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Digestibility and growth performance of sheep fed alfalfa hay treated with fibrolytic enzymes and a ferulic acid esterase producing bacterial additive

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ABSTRACT

This study was conducted to determine the effects of fibrolytic enzymes applied at baling, with or without ferulic acid esterase (FAE) producing bacterial inoculant, or to hay at feeding on digestibility and growth performance of lambs. Prior to starting the animal studies, two runs of replicated 24- and 48-h batch culture *in vitro* incubations were conducted using control alfalfa hay to select a suitable enzyme and dose. Eleven replicate bales of alfalfa-grass (93.8:6.2) hay (~500 kg) were produced with the application of one of three treatments: control (water), enzyme applied at baling (Eb; Econase RDE-L, AB Vista, Wiltshire, UK) and enzyme plus ferulic acid esterase producing bacterial additive applied at baling (EIb; 11 GFT, Pioneer HI-Bred Ltd., Chathan, ON, Canada). The mean internal bale temperature after 50 days of storage was greater ($P<0.001$) for Eb than control and EIb, as was the post-storage hemicellulose concentration ($P<0.05$). Two animal experiments using lambs were conducted after bales were stored for at least 90 days. The digestibility study was a replicated 4×4 Latin square design with 16 lambs and the animal performance study consisted of 32 lambs (8 per treatment) in a randomized complete block design. In both studies lambs received one of four treatments: control, Eb, EIb and enzymes added to control hay at feeding (Ef). In the digestibility study, total tract apparent organic matter (OM) ($P=0.07$) digestibility tended to be affected by treatment, with OM digestibilities greater for lambs fed Ef compared with lambs fed the other treatments, although differences were small (Ef *vs.* others; OM, 0.658 *vs.* 0.646). However, neutral detergent fiber (aNDF) and hemicellulose digestibilities were greatest ($P<0.05$) for lambs fed Eb, with no differences among the other treatments (aNDF, Eb = 0.480, control = 0.437, Ef = 0.430, EIb = 0.430; hemicellulose, Eb = 0.524, control = 0.460, Ef = 0.458, EIb = 0.446). In both studies there was no effect ($P>0.05$) of treatment on OM intakes. Average daily gain (ADG, g/d) of lambs in the performance study was greater ($P=0.048$) for EIb (233) than control (192) and Ef (202), and intermediate for Eb (206). Feed efficiency tended to be affected ($P=0.07$) by treatment; gain:feed for EIb was 18% greater than control and Eb and Ef were similar to the control.

Abbreviations: ACT = 50 days, accumulated difference in daily temperatures of bales and ambient temperatures within 50 days of storage; ADF, acid detergent fiber inclusive of residual ash; ADG, average daily gain; ADICP, acid detergent insoluble crude protein; aNDF, neutral detergent fiber inclusive of residual ash; BW, body weight; DM, dry matter; DMD, dry matter degradability; DMI, dry matter intake; Eb, fibrolytic enzyme product added at baling; ENZ1, Econase RDE-L, AB Vista, Wiltshire, UK; EIb, fibrolytic enzyme plus ferulic acid esterase producing bacterial inoculant added at baling; Ef, fibrolytic enzyme added at feeding; FAE, ferulic acid esterase; FE, feed efficiency; OM, organic matter; ENZ2, Rovabio Excel LC, Adisseo, Alpharetta, GA, USA.

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This study showed that applying enzymes to alfalfa hay at baling decreased aerobic stability, and increased fiber content and its digestibility, but ADG and gain:feed of lambs were not improved. Adding FAE producing bacterial inoculant with enzymes at baling improved aerobic stability of hay and ADG and gain:feed of lambs were increased relative to lambs fed control and enzymes applied at feeding. Applying enzymes at feeding increased apparent OM digestibilities but not fiber digestibilities, and had no effect on animal performance. We conclude that fibrolytic enzyme application with FAE producing bacterial inoculant at baling was the most promising method for enhancing performance of lambs fed baled alfalfa hay.

1. Introduction

Forage is fundamental to ruminant production so forage conservation with minimal loss of dry matter (DM) and nutrients is paramount. Alfalfa is a widely used forage crop that is grown and preserved as hay, haylage, or silage in North America, Europe, and elsewhere. Its digestible energy content is largely determined by the digestibility of fiber. In recent years, the use of exogenous fibrolytic enzymes to improve the digestibility of forages has been the focus of considerable research, as reviewed by Beauchemin et al. (2003) and Adesogan et al. (2014). Some experiments conducted *in vitro* have reported an increase in DM and fiber degradability when ground forage was supplemented with enzymes during incubation (Colombatto et al., 2007; Gallardo et al., 2010). Consistent with these *in vitro* studies, some *in vivo* studies with sheep (Titi, 2004) and cattle (Feng et al., 1996) reported that enzyme supplementation of diets at the time of feeding improved intake and digestibility, resulting in greater average daily gain (ADG) of cattle (Beauchemin et al., 1999). However, other studies in sheep (McAllister et al., 2000; Rojo et al., 2005) and in cattle (ZoBell et al., 2000; Krueger et al., 2008b) reported no effects of enzyme treatments on ADG when applied at feeding. Similarly, other experiments reported increases in intake and digestibility of DM in lambs (Giraldo et al., 2008) and steers (Krueger et al., 2008b) with the use of enzymes applied to the diet, while others reported no effect in lambs (Miller et al., 2008; Awawdeh and Obeidat, 2011).

Inconsistencies in animal responses to added enzymes are multifactorial, and can possibly be attributed to four main factors: enzyme characteristics (*e.g.*, differences in enzyme preparations, enzymic activities, units of activity added, pH, and temperature effects on activity), forage (*e.g.*, type, maturity), animal (*e.g.*, species, age) and management (*e.g.*, diet, mode of enzyme application, application rate, interaction time of enzymes applied to feed) (Beauchemin et al., 2003). In most feeding studies, enzymes were applied to forage or to the total mixed ration immediately prior to feeding to promote interaction between the enzyme and substrate before ruminal fermentation. Association of the enzyme to the feed can cause the release of soluble sugars for use by the rumen microbes and the initial hydrolysis can create sites for microbial attachment to feed particles (Adesogan, 2005).

Applying enzymes to forage at the time of preservation could increase the period in which enzymes interact with fiber and may prevent competitive effects between rumen microbial enzymes and exogenous enzymes (Adesogan, 2005). Recently, Lynch et al. (2013) reported increased *in vitro* fiber degradability when wilted forage was supplemented with fibrolytic enzymes at baling and stored for 90 days, compared to an untreated control. Furthermore, applying enzymes to forage at baling could improve uniformity of application (Adesogan, 2005) and eliminate the labor associated with daily application of enzymes at feeding. However, few studies have compared the relative merits of applying enzymes at feeding compared with application at baling.

Many commercial enzyme products used to improve feed digestion by ruminants contain cellulases and xylanases (Adesogan et al., 2014). However, the extent of cell wall digestion is largely controlled by ferulic acid, which is linked by ether and ester bonds with lignin and by ester bonds with polysaccharides (Jung and Deetz, 1993; Ralph and Helm, 1993; Yu et al., 2005). Thus, use of cellulases and xylanases in combination with ferulic acid esterase (FAE) may be an effective way of hydrolyzing the cross linkages of the cell wall. Applying these additives at the time of baling the forage may provide ample time for their interaction with the cell wall. A third-generation FAE producing bacterial inoculant that breaks the ester linkages between ferulic acid and more digestible carbohydrates has been reported to improve feed value of barley silage as well as feed efficiency of feedlot cattle (Addah et al., 2012). Also Lynch et al. (2013) reported that the application of this inoculant together with a cellulase/xylanase enzyme product at baling increased *in vitro* fiber digestibility when compared to an untreated control of alfalfa hay after 90 days of storage. Improvement in *in vitro* fiber digestibility, with the use of enzymes and FAE inoculant applied at baling could lead to improved animal performance.

The objectives of this study were to: (1) determine the effects of using exogenous fibrolytic enzymes applied to alfalfa hay at baling compared with at feeding on feed intake, digestibility, and growth of lambs; and (2) determine if the effects of exogenous enzymes applied at baling could be enhanced when used in combination with a FAE producing bacterial inoculant. The hypothesis of the study was that an enzyme additive, applied at the time of baling, would increase fiber digestibility of hay and therefore the intake, growth, and feed efficiency of lambs, and that the response would be further increased when enzymes were used in combination with FAE.