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Effect of citron by-product fermented with beneficial bacteria as a functional feed additive for Korean native steers

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This study was conducted to develop a functional feed additive, citron (*Citrus junos* Sieb. ex Tanaka) probiotics (CPB) for beef cattle, using citrus junos by-product (CJ) with probiotics. A two-step fermentation process was developed for the production of CPB and the effects of CPB on growth performance, immune status, carcass characteristics and fatty acid profile in Korean native (Hanwoo) steers were investigated. Twenty (20) Hanwoo steers (22 months old; 619.00±10 kg BW) were randomly assigned to two dietary treatments in a completely randomized design: control (basal diet) and 1.0% CPB (basal diet + CPB 1.0% DM basis). At the end of the trial, steers were slaughtered and carcasses were evaluated. Dietary CPB supplementation significantly increased the average daily gain (ADG) and feed efficiency (kg gain/kg DMI) of Hanwoo steers as compared to the control ($P < 0.05$). Additionally, serum IgG and carcass weight of Hanwoo steers were increased in response to CPB dietary supplementation ($P < 0.05$). No difference ($P > 0.05$) was observed in muscle composition while cholesterol concentration reduced in CPB supplementation group ($P < 0.05$). Overall, the concentrations of n-3 fatty acids were increased, while the ratio of n-6/n-3 decreased in the CPB dietary group ($P < 0.05$). In conclusion, dietary CPB improved growth, immunity and carcass weight of Hanwoo steers while reducing muscle cholesterol concentration with an elevated n-3 fatty acids concentration, indicating that CPB can be used as a functional feed additive for beef cattle.

Key words: Citron probiotics, Hanwoo steer, growth performance, immunity, carcass characteristics, fatty acid profile.

INTRODUCTION

Consumers prefer high-quality beef, which is primarily determined by the appearance, freshness, nutritional value and eating quality. Despite their slow growth rate, Korean Hanwoo cattle consistently produce highly palatable and well-marbled beef (Cho et al., 2010). However, it is widely known that the incidence of cardiovascular diseases (CVD) is closely related to the dietary intake of cholesterol and saturated fatty acid (SFA) contents. Accordingly, the meat industry is looking for dietary strategies to modulate cholesterol and SFA while enriching meat with bioactive compounds such as antioxidants to improve product quality and protect consumers from oxidant-mediated diseases.

Citron (*Citrus junos* Sieb. ex Tanaka), which is also known

as Yuza in Korea, is a citrus fruit of the Rutaceae family commonly used as preserved tea or in tablet form as an herbal antioxidant. Citrus junos by-product (CJ) is the residues of the juice processing industries in Korea. About 10-15% of Yuza fruit is generated as by-product from Yuza juice extraction (Lee et al., 1987) and approximately 1800 tons per year have been produced (Kim et al., 2010a). These residues are however discarded as processing by-products, causing environmental and economic problems for waste disposal. CJ are mainly composed of peel, pulp and seed. Especially, peel includes valuable compounds such as terpenoid, pectin and flavonoid. Fresh or dehydrated citrus pulp is primarily used in ruminant feeding (Fegeros et al., 1995). Fermentation of feed silage for ruminants using beneficial bacteria has been practiced for many years. Citrus by-products have been reported to have characteristics required for use as a substrate for the growth of probiotics during fermentation (Contreras Esquivel et al., 1999).

Ruminant feeding systems based on locally available by-product feed stuffs are often a practical alternative as the rumen microbial ecosystem can utilize food by-products, which often contain high levels of structural fiber, to meet their nutrient requirements for maintenance, growth, production and reproduction (Bampidis and Robinson, 2006). Probiotics also have pseudo-antibiotic properties that improve feed efficiency, growth performance and health of animals (Hossain et al., 2012). In ruminants, dietary probiotics supplementation has been found to reduce acidosis and improve energy utilization in the rumen, while maintaining the intestinal microbial balance and improve animal performance (Krehbiel et al., 2003). They also reported increased daily gain and feed efficiency in feedlot cattle, enhanced milk production in dairy cows and improved immune response in stressed calves. Dietary yeast cultures (*Saccharomyces cerevisiae*) supplementation increased weight gain and feed conversion efficiency of bulls (Mutsvangwa et al., 1992); increased ADG and feed to gain ratio in Awassi lambs (Haddad and Goussous, 2005). However, the combined effects of CJ and probiotics in ruminant feeding systems have not been evaluated to date. Hence, this study was conducted to verify the collective effect of CJ fermented with beneficial probiotics strain on growth performance, immune status, carcass characteristics and fatty acid profile of Hanwoo steers.

MATERIALS AND METHODS

Steers were handled in accordance with the guidelines of animal care and use of animals in research (Korean Ministry for Food Agriculture Forestry and Fisheries, 2008). All experimental procedures used in this study were approved by the Animal Care and Use Committee of Sunchon National University.

Preparation of CPB

Candidate probiotics strains consisting of 14 strains, *Lactobacillus acidophilus* (3111, 3146, 3150), *Lactobacillus plantarum* (KCTC 3104, 3107), *Enterococcus faecium* (KCTC 2022, 3078, 3080), *Bacillus subtilis* (KCTC 1022, 1103, 3239) and *S. cerevisiae* (KCTC 7107, 7915, 7928), were obtained from the Korean collection for type cultures (KCTC). The KCTC microbial strains were collected from the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. From the above strains, *L. acidophilus* KCTC 3111, *B. subtilis* KCTC 3239, and *S. cerevisiae* KCTC 7915 were selected as starter cultures based on acid, bile and heat tolerance level according to Hossain et al. (2012).

CJ (30%), defatted rice bran (58%), ground barley stone (10%) and molasses (2%) were used as the fermented solid media. A two-step fermentation procedure was conducted using a commercial fermenter (W-1000; Wonbalhyo Industry Co., Icheon, South Korea). First, 0.5% *L. acidophilus* KCTC 3111 was added to solid substrate media and fermented at 40°C for two days under repeating cycles of 5 h of anaerobic and 3 h of aerobic conditions. The second fermentation was performed with 0.5% *B. subtilis* KCTC 3239 and 0.5% *S. cerevisiae* KCTC 7915 strains for 2 days at 40°C under aerobic conditions. The formulated probiotics mixtures were then dried for 2 days until the moisture level was less than 15% using a dry oven (DooriTech FA, Co., Ltd, Korea). To determine the number of cells, 1 g of CPB was diluted with sterilized distilled water (10 ml) at room temperature. Approximately 10 min later, 1 ml of this mixture was serially diluted 10-fold in 0.85% NaCl solution and was cultured in agar media. The culture plate was then incubated at 37°C for 24-48 h, after which the number of colonies was counted. The chemical compositions of CJ and CPB were determined by the method described by the Association of Official Analytical Chemists (AOAC, 2000). The microflora concentration and chemical composition of CJ and CPB are shown in Table 1.

Animals, experimental design and diet

The study was conducted at the experimental farm of Sunchon

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Abbreviations: ADG, Average daily gain; **AOAC,** Association of Official Analytical Chemists; **BW,** body weight; **CJ,** citrus junos by-product; **CPB,** citron (*Citrus junos* Sieb. ex Tanaka) probiotics; **CVD,** cardiovascular diseases; **DFM,** direct fed microbial; **DM,** dry matter; **DMI,** dry matter intake; **GLM,** general linear model; **IgG,** immunoglobulin G; **IgM,** immunoglobulin M; **KAPE,** Korea Institute for Animal Products Quality Evaluation; **KCTC,** Korean collection for type culture; **MUFA,** monounsaturated fatty acids; **NRLSI,** National Rural Living Science Institute; **PUFA,** polyunsaturated fatty acid; **SAS,** statistical analysis system; **SFA,** saturated fatty acid; **UFA,** unsaturated fatty acid.

Table 1. Microflora concentration and chemical composition of *Citrus junos* by-product (CJ) and citron probiotics (*Citrus junos* Sieb. ex Tanaka) (CPB).

Item	CJ	CPB
Microbial strains in CPB (cfu/g)		
Lactobacillus acidophilus KCTC 3111		2.0×10^7
Bacillus subtilis KCTC 3239		1.0×10^7
Saccharomyces cerevisiae KCTC 7915		2.0×10^7
Chemical composition (% DM)		
Moisture	76.02	13.09
Crude protein	3.53	14.84
Crude fat	0.45	1.64
Crude fiber	3.73	8.18
Crude ash	0.83	17.21

Korean collection for type culture (KCTC) strains obtained from the Korean Research Institute of Bioscience and Biotechnology.
DM = Dry matter.

Steers were weighed using a platform balance and body weights were measured before feeding and watering at the onset and end of the experiment. Feed bunks were cleaned and residues were collected daily and weighed at intervals corresponding to weigh dates. Feed efficiency was calculated as the ratio between body weight gain and average feed intake.

Immunoglobulin (IgG and IgM) determinations

To determine the IgG and IgM levels, approximately 5.0 ml of blood was collected from the jugular vein. Samples were then centrifuged at 1,610 x*g* for 15 min in a cold chamber (4°C), after which the serum was collected. The separated serum was then carefully removed to plastic vials and stored at -20°C for further analysis. Serum Ig concentrations were assayed using bovine IgG (Cat. No. E10-118) and IgM (Cat. No. E10-101) ELISA Quantification Kits (BETHYL Laboratories Inc., USA) according to the manufacturer's instructions. Tests were performed in duplicate and results were presented as the mean value of three replications.

Carcass grading and meat quality

At the end of the trial, steers were slaughtered in a commercial slaughterhouse in Suncheon, South Korea. Carcasses were graded for quality and yield factors by a trained carcass evaluator in accordance with Korean beef carcass grading standards (KAPE, 2012). The beef carcasses were graded as 1++, 1+, 1, 2, or 3, which were principally based on marbling scores and then adjusted according to other carcass traits such as meat color, fat color, texture of lean meat and maturity. One of three yield grades (A, B or C) was determined by assessing the live weight, carcass weight and back fat thickness in the rib eye area.

Muscle composition and cholesterol determination

Within approximately 2 h of slaughter, samples were stored at -20°C until required for analysis. To investigate the meat chemical compositions, longissimus muscles from the loin area were selected and ground using a meat grinder. The moisture, crude protein, crude fat and crude ash contents were determined using the AOAC methods (AOAC, 2000). The cholesterol concentration was determined according to the slightly modified method of King et al. (1998). One gram of ground meat, 100 µg of 5α-cholestane and 15 ml water were homogenized with 200 ml of Folch solution (chloroform : methanol, 2:1 v/v) and filtered. The filtrate was added to 50 ml of 0.5% sodium hydroxide. The sample was saponified at 85°C for 60 min with 10 ml of 2 M ethanolic potassium hydroxide solution (Adams et al., 1986). After cooling to room temperature, cholesterol was extracted with 1 ml of hexane. The process was repeated four times. The hexane layers were transferred to a round-bottomed flask and dried under vacuum. The extract was redissolved in 1 ml of hexane and was stored at -20°C until analysis. Cholesterol content was determined by gas chromatography (DS 6200, Donam Co., Seongnam, Gyeonggi-do, Korea) as shown in Table 3.

Fatty acid composition

Meat fatty acids were determined by the methyl ester extraction method described by Yang et al. (2003). One gram of ground meat sample was dissolved separately into 100 ml of Folch solution (chloroform : methanol 2:1 v/v) for 15 min. The samples were then flushed with nitrogen gas for 30 min in an evaporator and filtered through a Buchner funnel. The filtrate was subsequently dissolved in 70 ml of double distilled water and kept at 5°C in a refrigerator until the fat layer separated. After phase separation, the bottom layer was evaporated at 35°C with nitrogen gas and dissolved in 3 ml of 5% sulfuric acid methanol. The tubes were then heated in a water bath at 95°C for 45 min, after which they were allowed to cool at room temperature. Next, fatty acid methyl ester was extracted three times with 3 ml of petroleum ether and dried with nitrogen gas and the fatty acid profile of meat was analyzed by gas chromatography (DS 6200, Donam Co., Korea). Samples were loaded onto the column via 1 µl splitless injections under the conditions described in Table 3. Fatty acids were identified by matching their retention times with those of their relative standards (polyunsaturated fatty acid (PUFA)-2; Supelco, USA) and the Food Composition Table (NRLSI, 2002).

Statistical analysis

Data were analyzed by a randomized block design, using the GLM procedure of SAS (SAS, 2003) based on the statistical model:

$Yij = \mu + Ti + eij$

Where, Yij = dependent variable of the jth animal on the ith treatment; µ = overall mean; Ti = the fixed effect of ith treatment effect (i = 1, 2); Eij = random residual (error) associated with the dependent variable from the jth animal on the ith treatment.

For growth performance parameters, a group of two steers served as the experimental unit. For carcass characteristics, meat composition, cholesterol concentration and fatty acid profile of meat, an individual steer served as the experimental unit. Variability in data was expressed as the pooled S.E. The α-level for analysis was < 0.05, with P-values > 0.05 and < 0.10 considered as tendencies.

RESULTS

Growth performance and immunity

The effects of dietary CPB supplementation on the growth

Table 2. Ingredients and chemical composition of total mixed ration feed.

Ingredient/chemical composition	**Experimental diet**
Ingredients (% fed basis)	
Concentrated feed	17.03
Corn, ground	18.44
Corn gluten feed	7.74
Wheat bran	11.36
Brewers grain	8.32
Whole crop barley silage	15.90
Italian ryegrass	11.36
Tall fescue	6.81
Salt	2.00
Vitamin-mineral mix[1]	0.23
Limestone	0.81
Calculated chemical composition (% DM)	
Moisture	24.28
Crude protein	8.48
Crude fat	1.55
Crude fiber	13.89
Crude ash	5.36
Acid detergent fiber	17.95
Neutral detergent fiber	41.90
Non-fibrous carbohydrate	42.71
Total digestible nutrients	72.30

[1]Premix provided the following nutrients per kg of diet: Vitamin (Vit) A, 9,000,000 IU; Vit. D3, 2,100,000 IU; Vit. E, 15,000 IU; Vit. K, 2,000 mg; Vit. B1, 1,500 mg; Vit. B2, 4,000 mg; Vit. B6, 3,000 mg; Vit. B12, 15 mg; Pan-Acid-Ca, 8500 mg; Niacin, 20,000 mg; Biotin, 110 mg; folic-acid, 600 mg; Co, 300 mg; Cu, 3,500 mg; Mn, 55,000 mg; Zn, 40,000 mg; I, 600 mg; Se, 130 mg.

performance of Hanwoo steers is shown in Table 4. The ADG and feed efficiency of Hanwoo steers were significantly increased in the CPB dietary group relative to the control ($P < 0.05$). Dietary CPB supplementation significantly increased the serum IgG level, whereas the IgM level remained unaffected relative to the control group ($P < 0.05$) (Table 5).

Carcass characteristics and muscle composition

As shown in Table 6, the carcass weight of Hanwoo steers increased significantly in response to CPB supplementation relative to the control group ($P < 0.05$). No significant variation was observed in dressing out percentage, meat quality grade and carcass yield grade between groups. The moisture, crude protein, crude fat and crude ash content of the longissimus muscle of Hanwoo steers was unaffected by CPB supplementation (Table 7). A significant reduction of meat cholesterol concentration was observed in the CPB dietary group as compared to the control group ($P = 0.01$) (Table 7).

Fatty acid profile

The fatty acid profiles of the longissimus muscle of Hanwoo beef are shown in Table 8. An increased concentration of stearic acid (C18:0) in CPB dietary group as compared to the control ($P < 0.05$) was seen.

Among the monounsaturated fatty acids (MUFA), myristoleic acid (C14:1) increased and eicosenoic acid (C20:ln-9) decreased in response to CPB dietary supplementation relative to the control ($P < 0.05$). Supplementation of CPB increased ω-3 (α-linolenic acid, C18:3n-3 and eicosatrienoic acid, C20:3n-3) and ω-6 (arachidonic acid, C20:4n-6) fatty acids ($P < 0.05$). No significant variation was observed in total SFA and unsatu-

Table 3. Conditions applied for gas chromatography analysis of cholesterol and fatty acid profile.

Item	Condition	
	Cholesterol	**Fatty acid**
Instrument	DS 6200 (Korea)	DS 6200 (Korea)
Detector	FID 270°C	FID 270°C
Injector	Capillary ING 250°C	Capillary ING 250°C
Column	HP-5 (J&W, 30 m × 0.32 mm, 0.25 μm film thickness)	HP-5 (J&W, 30 m × 0.32 mm, 0.25 μm film thickness)
Carrier gas flow	Nitrogen (1.0 mL/min)	Nitrogen (1.0 mL/min)
Make up gas flow	H_2 (3.0 mL/min)	H_2 (3.0 mL/min)
Oven temperature	250°C	140°C
Detector temperature	280°C	270°C
Injector temperature	280°C	250°C
Temperature program	250°C (2 min) - 15°C/min - 290°C (10min) - 10°C/min - 310°C (10 min)	140°C (1 min) -10°C /min - 220°C (2min) -2°C /min - 240°C (9 min)
Split ratio	50:1	50:1
Injection volume	2 μL	1 μL

Table 4. Effect of citron (*Citrus junos* Sieb. ex Tanaka) probiotics (CPB) on growth performance of Hanwoo steers over 180 days[1].

Parameter	Treatment		Pooled S.E.[2]	P-value
	Control	**CPB**		
Initial body weight (kg/steer)	619.18	621.73	6.45	0.79
Final body weight (kg/steer)	750.30	762.52	5.32	0.13
ADG (kg/steer)	0.73	0.78	0.02	0.04
ADFI (kg DM/steer)	11.34	11.42	0.16	0.74
Feed efficiency (kg gain/kg DMI)	0.06	0.07	0.002	0.03

Significant difference ($P < 0.05$) or tend to differ ($P < 0.1$). [1]Data presented as the mean value of five replicate groups with two steers per replication (n=10). ADG = average daily gain; ADFI = average daily feed intake; [2]Pooled standard error.

Table 5. Effect of citron (*Citrus junos* Sieb. ex Tanaka) probiotics (CPB) on serum immunoglobulin level of Hanwoo steers[1].

Parameter (ng/ml)	Treatment		Pooled S.E.[2]	P-value
	Control	**CPB**		
IgM	3.55	3.35	0.23	0.54
IgG	184.82	243.11	4.34	<0.0001

Significant difference ($P < 0.05$) or tend to differ ($P < 0.1$). [1]Data presented as the mean value of five replicate steers for each treatment (n=5); IgM = immunoglobulin M; IgG = immunoglobulin G; [2]Pooled standard error.

rated fatty acid (UFA) concentration between treatments. Dietary CPB supplementation elevated the total concentration of n-3 PUFA ($P = 0.01$), while it reduced the ratio of n-6/n-3 fatty acids ($P < 0.05$).

Table 6. Effect of citron (*Citrus junos* Sieb. ex Tanaka) probiotics (CPB) on carcass characteristics and meat quality of Hanwoo steers[1].

Parameter	Treatment		Pooled S.E.[4]	P-value
	Control	CPB		
Carcass weight (kg)	454.10	476.18	6.68	0.04
Dressing out percent	60.56	62.46	1.02	0.21
Meat quality grade[2]	3.75	2.88	0.36	0.11
Carcass yield grade[3]	1.25	1.38	0.17	0.62

Significant difference (P < 0.05) or tend to differ (P < 0.1). [1]Data presented as the mean value of five replicate steers for each treatment (n=5); [2]Meat quality grades: 5 = 1++; 4 = 1+; 3 = 1; 2 = 2; 3 = 1 (According to KAPE, 2012); [3]Carcass yield grades: 3 = A; 2 = B; 1 = C grade; [4]Pooled standard error.

Table 7. Effect of citron (*Citrus junos* Sieb. ex Tanaka) probiotics (CPB) on longissimus muscle chemical composition (g/100 g) and cholesterol content of Hanwoo carcass[1].

Parameter	Treatment		Pooled S.E.[2]	P-value
	Control	CPB		
Moisture	61.94	60.67	1.87	0.65
Crude protein	20.91	19.18	0.93	0.25
Crude fat	16.39	19.27	2.10	0.37
Crude ash	0.90	0.88	0.04	0.80
Cholesterol (mg/100 g meat)	66.06	52.00	2.66	0.01

Significant difference (P < 0.05) or tend to differ (P < 0.1). [1]Data presented as the mean value of five replicate steers for each treatment (n=5); [2]Pooled standard error.

DISCUSSION

Growth performance and immunity

The use of feed additives of natural origin has been encouraged in human and animal nutrition over the last decade. The new approach of feeding Hanwoo steers with a combination of CJ fermented with beneficial probiotics strains (*L. acidophilus* KCTC 3111*, B. subtilis* KCTC 3239, S. cerevisiae KCTC 7915) is believed to adapt adapt the rumen to the presence of large quantities of lactic acid, either directly by feeding lactate-utilizing bacteria, or indirectly by feeding lactate-producing bacteria, which in turn stimulates the growth of lactate-utilizers (Krehbiel et al., 2003).

In the present study, dietary CPB supplementation significantly increased the ADG and feed efficiency of Hanwoo steers as compared to the control (Table 4). These findings are in agreement with those of Bueno et al. (2002), who reported the increased daily gain, dry matter intake and feed conversion efficiency in response to supplementation of the diets of growing kids with dehydrated citrus pulp. Supplementation of lactate-producing and/or lactate-utilizing bacteria has been shown to improve daily gain and feed efficiency of feedlot cattle (Swinney-Floyd et al., 1999; Galyean et al., 2000). During the fermentation process, lactic acid bacteria act on water-soluble carbohydrates in feed particles to produce a number of products, primarily lactic acid. This results in a rapid reduction of pH, protecting the microbes and preserving the maximum level of nutrients in the products (Merry and Davies, 1999).

The yeasts *S. cerevisiae* (Kim et al., 2010b) and *B. subtilis* (Nimker et al., 2010) can produce extracellular enzymes such as α-amylase, which stimulate starch hydrolysis and the growth of lactic acid bacteria. Direct fed microbial (DFM) has been reported to alter the efficiency of production as well as proportional concentrations of volatile fatty acids in the rumen (Kmet et al., 1993). If propionate production is both energetically and proportionately increased by DFM, then it is likely that the energy available to the animal also increase (Elam et al.,

Table 8. Effect of citron (*Citrus junos* Sieb. ex Tanaka) probiotics (CPB) on longissimus muscle fatty acid compositions in Hanwoo steers[1].

Fatty acid (% total fatty acid)	Treatment		Pooled S.E.[2]	P-value
	Control	CPB		
Myristic acid (C14:0)	3.02	3.65	0.26	0.14
Palmitic acid (C16:0)	27.21	26.32	1.72	0.72
Stearic acid (C18:0)	10.68	14.7	1.12	0.05
Myristoleic acid (C14:1)	0.29	1.14	0.04	<.0001
Palmitoleic acid (C16:ln7)	4.45	4.32	0.14	0.60
Oleic acid (C18:l n-9)	51.27	46.73	2.30	0.22
Eicosenoic acid (C20:l n-9)	0.51	0.27	0.06	0.03
Linoleic acid (C18:2 n-6)	2.32	2.41	0.14	0.69
α-Linolenic acid (C18:3 n-3)	0.04	0.09	0.01	0.04
Linolenic acid (C18:3 n-3)	0.10	0.16	0.03	0.24
Eicosatrienoic acid (C20:3 n-3)	0.06	0.11	0.01	0.05
Arachidonic acid (C20:4 n-6)	0.05	0.10	0.01	0.01
ΣSFA	40.92	44.67	2.71	0.36
ΣUFA	59.09	55.34	2.10	0.26
UFA/SFA	1.46	1.27	0.12	0.31
ΣMUFA	56.23	51.33	2.22	0.17
ΣPUFA	2.57	2.88	0.14	0.19
Σn-3	0.20	0.36	0.02	0.01
Σn-6	2.37	2.51	0.15	0.54
n-6/n-3	12.25	7.11	1.28	0.04
MUFA/SFA	1.38	1.17	0.12	0.26
PUFA/SFA	0.06	0.06	0.00	0.85

Significant difference ($P < 0.05$) or tend to differ ($P < 0.1$). [1]Data presented as the means of five replicate steers for each treatment (n=5). ΣSFA = total saturated fatty acid; ΣUFA = total unsaturated fatty acids; ΣMUFA = total monounsaturated fatty acids; ΣPUFA = total poly unsaturated fatty acids; Σn-3 = total n-3 PUFA; Σn-6 = total n-6 PUFA. [2]Pooled standard error.

2003). Accordingly, the growth performance observed in the present study may have been due to the synergistic effects of CJ components and probiotics metabolites (Hossain et al., 2012). The increased feed efficiency might be the result of increased ADG in CPB dietary group.

The increased IgG concentration in the CPB dietary group relative to the control (Table 5) might have been due to the combined effects of CJ immunomodulatory compounds and fermentation output with beneficial probiotics strains. Flavonoids, a major bioactive component of citrus fruits can act on immune responses via different mechanisms such as protein binding, active site interference or antioxidant effects (Provenza and Villalba, 2010). Besides, DFM have been shown to affect the innate, humoral and cellular arms of the host immune system (Krehbiel et al., 2003). A bi-directional communication pathway between the immune and endocrine system supports the health and optimal growth of animals (Carroll, 2008). Feed fermented with probiotics showed increased levels of lactic acid and other organic acids, stimulating the specific and non-specific immune function and modulating the composition of the intestinal microbial population against harmful organisms (Van der Wielen et al., 2000).

Supplementation of the diets with CPB resulted in an increased carcass weight of Hanwoo steers as compared to the control (Table 6). The heavier carcass weight in the CPB dietary group was likely due to the increased slaughter weight of animals, since carcass measurements are significantly affected by the slaughter weight (Mohammed et al., 2007). No significant effect of dietary CPB was observed on dressing out percent, meat quality grade and carcass yield grade in the present study (Table 6), which is consistent with the findings of Henrique et al. (2006). Caparra et al. (2007) also reported that lambs fed diet containing solar-dried citrus pulp had similar carcass weight and meat quality grade as the control.

Supplementation of CPB had no significant effect on proximate components of longissimus muscle in the present study (Table 7). The results of our study are in agreement with the findings of Henrique et al. (2006) who also found no significant variation in beef chemical composition when supplemented with dehydrated citrus pulp pellets in finisher bull. In the present study, CPB supplementation significantly reduced the meat cholesterol concentration as compared to the control (Table 7), which might have been due to the combined effect of flavonoids in CJ and multiple probiotics strains activity. Paengkoum et al. (2011) observed a reduced meat cholesterol concentration when *Lactobacillus* and *Saccharomyces* probiotics fermented feed were supplemented in broilers and goats.

Fatty acid profile

Tissue fatty acid composition is affected by the amount and structure of dietary fat, *de novo* fatty acid synthesis, rate of conversion to other fatty acids and metabolites, and the proportion of oxidation used for energy consumption (Nuernberg et al., 2005). Meat fatty acid profile and cholesterol concentration are considered as the pivotal role in human diet as they have hypercholesterolemic properties, which are associated with coronary heart diseases (Daley et al., 2010), while dietary n-3 fatty acids including α-linolenic acid and its metabolites reduce the risk of heart disease (De Lorgeril et al., 1994). The increased concentrations of SFA (stearic acid) and UFA (myristoleic acid, α-linolenic acid, eicosatrienoic acid, arachidonic acid) in CPB dietary group (Table 8) supported the findings of Mourão et al. (2008). They reported palmitic and stearic acids as the predominant fatty acids found in chicken meat as SFA, whereas linoleic and arachidonic acid as PUFA when supplied citrus by-product in broiler diet.

The increased concentration of stearic acid of our study may have little effect on human health. Hu et al. (1999) reported that the major SFA within beef (myristic acid, palmitic acid and stearic acid) is significantly associated with coronary heart disease, however others argue that a distinction should be made for stearic acid which has been found to have little cholesterol-raising effects in humans as compared to myristic and palmitic acid (Grande et al., 1970; Kelly et al., 2002; Mensink et al., 2003). Zock and Katan (1992) also reported that stearic and linoleic acids to be more or less equivalent with respect to their cholesterolemic effect. The increased concentration of total n-3 PUFA and the decreased ratio of n-6/n-3 in CPB dietary supplementation might have been due to the synergism of probiotic strains and CJ (Table 8). Supplementation of dietary citrus pulp increased meat PUFA concentration in broiler (Mourão et al., 2008).

Paengkoum et al. (2011) found a reduced ratio of n-6/n-3 fatty acid in meat when supplemented with *Lactobacillus* and *Saccharomyces* fermented feed in broilers and goats. Dietary probiotics such as *Lactobacillus* and *Saccharomyces* can reduce the SFA concentration and increase the linolenic and total PUFA contents in pig meat through a positive effect on the intestinal flora (Ross et al., 2012).

Conclusion

Based on the results of this trial, the use of CPB significantly improved the daily gain and feed efficiency of Korean native steers. Dietary CPB increased body immunity and carcass weight of the Hanwoo steers, and significantly reduced the meat cholesterol concentration without any significant variation in total SFA or UFA concentration between treatments. The increased content of n-3 PUFA and a reduced ratio of n-6/n-3 PUFA will build up the confidence of beef consumers because it will minimize the risk of cardiovascular diseases. Therefore, CPB can be used as a functional feed additive for Hanwoo steers to improve growth performance and immunity, as well as to produce functional beef for human consumption.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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