Korean J. Org. Agric.
Volume 24, Number 3: 485-495, August 2016
http://dx.doi.org/10.11625/KJOA.2016.24.3.485

ISSN 1229-3571 (Print)
ISSN 2287-819X (Online)

Effect of Supplementing Cultured Wild Ginseng Roots in the Diet of Organic Saanen Dairy Goats on Milk Composition and Ginsenoside Profiles in Blood and Milk*

Bae, Gui-Seck**

유기농 산양유 사료에 산삼배양근 첨가가 산양유와 혈액 내 진세노사이드 함량 및 조성에 미치는 영향

배 귀 석

The aim of the present study was to determine the effect of dietary cultured wild ginseng root (CWGR) supplementation on goat milk composition and ginsenoside profiles. Sixteen Saanen dairy goats were allocated to two balanced groups based on lactation period, body weight (38.6 ± 3.2 kg), and dairy milk yield (2.85 ± 1.2 kg), and were kept in separate pens. Goats were fed a total mixed ration (TMR) feed (2.3 kg/d, dry matter basis) and 1.5 g of CWGR powder was supplemented in the experimental diet. The total feeding period was 3 weeks, and milk and blood samples were collected on the last three days of the experimental period. There was no effect of CWGR on daily milk yield and milk composition (fat, protein, lactose, and solid-not-fat). However, the CWGR-treatment group had significantly higher plasma IgG and protein contents than the control group ($P < 0.05$). Significant amounts of ginsenosides were observed in the milk of the CWGR-treatment group, whereas ginsenosides were not detected in the milk of the control group. In conclusion, dietary CWGR was a useful regimen to produce functional goat milk enriched in ginsenosides.

Key words : *cultured wild ginseng roots, ginsenosides, goat milk*

* 본 연구는 농림수산식품부 고부가가치식품기술개발사업(과제관리번호 314075-3)에 의해 이루어진 것임.

** First author and Corresponding author, 중앙대학교 연구교수(gsbae@cau.ac.kr)

Ⅰ. Introduction

Panax species have been widely used as a major pharmaceutical component of health food for humans. Ginsenosides in the *Panax* species promote efficient protein metabolism, and enhance physiological activities including reproduction, antioxidants, neurotransmitters, and antagonists to muscle fatigue (Chen and Lee, 1995; Kim et al., 1996; Zhang et al., 2001). Recently, wild ginseng has been cultivated in large quantities in a bioreactor. Previous studies using cultured wild ginseng roots (CWGR) incubated in a bioreactor confirmed the specific genetic homogeneity, such as *rbcL* and *psbD*, in the wild ginseng using a genomic DNA fingerprinting technique (Bae et al., 2003). More than 30 different kinds of ginsenosides in ginseng, especially wild ginseng, contain Ra_1, Ra_2, Rb_1, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, and Rg_3 of protopanaxadiol form, Rh_1 of protopanaxatriol form, and RO of oleanolic acid form (Tanaka et al., 1966; Shibata, 1967). Several studies have been conducted to determine the effectiveness of Rh_2, Rh_1, and Rk_1, which were produced by b-galactosidase from Rg_3 by intestinal microorganisms, in preventing cancer (Lee et al., 1998; Zhang et al., 2001). Saponin, as a secondary metabolite of plant compounds, potentially suppresses infections, acting as phytoanticipins, anti-insect, and phytoprotectants (VanEtten et al., 1994). Glycosylated terpenoid saponin effectively inhibited diguanylate cyclase, which is required for plant fiber synthesis (Ohana et al., 1998).

Supplemented saponins in forage and cereals fed to ruminants were degraded to monosaccharides by microbial fermentation in the rumen (Francis et al., 2002). Therefore, the saponin of alfalfa, oats, corn, and cottonseed in diets, was decreased in the rumen (Bird and Leng, 1978; Demeyer and Van Nevel, 1979; Makkar and Becker, 1996; Pell et al., 2000) and protozoa activity increased with cross-feeding of rumen bacteria (Hill et al., 1991; Wang et al., 1996; Wang et al., 1998). Therefore, the purpose of this study was to investigate the effect of feeding CWGR to organic Saanen dairy goats on the ginsenoside profiles, milk composition, and enhancement of immunity parameters in the blood.

Ⅱ. Materials and Methods

The present study was approved by the Institutional Animal Care and Use Committee at the Chung-Ang University in Seoul, Korea (NO: 2013-0047).

1. Preparation of cultured wild ginseng root (CWGR)

Primary roots of wild ginseng (70-120 years old, approximately 50 cm in length, collected during hibernation) were used as experimental material, and the rest of the wild ginseng was stored at 4°C for further analysis. Modified wood plant medium (WPM) (Lloyd and McCown, 1980; Owen and Miller, 1992) was used as a basal medium, and 30 g/L sugar, 7 g/L Agar, and 1-5 ppm 3-indole acetic acid (IAA) plant growth regulator were added; organ differentiation was induced in 40-60 days in the petri dish (pH 5.8, 25℃ dark room). Organ differentiation of CWGR was sub-cultured to the next step in a 20-L bioreactor. CWGR was cultured using WPM for 30 days in a 500-L bioreactor for mass production. After freeze-drying, CWGR was crushed to about 1 mm in diameter for further experiments.

2. Experimental animals and design

Sixteen dairy goats (Saanen, average body weight 38.6 ± 3.2 kg, average daily milk yield 2.85 ± 1.2 kg/d, and average parity 3.0) were kept in two groups throughout the experimental period. During the experimental period, the feed supplement was followed by 2.3 kg/d total mixed ration (TMR) of dry matter intake (DMI) twice a day at 08:00 and 18:00 (Table 1). The TMR diet was supplemented twice daily at milking time with 1.5 g/DMI/d CWGR powder. The experimental period comprised 24 days, and the goat milk and blood was sampled 3 days after an adaptation period of 21 days.

Table 1. Ingredients and chemical composition (% DM) of the TMR used in this experiment

Items	% in DM	Items	% in DM
Ingredient composition		Chemical composition	
Alfalfa (hay bale)	6.21	Dry matter	76.81
Tall fescue (straw)	9.31	Crude protein (CP)	14.60
Klein (hay)	6.21	Undegradable protein (%, CP)	35.70
Oats (hay)	7.76	Degradable protein (%, CP)	64.30
Beet pulp	3.10	Soluble protein (%, CP)	30.30
Whole cotton seed	3.10	Ether extract	4.21
$CaCO_3$	6.55	Crude Fiber	17.09
$NaHCO_3$	10.00	NDF[2)]	40.98

Items	% in DM	Items	% in DM
Corn (mash)	5.92	ADF[3)]	25.88
Corn Silage	8.45	eNDF (%, NDF)[4)]	73.30
Concentrate mix[1)]	33.39	TDN[5)]	70.00
		NEl (Mcal)[6)]	37.96

[1)] TMR concentrate mixes contained on dry matter basis, 11.5% ground corn, 10.2% DDGS (dried distillers grains with soluble), 8.8% corn gluten feed, 7.1% corn germ meal, 7.0% palm kernel meal, 6.2% wheat bran, 6.2% rapeseed meal, 6.2% wheat flour, 5.3% wheat, 3.8% soybean meal (44% CP), 3.7% coconut meal, 2.3% full-fat soy, 2.7% perilla meal, 0.4% bypass protein, 7.29% etc. & vitamin & mineral.

[2)] Neutral detergent fiber.

[3)] Acid detergent fiber.

[4)] Effective NDF.

[5)] Total digestible nutrient.

[6)] Net energy of lactation.

3. Sample preparation

Milk yield was measured with a milk meter twice a day using a mobile milking machine (LT 80, Shinil Co. Ltd., Korea) at 08:00 and 17:00. Milk was analyzed for milk composition and somatic cell counts (SCC) by a Milko-Scan (FOSS 4000, Foss, Denmark). Blood was taken from a jugular vein into a vacutainer tube without heparin. The serum was separated to measure IgG and plasma proteins by the radial immunodiffusion test (Mancini et al., 1965). CWGR using experimental feed additive contained an amount of ginsinoside Rg_1, Re, Rf, Rb_1, Rc, Rb_2, Rd, Rg_2 and Rg_3 concentration on the dry matter basis was 0.35, 0.15, 0.10, 0.20, 0.06, 0.08, 0.15, 0.06 and 0.01 mg/g, respectively.

4. Analysis of saponin, CWGR ginsenoside profiles, blood, and milk

Each sample was pretreated with 5 mL 70% ethanol for thin layer chromatography (TLC), mixed, and then preserved at 4℃ for 60 min. The samples were centrifuged at 800 × g for 15 min and 3 mL supernatant was added to 3 mL hexane and mixed. The mixed supernatant was centrifuged at 1,500 × g for 15 min and the supernatant was removed. The other phase was added to 3 mL butanol and 9 mL water, and concentrated by an evaporator (EYELA N-N series, Rikakikai Co. Ltd., Japan); subsequently, 1 mL methanol was added, and the standard was prepared. The CWGR (0.1%, 0.01%) was prepared in the same way. A TLC plate of 5 × 6 cm (Silica gel 60 F245, Merck Co. Ltd., Japan) was analyzed using a plain capillary tube (Chase

Ins., USA), which was wetted at the same point with each concentrated sample. The concentrated TLC plate was placed in a chamber of chloroform (C) : methanol (M) : water (W) ratio of 65 : 25 : 10 for band formation. Then, non-saponin bands were selected by UV lamp when the agar dried. Non-saponin was separated from the standard and treatment, which were wetted with 5% ethanol with sulfuric acid and heated.

For the analysis of the saponin content of goat milk, sample preparation was performed in the same way as that of the TLC, and the final sample was filtered through a 0.2-μm filter. The solvent composition for TLC development was a C:M:W ratio of 65:25:10. For the analysis of the ginsenosides of goat milk, the analytical column used was a Bondapak C18 (3.9 × 150 mm, Waters, USA) were used at a column temperature of 40°C. The optimal conditions included of HPLC a gradient elution of water(A) and acetonitrile (B) (80.5:19.5, v/v) using the following gradient program: 0-30 min, 19-19% B; 30-40 min, 19-31% B; 40-60 min, 31-56% B. The detection wavelength was set at 1 mL/min. The intensities of the UV detector (203 nm) were compared with those of the ELSD detector. Each standard ginsenoside Rg_1, Re, Rf, s-Rh_3, Rb_1, Rc, Rd, s-Rg_3, r-Rg_3 (BTGin Co. Ltd., Korea) was qualitatively analyzed individually and in a mixture. The quantitative analysis of the Re and Rg_3 concentration was quantified by a peak of 0.025, 0.05, 0.1 mg/mL, respectively.

5. Statistical analysis

Results of the goat milk composition, ginsenoside profiles, and immune responses were analyzed using an analysis of variance with a mixed procedure by SAS (SAS, 1999). Mean comparison between control and treatment was performed using the LSMEANS option, and significance was declared at $P < 0.05$.

Ⅲ. Results and Discussion

A difference was observed in the average daily feed intake of goats (Saanen) between the control (2,161 g DMI/d) and treatment (2,133 g DMI/d) groups, respectively. However, there was no significant difference in the average milk composition (fat, protein, lactose, and solid-not-fat) between the control and treatment group (Table 2). These results are similar to those of a study wherein saponin-rich yucca extract was supplemented in the diet of dairy cows, and milk production and feed intake were not compromised (Benchaar et al., 2008).

Table 2. Effect of artificially cultured wild ginseng roots on goat milk composition

Items	Control	Treatment[1)]	SEM[2)]	P < 0.05
Dry matter intake, kg/d	2.17	2.13	0.034	0.498
Milk yield, kg/d	2.16	2.13	0.43	0.757
Milk fat, %	3.71	3.69	0.04	0.711
Milk protein, %	2.79	2.76	0.30	0.473
Milk lactose, %	4.30	4.28	0.02	0.572
Milk solid not fat, %	7.89	7.91	0.03	0.545

[1)] The feed supplement was followed by 2.3 kg/d total mixed ration (TMR) of dry matter intake twice a day at 08:00 and 18:00. The TMR diet was supplemented twice daily at milking time with 1.5 g/DMI/d cultured wild ginseng root powder.

[2)] Standard error of the mean.

Plasma IgG concentration was higher ($P < 0.05$) in the treatment (12.12 mg/mL) than the control (10.34 mg/mL) group. Plasma protein concentration was also higher ($P < 0.05$) for the treatment (27.51 mg/mL) than the control (21.34 mg/mL) group (Table 3). These results were consistent with previous studies that reported increased blood IgG and IgM concentration by ginseng components and saponin (Fukuda et al., 2000; Song et al., 2009; Endale et al., 2014). Indeed, artificially inoculated *Staphylococcus aureus* with ginsenoside in mammary glands prevented mastitis by enhancing the immune responses (Hu et al., 2001). Therefore, it may be concluded that increased concentrations of plasma IgG and protein, as observed in this experiment, are possibly caused by CWGR supplementation in goat diets.

Table 3. Effect of supplementation of cultured wild ginseng roots[1)] on plasma IgG and plasma protein in goats

Items	Control	Treatment	SEM[2)]	P < 0.05
Plasma IgG (mg/mL)	10.34^{B}	12.15^{A}	0.1143	0.0004
Plasma protein (mg/mL)	21.34^{B}	27.51^{A}	0.3044	0.0001

[1)] The feed supplement was followed by 2.3 kg/d total mixed ration (TMR) of dry matter intake twice a day at 08:00 and 18:00. The TMR diet was supplemented twice daily at milking time with 1.5 g/DMI/d cultured wild ginseng root powder.

[2)] Standard error of the mean.

[A, B] Within a row, values with different superscripts differ ($P < 0.05$)

Isolated ginsenoside by TLC in goat milk from the control group showed no band, which ginsenosides of CWGR are not present. However, in the treatment group, ginsenosides S-Rh_1, R-Rh_1, Rd, and Rb_1 were detected in goat milk (Fig. 1).

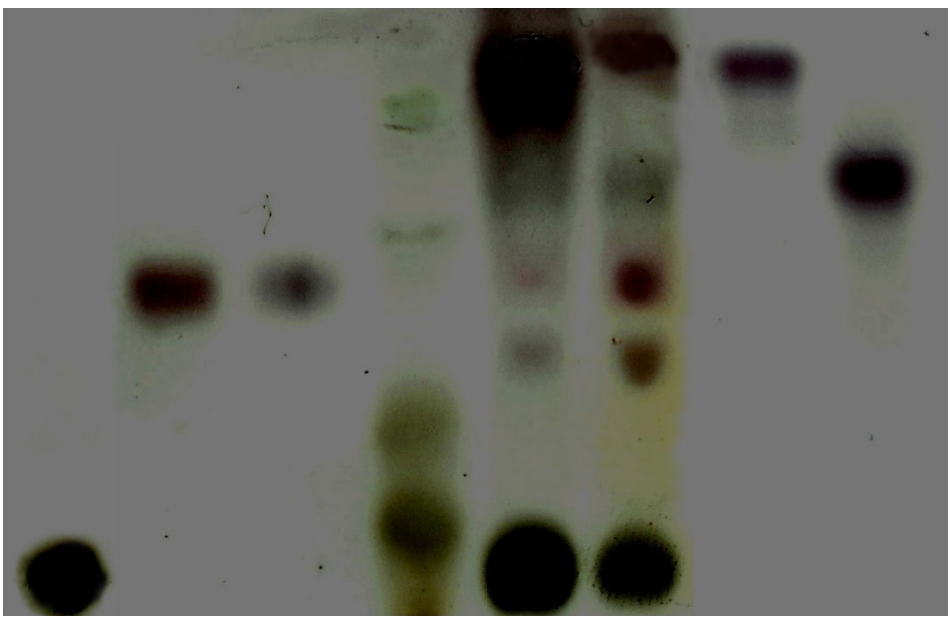

Fig. 1. Thin layer chromatography separation of ginsenoside S-Rh_1, R-Rh_1, Rd and Rb_1. Control was not supplemented cultured wild ginseng roots (CWGR), Standard (Std.) was supplemented 1.5 g/d CWGR powder. Goat milk was supplemented with artificially cultured wild ginseng roots of the lactating goats at day 11 and day 21.

An HPLC analysis, ginsenoside concentrations in goat milk were 355, 348, 1,672, 319, and 524 μM, respectively (Table 4). This result supports the TLC results, and is consistent with previous studies on the degradation and transformation of ginsenoside and internal degradation by pH, inherent digestive enzymes, and the enzymes of intestinal microorganisms (Odani et al., 1983; Strombom et al., 1985; Hasegawa et al., 1996; Akao et al., 1998; Lee et al., 2000). Moreover, rumen microbial fermentation of CWGR could be increased by β-glucosidase from rumen cellulolytic bacteria. Ginsenoside in the small intestine of goats enhanced the level of neutrophilic leukocytes and peripheral blood lymphocytes (Hu et al., 1995; Concha et al., 1996). Therefore, CWGR ginsenosides and ginsenosides from microbial degradation in the rumen were possibly transferred to goat milk, resulting in enhanced immunity of the goat milk.

Table 4. Effect of supplementation of artificially cultured wild ginseng[1)] roots on ginsenoside profiles in goat milk

Items	Control	Treatment[1)]
	---------- Ginsenosides (mM) ----------	
Rf	ND[2)]	335 ± 9.62
S-Rh_1	ND	348 ± 11.67
R-Rh_1	ND	1,672 ± 12.93
Rd	ND	319 ± 9.41
Rb_1	ND	524 ± 8.47

[1)] The feed supplement was followed by 2.3 kg/d total mixed ration (TMR) of dry matter intake twice a day at 08:00 and 18:00. The TMR diet was supplemented twice daily at milking time with 1.5 g/DMI/d cultured wild ginseng root powder.

[2)] ND = not detected.

[Submitted, April. 25, 2016; Revised, July. 7, 2016; July. 19, 2016]

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