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The effect of different additives on the fermentation quality, *in vitro* digestibility and aerobic stability of a total mixed ration silage

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ABSTRACT

Fermented total mixed ration (TMR) is novel feed for ruminants in Tibet. Studies were conducted to compare the effects of using microbial inoculants, molasses and ethanol on the fermentation quality, nutritive value and aerobic stability of TMR silage. TMR were ensiled in laboratory silos (5 L) and treated with (1) no additive (control), (2) ethanol (E), (3) molasses (M); (4) *Lactobacillus plantarum* (L); (5) ethanol + molasses (EM); (6) ethanol + *L. plantarum* (EL). Ethanol was applied at 25 ml/kg fresh weight (FW), molasses was applied at 30 g/kg FW, and *L. plantarum* was applied at 10^6 cfu/g FW. All silos were opened after 45 days of ensiling, three silos per treatment were used for fermentation quality study and 18 silos for aerobic stability test. After 45 days of ensiling, TMR silages treated with ethanol had highest pH ($P < 0.05$), inoculant with or without ethanol significantly ($P < 0.05$) increased lactic acid (LA) concentration, decreased pH and ammonia nitrogen (AN) compared with control. Butyric acid (BA) concentration in L, EM and EL silages were significantly lower ($P < 0.05$) than that of control. Ethanol significantly ($P < 0.05$) decreased the population of lactic acid bacteria (LAB), aerobic bacteria and yeast. Ethanol alone or in combination with molasses or inoculant improved aerobic stability of TMR silages, indicated by higher and more stable LA content, smaller rise in pH, aerobic bacteria and yeast count than silages without ethanol. The pH for EL and EM remained at 3.91–4.41, whereas that in control, M and L gradually increased to exceed 4.80 after 9 days of aerobic exposure. Aerobic bacteria and yeast counts in control, M and L silages were increased to significantly ($P < 0.05$) higher value than those of E, EM and EL silages after 9 days of aerobic exposure. These results indicate that ethanol played an important role in the inhibition of aerobic bacteria and yeast growth and improvement of aerobic stability of TMR silages.

Abbreviations: AA, acetic acid; ADF, acid detergent fibre; aNDF, neutral detergent fibre; AN, ammonia nitrogen; BA, butyric acid; BC, buffer capacity; cfu, colony-forming units; DM, dry matter; E, ethanol; EM, ethanol + molasses; EL, ethanol + *Lactobacillus plantarum*; FW, fresh weight; DM-D, *in vitro* dry matter digestibility; NDF-D, *in vitro* neutral detergent fiber digestibility; LAB, lactic acid bacteria; L, *Lactobacillus plantarum*; M, molasses; TMR, total mixed ration; PA, propionic acid; TN, total nitrogen; WSC, water soluble carbohydrates; VFAs, volatile fatty acids.

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1. Introduction

Total mixed rations (TMR) have been widely used for dairy cattle because they combine forages, grains, protein feeds, minerals, vitamins and feed additives formulated into a single feed mix to satisfy the nutrient requirement of animal. TMR are highly deteriorative feedstuffs, needs to be prepared near to time of use. This rapid deterioration restricts its use on some farms due to labor shortage. Ensiling TMR avoids daily labor for TMR preparation, it also improve preservation and facilitate long distance transportation. Ensiling industrial and agricultural by-products with concentrate as TMR silage could provide year-round nutrition balance feed, and also could improve the palatability by altering odors and flavors from by-products through silage fermentation (Nishino et al., 2003).

Molasses is a by-product of sugar-cane and sugar-beet industries and has a dry matter (DM) content of 700–750 g/kg and a soluble carbohydrate content of about 650 g/kg DM, of which sucrose is the main component (McDonald et al., 1991). Many researchers reported that molasses addition could stimulate fermentation by supplying substrates for the growth of lactic acid bacteria (Guo et al., 2014). During ensiling, lactic acid bacteria (LAB) have been used as additives to increase the preservation of nutritive value of forages by reducing plant respiration and enzyme activity and by inhibiting deleterious epiphytic microbial populations. *L. plantarum*, inoculated in this study, is facultative heterofermentative lactic acid bacteria and could ferment a wide variety of substrates and quickly produce large amounts of lactic acid.

In Tibet, it is necessary to relocate TMR silages before feeding to ruminants. TMR silages are prepared in the south where surplus agricultural crop are available and later moved north to the dairy farm for utilization. The inconvenient transportation in Tibet decelerated the relocation of silage, which might take 3–5 d, and silages are inevitably exposed to air, opportunistic aerobic microbes proliferate further aggravating the spoilage in the silage mass.

Ethanol has been added to Napier grass (*Pennisetum purpureum*) silage and shown some inhibition of undesirable bacteria that resulted in lower silage losses during the early stage of ensiling (Zhang et al., 2011). To the best of our knowledge, scant information is available about the effect of ethanol on aerobic stability of TMR silages. Much residual ethanol is contained in wet hulless-barley distillers' grains (WHDG), which are abundant in Tibet as an animal feed and the addition of ethanol could provide theoretical basis for using of WHDG.

The objectives of this study were to determine the effect of molasses, *Lactobacillus plantarum*, ethanol and their combined application on the fermentation quality and aerobic stability of TMR silage prepared with forage, agricultural residue and concentrate in Tibet.

2. Materials and methods

2.1. Total mixed ration silage preparation

Whole-plant corn (*Zea mays* L.), alfalfa (*Medicago sativa* L.) and whole-crop oat (*Avena sativa* L.) were cultivated in the experimental field of Rikaze Grassland Station (29°16′ latitude N, 88°53′ longitude E, 3836 m above sea level, Tibet, China). First cutting alfalfa was harvested at 75% bloom, whole-plant corn was harvested at the one-half milk line stage and oat was harvested at the milky stage on 25 September 2013. Hulless-barley (*Hordeum vulgare* L.) and wheat (*Triticum vulgaris* L.) straw were the residues remaining after grain harvested. All of them were chopped to the length of 2–3 cm with manual forage chopper.

The concentrates were obtained from a private small-scale dairy farm in Rikaze Tibet China. The chemical compositions of materials are shown in Table 1, ingredients and chemical composition of total mixed ration are shown in Table 2. TMR mixtures (3.2 kg) were put in plastic box and well mixed to get a uniform distribution of each ingredient, and then packed into 5 L plastic laboratory silo (17.3 cm diameter × 26.5 cm height) equipped with a lid that only enabled gas release, there were 24 silos for each treatment. TMR were treated with (1) no additive (control), (2) ethanol (E; AR grade, 95%, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) applied 25 ml/kg fresh weight (FW), (3) molasses (M; industrial by-product and obtained from a private company at Rikaze) applied at 30 g/kg FW; (4) *L. plantarum* (L; strain G18, Biogrowing Co., Ltd., Shanghai, China) applied at 6 log cfu/g FW; (5) ethanol at 25 ml/kg FW combined with molasses at 30 g/kg FW (EM); (6) ethanol at 25 ml/kg FW combined with *L. plantarum* at 10^6 cfu/g FW (EL). Additives were dissolved in water and added in

Table 1
Chemical composition of ingredients used for the formulation of total mixed ration.

Items[a]	Whole-crop corn	Alfalfa	Whole-crop oat	Hulless-barley straw	Wheat straw	Concentrate
Dry matter (g/kg FW)	219	323	347	910	840	895
Crude protein (g/kg DM)	46.0	217	107	54.3	42.1	189
Neutral detergent fiber (g/kg DM)	512	382	529	744	696	401
Acid detergent fiber (g/kg DM)	247	214	285	312	407	168
Water soluble carbohydrate (g/kg DM)	329	53.7	171	67.1	88.3	121
Ether extract (g/kg DM)	54.9	65.3	58.5	53.9	34.7	81.9
Ash (g/kg DM)	45.6	79.1	88.3	46.4	99.8	82.1
Buffering capacity (mEq/kg DM)	237	543	262	46.2	113	213

[a] DM, dry matter; FW, fresh weight.