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Effects of *Taraxacum mongolicum* on *in vitro* response of milk somatic cells stimulated by lipopolysaccharide and subclinical mastitis in dairy cows *in vivo*

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The anti-inflammatory effects of *Taraxacum mongolicum* (TM) were investigated in Holstein-Friesian dairy cows, *in vitro* and *in vivo*. *In vitro*, isolated milk somatic cells were pretreated with various concentrations (31 to 500, µg/ml) of TM extract (TME) and subsequently incubated with lipopolysaccharide (LPS, 1 µg/ml). The results show that TME treatment had no effect on cell viability; however, it significantly suppressed LPS-induced expression of nitric oxide (NO), interleukin(IL)-8, IL-1β, and tumor necrosis factor (TNF)-α in milk somatic cells, in a dose-dependent manner. *In vivo*, 14 lactating Holstein-Friesian cows, with subclinical mastitis, were randomly assigned to two groups and fed a diet with (treatment group, n=7, 150 g TM powder per head per day) or without (control group, n=7) TM supplementation for 14 days. Cows fed with TM powder had a significantly (P<0.05) reduced somatic cell count, total bacteria count and IL-8 in milk compared to the control group. In conclusion, the anti-inflammatory effects of TM were associated with down-regulation of NO and pro-inflammatory cytokines. Addition of TM as a dietary supplement might minimize the impact of subclinical bovine mastitis.

Key words: Cytokine, mastitis, somatic cell count, *Taraxacum mongolicum,* traditional Chinese medicine.

INTRODUCTION

Mastitis, defined as inflammation of the mammary gland, is usually caused by bacterial invasion into the udder. The inflammatory response is usually initiated when bacteria enter the mammary gland through the teat canal and multiply in the milk. Moreover, mastitis is one of the most common and costly infectious diseases in the dairy industry, it is responsible for significant economic loss associated with decreased milk production and quality, therapeutic interventions, loss of antibiotic-contaminated milk and additional labor (Degraves and Fetrow, 1993). Mastitis may exist in different forms. Clinical mastitis is an

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abrupt and severe inflammation of the udder with clinical signs. By contrast, subclinical mastitis is associated with moderate and persistent inflammation without noticeable clinical signs (such as fever, swollen glands or changes in the appearance of milk) (Jain, 1979) and it can only be diagnosed by microbiological methods or analysis of the somatic cell count (SCC) in milk (Mercer et al., 1976). A common practice in treating bovine mastitis is the intramammary infusion of antibiotics. However, the efficacy of this treatment is only moderate and the residue of antibiotics in milk has become a public health concern (Daley and Hayes, 1992). Moreover, the biggest challenge facing the modern dairy industry is the pressure to reduce the use of antibiotics in food-producing animals. Therefore, using therapeutic agents other than antibiotics for prevention or treatment of clinical and subclinical mastitis is an important research subject in the dairy industry.

The milk SCC is composed of lymphocytes, macrophages, polymorphonuclear cells (PMNs), and epithelial cells, and may be the second line of defense against mammary gland infection (Paape et al., 1979; Kehrli and Shuster, 1994; Sarikaya et al., 2005). Many studies have indicated that somatic cells play an important role in regulating inflammation by the release of inflammatory mediators such as nitric oxide (NO) and pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-8 after contact with pathogens or E. coli-derived lipopolysaccharide (LPS) (Burvenich et al., 1994; Boutet et al., 2007; Wittmann et al., 2004; Yu et al., 2010).

Plant-based products constitute a major source of alternative therapies for a wide spectrum of diseases in both human beings and animals. Herbs with anti-inflammatory properties that are used in traditional Chinese medicine, such as *Taraxacum mongolicum* (TM), may be potential candidates for a variety of treatments. The herb is frequently used to treat hepatic and inflammatory disorders, and certain diseases common to women associated with lactation, as diuretics and anti-inflammatory remedies in the Pharmacopoeia Chinensis. In Taiwan, the amount of *Taraxacum Mongolicum* (TM), a local species of Taraxacum, used on animals far exceeds that used on human beings (Kaphle et al., 2006). This suggested that TM may be a very potential alternative for preventing and treating subclinical bovine mastitis. Therefore, the anti-inflammatory effects associated with TM were examined in milk somatic cells (*in vitro*) from Holstein-Friesian cows with subclinical mastitis (*in vivo*) in the present study.

MATERIALS AND METHODS

Preparation of *T. mongolicum in vitro* and *in vivo* trials

T. mongolicum Hand.-Mazz. (Herb. No. 421407502; *Asteraceae*) powder was purchased from Koda Pharmaceutics Ltd (Taoyuan, Taiwan). TM powder was used for preparing the extract and fed as

a dietary supplementation in the feeding trial (*in vivo*). The TM powder was extracted with 10 volumes of distilled water in Soxlet apparatus for up to four cycles. The extracted material was filtered through sterile muslin cloth and the filtrate was vacuum dried at temperatures below 40°C. The yield was 9.25% w/w of the raw TM extract (TME) powder. The extract powder was weighed and reconstituted in sterile phosphate buffer saline (PBS, pH 7.4, 0.01 M) to a final concentration of 20 mg/ml. The TME solution was filtered through a 0.2-mm filter (Microgen, Laguna Hills, CA, USA), aliquoted, and stored at -20°C until used in the *in vivo* trial.

In vitro trial

Culture and treatment of milk somatic cells in vitro

Milk somatic cells were collected from three lactating Holstein-Friesian cows (4 to 6 years old) free of mastitis as determined by milk SCC (< 100,000/ml) and negative for intramammary infection without any clinical signs. After cleaning the teat orifice with 70% ethyl alcohol and discarding a few streams of foremilk, milk samples from the four quarters of a cow were collected and mixed (total volume 50 ml). Samples were maintained on ice and shipped to the laboratory immediately. Isolation of somatic cells was carried out as described by Sarikaya et al. (2002). Briefly, 15 ml of diluted milk sample (30%, vol/vol dilution with cold PBS) was centrifuged for 10 min at $180 \times g$ to isolate somatic cells. The somatic cells were removed and washed three times in PBS (pH7.5). Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 1% glutamine, 10% fetal calf serum, streptomycin (50 µg/ml), and penicillin (50 IU/ml) (all from Gibco Invitrogen) was used to prepare various concentrations of TME (0, 31, 63, 125, 250, 500 ug/ml, respectively) that were used to re-suspend isolated somatic cells (2 $\times 10^6$ cells/ml). The cells were incubated in humidified 5% CO_2 atmosphere at 37°C for 4 h before the addition of LPS (Sigma Aldrich, St. Louis, MO). The final working concentration of LPS was 1 µg/ml.

Viability of somatic cells

Isolated somatic cells were plated (at a density of 5×10^4 cells per well per 100 µl of medium) with the indicated concentrations of TME at 37°C under 5% CO_2 atmosphere in a 96-well plate for 24 h. Cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylthiazolium bromide (MTT) assay (Kao et al., 2001) and is expressed as a percentage of the control without TME. One-tenth volume (10 ul) of 5 mg/ml MTT (Sigma Aldrich, St. Louis, MO) was added to the culture medium. After 4 h incubation at 37°C, an equal volume of 0.04 N HCl in isopropanol was added to dissolve the MTT formazan and the absorbance was measured at 570 nm using a microplate reader. The absorbance of the formazan formed by the untreated cells was defined as 100%.

NO analysis

The culture medium of milk somatic cells were pretreated with the indicated concentrations of TME for 4 h and treated with LPS (1 µg/ml). After 24 h, the amounts of NO in the culture supernatants were measured using the Griess reaction assays. In brief, 100-µl samples were incubated with 100-µl of Griess reagent [equal volumes of 1% (wt/vol) sulfanilamide in 5% [vol/vol] phosphoric acid and 0.1% (wt/vol) naphthylethylenediamine-HCl, Sigma] at room temperature for 10 min, and then the absorbance at 550 nm was measured in an enzyme-linked immunosorbent assay (ELISA) microplate reader. Fresh culture medium was used as the blank in all the experiments. The amount of nitrite in the samples was

Table 1. Sequences of primers for RT-PCR.

Item	Primer sequence	Predicted size (bp)	Annealing temperature (°C)
IL-1β	5′-CCATGGCAACCGTACCTGAACCCA-3′ 5′-AATGGAAACCCTCTCTCCCTAAAG-3′	804	55
TNF-α	5′-GGACCCTTCTGAAAAAGACACC-3′ 5′-GGGTGCAGGACATGCATCTTC-3′	702	52
β-actin	5′-CCAGACAGCACTGTGTTGGC-3′ 5′-GAGAAGCTGTGCTACGTCGC-3′	270	55

calculated from a sodium nitrite standard curve freshly prepared in culture medium (Yeh et al., 2007).

Determination of IL-8 levels

Cultured milk somatic cells were pretreated with the indicated concentrations of TME for 4 h and treated with LPS (1 µg/ml). After 24 h, the amounts of IL-8 in the culture supernatants were measured using enzyme-linked immunosorbent assays (ELISA). The IL-8 levels in the culture medium of somatic cells were determined using a commercially available human IL-8 ELISA kit (R&D Systems, Inc., Minneapolis, MN), which has been previously shown to cross-react with bovine IL-8 (Bannerman et al., 2003). The assay was carried out according to the manufacturer's instructions.

Reverse transcription-polymerase chain reactions

Cultured milk somatic cells (2×10^6 cells/ml) in 100-mm dishes were pre-incubated with and without indicated concentrations of TME for 4 h, and then incubated with LPS (1 µg/ml) for 24 h. Total RNA was extracted from cells using the RNeasy Mini kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Reverse transcription-polymerase chain reaction (RT-PCR) was performed using the one-step RT-PCR kit (GeneMark, Taiwan). PCR amplification was performed using the primer sequences, predicted size, and annealing temperature for IL-1β, TNF-α, and β-actin were listed in Table 1. A standard PCR mix was used with 2.5 units of Taq polymerase and supplied buffer (Invitrogen Co., Carlsbad, CA, USA), using 35 cycles of the following steps: 95°C for 45 s, annealing temperature (as listed in Table 1) for 45 s, 72°C for 1 min and subsequently concluded with 5 min at 72°C. Expression of the β-actin served as the control. The amplified products were resolved by electrophoresis in 2% agarose gels. Agarose gels were stained with 0.5 mg/ml ethidium bromide in Tris/borate/EDTA buffer (ICN, Costa Mesa, CA) for visualization. Data were quantified using Gel digitizing software system (Silk Scientific Co., USA). All signals were normalized to mRNA levels of housekeeping gene β-actin, and expressed as the ratio.

In vivo trial

Selection of animals

Fourteen (14) Holstein-Friesian cows, between 2 and 7 years of age (1st lactation: n=2, 2nd lactation: n=6, 3rd lactation: n=4, 5th lactation: n=2) and had been lactating for 1 to 8 months, with subclinical mastitis were used in this study. The presence of subclinical mastitis was confirmed by persistently elevated SCC (> 5×10^5 cells/ml) and positive for intramammary infection without any clinical signs. The cows did not receive any treatment during the month prior to the beginning of the experiment. These cows were housed in the animal shed at the farm of Hsin-Chu Branch Station, COA-TLI, Hsin-Chu, Taiwan under identical environmental conditions. The use of animals was approved by the Institutional Animal Care and Use Committee (IACUC).

Experimental design of animal trial

The 14 lactating Holstein cows with subclinical mastitis were randomly assigned to two groups, control (n=7, basal diet, Table 2) and treatment (n=7, basal diet with the supplementation of TM herb powder, 150 g per day). Cows were fed daily with total mixed ration, and hay and water were given *ad libitum*. The TM (150 g per day) was added to the concentrates for cows in the treatment group for 14 days (D1 to D14). Milk and blood samples were collected one day before (D0) and one day after (D15) the experimental period.

Milk sample collection and analysis of TBC, SCC, IL-8

Cows were milked twice (5:00 am and 5:00 pm) daily. After cleaning the teat orifice with 70% ethyl alcohol and discarding a few streams of foremilk, milk samples from the four quarters of a cow were collected and mixed (total volume 50 ml). Samples were maintained on ice and shipped to the analytical laboratory immediately. The total bacterial count (TBC) was performed by plating milk samples on 5% bovine blood agar plates followed by the method described by Griffin et al. (1997). Analysis of milk SCC was conducted using a Fossmatic 5000 cell counter (Foss, Hillerd, Denmark). The concentration of IL-8 in milk samples was determined by a commercially available human IL-8 ELISA kit as described in the *in vitro* trial.

Blood sample collection and analysis of biological parameters

Blood samples were collected following the procedure described previously (Paape et al., 1972) with modifications. Briefly, 9 ml of blood were collected aseptically from the jugular vein using an 18 gauge needle into a vacuum tube. The tubes were kept at room

Table 2. TMR formulation and compositions fed to Holstein lactating cows in the experiment (%, DM basis)[1].

Ingredient[2]	DM% In TMR	Chemical compositions and *in vitro* digestibility, %[3]									
		DM	CP	ADF	ADL	NDF	EE	Ash	Ca	P	IVDMD
Corn silage	31.1	26.8	10.9	36.8	3.36	56.6	3.48	7.9	0.35	0.24	56.7
PG hay	4.3	89.6	4.5	43.4	5.66	75.4	1.22	5.4	0.24	0.06	47.8
Alfalfa hay	12.8	88.2	17.7	37.7	7.17	45.7	1.66	8.2	1.13	0.19	63.5
Dehy AP	6.8	93.0	20.8	32.1	7.59	43.7	2.69	10.7	1.90	0.27	65.6
Fish meal	0.87	89.5	72.2	1.1	0.19	12.4	8.50	17.5	4.10	2.56	81.2
Concentrate [4]	44.2	91.2	23.2	5.6	0.49	14.3	1.82	8.1	0.87	0.65	89.2
TMR		51.5	17.6	24.4	3.20	37.7	2.67	7.7	0.82	0.43	71.2

[1]Same TMR was offered to both groups (control and treatment) *ad libitum*; [2] PG hey, pangolagrass hay; Dehy AP, denydrated alfalfa pellet; TMR, totally mixed ration; [3]DM, dry matter; CP, crude protein; ADF, acid-detergent fiber; ADL, acid-detergent lignin; NDF, neutral-detergent fiber; EE, Ether extract; IVDMD, *in vitro* dry matter digestibility; [4]each metric ton of concentrate was constituted by 575 kg of ground corn, 305 kg of soybean meal, 20 kg of fish meal, 50 kg of molasses, 4 kg of limestone, 8 kg of dicalcium phosphate, 25 kg of sodium bicarbonate, 5 kg of magnesium oxide, 3 kg of salt and 5 kg of vitamin and mineral premix (as fed basis).
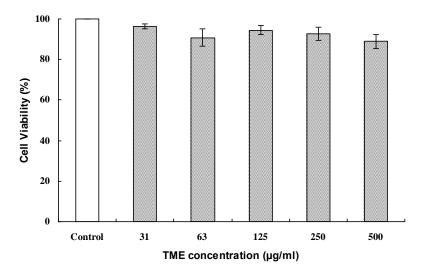

Figure 1. Effect of *Taraxacum mongolicum* extract (TME) on cell viability in milk somatic cells. Milk somatic cells were cultured with the indicated concentrations of TME at 37℃ in a 96-well plate for 24 h. Cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylthiazolium bromide (MTT) assay and expressed as the percentage over the medium control. Data are expressed as mean ± SEM of three independent experiments.

temperature for 10 min, and then centrifuged at 1200 × *g* for 15 min. The sera were collected and stored at -20°C until analysis. The concentrations of aspartate aminotransferase (AST) and blood urea nitrogen (BUN) were determined by VITROS Chemistry System DT60 and DTSC (Johnson & Johnson Inc., USA) to evaluate the effect of TM on liver and kidney functions.

Statistical analysis

In vitro data were expressed as the mean ± standard error of the mean (SEM). The statistical analysis was performed by analysis of variance, followed by the Dunnet test. Values of $P < 0.05$ or 0.01 were considered to be statistically significant. *In vivo* data were expressed as the mean ± standard error of the mean (SEM). The statistical analysis was performed by the Student's *t* test to compare the treatment and control groups with the difference between pre-

and post- experiment observations (SAS, 2002). Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

***In vitro* trial**

Effects of TME on cell viability and LPS-induced NO in cultured somatic cells

The results of the MTT assay, after exposure of milk somatic cells to various concentrations of TME for 24 h, showed no significant effect on cell viability (Figure 1). The inhibitory effects of TME on LPS-induced NO
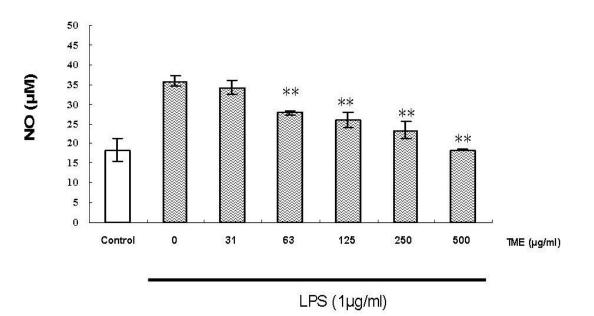
Figure 2. Effect of *Taraxacum mongolicum* extract (TME) on LPS-induced nitrite (NO) in milk somatic cells. Milk somatic cells were pretreated with the indicated concentrations of TME for 4 h and treated with LPS (1 µg/ml). After 24 h, the amounts of NO in the culture supernatants were measured using the Griess reaction assays. Data are expressed as mean ± SEM values of three independent experiments. ** $P < 0.01$ as compared with the LPS group (TME 0 µg/ml).

production was examined in milk somatic cells. The accumulated nitrite, determined by the Griess method, in the medium, was used as an index of the NO level. After treatment with LPS, the nitrite content markedly increased (Figure 2). In the somatic cells treated with 125, 250 and 500 µg/ml TME, NO production induced by LPS, was significantly suppressed in a dose dependent manner: 27.8 ± 0.6, 26.0 ± 1.8, 18.4 ± 0.3 µM, respectively, compared to the control (35.8 ± 1.3 µM) (Figure 2).

TME decreased LPS-induced expression of IL-8, TNF-α, and IL-1β in cultured somatic cells

The production of inflammatory cytokines, in LPS-treated somatic cells, was examined to elucidate the anti-inflammatory effects of TME. In the resting state, the secretion of IL-8 from somatic cells was low (30.0 ± 12.0 pg/ml) and TME treatment did not increase secretion. After LPS stimulation, the IL-8 concentration increased by approximately 14-fold (421.3 ± 31.6 pg/ml) in the medium compared to the control. TME pretreatment had an inhibitory effect on LPS-induced IL-8 production, in a dose-dependent manner (Figure 3). TME effectively suppressed IL-8 production, with values of 265.4 ± 29.8, 180.6 ± 7.8, 128.5 ± 17.9 and 100.4 ± 8.5(pg/ml) for TME concentrations of 31, 63, 125, 250, and 500 (µg/ml), respectively.
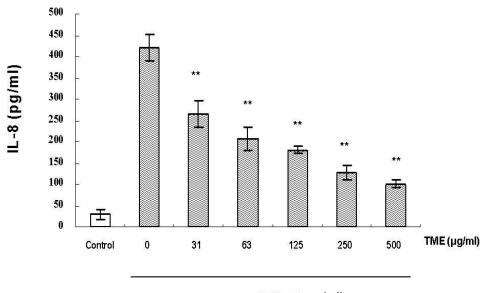
In addition, the expression of TNF-α mRNA was

markedly increased in response to LPS, which was significantly inhibited by TME pretreatment in a dose-dependent manner (Figure 4). A similar trend was observed in LPS-induced IL-1β mRNA expression. TME treatment did not affect the expression of the housekeeping gene, β-actin, even at the highest concentration.

In vivo trial

Dietary supplementation with TM reduced the bacterial count in milk SCC and the IL-8 concentration in cows with subclinical mastitis

Dietary supplementation with TM powder, for 14 days, significantly reduced the number of bacteria in milk (Table 3). In the treatment group, the TBC (log$_{10}$ CFU/ml) in milk averaged 4.33 ± 0.62 on D0 and significantly ($P < 0.05$) decreased to 3.60 ± 0.40 on D15. By contrast, the TBC in the milk of cows, in the control group, remained unchanged during the same period. Dietary supplementation with TM powder significantly reduced the absolute number of SCC in milk (Table 3). The initial SCC in the milk, of the treatment group, averaged 137.6 ± 27.0 × 10^4 cells/ ml and decreased to 61.9 ± 11.0 × 10^4 cells/ml after 14 days of treatment. By contrast, the SCCs in the milk, of the control group, increased from 129.6 ± 29.2 × 10^4 cells/ml to 155.0 ± 28.3 × 10^4 cells/ml during the same period. After conversion to the SCC score

Figure 3. Effect of *Taraxacum mongolicum* extract (TME) on LPS-induced interleukin (IL)-8 in milk somatic cells. Milk somatic cells were pretreated with the indicated concentrations of TME for 4 h and treated with LPS (1 µg/ml). After 24 h, the amounts of IL-8 in the culture supernatants were measured using enzyme-linked immunosorbent assays. Data are expressed as mean ± SEM of three independent experiments. ** $P < 0.01$ as compared with the LPS group (TME 0 µg/ml).
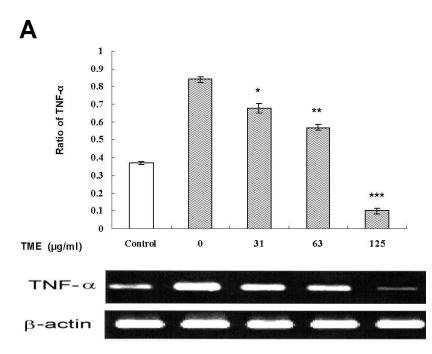

Figure 4. Effects of *Taraxacum mongolicum* extract (TME) on TNF-α and IL-1β mRNA expression in LPS- stimulated milk somatic cells. Milk somatic cells were pretreated with the indicated concentrations of TME for 4 h and treated with LPS (1 µg/ml). After 24 h, the mRNA expression levels of TNF-α (Panel A) and IL-1β (Panel B) were measured using RT-PCR assay. The β-actin was used as an internal control. All signals were normalized to mRNA levels of β-actin and expressed as a ratio. Data are expressed as mean ± SEM of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared with the LPS group (TME 0 µg/ml).
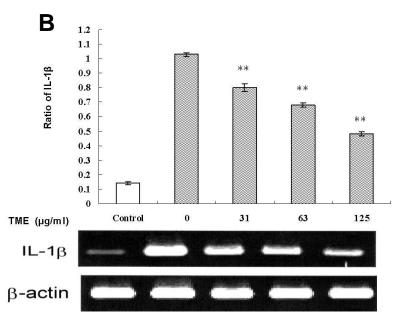
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Figure 4. Continued.

[SCCS, log$_2$ (somatic cell count /100,000)+3], the treatment group had a reduction of 1.0 ± 0.3, which was significantly different (P < 0.05) from the control group (an increase of 0.4 ± 0.1). In addition, the IL-8 concentration, of the milk in the treatment group, but not the control group, significantly (P < 0.05) decreased from 410.6 ± 27.4 pg/ml to 165.9 ± 7.9 pg/ml after the feeding trial (Table 3).

Effects of TM on liver and kidney function

To verify the safety of TM powder, the AST and BUN were analyzed to monitor the health of the liver and kidneys. For cows fed with the dietary supplemented TM powder for 14 days, the bioactivity index for liver (AST) and kidney (BUN) function in serum were measured before and after the experiment (Table 3). The levels of BUN and AST were not significantly different in comparisons between the two groups. All AST and BUN values were within the normal range [AST (58-100 U/l) and BUN (6-22mg/dl)].

DISCUSSION

Mastitis is commonly observed in milking cows. None of the available antibiotics have been proven to have more than 60% efficacy under field conditions against the major pathogens that cause mastitis (Radostits et al., 1994). Regulation of polymorphonuclear cell (PMNs) migration from the blood stream to an inflamed udder is of great importance with regard to further development of

fundamental methods that are effective for the treatment of mastitis. In previous studies, TM showed anti-inflammatory activity, which suggested that TM might be a potential alternative treatment for preventing and treating bovine mastitis. Therefore, the anti-inflammatory effects of TM in LPS-stimulated somatic cells of dairy cows with subclinical mastitis were studied.

Milk somatic cells are composed of lymphocytes, macrophages, PMNs and epithelial cells; they play a pivotal role in the defense of the mammary glands and regulation of inflammation (Sordillo et al., 1997; Sarikaya et al., 2005). Stimulation of somatic cells by LPS triggers a cascade of intracellular signaling effects that ultimately lead to the expression of cytokines and other inflammatory mediators that constitute the pro-inflammatory response (Meng and Lowell, 1997). Production of inflammatory mediators, in somatic cells, such as LPS-induced NO and pro-inflammatory cytokines (TNF-α, IL-1β and IL-8), were analyzed using TME.

In the present study, TME significantly inhibited LPS-stimulated NO production in somatic cells. Excessive NO production, in mammary glands, is believed to contribute to inflammation (Boutet et al., 2007; Nathan, 1992). Therefore, TME that inhibits NO production may have therapeutic potential against inflammation (Shan et al., 2009). TME, at concentrations of 125 to 500 µg/ml, inhibited 22 to 48% of LPS-stimulated NO production in somatic cells. Furthermore, TME also significantly inhibited the expression of TNF-α, IL-1β and IL-8 in LPS-stimulated somatic cells. The clinical severity of bovine mastitis has been positively correlated with these cytokines (Dinarello,1997). The presence of TNF-α and IL-1β have been associated with severe clinical coliform

Table 3. Data of total bacterial count, SCC, SCCS, IL-8, AST, and BUN, in the feeding trial.

Item	Control (n=7)			Treatment (n=7)		
	Before trial	After trial	Difference[1]	Before trial	After trial	Difference
TBC (log$_{10}$ [CFU/ml])	4.36 ± 0.66	4.44 ± 0.69	0.07 ± 0.02 (1.6 ± 0.2 %)	4.33 ± 0.62	3.60 ± 0.40	-0.73 ± 0.07* (-16.7 ± 0.9 %)
SCC (10^4 cells/ml)	129.6 ± 29.2	155.0 ± 28.3	25.4 ± 31.7	137.6 ± 27.0	61.9 ± 11.0	-68.5 ± 30.6
SCCS[2]	6.5 ± 0.2	6.9 ± 0.3	0.4 ± 0.1 (5.8 ± 0.7 %)	6.6 ± 0.3	5.6 ± 0.3	-1.0 ± 0.3* (-13.7 ± 3.2 %)
IL-8 (pg/ml)	452.4 ± 28.8	502.6 ± 31.3	50.2 ± 29.7 (11.4 ± 1.0 %)	410.6 ± 27.4	165.9 ± 7.9	-244.7 ± 17.5* (-59.3 ± 2.1 %)
AST (U/ dL)	76.5 ± 3.1	82.3 ± 4.0	5.8 ± 0.3	75.4 ± 2.9	57.8 ± 3.4	-17.6 ± 0.5
BUN (µg/dL)	12.2 ± 1.2	12.8 ± 1.4	0.6 ± 0.2	12.8 ± 0.7	13.0 ± 0.3	0.2 ± 0.1

[1] Difference = After trial – Before trial. [2] SCCS = log$_2$（somatic cell count /10^5）+3. * Significantly (P < 0.05) different from the control. TBCTotal cell count; SCC, somatic cell count; SCCS, somatic cell count score; IL-8, interleukin-8; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

mastitis, where they might play a role in generating inflammation of the mammary glands (Nakajima et al., 1997). IL-8 is produced by somatic cells (Boudjellab et al., 1998; Strandberg et al., 2005), and plays an important role in neutrophil migration across the blood/milk barrier during the course of mastitis (Lee and Zhao, 2000). The concentration of IL-8 is dramatically increased in mammary secretions during the course of coliform mastitis. Therefore, the anti-inflammatory effects of TME might be associated with the down-regulation of TNF-α, IL-1β and IL-8, which reduces local inflammatory responses and the excessive influx of polymorphonuclear leukocytes.

In order to consider TM powder as a therapeutic alternative, the anti-inflammatory effects of TM powder was examined in Holstein cows with subclinical mastitis. Animals were fed with a diet supplemented with TM powder (150 g/head/day) for 14 days and several parameters were analyzed before (D0) and after (D15) the feeding

trial. The results show that the SCC, TBC and IL-8 in the milk from cows with subclinical mastitis were all significantly reduced after diet supplementation with TM powder for 14 days. The SCC is a fast and reliable analytical tool that reflects the immunological status of the udder in response to bacterial infections (Leitner et al., 2000). From the SCC, the results indicate that udder health was improved after feeding the cows a diet supplemented with TM powder for two weeks. In addition, a reduced TBC and IL-8 level in milk was observed. These results suggest that dietary supplementation with TME has an anti-inflammatory effect on dairy cows with mastitis. The components of TME may activate the anti-inflammatory effects locally or systemically to reduce inflammatory cytokines, such as IL-8, so that the migration of PMNs is decreased. As a consequence, the milk SCC was significantly decreased in the cows in the treatment group.

In addition to anti-inflammatory substances, antioxidants in *Taraxacum* might contribute to the

effects of TME. Numerous studies have attempted to isolate and evaluate the bioactive compounds in *Taraxacum*, including luteolin, chicoric acid, chlorogenic acid, quercetin, and chrysoeriol acid (Hu and Kits, 2005; Schutz et al., 2006). Luteolin and chicoric acid play diverse roles as antioxidants and the prevention of inflammation (Lima et al., 2007). The eudesmanolide from TM has been associated with the inhibition of the production of NO *in vitro* (Kim et al., 2011).

In order to confirm the safety of TME, milk somatic cells were exposed to various concentrations of TME for 24 h; no significant effects on cell viability were found. *Taraxacum* was found to be free of significant toxins and alkaloids (Schutz et al., 2006). Rabbits orally treated with dried whole dandelion plants at3 to 6 g/kg body weight showed no visible signs of acute toxicity (Akhtar et al., 1985). Data from the *in vivo* trial of this study supported this finding. Finally, the AST and BUN in serum were measured as the bioactivity index for liver and kidneys, to assess

the toxicity of TM powder as a dietary supplement in cows. The results indicate that dietary supplementation with TM powder did not affect liver and kidney function neither the AST nor BUN levels in serum were significantly affected by the TM powder treatment for 14 days.

In summary, TME minimized the expression of NO and pro-inflammatory cytokines (TNF-α, IL-1β, and IL-8) in LPS-stimulated milk somatic cells (*in vitro*). During the feeding trial (*in vivo*), dietary supplementation with TM powder for 14 days decreased the TBC, SCC and IL-8 in milk from cows with subclinical mastitis, and did not have a negative impact on kidney or liver function. These results suggest that TM might be an affordable alternative with anti-inflammatory properties effective for the prevention and treatment of mastitis in dairy cows. Further investigations are needed to examine the underlying molecular mechanisms and their application in treating bovine mastitis.

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