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Effect of supplementation of rumen-protected choline with soluble vitamins on hepatic function and carcass characteristics in Hanwoo steers

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ABSTRACT. This study was conducted to investigate the effects of supplementing rumen-protected choline with soluble vitamins (RPCSV) on growth performance, plasma profiles, and carcass characteristics in Hanwoo steers. Forty-five steers were fed 0 (T0), 50 (T50), or 100 g (T100) of RPCSV daily for 6 months. The average daily gain was not affected by RPCSV supplementation. The concentrations of total bilirubin ($P < 0.05$), aspartate glutamyltransferase ($P < 0.01$), alanine aminotransferase ($P < 0.01$), glucose ($P < 0.01$), non-esterified fatty acids ($P < 0.05$), blood urea nitrogen ($P < 0.01$), and triglycerides ($P < 0.01$) differed depending on the RPCSV supplementation period in each treatment group. Backfat thickness ($P < 0.05$) and yield index ($P < 0.01$) were affected by RPCSV supplementation. Thus, the results of this study indicated that plasma profiles related to energy, protein, and lipid metabolism increased due to improved liver function with RPCSV supplementation.

Introduction

Long-term fattening periods with high energy feeds are used to produce high-quality Hanwoo beef in Korea. However, these methods have a negative energy balance because they are insufficient to meet high-energy requirements associated with reduced feed intake (Grummer, 2008). This leads to the mobilisation of fat from adipose tissue mainly in the form of non-esterified fatty acids (NEFA), which are released into the blood as an energy source (Bobe et al., 2004). NEFA may be the cause of fatty liver, a metabolic dysfunction in cattle that affects health of this organ. It is characterized by fat deposition in the liver, which leads to hepatocyte dysfunction, thereby affecting the liver's ability to undergo ureagenesis and gluconeogenesis, ultimately leading to ketosis and decreased productivity (Aparna and Hundal, 2019).

Choline (tri-methyl ethanolamine) is a vitamin-like substance and part of the multifunctional vitamin B complex (Zeisel and Holmes-McNary, 1991). Choline can positively increase the utilisation of fatty acids in the liver, promote the transport of fatty acids such as lecithin, as well as enhance the synthesis of very low-density lipoprotein (VLDL) components, to increase their secretion from the liver, and metabolism of ketones by peripheral tissues to prevent abnormal fat accumulation (Aparna and Hundal, 2019). In addition, choline works in concert with methionine, vitamin B12, and folic acid, and has been found to play a role in animal production, reproduction, and health; therefore, it is an essential component in ruminant diets (Aparna and Hundal, 2019).

Although choline requirements for beef cattle have not been established (NRC, 2016), providing rumen-protected choline (RPC) is one method to

improve hepatic function in the feeding systems involving long-term fattening periods with high energy levels (Shahsavari et al., 2016; Humer et al., 2019). This is because choline chloride is extensively degraded by more than 80% of the rumen microbial population and little choline is available for absorption (Pinotti et al., 2020). A previous study has established that, from a technical or nutritional point of view, choline must be rumen-protected to be effective in ruminant nutrition (Pinotti et al., 2020).

Supplemental RPC affects liver function and performance (Shahsavari et al., 2016), milk production (Pinotti et al., 2003; 2005), and immunometabolic status (Zhou et al., 2016) in dairy cows. However, only one study has reported that supplemental RPC influenced growth performance in beef cattle (Pinotti et al., 2009). Moreover, to the best of our knowledge, supplemental rumen-protected choline with soluble vitamins (RPCSV) has not been studied in beef cattle to date. Therefore, the purpose of this study was to investigate whether growth performance, blood metabolites, hepatic function, and carcass characteristics could be improved in Hanwoo steers fed supplemental RPCSV.

Material and methods

This study followed all animal experimental procedures as indicated by the Kangwon National University Animal Experimental Ethics Committee (Institutional Animal Care and Use Committee, IACUC).

RPCSV composition

Liverdoctor® RPCSV supplement (Busanbio, Nonghyup Feed, Co., Ltd, Busan, Republic of Korea) was used, which contained 25 000 mg/kg RPC, 2 000 mg/kg taurine, 300 mg/kg vitamin B2, 3 000 mg/kg vitamin B6, 5 000 mg/kg pantothenic acid, 2 000 mg/kg niacin, and 100 mg/kg biotin. The carrier contained 40% cornflake powder, 30% yeast culture, and 10% silicates. RPC was supplied by Metvita® (Morningbio, Cheonan, Republic of Korea) and was manufactured with joint matrix coating technology using choline chloride and vegetable fats; the vitamin B group and taurine was coated by ethyl cellulose for dissolution in water.

Animals, treatment, and management

A total of 45 Hanwoo steers (average body weight, 596.5 ± 17.0 kg) were randomized into three groups of 15 steers each to reduce the experimental variable, and to adhere to the recommended number of breeding steers (7 m^2 per head) according to the livestock industry act in Korea. The T0 group was fed a formula feed without RPCSV supplementation, the T50 group was fed a formula feed with 50 g RPCSV supplementation, and the T100 group was fed a 100 g RPCSV-supplemented formula feed. RPCSV was given in the powder form in an oral feed formula to the steers.

The steers were maintained in the same environment and fed a diet based on the fattening feeding program throughout the experiment. They were acclimatized to the experimental diets for 14 days. Five steers were allocated per pen (8 m × 10 m) containing an approx. 20-cm layer of sawdust as bedding. The formula feed was administered using an automatic feeding system (SEOCHANG 65M/M, Seochang Co., Ltd, Cheonan, Republic of Korea) at 08:30, 13:00, and 17:00. The steers had free access to rice straw, water, and mineral blocks. Other feeding management procedures were conducted in accordance with the practices of the experimental farm. The chemical compositions of the experimental diets were analysed in accordance with the methods of the AOAC International (2005), and the neutral detergent fibre content was determined according to the method described by Goering and Van Soest (1970). The ingredients and chemical compositions of the experimental diets are listed in Table 1.

Table 1. Ingredients and chemical composition of experimental diets

Items	Formula feed		Rice straw
	Early fattening	Late fattening	
Ingredients composition			
corn grain (4-02-854)	26.2	28.4	–
wheat grain (4-13-245)	17.0	17.0	–
corn gluten feed (5-28-243)	10.0	10.0	–
distillers' grain (5-28-236)	10.0	10.0	–
palm kernel meal (5-0)	11.0	8.8	–
wheat bran (4-00)	6.0	5.0	–
coconut meal (5-01)	5.0	2.0	–
rapeseed meal (5-085)	4.0	4.0	–
cane molasses (4-04-696)	4.0	4.0	–
lupin, %	3.0	3.0	–
tapioca residue, %	2.0	6.0	–
cotton seed, %	1.0	1.0	–
sodium bicarbonate, %	0.6	0.6	–
vitamin premix[1], %	0.1	0.1	–
trace mineral premix[2], %	0.1	0.1	–
Chemical composition (as-fed basis)			
dry matter, %	88.95	88.68	90.18
crude protein, %	13.90	12.90	3.65
ether extract, %	3.31	3.76	1.02
crude ash, %	7.43	6.62	10.58
calcium, %	1.19	0.91	0.09
phosphorus, %	0.54	0.41	0.05

[1] supplied per kilogram of diet: IU: vit. A 4 000, vit. D_3 800, vit. E 25; [2] supplied per kilogram of diet: mg: Fe 50, Cu 10, Zn 40, Mn 20, I 0.50, Se 0.15, Co 0.15

Growth performance

Body weight was measured at the beginning (0 d), middle (90 d), and end (180 d) of the experimental period, and average daily gain (ADG) was calculated by dividing the total weight gain by the number of experimental days. Dry matter intake (DMI) was calculated by measuring the amount of residual feed per pen before morning feeding and dividing by 1/5 to calculate individual intake. Feed conversion ratio (FCR) was calculated using the DMI and ADG values.

Plasma profiles

Blood samples were collected at the beginning (0 d), middle (90 d), and end (180 d) of the experimental period. Samples were collected from the jugular vein using a 10 ml vacutainer (Becton Dickinson, Franklin Lakes, NJ, USA) before feeding at 08:30. To analyse blood metabolites, heparinized blood samples were centrifuged at 2 000 *g* for 15 min to separate the plasma. Plasma metabolites were analysed using an automatic blood analyser (Hitachi 7020, Hitachi Ltd, Tokyo, Japan). The following plasma parameters were determined: total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol (CHOL), triglyceride (TG), total protein (TP), globulin (GLO), blood urea nitrogen (BUN), albumin (ALB), and creatinine (CREA).

Carcass characteristics

All steers were slaughtered at a local slaughterhouse. Carcass yield (carcass weight, backfat thickness, rib eye area, and yield index) and quality (marbling score, meat colour, fat colour, texture, and maturity) were examined according to the criteria of the Korean carcass grading system (MAFRA, 2018). The carcasses were chilled for 24 h and the weight of cold carcasses was measured. The left side of each carcass was then cut between the thirteenth rib and the first lumbar vertebra and used to determine yield and quality grades. The rib eye area was measured from the longissimus muscle on the thirteenth rib. Backfat thickness was measured at the thirteenth rib. The yield index was calculated as follows: yield index = {68.184 − [0.625 × backfat thickness (mm)] + [0.130 × rib eye area (cm^2)] − [0.024 × carcass weight (kg)]} × 3.23. Yield was classified as grade A (best, yield index > 67.20), grade B (yield index = 63.30 – 67.20), and grade C (worst, yield index < 63.30) based on the yield index.

The quality class was determined by assessing the degree of marbling on the cut surface of the rib eye based on the maturity, texture, meat and fat colour of the carcass. Marbling was graded on a scale of 1 to 9, with higher numbers indicating better quality (1 = devoid, 9 = abundant). Additional scores included assessment of meat colour (1 = bright red, 7 = dark red), fat colour (1 = creamy white, 7 = yellowish), maturity (1 = young, 9 = old), and texture (1 = soft, 5 = firm). Quality was evaluated as follows: 1^{++} (excellent quality), 1^{+}, 1, 2, and 3 (low quality).

Statistical analyses

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS)/ Windows 24 (SPSS Inc., Chicago, IL, USA). The mean values of individual groups were compared using one-way analysis of variance followed by Duncan's multiple range test. Differences were considered statistically significant at $P < 0.05$.

Results

Growth performance

Table 2 shows the effects of RPCSV supplementation on growth performance of Hanwoo steers. Supplementation with RPCSV did not significantly affect ADG, formula feed intake, rice straw intake or FCR in Hanwoo steers.

Table 2. Effects of supplementation of rumen-protected choline with soluble vitamins (RPCSV) on growth performance in Hanwoo steers

Items	T0	T50	T100	SEM	*P*-value
Early fattening period					
IBW, kg	629.1	581.5	578.8	17.063	0.097
FBW, kg	783.2	751.3	723.1	21.134	0.162
ADG, kg/day	0.82	0.90	0.77	0.047	0.131
DMI, DM kg/day	9.0	9.0	9.0	–	–
FFI, DM kg/day	7.5	7.5	7.5	–	–
roughage, DM kg/day	1.5	1.5	1.5	–	–
FCR	11.28	10.29	12.05	0.645	0.175
Late fattening period					
IBW, kg	783.2	751.3	723.1	21.102	0.162
FBW, kg	836.4	794.7	777.9	21.042	0.170
ADG, kg/day	0.61	0.48	0.59	0.058	0.260
DMI, DM kg/day	10.0	10.0	10.0	–	–
FFI, DM kg/day	8.5	8.5	8.5	–	–
roughage, DM kg/day	1.5	1.5	1.5	–	–
FCR	17.35	22.78	22.18	2.705	0.402
Whole experimental period					
IBW, kg	629.1	581.5	578.8	17.041	0.097
FBW, kg	836.4	794.7	777.9	21.063	0.170
ADG, kg/day	0.75	0.76	0.71	0.038	0.387
DMI, DM kg/day	9.5	9.5	9.5	–	–
FFI, DM kg/day	8.0	8.0	8.0	–	–
roughage, DM kg/day	1.5	1.5	1.5	–	–
FCR	12.91	12.84	13.69	0.702	0.413

IBW – initial body weight, FBW – final body weight, ADG – average daily gain, DMI – dry matter intake, FFI – formula feed intake, FCR – feed conversion ratio, SEM – standard error of the mean; $P > 0.05$

Plasma profiles related to hepatic function

Table 3 shows the effects of RPCSV supplementation on plasma levels of liver function markers in Hanwoo steers. TB concentrations were affected by the supplementation period (P = 0.014), but neither the supplementation level nor the combination of supplementation period × supplementation level caused significant differences in the results (P = 0.227 and P = 0.085, respectively). TB levels at 0 d were higher (P = 0.050) in the T50 group than in the T0 group, and its concentrations in the T0 group were higher (P < 0.001) at 180 d than at 0 d. AST levels were also affected by the supplementation period (P = 0.004), but differences between individual supplementation levels or the combination of supplementation period × supplementation level were not significant (P = 0.074 and P = 0.337, respectively). AST concentrations at 0 d were higher (P = 0.044) in the T50 and T100 groups compared to the T0 group, while AST levels in the T50 and T100 groups were lower (T1, P = 0.042; T2, P = 0.001) at 180 d than at 0 d. ALT concentrations were also affected by the supplementation period (P < 0.001), but differences in supplementation levels or the combination of supplementation period × supplementation level were not significant (P = 0.081 and P = 0.371, respectively). ALT concentrations in the T50 and T100 groups were lower (P < 0.001) at 180 d than at 0 d.

Plasma profiles of energy and lipid metabolism markers

Table 4 shows the effects of RPCSV supplementation on the levels of plasma energy and lipid metabolism markers in Hanwoo steers. GLU concentrations were affected by the supplementation period (P = 0.001), but differences between individual supplementation levels were not significant (P = 0.617). GLU level in the T0 group was lower (P = 0.003) at 180 d than at 0 d, while that at 180 d was higher (P = 0.047) in the T100 group compared to the T0 group. NEFA concentration was affected by the supplementation period (P = 0.010) and supplementation period × supplementation level (P = 0.007), but individual supplementation levels did not result in significant differences (P = 0.262). NEFA concentration in the T0 group was higher (P = 0.023) at 180 d than at 0 d, whereas its levels in the T100 group were lower (P = 0.017) at 180 d compared to 0 d and 90 d. CHOL concentrations were not affected by the supplementation period (P = 0.098),

Table 3. Effects of supplementation of rumen-protected choline with soluble vitamins (RPCSV) on plasma levels of liver function markers in Hanwoo steers

Items		T0	T50	T100	SEM	*P*-value
TB, mg/dl	0 d	0.110^{bB}	0.170^{a}	0.150^{ab}	0.016	0.050
	90 d	0.100^{B}	0.120	0.130	0.010	0.201
	180 d	0.167^{A}	0.150	0.140	0.017	0.534
	SEM	0.009	0.017	0.016	–	–
	P-value	<0.001	0.144	0.684	–	0.085
AST, IU/l	0 d	81.00^{b}	95.50^{abA}	101.40^{aA}	5.930	0.044
	90 d	87.90	87.70^{AB}	89.10^{A}	8.028	0.992
	180 d	83.67	73.80^{B}	75.70^{B}	6.437	0.556
	SEM	9.86	6.20	4.33	–	–
	P-value	0.884	0.042	0.001	–	0.337
ALT, IU/l	0 d	23.50	27.80^{C}	26.50^{A}	1.480	0.146
	90 d	20.40	23.90^{B}	21.70^{AB}	1.181	0.129
	180 d	19.44	18.90^{A}	18.80^{B}	1.070	0.911
	SEM	1.362	1.204	1.166	–	–
	P-value	0.127	<0.001	<0.001		0.371
GGT, mg/dl	0 d	37.10	36.00	36.90	4.546	0.984
	90 d	42.70	31.40	34.80	5.453	0.356
	180 d	45.11	30.90	33.40	5.674	0.198
	SEM	5.205	4.813	5.655	–	–
	P-value	0.545	0.720	0.911	–	0.744

TB – total bilirubin, AST – aspartate-amino-transferase, ALT – alanine-amino-transaminase, GGT – γ-glutamyl-transferase, SEM – standard error of the mean; ^{ab} – means followed by different letters in the same row are significantly different (P < 0.05); ^{AB} – means followed by different letters in the same column are significantly different (P < 0.05)

Table 4. Effects of supplementation of rumen-protected choline with soluble vitamins (RPCSV) on plasma levels of energy and lipid metabolism markers in Hanwoo steers

Items		T0	T50	T100	SEM	*P*-value
GLU, mg/dl	0 d	82.50^{A}	72.80	74.90	2.723	0.060
	90 d	86.20^{A}	83.70	81.30	3.579	0.652
	180 d	70.56^{bB}	75.50^{ab}	78.70^{a}	2.104	0.047
	SEM	2.590	3.591	2.222	–	–
	P-value	0.003	0.111	0.142	–	0.058
NEFA, uEq/l	0 d	135.3^{bB}	208.1^{a}	224.4^{aA}	15.39	0.001
	90 d	178.1^{AB}	204.2	206.0^{A}	19.53	0.998
	180 d	205.1^{aA}	159.9^{b}	151.3^{bB}	14.43	0.040
	SEM	16.69	15.33	17.33	–	–
	P-value	0.023	0.064	0.017	–	0.007
CHOL, mg/dl	0 d	135.3^{bB}	208.1^{a}	224.4^{aA}	15.39	0.001
	90 d	178.1^{AB}	204.2	206.0^{A}	19.53	0.998
	180 d	205.1^{aA}	159.9^{b}	151.3^{bB}	14.43	0.040
	SEM	16.69	15.33	17.33	–	–
	P-value	0.023	0.064	0.017	–	0.007
TG, mg/dl	0 d	16.40	16.60^{B}	17.00^{B}	1.152	0.934
	90 d	19.50	21.60^{A}	22.40^{A}	1.480	0.373
	180 d	18.33	21.70^{A}	21.40^{A}	1.306	0.211
	SEM	1.263	1.380	1.294	–	–
	P-value	0.233	0.022	0.022	–	0.744

GLU – glucose, NEFA – non esterified fatty acid, CHOL – cholesterol, TG – triglycerides, SEM – standard error of the mean; ^{ab} – means followed by different letters in the same row are significantly different (P < 0.05), ^{AB} – means followed by different letters in the same column are significantly different (P < 0.05)

supplementation level (P = 0.662), or the combination of supplementation period × supplementation level (P = 0.346). CHOL concentration in the T100 group was higher (P = 0.037) at 180 d than at 0 d. TG levels were affected by the supplementation period (P = 0.0002); however, differences caused by individual supplementation levels (P = 0.146) or the combination of supplementation period × supplementation level were not significant (P = 0.146 and P = 0.7435, respectively). TG concentrations in the T50 and T100 groups were higher (T50, P = 0.022; T100, P = 0.022) at 90 d and 180 d than at 0 d.

Plasma profiles related to protein metabolism

Table 5 shows the effects of RPCSV supplementation on plasma levels of protein metabolism markers in Hanwoo steers. TP levels were affected by the supplementation level (P = 0.024), but the supplementation period (P = 0.623) or the combination of supplementation period × supplementation level variables did not show significant differences (P = 0.541). TP levels at 180 d were higher (P = 0.019) in the T50 and T100 groups compared to the T0 group. GLO concentrations were affected by the supplementation period (P = 0.001), however the results for individual supplementation levels did not differ significantly (P = 0.617). GLO levels in the T0 group were lower (P = 0.003) at 180 d than at 0 d and 90 d, while those at 180 d were higher (P = 0.047) in the T100 group than in the T0 group. BUN concentrations were affected by the supplementation period (P < 0.001) and supplementation level (P < 0.001), but individual combinations of supplementation period × supplementation level did not differ significantly (P = 0.228). BUN levels in the T0 group were lower (P < 0.001) at 180 d than at 90 d, whereas its concentrations in the T100 group were lower (P < 0.001) at 90 d and 180 d compared to 0 d. The concentrations of ALB and CREA were not affected by the supplementation period (ALB, P = 0.320; CREA, P = 0.431), supplementation level (ALB, P = 0.749; CREA, P = 0.529) or supplementation period × supplementation level (ALB, P = 0.471; CREA, P = 0.442).

Table 5. Effects of supplementation of rumen-protected choline with soluble vitamins (RPCSV) on plasma levels of protein metabolism markers in Hanwoo steers

Items		T0	T50	T100	SEM	*P*-value
TP, g/dl	0 d	6.830	7.060	7.170	0.105	0.088
	90 d	6.840	6.830	7.020	0.202	0.762
	180 d	6.600^{b}	7.140^{a}	7.120^{a}	0.137	0.019
	SEM	0.149	0.159	0.134	–	–
	P-value	0.496	0.418	0.743	–	0.541
GLO, mg/dl	0 d	82.50^{A}	72.80	74.90	2.723	0.060
	90 d	86.20^{A}	83.70	81.30	3.579	0.652
	180 d	70.56^{bB}	75.50^{ab}	78.70^{a}	2.104	0.047
	SEM	2.593	3.591	2.222	–	–
	P-value	0.003	0.111	0.142	–	0.058
BUN, mg/dl	0 d	15.96^{bB}	19.32^{a}	15.10^{bB}	0.771	0.010
	90 d	14.45^{B}	17.21	19.11^{A}	0.749	0.059
	180 d	18.70^{A}	19.71	20.61^{A}	0.732	0.220
	SEM	0.527	1.004	0.721	–	–
	P-value	<0.001	0.192	<0.001	–	0.228
ALB, g/dl	0 d	3.820	3.780	3.790	0.061	0.896
	90 d	3.800	3.760	3.790	0.103	0.962
	180 d	3.756	3.990	3.890	0.072	0.111
	SEM	0.072	0.093	0.072	–	–
	P-value	0.833	0.193	0.558	–	0.471
CREA, mg/dl	0 d	1.380	1.530	1.490	0.049	0.106
	90 d	1.390	1.430	1.410	0.057	0.892
	180 d	1.456	1.400	1.450	0.047	0.706
	SEM	0.035	0.059	0.059	–	–
	P-value	0.304	0.303	0.637	–	0.442

TP – total protein, GLO – globulin, BUN – blood urea nitrogen, ALB – albumin, CREA – creatinine, SEM – standard error of the mean; ^{ab} – means followed by different letters in the same row are significantly different (P < 0.05), ^{AB} – means followed by different letters in the same column are significantly different (P < 0.05)

Carcass characteristics

Table 6 shows the effects of RPCSV supplementation on carcass characteristics of Hanwoo steers. RPCSV supplementation did not significantly affect

Table 6. Effects of supplementation of rumen-protected choline with soluble vitamins on carcass characteristics in Hanwoo steers

Items	T0	T50	T100	SEM	*P*-value
Yield traits[1]					
carcass weight, kg	511.3	486.0	481.5	13.501	0.284
backfat thickness, mm	11.60^{b}	18.10^{a}	14.70^{ab}	1.563	0.028
rib eye area, cm^2	103.7	99.1	109.7	3.204	0.093
dressing, %	63.60	63.85	61.60	1.330	0.132
yield index, %	65.37^{a}	61.32^{b}	64.93^{a}	1.061	0.025
yield grade score	2.00	1.30	1.90	0.254	0.122
Quality traits[2]					
marbling score	5.00	5.40	5.80	0.512	0.556
meat color	4.30	4.20	4.40	0.154	0.647
fat color	3.00	3.00	3.20	0.041	0.124
texture	1.10	1.10	1.00	0.073	0.612
quality grade score	3.20	3.30	3.70	0.264	0.360

SEM – standard error of the mean; [1] area was measured from the rib eye area taken at the 13th rib and backfat thickness was also measured at the 13th rib, yield index was calculated using the following equation: 68.184 − [0.625 × backfat thickness (mm)] + [0.130 × rib eye area (cm^2)] − [0.024 × dressed weight amount (kg)], carcass yield grades from C (low yield) to A (high yield), yield scores from 1 (grade A) to 3 (grade C); [2] grading ranges are 1 to 9 for marbling score, with higher numbers indicating better quality (1 – devoid, 9 – abundant), meat colour (1 – bright red, 7 – dark red), fat colour (1 – creamy white, 7 – yellowish), texture (1 – soft, 3 – firm), maturity (1 – youthful, 9 – old), quality grades from 3 (low quality) to 1^{++} (very high quality); quality scores from 0 (grade 1^{++}) to 4 (grade 3); ^{ab} – means followed by different letters in the same row are significantly different (P < 0.05)

carcass weight ($P = 0.284$), rib eye area ($P = 0.093$), dressing ($P = 0.132$) or yield score ($P = 0.122$). Backfat thickness was higher ($P = 0.028$) in the T50 group than in the T0 group, and the yield index was lower ($P = 0.028$) in the T50 group compared to the T0 and T100 groups. RPCSV supplementation had no effect on quality traits such as marbling score ($P = 0.556$), meat colour ($P = 0.647$), fat colour ($P = 0.124$), texture ($P = 0.612$) or quality score ($P = 0.360$) in Hanwoo steers.

Discussion

Bindel et al. (2005) reported that RPC supplementation improved growth performance without negatively affecting carcass characteristics in finishing beef cattle. Moreover, Pinotti et al. (2009) reported that supplementation of 5 g of RPC to Charolais steers increased ADG up to day 89, but not in the later part of the experiment. Growth performance results in the last part of the experiment could be attributed to choline deficiency, with animals receiving approximately 30% less choline per kilogram BW at 180 d compared to 0 d. In contrast to the results of the previous study, RPCSV supplementation did not affect ADG and feed intake of Hanwoo steers in the present study. Although choline requirements have not been established in beef cattle (NRC, 2016), Previous studies have shown that the demand for protected choline increases as BW of beef cattle. Therefore, the findings of the present study, which attributed a low RPCSV dose to the increased growth performance of Hanwoo steers were supported by earlier studies.

Bilirubin, AST, ALT, and GGT are the most commonly used metabolites in the blood to determine liver function in livestock. Blood levels of these enzymes increase when liver cells are damaged, and thus they are used as indicators of hepatic function in livestock. High plasma bilirubin levels reflect liver disease and hepatocyte damage and are used as hepatic function markers (Hamoud et al., 2018). Blood AST and ALT levels are increased by fungal toxins in the feed, reproductive failure and elevated rumen ammonia levels due to excessive formula feed.

In this study, RPCSV supplementation improved liver function, as indicated by TB, AST, and ALT concentrations in each treatment group. Similar to the current work, previous studies reported that choline as a type of RPC improved metabolic health and hepatic function in ruminants (Piepenbrink and Overton, 2003; Pinott et al., 2003; Cooke et al., 2007; Chung et al., 2009). Thus, dietary choline seems to improve fat metabolism in the liver and increase the apparent absorption of TG in the bloodstream, further confirming the beneficial effects of supplemental RPC for adult ruminants (Zenobi et al., 2018). Our results demonstrated that although the long-term fattening period with high-energy formula feed increased TB, AST, and ALT concentrations, these profiles did not differ in Hanwoo steers fed RPCSV for 3 months. Thus, the results of this study imply that RPCSV supplementation can improve liver function without negatively affecting the growth performance of Hanwoo steers.

Vernon (1992) reported that GLU maintained blood homeostasis in fattening cattle and played an important role in the biosynthesis of intramuscular fat in adipose tissue. Lager and Jordan (2012) found that although ruminants did not absorb large amounts of GLU from their digestive system, they synthesized large amounts of GLU in their liver from volatile fatty acids, especially propionic acid, absorbed from the rumen, but also from amino acids. Meanwhile, Beever (2006) demonstrated that NEFA could also be used as an indicator of excessive or insufficient energy intake and to detect subclinical ketosis, with NEFA concentrations generally increasing due to insufficient energy availability in the livestock body. Jayaprakash et al. (2016) reported that increased NEFA uptake by the liver could lead to the development of fatty acids, such as hepatic lipidosis, caused by increased accumulation of triacylglycerol in the liver parenchyma. Excessive TG accumulation in the liver reduces the ammonia-to-urea detoxification capacity, which in turn may disturb gluconeogenesis based on propionate, i.e. the predominant glucogenic precursor.

In this study, RPCSV supplementation increased GLU and decreased NEFA levels at day 180. Similarly, Pinotti et al. (2004) found that dietary RPC reduced plasma beta-hydroxybutyrate concentrations by over 30% in dairy cows, which supported the role of choline in fatty acid metabolism. Moreover, Piepenbrink and Overton (2003) reported that RPC supplementation in dairy cows during the periparturient period reduced NEFA accumulation in the liver and increased its glycogen content. Earlier studies showed that RPC supplementation reduced blood NEFA levels (Pinotti et al., 2003; Cooke et al., 2007), while increasing the concentration of blood GLU (Cooke et al., 2007; Chung et al., 2009) in dairy cows. Similarly to previous studies, the results of the present work demonstrated that RPCSV

supplementation improved energy availability by increasing blood GLU and decreasing blood NEFA levels through improved liver function, as shown by reduced AST and ALT levels in Hanwoo steers.

Shahsavari et al. (2016) reported that NEFA caused fatty liver, a metabolic dysfunction, and ketosis, which affects liver health. Esterified NEFA is converted to triacylglycerol in the liver, and when the concentration of NEFA exceeds the hepatic capacity for hepatic oxidation, it negatively affects liver health and secretion of triacylglycerols and VLDL. Lager and Jordan (2012) have argued that the liver is under greater stress after calving because the cow requires more energy to maintain milk production, resulting in body fat mobilisation. This fat reaches the liver and is transported by VLDL.

Choline is necessary for the transport and metabolism of CHOL, which explains higher blood CHOL levels (Zeisel and Da Costa, 2009), and has the potential to decrease the incidence of metabolic disorders in cows by reducing liver triacylglycerol levels beyond the fatty liver itself (Zom et al., 2011). The mechanism by which hepatic CHOL synthesis is stimulated by choline in ruminants is not fully understood; however, in other species, choline is required for the synthesis of the phosphatidylcholine portion of the VLDL (Yao and Vance, 1988). Thus, the result of the present study were consistent with those of Humer et al (2019), who reported that RPC supplementation improved lipid metabolism and health of dairy cows by increasing phosphatidylcholine and VLDL synthesis. The latter authors also hypothesised that RPC facilitated triacylglycerol production in the liver of transition dairy cows as their results showed that RPCSV supplementation increased CHOL levels in the T100 group and TG levels in the T50 and T100 groups in Hanwoo steers.

Otto et al. (2000) have reported that TP in the blood contains ALB and GLO, and there are more than 100 types of proteins in the serum that contain 60% ALB, while the rest are globulin-based proteins. TP is involved in important functions such as metabolite transport, blood clotting, maintenance of osmotic pressure and materials for immune antibodies in livestock; its concentration also increases with dietary protein levels.

Blood urea levels provide important information whether rumen degradable and undegradable protein are coordinated with starch degradation to optimise rumen microbial protein synthesis, as an imbalance in protein and carbohydrate degradability can result in suboptimal animal health and production (Lager and Jordan, 2012). In addition, blood BUN levels tend to increase with raising protein intake in beef cattle. Thus, it can be assumed that BUN concentration in this study increased with the amount of protein intake rather than due to the dietary effect of RPCSV. Although RPCSV supplementation slightly increased TP and GOL concentrations over 180 d, we did not observe any effect of RPCSV supplementation on plasma profiles related to protein metabolism in ruminant animals.

Lee et al. (1997) reported that carcass weight and rib eye area were higher in the group of Hanwoo steers with the highest live weight (11 601), while backfat thickness was high and meat yield low, which was consistent with the results of this study. Backfat thickness was most strongly correlated with decreases in carcass yield grade. There was no literature reference stating that protected choline supplementation affected backfat thickness in beef cattle. However, it was estimated that supplementation with 50 g RPCSV increased energy and fat metabolism by improving liver function, thereby reducing backfat thickness or decreasing carcass yield index. In this study, supplementation with 50 g RPCSV increased backfat thickness and decreased carcass yield of Hanwoo steers.

Park et al. (2020) reported that the marbling score of Hanwoo steers was increased by supplementation of rumen-protected methionine with vitamin E, as methionine is a methyl donor in the transmethylation reaction in lipid transport and biosynthesis. Although no statistically significant difference was observed in the latter study, the marbling score increased with RPCSV supplementation, which was similar to the results of the present work. In addition, Liu et al. (1995) reported that supplementation with alpha-tocopherol increased marbling and backfat thickness in beef cattle, which was consistent with the results of this study.

Conclusions

The results of this study demonstrated that supplementation with RPCSV exerted positive effects on liver function, as indicated by beneficial changes in plasma TB, AST, and ALT levels in Hanwoo steers during the experimental period. In addition, supplementation with RPCSV is expected to improve plasma profiles related to energy, protein, and lipid metabolism as a result of enhanced hepatic function, without adversely affecting growth performance and carcass characteristics in Hanwoo steers.

Conflict of interest

The Authors declare that there is no conflict of interest.

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