


The effects of supplementation of noni (*Morinda citrifolia* L.) fruit polysaccharides-rich extract on antioxidant status and immune function in cashmere goats

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Abstract

This experiment was designed to examine the effects of a dietary supplementation of polysaccharides-rich noni (*Morinda citrifolia* L.) fruit extract (NFP) on the anti-oxidant enzyme activities, cytokines level, and expression of corresponding genes in blood of cashmere goats. Twelve castrated, 2-yr-old male cashmere goats (45.44 ± 3.30 kg of BW ± SD) were used in a 2 × 2 crossover design: the basal diet with or without (CON) supplementation of NFP at 4 g per kg DM (0.4%). Each period lasted for 29 d, including 1 wk for diet transition, 20 d for adaptation, and the last 2 d for sampling. The results showed that NFP supplementation increased ($P < 0.05$) the levels of nitric oxide, interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), and the activities of catalase (CAT), glutathione peroxidase (GPx), thioredoxin reductase (TrxR), and total superoxide dismutase (T-SOD) in serum. The expressions of *CAT*, *GPx4*, *TrxR*, *SOD1*, *IL-6*, and *TNF- α* genes were upregulated ($P < 0.05$), whereas the levels of malondialdehyde ($P = 0.015$) and reactive oxygen species ($P = 0.051$) in serum were reduced. The body weight gain of goats was increased ($P = 0.006$) with a nonsignificant increase of feed intake with NFP supplementation. In conclusion, dietary NFP supplementation enhanced the antioxidant status and immune function in blood of cashmere goats.

Lay Summary

Due to the limited pasture supply and the seasonal imbalance of nutrients in grazed pastures in China, cashmere goats are commonly raised in a confined yard-feeding system, which may result in oxidative stress from a lack of green pastures. Noni (*Morinda citrifolia* L.) fruit polysaccharides contain various biological compounds that function as anti-inflammatory, antitumor, and to enhance immune responses, hence likely to relieve oxidative stress in animals. Previous researches in our laboratory have shown that polysaccharides-rich extract from noni fruit (NFP) enhanced rumen fermentation in cashmere goats. This experiment was designed to evaluate the effect of NFP supplementation on serum antioxidant status and immune function in cashmere goats. The results showed that dietary supplementation of 0.40% NFP enhanced the immune signaling molecule levels and antioxidant enzyme activities by upregulating the expression of related genes in blood and reduced the levels of lipid peroxides and free radicals in serum, while mature goats improved body weight. Therefore, NFP could be a viable source of antioxidants for cashmere goats.

Key words: antioxidant, cashmere goat, immune, noni, polysaccharide

Abbreviations: ABTS, 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); ADF, acid detergent fiber; ADG, average daily gain; CAT, catalase; CON, control treatment; CP, crude protein; DM, dry matter; DMI, dry matter intake; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EE, ether extract; GPx, glutathione peroxidase; IL, interleukin; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; NDF, neutral detergent fiber; NFP, polysaccharides-rich extract of noni fruit; NO, nitric oxide; qRT-PCR, quantitative real-time PCR; ROS, reactive oxygen species; T-AOC, total anti-oxidant capacity; TNF- α , tumor necrosis factor- α ; TrxR, thioredoxin reductase; T-SOD, total superoxide dismutase

Introduction

Inner Mongolia cashmere goat is a dual-purpose breed used for cashmere and meat production in the Inner Mongolian region of China. The fine and soft cashmere fiber it produces is known as “soft gold”, and meat products are also of economic importance for local farmers. Recently, about 700,000 cashmere goats are slaughtered every year, producing nearly 10,000 tons of meat (Xie et al., 2021), and the annual production of over 6,000 tons of cashmere fiber was the main source of income for farmers (Duan et al., 2019). Cashmere goats are predominantly farmed on semidesert and desert

grassland, and used to graze on natural grasses with supplementary feeding in the cold winter (the lowest temperature about -30 °C, which was acquired from the China Meteorological Data Sharing Service System [<http://data.cma.cn/>]) and early spring seasons (Wang et al., 2019). However, due to the limited pasture supply and imbalance of nutrients in deteriorated pastures, intensive and semi-intensive yard-farming systems for cashmere goats have been gradually adopted. Goats are confined in fenced yards with feeders, water supply, and sheds facilitated and no grazing. However, there are some concerns that relate to intensive feeding systems such

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as poor animal health, and development of metabolic and specific infectious diseases (Tiezzi et al., 2019). It has been found that improving the body's anti-oxidant defenses can mitigate the negative effects of intensive rearing conditions on animals (Shourbela et al., 2021; Ogbuwu et al., 2022). Therefore, it is important to improve the anti-oxidant capacity and immune-regulation of cashmere goats under intensive rearing conditions.

Noni (*Morinda citrifolia* L.) is a traditional ethnic medicine in Polynesia with a long history of use both for medicinal and edible purposes. There are abundant noni germplasm resources in south China. In recent decades, noni fruit has attracted much research attention because of its extensive biological and pharmacological properties including anti-cancer (Ma et al., 2020), anti-inflammatory (Kharaeva et al., 2022), anti-oxidant (Wang et al., 2022), and immune modulatory effects (Ezzat et al., 2021). Dietary supplementation of noni fruit has been found to strengthen humoral immune response in broiler chickens (Sunder et al., 2015), to enhance the anti-oxidant enzyme activities in serum of calves (Anantharaj et al., 2017), and to counteract the adverse effect of high-fat intake on yellow catfish growth (Wang et al., 2021).

Fresh noni fruit is difficult to transport and store. Therefore, fruit processing, such as juicing and extracting to improve product life has attracted much attention (Kongpuckdee et al., 2020; Ezzat et al., 2021; Wang et al., 2021; Zhang et al., 2021). Fruit extracts contain polysaccharides that have been found to exhibit various biological activities such as anti-inflammatory (Jin et al., 2021), antitumor (Furusawa et al., 2003), and enhancing cell-mediated immune responses (Nayak and Mengi, 2010). The extract also protected the intestinal mucosal barrier in mice from inducing inflammatory bowel disease (Jin et al., 2019), and reverse edema, leukocyte migration, and inflammatory nociception in mice (Sousa et al., 2018). Ultrasonic-assisted extracts of noni fruit demonstrated a dose response scavenging of free radicals, such as hydroxyl radical, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Li et al., 2020). The polysaccharide can also enhance the activity of catalase (CAT), superoxide dismutase-1 (SOD-1), and glutathione peroxidase (GPx) in oxidative stress-damaged neuronal cells of rats (Chen et al., 2018). In rats fed a high-fat diet, the polysaccharide increased the levels of anti-oxidant enzymes in liver and reduced hepatic lipid peroxidation (Yang et al., 2020). In addition, noni fruit extract played an immunostimulatory role by activating innate and adaptive immune responses in mice (Hong et al., 2019).

So far, the research on noni fruit polysaccharides is focused on the extraction procedure, and analyses of chemical structures and pharmacological activity of effective compounds, mainly in humans and mice (Chen et al., 2018; Li et al., 2020; Yang et al., 2020; Jin et al., 2021). In view of the functions of anti-oxidants and immune regulation of noni fruit in humans and other species of animals, we hypothesized that noni fruit polysaccharides could improve oxidative defense and immune regulation in cashmere goats to counteract oxidative stress that may incur under intensive-rearing conditions. Our laboratory has shown that polysaccharides-rich extract from noni fruit (NFP) could raise the rumen fermentation rate in cashmere goats (unpublished data, Zhang Q et al, Inner Mongolia Agricultural University, China). In this study, we examined the effect of dietary supplementation of

NFP on the anti-oxidant status and immune signaling molecules in serum as well as growth productivity in cashmere goats.

Materials and Methods

The goat experiment was carried out in the Experimental Farm of Inner Mongolia Agricultural University (Hohhot, China). The use of the animals and all experimental procedures were approved by Animal Ethics and Welfare Committee of Inner Mongolia Agricultural University in accordance to the Laboratory Animal Sciences and Technical Committee of the Standardization Administration of China (SAC/TC281).

Preparation of NFP

Detailed information about the preparation of NFP is described elsewhere by Zhang (unpublished data, Zhang Q et al, Inner Mongolia Agricultural University, China). In brief, ripen noni fruit from Wuzhishan City (18.7751°N, 109.5170°E), China was dried at 65 °C and ground by an electric pulverizer (CH-200A, Chenhe Shengfeng Industry and Trade Co., Ltd., Yongkang, China). The powder was then soaked in distilled water at 70 °C for 12 h, and filtered through filter paper. The filtrate was concentrated in a rotary evaporator at 65 °C. The concentrate was dissolved in absolute ethanol (1:4, v/v) at 4 °C for 48 h to separate polysaccharides, and the extract was centrifuged and lyophilized to obtain NFP. The yield of NFP was 11.02% of the dried powder, and the chemical ingredients of NFP are listed in [Supplementary Table S1](#).

Animals, diets, and experimental design

Twelve healthy, castrated male, 2-yr-old cashmere goats (45.44 ± 3.30 kg of BW ± SD) were paired to two groups based on their weights. Two groups were then assigned, in a 2 × 2 crossover design, to two treatments: a basal diet without supplementation (CON) or with supplementation with 4 g/kg DM (0.40% NFP), so each treatment had 12 animal replicates over two periods. The NFP dose was determined according to our preliminary experiment (unpublished data, Zhang Q et al, Inner Mongolia Agricultural University, China). Each period lasted 29 d, including 1 wk for feeding the basal diet for transition, 20 d on the treatment diets (either CON or NFP diet), and the last 2 d for weighing and sampling. Goats were individually penned in an animal house and ad libitum accessed to feed and water. The feed ingredients and chemical composition of the basal diet are shown in [Table 1](#). The diet was offered to the goats twice daily at 0800 and 1500 hours, respectively.

Sampling and measurements

Goats were weighed before the morning feeding on the first two days after the transition period and the adaption period, respectively. The amounts of feed supplied and refusal (about 5% to 10%) were daily weighed during the adaption and sampling periods to calculate daily feed intake. Blood samples were collected by jugular puncture on the last day before the final weighing. An aliquot was stored in a cryogenic vial (Corning, New York, USA), snap-frozen in liquid nitrogen, and stored at -80 °C until mRNA extraction. A second aliquot was centrifuged at 2,500 × g at 4 °C for 15 min to obtain serum. Serum was stored at -20 °C until analyzed for anti-oxidant and immune indicators.

Table 1. Ingredients and chemical composition of the basal diet

Item	Content
Ingredient, g/kg air-dry basis	
Millet straw	589.6
Alfalfa hay	29.6
Oat grass hay	80.8
Corn grain	145.8
Soybean meal	53.0
Distillers dried grains with solubles	33.0
Flax cake	53.0
Limestone meal	1.2
Calcium bicarbonate	1.2
Premix ¹	5.0
Sodium chloride	3.0
Sodium bicarbonate	4.8
Nutrient composition (DM basis)	
Digestible energy, MJ/kg ²	9.95
CP, g/kg	100.4
EE, g/kg	22.4
Calcium, g/kg	6.3
Phosphorus, g/kg	2.9
aNDFom ³	547.1
ADFom ⁴	309.8

¹Provided per kilogram of premix: iron 4 g, copper 0.8 g, zinc 5 g, manganese 3 g, iodine 30 mg, selenium 30 mg, cobalt 25 mg, vitamin A 600,000 IU, vitamin D 250,000 IU, vitamin E 1,250 IU, vitamin K 180 mg, vitamin B1 35 mg, vitamin B2 850 mg, vitamin B6 90 mg, nicotinic acid 2,200 mg, D-pantothenic acid 1,700 mg, vitamin B12 3 mg, biotin 14 mg, folic acid 150 mg.

²Digestible energy was calculated based on the ingredients of the diet and their digestible energy content, not based on the actual dry matter intake.

³Neutral detergent fiber assayed with a heat-stable amylase and expressed exclusive of residual ash.

⁴Acid detergent fiber expressed exclusive of residual ash.

Determination of feed chemical composition

Feed samples were analyzed for DM (method 930.15), ether extract (EE; method 973.18), CP (method 976.05), calcium, and phosphorus (method 935.13) according to the Association of Official Analytical Chemists (AOAC, 2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods of Van Soest et al. (1991) with an Ankom 200I Fibre Analyser (Ankom Technology Co., NY, USA), and sodium sulfite and heat-stable alpha-amylase were used and expressed exclusive of residual ash.

Analyses of antioxidant enzyme activities and immune signaling molecules

The activity of total superoxide dismutase (T-SOD), GPx, and thioredoxin reductase (TrxR), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) concentration in serum were determined using commercial antioxidant kits (Nanjing Jiancheng Bioengineering Institute of China, Nanjing, China) according to the manufacturer's protocols. The concentrations of reactive oxygen species (ROS), interleukin (IL)-1 β , IL-6, tumor necrosis factor-alpha (TNF- α), nitric oxide (NO), and inducible nitric oxide synthase (iNOS) were assayed using commercial ELISA kits (Ruixin Biological Technology Co., Ltd. Quanzhou, China) following manufacturer's protocols.

Total RNA extraction and quality determination

Total RNA was extracted from a 0.5-mL sample of thawed blood using TRIzol reagent (TaKaRa, Dalian, China) according to the manufacturer's protocol. The concentration and purity of the RNA were assessed spectrophotometrically using a microplate reader (Synergy H4, BioTek, USA) at 260/280 nm (OD260/OD280 = 1.8 to 2.0). The integrity of the RNA was evaluated with 2% agarose gel. Subsequently, the total mRNA was treated with a gDNA digester to remove gDNA, and then reverse-transcribed into cDNA on 2 \times Haifai II SuperMix plus using a Haifai II 1st Strand cDNA Synthesis SuperMix kit (YEASEN Biotechnology Co. Ltd, Shanghai, China) according to the manufacturer's protocols.

Quantitative real-time PCR analysis

Quantitative real-time PCR (qRT-PCR) reactions were used to generate cDNAs. *YWHAZ* and β -*actin* by geNorm (Vandesompele et al., 2002) were used as the internal control in the present study. The specific primers used for the target genes are shown in Table 2. The primers for *CAT*, *GPx1*, *GPx4*, *SOD1*, *SOD2*, *IL-1 β* , *IL-6*, *TNF- α* , and *iNOS* genes, and for *YWHAZ* and β -*actin* as reference genes were designed using the NCBI primer blast online software. Besides, the primers for *TrxR* were designed by Gene bank database sequences from Sangon Biotech (Shanghai) Co. Ltd (Shanghai, China). The qRT-PCR reactions were carried out in 10- μ L reactions containing 5 μ L of Haifai qPCR SYBR Green Master Mix (YEASEN Biotechnology Co. Ltd, Shanghai, China), 1 μ L of cDNA template, 0.2 μ L each of 10 μ mol/L forward and reverse primers, and 3.6 μ L of RNase-free water, using a LightCycler 480 Real-Time PCR Design & Analysis System (ROCHE Ltd, Basel, Switzerland). The cycling conditions were set as followings: 95 $^{\circ}$ C for 30 s (hold stage), followed with 40 cycles of 95 $^{\circ}$ C for 30 s (denaturation), 60 $^{\circ}$ C for 30 s (annealing), and 72 $^{\circ}$ C for 20 s (amplification). The relative quantity of target gene mRNA was expressed as $2^{-\Delta\Delta CT}$ using the relative comparative threshold cycle method as described previously (Livak and Schmittgen, 2001). The geometric mean Ct of the two reference genes was used to normalize the qRT-PCR data (Vandesompele et al., 2002).

Statistical analysis

All of the data were analyzed using the PROC MIXED procedure of SAS (version 8.1, SAS Institute Inc., Cary, NC). The statistical model was performed with the treatment (CON, NFP) considered as a fixed effect, and goats nested within crossover design and the crossover period as a random effect. Comparisons among the least square means were performed with the pdiff option and considered as significant with $P \leq 0.05$.

Results

Growth performance

The influence of dietary NFP on growth performance in goats is presented in Table 3. Compared with the CON group, ADG was increased ($P = 0.006$) on 0.40% NFP diet.

Antioxidant activities

Table 4 shows the antioxidant enzyme activities and MDA concentration in serum. Supplementation of 0.40% NFP increased ($P < 0.05$) the activities of CAT, GPx, TrxR, and

Table 2. Primers for quantitative real-time PCR

Target	Primer sequences (5'-3') ¹	GenBank no.	Length (bp)
<i>CAT</i>	F: CACTCAGGTGCGGGATTCT R: ATGCGGGAGCCATATTCAGG	GQ_204786.1	159
<i>GPx1</i>	F: ACATTGAAACCCCTGCTGTCC R: TCATGAGGAGCTGTGGTCTG	XM_005695962.2	216
<i>GPx4</i>	F: TTCCCTTGCAACCAGTTTGG R: TCATCCATTTCCACAGAGGGT	NC_030814.1	105
<i>TrxR</i>	F: TGTAGATGCTGCCGCCTAGACTC R: GTGCCAAACCCGAGAGCCTTTC	XM_005680564.3	91
<i>SOD1</i>	F: ATCCACTTCGAGGCAAAGGG R: GCACTGGTACAGCCTTGTGTA	NM_001285550.1	122
<i>SOD2</i>	F: TCAATAAGGAGCAGGGACGC R: AGCAGGGGATAAGACCTGT	XM_005684984.1	85
<i>IL-1β</i>	F: CATGTGTGCTGAAGGCTCTC R: AGTGTGCGCGTATCACCTTT	D63351.1	173
<i>IL-6</i>	F: GGGCTGCTCCTGGTGATGACTT R: CGATGTGCTTAATGAGAGCTTCGG	HM_565937.1	133
<i>TNF-α</i>	F: CAACAGGCCTCTGGTTCAGAC R: GGACCTGCGAGTAGATGAGG	NC_030830.1	209
<i>iNOS</i>	F: CCAGCCCAAGGTCTATGTTC R: TAGTCCTCCACCTGCTCCTC	XM_013971952.2	189
<i>β-actin</i>	F: ACTGGGACGACATGGAGAAGA R: GCGTACAGGGACAGCACAG	U39357	199
<i>YWHAZ</i>	F: TGTAGGAGCCCGTAGGTCATCT R: TTCTCTCTGTATTCTCGAGCCATCT	AY970970	102

¹ F = Forward primer; R = Reverse primer.

Table 3. Effects of NFP supplementation on growth performance of cashmere goats

Item	CON ¹	NFP ²	SEM	P-value
DMI, kg/d	1.45	1.60	0.059	0.082
Initial body weight, kg	46.2	46.4	1.118	0.633
Final body weight, kg	47.3	48.0	1.141	0.141
ADG, kg/d	0.057	0.082	0.006	0.006

¹Diet without NFP supplementation.

²Diet supplemented with 0.4% NFP.

T-SOD, and reduced ($P = 0.015$) the MDA concentration compared with the CON. There were no significant changes in ROS concentration ($P = 0.051$) and T-AOC ($P = 0.069$) between the two groups.

Immune signaling molecule levels

The results in Table 5 show the concentrations of immune indicators in serum. Supplementation of NFP raised ($P < 0.05$) IL-6, TNF- α , and NO levels, and did not influence the iNOS concentration ($P = 0.095$) and IL-1 β concentration ($P = 0.177$) compared with the CON.

The mRNA expression

The relative mRNA expressions of genes in blood are presented in Table 6. In comparison with the CON, NFP supplementation upregulated the mRNA expression of *CAT*,

GPx4, *TrxR*, *SOD1*, *IL-6*, and *TNF-α* genes ($P \leq 0.05$). There were no significant changes in the expression of *GPx1*, *IL-1β*, *iNOS* genes ($0.05 \leq P \leq 0.10$) and *SOD2* gene ($P = 0.169$) between the two groups.

Discussion

Antioxidant activities in serum

In response to external stress and the processes of nutrient digestion and metabolism, animals are prone to produce free radicals (such as ROS) and lipid peroxides that are catabolized to MDA (Kosaka et al., 1996). The excessive levels of the free radicals and peroxides can cause oxidative stress in the body. Antioxidant enzymes, including *CAT*, *TrxR*, *GPx*, and *SOD* will scavenge the free radicals and peroxides and maintain the redox homeostasis (Adam, 2017; Mahaseth and Kuzminov, 2017), as indicated by an increase in anti-oxidant enzyme activity and a decrease of the MDA and ROS concentrations (Inal et al., 2001). In the present study, we demonstrated that a dietary supplement of 0.40% NFP to cashmere goats enhanced the activity of anti-oxidant enzymes (*CAT*, *GPx*, *TrxR*, and T-SOD) and up-regulated the expressions of the related genes (*CAT*, *GPx4*, *TrxR*, and *SOD1*) in blood, indicating improvement of antioxidant capacity in the body. As a result, serum levels of MDA and ROS were lowered in cashmere goats. Our results are supported by the observation of Chen et al (2018) that polysaccharides rich aqueous extract of noni juice exhibited anti-oxidant defense in vivo by restoring cellular antioxidant enzyme activities,

Table 4. Effects of NFP supplementation on serum anti-oxidant status of cashmere goats

Item	CON ¹	NFP ²	SEM	P-value
CAT, U/mL	2.87	3.65	0.250	0.043
GPx, U/mL	105.90	147.54	11.284	0.022
TrxR, U/mL	12.14	16.14	1.295	0.045
MDA, nmol/mL	2.84	2.40	0.109	0.015
ROS, IU/mL	95.06	68.98	7.596	0.051
T-SOD, U/mL	89.05	91.80	0.949	0.012
T-AOC, U/mL	1.92	2.66	0.247	0.069

¹Diet without NFP supplementation.²Diet supplemented with 0.4% NFP.**Table 5.** Effects of NFP supplementation on immune responses in serum of cashmere goats

Item	CON ¹	NFP ²	SEM	P-value
IL-1 β , pg/mL	24.44	29.70	1.892	0.177
IL-6, pg/mL	33.62	45.99	2.449	0.011
TNF- α , pg/mL	52.45	64.83	2.296	0.012
NO, μ mol/mL	8.31	10.11	0.395	0.025
iNOS, ng/mL	9.16	11.36	0.657	0.095

¹Diet without NFP supplementation.²Diet supplemented with 0.4% NFP.**Table 6.** Effects of NFP supplementation on gene expression of anti-oxidant enzymes and immune cytokines in blood of cashmere goats

Item	CON ¹	NFP ²	SEM	P-value
CAT	0.704	2.351	0.392	0.010
GPx1	0.870	1.127	0.170	0.060
GPx4	0.983	1.369	0.140	0.022
TrxR	0.864	0.962	0.022	0.033
SOD1	0.829	1.226	0.128	0.050
SOD2	0.664	0.871	0.096	0.169
IL-1 β	0.978	1.568	0.215	0.082
IL-6	0.661	1.345	0.189	0.037
TNF- α	1.946	2.296	0.908	0.032
iNOS	0.449	1.335	0.292	0.071

¹Diet without NFP supplementation.²Diet supplemented with 0.4% NFP.

up-regulating expression of *heme oxygenase-1*, *CAT*, and *SOD1*, and increasing the nuclear accumulation of nuclear factor erythroid 2 related factor 2 in rat SH-SY5Y cell damaged by tert-butyl hydroperoxide.

The present study showed that the expression of *GPx4* and *GPx1* in blood were up-regulated by supplementation of 0.40% NFP in the diet for cashmere goats. In the GPx family, GPx1 uses glutathione as a cofactor to reduce hydroperoxides to their corresponding alcohols (Brigelius-Flohe, 2006), and GPx4 is associated with the gene expression of cytokines (Hugejiletu et al., 2013) and is the major antioxidant enzyme that directly reduces phospholipid hydroperoxides within membranes and lipoproteins (Brigelius-Flohe, 2006). The reduction of MDA in serum agrees with the up-regulation of *GPx4* expression in blood by NFP supplementation in the

present study. In the future, we will investigate the upstream pathway mediating phase II detoxifying enzyme genes such as *GPx* isomers and the downstream corresponding peroxides in cashmere goats supplemented with NFP to define specific metabolic pathways affected by NFP.

The strengthening of antioxidant defense in goats by NFP may be related to the number of hydroxyl groups complexed with metal ions (such as Fe²⁺ and Cu²⁺) in polysaccharides, and these complexes are necessary for scavenging free radicals and reducing lipid peroxides (Volpi and Tarugi, 1999). Sousa et al. (2018) showed that heteropolysaccharides from noni fruit, composed mainly of homogalacturonan and rhamnogalacturonan, contained a large number of hydroxyl groups. The NFP used in the present study contained 44.52% saccharides (Supplementary Table S1), indicating

that the reduction of MDA and ROS in serum of cashmere goats might be related to the number of hydroxyl groups in NFP saccharides. The monosaccharide concentration of NFP was not determined in the present study, so the pathways involved needs further clarification. In addition, the NFP extract obtained after lyophilization in this study was friable and easily crushed. He et al. (2018) found that the friable structure of polysaccharides was attributed to their smaller molecular weights and related to stronger anti-oxidant activities as the larger surface area of a small size particle enables easier access by free radicals (Zhang et al., 2016). Therefore, the relationships between saccharide composition and structures of NFP with influences on anti-oxidant activity warrants further investigations.

Immune responses in serum

We hypothesized that NFP would have an immune-regulatory role in intensively-reared cashmere goats. Our results in the present study showed that NFP supplementation upregulated the expression of *IL-6* and *TNF- α* in blood, and their corresponding concentrations in serum were increased, while the *IL-1 β* expression and concentration were not statistically significant although the values were higher compared with those for the CON, indicating that the NFP extract can modulate some components of immune cytokines. Our results support the hypothesis. This is the first report on the effect of NFP on cytokines in cashmere goats. Cytokines are the core of immune cell communication and activity (Bernstein & Murasko, 1998), and their variations are usually used to evaluate the immune function of the body. *IL-1 β* , *IL-6*, and *TNF* are three of the most prominent cytokines associated with the innate immune response (Schroder et al., 2010). *IL-1* can be rapidly produced by monocytes and macrophages activated by microbial products (Lin et al., 2002), and promote the adhesion of neutrophils, eosinophils, basophils, and other leukocytes to endothelial cells, which is a key prerequisite step for local inflammatory infiltration (Ulich et al., 1991; Dinarello, 1996). *IL-6* can cooperate with *IL-1* to promote the proliferation of T-lymphocytes, and *TNF- α* is a helper T-cell 1 (Th1) cytokine and a vital cell-derived inflammatory mediator, participating in the pro-inflammatory response like *IL-1 β* and *IL-6* (Beutler and Cerami, 1986; Vielhauer and Mayadas, 2007). Our results with goats are similar to those reported in mice. In a rat model, polysaccharides in noni fruit extract could stimulate effector cells to release various mediators, such as *TNF- α* , *IL-1 β* , *IL-10*, *IL-12*, *p70*, *interferon- γ* , and *NO* (Sasmito et al., 2015).

In an in vivo study, noni fruit water extract played an immunostimulatory role by inducing *NO* production and the expression of inflammatory cytokines including *IL-1 β* , *IL-6*, *IL-12*, *TNF- α* , and *interferon- γ* , without cyto-toxicity (Hong et al., 2019). Another in vitro immunological activity assay revealed that polysaccharides from *Sambucus adnata* Wall could induce the secretion of *NO* and increase the *iNOS* in macrophages (Yuan et al., 2019). We found in the present study that NFP supplementation increased the *NO* concentration. *NO* is a gas signal molecule that can regulate a variety of physiological functions, including killing virus-infected cells, tumor cells, and pathogens, and plays an important role in inflammation and immune responses (Macmicking et al., 1997; Xu et al., 2012). The role of *NO* production in the immune cell changes in response to NFP supplementation to cashmere goats needs a further experimental confirmation.

Growth performance

We report that goats on the NFP diet gained more body weight compared with goats on the basal diet, apparently associated with the increased antioxidant capacity. The effect of dietary supplementation of NFP on the growth of cashmere goats has not been previously reported. In cattle, noni fruit supplementation decreased the DMI to ADG ratio in a dose-dependent manner (Yancey et al., 2013). In broilers, noni fruit supplementation improved the body weight gain, feed conversion ratio, and immunity (Sunder et al., 2015). A strong antioxidant capacity is beneficial to the transportation of nutrients through epithelial cells of the gastrointestinal tract (Papas, 1996). Strengthening antioxidant capacity may have contributed to the improved growth performance of cashmere goats in the present study. The increase in the rumen fermentation rate could also contribute to the improvement of body weight gain (unpublished data, Zhang Q et al, Inner Mongolia Agricultural University, China). This needs to be verified in future studies with growing kids.

Conclusion

To sum up, diet supplementation with 0.40% NFP raised serum levels of *NO*, *IL-6*, and *TNF- α* and the activities of *CAT*, *GPx*, *TrxR*, and *T-SOD* by up-regulating related gene expressions in blood, and reduce the serum levels of *MDA* and *ROS* in cashmere goats. The change in the anti-oxidant status may have contributed to body weight gain of goats.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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