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Original Research Article

Probiotic effect of ferulic acid esterase-producing *Lactobacillus plantarum* inoculated alfalfa silage on digestion, antioxidant, and immunity status of lactating dairy goats

Fuhou Li [a, b], Baibing Zhang [a, b], Yixin Zhang [a, b], Xia Zhang [a, b], Samaila Usman [a], Zitong Ding [a, b], Lizhuang Hao [c], Xusheng Guo [a, b, *]

[a] State Key Laboratory of Grassland Agro-ecosystems, School of Life Sciences, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730000, China
[b] Probiotics and Biological Feed Research Centre, Lanzhou University, Lanzhou, 730000, China
[c] Key Laboratory of Plateau Grazing Animal Nutrition and Feed Science of Qinghai Province, The Academy of Animal and Veterinary Sciences, State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, 810016, China

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ABSTRACT

A feeding experiment was conducted to determine the effects of inoculating alfalfa silage with a ferulic acid esterase-producing inoculum on feed digestibility, rumen fermentation, antioxidant, and immunity status of lactating dairy goats. Twenty dairy goats were distributed into 2 experimental groups consisting of control diet (Lp MTD/1, including *Lactobacillus plantarum* MTD/1 inoculated silage) against diet containing silage treated with ferulic acid esterase-producing *L. plantarum* A1 (Lp A1). Alfalfa silage inoculated with a ferulic acid esterase-producing Lp A1 had better fermentation quality than the Lp MTD/1 inoculation. The application of Lp A1 improved silage antioxidant capacity as indicated by greater total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and glutathion peroxidase (GSH-Px) activities in Lp A1 treated silage versus Lp MTD/1 treatment. Compared with Lp MTD/1 treated group, inoculation of silage with Lp A1 increased apparent total tract digestibility of dietary dry matter, organic matter and crude protein, and ruminal concentrations of total volatile fatty acids, acetate, propionate and isobutyrate as well. The results of current study also demonstrated improved antioxidant capacity and immune performance of dairy goats with Lp A1 inoculation. Feeding Lp A1-treated silage increased dairy goats' serum antioxidase activity, such as T-AOC, SOD, GSH-Px and catalase, and the serum concentration of immunoglobulin A, while decreased tumor necrosis factor α, interleukin (IL)-2 and IL-6. In addition, compared with Lp MTD/1, diet containing alfalfa silage inoculated with Lp A1 endowed dairy goats' milk with greater fat and protein contents, improved dairy goat milk quality without affecting feed efficiency.

1. Introduction

Silage is a major source of energy, nutrients, and digestible fiber and a primary feed ingredient for ruminants, especially dairy cows, which makes up over 50% of daily ruminants' ration (Zhu et al., 2013; Li et al., 2021). Studies have shown that increasing the ruminal fermentability of high-quality forage fed to ruminants would have the same benefits as feeding concentrate, because it could increase the energy supply from volatile fatty acid (VFA) and protein from microbial protein synthesis (Broderick et al., 1999; Cantalapiedra-Hijar et al., 2009; Zhu et al., 2013). In addition,

* Corresponding author.
E-mail address: guoxsh07@lzu.edu.cn (X. Guo).
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numerous studies confirmed that the digestible energy content of forage is largely determined by the digestibility of dry matter (DM) or neutral detergent fiber (NDF) (Aboagye et al., 2015; Abdelraheem et al., 2019). Therefore, ensuring the long-term quality of green forage and improving its digestibility have received a great deal of attention in the dairy industry of the world.

Alfalfa is the most cultivated herbaceous legume that provides a major forage protein source for livestock worldwide (Agarussi et al., 2019). A wide variety of silage additives containing fibrolytic enzymes, primarily consisting of xylanases and cellulases, are available for improving silage fermentation and digestibility (Dean et al., 2005; Kang et al., 2009). However, there may exist a greater impact on the nutritive value of silage when cellulase acts on forage for a long-time duration with no indication that the nutritive value of silage is improved (Muck et al., 2018). The efficiency of fibrolytic enzyme is not always satisfactory owing to the lack of effect or a negative effect on the DM and NDF degradability of forage despite the improvements in silage fermentation (Lynch et al., 2014; Jin et al., 2015). The reason could be that enzyme action is limited to more digestible components during ensiling (Adesogan et al., 2019). Besides, the ferulic acid bridge cannot be hydrolyzed by rumen microorganisms due to the cross-link between arabinoxylans and lignin with the cell wall phenolic acids, especially ferulic acid, by ether or ester linkages (Rakotoarivonina et al., 2014), resulting in a decline in in vitro digestibility of DM and NDF (Jin et al., 2015; Adesogan et al., 2019).

Extensive literatures have elucidated the positive effects of carboxylesterase in degrading ferulic acid ester linkages and changing structure matrix of forage lignocellulose (Addah et al., 2012; Li et al., 2019, 2020). Breakage of the link between lignin and cell wall carbohydrates facilitates further degradation of forage in the rumen (Nsereko et al., 2008). This provides more substrates for the fermentation of rumen microorganisms and improves the rate of ruminal digestion of forage. Based on these, we hypothesized that inoculating alfalfa silage with ferulic acid esterase-producing lactic acid bacteria could also improve the nutritive value and utilisation efficiency of silage fed to animals. Furthermore, small ruminants represented by dairy goats are more susceptible to oxidative stress due to their intensive metabolic requirements for maintenance and production, resulting in metabolic and infectious diseases (Sordillo and Aitken, 2009; Tian et al., 2019). Previous studies indicated that ferulic acid is a proven phenolic compound with antioxidant activity, which could be used as a feed additive to alleviate oxidative stress and improve antioxidant capacity of ruminants (Soberon et al., 2012a, b; Wang et al., 2019; Valadez-García et al., 2021). Our previous study showed that inoculation of ferulic acid esterase-producing lactic acid bacteria during ensiling enhanced the forage lignocellulose degradation with a concomitant increase of free ferulic acid concentration in silage compared with the same type of inoculant treatments (Li et al., 2020, 2021). Moreover, the free ferulic acid released from lignocellulose degradation during ensiling improved the antioxidant activity of silage (Li et al., 2021). Therefore, feeding the silage inoculated with ferulic acid esterase-producing lactic acid bacteria probably also show a health benefit for the ruminants. However, to date, there have been no study verified the effect of ferulic acid esterase-producing lactic acid bacteria as a silage inoculant on the in vivo apparent total tract digestibility of silage, as well as the effect on animal antioxidant and immunity status as a consequence of the improvement on fiber degradation of the inoculated silage. Therefore, the experiment was aimed at studying the fermentation quality of alfalfa silage inoculated with ferulic acid esterase-producing lactic acid bacteria, and the in vivo nutrients apparent digestibility, rumen fermentation, blood and milk biochemical indexes, as well as milk yield and composition of dairy goats fed with the ferulic acid esterase-producing lactic acid bacteria treated alfalfa silage as a basal diet.

2. Materials and methods

2.1. Experimental site and animal ethics

The feeding trial was conducted at Dingxi Jupen Forage Co., Ltd. (35.43 °N, 104.52 °E, 2,000 m a.s.l.), located in the Liangmao gully area of the Loess Plateau in China. The area belongs to the mid-temperate semi-arid climate, with an average annual temperature of 5.9 °C. The temperature was changed from −8.4 °C in January to 17.2 °C in July. In addition, the average annual growing period is 93 d, and the annual average precipitation is 500 mm.

All study procedures involving animals were reviewed and approved by the Animal Ethics Committee of Lanzhou University (file no. 2010-1 and 2010-2), and confirming that all experiments were performed in accordance with relevant guidelines and regulations.

2.2. Dairy goats, experimental design, and diets

A total of 20 multiparous lactating Guanzhong dairy goats (body weight [BW], 37.9 ± 3.43 kg; mean ± standard deviation [SD]) averaging from 75 ± 9 (mean ± SD) d in milk (DIM) were used in current study. Dairy goats were randomly assigned into 2 groups of 10 according to the BW, milk yield, and parity exhibited previously to the experiment start. The dairy goats in group 1 averaged 0.82 ± 0.12 kg milk yield per day at the start of the experiment with an average BW of 37.8 ± 3.4 kg, while the average milk yield of the dairy goats in group 2 was 0.84 ± 0.09 kg per day at the start of the experiment with an average BW of 38.1 ± 3.7 kg.

To allow for individual feed intake measurements and excrement collection, each dairy goat was housed individually in a clean metabolic cage with free access to water and the cage was regularly cleaned. The metabolic cage had an area of 0.8 m^2 (1 m height), iron mesh walls, grid raised floor, and a bucket for diet and another for water. A nylon net (aperture 2 mm) that fixed under the grid raised floor was used to separate feces and urine. Within each group, dairy goats were randomly assigned to 1 of 2 experimental diets ($n = 10$), which were formulated to meet the requirements for dairy goats having a BW of 40 kg according to the criteria described of NRC (2007). The experimental diet consisted of 55% alfalfa silage and 45% concentrate mixture on DM basis (concentrate [g/kg product basis]: 550 maize, 100 wheat bran, 300 flaxseed residue, 10 sodium bicarbonate and 40 commercial premixes (Beijing Longyue Xingmu Biotechnology Co., Ltd., Beijing, China)). The experimental diets only differed in the silage inoculants, which silage was inoculated with either *Lactobacillus plantarum* MTD/1 (Lp MTD/1) or *L. plantarum* A1 (Lp A1). In the current study, Lp MTD/1 is a widely adopted commercial inoculant, used to improve the fermentation quality of silages, and it has neither antioxidant nor ferulic acid esterase activity (Ecosyl Products Ltd., Stokesley, UK) (Li et al., 2019, 2021). Whereas Lp A1 is a strain that isolated and screened from the ensiled grass of *Elymus nutans* harvested from the Qinghai-Tibet Plateau, possess ferulic acid esterase and antioxidant activities in addition to improving silage fermentation quality, which has been proved in our previous study (Li et al., 2021). Lp MTD/1 was employed as a control group, and Lp A1 was regarded as an experimental group. During the silage preparation, both strains were provided in the form of freeze-dried powder.

Both groups of dairy goats were fed ad libitum (at least 10% refusals on an as-fed basis) divided into 2 daily meals fed at 07:30 and 17:30. Silage and concentrate were fed separately. The dairy goats were milked twice a day at 08:00 and 18:00 via a portable

milking machine, and the individual goat milk yield was recorded throughout the experiment. Prior to each milking, the udder of the dairy goats was inspected to ensure that they are free from inflammation and there is no milk clot around the teats. During each milking, the teat was cleaned and disinfected by 0.85% sodium chloride solution and 1.0% sodium hypochlorite solution. The experiment lasted for 8 weeks, where the first 2 weeks served as an adjustment period, followed by 6 weeks of feed evaluation and 2 weeks of sample collection (including digestion, rumen fluid, milk, and blood samples). During the adaptation period, all goats were adapting to individual cage and experimental diets, with concomitant sanitary management.

2.3. Silage preparation

The forage for the experimental silages was harvested from a 3-ha plot of perennial cultivated alfalfa (*Medicago sativa* L.) on July 10, 2020. The growth stage of alfalfa was full-bloom and it was a first cut in summer harvest. The forage was harvested in the afternoon, left to wilt for 1 d, and then chopped to 2 to 4 cm filaments in length by a fresh grass shredding machine (9Z-12.0, Zhengzhou Yike Heavy Industry Machinery Manufacturing Co., Ltd., Zhengzhou, China). Immediately after that, the forage was inoculated and baled using a bale wrapper machine with front-conveyor (RX-DK5252C, Zhengzhou Muchang Agricultural Machinery Manufacturing Co., Ltd., Zhengzhou, China). The chemical composition of alfalfa before ensiling is presented in Table 1. After that, bales were wrapped with 4 to 6 layers of stretch film (MY-100, green forage stretch film, Zhengzhou, China; 250 mm wide and 25 μm thick) and stored on a well-drained grass shed for 1 month fermentation (approximately 25 °C), until the onset of the animal trial (Li et al., 2021). For distribution of 2 *Lactobacilli* into the bales, a 20-L spray equipment with 2 nozzles (3WBD-20, Taizhou Luqiao the Ming Hui Electric Sprayer Co., Ltd., Taizhou, China) was used during ensiling, and the nozzles were directly fixed at 20 cm above the conveyor. Prior to the onset of experiment, the number of viable bacteria of both strains were detected by using plate counting method. Before baling commenced, the inoculants were weighed and dissolved directly to the water immediately to achieve an application rate of 5×10^{10} colony forming units (cfu) of viable cells per liter. Based on calibration data, an application rate of 10 L of liquid was applied per metric ton of fresh forage. Thus, the inoculant was applied in a water solution during the loading process to achieve a standard of 5×10^5 cfu/g of grass as applied in current practice. The cylindrical bales were of 0.6 m tall × 0.6 m diameter and weighed approximately 50 kg. Thirty bales were prepared for each treatment.

2.4. Sampling and measurements

During the experimental period, amounts of feed offered and refused of each goat were recorded daily to calculate DM intake (DMI). Approximately 100 g of the silage and concentrate (with 6 replications) were collected once weekly and freezed immediately at −20 °C. The samples of silage and concentrate were pooled by treatments, respectively, after total collection. Each of silage sample was divided into 2 parts. One part was used to determine the fermentation indicators (including pH, organic acids and ammonia nitrogen [NH_3-N]) and antioxidant-related enzyme activity exactly according to the methods as described earlier (Li et al., 2021). Another part of the silage and the total concentrate were dried at 65 °C in a forced air oven for 72 h, and ground to pass through a 1-mm sieve for chemical analyses.

Before the morning feeding from d 43 to 47 (week 7), the total feces of each dairy goat were collected, weighed and recorded daily to determine the apparent total-tract digestibility of nutrients. About 10% of the total feces were subsampled daily into a plastic container, mixed thoroughly by goat after total collection. Subsequently, the feces were acidified by 10% (wt/wt) hydrochloric acid solution with an application rate of 10 mL/kg feces. The feces samples were dried in an oven at 65 °C for 72 h, and then ground through a 1-mm sieve for further chemical analyses.

Dry matter (AOAC method 943.01) and ash (AOAC method 942.05) in samples of offered and refused diet, feces were determined according to AOAC (1999). The nitrogen in the feeds, refusals and feces were measured using Kjeldahl automated apparatus (K9805, Shanghai Analytical Instrument Co., Ltd, Shanghai, China) and crude protein (CP) was computed by multiplying the Kjeldahl nitrogen with 6.25 (Li et al., 2021). Fiber bag (ANKOM F57, 25 μm porosity) technique (ANKOM Technology, Fairport, NY, USA) was used to determine the fiber fractions (neutral detergent fiber [aNDF], acid detergent fiber [ADF]) of the feed and feces according to the method of McRoberts and Cherney (2014). During the aNDF analysis, heat stable alpha-amylase and sodium sulfite were added to eliminate starch and protein effects, respectively, while the aNDF content is inclusive of the residual ash. The method of Zhao et al. (2014) with modification was used for the extraction and measurement of silage ferulic acid concentration. Colorimetric method was used to quantify the water-soluble carbohydrate (WSC) concentration of samples after fully reacting with anthrone reagents (Thomas, 1977).

On d 48, rumen fluid was sampled from each of 20 dairy goats prior to the morning feeding by a clean, flexible esophageal tube. To further avoid saliva contamination, the initial 20 to 30 mL of rumen fluid was discarded and 40 mL rumen fluid was then sampled from each goat using 50 mL screw-cap centrifuge tubes. Ruminal pH was measured by a glass electrode pH meter (pH6220, Sunny Hengping Instrument Co., Ltd, Shanghai, China) immediately after sampling, and the rumen fluid was squeezed through 4 layers of medical gauze. Then, the rumen fluid samples were frozen at −80 °C for subsequent determination of VFA concentration. About 250 g/L (wt/vol) HPO_3 was mixed with ruminal fluid at a ratio of 1:5 and left overnight at 4 °C for the precipitation of true proteins. After centrifugation (4 °C; 15,000 × *g*; 15 min) and filtration with an 0.22-μm dialyzer, the VFA concentration of ruminal fluid was determined by a gas chromatograph (SP-3420A, Beijing Beifen-Ruili Analytical Instrument Co., Ltd, Beijing, China) that equipped with an AT-FFAP type capillary column (30 m × 0.32 mm × 0.5 μm) and a Flame Ionization Detector (Li et al., 2018).

About 100 mL of raw milk samples were collected twice daily from each dairy goat after morning and afternoon milking from d 50 to 55 (week 8), respectively. The first 20 mL of collected milk at each time was discarded, and the milk samples were mixed according to the ratio of 6:4 (vol/vol, morning: afternoon) daily. Thoroughly mixed milk samples were subsampled daily and mixed by goat after total collection. One part of mixed milk was preserved with saturated potassium dichromate solution (Thomson et al.,

Table 1
Chemical composition of alfalfa before ensiling (g/kg DM).

Item[1]	*n*	Mean	SD[2]
DM, g/kg FW	6	449	7
OM	6	909	1
WSC	6	55.7	1.0
aNDF	6	445	1
ADF	6	337	1
CP	6	177	3

[1] DM = dry matter; FW = fresh weight; OM = organic matter; WSC = water soluble carbohydrates; aNDF = neutral detergent fiber, assayed with a heat-stable amylase and expressed inclusive of residual ash; ADF = acid detergent fiber; CP = crude protein.
[2] SD = standard deviation.

2018) at 4 °C for milk fat, protein, lactose, total solids, urea and free fatty acid content determination by an infrared milk analyzer (MilkoScan FT1, Foss Analytical Instruments, United States) within 4 to 7 d after sampling. The remaining part of the milk was stored at −20 °C for the future milk antioxidase activity analysis. The thawed goat milk was centrifuged at 12,000 × *g* for 30 min and the supernatant was used to determine the antioxidase activity. The enzymatic activities of total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were measured using the commercial assay kits, which purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China (A015-2-1, A001-3-2, A005-1-2, and A007-1-1, respectively).

Blood samples were taken from the jugular vein of all goats before morning feeding at d 56 of the experimental period. Vacutainer tubes without any additive (5 mL, Kangjian Medical Products Co., Ltd., Jiangsu, China) were used to collect blood and about 4 mL of blood sample was obtained from each goat. The serum was transferred into a 1.5-mL tube after centrifugation at 1,800 × *g* for 15 min at 4 °C, and stored at −80 °C until further analysis of antioxidase activity, immunoglobulin (Ig)A, IgM, and IgG levels and the concentrations of interleukin (IL)-2, IL-6, IL-1β and tumor necrosis factor-α (TNF-α). The determination of serum antioxidase (including T-AOC, SOD, GSH-Px and CAT) was performed by the commercially available assay kits as above mentioned (Jin et al., 2014). The determination of serum IgA, IgM, and IgG levels was conducted using goat IgA, IgM and IgG ELISA kits (Montgomery, TX, USA) following the manufacturer's instructions. The concentrations of IL-2, IL-6, IL-1β and TNF-α in the serum were determined with the goat enzyme-linked immunosorbent assay kit (Groundwork Biotechnology Diagnosticate Ltd, San Diego, CA) according to the manufacturer's instructions.

2.5. Calculations and statistical analysis

The chemical composition of the wilted alfalfa before ensiling was showed as means ± standard deviation (SD) of the replicates. Apparent digestibility of nutrients was calculated following the formula: nutrient digestibility (%) = 100% − [(feces weight, kg) × (fecal nutrient content, %)/(DMI, kg) × (feed nutrient content, %)] as described earlier (Chen et al., 2020). Data were analyzed using the general linear model procedure of SPSS 21.0 (SPSS, Inc., Chicago, IL, United States) for a randomized complete design. Treatment was the fixed effect, and the dairy goat was the random effect in the model. Tukey's test was used to distinguish the difference between Lp A1 and Lp MDT/1 treatment groups. Levels of significance and extreme significance were set at $P < 0.05$ and $P < 0.01$, respectively.

3. Results

3.1. Silage and diet composition

Chemical composition, ensiling characteristics and antioxidase activity of the experimental alfalfa silages are summarized in Table 2. The Lp A1 treated-silage was slightly lower in aNDF and ADF ($P > 0.05$), but greater in WSC and free ferulic acid ($P < 0.001$), compared with the Lp MTD/1 treated-silage. The pH of Lp A1 treated-silage was lower, but lactic acid, acetic acid and propionic acid concentrations were greater than that of the Lp MTD/1 treated-silage ($P < 0.01$). Compared with Lp MTD/1 treated-silage, Lp A1 treated-silage had greater T-AOC, SOD and GSH-Px activities ($P < 0.05$). The CAT activity of Lp A1 treated-silage was lower than that of Lp MTD/1 treated-silage ($P < 0.001$).

Diet containing Lp A1 treated-silage was slightly lower in aNDF ($P = 0.052$) and ADF ($P = 0.084$) concentrations compared with the diet formulated with Lp MTD/1 treated-silage (Table 3). The OM and CP contents of diet with Lp A1 treated-silage did not differ from the diet with Lp MTD/1 treated-silage ($P > 0.05$).

Table 2
Chemical composition, ensiling characteristics and antioxidase activity of alfalfa silage.

Item[1]	Silage treatment[2]		SEM[3]	*P*-value
	Lp MTD/1	Lp A1		
n	6	6	—	—
Chemical composition, g/kg DM				
DM, g/kg FW	450	452	0.6	0.093
OM	903	901	1.3	0.582
CP	167	169	1.9	0.426
aNDF	453	430	6.7	0.078
ADF	345	328	5.4	0.135
WSC	4.83	6.66	0.285	<0.001
FA	4.61	5.19	0.094	<0.001
Fermentative characteristics, g/kg DM				
pH	4.67	4.54	0.021	<0.001
Lactic acid	70.5	88.2	3.21	0.001
Acetic acid	13.9	20.2	1.02	<0.001
Propionic acid	4.28	6.04	0.287	<0.001
NH_3-N, g/kg of total N	66.6	63.3	1.55	0.300
Antioxidase activity, U/g FM				
T-AOC	158	181	5.6	0.035
SOD	617	636	4.9	0.048
GSH-Px	788	839	9.2	0.002
CAT	14.6	7.82	1.046	<0.001

[1] DM = dry matter; FW = fresh weight; OM = organic matter; WSC = water soluble carbohydrates; aNDF = neutral detergent fiber, assayed with a heat-stable amylase and expressed inclusive of residual ash; ADF = acid detergent fiber; CP = crude protein; NH_3-N = ammonia nitrogen; FA = ferulic acid; T-AOC = total antioxidant capacity; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; CAT = catalase.
[2] Lp MTD/1 = alfalfa silage inoculated with *Lactobacillus plantarum* MTD/1; Lp A1 = alfalfa silage inoculated with *Lactobacillus plantarum* A1.
[3] SEM = standard error of the mean.

Table 3
Ingredient proportions and nutrient compositions of the treatment diets.

Item[1]	Treatment diet[2]		SEM[3]	*P*-value
	Lp MTD/1	Lp A1		
Ingredient, % of DM				
Lp MTD/1 treated-silage	55	—	—	—
Lp A1 treated-silage	—	55	—	—
Corn	25	25	—	—
Wheat bran	4.5	4.5	—	—
Flaxseed residue	13	13	—	—
Sodium bicarbonate	0.5	0.5	—	—
Commercial premix[3]	2	2	—	—
Chemical composition, g/kg DM				
OM	922	921	1.7	0.865
CP	178	181	1.5	0.442
aNDF	339	326	3.6	0.052
ADF	224	215	2.7	0.084

[1] DM = dry matter; OM = organic matter; aNDF = neutral detergent fiber, assayed with a heat-stable amylase and expressed inclusive of residual ash; ADF = acid detergent fiber; CP = crude protein.
[2] Lp MTD/1 = alfalfa silage inoculated with *L. plantarum* MTD/1; Lp A1 = alfalfa silage inoculated with *L. plantarum* A1.
[3] Premix contained (per kilogram of DM): vitamin A, 600 kIU; vitamin D_3, 180 kIU; VE, 1,100 IU; biotin, 50 mg; β-carotene, 300 mg; Cu, 600 mg; Fe, 1,500 mg; Zn, 1,600 mg; Mn, 1,800 mg; I, 49 mg; Se, 15 mg; Co, 30 mg.

3.2. Intake, diet apparent digestibility and rumen fluid fermentation parameters

The total DM, OM, and CP intake of diets applied with Lp MTD/1 and Lp A1 treated-silages were similar (Table 4). However, the totals of aNDF and ADF intakes were lower in diet with Lp A1 treated-

Table 4
Intake and apparent digestibility in dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* MTD/1 or *L. plantarum* A1.

Item[1]	Treatment diet[2]		SEM[3]	*P*-value
	Lp MTD/1	Lp A1		
n	10	10	—	—
Total intake, g/d				
DM	1370	1381	4.1	0.213
OM	1263	1271	3.7	0.285
CP	247	250	1.8	0.396
aNDF	467	452	2.2	<0.001
ADF	310	298	1.6	<0.001
Silage intake, g/d				
DM	763	765	2.2	0.686
OM	689	690	2.0	0.972
CP	128	129	1.4	0.535
aNDF	346	329	2.2	<0.001
ADF	263	251	1.6	<0.001
Concentrate intake, g/d				
DM	609	616	2.6	0.191
OM	576	581	2.4	0.226
CP	119	121	0.5	0.100
aNDF	121	123	0.5	0.145
ADF	46.5	47.2	0.20	0.201
Apparent total tract digestibility, %				
DM	60.1	63.7	0.49	<0.001
OM	62.3	65.6	0.46	<0.001
CP	70.3	72.9	0.56	0.013
aNDF	37.8	39.0	1.03	0.582
ADF	32.3	32.5	1.11	0.921

[1] DM = dry matter; OM = organic matter; aNDF = neutral detergent fiber, assayed with a heat-stable amylase and expressed inclusive of residual ash; ADF = acid detergent fiber; CP = crude protein.
[2] Lp MTD/1 = alfalfa silage inoculated with *L. plantarum* MTD/1; Lp A1 = alfalfa silage inoculated with *L. plantarum* A1.
[3] SEM = standard error of the mean.

silage versus the diet formed with Lp MTD/1 treated-silage ($P < 0.001$). Dietary DM, OM, and CP digestibilities were greater in Lp A1 treated group versus Lp MTD/1 treated group ($P < 0.05$). But the aNDF and ADF digestibilities were comparable between diets containing Lp MTD/1 and Lp A1 treated-silages.

The pH of the rumen fluid was similar among treatments ($P > 0.05$; Table 5). However, the total VFA concentration of rumen fluid was significantly greater in Lp A1 inoculated silage compared with Lp MTD/1 treatment ($P < 0.001$). Similarly, rumen fluid acetate, propionate, and isobutyrate concentrations were greater in Lp A1 treated group versus Lp MTD/1 inoculated silage treatment ($P < 0.05$). But the concentrations of butyrate, valerate, and isovalerate were similar among treatments. There was a tendency for greater total branched chain VFA (BCVFA) with Lp A1 silage inoculation ($P = 0.070$). In addition, the molar proportion of acetate was greater ($P < 0.05$) and that of butyrate was lower ($P < 0.05$) in the Lp A1 treatment group than in the Lp MTD/1 treatment group. Lp A1 silage inoculation had no effects on the molar proportion of propionate and the ratio of acetate to propionate in the rumen fluid.

Table 5
Ruminal fermentation characteristics of dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* MTD/1 or *L. plantarum* A1.

Item[1]	Treatment diet[2]		SEM[3]	*P*-value
	Lp MTD/1	Lp A1		
pH	6.42	6.36	0.025	0.329
VFA concentration, mM				
Total VFA	45.1	63.8	2.83	<0.001
Acetate	31.4	46.4	2.25	<0.001
Propionate	6.14	8.76	0.436	0.001
Butyrate	5.19	5.89	0.358	0.342
Valerate	0.39	0.47	0.025	0.146
Isobutyrate	0.83	0.97	0.035	0.048
Isovalerate	1.13	1.30	0.050	0.103
Total BCVFA	1.97	2.27	0.083	0.070
VFA composition, %				
Acetate	69.7	72.6	0.64	0.021
Propionate	13.5	13.8	0.44	0.719
Butyrate	11.5	9.22	0.53	0.029
Acetate/Propionate	5.27	5.34	0.185	0.850

[1] VFA = volatile fatty acid; BCVFA = branched chain volatile fatty acid.
[2] Lp MTD/1 = alfalfa silage inoculated with *L. plantarum* MTD/1; Lp A1 = alfalfa silage inoculated with *L. plantarum* A1.
[3] SEM = standard error of the mean.

3.3. Serum and milk serum antioxidase activity of dairy goats

Silage inoculated with Lp A1 significantly affected serum antioxidase activity of dairy goats ($P < 0.05$; Table 6). The diet containing Lp A1 treated-silage had greater serum T-AOC, SOD, GSH-Px, and CAT activities compared with Lp MTD/1 treatment ($P < 0.05$). However, compared with Lp MTD/1 treated group, the inclusion of Lp A1 treated-silage in the diet had a minor effect on milk serum antioxidase activity, except for GSH-Px activity which was obviously greater in Lp A1 treated group versus Lp MTD/1 treatment ($P = 0.021$).

3.4. Serum immunoglobulin and inflammatory cytokines of dairy goats

As shown in Fig. 1, the addition of Lp Al-treated silage to the diet resulted in a greater concentration of IgA in the serum compared with the Lp MTD/1 treated-silage group ($P < 0.05$). However, no difference was found between the concentrations of IgG or IgM in the serum from the 2 treatments.

The concentrations of TNF-α ($P = 0.007$), IL-2 ($P = 0.036$), and IL-6 ($P = 0.041$) in the serum were lower in goats fed with diet containing Lp A1 inoculated silage compared with the diet with Lp MTD/1 inoculated silage (Fig. 2), but the concentration of IL-1β in the serum was not affected by the silage inoculation of Lp A1.

3.5. Milk production and composition of dairy goats

Milk yields and composition are presented in Table 7. Milk yield, fat, and lactose yields were similar among Lp A1 and Lp MTD/1 treatments. However, milk protein ($P = 0.021$) and total solids ($P = 0.027$) yields were greater with Lp A1 inoculation in the silage.

Table 6
Serum and milk serum antioxidase activity in dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* MTD/1 or *L. plantarum* A1.

Item[1]	Treatment diet[2]		SEM[3]	*P*-value
	Lp MTD/1	Lp A1		
Serum, U/mL				
T-AOC	11.6	13.7	0.36	0.040
SOD	96.3	120	8.32	0.007
GSH-Px	723	791	12.2	<0.001
CAT	4.14	5.03	0.496	0.032
Milk serum, U/mL				
T-AOC	6.80	7.98	0.645	0.443
SOD	107	118	7.4	0.386
GSH-Px	167	214	9.6	0.021
CAT	4.31	4.70	0.322	0.275

[1] T-AOC = total antioxidant capacity; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; CAT = catalase.
[2] Lp MTD/1 = alfalfa silage inoculated with *L. plantarum* MTD/1; Lp A1 = alfalfa silage inoculated with *L. plantarum* A1.
[3] SEM = standard error of the mean.

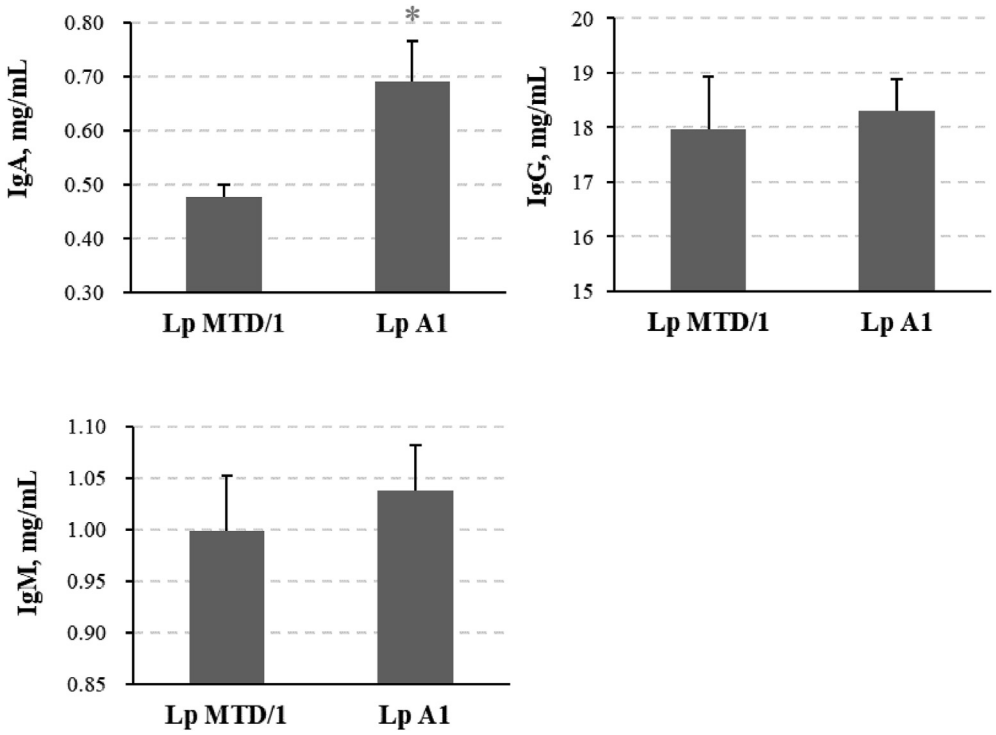

Fig. 1. Serum immunoglobulin concentration in dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* MTD/1 or *L. plantarum* A1. Treatment: Lp MTD/1 = alfalfa silage inoculated with *L. plantarum* MTD/1; Lp A1 = alfalfa silage inoculated with *L. plantarum* A1. *, $P < 0.05$.

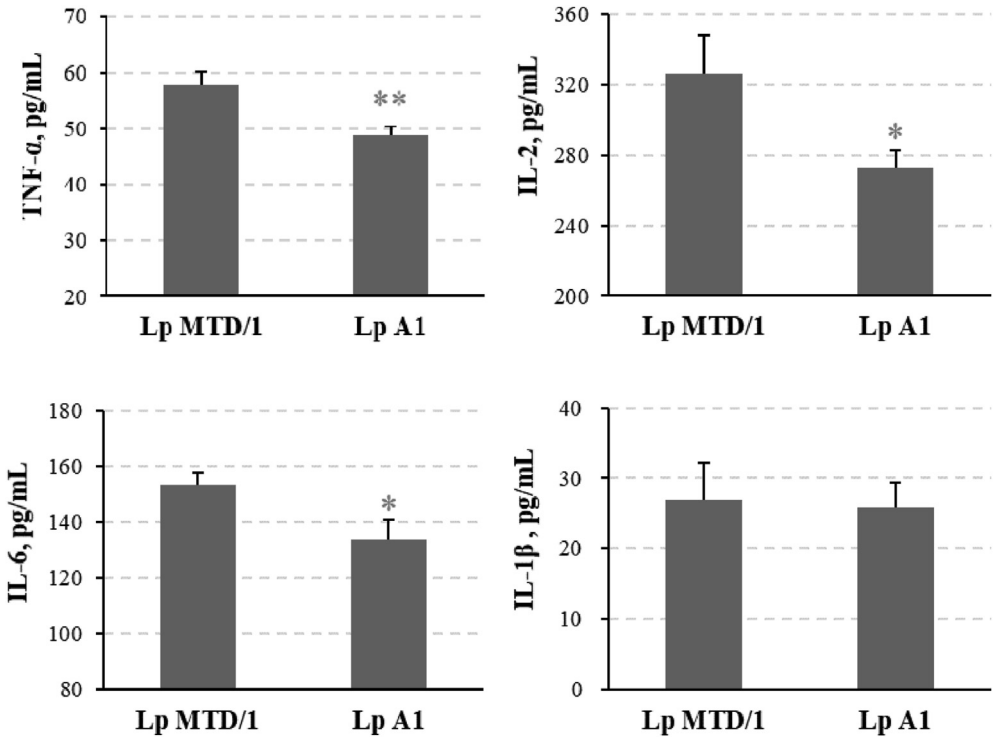

Fig. 2. Serum proinflammatory factors concentration in dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* MTD/1 or *L. plantarum* A1. Treatment: Lp MTD/1 = alfalfa silage inoculated with *L. plantarum* MTD/1; Lp A1 = alfalfa silage inoculated with *L. plantarum* A1. *, $P < 0.05$, **, $P < 0.01$.

Similarly, the milk content for fat ($P = 0.041$), protein ($P = 0.046$), and total solids ($P = 0.039$) were greater for the dairy goats fed the diet containing Lp A1 treated-silage than the diet that contains Lp MTD/1 treated-silage. The milk lactose and free fatty acid contents were similar between treatments ($P > 0.05$). Additionally, feeding efficiency expressed as milk/DMI tended to be greater ($P = 0.060$) in Lp A1 treatment's dairy goats than in dairy goats of Lp MTD/1 treatment.

Table 7
Milk yield and composition in dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* MTD/1 or *L. plantarum* A1.

Item	Treatment[1]		SEM[2]	*P*-value
	Lp MTD/1	Lp A1		
Yields, g/d				
Milk, kg/d	0.74	0.78	0.030	0.565
Fat, g/d	32.5	33.4	0.61	0.106
Protein, g/d	33.5	36.4	0.10	0.021
Lactose, g/d	33.4	35.0	0.40	0.246
Total solids, g/d	100	109	1.5	0.027
Milk composition, g/100g				
Fat	4.19	4.35	0.035	0.041
Protein	4.51	4.67	0.036	0.046
Lactose	4.49	4.50	0.099	0.955
Total solids	13.5	13.9	0.04	0.039
Milk free fatty acid, mM	0.79	0.79	0.064	0.599
Feed efficiency (milk yield/ dry matter intake)	0.54	0.57	0.032	0.060

[1] Lp MTD/1 = alfalfa silage inoculated with *L. plantarum* MTD/1; Lp A1 = alfalfa silage inoculated with *L. plantarum* A1.
[2] SEM = standard error of the mean.

4. Discussion

4.1. Fermentation characteristics and antioxidant activity of silage

In the current study, the fiber fractions and fermentation characteristics of the wrapped silages were similar to those observed for silages ensiled in the mini silos (Li et al., 2021). The fermentation quality of alfalfa silage inoculated with *L. plantarum* A1 was obviously better than that of Lp MTD/1 treatment, which further proved to be consistent with the results of our previous study (Li et al., 2021). The excessive accumulation of organic acids (e.g., lactic, acetic, and propionic acids) in Lp A1 treated-silage caused a substantial decrease in pH (Hu et al., 2009), and this result indicates that more soluble carbohydrates have been transformed into organic acids by microorganisms in the Lp A1 treated-silage compared with Lp MTD/1 treatment. The superiority of residual WSC concentration in Lp A1 treated-silage appears to be justified because aNDF and ADF concentrations decreased numerically by Lp A1 inoculation (Li et al., 2019). The decreases in silage aNDF and ADF with Lp A1 inoculation is generally attributed to the ferulic acid esterase produced by this strain (Li et al., 2019, 2020, 2021). The ferulic acid esterase produced by Lp A1 reduced the crystallinity of the fiber and increased the accessibility of the microbial enzymes or organic acids to the structural polysaccharide by hydrolyzing the ferulic acid ester linkages (Addah et al., 2012). As a result, microbial enzymes or acids increased the amount of WSC available to lactic acid bacteria via fiber degradation (He et al., 2019). This was also demonstrated by the greater free ferulic acid concentration in Lp A1 treated-silage, because further free ferulic acid can be released in silage when it is inoculated with a ferulic acid esterase-producing *Lactobacillus* (Adesogan et al., 2019; Li et al., 2020, 2021).

Both the present investigation and our previous study showed that the addition of Lp A1 in silage significantly increased silage T-AOC as well as GSH-Px activity, and reduced CAT activity (Li et al., 2021). Furthermore, compared with Lp MTD/1 treated-silage, the Lp A1 treated-silage exhibited a greater SOD activity, which was contradictory with our previous study (Li et al., 2021). The underlying mechanism of the greater SOD activity in Lp A1 treated-silage of the current study was probably related to the greater concentration of ferulic acid released from lignocellulose degradation during ensiling, and act as an antioxidant, greater concentration of ferulic acid in the silage enhanced the scavenging ability of O_2^- (Itagaki et al., 2009; Li et al., 2021). Additionally, ferulic acid probably terminated oxidation via providing protons or electrons to free radicals, thereby improving the silage's antioxidant-related enzyme activity (Tian et al., 2019). This result indirectly indicated that the concentration of O_2^- was lower in Lp A1 treated-silage relative to the Lp MTD/1 treated-silage. Compared with Lp MTD/1 treated-silage, the lower activity of CAT in Lp A1 treated-silage might be due to the lower pH in the silage, which inhibited CAT activity (Garcia-Molina et al., 2005). In addition, the low pH of Lp A1 treated-silage might further limit the synthesis and assembly of CAT protein during ensiling. The high antioxidase activity would likely endow alfalfa silage with acceptable antioxidant status on the premise of improving fermentation quality (He et al., 2019). Therefore, based on these results, it could be concluded that alfalfa silage inoculated with Lp A1 might be used as a functional feed to improve the antioxidant status of ruminants and their products. Additionally, the superiority of free ferulic acid concentration in Lp A1 treated-silage may have a positive effect on animal health.

4.2. Apparent digestibility and ruminal fermentation

Lactic acid bacteria inoculants generally had a little impact on fiber degradation and nutrient digestibility of silage due to lack of any enzymatic capability (Weinberg et al., 2007). Although studies have shown that ferulic acid esterase-producing lactic acid bacteria exhibited a positive response on silage in vitro degradation characteristics (Kang et al., 2009; Addah et al., 2012; Jin et al., 2015), to date, there is none of study that verified the effect of ferulic acid esterase-producing lactic acid bacteria on in vivo apparent digestibility of silage. Thus, this study was conducted to investigate the effect of ferulic acid esterase-producing lactic acid bacteria inoculants on the digestibility of diets through feeding experiments, and some comparisons had been made with a homolactic inoculant that is widely used in silage (Hu et al., 2009). The result showed that there was no difference on DM intake between the 2 silage inoculations. However, inoculation of Lp A1 in silage resulted in substantial increases in diet DM, OM, and CP digestibilities as compared with control (Lp MTD/1) group. Based on the present results, it is easy to distinguish clearly that the difference in digestibility was caused by the nutritional differences of the silages because a difference in nutrient compositions was observed between 2 silages, particularly the fiber components (Hosoda et al., 2012; Miller et al., 2021). This should be explained by the fact that the changes of cell wall structure by Lp A1, increased the potentially degradable fraction of the silage, which dramatically improved the nutrients availability of the silage by ruminants (Kang et al., 2009; Addah et al., 2012). The silage accounted for 55% (DM basis) of the total diet in current study. Therefore, the current result of the diets digestibility is considered to potently reflect the nutritional characteristics of different treatments of the alfalfa silages. In situ digestibility studies have shown that complete or partial hydrolysis of ferulic acid ester linkages in normal forage may consequently improve ruminal digestion or increase the susceptibility of cell walls to ruminal digestion (Nsereko et al., 2008; Kang et al., 2009). Despite this, the lack of effect of Lp A1 inoculation on the diet aNDF and ADF digestibilities disagreed with Nsereko et al. (2008), Kang et al. (2009) and Jin et al. (2015), who found an improvement in fiber digestibility of silages treated with ferulic acid esterase-producing inoculants. The reason for the minor effect of ferulic acid esterase-producing Lp A1 on fiber digestibility in our study was unknown. The possibility can be attributed to several facts. Firstly, by degrading the more readily fermented fiber (e.g., hemicellulose), extensive fermentation of forage before feeding lead to greater losses of digestible substrate during the silage preservation, which may improve DM digestibility, but not change or even decrease the digestibility of the residual aNDF and ADF

(Comino et al., 2014; Jin et al., 2017). Secondly, recent research suggested that the reduction in the structural carbohydrate due to fiber degradation during ensilage can result in aggregation of the residual fiber structure, which can increase the fiber strength and subsequently resist the rumen microbes and enzymatic degradation (Desta et al., 2016). Thirdly, the role of rumen microorganisms in the rumen is weaker than that of directly adding enzymes in vitro (Li et al., 2019, 2020). Similar results have also been reported by Tabacco et al. (2011), who found that inoculation with ferulic acid esterase-producing *Lactobacillus buchneri*, alone or in combination with a homofermentative inoculant, did not affect the NDF degradability of maize silages.

Acetate, propionate and butyrate are the key VFA formed in the rumen from the fermentation of dietary carbohydrates, and these could provide ruminants with up to 70% to 80% of all their energy requirements (Van Houtert, 1993). Based on the fermentation results, the inoculant treatments, particularly in Lp A1 treatment, would have facilitated rumen fermentation but hardly affected rumen pH. The greater total VFA concentration in dairy goats' rumen of the Lp A1 treatment probably resulted from the greater OM digestibility of the silage (Cantalapiedra-Hijar et al., 2009; Hosoda et al., 2012), which supplied more fermentation substrate for rumen microbes compared with Lp MTD/1 treatment. Generally, there is extensive acetate production in the rumen of animals fed roughages, while propionate fermentation takes prominence in the rumen receiving high levels of concentrates (Van Houtert, 1993). In the current study, the dominant VFA product of ruminal fermentation from dairy goats in the Lp A1 and Lp MTD/1 treatments was acetate. The greater concentration and molar rate of acetate in Lp A1 treatment indicated that the efficiency of rumen fermentation for the diet was greater in the Lp A1 treatment than in the control (Lp MTD/1 treatment) (Li et al., 2018). Moreover, there was no difference in the ratio of acetate to propionate between the 2 treatment groups, which might be related to the consistency of the composition of the 2 experimental diets (Fimbres et al., 2002).

4.3. Antioxidant capacity and immune performance of dairy goats

In the current study, inoculation with Lp A1 not only improved fermentation quality and antioxidant status of the silage, but also increased nutrient value of the experimental diet. The diet containing the silage inoculated with Lp A1 also increased the serum antioxidase (including T-AOC, SOD, GSH-Px and CAT) activity of dairy goats, thereby enhancing the body's antioxidant capacity of the animals. Studies where plasma was frequently sampled from lactating cows or ram lambs fed free ferulic acid, showed greater ferulic acid concentration in the plasma compared with control (Soberon et al., 2012a, b). Thus, the reason for the greater antioxidase activity in serum of Lp A1 treatment dairy goats could probably be explained by the greater ferulic acid level of the silage, and the greater ferulic acid concentration in the serum when dairy goats were fed diet containing the Lp A1 treated-silage because ferulic acid could be absorbed from rumen almost immediately (Soberon et al., 2012a). In line with the results of our study, Wang et al. (2019) reported that addition of 80 mg ferulic acid/kg diet increased plasma GSH-Px and CAT activities, which alleviated oxidative stress of lambs in cold environment. Similarly, as an antioxidant, Jin et al. (2014) reported that vitamin A supplementation significantly increased the activities of GSH-Px, SOD, CAT, and T-AOC in serum of dairy cows. In lactating dairy cows, Kotsampasi et al. (2017) also found that supplementation of antioxidant-rich pomegranate pulp silage in diet improved blood plasma glutathione-S-transferase (GST) and SOD activities. Moreover, a recent study where lactating dairy goats received anthocyanin-rich purple corn stover silage exhibited a greater level of SOD activity in plasma and milk relative to the sticky corn stover silage (Tian et al., 2019). Based on the result that ferulic acid can enter into circulation intact, which leads us assume that when included in the diet, ferulic acid are probably absorbed into the milk and endowed milk with a desired antioxidant performance. However, there was limited impact of Lp A1 inoculation on milk serum antioxidase activity, except that the GSH-Px activity of Lp A1 treatment was greater than that of Lp MTD/1 treated group. This might be accounted for by the partial excretion of ferulic acid in the urine during the transportation of ferulic acid from the bloodstream to the peripheral tissues (e.g., mammary gland), which ultimately resulted in a comparable level of ferulic acid between the 2 groups of goat milk (Soberon et al., 2012b). In addition, this also could be related to the lesser ingestion of ferulic acid by dairy goats throughout the experiment (Li et al., 2021). In the current study, the intake of ferulic acid from silages for the 2 groups of dairy goats was approximately 0.23 to 0.26 g/kg $BW^{0.75}$ per day, which was below the minimum (0.5 g/kg $BW^{0.75}$ per day) of the ferulic acid intake that could be detected in the milk (Soberon et al., 2012a). Anyhow, feeding the diet that contain Lp A1 treated-silage to dairy goats had a positive effect on improving the milk antioxidant properties.

To investigate the effect of Lp A1 inoculation on the immune status of the experimental animals, 3 principal immunoglobulins and 4 important cytokines in serum were investigated in the current study, which are known to be involved in the natural defence mechanisms for various inflammatory disease (Schmitz et al., 2004; Jin et al., 2014; Zhao et al., 2018). Immunoglobulins are antibodies that produced by the plasma cells and they take a vital role in specific and non-specific immunity (Wei et al., 2021). Cytokines are proteins or small molecular peptides produced by immune cells that can transmit information between cells and play an important role in the regulation and effective immune responses (Zhao et al., 2018). However, cytokines (e.g., TNF-α, IL-2, IL-6 and IL-1β) are also regarded as key pro-inflammatory factors, and their serum concentrations can be used as an indicator for inflammatory reaction (Zhao et al., 2018; Cai et al., 2020; Chen et al., 2021). In the current study, silage inoculated with Lp A1 increased the concentration of serum IgA, but had no effect on the concentration of IgG and IgM. In addition, there exhibited a decrease in the concentration of serum cytokines (such as TNF-α, IL-2 and IL-6) of Lp A1 treatment dairy goats. The greater concentration of IgA in serum, and the numerical increase of IgG and IgM generally indicated an improved immune status in Lp A1 treated group dairy goats (Jin et al., 2014; Chen et al., 2020; Wei et al., 2021). In dairy cows, Jin et al. (2014) reported that feeding greater concentration of vitamin A increased the contents of IgM, IgG, IgA, TNF-α in the serum. Similarly, Wei et al. (2021) found that astragalus root extract could increase the serum concentrations of IgG and cytokine (e.g., TNF-α, IL-2 and IL-6) of early weaned and healthy yak calves. However, compared with the control, the reduction of serum TNF-α, IL-2 and IL-6 in Lp A1 treated group was inconsistent with previous studies (Jin et al., 2014; Wei et al., 2021). The reason is not clearly known but could be probably attributed to the cold weather of the experimental site, because cold environment may induce varying degrees of oxidative stress in dairy goats (Wang et al., 2019). During the experiment, the local daily average temperature was about 10 °C, and a lower temperature was often observed at night. Considerable evidence showed that oxidative stress on ruminants could inhibit the production of serum immunoglobulins and promote an increase of serum cytokine (Sordillo and Aitken, 2009). Hence, low concentrations of TNF-α, IL-2 and IL-6 in serum were noteworthy because this could indicate that feeding Lp A1 treated diets to dairy goats could alleviate the oxidative stress caused by cold environments (Wang et al., 2019). Moreover, Valadez-García et al. (2021) observed that free ferulic acid supplementation to heat-stressed hair ewe lambs reduced the oxidative stress index in

serum. These previous reports led us to presume that high ferulic acid level in serum of the dairy goats fed diet containing Lp A1 treated-silage might have also promoted an increase in cell-mediated immunity and systemic humoral immunity in the dairy goats under the current experimental conditions.

4.4. Milk yield and composition

In the current study, milk yield and composition remained unaffected, except protein and total solids, which were greater in Lp A1 inoculation group versus Lp MTD/1 treated group. Because of the limited numbers of animals and the duration time of experiment, the milk yield result is not validated. Under the experimental conditions of the current study, the digestibility of CP was greater for dairy goats fed Lp A1 treated experimental diet than the Lp MTD/1 treatment. Thus, the large increase in milk protein yield and protein content is probably related to the increase in CP digestibility of the diet, because this may result in the dairy goats of the Lp A1 treatment to synthesize more rumen microbial protein (Gado et al., 2009). Kholif et al. (2014) also reported that elevated milk protein content was related to greater DM, OM and CP digestibility. Propionic acid is considered the precursor of glucose and lactose, commonly resulting in linear elevated milk protein content (Rigout et al., 2003). Thus, the greater milk protein content of the dairy goats in the Lp A1 treatment group may also be related to the greater concentration of propionic acid in the rumen (Rojo et al., 2015). It has been reported that the rumen acetate is the major component required for the synthesis of milk short-chain fatty acid (Rojo et al., 2015). Therefore, greater concentration of acetate in the ruminal fluid of the dairy goats in the Lp A1 treatment group might be the reason for the greater milk fat content (Gado et al., 2009). Zhao et al. (2021) also reported that acetate in the rumen could stimulate milk fat synthesis by increasing de novo fatty acid synthesis in lactating dairy cows. These results suggest that feeding diet containing the Lp A1 treated-silage to dairy goats may improve the nutritive composition of milk compared with feeding a diet based on Lp MTD/1 treated silage. However, further research is required to validate these results under long-term feeding experimental conditions.

5. Conclusion

Alfalfa silage inoculated with the ferulic acid esterase-producing Lp A1 improved silage fermentation as well as apparent DM, OM and CP digestibilities when fed to dairy goats as a basal diet. Feeding the diet containing Lp A1 treated silage also resulted in the improvement of rumen fermentation and greater concentrations of rumen total VFA. Inoculating alfalfa with Lp A1 during ensiling promoted greater T-AOC, SOD and GSH-Px activities, which implies an improved antioxidant capacity of the silage, and consequently increased the antioxidase activity of T-AOC, SOD and GSH-Px in the dairy goats' serum. Feeding the Lp A1 treated silage as a basal diet increased serum IgA and decreased serum TNF-α, IL-2 and IL-6 concentrations, which have a potential benefit on passive immunity of dairy goats. In addition, dairy goats fed alfalfa silage inoculated with Lp A1 had greater milk's fat and protein contents. These findings indicated that inoculating alfalfa silage with Lp A1 is a feasible dietary strategy in dairy goats' husbandry, which can act as a functional feed to improve the digestion, antioxidant capacity and immune performance, as well as milk nutritional composition of the dairy goats.

Author contributions

Fuhou Li: Conceptualization, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft preparation. **Baibing Zhang:** Investigation, Methodology, Software, Validation. **Yixin Zhang:** Investigation, Methodology, Software, Validation. **Xia Zhang:** Investigation, Methodology, Validation. **Samaila Usman:** Investigation, Methodology, Validation, Visualization. **Zitong Ding:** Data curation, Formal analysis, Methodology, Validation, Visualization. **Lizhuang Hao:** Investigation, Methodology, Software. **Xusheng Guo:** Conceptualization, Investigation, Data curation, Funding acquisition, Project administration, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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