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Inoculation of corn silage with *Lactobacillus plantarum* and *Bacillus subtilis* associated with amylolytic enzyme supply at feeding. 2. Growth performance and carcass and meat traits of lambs

Erika C. Lara*, Uly C. Bragiato, Carlos H.S. Rabelo, Juliana D. Messana, Américo G.S. Sobrinho, Ricardo A. Reis

UNESP, São Paulo State University, Department of Animal Sciences, 14884-900, Jaboticabal, SP, Brazil

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ABSTRACT

The objective of this research was to investigate the effects of inoculation of corn silage and dietary supplementation of amylolytic enzymes, and their interaction, on lamb growth performance and carcass and meat traits. Whole-crop corn forage was treated either with water (untreated) or with *Lactobacillus plantarum* MA 18/5U at 1×10^5 cfu/g fresh forage combined with *Bacillus subtilis* AT553098 at 1×10^5 cfu/g fresh forage (inoculated) and ensiled for 170 d. Forty non-castrated Texel × Dorper male lambs were arranged in a randomized block design ($n = 10$) and then assigned to one of four diets, as follows: 1) untreated corn silage with no amylolytic enzyme supply (NI-NA); 2) untreated corn silage with amylolytic enzyme supply (NI-WA); 3) inoculated corn silage with no amylolytic enzyme supply (WI-NA); and 4) inoculated corn silage with amylolytic enzyme supply (WI-WA). α-Amylase (602 dextrinizing units/kg of dry matter (DM)) was supplied to the lambs at total mixed ration delivery. Inoculation of corn silage increased ($P = 0.003$) both digestible energy (DE) and metabolizable energy (ME) intake by 1.4 and 1.2 MJ/kg DM, respectively, and also resulted in higher average daily gain (ADG; $P = 0.023$) of lambs (233 vs. 212 g/d in lambs fed NI diet). In contrast, dietary supplementation with amylolytic enzymes had no effect on the DM intake ($P = 0.90$) or ADG ($P = 0.15$) of lambs. Moreover, inoculation, enzyme supplementation, and their interaction had no significant effects on carcass traits ($P \geq 0.06$), commercial cut yield ($P \geq 0.26$), chemical composition of meat ($P \geq 0.13$), and meat tenderness and color ($P \geq 0.11$). In terms of the fatty acid composition of *longissimus* muscle, inoculation of corn silage increased concentrations of saturated fatty acids (SFA; $P = 0.035$) from 46.2% to 47.6%, and reduced concentrations of unsaturated fatty acids (UFA; $P = 0.035$) from 53.8% to 52.5%, compared to the NI diet. Inoculation also reduced the UFA:SFA ratio ($P = 0.031$) from 1.17 to 1.11, a small but significant difference. Dietary supplementation with amylolytic enzymes did not alter ($P \geq 0.09$) SFA, UFA, and polyunsaturated

Abbreviations: ADFom, acid detergent fiber expressed exclusive of residual ash; ADG, average daily gain; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; BW, body weight; CCW, cold carcass weight; CCY, cold carcass yield; CFU, colony-forming units; CL, cooking loss; CLA, conjugated linoleic acid; CP, crude protein; DE, digestible energy; DM, dry matter; DMI, dry matter intake; DU, dextrinizing unit; EE, ether extract; FA, fatty acid; FFA, free fatty acid; GE, gross energy; HCW, hot carcass weight; HCY, hot carcass yield; LDL, low-density lipoprotein; LMA, *longissimus* muscle area; ME, metabolized energy; OM, organic matter; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TMR, total mixed ration; TN, total nitrogen; UFA, unsaturated fatty acid; VFA, volatile fatty acid; WBSF, Warner-Bratzler shear force; WHC, water-holding capacity

* Corresponding author at: Departamento de Zootecnia, Universidade Estadual Paulista (UNESP), Via de acesso Prof. Paulo Donato Castellane, km 5, 14884-900, Jaboticabal, SP, Brazil.

E-mail address: erika.lara@agroceres.com (E.C. Lara).

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fatty acid concentrations, or their ratios. In conclusion, our results indicated that the silage inoculant examined in the present study is recommended for use in improving lamb production at the farm scale, whereas inclusion of amylolytic enzymes is not required under our experimental conditions.

1. Introduction

Demand for animal products may double in developing countries by 2030 (FAO, 2015). Associated with this increased demand, consumers may play an important role in the meat production chain due to growing interest in how livestock diets can improve the meat quality of ruminants, thus promoting human health and disease prevention (Furnols et al., 2011; Scollan et al., 2006). As such, development of livestock management strategies that improve meat quality, such as the supplementation of ruminant diets, is essential. For instance, amylolytic enzymes are commonly used as direct-fed additives in order to improve starch utilization in high-grain diets (Nozière et al., 2014). In Brazil, this strategy is particularly important because hard-texture corn hybrids are mostly used, in which high vitreousness endosperms are predominant (Pereira et al., 2004). The vitreous endosperm possesses a continuous and abundant protein matrix, which limits the enzymatic digestion of starch and then reduces ruminal degradability of grains (Kotarski et al., 1992; Philippeau and Michalet-Doreau, 1997), compromising the total-tract starch digestibility (Bal et al., 1997). In this regard, dietary supplementation with amylolytic enzymes leads the starch hydrolysis by cleaving starch polymers to low molecular weight oligosaccharides. In turn, the availability of starch hydrolysis products might increase in the rumen, shifting the ruminal fermentation (e.g., increasing propionate) process with gains in digestibility and efficiency of dietary utilization (Tricarico et al., 2008). Improvements in digestibility of starch and other feed components (likely by cross-feeding effect) might result in increased dry matter intake (DMI), the way so that livestock production could be enhanced (Tricarico et al., 2008).

Furthermore, enhanced ruminal degradation of starch alters ruminal fermentation by increasing the molar proportion of propionate and concentrations of total volatile fatty acids (VFA; Church, 1993). Increased concentration of VFA (accounts for up 70% of the energy requirements of ruminants) may result in higher gluconeogenesis due to metabolism of propionate in the liver, whereas a higher synthesis of long-chain fatty acids (FA) in adipose tissue is expected due to metabolism of acetate (Bergman, 1990; Ingle et al., 1972). Thereby, increasing total amount of VFA production might increase FA synthesis in adipose tissue. Although previous research has suggested that dietary supplementation with amylolytic enzymes have no effect on the carcass traits of lambs (McAllister et al., 2000; Prado et al., 2015), to the best our knowledge, meat quality and FA composition has not been appropriately examined in finishing feedlot lambs fed diets supplemented with amylolytic enzymes.

In addition to amylase supplementation, silage inoculants have been used to improve fermentation and aerobic stability of corn and other crops silages worldwide (Kung et al., 2003), so that the nutritional value of livestock diets could be improved as well. Regarding the silage inoculants, *Lactobacillus plantarum*, a facultative heterofermentative lactic acid bacterium, is the most common microorganism used to preserve the nutritional quality of ensiled forages by improving lactic acid fermentation and inhibiting deleterious epiphytic microbes (McDonald et al., 1991; Oliveira et al., 2017). Moreover, the *Bacillus subtilis*, a bacterium commonly found in the soil, was proposed to be used as a silage additive because of its potential to increase the aerobic stability of corn silage (Basso et al., 2012) by producing antifungal compounds (Zuber et al., 1993). The combination of the bacteria described above was tested in corn silage by Lara et al. (2016), who reported that inoculation improved silage digestibility. Improvements in corn silage preservation with gains in digestibility is particularly important because this feed is generally a component of the total mixed ration (TMR) used for livestock production in Brazil (Oliveira and Millen, 2014). Nevertheless, inoculation with *Lactobacillus plantarum* and *Bacillus subtilis* had no effect on dry matter intake (DMI) and average daily gain (ADG) of finishing feedlot beef cattle (Rabelo et al., 2018). However, to date few studies have focused on the effects of silage inoculation with these bacteria on the performance of livestock, and to the best of our knowledge there have been no studies on the effects of silage inoculation with these bacteria on meat quality.

It was hypothesized that both inoculation of corn silage and dietary supplementation with amylolytic enzyme could be used to manipulate feed utilization in order to improve performance and meat traits of lambs. As such, the objective of the current study was to examine the effects of bacterial inoculation of corn silage and dietary supplementation with amylolytic enzymes, and their interaction, on the growth performance and carcass and meat traits of lambs.

2. Material and methods

2.1. Ethics statement

Animal care and handling procedures were carried out in accordance with the Brazilian College of Animal Experimentation (COBEA) guidelines and were approved by the Ethics, Bioethics, and Animal Welfare Committee (CEBEA) of the FCAV/UNESP-Jaboticabal campus, Brazil (protocol no. 1.754/15).

2.2. Crop harvest and silage preparation

A flint corn (hybrid 2B710, Dow AgroSciences Cravinhos, SP, Brazil) was planted on December 18, 2013, on the grounds of São