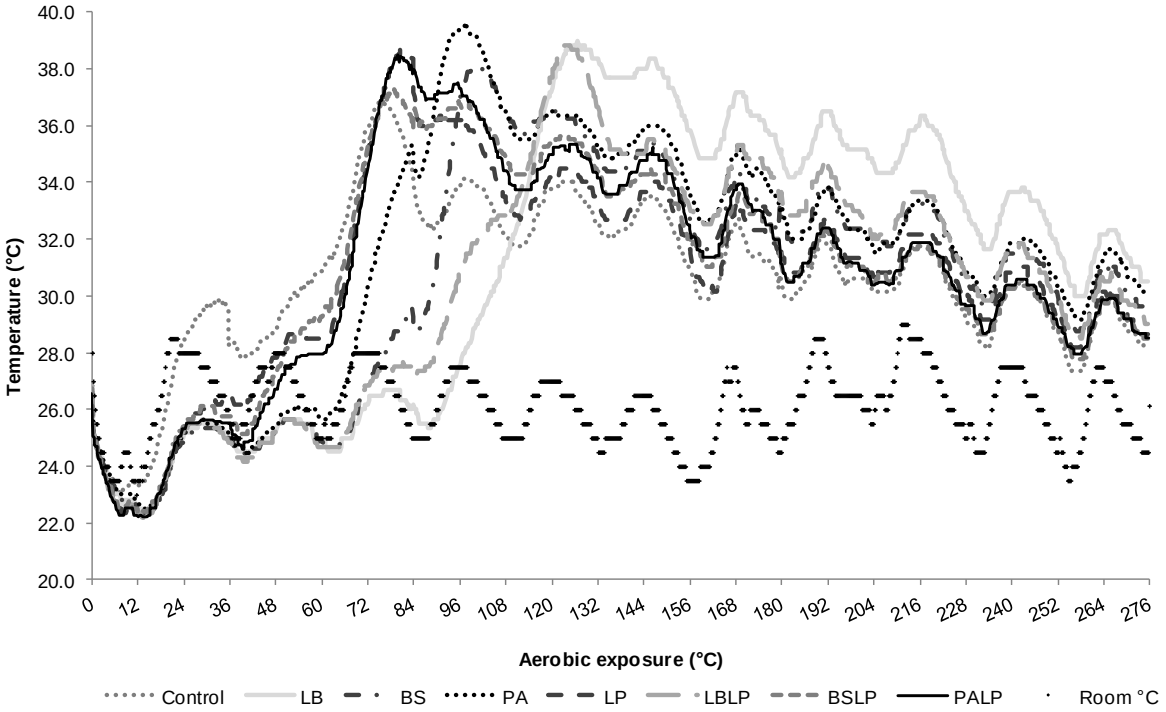

Figure 2. Changes in the temperature of the corn silages inoculated with microbial additives during aerobic exposure for 12 days.

[1]Silages: Control – without inoculant; LB: *L. buchneri*; BS: *B. subtilis*; PA: *P. acidipropionici*; LP: *Lactobacillus plantarum*; LBLP: *L. buchneri* and *L. plantarum*; BSLP: *B. subtilis* and *L. plantarum*, PALP: *P. acidipropionici* and *L. plantarum*.

4. Discussion

The higher losses of DM in the inoculated silages could be due to extensive fermentation promoted by the microorganisms that were inoculated. Although the LBLP silage presented the highest DM loss, this percentage (7.8%) could also be deemed low; according to Kung Jr. (2009), heterolactic fermentation results in variable DM losses, from 24 to 5%, for the fermentation of glucose and fructose, respectively.

The silages inoculated with *L. plantarum* alone or in combination with other microorganisms had a greater acid lactic content, because of higher lactic acid production by this microrganism (McDonald et al. 1991). The highest acetic acid content in the inoculated silages was due to heterolactic fermentation caused by the microorganisms that were inoculated (McDonald et al. 1991, Pahlow et al. 2003, Kung Jr. 2009). Higher concentrations of butyric acid in the LP and LBLP silages

could be related to secondary fermentation by clostridia and enterobacteria, since the NH_3/TN content was also higher in these silages.

The pH values and NH_3 TN^{-1} were higher in the inoculated silages, mainly in LB, LP and LBLP silages. According to Driehuis et al. (2001), the *L. buchneri*-treated corn silage is associated with a relatively increase in the pH during the storage phase because of the high metabolic activity of *L. buchneri* in these silages. Therefore, in the treated silages the decrease in pH can have been less pronounced than control silage which provided greater proteolysis resulting in higher concentrations of NH3 TN-1 in treated silages. However, both the pH values and NH_3 TN^{-1} content were within the limits provided by Kung Jr. and Shaver (2001), from 3.7 to 4.2 and 5 to 7% TN, respectively, for corn silage that has been suitably preserved. The inoculated silages were well preserved, in terms of the traditional indicators of silage quality (high lactic acid concentrations, low pH values, and low ammonia N content).

Inoculation changed the fiber composition of the silages. All inoculated silages had reduced NDF and hemicellulose content and increased NFC; these fiber composition changes resulted in higher DM and OM digestibility than the control silage. Likewise, the gas production volume was higher in the inoculated silages evaluated at 24 h of incubation. In the beginning ensiling process many enzymes are in action until the decrease the pH below 4.0 (McDonald et al., 1991). In present study is possible that the decrease in pH can have been less pronounced than control silage. Thus, enzymes as hemicellulase, can have acted in reducing hemicelluloses content. Filya and Sucu (2010) found increases in the *in vitro* DM digestibility of corn silages inoculated with LAB and attributed to some solubilization of the hemicellulose during ensiling.

Following aerobic exposure, the inoculated silages, especially those inoculated with *L. buchneri* alone or in combination with *L. plantarum* and the silages inoculated with *P. acidipropionici* or *B. subtilis* alone, had lower yeast and mold counts presumably due to higher concentrations of acetic acid (Table 1), which has an antifungal effect (Moon 1983). Lactate-assimilating-yeast has been implicated in the aerobic deterioration of ensiled feed because the lactic acid is consumed during aerobic exposure; hence, the silage pH rises becoming a favourable environment for other undesirable microorganisms, such as molds and aerobic bacteria. The

metabolic activity of these microorganisms causes the silage temperature to rise (Lindgren et al. 1985). Thus, in the present study, these silages had improved aerobic stability and more stable pH values during aerobic exposure. The pH value and the temperature are indicators of aerobic deterioration of the silage (Lindgren et al. 1985).

These results are in agreement with Basso et al. (2012a) who found a lower occurrence of yeast and improved aerobic stability in corn silage inoculated with *L. buchneri*. Filya (2003) also observed lower yeast and mold counts and improved aerobic stability of corn and sorghum silages. Filya et al. (2004) found that inoculation of *P. acidipropionici* can improve the aerobic stability of wheat, sorghum and corn silages, but inoculation with both *P. acidipropionici* and *L. plantarum* was not efficient. Basso et al. (2012b) observed a lower occurrence of yeast and improved aerobic stability in corn silage inoculated with *B. subtilis*. Phillip and Fellner (1992) found that inoculation of high-moisture ear corn with *B. subtilis* and *L. plantarum* could improve its aerobic stability.

5. Conclusions

The microbial inoculation increased the concentration of lactic and acetic acid, decreased the hemicellulose content and improved the *in vitro* digestibility of corn silages.

The inoculation of corn silage with *Lactobacillus buchneri*, *Bacillus subtilis* and *Propionibacteria acidipropionici* and just *Lactobacillus buchneri* combined with *Lactobacillus plantarum* decreased the occurrence of yeasts and molds and enhanced the aerobic stability.

6. Acknowledgment

The authors wish to express their appreciation to São Paulo Research Foundation – FAPESP for their financial support.

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CHAPTER 3 - EFFECTS OF FEEDING CORN SILAGE INOCULATED WITH MICROBIAL ADDITIVES ON THE *IN VIVO* DIGESTIBILITY, MICROBIAL PROTEIN YIELD, N BALANCE AND PERFORMANCE OF LAMBS

Abstract

This study aimed to examine the effects of feeding corn silage inoculated without or with either *Lactobacillus buchneri* (LB) alone or a combination of LB and *Lactobacillus plantarum* (LBLP) on the *in vivo* apparent digestibility, ruminal fermentation parameters, microbial protein synthesis and performance of lambs. Thirty Santa Inês × Dorper crossbred intact males lambs weighing 20.4 ± 3.8 kg were blocked by weight into ten groups. Lambs in each group were randomly assigned to one of the following three dietary treatments: Untreated (Control), LB and LBLP silage. Lambs were fed experimental diets for 61 d. *In vivo* digestibility was indirectly estimated from fecal output measured on d 57 to 59. *Spot* urine samples were collected from all animals on d 59 to estimate microbial protein synthesis and N utilization. Lambs were slaughtered for carcass evaluation on d 61 when they weighed 32.4 ± 5.2 kg. Six additional ruminally-cannulated wethers were used to examine dietary effects on ruminal fermentation parameters. The LBLP silage had the highest lactic acid concentration and, both inoculated silages had higher acetic acid concentrations than the control silage. Inoculation of corn silage increased intake of DM, OM, CP, NDF, CHO and GE by the lambs. Lambs fed inoculated silages had the highest microbial N supply and N retention. The acetate to propionate ratio was lower in rumen fluid of wethers in LBLP treatment than LB and Control treatment and ruminal pH tended to be higher in LB lambs than in LBLP and Control wethers. Average daily gain was increased when lambs were fed LBLP silage, but not LB silage. In conclusion, inoculation increased the lactic and acetic acids concentration in the silages but did not affect other chemical components. Feeding corn silage inoculated with both microbial additives increased intake, ruminal fermentation, microbial N supply and N retention. Inoculation of corn silage with *L. buchneri* combined with *L. plantarum* enhanced the daily gain of lambs.

Keywords: digestibility, heterofermentative, inoculants, ruminal fermentation, sheep

1. Introduction

Aerobic spoilage by yeasts and molds is a major cause of reduced nutritional value and wastage of silage. This problem can be severe in corn silage that is not preserved with additives or fed out at an appropriate rate, especially in warm weather (Bernardes and Adesogan, 2012; Queiroz et al., 2012). Yeasts utilize soluble carbohydrates and lactic acid produced by lactic acid bacteria (LAB) during silage fermentation as a source of energy. The depletion of lactic acid increases the pH, which makes the silage conducive for the growth of molds and aerobic bacteria that require moderate to high pH values for growth.

An obligatory heterofermentative LAB, *Lactobacillus buchneri* (LB), has been successfully used as an additive to improve the aerobic stability of silages (Queiroz et al., 2012; Basso et al., 2012). This bacteria converts glucose and fructose to lactic acid, acetic acid and other end products (McDonald et al., 1991) and also converts lactic acid to acetic acid and 1,2 propanediol, which may be converted into propionic acid if *L. diolivorans* is present (Oude Elferink et al., 2001). The presence of acetic and or propionic acid protects the silage against spoilage by aerobic microorganisms (Moon, 1983). However, heterolactic fermentations are less desirable than homolactic fermentations because of the greater loss of dry matter (DM) in the former (McDonald et al., 1991).

Lactobacillus plantarum (LP), a facultative heterofermentative lactic acid bacteria (Pahlow et al., 2003) has been combined with *L. buchneri* in silage inoculants to reduce DM losses and protein degradation during the fermentation by faster and greater lactic acid production, while retaining the antifungal, aerobic stability-enhancing characteristic of *L. buchneri* (Filya, 2003; Nkosi et al., 2009).

Moreover, Weinberg et al. (2004a, 2004b, 2007) suggesting that LAB used in inoculant for silage could survive in rumen fluid, to interact with rumen microorganisms, to change ruminal fermentation and enhance rumen functionality, as well as provide a probiotic effect.

However, only a few studies have examined effects of feeding silage treated with LB alone or LBLP on the ruminal fermentation and performance of lambs. Therefore, this study was aimed at evaluating the *in vivo* apparent digestibility, ruminal fermentation parameters, microbial protein synthesis, N retention and

performance of lambs fed corn silage inoculated with *L. buchneri* alone and *L. buchneri* combined with *L. plantarum.*

2. Material and Methods

The trial was conducted at Faculdade de Ciências Agrárias e Veterinárias, Univ Estadual Paulista - UNESP (Jaboticabal, São Paulo, Brazil), located at 21º14'14.04" S and 48°17'27.92" W. According to the Köppen classification (Setzer, 1966), the climate is of the “Aw” type characterized as tropical wet and dry, rainy in summer and dry in winter season. Humane animal care and handling procedures were followed according to the Sao Paulo State University’s Animal Care Committee (Project no. 022704/09).

2.1. Silage production

A corn hybrid (cv. Maximus, Syngenta, Matão, Sao Paulo, Brazil) sown on December 21st 2009 was harvested on March, 22 2010 using a Premium Flex forage harvester (Menta Mit, Cajuru, Sao Paulo, Brazil) and chopped to achieve a theoretical length of cut of 10 mm. The corn plants were treated with water (2 L t^{-1}; Control) or with either 1 × 10^5 cfu g^{-1} of fresh forage of *Lactobacillus buchneri* NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI, USA; LB) or LB combined with 1 × 10^5 cfu g^{-1} of fresh forage of *L. plantarum* MA18/5U (LBLP). The inoculants were dissolved in water (2 L t^{-1}) and then sprayed on piles of fresh forage under constant mixing. The application rate of the inoculant was determined in accordance to Basso et al. (2012) in tropical conditions. Ten tons of forage from each treatment was conserved in stack silos. Each silo was sealed with plastic black-on-white sheeting (200 µm thick), which were weighted down with sand bags. Samples were taken to characterize the chemical composition of the corn plant at silo filling (Table 1).

The chemical composition of the corn plants was typical of those harvested in previous trials (Basso et al., 2012). Inoculation did not seem to affect the chemical composition of the forages but DM concentrations differed slightly among treatments. After 165 d of ensiling at ambient temperature (21°C), silos were opened and fed out at a rate of approximately 10.5 cm d^{-1}. Samples of all silages were collected weekly and stored at -20°C for later analysis or stored after air drying for 72-h.

Table 1 Chemical composition of untreated and inoculated corn plants at silo filling.

Item[1]	Control	LB	LBLP	SEM[2]
DM (g kg^{-1})	320	355	314	0.69
Ash (g kg^{-1}DM)	48	51	53	2.30
CP (g kg^{-1}DM)	87	80	89	2.03
EE (g kg^{-1}DM)	27	26	27	1.88
NDF (g kg^{-1}DM)	580	569	587	12.61
ADF (g kg^{-1}DM)	272	261	284	8.78
Hemicellulose (g kg^{-1}DM)	309	308	303	11.03
Cellulose (g kg^{-1}DM)	219	219	205	12.10
Lignin (g kg^{-1}DM)	54	42	59	2.36
pH	5.1	5.2	5.3	0.05
Ammonia N (%TN)	2.3	2.4	2.3	0.12

Treatments: Control - without inoculant; LB - 1 × 10^5 cfu g^{-1} of *Lactobacillus buchneri* NCIMB 40788; LBLP - LB combined with 1 × 10^{5}cfu g^{-1} of *Lactobacillus plantarum* MA18/5U. [1]DM: Dry matter; CP: crude protein; EE: extract ether; NDF: neutral detergent fiber; ADF: acid detergent fiber; TN: Total nitrogen.[2]SEM: Standard error of the mean.

2.2. Animal performance study

The study was conducted from September to November 2010. Average ambient temperature during the trial ranged from 23.5 to 24°C. Thirty Dorper × Santa Ines crossbred intact male, weaned, lambs with average initial body weight (BW) of 20.4 $\pm$ 3.8 kg were blocked by weight into ten groups. Lambs in each group were randomly assigned to one of the following three dietary treatments: Control, LB and LBLP silage.

All diets were formulated (Table 2) to meet nutrient requirements of lambs gaining 200 g d^{-1} (NRC, 2007). Diets consisted of 80% of the respective corn silage, 11.5% of soybean meal, 1.0% of cottonseed meal (380 g/kg of CP), 1.4% of wheat meal, 3.2% of corn meal, 2.5% of citrus pulp and 0.4% of mineral supplement (80 g kg^{-1} of P, 130 g kg^{-1} of Ca, 1500 mg kg^{-1} of Na, 1000 mg kg^{-1} of Mg, 60 mg kg^{-1} of Zn, 5 mg kg^{-1} of Cu, 40 mg kg^{-1} of Mn, 42 mg kg^{-1} of Fe, 1.50 mg kg^{-1} of I) on a DM basis.

Lambs were adapted to diets for 14 d and fed for 47 d. Diets were fed *ad libitum* (10% of refusals) twice daily (0700 and 1700 h). Refusals were weighed daily before the morning feeding and DM intake was calculated. Samples of offered feed (silage and concentrate) and refusals were collected twice a week, and stored at -20°C for later analysis. Lambs were housed in individual wooden pens (0.5 m^2) with slatted floored, each fitted with a feed and water container in a well-ventilated covered barn.

Initial and final BW was measured after a 16-h fast solid and average daily gain (ADG) was calculated by subtracting the initial BW from final BW and dividing the difference by the trial duration of 61 days. The feed efficiency ratio (FER) was calculated by dividing daily ADG by DM intake.

Table 2 Chemical composition of the experimental diets.

	Control	LB	LBLP
DM (g kg^{-1})	440	454	426
OM (g kg^{-1}DM)	949	943	943
Ash (g kg^{-1}DM)	52	57	59
CP (g kg^{-1}DM)	130	135	130
EE (g kg^{-1}DM)	26	27	27
NDF (g kg^{-1}DM)	436	436	441
ADF (g kg^{-1}DM)	260	253	264
CHO (g kg^{-1}DM)	792	780	785
NFC (g kg^{-1}DM)	382	375	374
GE (MJ kg^{-1})	16.5	16.3	16.5
DE (MJ kg^{-1})	11.8	11.0	11.0
ME (MJ kg^{-1})	9.7	9.0	9.0

Treatments: Control - without inoculant; LB - 1 × 10^5 cfu g^{-1} of *Lactobacillus buchneri* NCIMB 40788; LBLP - LB combined with 1 × 10^5cfu g^{-1} of *Lactobacillus plantarum* MA18/5U. [1]DM: Dry matter; OM: organic matter; CP: crude protein; EE: extract ether; NDF: neutral detergent fiber; ADF: acid detergent fiber; CHO: total carbohydrates; NFC: non-fibrous carbohydrates; GE: gross energy; DE: digestible energy (obtained in digestibility trial); ME: metabolizable energy (ME= DE x 0.82).

2.3. Digestibility and N utilization study

In vivo apparent digestibility was calculated indirectly using indigestible NDF (iNDF) as a marker to estimate fecal output (Valente et al., 2011). Fecal grab samples were collected from each lamb every 26 h from d 57 to 59 (Pina et al., 2006). Samples of silage, concentrate and refusals were also collected daily. Fecal output and *in vivo* apparent digestibility were calculated using the following respective formulas:

Fecal DM output = *iNDF intake* (g) / *iNDF fecal* (%) × 100 (1)

Apparent digestibility = [*DM intake* (g) – *fecal output* (g)] / [*DM intake* (g)] × 100 (2)

On d 59, spot urine samples were collected with collection bags from all animals 4 h after the morning feeding. A harness was used to fit the collection bags onto the sheep. Subsamples (10 and 5 mL) of urine were collected and stored at -

20°C for later analysis of purine derivatives (PD) and total N, respectively, to estimate microbial protein synthesis and N utilization. The 10-mL urine sample was and diluted with 40 mL of a 0.036 N solution of H_2SO_4 prior to storage.

Blood samples were collected 4 h after the morning feeding from every sheep via jugular venipuncture into EDTA tubes (5 mL; BD, Sao Paulo, Brazil) on d 59. These samples were centrifuged at 10,000 × *g* for 5 min at 4ºC and plasma was frozen (-20ºC) for subsequent determination of urea-N.

2.4. Carcass measurements

After the 61-d trial, the animals were anesthetized by electronarcosis (220 V/ 0.5 A) and jugular veins and carotid arteries were severed. After evisceration, carcasses were weighed to measure the hot carcass weight (HCW) to calculate the hot carcass yield (*HCY = HCW*/*BW at slaughter (BWS)* × 100). Carcasses were hung by the gastrocnemius tendons at -4°C for 24 h. Subsequently, cold carcass weight (CCW) was measured and cold carcass yield calculated (*CCY = CCW/BWS* × 100). Losses due to cooling (LC) were also calculated (*LC = HCW – CCW/HCW* × 100). The subcutaneous fat thickness (FT) on the left side carcasses was measured with a caliper rule over the loin-eye muscle between the 12th and 13th ribs. Average daily gain (ADGc) and feed efficiency ratio (FERc) also were expressed relative to hot carcass yield, since initial HCY was estimated at 47.0% as found by Cartaxo et al. (2009) in Santa Ines x Dorper lambs in this weight range.

2.5. Ruminal parameters study

Six Dorper × Santa Ines crossbred ruminally-cannulated wethers, each fitted with a silicone, 2.5-inch ruminal cannula were used. The initial BW of the wethers was 40.5 $\pm$ 1.8 kg and they were housed individually in (0.9 × 2.0 m) pens, each fitted with individual feed and water containers. These sheep received a similar diet to the others and the diets were fed *ad libitum* once-a-day (0700 h).

Ruminal measurements were taken over 3 experimental 10-d periods, each consisting of 9 d for diet adaptation and 1 d for rumen fluid collection. Ruminal fluid was collected before feeding, 3, 6, 9 and 12 h after feeding and pH and concentrations ammonia nitrogen and volatile fatty acids (VFA) were analyzed.

2.6. Laboratory analysis

A water extract was made from undried silage samples according to Kung Jr. Jr. et al. (1984) and an electrode (MA522 model, Marconi Laboratory Equipments, Piracicaba, SP, Brazil) was used to measure the pH. Volatile fatty acids were measured in a gas chromatograph (GC 2014, Shimadzu Corporation, Kyoto, Japan) using a HP-INNOwax capillary column (30 m × 0.32 mm; Agilent Technologies, Colorado, USA) at an initial temperature of 80ºC and a final temperature of 240ºC. Lactic acid was determined by a colorimetric method (Barker and Summerson, 1941). Ammonia N was measured by distillation (AOAC, 1996, method no. 941.04).

Samples of silage, concentrate, refusals and feces were oven dried (55°C for 72 h) and ground in a knife mill (Wiley, A. H. Thomas, Philadelphia, PA, USA) to pass through a 1-mm screen, and analyzed for DM (105°C for 12 h) and ash (500°C for 5 h). Organic matter (OM) was calculated (*OM = 100 – Ash*) on a DM basis. Neutral (NDF) and acid detergent fiber (ADF) concentrations were determined using the method of Van Soest et al. (1991) in an ANKOM 2000 Fiber Analyzer (ANKOM Technologies, Macedon, NY, USA) without sodium sulfite. Heat-stable α-amylase was used in the NDF assay. Separate samples were used for NDF and ADF analysis and both included residual N.

Indigestible NDF (iNDF) was measured by weighing feed samples into non-woven textile (100 g m^{-2}) bags (25 cm^2) (Valente et al., 2011) at the ratio of 20 mg DM cm^{-2} of surface area (Nocek, 1988) and incubating the bags in the rumens of two bulls for 264-h (Casali et al., 2008). The bulls were fed diets consisting of 60% of corn silage and 40% of concentrate on a DM basis. The iNDF concentration was determined using an autoclave at the temperature of 110°C for 40 min (Senger et al., 2008). Potentially digestible NDF (pdNDF) was estimated as NDF – iNDF, according to Huhtanen et al., 2010.

Lignin concentration was measured after hydrolysis of the cellulose in ADF residues in a 72% H_2SO_4 (Van Soest and Robertson, 1985). Extract ether (EE) was measured according to AOAC (1996; method no. 920.39). The N concentration of N NDF and ADF residues as well as feed, feces and urine samples was determined by rapid combustion using a Leco F528 N analyzer (LECO Corporation, St. Joseph, MI,

USA). Crude protein (CP) concentration was calculated as N × 6.25. Gross energy (GE) was determined with a bomb calorimeter (PARR 6200, Parr Instrument Company, Illinois, USA). Digestible energy (DE) was calculated by subtracting the GE excreted in feces from the GE consumed. Metabolizable energy (ME) was estimated as DE × 0.82 (NRC, 1985).

Total carbohydrate (CHO) (Eq. (1)) and non-fibrous carbohydrates (NFC) (Eq. (2)) concentrations were estimated according to Sniffen et al. (1992) and Detmann and Valadares Filho (2010) respectively, using the following equations:

CHO (g kg^{-1} of DM) = 100 – (CP + EE + Ash) (3)

NFC (g kg^{-1} of DM) = 100 – ($NDFap$ +CP + EE + Ash] (4)

where NDFap = NDF corrected for ash and protein.

Purine derivatives (PD) were measured as the sum of allantoin, uric acid, xanthine and hypoxanthine according to Chen and Gomes (1995a). Daily PD excretion (Eq. (5)) was calculated as follows according to Chen and Gomes (1995b):

PDC index = *daily PD* (mmol d^{-1}) / *daily Creatinine* (mmol d^{-1}) × BW$^{0.75}$ (5)

where *PDC index*= *PD* (in spot urine sample; mmol)/ [*Creatinine* (in spot urine sample; mmol) × $BW^{0.75}$].

To obtain the concentration of daily creatinine in the urine, prior to the experiment, total urine was collected from six crossbred Dorper × Santa Ines lambs (40.9 $\pm$ 1.4 kg) for 6 d after they received a diet consisting of corn silage (60%) and concentrate (40%) (DM basis) for 21 d. Creatinine concentration in the urine was determined to be 0.370 $\pm$ 0.150 using a commercial kit (Labtest, Lagoa Santa, MG, Brazil).

Microbial N supply (Eq. (6)) and intestinal flow of microbial N (Eq. (7)) were according to Chen and Gomes (1995a):

Daily PD (mmol d^{-1}) = 0.84 × AP + (0.150 × $BW^{0.75}exp^{-0.25AP}$) (6)

where AP = absorption of microbial purines (mmol/d); 0.84 represents the recovery of absorbed purines as PD in urine; the component within parenthesis represent the endogenous contribution which decreases as exogenous purines become available for utilization by the animal.

Microbial N (g N d^{-1}) = (*AP* × 70)/ (0.116 × 0.83 × 1000) = 0.727 x *AP* (7)

where the N concentration of purines is 70 mg N/mmol; the ratio of purine-N: total N in mixed rumen microbes is taken as 11.6:100; the digestibility of microbial purines is assumed to be 0.83.

The efficiency of microbial N synthesis was expressed as grams of microbial N per kilogram of digestible OM fermented in the rumen (DOMR: calculated as Digestible organic matter intake (DOMI) × 0.65, according to ARC (1984)).

Blood urea was measured using a commercial kit (Labtest, Lagoa Santa, MG, Brazil). The conversion of urea into blood urea-N (BUN) was done multiplying by a factor 0.4667.

Absorption (g d^{-1}) (Eq. (8)) and retention of N (g d^{-1}) (Eq. (9)) were calculated as follows:

N absorbed = N intake – N fecal (8)

N retention = N intake – (*N fecal* + *N urinary*) (9)

The pH of rumen fluid was measured using a pH meter (MA522 model, Marconi Laboratory Equipments, Piracicaba, SP, Brazil). Subsequently, 1mL of H_2SO_4 (1:1) was added to the ruminal fluid and it was stored at -20 °C for analysis of concentrations of rumen ammonia N by distillation with 2N KOH according to Fenner (1965) and for analysis of VFA by gas chromatography as described earlier.

2.7. Statistical analysis

The corn silage chemical composition data were as analyzed as a completely randomized design with 8 replicates. Data on the effects of treatments on digestibility, microbial protein synthesis and performance were analyzed as a randomized block design (with 10 replicates). Ruminal parameter data were analyzed as a replicated 3 × 3 Latin square design. All data were analyzed using the MIXED procedure of SAS (v. 9.0 SAS Institute Inc., Cary, NC). The effect of treatments was considered fixed and the animals considered random. The initial BW was used as a covariate for analyzing performance and carcass data. The model for analyzing ruminal fermentation data included a repeated measures statement; the treatments were the fixed effect and the animals and periods were considered random effects.

Banded toeplitz (TOEP) and autoregressive (AR (1)) were the best covariance structures for the data as these had the lowest Akaike information criterion scores. Differences between means were determined using the PDIFF, which differentiates means based on Fisher´s *F*- protected least significant difference test. Significance was declared at $P < 0.05$.

3. Results

3.1. Chemical composition and fermentation characteristics of corn silage

As for the fresh forages (Table 1), the DM concentration of the LB silage was greater than those of the other silages (Table 3).

Table 3 Chemical composition and fermentation characteristics of corn silage (sampled weekly over 8 weeks).

	Control	LB	LBLP	SEM[2]	*P* value
Chemical composition[1]					
DM (g kg^{-1})	317^{b}	334^{a}	299^{c}	6.27	0.002
OM (g kg^{-1}DM)	965^{a}	958^{b}	956^{b}	1.34	0.001
Ash (g kg^{-1}DM)	34^{b}	42^{a}	44^{a}	1.34	0.001
CP (g kg^{-1}DM)	82	88	82	2.27	0.083
EE (g kg^{-1}DM)	27	28	28	1.03	0.758
NDF (g kg^{-1}DM)	507	492	498	8.41	0.467
NDFap (g kg^{-1}DM)	466	455	461	6.27	0.458
iNDF (g kg^{-1}DM)	143^{b}	143^{b}	157^{a}	3.03	0.020
pdNDF (g kg^{-1}DM)	364	349	341	8.41	0.177
ADF (g kg^{-1}DM)	292^{b}	280^{b}	300^{a}	5.32	0.040
Cellulose (g kg^{-1}DM)	242	230	250	5.82	0.070
Hemicellulose (g kg^{-1}DM)	215	212	198	6.60	0.191
Lignin (g kg^{-1}DM)	43	41	44	1.96	0.472
NDIN (g kg^{-1}TN)	250	224	221	22.70	0.621
ADIN (g kg^{-1}TN)	96	81	94	9.01	0.451
CHO (g kg^{-1}DM)	856^{a}	841^{b}	846^{b}	3.16	0.008
NFC (g kg^{-1}DM)	394	386	385	5.92	0.555
Fermentation characteristics					
Lactic acid (g kg^{-1}DM)	67^{b}	62^{b}	93^{a}	7.50	0.018
Acetic acid (g kg^{-1}DM)	27^{b}	42^{a}	55^{a}	5.92	0.011
Lactate:acetate ratio	2.6	1.5	2.9	0.40	0.169
pH	4.1	4.0	4.1	0.02	0.197
Ammonia N (%TN)	2.6	2.4	2.9	0.17	0.076

Treatments: Control - without inoculant; LB - 1×10^5 cfu g^{-1} of *Lactobacillus buchneri* NCIMB 40788; LBLP - LB combined with 1×10^5cfu g^{-1} of *Lactobacillus plantarum* MA18/5U. [1]DM: Dry matter; OM: organic matter; CP: crude protein; EE: extract ether; NDF: neutral detergent fiber; NDFap: neutral detergent fiber corrected for ash and protein; iNDF: indigestible neutral detergent fiber; pdNDF: potentially digestible neutral detergent fiber; ADF: acid detergent fiber; NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen; CHO: total carbohydrates; NFC: non-fibrous carbohydrates; TN: total nitrogen. [2]SEM: Standard error of the mean.

Total CHO concentration was lower in inoculated silages. Inoculated silages had lower OM concentrations than the Control silage. For unknown reasons, the ADF and iNDF concentrations were slightly greater in the LBLP silage than the others but cellulose and lignin concentrations were unaffected by treatment (Table 3).

Inoculation with LBLP increased the lactic and acetic acid concentrations for the silage, whereas inoculation with LB only increased the acetic acid concentration. Inoculation had no effect on other silage fermentation characteristics (Table 3).

3.2. Intake and digestibility

Inoculation of corn silage with both inoculants increased the intake of DM (g d^{-1}, % of BW, % of $BW^{0.75}$), OM, CP, NDF, CHO and gross energy of the lambs (Table 4). These measures of intake did not differ among inoculant treatments except that lambs fed LBLP silage had greater CP intake than those fed LB. However, *in vivo* apparent digestibility of DM, OM, CP, and CHO, NSC, and concentrations of GE, DE and ME were less in inoculated versus Control silages. Although, lambs fed LBLP silage also had lower NDF digestibility than those fed the Control silage, lambs fed LB silage had similar NDF digestibility to those fed the Control silage and 4.6% higher values than those fed LBLP silage. Hence, lambs that consumed LB silage had higher digestible NDF intake than those in other treatments, which had similar values. Moreover, intake of digestible OM and CHO were greater in lambs fed inoculated *versus* Control silage, yet DE intake was lower in lambs fed inoculated *versus* Control silages (Table 4).

3.3. Ruminal fermentation

Inoculation of corn silage did not affect most ruminal fermentation indices (Table 5; Figure 1). However, acetate to propionate ratio was lower in the rumen fluid of wethers in the LBLP treatment *versus* other treatments. Unlike those of other wethers, ruminal fluid pH values of wethers fed LB silage remained above 6 throughout the monitoring period (Figure 1 – E).

Table 4 Effect of microbial inoculation of corn silage on intake and apparent digestibility of nutrients and energy in lambs.

	Control	LB	LBLP	SEM[2]	*P* value
			Intake of nutrients (g d^{-1})		
DM[1]	1001[b]	1056[a]	1058[a]	53.86	0.001
DM (% BW)	3.8[b]	4.0[a]	4.0[a]	0.09	0.001
DM (g $BW^{-0.75}$)	86.9[b]	90.1[a]	90.2[a]	1.90	0.001
OM	958[b]	1015[a]	1023[a]	46.75	0.022
CP	134[c]	141[b]	148[a]	7.46	0.001
EE	30	31	31	1.57	0.206
NDF	411[b]	442[a]	446[a]	21.83	0.002
CHO	783[b]	822[a]	840[a]	41.47	0.026
NSC	405	421	423	21.66	0.212
GE (MJ d^{-1})	16.5[b]	17.2[a]	17.3[a]	0.88	0.001
			Aparent nutrient digestibility (%)		
DM	71.9[a]	68.3[b]	67.2[b]	0.56	0.001
OM	72.5[a]	70.0[b]	68.1[c]	0.64	0.001
CP	72.2[a]	66.5[b]	66.8[b]	1.25	0.006
EE	88.9	87.5	87.0	0.67	0.052
NDF	54.2[a]	54.9[a]	50.3[b]	0.64	0.001
CHO	72.1[a]	69.8[b]	67.5[c]	0.53	0.001
NSC	87.7[a]	86.2[b]	85.2[b]	0.36	0.001
GE	71.2[a]	67.9[b]	66.5[b]	0.57	0.001
DE (MJ kg^{-1})	11.7[a]	11.0[b]	11.0[b]	0.09	0.001
ME (MJ kg^{-1})	9.6[a]	9.1[b]	8.9[b]	0.09	0.001
			Intake of digestible nutrients (g d^{-1})		
DM	720	721	711	35.97	0.131
OM	674[b]	703[a]	696[a]	31.95	0.022
CP	99	94	97	5.11	0.107
EE	27	27	27	1.41	0.668
NDF	222[b]	242[a]	225[b]	5.32	0.002
CHO	548[b]	573[a]	567[a]	25.05	0.032
NSC	367	357	360	19.50	0.152
DE (MJ d^{-1})	11.9[a]	11.7[b]	11.7[b]	0.61	0.001
ME (MJ d^{-1})	9.7	9.6	9.6	0.50	0.115

[*]Means following of the same letter did not differ (P>0.05). Treatments: Control - without inoculant; LB - 1 × 10^5 cfu g^{-1} of *Lactobacillus buchneri* NCIMB 40788; LBLP - LB combined with 1 × 10^5cfu g^{-1} of *Lactobacillus plantarum* MA18/5U. [1]DM: dry matter; OM: organic matter; CP: crude protein; EE: extract ether; NDF: neutral detergent fiber; CHO: total carbohydrates; NFC: non-fibrous carbohydrates; GE: Gross energy; DE: Digestible energy; ME: Metabolizable energy (ME= DE x 0.82); BW: body weight; $BW^{0.75}$: metabolic body weight. [2]SEM: Standard error of the mean.

Table 5 Effect of microbial inoculation of corn silage on ruminal fermentation parameters of wethers.

	Control	LB	LBLP	SEM[2]	*P* value		
					Silage	Time	Silage x Time
Acetate (mmol L^{-1})	42.82	42.75	43.70	4.567	0.882	0.009	0.10
Propionate (mmol L^{-1})	7.88	8.00	9.00	1.097	0.189	0.007	0.18
Butyrate (mmol L^{-1})	3.52	2.29	2.98	0.585	0.080	0.203	0.51
Total VFA (mmol L^{-1})[1]	54.24	53.02	55.69	5.862	0.662	0.012	0.16
Acetate:propionate ratio	5.92[a]	6.33[a]	5.20[b]	0.430	0.033	0.030	0.08
pH	6.04	6.13	6.07	0.098	0.135	0.001	0.12
Ammonia N (mg dL^{-1})	13.86	13.69	13.98	1.435	0.963	0.001	0.43

[*]Means following of the same letter did not differ (P>0.05). Treatments: Control - without inoculant; LB - 1 × 10^5 cfu g^{-1} of *Lactobacillus buchneri* NCIMB 40788; LBLP - LB combined with 1 × 10^5cfu g^{-1} of *Lactobacillus plantarum* MA18/5U. [1]Total of volatile fatty acids. [2]SEM: Standard error of the mean.

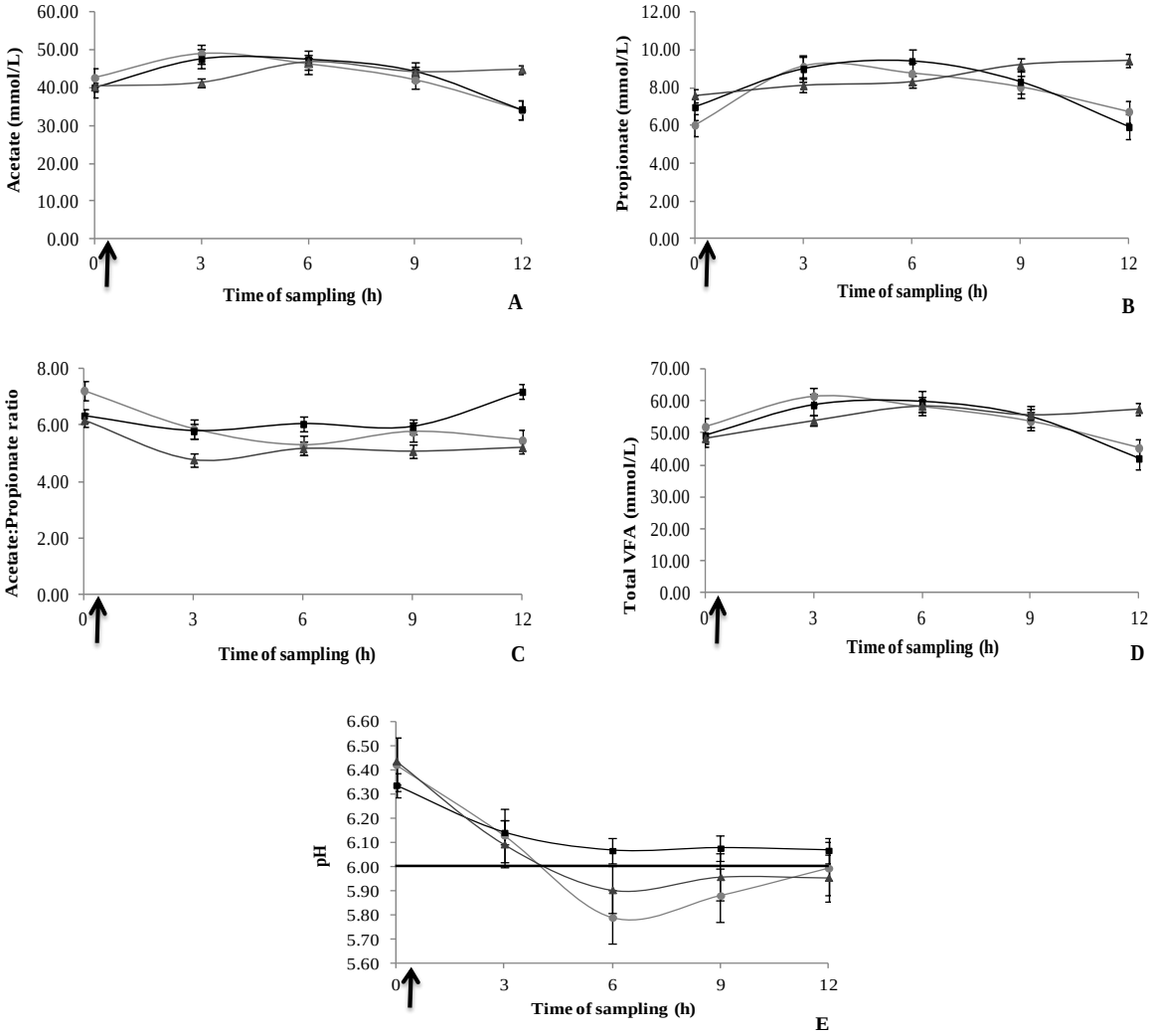

Figure 1 Effect of microbial inoculation of corn silage on changes in ruminal fermentation parameters of wethers fed the silage. Key: ● Control silage; ■ LB silage; ▲ LBLP silage. Feeding (→).

3.4. Nitrogen utilization and microbial protein synthesis

Lambs fed LB silage had higher concentrations of urinary allantoin, PD, DOMR, and microbial N supply than those fed Control silage (Table 6). Lambs fed LBLP silage also had greater DOMR than those fed Control silage but their PD and microbial N supply values did not differ from those of the other treatments.

Nitrogen absorption was less in lambs fed inoculated versus Control silages but N retention (% of N absorbed) was greater in the lambs fed inoculated versus Control silages. Nitrogen retention (% of N intake) did not differ between lambs fed Control and LB silage, but it was greater in lambs fed LBLP silage than those fed others largely due to greater N intake by lambs fed LBLP silage.

Table 6 Effect of microbial inoculation of corn silage on ruminal microbial N synthesis and N utilization by lambs.

	Control	LB	LBLP	SEM[5]	*P* value
	Microbial N synthesis				
Allantoin (mmol d^{-1})	8.5[b]	8.9[a]	8.6[b]	0.41	0.001
Uric acid (mmol d^{-1})[1]	1.9	1.9	1.9	0.10	0.855
PD (mmol d^{-1})[2]	10.4[b]	10.7[a]	10.5[ab]	0.46	0.018
Microbial N supply (g d^{-1})	8.9[b]	9.2[a]	9.0[ab]	0.41	0.030
DOMR (g d^{-1}) [3]	437[b]	457[a]	452[a]	20.77	0.022
Microbial N kg^{-1} of DOMR	20.6	20.4	20.1	0.97	0.525
	N utilization				
N Intake (g d^{-1})	21.6[c]	22.7[b]	25.8[a]	1.19	0.002
Faecal N (g d^{-1})	6.6[b]	8.2[a]	8.1[a]	0.46	0.001
Urinary N (g d^{-1})	12.8[a]	11.8[ab]	10.9[b]	0.47	0.006
N absorbed (g d^{-1})	15.0	14.4	15.6	0.92	0.131
N retention (g d^{-1})	2.7[c]	3.2[b]	4.7[a]	0.66	0.019
N absorbed (% of N intake)	69.3[a]	63.2[b]	65.6[b]	1.38	0.015
N retention (% of N intake)	11.6[b]	13.8[b]	19.6[a]	2.44	0.027
N retention (% of N absorbed)	16.3[b]	21.7[a]	29.3[a]	3.31	0.008
BUN (mg dL^{-1})[4]	15.2	14.2	14.2	0.71	0.426

[*]Means following the same letter did not differ (P>0.05). Treatments: Control - without inoculant; LB - 1 × 10^5 cfu g^{-1} of *Lactobacillus buchneri* NCIMB 40788; LBLP - LB combined with 1 × 10^5cfu g^{-1} of *Lactobacillus plantarum* MA18/5U. [1]Uric acid: xanthine and hypoxanthine were converted into uric acid (xantine oxidase enzyme); [2]PD: purine derivatives - sum of allantoin and uric acid; [3]DOMR: digestible organic matter fermented in the rumen. [4]BUN: Blood urea nitrogen. [5]SEM: Standard error of the mean.

3.5. Growth performance and carcass yield

Average daily gain was increased by 4% in lambs fed LBLP silage instead of the Control silage (Table 7).

Table 7 Effect of microbial inoculation of corn silage on growth performance and carcass yield of lambs.

	Control	LB	LBLP	SEM[2]	*P* value
IBW (kg)[1]	20.2	20.6	20.4	1.25	0.350
ADG (g d^{-1})	198[b]	199[b]	206[a]	6.21	0.030
FER (gain/feed)	0.199[b]	0.190[a]	0.195[ab]	0.01	0.017
SBW (kg)	32.0	32.5	32.7	0.39	0.373
HCW (kg)	13.9	14.2	14.6	0.23	0.139
CCW (kg)	13.5	13.7	14.2	0.23	0.126
HCY (%)	43.5	43.8	44.7	0.75	0.412
CCY (%)	42.0	42.4	43.5	0.86	0.434
LC (%)	2.9	3.5	2.7	0.07	0.198
ADGc (g d^{-1})	72[b]	75[b]	83[a]	2.65	0.017
FERc (gain/feed)	0.071	0.073	0.078	0.01	0.145
FT (mm)	1.9	1.8	1.8	0.15	0.905

[*]Means following the same letter did not differ (P>0.05). Treatments: Control - without inoculant; LB - 1 × 10^5 cfu g^{-1} of *Lactobacillus buchneri* NCIMB 40788; LBLP - LB combined with 1 × 10^5cfu g^{-1} of *Lactobacillus plantarum* MA18/5U. [1]IBW: initial body weight; ADG: average daily gain; FER: feed efficiency ratio; SBW: shrunk body weight; HCW: hot carcass weight; CCW: cold carcass weight; HCY: hot carcass yield; CCY: cold carcass yield; LC: losses due to cooling; ADGc: average daily gain expressed in relation to HCY; FT: fat thickness. [2]SEM: Standard error of the mean.

This increase was also evident when ADG was expressed in relation to hot carcass yield. Lambs fed Control silage had higher feed efficiency ratio (ADG/DMI; FCR) than those fed LB silage and similar values to lambs fed LBLP silage. However, when FCR was expressed relative to hot carcass yield, no differences among treatments were evident. Other carcass yield measures were unaffected by treatment (Table 7).

4. Discussion

4.1. Chemical composition and fermentation characteristics of corn silage

The DM concentrations of silages differed among treatments reflecting similar differences in the DM concentrations of the forages at ensiling. However, the reduction in DM concentration between that in the plant and the silage (Tables 1 and 3) seemed greater in inoculated silages (15 to 20 g kg^{-1} of DM), presumably reflecting the increased water production due to greater fermentative activity (McDonald et al., 1991).

Inoculation with LB or LBLP resulted in the lowest OM and CHO concentrations. These responses likely reflect greater fermentation of carbohydrates in inoculated silages. Lactic acid bacteria need carbohydrates as energy and carbon sources (Pahlow et al., 2003) and these microorganisms metabolize nonstructural carbohydrates into organic acids (Rooke and Hatfield, 2003).

As expected, when *L. buchneri* was combined with facultative heterofermentative *L. plantarum*, lactic acid concentration increased. The latter increased the supply of substrates for conversion into acetic acid by *L. buchneri*, hence the LBLP silage had the highest acetic acid concentration. In agreement, Weinberg et al. (2002), Filya (2003) and Hu et al. (2009) also found increases in the lactate concentration of corn silage inoculated with *L. buchneri* and *L. plantarum* and increases in the acetic acid concentration of corn silage inoculated with *L. buchneri* alone or combined with *L. plantarum*.

All pH values were low as they fell within the range of 3.7 to 4.2. Low pH values inhibit protein degradation in silage (McDonald et al. 1991) and ammonia N concentrations were below the upper limit of the range (5 to 7% TN) beyond which unsuitable preservation of silage occurs (Kung Jr. and Shaver, 2001).

Although pH values during feedout were similar between silages and no difference was observed among concentration of NDF, the LBLP silage had a reduction in the NDF concentration of 89 g kg^{-1} of DM from corn plant compared to decrease of 77 and 73 g kg^{-1} of DM of LB and Control silage respectively. This may be related to partial acid hydrolysis of hemicellulose (McDonald et al., 1991), which might have resulted in higher iNDF concentration in this silage since hemicellulose is the potentially digestible fraction of forage NDF.

4.2. Lambs study

Despite the fact that inoculated silages had higher levels of acetic acid, intake of such silages was greater than that of Control silages (Table 4). According to Muck (2010) levels of acetic acid above 50 g kg^{-1} of DM might negatively affect silage intake. However, Charmley (2001) reported that silage intake is not affect by organic acids itself, but ammonia N and protein solubility of silage also contribute to low intake responses.

Our findings in this study are in agreement with those of Nkosi et al. (2009, 2011) who found higher DM intake in lambs fed corn silage inoculated with *L. buchneri* than animals fed Control silages. However, these authors attributed the greater DM intake to higher levels of water-soluble carbohydrates in the inoculated corn silage, which did not occur in the present study. The higher nutrient intake of inoculated silages by lambs may be due to survival of LAB in rumen fluid and interact positively with rumen microorganisms, alter ruminal fermentation, increase ruminal microbial biomass yield, enhancing the functionality of the rumen as reported by Weinberg et al. (2004a, 2004b) and Contreras-Govea et al. (2011, 2013) in *in vitro* studies.

In vivo apparent nutrient digestibility was decreased in lambs fed inoculated silages (Table 4). However, as noted by Chen et al. (1992), this is likely a response to the large increase in intake, because there are linear negative relationships between intake and digestibility in lambs. These authors verified that the *in vivo* apparent digestibility of DM and OM decreases as DM intake increases, or as retention time decreases and rate of passage increases (Mertens and Ely, 1982).

Rowghani et al. (2008) observed no effects of inoculation of corn silage on nutrient digestibility in sheep. However, Aksu et al. (2004), Nkosi et al. (2010; 2011) reported increased silage digestibility in sheep due to inoculation of the silage. These contrasting results, could be due to the magnitude of the intake response among the different experiments as well as differences among animals, because the rate of passage varies among animals fed similar diets, and younger or smaller animals of a species chew more per kilogram than do older larger animals (Mertens and Ely, 1982).

That NDF digestibility in lambs fed LB silage was higher than that of lambs fed LBLP silage is probably related to the fact that ruminal pH did not drop below 6 in lambs fed LB silage but did in lambs fed LBLP silage. Ruminal pH below 6 is detrimental to the growth cellulolytic bacteria (Russell and Wilson, 1996). In addition, greater concentrations of iNDF in LBLP may have limited the NDF digestibility of this silage.

Although the average ruminal pH values were similar among treatments, the pH of ruminal fluid of lambs in LB silage was greater than those of other treatments from 6 to 9 h after feeding. These findings agree with results obtained *in vitro* by Weinberg et al. (2004a, 2004b and 2007) that showed higher pH values in ruminal fluid of lambs fed diets inoculated with LAB. According to Weinberg et al. (2007), LAB might survive in the rumen fluid and are more tolerant to acidity than lactate-producing microorganisms, thus, they compete more efficiently for readily fermentable substrates resulting in less lactic acid causing a higher ruminal pH, which in turn could be favorable for fibrolytic bacteria in the rumen.

Acetate, butyrate and total VFA concentrations of rumen fluid were not affected by silage inoculation. Keles and Demirci (2011) did not find differences in the ruminal parameters of lambs fed a triticale – Hungarian vetch herbage inoculated with *L. plantarum* or *L. buchneri*.

On the other hand, a numerically greater ruminal propionate concentration and lower acetate to propionate ratio was found in ruminal fluid of lambs fed LBLP silage relative to the others. This can be due to higher lactic acid content of this silage, because in the rumen, silage lactate is metabolized primarily to propionate (Charmley, 2001). Keady and Steen (1994) also found lower acetate to propionate

ratio in the rumen fluid of steers fed grass silage inoculated with *L. plantarum* than in the animals that consumed control silage.

The ruminal ammonia N concentration was similar between treatments. Bayatkousar et al. (2011) also found no differences in the ruminal ammonia N (average of 15.85 mg/dL) of cows fed corn silage inoculated with or without LAB. The ruminal ammonia N of the lambs in all treatments was above the minimum of 5 mg/dL required to maximize microbial protein synthesis according to Satter and Slyter (1974). Van Soest (1994) reported that the optimum level of rumen ammonia is 10 mg/dL but noted that the value is not a constant because the capacity of bacteria for protein synthesis and ammonia uptake depends on the rate of carbohydrate fermentation, and faster rates elicit greater efficiency and relatively higher ammonia tolerance.

Inoculations of the corn silage with LB resulted in greater microbial N supply and inoculation with LBLP resulted in a similar response. Consequently lambs fed silages treated with both inoculants had greater N retention (% of N absorbed) than those fed the Control silage. Rooke et al. (1988) also found improvements in N retention due to improved microbial N synthesis within the rumen of sheep fed grass silage inoculated with LAB.

The efficiency microbial N synthesis (EMNS) was similar among treatments, because the intake of digestible OM was greater in the lambs in fed LB and LBLP silage than Control silage (Table 6). The values of EMNS observed in the present study are within the range of values (14 to 49 g kg^{-1} of DOMR) reported by ARC (1984). Verbič et al. (2005) reported an EMNS of 24.5 g kg^{-1} of DOMR in sheep fed corn silage alone.

Contreras-Govea et al. (2011; 2013) found higher microbial biomass yield (MBY) in silage inoculated with LAB in *in vitro* studies. Contreras-Govea et al. (2013) suggested that the improvements in MBY are due to enhanced protein preservation during ensiling. Nkosi et al. (2010; 2011) found greater N retention in lambs fed silage inoculated with LAB and attributed this to better digestibility of CP, which resulted in greater N absorption and N use efficiency in lambs.

We agree with the postulations by Contreras-Govea et al. (2011), the fact that LAB could survive in the ruminal fluid, suggest that these bacteria may directly affect

ruminal fermentation in a manner that improves ruminal MBY (Weinberg et al., 2003; 2004a; 2004b). Although Chen et al. (1992) reported that increasing DM intake may increase microbial N supply, in our findings a likely interaction between rumen microorganism and LAB consumed from silage have benefited microbial biomass yield resulting in a possible probiotic effect, i.e, improvement of nutrients intake by lambs as well as an increase in ruminal pH favoring cellulolytic bacteria as reported by Gollop et al. (2005).

Although microbial inoculation of corn silage with a heterofermentative LB alone or with LB combined with facultative heterofermentative LP promoted similar responses in nutrient intake and digestibility and resulted in similar trends in microbial N supply and N retention, ADGc was greater in lambs fed the LBLP silage. This is attributable to the greater efficiency of ruminal energy utilization by lambs due to metabolism of the higher lactate concentrations in the LBLP silage to propionate in the rumen, resulting in lower acetate to propionate ratio. Keady and Steen (1994) reported that animal performance increases due to feeding inoculated silage might be caused by improved efficiency of energy utilization due to higher levels of propionate in the rumen caused by the inoculant.

The average FER in the present study was 0.192, which indicates relatively efficient feed utilization by the lambs fed diets containing 0.80 of forage. Nkosi et al. (2009) reported FER values near 0.200 in lambs fed corn silage inoculated with or without LAB in diets with forage to concentrate ratios of 50:50.

Microbial inoculation did not affect carcass yield measures. Our findings support those of Fugita et al. (2012) who found no differences in the hot carcass yield and fat thickness of crossbred bulls fed corn silage inoculated with facultative heterofermentative LAB or a Control silage. Our yields of hot (45.5%) and cold carcass (43.8%) and fat thickness (1.9 mm) were close to those reported by Cartaxo et al. (2009) in Santa Inês x Dorper crossbred lambs fed a diet with a forage to concentrate ratio of 30:70 (HCY = 46.5%; CCY = 45.6%; FT = 2.0 mm), whereas our cooling losses (3.0%) were higher than those found by these authors (1.9 %).

Silva Sobrinho et al. (2008) claimed that lamb carcasses of specialized meat breeds have carcass yields that range from 40 to 50%. The mean hot carcass yield of 45.5% obtained in the present study is within this range. The mean cooling losses

of 3.1% are within of range (3.0 to 4.0%) that denotes acceptable levels of loss, (Sañudo et al. 1991). The cooling by are directly related to fat thickness because the fat layer has a protective effect on the carcass forming a barrier against water loss (Silva Sobrinho et al., 2008). Thus, although the FT in this present study was regarded low (Silva Sobrinho et al., 2008) it was enough to retain the LC within acceptable levels.

5. Conclusions

Inoculation increased concentration of lactate and acetate but did not affect other chemical components of silage. Both microbial inoculants, heterofermentative alone or combined with a facultative heterofermentative, retain in terms of fermentation characteristics suitable to the preservation of corn silage.

Improvements in the intake, ruminal fermentation, microbial N supply and N retention occurred when lambs were fed corn silage inoculated with *Lactobacillus buchneri* alone or combined with *Lactobacillus plantarum*. Inoculation with both combined microbial additives enhanced the daily gain of lambs.

6. Acknowledgment

The authors wish to express their appreciation to São Paulo Research Foundation – FAPESP for their financial support.

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CHAPTER 4 - UTILIZATION EFFICIENCY OF CORN SILAGE INOCULATED WITH *Lactobacillus buchneri* ASSOCIATED TO THE LOWEST LEVEL OF CONCENTRATE

Abstract

This study is aimed at evaluating the digestibility, ruminal parameters, microbial N synthesis and performance of lambs fed corn silage inoculated with *L. buchneri* (LB) associated to levels of concentrate. Twenty eight crossbred intact male lambs with average initial body weight of 25.7 $\pm$ 4.3 kg were blocked into seven groups. Lambs in each group were randomly assigned to one of the four treatments. The treatments were two silages (Control and LB) associated to two levels of concentrate (40 and 60%). The digestibility study occurred from d 35 to 37, and on d 37 *spot* urine samples were collected from all animals to estimate microbial protein synthesis and N utilization. The lambs were slaughtered with 38.0 ± 0.9 kg of shrunk body weight for carcasses evaluations. Eight ruminally-cannulated crossbred wethers weighting 50.7 $\pm$ 4.6 kg were used were used to examine dietary effects on ruminal fermentation parameters. The inoculation of corn silage with *L. buchneri* reduced concentrations of fiber fraction. Lambs fed Control silage associated to 40% of concentrate had lower nutrients intake. However, this was not observed in the lambs fed inoculated corn silage. Lambs fed LB silage associated to lower level of concentrate had similar intake to those fed LB silage associated to 60% of concentrate. This resulted in higher microbial N synthesis and daily weight gain than animals fed Control silage associated to 40% of concentrate. Wethers fed LB silage associated to 60% of concentrate had higher rumen propionate concentrations and lower pH values in rumen fluid, which decreased microbial N synthesis, but it did not affect daily weight gain. Inoculation with *L. buchneri* reduced the corn silage cell wall content. Diet composed by corn silage inoculated *L. buchneri* associated to 40% of concentrate increased the efficiency silage utilization, improving the DM intake, microbial N synthesis and daily weight gain of lambs.

Keywords: daily gain, digestibility, heterofermentative bacteria, inoculants, rumen fluid, sheep

1. Introduction

The *Lactobacillus buchneri*, a obligatory heterofermentative lactic acid bacteria (LAB), has been suggested as an additive to improve the aerobic stability of silages (Mari et al., 2009; Queiroz et al., 2012; Basso et al., 2012). This bacteria converts glucose and fructose to lactic acid, acetic acid and other end products (McDonald et al., 1991) and also converts lactic acid to acetic acid and 1,2 propanediol, which may be converted into propionic acid if *L. diolivorans* is present (Oude Elferink et al., 2001). The presence of organic acids protects the silage against spoilage by aerobic microorganisms (Moon, 1983) because of their antifungal nature. However, heterolactic fermentations are less desirable than homolactic fermentations because of the greater loss of dry matter (DM) in the former (McDonald et al., 1991). But, improvements in the aerobic stability during the prolonged air exposure and feeding phase may be beneficial; thus, small losses of dry matter caused by heterofermentation become less important (Kung Jr. Jr. and Ranjit, 2001).

Furthermore, *in vitro* studies show that LAB inoculated on silage can survive in rumen fluid (Weinberg et al., 2004a, 2004b; Contreras-Govea et al., 2011, 2013). Weinberg et al. (2004a, 2004b, 2007) suggesting that LAB used in inoculant for silage could survive in rumen fluid, mainly in presence of starch (simulating the concentrate in the diet), to interact with rumen microorganisms, to change ruminal fermentation and enhance rumen functionality, as well as provide a probiotic effect. Moreover, treating silages with LAB could have advantages of imparting bacteriocins to the silages which might inhibit detrimental microorganisms in the silage or even in the rumen and thus, enhancing animal health and performance (Gollop et al., 2005).

However, no study evaluated the ruminal fermentation, the efficiency of microbial N supply and performance of lambs fed corn silage inoculated with *L. buchneri* associated to levels of concentrate. Therefore, this study was aimed at evaluating the digestibility, ruminal fermentation parameters, microbial protein synthesis and performance of lambs fed corn silage inoculated with *Lactobacillus buchneri* associated to two levels of concentrate.

2. Material and Methods

The trial was conducted at Faculdade de Ciências Agrárias e Veterinárias, Univ Estadual Paulista - UNESP (Jaboticabal, São Paulo, Brazil), located at 21º14'14.04" S and 48°17'27.92" W. According to the Köppen classification (Setzer, 1966), the climate is of the "Aw" type characterized as tropical wet and dry, rainy in summer and dry in winter season. Humane animal care and handling procedures were followed according to the Sao Paulo State University's Animal Care Committee (Project no. 022704/09).

2.1. Silage Production

A corn hybrid (cv. 2B688 Hx, Dow AgroSiences, Cravinhos, SP) sown on November 18 2010 was harvested on February, 21 2011 using a Premium Flex forage harvester (Menta Mit, Cajuru, SP) and chopped to achieve a theoretical length of cut of 10 mm. The corn plants were treated with water (0.7 L t^{-1}; Control) or with either 1 × 10^5 cfu g^{-1} of fresh forage of *Lactobacillus buchneri* NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI, USA; LB). The inoculants were dissolved in water (0.7 L t^{-1}) and then sprayed on piles of fresh forage under constant mixing. The application rate of the inoculant was determined in accordance to Basso et al. (2012) in tropical conditions.

Two bunker silos were filled with approximately 60 tons of forage each one (face area of silo was of 5 and 7 m^2 for control and LB, respectively). Each silo was sealed with plastic black-on-white sheeting (200 µm thick). During the filling of the silos, after inoculation, samples (12 of each treatment) were taken to characterize the corn plant. The silos remained closed for 70 d at room temperature (23° C). Samples of all silages were collected weekly and stored at -20°C for later analysis or stored after air drying for 72-h.

Although these silages have been used for feeding cattle and sheep, the amount of approximately 220 kg d^{-1} was not enough to remove all face silage from the silo, the remaining part of silage exposed to the air had to be removed in the next day, i.e a homogenous feedout rate was not possible.

2.2. Animal Performance Study

The study was conducted from May to July 2011. Average ambient temperature during the trial ranged from 17 to 22°C. Twenty eight Dorper × Santa Ines crossbred intact males, weaned, lambs with an average initial body weight (IBW) of 25.7 ± 4.3 kg were blocked by weight into seven groups. Lambs in each group were randomly assigned to one of the four dietary treatments. The treatments were two silages (Control and LB) associated with two levels of concentrate (40 and 60%) on a dry matter basis. All diets were formulated (Table 1) to meet nutrient requirements of lambs gaining 300 g (NRC, 2007).

Table 1 Ingredient (g kg^{-1}) and chemical composition (g kg^{-1} of DM) of the experimental diets.

	Levels of concentrate	
Ingredient composition	40%	60%
Corn Silage	600	400
Corn meal	240	414
Soybean meal	100	96
Wheat bran	40	60
Mineral supplement[1]	20	30

	Silage			
	Control		*Lactobacillus buchneri*	
Chemical Composition	40%	60%	40%	60%
DM[2]	517	629	524	634
Ash	51	55	49	54
OM	946	939	947	940
CP	139	142	141	143
RDP (g kg^{-1} of CP)	665	652	677	664
RUP (g kg^{-1} of CP)	402	457	409	466
EE	39	42	38	46
NDF	309	198	292	186
ADF	246	134	227	125
CHO	767	756	768	756
NFC	489	512	505	522
GE (Mcal kg^{-1})	4.1	4.1	4.1	4.0
DE (Mcal kg^{-1})	3.1	2.7	2.8	2.5
ME (Mcal kg^{-1})	2.5	2.2	2.3	2.0

[1]Mineral supplement: P - 65 g kg^{-1}; Ca - 180 g kg^{-1}; Na - 70 g kg^{-1}; Cl - 100 g kg^{-1}; Mg - 80 g kg^{-1}; S - 38 g kg^{-1}; Zn - 4,000 mg kg^{-1}; Cu - 100 mg kg^{-1}; Mn - 1,500 mg kg^{-1}; Fe - 1,100 mg kg^{-1}; Co - 100 mg kg^{-1}; I - 150 mg kg^{-1}; Se - 25 mg kg^{-1}.
[2]DM: Dry matter; OM: organic matter; CP: crude protein; RDP: rumen degradable protein; RUP: rumen undegraded protein; EE: extract ether; NDF: neutral detergent fiber; ADF: acid detergent fiber; CHO: total carbohydrates; NFC: nonstructural carbohydrates; GE: Gross Energy; DE: digestible energy; ME: metabolizable energy (ME = DE × 0.82).

Lambs were adapted to diets for 14 d. Diets were fed *ad libitum* twice daily (0700 and 1700 h). Refusals were weighed daily before the morning feeding and DM intake was calculated. Samples of offered feed (silage and concentrate) and refusals

were collected twice a week, and stored at -20°C for later analysis. Lambs were housed in individual wooden pens (0.5 m^2) with slatted floored, each fitted with a feed and water container in a well-ventilated covered barn.

Initial and final BW was measured after a 16-h fast and average daily gain (ADG) was calculated by subtracting the initial BW from final BW and dividing the difference by the trial duration of time that the lambs taken to be slaughtered with 38.0 ± 0.9 kg of body weight. The feed efficiency ratio (FER) was calculated by dividing daily DM intake by ADG.

2.3. Digestibility and nitrogen utilization study

In vivo apparent digestibility was calculated indirectly using indigestible NDF (iNDF) as a marker to estimate fecal output (Valente et al., 2011). Fecal grab samples were collected from each lamb every 26 h from d 35 to 37 (Pina et al., 2006). Samples of silage, concentrate and refusals were also collected daily. Fecal output and *in vivo* apparent digestibility were calculated using the following respective formulas:

Fecal DM output = *iNDF intake* (g) / *iNDF fecal* (%)× 100 (1)

Apparent digestibility = [*DM intake* (g) – *fecal output* (g)] / [*DM intake* (g)] × 100 (2)

On d 38, spot urine samples were collected with collection bags from all animals 4 h after the morning feeding. A harness was used to fit the collection bags onto the sheep. Subsamples (10 and 5 mL) of urine were collected and stored at -20°C for later analysis of purine derivatives (PD) and total N, respectively, to estimate microbial protein synthesis and N utilization. The 10-mL urine sample was and diluted with 40 mL of a 0.036 N solution of H_2SO_4 prior to storage.

2.4. Carcass measurements

The animals were anesthetized by electronarcosis (220 V/ 0.5 A) and jugular veins and carotid arteries were severed. After evisceration, carcasses were weighed to measure the hot carcass weight (HCW) to calculate the hot carcass yield (*HCY* = *HCW*/*BW at slaughter (BWS)* × 100). Carcasses were hung by the gastrocnemius

tendons at -4°C for 24 h. Subsequently, cold carcass weight (CCW) was measured and cold carcass yield calculated (*CCY = CCW/BWS* × 100). Losses due to cooling (LC) were also calculated (*LC = HCW – CCW/HCW* × 100). The subcutaneous fat thickness (FT) on the left side carcasses was measured with a caliper rule over the loin-eye muscle between the 12th and 13th ribs. Average daily gain (ADGc) and feed efficiency ratio (FERc) also were expressed relative to hot carcass yield, since initial HCY was estimated at 47.0% as found by Cartaxo et al. (2009) in Santa Ines x Dorper lambs in this weight range. Feed cost per kilogram gain was calculated as a ratio of the cost of the feed consumed per day to ADG and ADGc.

2.5. Ruminal Parameters Study

Eight Dorper × Santa Ines crossbred ruminally-cannulated wethers, each fitted with a silicone, 2.5-inch ruminal cannula were used. The initial BW of the wethers was 50.7 $\pm$ 4.6 kg and they were housed individually in (0.9 × 2.0 m) pens, each fitted with individual feed and water containers. These sheep received a similar diet to the others and the diets were fed *ad libitum* twice daily (7 am and 5 pm).

Ruminal measurements were taken over 3 experimental 10-d periods, each consisting of 9 d for diet adaptation and 1 d for rumen fluid collection. Ruminal fluid was collected before feeding, 3, 6, 9 and 12 h after feeding and pH and concentrations ammonia nitrogen and volatile fatty acids (VFA) were analyzed.

2.6. Laboratory Analysis

A water extract was made from undried silage samples according to Kung Jr. Jr. et al. (1984) and an electrode (MA522 model, Marconi Laboratory Equipments, Piracicaba, SP, Brazil) was used to measure the pH. Volatile fatty acids were measured in a gas chromatograph (GC 2014, Shimadzu Corporation, Kyoto, Japan) using a HP-INNOwax capillary column (30 m × 0.32 mm; Agilent Technologies, Colorado, USA) at an initial temperature of 80ºC and a final temperature of 240ºC. Lactic acid was determined by a colorimetric method (Barker and Summerson, 1941). Ammonia N was measured by distillation (AOAC, 1996, method no. 941.04).

Samples of silage, concentrate, refusals and feces were oven dried (55°C for 72 h) and ground in a knife mill (Wiley, A. H. Thomas, Philadelphia, PA, USA) to pass

through a 1-mm screen, and analyzed for DM (105°C for 12 h) and ash (500°C for 5 h). Organic matter (OM) was calculated (*OM = 100 – Ash*) on a DM basis. Neutral (NDF) and acid detergent fiber (ADF) concentrations were determined using the method of Van Soest et al. (1991) in an ANKOM 2000 Fiber Analyzer (ANKOM Technologies, Macedon, NY, USA) without sodium sulfite. Heat-stable α-amylase was used in the NDF assay. Separate samples were used for NDF and ADF analysis and both included residual N.

Indigestible NDF (iNDF) was measured by weighing feed samples into non-woven textile (100 g m^{-2}) bags (25 cm^2) (Valente et al., 2011) at the ratio of 20 mg DM cm^{-2} of surface area (Nocek, 1988) and incubating the bags in the rumens of two bulls for 264-h (Casali et al., 2008). The bulls were fed diets consisting of 60% of corn silage and 40% of concentrate on a DM basis. The iNDF concentration was determined using an autoclave at the temperature of 110°C for 40 min (Senger et al., 2008). Potentially digestible NDF (pdNDF) was estimated as NDF – iNDF, according to Huhtanen et al., 2010.

Lignin concentration was measured after hydrolysis of the cellulose in ADF residues in a 72% H_2SO_4 (Van Soest and Robertson, 1985). Extract ether (EE) was measured according to AOAC (1996; method no. 920.39). The N concentration of N NDF and ADF residues as well as feed, feces and urine samples was determined by rapid combustion using a Leco F528 N analyzer (LECO Corporation, St. Joseph, MI, USA). Crude protein (CP) concentration was calculated as N × 6.25. Gross energy (GE) was determined with a bomb calorimeter (PARR 6200, Parr Instrument Company, Illinois, USA). Digestible energy (DE) was calculated by subtracting the GE excreted in feces from the GE consumed. Metabolizable energy (ME) was estimated as DE × 0.82 (NRC, 1985).

Total carbohydrate (CHO) (Eq. (3)) and non-fibrous carbohydrates (NFC) (Eq. (4)) concentrations were estimated according to Sniffen et al. (1992) and Detmann and Valadares Filho (2010) respectively, using the following equations:

$$CHO \text{ (g kg}^{-1}\text{ of DM)} = 100 - (CP + EE + Ash) \quad (3)$$

$$NFC \text{ (g kg}^{-1}\text{ of DM)} = 100 - (NDFap + CP + EE + Ash] \quad (4)$$

where NDFap = NDF corrected for ash and protein.

Feed protein fractions were partitioned (A = non-protein nitrogen – NNP; B1 = soluble protein rapidly degraded in the rumen; B2 = true protein intermediately degraded in the rumen; B3 = true protein slowly degraded in the rumen; C = unavailable nitrogen) according to Sniffen et al. (1992).

Rumen degradable protein (RDP) (Eq. (5)) and rumen undegraded protein (RUP) (Eq. (6)) were calculated as:

$$RDP\ (g\ kg^{-1}\ of\ CP) = A + Bi \times [kdBi/ (kdBi + kp)] \quad (5)$$

$$RUP\ (g\ kg^{-1}\ of\ CP) = Bi \times [kdBi/ (kdBi + kp)] + C \quad (6)$$

Where Bi = true protein in the three B fractions (B1, B2 e B3); kd = rates of degradation estimated according to *Small Ruminant Nutrition System* (SRNS) *version 1.9.4468* and kp = rates of passage calculated as reported by Cannas and Van Soest (2000).

Purine derivatives (PD) were measured as the sum of allantoin, uric acid, xanthine and hypoxanthine according to Chen and Gomes (1995a). Daily PD excretion (Eq. (7)) was calculated as follows according to Chen and Gomes (1995b):

$$PDC\ index = daily\ PD\ (mmol\ d^{-1}) / daily\ Creatinine\ (mmol\ d^{-1}) \times BW^{0.75} \quad (7)$$

where *PDC index= PD* (in spot urine sample; mmol)/ [*Creatinine* (in spot urine sample; mmol) × $BW^{0.75}$].

To obtain the concentration of daily creatinine in the urine, prior to the experiment, total urine was collected from six crossbred Dorper × Santa Ines lambs (40.9 ± 1.4 kg) for 6 d after they received a diet consisting of corn silage (60%) and concentrate (40%) (DM basis) for 21 d. Creatinine concentration in the urine was determined to be 0.370 ± 0.150 using a commercial kit (Labtest, Lagoa Santa, MG, Brazil).

Microbial N supply (Eq. (8)) and intestinal flow of microbial N (Eq. (9)) were according to Chen and Gomes (1995a):

$$Daily\ PD\ (mmol\ d^{-1}) = 0.84 \times AP + (0.150 \times BW^{0.75} exp^{-0.25AP}) \quad (8)$$

where AP = absorption of microbial purines (mmol/d); 0.84 represents the recovery of absorbed purines as PD in urine; the component within parenthesis represent the endogenous contribution which decreases as exogenous purines become available for utilization by the animal.

$$Microbial\ N\ (g\ N\ d^{-1}) = (AP \times 70)/ (0.116 \times 0.83 \times 1000) = 0.727 \times AP \quad (9)$$

where the N concentration of purines is 70 mg N/mmol; the ratio of purine-N: total N in mixed rumen microbes is taken as 11.6:100; the digestibility of microbial purines is assumed to be 0.83.

The efficiency of microbial N synthesis was expressed as grams of microbial N per kilogram of digestible OM fermented in the rumen (DOMR: calculated as Digestible organic matter intake (DOMI) × 0.65, according to ARC (1984)).

Absorption (g d^{-1}) (Eq. (10)) and retention of N (g d^{-1}) (Eq. (11)) was calculated as follows:

N absorbed = N intake – N fecal (10)

N retention = N intake – (*N fecal* + *N urinary*) (11)

The pH of rumen fluid was measured using a pH meter (MA522 model, Marconi Laboratory Equipments, Piracicaba, SP, Brazil). Subsequently, 1mL of H_2SO_4 (1:1) was added to the ruminal fluid and it was stored at -20 °C for analysis of concentrations of rumen ammonia N by distillation with 2N KOH according to Fenner (1965) and for analysis of VFA by gas chromatography as described earlier.

2.7. Statistical Analysis

The corn silage chemical composition data were as analyzed as a completely randomized design with 14 replicates. Data on the effects of treatments on digestibility, microbial protein synthesis and performance were analyzed in a randomized block design in a 2 × 2 factorial arrangement (with 7 replicates) and on the effects of treatments on ruminal parameters with a 4 × 4 double latin square design with a 2 × 2 factorial arrangement. All data was analyzed using MIXED procedure of SAS (v. 9.0 SAS Institute Inc., Cary, NC). The effect of the treatments was considered fixed and animals considered random. The BWI was used as a covariate for analyzing ADG and carcass data. The model for analyzing ruminal fermentation data included a repeated measures statement; the treatments were the fixed effect and the animals and periods were considered random effects. Unstructured (UN), compound symmetric (CS), toeplitz (TOEP), banded toeplitz , factor analytic (FA) and Huynh Feldt (HF) were the best covariance structures for the data as these had the lowest Akaike information criterion scores. Differences

between means were determined using the DIFF, which differentiates means based on Fisher´s *F* protected least significant difference test. Significance was declared at $P < 0.05$ and tendencies at $0.05 > P < 0.10$.

3. Results

3.1. Characterization of corn plant

The average DM content was of 313 g kg^{-1}. The concentrations of ash, CP, EE, NDF, ADF and hemicellulose were: 52; 111; 16; 426; 186 and 240 g kg^{-1} of DM respectively, whereas the pH value was 5.4 and ammonia N 0.9 % of total nitrogen.

3.2. Chemical composition and fermentation characteristics of corn silage

Inoculation of corn silage with *L. buchneri* reduced concentrations of NDF, pdNDF, ADF and cellulose ($P < 0.05$); consequently the NFC concentration was higher than Control silage. Reduction of NDF concentration of corn plant compared to silage was 36 g/kg of DM in Control silage and 65 g/kg of DM in LB silage (Table 2).

The N content associated to NDF fractions (NDIN) also was lower in the LB silage. Lactic acid concentration and lactic to acetic acid ratio were higher in the Control silage than LB silage ($P < 0.05$), whereas the acetic acid concentrations, pH values and ammonia N content were not different between the silages (Table 2).

3.3. Intake and Digestibility

There was significant interaction ($P < 0.05$) between silages and levels of concentrate on DMI, EEI and GEI and there was a tendency of interaction on OMI, CPI, CHOI and NFCI. Nutrients and gross energy intake of the lambs fed with inoculated silage were similar in both concentrate levels. Otherwise, when the lambs were fed untreated silage the nutrient intake, except NDFI, was greater with more concentrate in the diet (Table 3).

The apparent digestibility of OM, CP, EE, CHO, NFC and GE was lower in the lambs fed LB silage compared to uninoculated silage. A greater amount of concentrate in the diet promoted a lower nutrient digestibility and gross energy ($P <$

0.05), except NDF and CHO digestibility, as well as decreased the DE and ME of these diets (Table 3).

There was interaction between silages and levels of concentrate on intake of digestible DM, OM, EE, CHO and NFC, and energy by the lambs. Lambs fed untreated silage associated to 40% of concentrate had the lowest intake of digestible nutrients and energy. However, lambs fed LB silage associated to 60% concentrate also consumed a lower amount of DE and ME than those fed uninoculated silage associated to 60% of concentrate. Digestible CP and NDF intake were not affected by treatments (Table 3).

Table 2 Chemical composition and fermentation characteristics of corn silage without inoculant (Control) and inoculated with *L. buchneri* (sampled weekly over 7 weeks).

	Control	*L. buchneri*	SEM[2]	*P value*
Chemical composition[1]				
DM (g kg^{-1})	315	326	5.50	0.169
OM (g kg^{-1}DM)	963	962	2.13	0.786
Ash (g kg^{-1} DM)	37	37	2.13	0.786
CP (g kg^{-1}DM)	82	84	1.19	0.459
EE (g kg^{-1}DM)	37	35	2.22	0.489
NDF (g kg^{-1}DM)	390	361	5.57	0.001
NDFap (g kg^{-1}DM)	353	328	5.91	0.006
iNDF (g kg^{-1}DM)	125	122	2.70	0.454
pdNDF (g kg^{-1}DM)	265	239	5.57	0.002
ADF (g kg^{-1}DM)	230	207	4.06	0.001
Cellulose (g kg^{-1}DM)	190	172	3.37	0.001
Hemicellulose (g kg^{-1}DM)	159	153	6.13	0.465
Lignin (g kg^{-1}DM)	29	25	1.90	0.217
NDIN (g kg^{-1}TN)	328	292	7.76	0.003
ADIN (g kg^{-1}TN)	125	115	6.00	0.289
CHO (g kg^{-1} DM)	844	843	2.81	0.872
NFC (g kg^{-1}DM)	491	514	6.59	0.016
Fermentation characteristics				
Lactic acid (g kg^{-1}DM)	106	74	9.77	0.028
Acetic acid (g kg^{-1}DM)	61	61	3.38	0.920
Lactic:acetic acid ratio	1.80	1.24	0.15	0.015
pH	3.60	3.60	0.02	0.784
Ammonia N (%TN)	5.05	4.35	0.46	0.292

[1]DM: Dry matter; OM: organic matter; CP: crude protein; EE: extract ether; NDF: neutral detergent fiber; NDFap: neutral detergent fiber corrected for ash and protein; iNDF: indigestible neutral detergent fiber; pdNDF: potentially digestible neutral detergent fiber; ADF: acid detergent fiber; NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen; CHO: total carbohydrates; NFC: non-fibrous carbohydrates; TN: total nitrogen. [2]SEM: Standard error of the mean.

Table 3 Effect of *Lactobacillus buchneri* inoculation of corn silage associated to two levels of concentrate (40 and 60%) on intake and apparent digestibility of nutrients and energy in lambs.

	Control		*L. buchneri*		SEM[2]	*P* value		
	40%	60%	40%	60%		Silages	Concentrate	S × C
Intake of nutrients (g d^{-1})								
DM[1]	1050	1330	1259	1291	63.76	0.096	0.002	0.012
DM (% BW)	3.3	4.1	3.9	4.0	0.15	0.090	0.002	0.019
DM (g $BW^{-0.75}$)	78.8	98.7	92.8	96.0	3.48	0.089	0.002	0.017
OM	995	1195	1192	1187	56.19	0.125	0.112	0.071
CP	156	186	180	188	6.20	0.026	0.002	0.053
EE	44	57	51	53	2.48	0.564	0.004	0.039
NDF	293	325	324	301	16.35	0.756	0.722	0.135
CHO	796	952	962	946	45.58	0.093	0.138	0.070
NFC	533	658	670	670	32.13	0.029	0.064	0.063
GE (Mcal d^{-1})	4.3	5.3	5.0	4.7	0.35	0.850	0.133	0.017
Apparent nutrient digestibility (%)								
DM	73.4	69.0	70.8	68.0	1.35	0.183	0.016	0.562
OM	75.1	70.8	72.2	68.6	1.45	0.097	0.014	0.808
CP	70.7	62.6	65.0	60.1	1.43	0.003	0.001	0.200
EE	89.8	89.6	87.1	83.5	1.16	0.001	0.073	0.092
NDF	36.8	36.3	34.8	33.0	1.82	0.158	0.540	0.732
CHO	75.3	72.8	72.3	71.5	1.05	0.053	0.136	0.424
NFC	91.1	88.4	89.3	87.7	0.66	0.019	0.004	0.309
GE	74.7	69.7	70.8	68.4	1.34	0.067	0.013	0.351
DE (Mcal kg^{-1})	3.1	2.7	2.8	2.5	0.19	0.119	0.038	0.859
ME(Mcal kg^{-1})	2.5	2.2	2.3	2.0	0.15	0.119	0.038	0.858
Intake of digestible nutrients (g d^{-1})								
DM	771	872	869	829	32.31	0.407	0.359	0.044
OM	745	840	862	805	33.50	0.207	0.553	0.025
CP	114	112	111	106	4.45	0.301	0.364	0.710
EE	39	51	44	44	2.05	0.608	0.008	0.008
NDF	116	108	111	96	7.21	0.269	0.142	0.610
CHO	598	680	702	662	28.00	0.106	0.419	0.028
NFC	482	584	601	593	26.90	0.023	0.085	0.048
DE (MJ d^{-1})	3.2	3.8	3.5	3.1	0.21	0.308	0.571	0.014
ME (MJ d^{-1})	2.6	3.1	2.8	2.6	0.17	0.310	0.574	0.014

*Silages - Inoculated vs. Control; Concentrate - 40% vs. 60%; S × C – Interaction between silages and concentrate. [1]DM: dry matter; OM: organic matter; CP: crude protein; EE: extract ether; NDF: neutral detergent fiber; CHO: total carbohydrates; NFC: nonstructural carbohydrates; GE: Gross energy; DE: Digestible energy; ME: Metabolizable energy (ME= DE x 0.82); BW: body weight; $BW^{0.75}$: metabolic body weight. [2]SEM: Standard error of the mean.

3.4. Ruminal fermentation

Some interactions between silages, levels of concentrate and sampling time were observed in ruminal parameters of wethers (acetate and total VFA concentrations, and ammonia N); however the most evident was in the propionate concentrations (Table 4). Corn silage inoculated with *L. buchneri* associated to a

larger amount of concentrate promoted a greater propionate ruminal concentration in all sampling times ($P < 0.05$; Table 4; Figure 1 – B); which resulted in a lower acetate to propionate ratio (Figure 1 – C), but without significant differences. Concentration of ammonia rumen N was the lowest in the lambs fed LB silage associated to 60% of concentrate (Interaction between *S* × *C*; $P < 0.05$; Table 4).

Regardless of the silage, the pH value was affected by larger amount of concentrate in the diet. Animals fed with 60% of concentrate had a lower ruminal pH than those fed with 40% of concentrate (Table 4).

3.5. Nitrogen utilization and microbial protein synthesis

There was an interaction between silages and levels of concentrate in the purine derivatives and microbial N synthesis (Table 5). Lambs fed LB silage associated to 40% of concentrate had higher microbial N supply (7.6 vs. 6.5 g d^{-1}) than those fed LB silage associated to 60% of concentrate; however microbial N synthesis was similar between the levels (13.6 vs. 12.4 g of N kg^{-1} of DOMR). Otherwise, lambs fed control silage associated to 40% of concentrate had a lower microbial N supply (5.8 *vs.* 8.7 g/d) and microbial N synthesis than those fed control silage associated to 60% of concentrate (12.1 vs. 16.1 g of N kg^{-1} of DOMR) in the diet ($P < 0.05$). However, when we compared the levels of concentrate, in the lower level, we observed that animals that consumed LB silage had a greater microbial N supply and similar microbial N synthesis than those fed untreated silage ($P < 0.05$). On the other hand, in the larger concentrate level, lambs fed control silage had higher microbial N supply and microbial N synthesis than those fed LB silage ($P < 0.05$).

Inoculation of corn silage with *L. buchneri* or a larger amount of concentrate in the diet increased the excretion of N on the feces; and decreased the N absorbed in % of N intake. Lambs fed LB silage associated to 60% of concentrate had the lowest excretion urinary N (Interaction S × C; $P < 0.05$). However, N retention was similar between treatments (Table 5).

Table 4 Effect of *Lactobacillus buchneri* inoculation of corn silage associated to two levels of concentrate (40 and 60%) on ruminal fermentation parameters of wethers.

Item	Control		*L. buchneri*		SEM[2]	*P* value				
	40%	60%	40%	60%		Silages	Levels	Time	S × C	S ×C× T
Acetate (mmol L^{-1})	39.5	36.8	39.0	36.9	2.95	0.819	0.253	0.001	0.945	0.038
Propionate (mmol L^{-1})	11.4	13.0	11.7	16.7	1.66	0.131	0.014	0.001	0.209	0.004
Butyrate (mmol L^{-1})	6.6	6.4	6.6	5.8	0.78	0.620	0.400	0.001	0.584	0.584
Acetic:propionic ratio	3.7	3.5	3.8	2.8	0.52	0.428	0.123	0.001	0.281	0.665
Total VFA (mmol L^{-1})[1]	58.6	56.2	57.4	59.4	4.94	0.785	0.965	0.001	0.535	0.022
pH	6.17	6.01	6.08	5.92	0.09	0.099	0.003	0.001	0.930	0.271
Ammonia N (mg dL^{-1})	23.4	24.6	24.9	21.8	1.99	0.559	0.337	0.001	0.031	0.050

[*]Silages (Inoculated vs. Control); Concentrate (40% vs. 60%); Time (Effect of sampling time); S × C (Interaction between silages and concentrate); S × L × T (Interaction between silages, levels and sampling time).[1]VFA: Volatile fat acid. [2]SEM: Standard error of the mean.

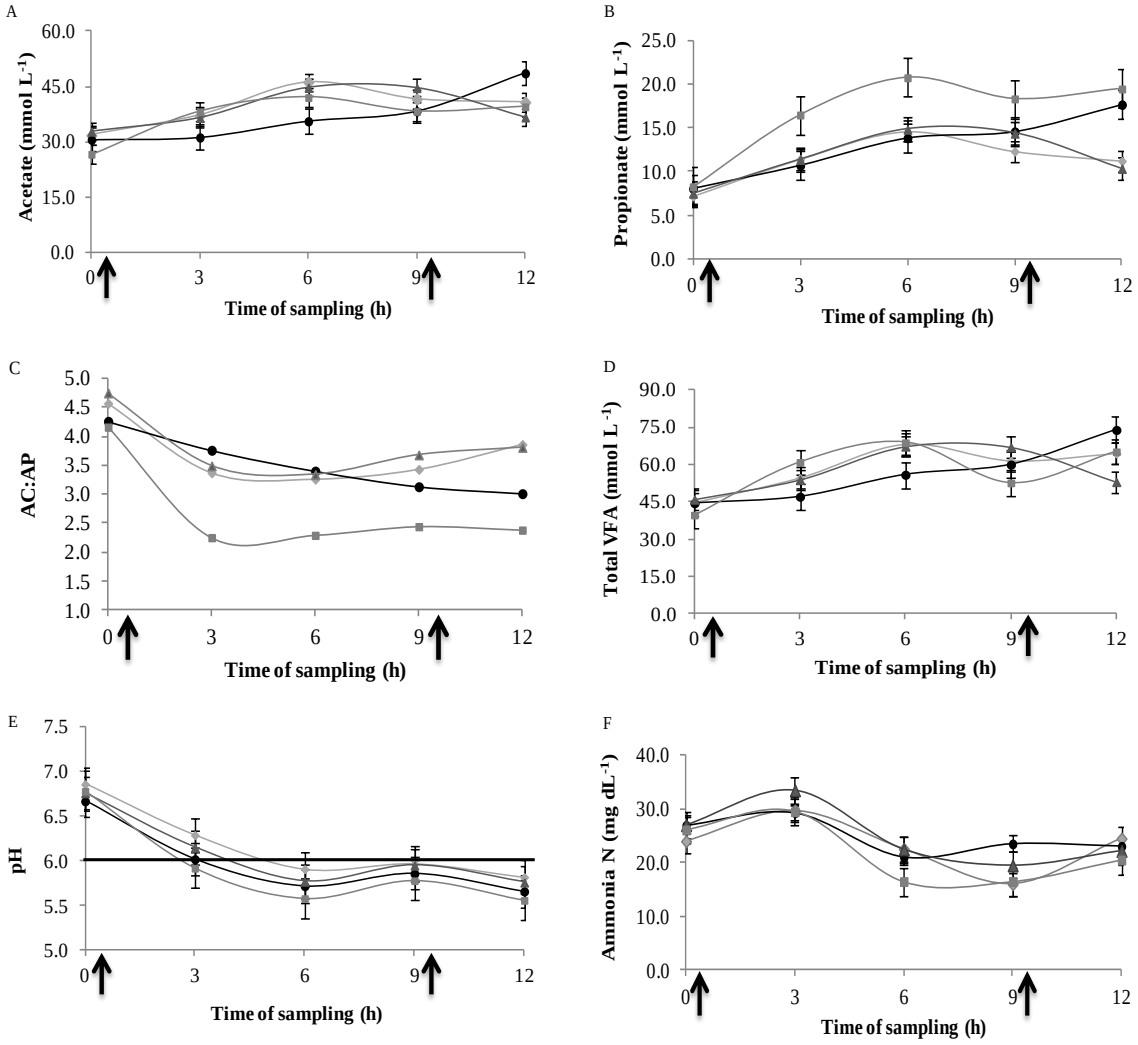

Figure 1. Effect of *Lactobacillus buchneri* inoculation of corn silage associated to two levels of concentrate (40 and 60%) on changes in ruminal fermentation parameters of wethers. Key: ◆ Control silage + 40% of concentrate; ● Control silage + 60% of concentrate; ▲ LB silage + 40% of concentrate; ■ LB silage + 60% of concentrate. Feeding (→).

Table 5 Effect of *Lactobacillus buchneri* inoculation of corn silage associated to two levels of concentrate (40 and 60%) on microbial N synthesis and N utilization by lambs.

	Control		*L. buchneri*		SEM[4]	*P* value		
	40%	60%	40%	60%		Silages	Concentrate	S × C
Microbial N synthesis								
Allantoin (mmol d^{-1})	5.3	6.0	6.6	5.3	0.40	0.342	0.322	0.008
Uric acid (mmol d^{-1})[1]	1.6	2.7	2.5	2.0	0.26	0.642	0.269	0.005
PD (mmol d^{-1})[2]	6.9	9.1	9.2	7.3	0.68	0.717	0.806	0.004
Microbial N (g d^{-1})	5.8	8.7	7.6	6.5	0.50	0.979	0.041	0.001
DOMR (g d^{-1})[3]	484	546	560	523	21.78	0.206	0.553	0.025
Microbial N kg^{-1} of DOMR	12.1	16.1	13.6	12.4	0.84	0.088	0.034	0.001
N utilization								
N Intake (g d^{-1})	24.9	29.9	28.4	30.2	1.00	0.045	0.001	0.082
Faecal N (g d^{-1})	7.2	11.4	10.5	13.2	0.67	0.001	0.001	0.433
Urinary N (g d^{-1})	10.3	11.1	11.5	10.1	1.00	0.639	0.277	0.001
N absorbed (g d^{-1})	17.2	18.5	17.8	17.0	1.02	0.622	0.800	0.249
N retention (g d^{-1})	6.9	7.4	6.3	6.8	1.39	0.474	0.500	0.995
N absorbed (% of N intake)	68.8	61.6	62.6	56.2	2.26	0.002	0.008	0.877
N retention (% of N intake)	26.9	24.6	21.8	22.6	4.76	0.199	0.785	0.565
N retention (% of N absorbed)	38.4	37.7	34.0	40.7	6.74	0.827	0.352	0.246

[*]Silages - Inoculated vs. Control; Concentrate - 40% vs. 60%; S × C – Interaction between silages and concentrate. [1]Uric acid: xanthine and hypoxanthine converted into uric acid (xantine oxidase enzyme); [2]PD: purine derivatives - sum of allantoin and uric acid; [3]DOMR: digestible organic matter fermented in the rumen (calculated as 0.65 × DOMI). [4]SEM: Standard error of the mean.

3.6. Growth performance and carcass yield

Lambs fed corn silage inoculated with *L. buchneri* (ADG: 262 vs. 252 g d^{-1}; ADGc: 135 vs. 113 g d^{-1}) or a greater level of concentrate (ADG: 262 vs. 252 g d^{-1}; ADGc: 128 vs. 120 g d^{-1}) had a higher ADG or AGDc than those fed control silage or a lower level of concentrate respectively. However, the feed efficiency ratio was improved in the animals fed with untreated silage associated to 40% of concentrate. On the other hand, when we evaluated the FER in relation to carcass we observed that the FER of lambs fed control silage associated to 40% of concentrate tended to be different from the FER of lambs fed control silage associated to 60% of concentrate, but it was similar to the FER of lambs that consumed LB silage associated to 40% of concentrate (Interaction S × C; $P = 0.090$). The carcass weight and yield were not influenced by treatments (Table 6). Fat thickness was higher in the lambs fed greater levels of concentrate.

Table 6 Effect of *Lactobacillus buchneri* inoculation of corn silage associated to two levels of concentrate (40 and 60%) on performance and carcass yield of lambs.

	Control		*L. buchneri*		SEM[2]	*P* value		
	40%	60%	40%	60%		Silages	Concentrate	S × C
IBW (kg)[1]	25.5	25.9	25.8	25.9	1.59	0.698	0.627	0.736
ADG (g/d)	245	258	258	265	9.33	0.022	0.030	0.384
FER (gain/feed)	0.232	0.192	0.208	0.204	0.02	0.579	0.036	0.048
Time of feedlot (d)	52	50	50	47	2.07	0.242	0.360	0.247
BWS (kg)	38.2	38.8	38.9	38.3	0.33	0.734	0.967	0.179
HCW (kg)	17.6	18.4	18.7	18.7	0.39	0.125	0.471	0.260
CCW (kg)	17.2	17.8	18.2	18.0	0.38	0.162	0.545	0.327
HCY (%)	46.1	47.4	48.2	48.5	0.97	0.111	0.412	0.660
CCY (%)	45.1	46.0	46.7	47.1	0.95	0.186	0.488	0.779
LC (%)	2.3	3.0	2.7	3.7	0.33	0.082	0.363	0.115
ADGc (g/d)	108	118	131	138	3.95	0.006	0.035	0.399
FERc (gain/feed)	0.103	0.091	0.104	0.106	0.05	0.830	0.103	0.090
FT(mm)	2.4	3.4	2.7	3.6	0.28	0.321	0.001	0.805

*Silages - Inoculated vs. Control; Concentrate - 40% vs. 60%; S × C – Interaction between silages and levels. [1]IBW: initial body weight; AGD: average gain daily; FER: feed efficiency ratio; BWS: body weight to slaughter; HCW: hot carcass weight; CCW: cold carcass weight; HCY: hot carcass yield; CCY: cold carcass yield; LC: losses by cooling; FT: fat thickness; ADGc: average daily gain expressed in relation to HCY. [2]SEM: Standard error of the mean.

Regardless of silage, feed cost of diets per kilogram of DM in the low (40%) or high (60%) level of concentrate were closed. The cost per animal per day was lower in the diet composed by control silage associated 40% of concentrate, as well as feed cost per kilogram of gain (Table 7).

Table 7 Feed cost of experimental diets.

	Control		*L. buchneri*	
	40%	60%	40%	60%
Cost kg^{-1} DM (U$)	0.22	0.26	0.23	0.27
Cost $animal^{-1}$ d^{-1} (U$)	0.23	0.35	0.29	0.35
Total cost $animal^{-1}$ time of $feedlot^{-1}$ (U$)	11.89	17.34	14.17	16.12
Feed cost kg^{-1} gain (U$)	0.94	1.35	1.10	1.30
Feed cost kg^{-1} gain of carcass (U$)	4.23	5.88	4.33	4.97

*Ingredients cost: Untreated silage – U$33.87 ton^{-1} of silage; LB silage – U$37.53 ton^{-1} of silage; Soybean meal – U$0.41 kg^{-1} DM; Corn meal – U$0.25 kg^{-1} DM; Wheat bran – U$0.24 kg^{-1} DM and Mineral supplement – U$0.60 kg^{-1} DM. 1.00 U$ = 2.00 R$

4. Discussion

4.1. Chemical composition and fermentation characteristics of corn silage

The lowest concentration of fiber fraction in the inoculated silage and the most intense reduction of NDF from corn plant to inoculated corn silage can be due *to L. buchneri*-treated corn silage is associated with a relatively increase in the pH during the storage phase because of the high metabolic activity of *L. buchneri* in these

silages (Driehuis et al., 2001). Therefore, in the treated silages the decrease in pH can have been less pronounced than Control silage. Thus, enzymes as hemicellulase and cellulase, can have acted in reducing hemicellulose and cellulose content.

Low lactic acid concentration of inoculated silage is possibly due to the conversion of this acid to acetic acid and carbon dioxide as reported by Oude Elferink et al. (2001). Lack of difference in the acetic acid content between silages might be related to the volatilization of VFA which occurs from active feeding faces of farm silos as cited by Mari et al. (2009). Since, in general the samples were collected from the faces of the silos that have been exposed to air for an entire evening or one day, as happened in the present trial.

Ranjit et al. (2002) reported higher concentrations of acetic and propionic acid in the corn silage inoculated with *L. buchneri* than in the untreated silage; however these authors did not find differences on the lactic acid content on farm silos. Queiroz et al. (2012) also found higher acetic acid concentrations and there were no differences detected in the lactic acid concentration in corn silage inoculated with *L. buchneri* and *Pediococcus pentosaceus* compared to uninoculated on farm silos. In laboratory studies, Nishino et al. (2003) reported that inoculation with 4.4 x 10^6 cfu *L. buchneri* per gram of forage reduced the lactic acid content from corn silage inoculated compared to untreated (3.47 vs 6.41% of DM). Filya (2003) also found declines from 4.04 to 2.76% of DM in the lactic acid content in corn silage inoculated with 1x10^6 cfu of *L. buchneri* per gram of forage.

All pH values were low as they fell within the range of 3.7 to 4.2. Low pH values inhibit protein degradation in silage (McDonald et al. 1991) and ammonia N concentrations were below the upper limit of the range (5 to 7% TN) beyond which unsuitable preservation of silage occurs (Kung Jr. and Shaver, 2001).

4.2. Lambs Study

The changes in the fiber fraction promoted by inoculation in the corn silage can have affected the nutrient intake by lambs because in general, with the increase of concentrate in the diet, the intake of nutrients also increases (Ramazin et al., 1997). However, regardless of concentrate levels, lambs fed inoculated corn silage

had similar nutrient intake, which was not observed in the animals fed untreated silage. One of the explanations for higher DM intake of LB silage associated to the lower level of concentrate by lambs compared to those fed control silage associated to the lower level is that corn silage inoculated with *L. buchneri* had greater NFC than Control silage, due to the decrease of fiber fraction. Thus, according to Addah et al. (2012) a reduction in NDF content should reduce ruminal bulk fill, allowing intake of diets rich in forage to increase. Nkosi et al. (2009, 2011) found higher DM intake in the lambs fed corn silage inoculated with *L. buchneri* than in the animals fed untreated silage and they attributed the greater DM intake to high levels of water-soluble carbohydrates in the inoculated corn silage.

The *in vivo* apparent nutrient digestibility was lower in the lambs fed LB silage than in Control silage. However, regardless of silage, lambs fed larger amounts of concentrate also had apparent nutrient digestibility impaired. Nevertheless, DM intake was generally superior in the animals fed inoculated silage and in the higher level of concentrate. According to Leaver et al. (1969), Robertson and Van Soest (1975), Chen et al. (1992) and Doreau et al. (2003) a large intake resulted in depression in the *in vivo* nutrient digestibility and there are linear negative relationships between intake and digestibility in sheep. These authors verified that the *in vivo* apparent digestibility of DM and OM decreases as DMI increases as well as retention time decreases and rate of passage increases.

Nkosi et al. (2010) found improvement of CP and NDF digestibility in rams fed potato hash silage inoculated with *L. buchneri*. Aksu et al. (2004) observed enhances in the DM and NDF digestibility in sheep fed corn silage inoculated with LAB. However, Rowghani et al. (2008) found no differences in the DM digestibility in rams fed corn silage inoculated with LAB and *Propionibacterium* or untreated silage. These contrasting results could be due to the magnitude of the intake between different experiments as well as differences between animals, because according to Mertens and Ely (1982) rate of passage varies among animals fed similar diets.

The intake of LB silage associated to higher level of concentrate promoted a greater ruminal propionate concentration, consequently lower acetate to propionate ratio and pH values in most sampling times in the ruminal fluid of lambs. This might be due to greater NFC content in this diet, because LB silage had NFC content

increased due to the actions of *L. buchneri*; and high levels of propionate are produced by starch-fermenting bacteria from high-concentrate diets (Russell, 1998).

However, beyond the effect of NFC content on the ruminal propionate concentration there seems to be an interaction of *L. buchneri* fed with silage and rumen bacteria, because although the diet composed by control silage associated to a greater level of concentrate also had high NFC concentration, the propionate production in the rumen fluid of lambs fed with it, was similar to animals fed lower NFC content in the diet until 9 h at sampling time (refeeding; Figure 1 B).

Many researchers suggest that the LAB might survive in the rumen, and these shifts in the ruminal fermentation that occurred in the present study may be an indication of an interaction of LAB with the rumen microbial population, but it is not totally clear, especially when there is an effect associated to levels of concentrate (Keady and Steen, 1994; Weinberg and Muck, 1996; Fellner et al., 2001; Weinberg et al., 2003; 2004a, 2004b and 2007).

The pH value of rumen fluid was affected by levels of concentrate, remaining below of 6.0 when the lambs were fed LB silage and a higher level of concentrate, which can impair activity of cellulolytic bacteria (Russell and Wilson, 1996); however, in our study the NDF digestibility was not affected. The low ruminal pH is result of fermentation of large amounts of available organic matter (Bach et al., 2005).

Lambs fed LB silage associated to a higher level of concentrate had a lower ammonia N content in the rumen fluid. This result might be related to higher N uptake by ruminal microorganism due to an increase in the available readily fermentable carbohydrates (Russell et al., 1983; Bach et al., 2005). Still, the lowest ruminal pH in the lambs fed this diet may have an effect on deamination of protein, because the decrease in pH can affect the proteolytic bacteria activity (Lana et al., 1998).

Lambs fed LB silage associated to 40% of concentrate and lambs fed control silage associated to 60% of concentrate had the greatest microbial N synthesis (MNS) and efficiency of microbial N synthesis (EMNS), whereas animals fed LB silage associated to 60% of concentrate and control silage associated to 40% of concentrate had lower MNS and MNSE. Some factors affecting microbial nitrogen synthesis are supply of fermentable energy, supply of nitrogen compounds, rumen outflow rate (related with dry matter intake) and rumen environment (ruminal pH) as

reported by Verbic (2002). Hoover and Stokes (1991) suggested that in a ruminal pH-controlled, maximum growth is attained with a 2:1 NFC:RDP ratio, highlighting the importance of supplying adequate amounts of available N when energy is not limiting, and the importance of synchrony at which nutrients become available (Bach et al., 2005).

Then, in the present study, the lower MNS and MNSE could be associated to sources and levels of N components, since if limited, can depress total CHO digested and affect microbial protein yield (Hoover and Stokes, 1991). However, the N sources from concentrate were similar to all diets and RDP (%CP) was similar between diets composed by LB silage and similar between diets composed by Control silage. Furthermore, it is important to highlight that the differences in rate of digestion among NFC and fibrous carbohydrates (FC) markedly affect the total CHO digested, which in turn influence microbial protein yield per day (Hoover and Stokes, 1991). Hence, the changes promoted by inoculation of *L. buchneri* on corn silage resulted in silage with a higher NFC content, which might have improved MNS in the lambs fed with a diet composed of LB silage associated to 40% of concentrate compared to those Control silage associated to 40% of concentrate. Likewise, the lower microbial yield in the lambs fed control silage associated to 40% of concentrate seems to be related to lower DM intake promoted by lower NFC compared to other diets. In the animals fed LB silage associated to 60% of concentrate, the lower MNS and MNSE can be related to rumen environment, since the ruminal pH was below of 6.0 and due to low pH value the energy within the rumen is diverted to non-growth functions, i.e. maintaining neutral pH in bacterial cell (Strobel and Russell, 1986).

Therefore, the shifts that occurred in the silage fiber composition resulted in changes in the ruminal fermentation, which promoted greater dry matter intake and MNS, resulting in the increase in the daily weight gain in the lambs fed inoculated silage. This is in agreement to results obtained by Keady and Steen (1994), McAllister et al. (1998), Fellner et al (2001) that found improvements in the daily gain of steers fed silage inoculated with lactic acid bacteria. Likewise, Nkosi et al. (2009, 2011) observed improved lamb performance occurred when the animals were fed corn silage inoculated with lactic acid bacteria.

Although lambs fed LB silage associated to 60% of concentrate have had MNS impaired their ADG was similar to those fed LB silage associated to 40% concentrate or lambs fed control silage associated to 60% of concentrate. This result can be due to a greater efficiency of energy utilization. Keady and Steen (1994) reported that na improvement on animal performance by feeding inoculated silage might be related to na improved efficiency of energy utilization due to higher levels of propionate in the rumen caused by an inoculant.

The FER in the present study presented average of 0.212 and 0.206 to lambs fed diets composed of control and LB silages respectively, and average of 0.220 and 0.198 to lambs fed diets composed of 40 and 60% of concentrate, which indicates relatively efficient feed utilization by the lambs. Nkosi et al. (2009) found values near 0.200 in the FER of lambs fed corn silage inoculated with LAB in forage to concentrate ratio of approximately 50:50. Papi et al. (2011) observed FER ranged from 0.105 to 0.136 in lambs fed with 90 to 30% of concentrate in the diet with hay as forage.

With respect to response of carcass characteristics to microbial inoculation of corn silage associated to levels of concentrate we did not find differences between treatments, except in FT. Our findings support Fugita et al., (2012) that found no differences in the hot carcass yield of crossbred bulls fed corn silage inoculated with facultative heterofermentative LAB or control silage. In the present study, the hot carcass (47.2%) and cold carcass (46.2%) yield were close to those reported by Cartaxo et al. (2009) in the Santa Inês x Dorper crossbred lambs fed in forage to concentrate ratio of 30:70 (HCY = 46.5%; CCY = 45.6%; FT = 2.0 mm), whereas the losses by cooling (2.9%) were higher than those found by these authors (1.9 %). Silva Sobrinho et al. (2008) claim that lambs carcasses of specialized breed for meat shows carcass yield that ranges from 40 to 50%. The mean of hot carcass yield of 47.2% obtained in the present study is within this range as well as the mean of losses by cooling of 2.8% are within the range from 3.0 to 4.0% that are acceptable levels of loss, according to Sañudo et al. (1991).

Fat thickness was higher in the lambs fed greater levels of concentrate. Since these diets promoted higher levels of propionate in the rumen that is the glucose precursor, and the glucose metabolism is closely associated with amino acids and

lipids metabolism through the endocrine action of insulin and glucagon (Huntington, 1997). Acetate and glucose are converted into lipids in adipose tissue from ruminants (Hanson and Ballard, 1967). Majdoub-Mathlouthi et al. (2013) also found higher FT in the lambs fed higher level of concentrate.

Regardless of silage, feed cost of diets per kg of DM in the low (40%) or high (60%) levels of concentrate were closed. However, when we regard the intake, the cost per animal per day it was lower in the diet composed by control silage associated 40% of concentrate, due to the lower DM intake this diet. This resulted in a lower total cost of diet control silage associated to 40% of concentrate. The feed cost per kilogram of gain was also lower in the animals fed control silage associated to 40% of concentrate. However, total feed cost per animal of diet composed by LB silage associated 40% of concentrate increased in U$2.28. If we regard that the application of *L. buchneri* in corn silage ensiled in farm silos decreases the occurrence of molds and increases the aerobic stability in 30 hours (Mari et al., 2009) as well as decreases the spoiled silage removed daily from silos by around to 44% (Queiroz et al., 2012), the feed cost could be recovered.

5. Conclusions

Fermentation characteristics were influenced by inoculation, but all silages were deemed suitable for preservation.

The inoculation with *Lactobacillus buchneri* reduced the cell wall of the corn silage.

The diet composed by corn silage inoculated with *Lactobacillus buchneri* associated to 40% of concentrate increased the efficiency of use of silage, improving in the dry matter intake, microbial N synthesis and daily weight gain of lambs.

6. Acknowledgment

The authors wish to express their appreciation to São Paulo Research Foundation – FAPESP for their financial support.

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