

Original Paper

Effect of Feeding Green Yeast Culture on the Growth Performance of Hanwoo Steers

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Abstract— To overcome the loss in terms of mortality and morbidity as the result of the restriction of in-feed antibiotics, many alternatives have been proposed. Yeast cultures as feed additives, have various physiological activities in ruminants. Supplementing yeast makes it possible to stimulate the growth of specific rumen microorganisms, which ultimately improves the growth of ruminants. This study was designed to analyze the potential effect of green culture, a feed supplement with fermented yeast, in growth performance and immune status of Hanwoo steers. A total of 30 animals were divided into two experimental groups: Control and Green Culture. For each group, five pens were used. Three 6 to 8 months old Hanwoo steers were housed per pen. In-vitro rumen fermentation by the green culture was done to generate base evidence to reflect the In-vivo field trial results. It was found out that, the average daily gain of the treatment group (0.93 ± 0.04 kg) supplemented with green culture was significantly higher (p-value less than 0.05) than that of the control group (0.84 ± 0.10 kg). In-vitro, ruminal fermentation analysis showed that the total volatile acid content increased with the addition of green yeast culture. These results suggested that the ruminal digestibility of nutrients improved, which is reflected in the increase in body weight. Moreover, there was an increase of acetic acid production in the treatment group supplemented with GC, which is reported to be related to the formation of lipid precursor cells. Therefore, green culture seems to improve the growth of Hanwoo steers as well as their meat quality.

Keywords—green yeast culture, Hanwoo, steers, rumen fermentation, meat quality

I. INTRODUCTION:

Antibiotics are extensively used in animal feeding not only as an anti-microbial agent but also as a growth-promoting agent. The animals supplemented with antibiotics have higher daily growth rate as compared to that of the animals without antibiotic supplementation [1]. However, the use of antibiotics in animal feed is considered as one of the underlying causes for the development of antibiotic-resistant strains [2]. Thus, injudicious use of antibiotics as a feed

supplement is banned in many countries [3]. It was banned in South Korea since July 2011. To overcome the loss in terms of mortality and morbidity as the result of the restriction of in-feed antibiotics, several alternatives have been proposed [4]. Various feed additives, with immune-stimulatory effects are believed to improve the innate defense of animals, providing resistance-against pathogens [5]. Several studies have exhibited health benefits of β -1, 3 /1, 6-glucan (from yeast cell walls) as a feed ingredient [6]. Supplementation of yeast culture is believed to improve the growth performances of ruminants by stimulating the growth of certain rumen microorganisms [7].

Dietary yeast supplementation increases dry matter (DM) intake and milk production [8] whereas in beef cattle it improves growth parameters like Average daily gain, DM intake, and feed: gain [9]. Besides, it has been reported that it reduces incidences of metabolic respiratory disease and improves energy metabolism which inhibits fat and protein degradation, leading to improvements in body weight and feed efficiency [10]. Therefore, the study was designed to analyze the potential effect of green culture, a feed supplement with fermented yeast, in growth performance and immune status of Hanwoo steers. Furthermore, to generate research-based evidence to promote the product.

II. MATERIALS AND METHODS

Yeast Culture:

The yeast culture was provided by Daeho Co., Ltd, Jeongmunsongsan-ro 241 beon-gil, Hwaseong-si, Gyeonggi-do, Korea. The product, named Green culture is a dried yeast culture composed of yeast and the media on which it was grown. The primary organism in starter culture is *Saccharomyces cerevisiae* ($>1 \times 10^7$ cfug), which undergoes a series of cultures in liquid media followed by solid media. The final product was prepared by drying the solid media in a controlled condition. The final product contains various enzymes (like α -amylase, Neutral protease), β -Glucan, Oligomanan, extruded corn along with the viable yeast cells with metabolic activity.

Experimental Design

Both the in-vivo (experimental feeding trial) and in-vitro batch culture was conducted to know the effect of green culture supplement on the growth performance of Hanwoo steers and its ultimate effect on the rumen of the animals respectively.

A. Experimental feeding experiment using Hanwoo steers

For, Experimental feeding trial, a beef cattle farm in Yangsung was selected. The animals were divided into two experimental groups: Control and Green Culture. For each group, five pens were used. Three 6 -8 months old Hanwoo steers were housed per pen. Each of the animals in “Green culture supplement” group was provided with 50gm of the supplement as a top dressing, over and above commercial feed (TABLE I). The feeding trial started from 2017.11.27 till 2018.05.25. The animals were provided with equal ration. Their ration comprised of commercial feed and roughages (hay).

For roughages, one-part oat and four parts alfa hay were used. Fresh feed was provided every day. The left-over feed from the previous day was weighed and removed. All of the animal care procedures were considered as per the Hankyong National University Animal Care and Use Committee. All the animals were subjected to standard farm management and procedures.

1) Growth performance:

Feed intake was measured daily and body weight was measured at the beginning and end of the experimental period in order to determine Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Efficiency (FE).

2) Blood parameters and serum immunoglobulin:

At the end of the experiment, 5-10 ml of blood samples were collected from jugular vein at the rate of ten heads per treatment. The samples were kept in EDTA vacutainers and transported to the lab in iceboxes. Then, the samples were centrifuged at 3000rpm for 10 min at 4°C to collect plasma. Plasma samples were stored at -80°C until use. The samples were analyzed for Immunoglobulin concentration, Tumor necrosis factor using ELISA kit (Cusabio, Biotech Co., Ltd, China).

3) Analysis of Biochemical parameters

The biochemical parameters like plasma glucose, total bilirubin, blood urea nitrogen (BUN), glutamate oxalate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were analyzed with SPOTCHEM SP 4430 (SPSS, Inc., Chicago, IL, USA) was used for the data analysis.

TABLE 1. EXPERIMENTAL DESIGN FOR DIET TREATMENTS

Treatments	Diets
Control (C)	Basal diet ¹
Treatment (GC)	Basal diet +50 gm green culture/ head

1) Basal diet: Commercial concentrate feed and roughage; the green culture supplement was added to concentrate diet.

TABLE II. GROWTH PERFORMANCE OF HANWOO STEERS FED DIET SUPPLEMENTED WITH GREEN CULTURE

Treatments	Initial Weight (kg)	Final weight (kg)	Weight gain (kg)	Average Daily weight gain(kg)	Dry Matter Intake (kg)	Feed Efficiency
Control	240.00±19.6	393.72±30.3	152.24±19.1 ^a	0.84±0.10 ^a	1081.56±5.07 ^a	0.13±0.02 ^a
Green culture	238.10±18.8	400.54±20.8	167.81±7.2 ^b	0.93±0.04 ^b	1084.35±3.27 ^b	0.15±0.01 ^b

All data are presented as means ± SD (n=15). The rows with different superscripts are significantly different ($P < 0.05$).

4) Statistical analysis

All experiments were replicated according to the requirement and data were expressed as means ± standard deviations (SD). The difference between control and treatments were evaluated using one-way ANOVA followed by Duncan's Multiple Range Test. A p-value of less than 0.05 was considered statistically significant.

B. Green culture in-vitro batch culture experiment

For the batch culture experiment rumen fluid was collected from an experimental farm in Anseong from a fistulated cow. The incubation inoculum was prepared by diluting the rumen fluid with the buffer in a 1:4 (vol/vol) ratio and stirring in a water bath at 40°C with purging CO₂ until its use (10 to 15 min later). For the feedstuff, commercial concentrate feed and Timothy grass were ground finely then mixed in the ratio of 3:2. 400mg of the mixture was placed in 35ml glass tubes to which 20 ml of the incubation inoculum was added. For, the green culture group, 400 mg of Green culture was also added. The tube was stoppered with a Bunsen valve. For both groups, each sample was incubated in three replicates. The samples were incubated at 40°C for 3,6,12 and 24 hours respectively. At the end of each incubation period, the samples were assessed for nutrient digestibility, pH, VFA, Total gas production, Ammonia Nitrogen analysis.

III. RESULTS AND DISCUSSIONS

A. Experimental feeding experiment using Hanwoo steers

1) Growth performances

Dry yeasts are extensively used in ruminant nutrition as feed additives in order to improve feed efficiency, productivity and prevent health disorders [11]. In this experiment, the overall weight gain and feed efficiency (G: F ratio) of the treatment group supplemented with green culture (GC) was significantly higher ($p < 0.05$) than the control group as shown in TABLE II. The previous studies have reported the similar effects of dry yeast supplementation in daily ration of small ruminants and young calves [12][13]. Most of the ruminant's diet comprises a considerable amount of cellulose and hemicellulose.

These cell wall constituents are insoluble, complex structurally and have low digestibility [14]. Feeding live *S. cerevisiae* cells daily increase the stable population of cellulolytic bacterial population in small ruminants [11]. According to [15], feeding sheep on yeast favors the growth of cellulolytic bacterial species (*Fibrobacter auccinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*) in the rumen. The high respiratory activity of *S. cerevisiae* scavenges O₂, which enters the rumen during feed and water intake, rumination and salivation; thus, protecting bacterial population of rumen which is strictly anaerobic [16]. Besides, yeast is a good source of thiamine for rumen fungi which improves fiber digestibility by colonizing the plant cell wall [17]. Moreover, inclusion of green culture in feed seems to improve the palatability of the feed as the Dry Matter (DM) intake in

TABLE III. PLASMA BIO-CHEMICAL PARAMETERS OF HANWOO STEERS FED DIET SUPPLEMENTED WITH GREEN CULTURE

Treatments	Glucose (mg/dl)	Total Cholesterol (mg/dl)	BUN (mg/dl)	Total Bilirubin (mg/dl)	GOT (IU/L)	GPT (IU/L)
Control	118.00 ±5.00	139.00±3.00	12.00±1.00	0.7±1.00	52.00±12.00	25.00±5.0
Green culture	106.00± 4.00	189.00± 3.4	13.00±1.09	0.3± 0.12	57.00± 8.08	24 ± 2.88

All data are presented as means ± SD (n=10).

treatment group was considerably higher.

Supplementation of yeast culture improves the bioavailability and retention of zinc and iron in lambs [18]. Dietary trace minerals like zinc and copper have a crucial role as they are the components of enzymes required for growth and lactation.

2) Plasma Biochemical parameters

To evaluate the overall health condition of the experimental animals, plasma biochemical analysis was conducted. As shown in TABLE III, all parameters were within the normal ranges and did not vary significantly between the control and treatment group. It indicates that green culture supplementation does not affect the physiology of the system related to the regulation of the parameters.

3) Cytokine levels

The immune system is responsible for protecting the animals against foreign substances and invasion by pathogenic organisms. Yeast culture supplementation has resulted in the amplification of serum IgM and IgA, and SIgA in the duodenum of broiler chicks [31]. β -D-Glucan, which comprises 50–60% of total yeast cell wall polysaccharides, which enhance the functional status of macrophages and neutrophils [19].

(1 → 3)- β -D-glucans mediate their immunomodulatory effect by binding to specific receptors leading to augmented production of monocytes and granulocytes, increased antibody titers, boosted cytokine release (including IL-1, IL-2, IL-6, and TNF- α), prostaglandin E₂ production, activation [20]. In this experiment, except for Interferon γ , cytokine level was not affected by the dietary inclusion of GC (TABLE IV). Interferon- γ is a vital cytokine that plays a central role in regulating the innate and adaptive immune responses. Our result opposes the findings of Pelizon and colleagues [21] I.P injections of β -glucan reduced IFN- γ production induced by concanavalin A in mice. The results might have varied due to the variety of sources, procedures involved during the commercial preparation.

B. Green culture in vitro batch culture experiment

Inclusion of green culture in the diet of Hanwoo beef cattle didn't affect apparent digestibility of nutrients, nitrogen balance and total volatile fatty acid production as the parameters didn't differ significantly between the control and treatment.

TABLE IV. CYTOKINE LEVELS OF HANWOO STEERS FED DIET SUPPLEMENTED WITH GREEN CULTURE

Treatments	IFN γ (pg/ml)	IgG (μ g/ml)	IL-1 β (pg/ml)	IL-6(pg/ml)	TNF α (ng/ml)
1 Control	16.46±1.46 ^a	1.14±0.88	10.23±0.32	2.80±1.35	2.74±1.29
2 Green culture	17.87±0.32 ^b	1.11±0.86	10.41±0.48	2.54±1.18	2.59±0.94

All data are presented as means ± SD (n=10). The rows with different superscripts are significantly different (P < 0.05).

1) Nutrient Digestibility

Digestibility was numerically greater for green culture supplementation compared to that of control (TABLE V). Digestibility is influenced by several factors related to the treatment and processing of feed ingredients. The increasing tendency in digestibility of the green culture supplemented group is an indicator of increased nutrient availability which might be the supporting factor for increased growth performance of the steers fed on green culture in the field study.

2) pH

pH is one of the most important factors that affects rumen performance. There were no statistically significant differences in pH values from green culture and control. However, the pH ranged from 7 to 6.57 during the experimental period (TABLE VI). The results suggested that green culture helped to maintain a stable pH in *in-vitro* ruminal fermentation. If the rumen pH drops below 6.0 the activity of fiber digesting bacteria will start to be reduced, leading to reduced feed utilization and feed intakes. The longer the period at low pH and the larger the fall in pH, the greater the impact on the performance of cattle [22]. Ingestion of readily fermentable carbohydrates increases Volatile fatty acid concentration (VFA) concentration in the rumen which ultimately leads to decrease in rumen pH. Low pH favors the growth of lactate producing bacterial species such as *Streptococcus bovis*, leading to the accumulation of lactate in the rumen [23]. Many animal studies have exhibited rumen pH stabilization effect of dietary live yeast [15].

TABLE V. EFFECT OF GREEN CULTURE ON NUTRIENT DIGESTIBILITY (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Treatments	Control	Green Culture
0	28.93 ± 1.1	26.94 ± 0.5
3	34.38 ± 0.9 ^a	39.31 ± 1.2 ^b
6	46.85 ± 2.3	47.42 ± 0.4
12	48.6 ± 1.8	49.84 ± 1.7
24	56.36 ± 4.2	58.08 ± 2.8

Digestibility (%) 43.02 ± 2.0 44.32 ± 2.9

All data are presented as means ± SD (n=3). The columns with different superscripts are significantly different (P < 0.05).

TABLE VI. EFFECT OF GREEN CULTURE ON RUMEN PH (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Treatments	Control	Green Culture
0	7.19 ± 0.0	7.09 ± 0.0
3	6.82 ± 0.1	6.78 ± 0.2
6	6.7 ± 0.1	6.69 ± 0.1
12	6.54 ± 0.0	6.51 ± 0.0
24	6.38 ± 0.0	6.35 ± 0.0
Average pH	6.7 ± 0.1	6.7 ± 0.1

All data are presented as means ± SD (n=3).

3) Ammonia, Methane and Total gas production

No significant differences were observed in ammonia, methane and total gas production (TABLE VII, VIII AND IX) between green culture and treatment group. In animal production, half of the dietary N is excreted in the form of Urea which is ultimately converted to nitrous oxide; a potential contributor to the global greenhouse effect. The N related parameter is assessed in terms of ruminal ammonia concentration [22]. In another study, daily yeast feeding in adult ruminants decreased ammonia concentration [23].

Varying amounts of formic acid, hydrogen (H₂) and carbon dioxide (CO₂) are produced as end products of fermentation [24]. Most of the methanogenic archaea in the rumen use H₂ to reduce CO₂ to produce methane (CH₄) [25]. In vitro studies have elucidated the increased H₂ utilization for acetate production by acetogenic bacteria isolated from a rumen of lambs, in spite of the presence of methanogens [26]. However, some yeast strains show no effect [7] to increase methane production in batch culture [27].

TABLE VII. EFFECT OF GREEN CULTURE ON AMMONIA (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Treatments	Control	Green Culture
3 hours	0.96±0.0	0.89±0.0
6 hours	0.95±0.1 ^b	0.89±0.0 ^a
12 hours	0.93±0.0	0.93±0.1
24 hours	0.95±0.0	1.01±0.0
Total Ammonia Production (ml/g DM)	4.67±0.0	4.61±0.0

All data are presented as means ± SD (n=3). The columns with different superscripts are significantly different (P < 0.05).

TABLE VIII. EFFECT OF GREEN CULTURE ON CH₄ (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Treatments	Control	Green Culture
3 hours	7.6 ± 0.3	8.0 ± 0.2
6 hours	14.0 ± 3.9	15.7 ± 3.4
12 hours	32.1 ± 3.4	31.5 ± 3.9
24 hours	46.3 ± 4.6	46.0 ± 5.0
Total CH ₄ Production (ml/g DM)	100.0 ± 3.1	101.2 ± 3.1

All data are presented as means ± SD (n=3).

TABLE IX: EFFECT OF GREEN CULTURE ON TOTAL GAS PRODUCTION (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Treatments	Control	Green Culture
3 hours	9.0 ± 0.4	8.8 ± 0.4
6 hours	22.3 ± 1.0	23.0 ± 1.0
12 hours	34.2 ± 3.0	32.7 ± 2.6
24 hours	44.3 ± 1.3	45.4 ± 2.1
Total gas production (ml/g DM)	109.8 ± 1.4	109.9 ± 1.5

All data are presented as means ± SD (n=3).

4) Volatile Fatty Acid production

The total concentrations of rumen Volatile fatty acid (VFA), as well as the concentrations of acetate, propionate and butyrate, remained unaffected by the supplementation of green culture (TABLE X to TABLE XII). Dawson reported similar results regarding the supplementation of live yeast culture [28]. However, inclusion of *Saccharomyces cerevisiae* yeast in the diet was found to increase acetate, propionate and butyrate concentrations [29]. The variation in the result might be due to the difference in feed used, variation in the production procedure of supplement, physiological status of animals. However, at 12 hours, the acetate production was significantly higher in green culture group. This indicates that the green culture has tendency to increase the production of acetate which

TABLE X: EFFECT OF GREEN CULTURE ON ACETATE PRODUCTION (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Sample	Acetate (mM)					Total Acetate Production (mM)
	0	3	6	12	24	
Control	11.6 ± 0.6	22.4 ± 0.3	27.6 ± 1.1	42.7 ± 1.3 ^a	57.5 ± 0.1	161.8 ± 0.7
Green culture	11.0 ± 0.1	22.2 ± 0.1	28.6 ± 0.1	50.3 ± 1.3 ^b	63.1 ± 0.6	175.1 ± 0.4

All data are presented as means ± SD (n=3). The rows with different superscripts are significantly different (P < 0.05).

might be underlying cause for the better growth performance of steers in the green culture supplemented group.

In addition, according to Wandita and colleagues [30], acetate improves marbling as it favors the expression of *Zfp423*; a critical regulator of the pre-adipocytes determination during proliferation stage of stromal vascular cells (SVC). Thus, green culture not only favors the growth performance of the steers but also tends to increase the number of pre-adipocytes in fat depots and intra-muscular regions which ultimately insures the intramuscular fat deposition in the growing stage.

TABLE XI. EFFECT OF GREEN CULTURE ON PROPIONATE PRODUCTION (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Sample	Propionate (mM)					Total Propionate Production (mM)
	0	3	6	12	24	
Control	0.7 ± 0.1	3.7 ± 0.4	7 ± 0.1	11.7 ± 0.3	19 ± 3.2	41 ± 0.8
Green culture	0.7 ± 0.0	3.2 ± 0.0	7 ± 0.2	12.0 ± 0.0	15 ± 1.0	38 ± 0.3

All data are presented as means ± SD (n=3).

TABLE XII. EFFECT OF GREEN CULTURE ON BUTYRATE PRODUCTION (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Sample	Butyrate (mM)					Total Butyrate Production (mM)
	0	3	6	12	24	
Control	2 ± 0.1	3.5 ± 0.1	4.2 ± 0.0	7.5 ± 0.0	13.5 ± 1.8	31 ± 0.4
Green culture	2 ± 0.0	3.5 ± 0.2	4.5 ± 0.1	9.2 ± 0.1	11 ± 0.5	30 ± 0.2

All data are presented as means ± SD (n=3).

TABLE XIII. EFFECT OF GREEN CULTURE ON TOTAL VFA PRODUCTION (0, 3, 6, 12 AND 24 HRS. OF INCUBATION)

Sample	VFA (mM)						Total VFA Production (mM)
	Acetate	Propionate	Butyrate	Isobutyrate	Valerate	Isovalerate	
Control	161.9 ± 0.7	40.9 ± 0.8	30.7 ± 0.4	5.5 ± 0.0	7.4 ± 0.1	6.6 ± 0.0	252.9 ± 0.3
Green culture	175.1 ± 0.4	37.6 ± 0.3	30.1 ± 0.2	5.5 ± 0.0	7.2 ± 0.0	6.5 ± 0.0	262.1 ± 0.2

All data are presented as means ± SD (n=3).

IV. CONCLUSION

Based on the results, we can conclude that supplementation of green culture promotes the growth performance of Hanwoo steers as the total weight gain and average daily gain of the treatment group were significantly ($p < 0.05$) higher than that of control. Similarly, it seems to improve the palatability of feed as average dry matter intake in green culture fed steers was significantly higher. In case of in vitro batch culture study, green culture supplemented group exhibited the tendency to increase nutrient digestibility and acetate production; which might have attributed to the improved growth performance of green culture fed steers. In pre-adipocyte determination and adipocyte differentiation point of view, it is interesting that the acetic acid production in green culture supplemented group was significantly higher than control group which might be practically related to the fine marbling and higher intramuscular fat deposition in beef cattle.

Altogether, dietary supplementation of green culture is beneficial for the growth performance in growing Hanwoo steers and showed the potential to improve the rumen environment. Further, increased acetic acid production out of VFAs during growing stage is favorable for high quality beef production.

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