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Effects of dietary protected fat and vitamin E on *in vitro* rumen fermentation characteristics and reproductive performances of Hanwoo cows

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Abstract

The present study investigated the effects of protected dietary fat and vitamin E on the reproductive performances of Hanwoo during the estrus period. The present study consisted of two experiments. Experiment 1 determined the effects of dietary supplements on the *in vitro* nutrient digestibility and fermentation characteristics in the rumen. Experiment 2 determined the effects of dietary supplements on blood fatty acid profiles, blood metabolites, and the pregnancy rate of Hanwoo cows. The basal diet was a total mixed ration, which was formulated for Hanwoo cows and was treated with different supplements as follows: without supplement (CON); supplemented 1% of protected fat (PF); supplemented 1% vitamin E (VE); and mixed PF and VE at 1:1 (MIX) based on dry matter (DM). The experimental diets were incubated in the rumen buffer for 72 h at 39℃ with four replications and three blanks for Experiment 1. In Experiment 2, forty Hanwoo cows on estrus (2.7 ± 0.15 of parity) were assigned to each dietary treatment. Each treatment consisted of ten Hanwoo cows placed in two pens and fed individually (5 steers per pen). The feeding period was conducted for 75 days, from 30 d before to 45 d after artificial insemination (AI). In Experiment 1, dietary treatment did not affect nutrient digestibility or fermentation characteristics in the rumen except for the concentration of total volatile fatty acid (VFA). Dietary PF had a higher ($p < 0.05$) total VFA concentration than CON. In Experiment 2, dietary PF and MIX had higher ($p < 0.05$) saturated fatty acid concentrations in the blood of Hanwoo cows, while dietary VE and MIX had higher ($p < 0.05$) vitamin E concentrations. Estrogen concentrations in the blood of Hanwoo cows were lower ($p < 0.05$) in all treatments with supplementary diets. All treatments

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Availability of data and material
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with supplementary diets had lower ($p < 0.05$) AI numbers, resulting in a higher pregnancy rate ($p < 0.05$) of Hanwoo cows. The present study found that the single and combo supplements with PF and vitamin E had beneficial effects on the reproductive performances of Hanwoo cows on estrus.

Keywords: Blood metabolites, Hanwoo cow, Pregnancy rate, Protected fat, Vitamin E

INTRODUCTION

Sustainable livestock production has become a global issue and concern for many countries due to the increased consumption of animal products, such as meat, milk, and eggs. Hanwoo is a Korean native beef cattle, and the demand for its meat has increased significantly over the past decade [1]. A successful breeding program to improve the pregnancy rate of Hanwoo is one of the keys to meeting the market's demand and ensuring a sustainable Hanwoo industry year by year. In general, diet directly influences the reproductive performances of animals [2–4]. With the fulfillment of required nutrients, both micro and macro-nutrients, an animal can express their natural behavior, for example, mature cows can have healthy reproduction cycles [2,5,6]. Malnutrition of cattle results in high service and day-to-conception rates, which increase the economic losses for farmers.

Recently, many studies have indicated that micronutrients, such as vitamins and fatty acids (FAs), play a significant role in maintaining animal performance and the quality of animal products [2,4]. The presence of these micronutrients in the diet are mandatory to support the optimum performance of cows, especially in terms of reproduction [3,5,6]. Studies regarding dietary unsaturated FA (UFA) on ruminants have been conducted widely and have resulted in beneficial effects on the growth performances, immune functions, and pregnancy rates of animals [7]. In addition to UFA, dietary saturated FA (SFA) can provide a high caloric value to increase dietary energy density. Supplementation of dietary SFA, mainly containing C16:0 and C18:0, increases milk yield and fat content, and reduces peak rectal temperatures in heat-stressed dairy cows in the mid-lactation period [8]. In addition, dietary SFA can replace fermentable carbohydrates in the diet without a change in glucose and insulin levels in the blood [8]. Meanwhile, dietary vitamin E also presents many beneficial effects on the immune functions and reproductive performances of cows [9]. Dietary vitamin E decreases the retention time for fetal membranes, reduces the incidence of inframammary infection and clinical mastitis, and enhances the macrophage function of cows [9]. The number of services and day-to-conception of cows is lower with supplementary vitamin E than without supplementation [9]. Moreover, dietary vitamin E in beef cattle also improves the extensions of lipid oxidation and shelf life [10]. The combination of protected fatty acids and vitamin E can be an alternative feed supplement, especially for Hanwoo cows. However, the study of dietary supplements for Hanwoo cows on estrus and successful artificial insemination (AI) is limited.

Therefore, this study consisted of two experiments. Experiment 1 aimed to determine the ruminal digestibility and fermentation characteristics of diets supplemented with protected FA and vitamin E. While Experiment 2 estimated the effect of protected FA and vitamin E on the metabolites, hormones, and FAs in the blood and the reproductive performances of Hanwoo cows during the estrus period.

MATERIALS AND METHODS

The animals used in the present study were reared according to the Animal Care and Use Committee guidelines at Gyeongsang National University, Jinju, Korea, under the animal care and

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use guidelines of the Animal Research Unit (GNU-200603-A0032).

Experimental diet

The basal diet used a total mixed ration (TMR) formulated to be isonitrogenous and isocaloric to meet the nutrient requirements of Hanwoo cows according to the Korean Feeding Standards for Hanwoo, National Livestock Research Institute, Rural Development Administration, Korea [11]. The ingredients and chemical compositions of the basal diet are presented in Table 1. Four dietary treatments were used during this study. They consisted of the basal diet without supplement (CON) and diets supplemented with either 1% protected fat (PF; Ca-salt, Bypass Mate, Emulsifying Industrial, Tokyo, Japan), 1% vitamin E (VE; α-tocopherol, 1,000 IU, Sigma-Aldrich, St. Louis, MO, USA), or a 1% mixture of PF and VE at 1:1 ratio (MIX) on a dry matter (DM) basis. The experimental diets were sub-sampled, then dried at 65℃ for 48 h, and ground using a cutting mill (Shinmyung Electric, Gimpo, Korea) to pass through a 1-mm screen for *in vitro* rumen incubation (2 kg) and chemical compositions (1 kg).

In vitro rumen incubation in Experiment 1

Before 2 h morning feeding, the rumen fluid was collected from two non-pregnant cannulated Hanwoo heifers fed rice straw and commercial concentrate for Hanwoo cows at a 4:1 ratio. The collected rumen fluid was composited, then filtered through two layers of cheesecloth, and mixed with a buffer solution at a 1:2 ratio as described by Adesogan et al. [12] for an anaerobic culture medium. The ground diet (0.5 g) with an anaerobic culture medium (40 mL) were placed in the incubation bottles in quadruplicate with two blanks. Then, all incubation bottles were gassed with CO_2, closed tightly to reach anaerobic conditions, and placed in an incubator at 39℃ for 72 h. Gas production was measured using an ANKOM^{RF} automatic gas production system (ANKOM Technology, Macedon, NY, USA) and recorded on a computer every 30 min for 72 h following the method described by Adesogan et al. [12]. After incubation, the bottle content was transferred to a 50 mL conical tube and centrifuged at 2,568 × g for 15 min (SUPRA21K, Hanil Electric, Gimpo, Korea) to separate the residue and supernatant for *in vitro* digestibility and rumen fermentation characteristics, respectively.

Animal and management in Experiment 2

Forty Hanwoo cows (2.71 ± 0.17 of parity; 50.8 ± 1.25 months of age) on estrus were grouped by parity and age and randomly assigned to one of four treatments (CON vs. PF vs. VE vs. MIX). Ten cows per treatment were housed in two pens (5 m × 8 m) and fed 3.6 kg of experimental diet (DM basis) twice daily at 08:00 and 17:00 h to meet their energy requirements using an individual feeder for 75 d, from 30 d before AI to 45 d after AI. Cows had free access to water and mineral blocks (Na, 380 g/kg; Mg, 5 g/kg; I, 150 mg/kg; Fe, 1.5 g/kg; vitamin A, 60,000 IU/kg; vitamin D3, 60,000 IU/kg; Tithebarn, Winsford, Cheshire, UK). The orts were collected daily before the morning feeding to estimate feed intake. The estrus of Hanwoo cows was confirmed by mounting behavior using a W-Tag estrus detector (Wuyang, Jeonju, Korea) and the body condition score (BCS) just before AI was recorded [13]. After 42 d of AI, the pregnancy was diagnosed using a pregnancy diagnosis kit (Alertys Rapid Visual Pregnancy Test, IDEXX, Westbrook ME, USA). On the day of AI, the cows were bled (10 mL) from the jugular vein into tubes containing clot activator-treated blood in a gel separator (Vacuette Z Serum Sep Clot Activator, Greiner Bio-One, Kremsmunster, Austria) at 3 h after the morning feeding and immediately stored on ice. Then, plasma samples were obtained by centrifuging the blood at 969×g for 15 min at 4℃ (SUPRA21K, Hanil Science Industrial, Incheon, Korea) and stored at −20℃ until subsequent analysis.

Laboratory analysis

Chemical composition

The DM content of the diet was determined by drying the sample (about 10 g) in a forced-air drying oven (OF-22GW, Jeio Tech, Deajeon, Korea) at 105℃ for 24 h. Approximately 500 g of sample was dried separately at 60℃ for 48 h and ground by a cutting mill (Shinmyung Electric) with a 1-mm screen to use for the chemical analysis. The procedures of Kjeldahl (B-324, 412, 435, and 719 S Titrino, BUCHI, Essen, Germany) and Soxhlet (OB-25E, JeioTech, Daejeon, Korea) were used to determine the concentrations of crude protein (CP) and ether extract (EE), respectively following [14]. The crude ash (CA) content was analyzed using a muffle furnace at 550℃ for 5 h. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using an Ankom^{200} Fiber Analyzer (ANKOM Technology) following the method of Van Soest et al. [15]. For the FA analyses, the fresh diet (20 g) was freeze-dried (FreeZone 12plus, LABCONCO, Kansas City, MO, USA) and methylated following the procedure described by Jenkins et al. [16] to prepare FA methyl esters. The methylated FA was determined using a gas chromatograph (450-GC, Bruker, Billerica, MA, USA) equipped with an auto-sampler (CP-8400, Varian, Palo Alto, CA, USA), a flame ionization detector, and a Varian capillary column (CP-Sil 88, 100 m × 0.25 mm × 0.2 μm). The carrier gas was nitrogen, and the injector and detector temperatures were maintained at 230℃. The oven temperature was initially set at 120℃ for 1 min, increased by 5℃/min up to 190℃, held at 190℃ for 30 min, increased again by 2℃/min up to 220℃, and held at 220℃ for 40 min. The FA concentrations were calculated based on the retention time and peak area of standards.

Ruminal digestibility and fermentation

In vitro digestibilities of DM (IVDMD) and NDF (IVNDFD) were measured by the method of Tilley and Terry [17] using an ANKOM DAISYII incubator (ANKOM Technology). For the rumen fermentation characteristics, the pH was measured by a pH meter (SevenEasy, Mettler Toledo, Greifensee, Switzerland), and the ammonia-N content was determined by the colorimetric method described by Chaney and Marbach [18]. For volatile fatty acid (VFA) analysis, the aerobic culture medium was centrifuged at 5,645×g for 15 min to separate the supernatant. The VFA concentration was measured using an HPLC (L-2200, Hitachi, Tokyo, Japan) fitted with a UV detector (L-2400, Hitachi) and a column (Metacarb 87H, Varian) according to the method described by Adesogan et al. [19].

Blood metabolites and reproductive hormones

From the collected blood plasma, the metabolite parameters, including vitamin E, blood urea nitrogen (BUN), and glucose, and the hormone parameters, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, and estrogen, were measured. The vitamin E concentration in the plasma was determined using an HPLC-MS (SCIEX API 5000^{TM}, AB Sciex, Woodlands, Singapore) fitted with a UV detector (SecurityGuard^{TM} ULTRA EVO C18, Phenomenex, Torrance, California, USA) and a column (Kinetex 5 μm EVO C18 100×2.1 mm, Phenomenex) according to Kamal-Eldin and Jastrebova [20]. The plasma concentration of the BUN was determined using a UREA/BUN kit (Roche, Mannheim, Germany). An enzymatic kinetic assay was used to determine the plasma concentrations of the glucose (GLU kit, Roche). An ELISA was used to determine the concentrations of plasma progesterone, estrogen, LH, and FSH (ELISA kit, Roche). In addition, the FA profiles of the blood plasma were analyzed using the same procedure used for the diet.

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Statistical Analysis

All data from Experiments 1 and 2 were analyzed using analysis of variance (ANOVA) [21]. Its model was Yij = m + Ti + eij, where Yij = response variable, m = overall mean, Ti = effect of treatment, and eij = error effect. Mean separation was performed by Tukey test, and the significant differences were declared at $p < 0.05$.

RESULTS

Experiment 1

Chemical compositions and fatty acid profiles of diet

The concentrations of DM, CP, EE, CA, NDF, and ADF from the basal diet were 80.62%, 7.42%, 4.48%, 8.27%, 61.0%, and 32.8%, respectively (Table 1). The dietary PF and MIX diets had high concentrations of C14:0 ($p < 0.001$; 6.18 and 6.01 vs. 4.20 and 4.29%), C16:0 ($p = 0.023$; 22.2 and 21.7 vs. 20.5 and 20.4%), C18:0 ($p < 0.001$; 16.0 and 15.5 vs. 6.32 and 6.49%), and C18:3n-3 ($p < 0.001$; 16.1 and 16.0 vs. 12.4 and 13.1%), but low concentrations of C18:1n-9 ($p < 0.001$; 22.8 and 23.2 vs. 34.7 and 34.4%) and C18:2n-6 ($p < 0.001$; 16.7 and 17.6 vs. 21.9 and 21.3%) compared to CON and VE diets (Table 2). The dietary PF and MIX resulted in a higher SFA concentration ($p < 0.001$; 44.4 and 43.2 vs. 31.0 and 31.2%) with lower UFA concentration ($p < 0.001$; 55.6 and 56.8 vs. 69.0 and 68.8%) compared to CON and VE.

Ruminal digestibility and fermentation

Dietary treatments did not affect the IVDMD and IVNDFD (Table 3). For the rumen

Table 1. Ingredients and chemical compositions of experimental diets (%, DM)

Item	Experimental diets
Ingredients	
Corn flake	10.0
Soybean meal	10.0
Wheat	10.0
Rice bran	8.00
Corn gluten	6.00
Distiller's dried grains with solubles	10.0
Palm kernel meal	3.00
Rice straw	20.0
Italian ryegrass	20.0
Molasses	2.50
Premix[1)]	0.50
Chemical composition	
Dry matter	80.6 ± 0.17[2)]
Crude protein	7.42 ± 0.13
Ether extract	4.48 ± 0.49
Crude ash	8.27 ± 0.28
Neutral detergent fiber	61.0 ± 0.85
Acid detergent fiber	32.8 ± 0.65

[1)]One kilogram contained the following: vitamin A, 450,000 IU; vitamin D3, 300,000 IU; vitamin E, 25,000 IU; vitamin K3, 500 mg; vitamin B1, 200 mg; vitamin B12, 13 mg; pantothenic acid, 40 mg; niacin, 30 mg; biotin, 20 mg; folic acid, 10 mg; $FeSO_4$, 3,500 mg; $CoSO_4$, 150 mg; CuSO4, 4,500 mg; $MnSO_4$, 2,000 mg; $ZnSO_4$, 2,500 mg; I, 400 mg; Se (Na), 150 mg.
[2)]Mean ± SD.

Table 2. Fatty acid profiles of experimental diets

Item	Treatment[1)]				SEM	*p*-value
	CON	PF	VE	MIX		
Total FA (mg/g)	18.1[b]	21.5[a]	18.6[b]	22.3[a]	0.385	< 0.001
C14:0 (% of total FA)	4.20[b]	6.18[a]	4.29[b]	6.01[a]	0.273	< 0.001
C16:0	20.5[b]	22.2[a]	20.4[b]	21.7[a]	0.741	0.023
C18:0	6.32[b]	16.0[a]	6.49[b]	15.5[a]	0.246	< 0.001
C18:1n-9	34.7[a]	22.8[b]	34.4[a]	23.2[b]	0.341	< 0.001
C18:2n-6	21.9[a]	16.7[b]	21.3[a]	17.6[b]	0.634	< 0.001
C18:3n-3	12.4[b]	16.1[a]	13.1[b]	16.0[a]	0.317	< 0.001
SFA	31.0[b]	44.4[a]	31.2[b]	43.2[a]	0.715	< 0.001
UFA	69.0[a]	55.6[b]	68.8[a]	56.8[b]	0.377	< 0.001

[1)]CON, without supplement; PF, supplemented 1% protected fat; VE, supplemented 1% vitamin E; MIX, supplemented 1% mixture of PF and VE at 1:1 ratio.

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

FA, fatty acid; SFA, saturated fatty acid; UFA, Unsaturated fatty acid.

Table 3. Effects of dietary protected fat and vitamin E on *in vitro* rumen digestibility and fermentation characteristics of experimental diets incubated with rumen buffer for 72 h

Item	Treatment[1)]				SEM	*p*-value
	CON	PF	VE	MIX		
In vitro digestibility (% DM)						
IVDMD	46.2	47.9	46.7	47.2	1.452	0.703
IVNDFD	35.9	38.5	36.9	38.0	1.424	0.371
Fermentation characteristics						
pH	6.54	6.62	6.59	6.59	0.032	0.068
Ammonia-N (mg N/dL)	27.8	27.5	27.8	28.5	0.535	0.241
Total VFA (mM)	67.1[b]	71.3[a]	69.4[ab]	69.7[ab]	0.906	0.011
Acetate (% molar)	64.1	62.8	64.1	62.9	0.719	0.107
Propionate (% molar)	20.3	20.8	19.0	20.5	0.769	0.149
Iso-butyrate (% molar)	0.81	1.04	0.99	1.02	0.101	0.087
Butyrate (% molar)	13.2	12.7	13.2	12.8	0.483	0.455
Iso-valerate (% molar)	1.67	2.15	2.04	2.10	0.178	0.158
Valerate (% molar)	0.16	0.35	0.37	0.53	0.115	0.172
Acetate : propionate	3.16	3.02	3.36	3.07	0.052	0.118

[1)]CON, without supplement; PF, supplemented 1% protected fat; VE, supplemented 1% vitamin E; MIX, supplemented 1% mixture of PF and VE at 1:1 ratio.

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

IVDMD, *in vitro* dry matter digestibility; IVNDFD, *in vitro* neutral detergent fiber digestibility; VFA, volatile fatty acid.

fermentation characteristics, dietary PE resulted in a higher total VFA concentration ($p = 0.011$; 71.3 vs. 67.1 mM) than CON, while dietary VE and MIX showed no differences among the treatments. The dietary treatments did not affect rumen pH, ammonia-N, or individual VFAs.

Experiment 2

Blood fatty acid profiles just before artificial insemination

The PF diet had a higher concentration of C18:0 ($p = 0.021$; 35.5 vs. 30.0%) but a lower concentration of C18:1n-9 ($p = 0.037$; 20.6 vs. 23.5%) than that of the CON diet (Table 4). Dietary

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Table 4. Effects of dietary protected fat and vitamin E on blood fatty acid profiles of Hanwoo cows just before artificial insemination

Item	Treatment[1)]				SEM	*p*-value
	CON	PF	VE	MIX		
Total FA (mg/mL)	30.7	32.3	29.5	30.1	4.002	0.683
C14:0 (% of total FA)	0.91	0.43	0.40	0.37	0.433	0.054
C16:0	12.5^{b}	12.7^{b}	13.6^{ab}	15.0^{a}	2.070	0.041
C18:0	30.0^{b}	35.0^{a}	30.2^{b}	32.0^{ab}	3.188	0.037
C18:1n-9	23.5^{a}	20.6^{b}	22.9^{ab}	20.8^{ab}	2.130	0.037
C18:2n-6	31.0	28.9	31.0	29.7	3.620	0.637
C18:3n-3	1.65	1.92	1.46	1.66	0.275	0.150
C20:4n-6	0.14	0.18	0.15	0.12	0.075	0.520
C22:5n-3	0.31	0.31	0.29	0.30	0.081	0.070
SFA	43.4^{b}	48.1^{a}	44.2^{b}	47.4^{a}	2.227	0.042
UFA	56.6^{a}	51.9^{b}	55.8a	52.6^{b}	3.162	0.031

[1)]CON, without supplement; PF, supplemented 1% protected fat; VE, supplemented 1% vitamin E; MIX, supplemented 1% mixture of PF and VE at 1:1 ratio.

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

FA, fatty acid; SFA, saturated fatty acid; UFA, Unsaturated fatty acid.

MIX presented a higher C16:0 concentration ($p = 0.041$; 15.0 vs. 12.5 and 12.7%) than CON and PF. The concentrations of C14:0, C18:2n-6, C20:4n-6, and C22:5n-3 were not affected by dietary treatments. The dietary PF and MIX had higher SFA concentration ($p = 0.042$; 48.1 and 47.4 vs. 43.4 and 44.2%) but lower UFA concentration ($p = 0.031$; 51.9 and 52.6 vs. 56.8 and 55.8%) than CON and VE.

Feed intake, blood metabolites and reproductive performance

Feed intakes were the same among the treatments, resulting in no orts from all cows (7.20 kg/d). And, the BCS just before AI were not affected by dietary treatments. However, dietary VE and MIX resulted in higher vitamin E concentration ($p = 0.001$; 11.8 and 10.9 vs. 7.08 and 7.97 μmol/L) in the blood of the cows just before AI than CON and PF (Table 5). The concentration of estrogen was decreased ($p = 0.001$; 31.2, 21.6, and 21.8 vs. 69.2 pg/mL) by dietary PF, VE, and MIX compared to CON. Dietary treatments did not affect the concentrations of BUN, glucose, LH, FSH, progesterone, or estrogen. The AI number of Hanwoo cows was lower in all dietary treatments compared to CON ($p < 0.041$; 1.33, 1.25, and 1.17 vs. 2.32). Therefore, the pregnancy rate of Hanwoo cows was higher in all dietary treatments compared to CON (70% vs. 60%).

DISCUSSION

In the present study, feed supplements consisting of PF and vitamin E did not affect the digestibility of diets. In general, Ca-salt for rumen-protected FAs has been widely applied in the livestock industry. Dietary unprotected FAs tended to modify the rumen fermentation and, in some cases, inhibit rumen microorganism activity [22]. The use of protected fat reduces and minimalize the adverse effects of FAs on rumen fermentation [16]. The results of the present study are similar to a previous study, in which protected FAs had no effect on nutrient digestibility in the rumen [23]. The effect of PF on nutrient digestibility in the rumen can be affected by the application level and oil source, which might result in a different response [22–24]. Ca-salt is stable at the normal pH conditions, such as in the rumen, but degrades in the acidic conditions, such as in the abomasum

Table 5. Effects of dietary protected fat and vitamin E on feed intake, blood metabolites, and reproductive performances of Hanwoo cows

Item	Treatment				SEM	*p*-value
	CON	PF	VE	MIX		
Feed intake (kg/d)	7.20	7.20	7.20	7.20	NA	NA
Body condition score	2.88	2.92	3.25	2.96	1.426	0.379
Blood metabolites before 30 d of AI						
Vitamin E (umol/L)	7.08[b]	7.97[b]	11.8a	10.9[a]	2.400	0.001
BUN (mg/dL)	10.9	10.8	9.95	10.2	2.504	0.807
Glucose (mg/dL)	52.9	60.6	52.1	57.0	8.316	0.069
LH (ng/mL)	1.43	1.28	1.13	1.01	0.607	0.538
FSH (ng/mL)	1.00	1.12	1.05	1.00	0.210	0.515
Progesterone (ng/mL)	> 30.0	> 30.0	> 30.0	> 30.0	NA	NA
Estrogen (pg/mL)	69.2[a]	31.2[b]	21.6[b]	21.8[b]	9.03	< 0.001
Reproductive performance after 42 d of AI						
AI number	2.32[a]	1.33[b]	1.25[b]	1.17[b]	0.513	0.041
Pregnancy rate (%)	60.0	70.0	70.0	70.0	NA	NA

[1)]CON, without supplement; PF, supplemented 1% protected fat; VE, supplemented 1% vitamin E; MIX, supplemented 1% mixture of PF and VE at 1:1 ratio.

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

NA, not applicable; AI, artificial insemination; BUN, blood urea nitrogen; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

[24]. However, the VFA concentration in the rumen increased because of dietary PF, which a small concentration of FA could still degrade in the rumen and might result in a minor effect on fermentation [24], such as the result of the present study. The FA profile of the diet presented that PF had a higher concentration of SFA than other diets. The supplementation of SFA in the rumen could partially replace the fermentable carbohydrates from the diet [8]. Based on the *in vitro* study, dietary PF, VE, and MIX had no significant effect on the nutrient digestibility and the fermentation characteristics in the rumen.

According to previous studies, supplementing required micronutrients, such as vitamins and FAs, has a big role in improving reproductive performance [3,5,6]. Moreover, those micronutrients also influence animal performance. Deficiencies in required micronutrients cause hormonal problems and reduce reproductive performance [3,5,6]. In the present study, dietary treatment presented effects on blood FAs. In general, the FA profile in the blood is affected by that of the diet. For example, the PF diet resulted in a lower concentration of C18:1n-9 but high concentrations of C18:0 and SFA, aligning with the concentrations found in the diet. Supplementary protected FAs directly affected the blood FA profile, especially the Ca-salt degradation in the abomasum, and then FA could be absorbed in the intestine. In general, vitamin E did not have a big impact on the FA profile of the blood. However, supplementation of MIX led to low UFA and SFA compared to CON, which presented a similar result to PF. High SFA concentrations in both PF and MIX diets were the main reason for this result.

Vitamin E is a fat-soluble antioxidant that protects the conversion of UFAs in the cell membrane into lipid peroxides and improves immune function [25]. In the present study, the vitamin E concentration in the blood increased according to the applied diet containing vitamin E, such as in VE and MIX. Supplementing vitamin E mainly presented oxidative properties in the blood and milk [9,26]. Similar to a previous study, supplementary vitamin E did not affect blood glucose or BUN [26]. Meanwhile, another study reported that SFA supplementation did not affect energetic

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blood metabolites [8]. The deficiency of dietary vitamin E could affect FSH or LH concentrations in the blood. The present study has shown that dietary vitamin E did not affect FSH and LH concentrations in the blood. This result is likely due to sufficient dietary vitamin E in all tested animals. The presence of estrogen causes the signs of estrus in cows [27]. One study reported that estrogen peaked before estrus and caused the release of LH in cows [28]. Furthermore, estrus estrogen and FSH concentrations decreased. During the ovulation period, the estrogen concentration should decrease after reaching its peak in the estrus period [28]. However, in the present study, CON still had a high concentration of estrogen at the peak of the estrus period, which could decrease the pregnancy rate of Hanwoo cows. In general, the blood metabolites and hormones of Hanwoo cows in the present study were within the normal range, according to Joo et al. [29].

Factors affecting the pregnancy rate include body weight, BCS, and parity [5]. However, the results of the present study indicated that dietary PF and vitamin E could increase the pregnancy rate even though feed intake did not differ. This study showed no significant difference in pregnancy rates among the different diet treatments. One study reported that the incidence of a reproductive disorder BCS in Hanwoo cows was low in the range of 2.5–3.0, and the reproductive disorder rate was high at 3.5 or greater [30]. The BCS result in the present study was in the normal range, which could reduce the potential for the reproductive disorders. A study indicated that a protected mixture of essential FA improved the embryo quality and pregnancy rate in lactating dairy cows [31]. PF has also been shown to increase the pregnancy rate of beef cows [7]. A previous study reported that dietary vitamin E reduced the incidence of retained fetal membranes and improved cow reproduction and pregnancy rates [9,26]. The results of the present study agreed with those of previous studies.

CONCLUSION

In vitro digestibility and fermentation characteristics were not affected by the dietary treatments. The PF and MIX diets increased the concentrations of SFA, such as C18:0 and C16:0, in the blood but decreased the concentrations of UFA, such as C18:1n-9 and C18:2n-6, during the estrus period of Hanwoo cows. All dietary treatments reduced the estrogen concentration in the blood during the estrus period. The VE and MIX diets increased the vitamin E concentration in the blood. The AI number decreased in all dietary treatments and improved the pregnancy rates. Therefore, the present study concluded that both single and mixture supplements could improve the reproductive performance of Hanwoo cows during the estrus period.

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