

Fermented Maesil (*Prunus mume*) with probiotics inhibits development of atopic dermatitis-like skin lesions in NC/Nga mice

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Conflict of interest

No conflict of interest has been declared.

Abstract

Maesil (*Prunus mume* Siebold & Zucc.), a potential source of free radical scavengers and inhibitor of pro-inflammatory mediators, is used in traditional Korean medical preparations as a remedy for skin disorders as have probiotics. The action of a probiotic fermented Maesil preparation on the development of atopic dermatitis (AD)-like skin lesions was determined in a NC/Nga mouse model as an initial step towards the development of a therapeutic feed supplement for use in dogs. Continuous ingestion of the experimental feed markedly inhibited the development of the AD-like skin lesions, as evidenced by a marked decrease in skin signs and reduced inflammation within the skin lesions. Efficacy was confirmed by significant decreases in eosinophil ratio and serum IgE concentration, and a reduction in the number of *Staphylococcus aureus* recovered from the ear. Relative mRNA expression levels of IL-4, interferon- γ and tumour necrosis factor- α in the spleens of the experimental animals were also decreased and there was an increased serum concentration of IL-10 with a concurrent decreased IL-4 concentration in comparison to a control group. Taken together, the results indicate that some component(s) of fermented Maesil have the ability to suppress the development of AD-like skin lesions, possibly by stimulation of IL-10. Beneficial effects of fermented Maesil may thus be expected in dogs with AD, although this and the nature of the active pathway remain to be explored.

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Introduction

Atopic dermatitis (AD), also known as atopic eczema, is one of the most common inflammatory skin diseases in humans and canines in which it exhibits comparable clinical features.^{1–3} Severe pruritus and chronic or relapsing eczematous skin lesions are major clinical features of this condition.⁴ Frequent characteristics include excessive infiltration of inflammatory cells such as lymphocytes, macrophages and granulated mast cells into the skin lesions^{5,6}, eosinophilia in peripheral blood and a high level of serum immunoglobulin (Ig) E.¹ Although the pathogenesis of AD is complex, involving genetic, environmental, pharmacological, skin barrier and immunological factors⁷, interleukin (IL)-4 and IL-5 produced by T-helper (Th) 2 cells could have an especially key role in its onset and development.^{8,9} IL-4 mediates an IgE isotype switch in B cells and IL-5 promotes IgE synthesis induced by IL-4. Mast cells stimulate the release of various chemical mediators in IgE-mediated allergic disorders. Subsequently, many types of inflammatory cells infiltrate into skin lesions.¹⁰ In addition, tumour necrosis factor (TNF)- α produced by macrophages also plays an important role in the acute phase of AD.¹¹ Although Th2 cells are dominant in this acute phase, interferon (IFN)- γ produced by Th1 cells is highly expressed and contributes to the pathogenesis in the chronic phase.^{12–14} IFN- γ increases the sensitivity of Fas-mediated apoptosis of keratinocytes by skin-infiltrating T cells.¹⁵ These results suggest that down-regulation of the responses of Th2 cells, macrophages, and Th1 cells may be effective strategies for the management of AD.

Maesil (*Prunus mume* Siebold & Zucc.) is a deciduous tree of the genus *Rosaceae* that is native to China. The flower, leaf, branch, seed and root of the plant are popular as traditional drug and health food supplements in Asian countries. In particular, the unripe fruit is used in traditional Korean medical preparations as a remedy for coughs, dyspepsia and skin disorders. Maesil possesses biological activities that include suppression of cancer cell proliferation¹⁶, inhibition of motility of *Helicobacter pylori*¹⁷, and improved blood fluidity.¹⁸ Of particular relevance to the present study, biological effects of Maesil that involve free radical scavengers¹⁹ and inhibition of pro-inflammatory mediators in lipopolysaccharide-stimulated macrophages have been described in relation to skin conditions.²⁰ These collective observations indicate that Maesil may be good candidate for control of AD. Probiotics also have therapeutic potential in the treatment of skin disorders.^{21–25}

Thus the present study was initiated to examine whether a fermented by-product of Maesil could

suppress development of AD-like skin lesions in NC/Nga mice, a suitable model for AD in humans and canines,^{26,27} with the aim of developing a therapeutic feed supplement for subsequent use in dogs.

Materials and methods

Maesil fermentation

Maesil flesh and juice were obtained as a by-product during commercial beverage preparation. Fruits were crushed, mixed thoroughly with nonfat rice bran, and the mixture (45–55% moisture, pH 4.5–5) was fermented with 1×10^7 CFU/mL *Saccharomyces cerevisiae* [Korea Collection for Type Cultures (KCTC) 7928; Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea] for 7 days at 25–30 °C. The fermented products (25–35% moisture, pH 5–6) were further fermented with 1×10^7 CFU/mL each of *Bacillus subtilis* (KCTC 1666) and *Lactobacillus acidophilus* (KCTC 3155) for 5 days at 45–50 °C. After fermentation, the products were air-dried for 20 h at 20 °C. The final product (10–15% moisture, pH 5.5–6.5) containing the soluble Maesil ingredients was dark brown in colour and possessed a sweet flavour.

Animals

Specific pathogen-free (SPF) female 6-week-old NC/Nga mice (SLC, Shizuoka, Japan), randomized into three groups each of five mice were initially acclimated for 2 weeks. The control group received a commercial, nutritionally complete, extruded dry rodent feed (Superfeed, Gangwon, Korea) containing 22.1% crude protein, 8.0% crude ash, 5.0% crude fibre, 3.5% crude fat, 1.2% phosphorus and 0.6% calcium. The experiment groups received the same extruded dry rodent feed supplemented with either 1% fermented Maesil (1% fermented Maesil feeding group; 1% FM group) or 2% (2% fermented Maesil feeding group; 2% FM group). All mice were housed in an air-controlled room in polypropylene cages and allowed free access to the particular diet and tap water from drinking bottles with stainless steel sipper tubes. All animal procedures were conducted in accordance with the guidelines of the local ethical committee (Chonnam National University).

Induction of dermatitis and experimental schedule

1-Chloro-2,4-dinitrobenzene (DNCB; Sigma-Aldrich, St Louis, MO, USA) was used to induce dermatitis in all three groups of NC/Nga mice through repeated cutaneous application as previously described²⁸ with some modification (Figure 1). Briefly, DNCB in an acetone/olive oil mixture (3 : 1) was used. Dorsal skin regions were shaved with an electric razor and these and the glabrous ears were sensitized epicutaneously with 200 µl of a 1% DNCB solution. Four days later dermatitis was induced with 150 µl of 0.2% DNCB solution and ears were challenged with 150 µl of 0.2% DNCB solution twice a week. Peripheral blood, skin and spleen samples were

collected post-mortem for analysis 3 days after the fifth application of 0.2% DNCB after hapten induction.

Evaluation of clinical skin severity

The severity of dermatitis was evaluated by scoring clinical signs on the face, ears, nose, neck and dorsal skin twice a week starting from day 1. The total clinical skin severity score was defined as the sum of the individual scores (none = 0, mild = 1, moderate = 2 and severe = 3). Skin severity was characterized according to the presence of erythema (haemorrhage), oedema, excoriation (erosion) and dryness (scaling) as described earlier.^{29–31}

Histological analysis

All mice were euthanized at the end of the experiment. Sampling was performed from dorsal skin lesions and ears as described earlier,³² and the samples were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin (H&E). All histological evaluations were performed in blinded fashion. General skin condition (epidermal hyperplasia, hyperkeratosis and inflammatory cell infiltration and dermal infiltration of inflammatory cells) was compared between groups. Toluidine Blue was used to identify mast cells in the sections and the number between the epithelium and panniculus carnosus was counted under a microscope at a magnification of $\times 400$ at five sites chosen at random as described earlier.³³

Measurement of eosinophil ratios in peripheral leucocytes

Blood was collected in tubes coated with ethylene diaminetetraacetic acid. Blood leucocyte and differential counts were determined using an automated Veterinary Haematology Analyzer (Oxford Science, Oxford, CT, USA) configured for mouse blood.

Measurements of IgE, IL-4, IL-10 and IFN-γ concentration in serum

Blood was collected from the retro-orbital plexus of each mouse at the end of the experiment. Serum was obtained by centrifugation and stored at -20 °C until used. Total serum IgE levels were measured using BD OptEIA™ Mouse IgE ELISA Set (BD, Sparks, MD, USA) and serum IL-4, IL-10 and IFN-γ levels were measured using the Mouse cytokines (IL-4, IL-10 and IFN-γ) Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Enumeration of *Staphylococcus aureus* on ear skin

Enumeration of *Staphylococcus aureus* was performed as described earlier³⁴ with some modification. Briefly, an area of ear skin of about 1 cm² was collected and suspended in 1 mL of sterile phosphate buffered saline. A total of 100 mL of serial tenfold dilutions of the suspensions were inoculated on Mannitol Salt medium (BD) and cultured for 48 h at 37 °C. Yellow colonies were counted and

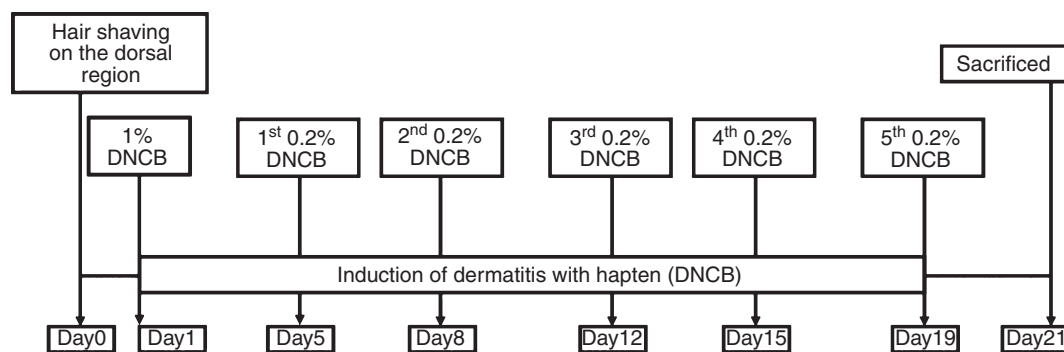


Figure 1. Protocol for Induction of dermatitis. Shaved dorsal regions and the glabrous ears of all the mice were sensitized epicutaneously with 200 µl of a 1% DNCB solution on day 1. Four days later dermatitis was induced with 150 µl of 0.2% DNCB solution at the intervals shown and the animals were euthanized at day 21.

expressed as log CFU/cm² (one ear). In addition, representative colonies were identified through gram staining and a coagulase test.³⁵

Evaluation of relative mRNA expression levels of IFN- γ , IL-4 and TNF- α in spleen

Spleenocytes were isolated under sterile conditions as previously described.³⁶ Total RNA extraction was performed using a RNeasy Mini kit (Qiagen, Valencia, CA, USA) and target RNA was reverse transcribed using Superscript II reverse transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. To minimize variations in reverse transcriptase efficiency, all samples were transcribed simultaneously. Primers and probes for murine IL-4, IFN- γ , TNF- α and β -actin were designed as described earlier^{37,38}; their sequences are shown in Table 1. Primer sets for IL-4, IFN- γ , TNF- α and β -actin yielded 95, 92, 175 and 148 base pairs, respectively. The probes were dual labelled with the reporter dye 6-carboxyfluorescein (FAM) at the 5' end and the quencher dye 6-carboxytetramethylrhodamine (TAMRA) at the 3' end. IL-4, IFN- γ and TNF- α mRNA levels were determined by a real-time polymerase chain reaction assay using a Rotor-gene 6000 (Corbett Research, Sydney, NSW, Australia) with 0.5 μ g of cDNA. The threshold cycle (C_t ; the cycle number at which the amount of amplified gene of interest reaches a fixed threshold) was determined subsequently. Relative quantitation of IL-4, IFN- γ and TNF- α mRNA expression was calculated by a comparative C_t method as described earlier.³⁹ The relative quantitation value of target (IL-4, IFN- γ or TNF- α) was normalized to an endogenous control β -actin gene and relative to a calibrator.⁴⁰ It was expressed as $2^{-\Delta\Delta C_t}$ (fold), where $\Delta C_t = C_t$ of target gene (IL-4, IFN- γ or TNF- α) - C_t of endogenous control gene (β -actin). $\Delta\Delta C_t = \Delta C_t$ of samples for target gene - ΔC_t of the calibrator for the target gene.

Statistical analysis

The data were expressed as mean $\pm$ standard deviation (SD) and the means of the different parameters were compared between groups by analysis of variance. Significant differences between the groups were evaluated by Tukey's multiple comparison tests using Minitab Statistical Software 13.20 (Minitab, State College, PA, USA), and $P < 0.05$ was considered as the level of significance.

Results

Clinical skin severity

Repeated cutaneous application of DNCB accelerates development of dermatitis in NC/Nga mice.²⁸ Consistent with this finding, clinical skin severity in the control group fed with control feed alone increased gradually with the number of cutaneous applications of DNCB and reached a peak at the end of experiment (clinical severity score:

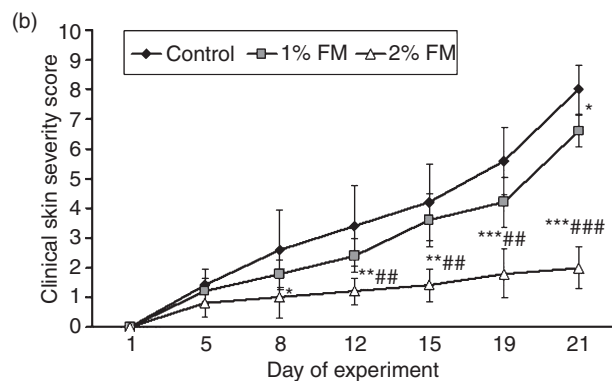
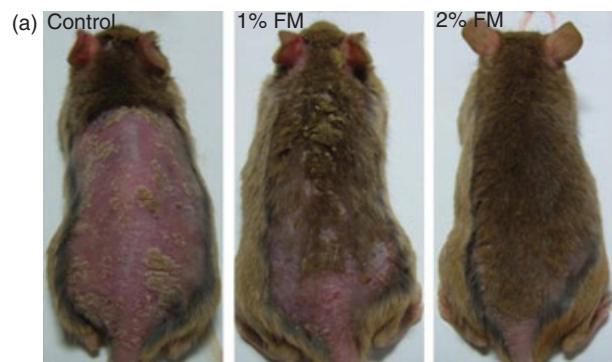


Figure 2. (a) Representative clinical skin features. These demonstrate visually a marked reduction in severity of the dermatitis and more rapid hair re-growth with increased fermented Maesil feeding. (b) Temporal changes in mean clinical skin severity score. These rise with time in the control group and also in the 1% FM group although the peak at 21 days was lower ($*P < 0.05$) in the latter. A significant improvement in the 2% group had occurred at day 8 ($P < 0.05$) and by day 12 was even more pronounced ($**P < 0.01$) compared with the controls.

8.0 ± 0.8 points; mean $\pm$ SD; $n = 5$) (Figure 2b). Most mice in this group exhibited severe dermatitis signs of erythema (haemorrhage), oedema, excoriation (erosion) and dryness (scaling), while mice in the 1% FM and 2% FM feeding groups presented with mild or moderate clinical skin severity (Figure 2a). A statistical difference in the clinical skin severity scores was observed between the control group and 1% FM feeding group only after the fifth application of 0.2% DNCB. The 2% FM feeding

Table 1. Primer and probe sequences for murine cytokines

	Sequence (5'–3')	Length (bp)*
IL-4	FW	ACAGGAGAAGGGACGCCAT
	RV	GAAGCCCTACAGACGAGCTCA
	TP	TCCTCACAGCAACGAAGAACACCACA
IFN- γ	FW	TCAAGTGGCATAGATGTGGAAGAA
	RV	TGGCTCTGCAGGATTTTCATG
	TP	TCACCATCCTTTTGCCAGTTCCTCCAG
TNF- α	FW	CATCTTCTCAAAATTCGAGTGACAA
	RV	TGGGAGTAGACAAGGTACAACCC
	TP	CACGTCGTAGCAAACCAAGTGGA
β -actin	FW	AGAGGGAATCGTGCCTGAC
	RV	CAATAGTGATGACCTGGCCGT
	TP	CAGTCCGCATCCTCTCTCC

FW, forward primer; RV, reverse primer; TP, TaqMan probe dual-labeled with 5'FAM (report dye) and 3'TAMRA (quencher dye); IFN- γ , interferon-gamma; IL-4, interleukin-4; TNF- α , tumour necrosis factor alpha; β -actin, beta-actin.

* Amplicon length in base pairs.

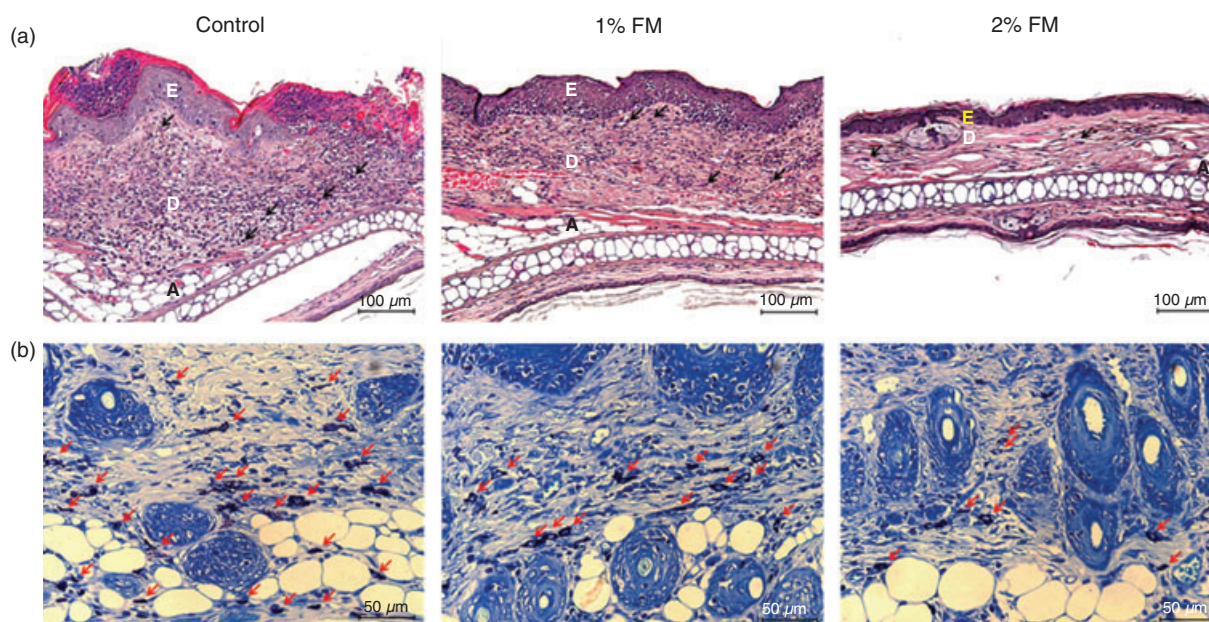


Figure 3. (a) Histological features of ear skin. Note the hyperplastic epidermis (E) and inflammatory cells (arrows) particularly in the dermis (D) of the control animal. (A) denotes the sub-dermal adipose layer. The inflammatory infiltrate (arrows) is less severe in the 1% FM group and negligible in the 2% FM group; in the latter hyperplasia of the epidermis (E) is not apparent (H&E). An identical result was obtained in the dorsal skin. (b) Dermal mast cell infiltration in dorsal skin. Note the more pronounced cellular degranulation (arrows) in the control group. (Toluidine blue).

group displayed a significantly lower clinical skin severity score compared with the control group even after only one application of DNCB (Figure 2b).

Histological analysis

Severe epidermal hyperplasia and hyperkeratosis and dense infiltration of inflammatory cells into both the epidermis and dermis were observed in both the dorsal and ear skin of the control group. However, supplementation with 2% fermented Maesil inhibited these inflammatory changes (Figure 3a). The 2% FM feeding group showed a significant decrease in the epidermal thickness of dorsal and ear skin compared with the control group (Table 2) ($P < 0.01$). Repeated cutaneous application of DNCB increased dermal mast cell number and degranulation in the control group. However, these features were significantly suppressed by 2% fermented Maesil supplement-

tation compared with the control group (Figure 3b; Table 2).

Peripheral eosinophil ratio

The ratio of eosinophils in peripheral leukocytes of the FM feeding groups was significantly lower than in the control group in a dose-dependent manner (Figure 4a).

Serum concentrations of IgE

Although no significant difference was observed in the serum IgE concentration between the control and 1% FM feeding groups, that of 2% FM feeding group was significantly lower than in the control group ($P < 0.05$) (Figure 4b).

Staphylococcus aureus on ear skin

The number of *Staphylococcus aureus* (log CFU/cm²) in both FM feeding groups showed a significant and dose-dependent decrease on the ear compared with the control group (Figure 4c).

Concentrations of serum IL-4, IL-10 and IFN- γ

No significant difference was observed in the concentration of serum IFN- γ between groups. However, the concentration of serum IL-4 in the FM feeding groups was significantly lower than in the control group in a dose-dependent manner ($P < 0.05$, control versus 1% FM feeding group; $P < 0.001$, control versus 2% FM feeding group; $P < 0.05$, 1% FM feeding group versus 2% FM feeding group). Conversely, the concentration of serum IL-10 in the 2% FM feeding group was significantly higher than in the control group ($P < 0.01$). Moreover, the concentration of serum IL-10 in the 1% FM feeding group also showed a tendency to increase compared with the

Table 2. Epidermal thickness and mast cell number in skin lesions of the control, 1% FM and 2% FM experimental groups

	Control	1% FM	2% FM
Thickness of epidermis (um) [†]			
Dorsal lesions	66.7 $\pm$ 12.0	54.2 $\pm$ 9.4	24.9 $\pm$ 3.2 ^{***,****}
Ear lesions	66.7 $\pm$ 12.1	60.1 $\pm$ 15.4	23.6 $\pm$ 7.0 ^{***,****}
Mast cell number (counts/site) [‡]	40.8 $\pm$ 3.1	37.0 $\pm$ 4.1	32.2 $\pm$ 1.9 [*]

Data are presented as mean $\pm$ SD ($n = 5$). ^{*} $P < 0.05$ versus control; ^{**} $P < 0.01$ versus control; ^{***} $P < 0.05$ versus 1% FM; ^{****} $P < 0.01$ versus 1% FM.

[†]Epidermal thickness was measured in five randomly chosen sites in sections of dorsal and ear skin lesions.

[‡]The number of mast cells in five sites chosen at random (at a magnification of $\times 400$) was counted.

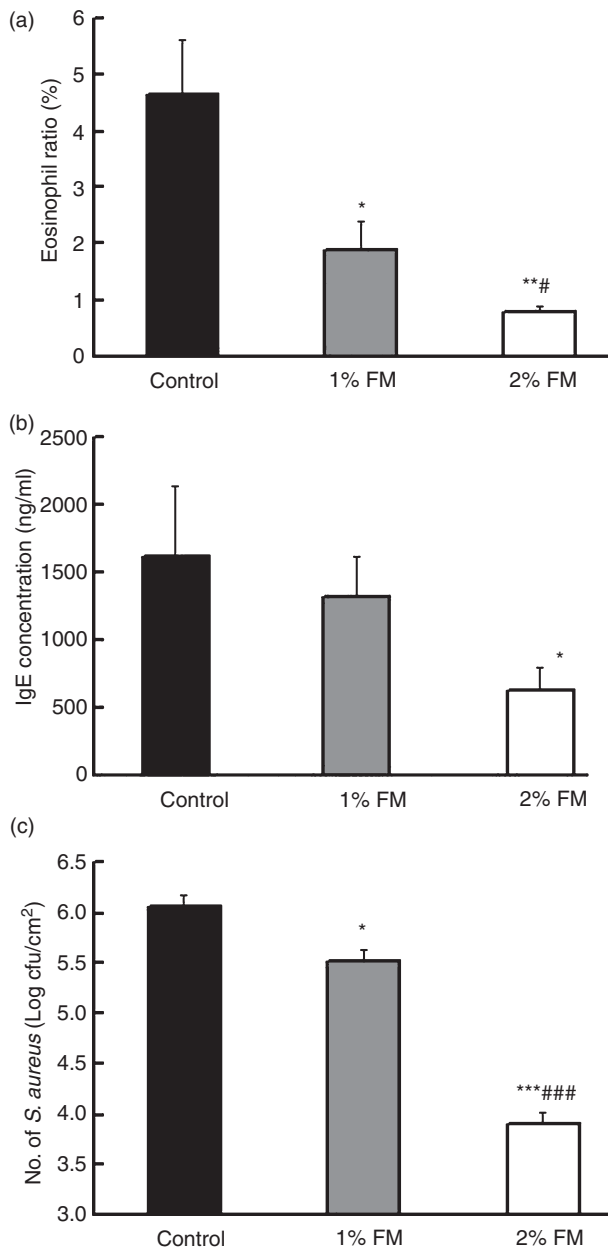


Figure 4. (a) Ratio of eosinophils in peripheral leukocytes, (b) concentration of IgE in serum and (c) number of *Staphylococcus aureus* on ear skin in the three groups [mean $\pm$ SD ($n = 5$)]. In every instance the control values were significantly higher than those in the 2% FM group (** $P < 0.01$, * $P < 0.05$ and *** $P < 0.001$, respectively). There was also a reduction in eosinophil ratio and surface *Staphylococcus aureus* count in the 1% FM group compared with control level (* $P < 0.05$). Differences between 1% FM and 2% FM were significant in eosinophil ratio and surface *Staphylococcus aureus* count (# $P < 0.05$ and ### $P < 0.001$, respectively).

control group, although in this instance the difference was not significant ($P = 0.056$) (Figure 5).

Relative mRNA expression level of IL-4, IFN- γ and TNF- α in spleen

Relative mRNA expression levels of IL-4, IFN- γ and TNF- α significantly decreased in the FM feeding groups compared with those in the control group, although no significant differences were observed in IFN- γ and TNF- α

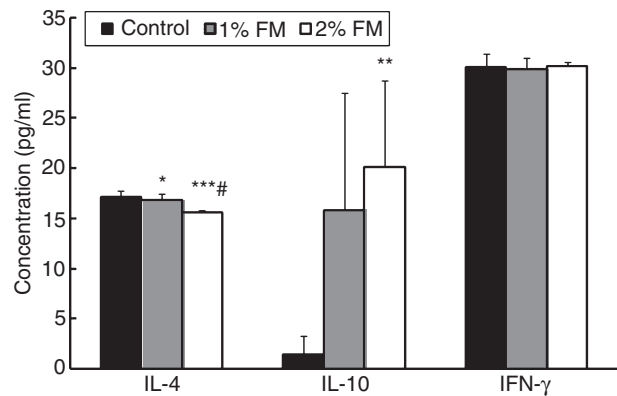


Figure 5. Concentrations of IL-4, IL-10 and IFN- γ in serum of the three groups [mean $\pm$ SD ($n = 5$)]. The concentration of serum IL-4 in the 1% and 2% FM feeding groups was significantly lower than in the control group (* $P < 0.05$ and *** $P < 0.001$, respectively). Conversely, the concentration of serum IL-10 in the 2% FM feeding group was significantly higher than in the control group (** $P < 0.01$), although there was no significant difference between the 1% FM feeding group and control group. No significant difference was observed in the concentration of serum IFN- γ between groups. Differences between 1% FM and 2% FM were significant for serum IL-4 concentration (# $P < 0.05$).

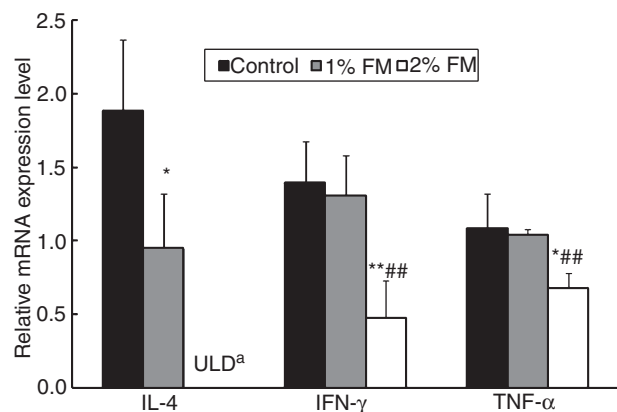


Figure 6. Relative mRNA expression levels of IL-4, IFN- γ and TNF- α in the spleens of the three groups [mean $\pm$ SD ($n = 5$)]. The relative expressions of the mRNAs of the three cytokines significantly decreased in the 2% FM feeding groups compared with those in the control group (^aUnder limit of detection (ULD), ** $P < 0.01$ and * $P < 0.05$, respectively). There was also a reduction in relative expression level of IL-4 in the 1% FM group compared with control level (* $P < 0.05$). Significant differences in the relative expression levels of IFN- γ and TNF- α (### $P < 0.01$) were found between the 1% FM and 2% FM groups.

between the 1% FM feeding group and the control group (Figure 6).

Discussion

Atopic dermatitis-like skin lesions in NC/Nga mice highly resemble those of human and canine AD,³ and show various grades of clinical features such as erythema and haemorrhage, followed by oedematous superficial erosion, deep excoriation, scaling, and dryness of the skin.³⁴ However, signs of dermatitis vary considerably under conventional conditions and clinical skin disorders do not

occur in this mouse strain under SPF conditions. Therefore, to reduce experimental variation and induce stable clinical dermatitis over a short period, investigators have adopted the use of hapten-induced dermatitis using repeated applications of 1-chloro-2,4,6-trinitrobenzene (TNCB); and successful development of dermatitis in NC/Nga mice under SPF conditions has been reported.^{27,29,30} In this instance, as importation of TNCB is banned from the Republic of Korea, DNCB was used as a rational alternative in view of its structural similarity and on the basis of previous evidence of its efficacy in creating AD-like skin lesions and IgE hyperproduction in NC/Nga mice.²⁸ In the present study, the control group of mice also developed other typical AD signs including erythema, oedema, excoriation and dryness of the skin lesions; hyperplasia and hyperkeratosis of the epidermis; dense infiltration of inflammatory cells and severe degranulation of dermal mast cells; peripheral blood eosinophilia and increased serum IgE concentration. The observation of increased colonization of the ear skin by *Staphylococcus aureus* in the control group accords with the view that massive colonization of *Staphylococcus aureus* on the skin coincides with the severity of dermatitis in NC/Nga mice.^{33,34}

Continuous ingestion of feed containing fermented Maesil with probiotics (*Saccharomyces cerevisiae*, *B. subtilis* and *L. acidophilus*) in DNCB-treated NC/Nga mice raised under SPF conditions inhibited development of AD-like skin lesions, especially at 2% FM supplement. Macroscopic and microscopic examinations revealed markedly decreased clinical skin severity and relatively mild epidermal and dermal inflammatory changes with 2% FM supplement compared with the ingestion of control feed alone. Moreover, significant decreases of the eosinophil ratio and serum IgE concentration, and a marked reduction in the number of *Staphylococcus aureus* colonies recovered from the ear confirmed the inhibitory effects of fermented Maesil on the development of AD-like skin lesions.

A noteworthy observation was an increased serum concentration of IL-10 concurrent with a decreased concentration of IL-4 in the FM feeding groups. Moreover, relative mRNA expression levels of IL-4, IFN- γ and TNF- α in the spleens of the FM feeding groups were decreased compared with those of the control group. These results are similar to previous prospective reports demonstrating that oral ingestion of probiotics influences the induction of plasma IL-10 in human patients with dermatitis^{22,25,41} and that Maesil inhibits pro-inflammatory mediators in lipopolysaccharide-stimulated macrophages.²⁰ IL-10 inhibits IFN- γ and IL-2 production by Th1 cells,⁴² and IL-4 and IL-5 production by Th2 cells, as well as eosinophil survival and IL-4-induced IgE synthesis.^{43,44}

Taken together, these findings suggest that some component(s) of fermented Maesil along with probiotics can delay the onset and suppress the development of AD-like skin lesions in NC/Nga mice, probably via the induction of IL-10 or suppression of T cell or macrophage responses. Hence, in combination with probiotics, fermented Maesil, which is readily obtained as a by-product during commercial beverage preparation, may be a good candidate for control of AD in dogs. However, fermented Maesil con-

tains a complex array of compounds; these and the probiotic microorganisms could be modified during the fermentation process. Therefore, precise knowledge of the major component(s) of fermented Maesil responsible for its inhibitory effects on AD-like skin lesions is needed, and studies are currently in progress. Confirmation of the prophylactic effect of fermented Maesil as a feed supplement is now required in dogs with AD.

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Résumé Le Maesil (*Prunus mume* Siebold & Zucc.), source potentielle de fixateurs de radicaux libres et inhibiteur de médiateurs pro-inflammatoires, est utilisé dans les préparations médicales traditionnelles coréennes comme probiotique en remède aux troubles cutanés. L'action d'une préparation probiotique de Maesil fermentée sur le développement de lésions dermatologiques de dermatite atopique (AD)-like a été déterminée dans un modèle de souris NC/Nga comme première étape du développement d'un supplément alimentaire pour chien. Une ingestion continue de la nourriture expérimentale a nettement diminué le développement des lésions cutanées de AD-like par la diminution des signes cutanés d'inflammation. L'efficacité a été confirmée par la diminution du ratio d'éosinophiles et de la concentration d'IgE sériques ainsi que la réduction du nombre de *Staphylococcus aureus* isolé à partir de l'oreille. Les niveaux d'expression relatifs d'IL-4, IFN- γ , et TNF- α dans la rate des animaux expérimentaux étaient diminués, les concentrations sériques d'IL-10 étaient augmentées en parallèle d'une diminution des concentrations d'IL-4 par comparaison avec le groupe contrôle. Pris ensemble, ces résultats indiquent que certains composants de Maesil fermenté ont la capacité de supprimer le développement des lésions cutanées d'AD-like, probablement par stimulation d'IL-10. Les effets bénéfiques du Maesil fermenté pourraient être reproduits chez les chiens atopiques bien que le mécanisme d'action reste encore à explorer.

Resumen El Maesil (*Prunus mume* Siebold & Zucc.), una fuente potencial de eliminador de radicales libres e inhibidor de mediadores proinflamatorios, es utilizado en preparaciones de medicina coreana tradicional como un remedio para enfermedades de la piel ya que tiene probióticos. Se determinó la acción de una preparación probiótica de Maesil fermentado en el desarrollo de lesiones similares a dermatitis atópica (AD) en un modelo de ratón NC/Nga, como un paso inicial para el desarrollo de un suplemento dietético

terapéutico en perros. La ingestión continua de la dieta experimental inhibió el desarrollo de lesiones de AD, evidenciado por una disminución de los signos clínicos y una disminución de la inflamación en las lesiones de la piel. La eficacia se confirmó mediante disminuciones significativas en la proporción de eosinófilos y en la concentración de IgE en el suero, y en la reducción del número de *Staphylococcus aureus* recuperados del oído. Los niveles de expresión relativa de mRNA de IL-4, IFN- γ , y TNF- α en los bazo de los animales de experimentación también estuvieron disminuidos y se observó un incremento de la concentración de IL-10 en suero con un descenso de la concentración de IL-4 en comparación con el grupo control. De forma conjunta, los resultados indican que algún componente(s) del Maesil fermentado tiene la capacidad de suprimir el desarrollo de lesiones similares a AD en la piel, posiblemente por la estimulación de IL-10. Se pueden esperar efectos beneficiosos del Maesil fermentado en perros con AD, aunque estos y la naturaleza de la vía de acción deben ser investigados.

Zusammenfassung Maesil (*Prunus mume* Siebold & Zucc.), eine mögliche Quelle an freien Radikale-Scavengers und Inhibitoren von pro-inflammatorischen Mediatoren, wird in traditionellen koreanischen Medizinpräparaten ebenso wie Probiotika als Heilmittel für Hautprobleme verwendet. Der Einfluss eines probiotisch fermentierten Maesil-Präparates auf den Verlauf von Hautveränderungen bei Atopikern ohne atopische Dermatitis (AD) wurde in einem NC/Nga Mausmodell untersucht, um einen ersten Schritt in Richtung der Entwicklung eines therapeutischen Futterzusatzstoffes zur Verwendung bei Hunden zu tun. Eine kontinuierliche Aufnahme des experimentellen Futters verhinderte deutlich die Entwicklung von AD-ähnlichen Hautveränderungen, was durch eine starke Reduzierung der Hautsymptomatik und eine reduzierte Entzündung innerhalb der Hautläsionen bestätigt wurde. Die Wirksamkeit wurde durch eine signifikante Abnahme des eosinophilen Anteils und der IgE Konzentration im Serum sowie durch eine reduzierte Keimzahl an *Staphylococcus aureus*, der im Ohr entdeckt wurde, bestätigt. Die relative mRNA Expression von IL-4, IFN- γ und TNF- α in den Milzen der Versuchstiere war ebenfalls erniedrigt und im Vergleich zur Kontrollgruppe bestand eine erhöhte Serumkonzentration an IL-10 bei gleichzeitig erniedrigter IL-4 Konzentration. Zusammenfassend weisen die Ergebnisse darauf hin, dass einige Komponenten im fermentierten Maesil die Fähigkeit haben, die Entwicklung von AD-ähnlichen Hautläsionen zu verhindern, möglicherweise durch die Stimulation von IL-10. Eine günstige Auswirkung von fermentiertem Maesil kann daher bei Hunden mit AD erwartet werden, obwohl das und die Art des aktiven Signalwegs noch untersucht werden müssen.