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# Effects of a Mixture of Eugenol, Thymol and Malate on Growth Performance, Beef Quality and Liver Function in Hanwoo Finishing Steers Fed a High-Concentrate Diet

Do Hyung Kim[1], Kyoung Hoon Kim[1], Chang Weon Choi[1], Eung Gi Kwon[1], Seong Koo Hong[1], Sang Cheol Lee[1], Yong Joo Seol[1], Joon Mo Yeo[2], Wan Young Kim[2], Jong Soo Keum[3], Sung Sill Lee[4] and Young Kyoon Oh[1]*

[1]National Institute of Animal Science, R.D.A., Suwon, 441-706, Korea,
[2]Korea National College of Agriculture and Fisheries, Suwon, 445-760, Korea,
[3]Dairy Cattle Genetic Improvement Center, NACF, Goyang, 412-030, Korea,
[4]Division of Applied Life Science, Graduate School of Gyeongsang National University, Jinju, 660-701, Korea.

**ABSTRACT**

Thirty six Hanwoo steers (average BW, 564.5 ± 25.7 kg; average age, approximately 23 months) were used to evaluate the effects of a mixture of eugenol (14%), thymol (1%) and malate (85%) on growth performance, beef quality and liver function in Hanwoo finishing steers fed ahigh-concentrate diet. A feeding trial was conducted for 7 months in National Agricultural Cooperative Federation farm located in Anseong, Korea. Steers were assigned randomly to one of three treatments: control (without additive supplementation), treatment 1 (0.05% additive of concentrate), treatment 2 (0.1% additiveof concentrate). The results of this study showed that initial and final BW averaged 564 and 755 kg, respectively, and BW gain was significantly higher ($P<0.05$) for steers fed the additive mixture than for those fed no mixture (0.78 and 0.79 vs. 0.69 kg/d, respectively). Serum aspartate aminotransferase in the T2 treatment was decreased during the 24 to 31 months of age. Although supplementation of additives resulted in no substantial effect on carcass characteristics, it had a potential effect to improve feed efficiency and AST concentration in Hanwoo finishing steers fed a high-concentrate diet. In conclusion, a mixture of eugenol, thymol and malate has shown promise in improving feed efficiency and liver function in the finishing phase of Hanwoo steers.
(**Key words :** Eugenol, Thymol, Malate, Growth performance, Beef quality, Liver function, Hanwoo steers)

## INTRODUCTION

Steers fed a high-concentrate diets are energetically more efficient in increasing beef quality compared with those fed a high-fiber diet, but it has been shown that a fattening system with the high-concentrate diets resulted in decreased and (or) fluctuating feed intake at finishing phage (Shin et al., 2002). The decreased and (or) erratic feed intake in the finishing phage might result from decreasing ruminal pH. In addition, the decrease in ruminal pH may cause the ruminal disturbance and increase the risk of acidosis (Nocek, 1997).

Antibiotics have been used in diets of beef cattle because of their ability to improve the efficiency of nutrient utilization and reduce the risk of ruminal disturbance such as acidosis, bloat and liver abscess (Chalupa et al., 1980; Bergen and Bates, 1984; Lechtenberg and Nagaraja, 1989). However, the use of antibiotics in animal feeds will be completely banned in the Republic of Korea from 2012. For this reason, livestock industry has become interested in evaluating other alternatives to modulate rumen fermentation, including the use of yeasts, organic acids, plant extracts, probiotics, and antibodies (Calsamiglia et al., 2007).

Plant extracts have been used for centuries for various purposes as traditional medicine and food preservatives due to their antimicrobial properties (Busquet et al., 2006; Davidson and Naidu, 2000). Essential oils are blends of secondary metabolites obtained from the plant volatile fraction by steam distillation (Gershenzon and Croteau, 1991). Beginning from the report of Borchers (1965), the potential benefit of essential oil have been evaluating on rumen microbial fermentation. Rumen microbial activity was affected by use of plant extracts and secondary plant metabolites (Bysquet et al., 2006). It has been reported that terpenoid and phenolic components were responsible for the anti-bacterial properties of many essential oils (Dorman and Deans, 2000; Benchaar et al., 2007). Among these essential

* Corresponding author : Young Kyoon Oh, Animal Nutrition & Physiology Team, National Institute of Animal Science, RDA, Suwon, 441-706 Korea. Tel: 031-290-1665, Fax: 031-290-1660, E-mail: oh665@korea.kr

oils, eugenol and thymol have been shown to alter the rumen fermentation by decreasing lactic acid and increasing pH in the rumen (Castillejos et al., 2006) by inhibiting the growth of *Streptococcus bovis* and *Lactobacillus* sp., producing large amounts of lactic acid.

Malate stimulates lactate uptake by *Selenomonas ruminantium*, the main bacteria using lactic acid in the rumen, so improves the ruminal pH and increases propionate production (Callaway and Martin, 1996; Martin, 1998). The stimulatory effects of malate on lactic acid fermentation have been clearly demonstrated *in vitro* (Callaway and Martin, 1996). As a key intermediate in the succinate-propionate pathway, malate enhances lactic acid fermentation by *Selenomonas ruminantium* (Montano et al., 1999).

In our previous study, eugenol, thymol and malate have contributed the stability of rumen microbial fermentation *in vitro* by decreasing lactic acid and increasing ruminal pH. However, eugenol and thymol inhibited the production of total VFA, acetate and propionate. The results of our previous study suggested that, when eugenol, thymol or malate were used at optimal doses, the rumen fermentation was likely to improve (Kim et al., 2009).

Therefore, the objective of this study was to evaluate the effects of a mixture of eugenol, thymol and malate on growth performance, beef quality and liver function in Hanwoo finishing steers fed a high-concentrate diet.

## MATERIALS AND METHODS

### 1. Experimental design

A feeding trial was conducted for 7 months in National Agricultural Cooperative Federation farm located in Anseong, Korea. Steers were assigned randomly to one of three treatments: control (without additive supplementation), treatment 1, and treatment 2. Dietary additive levels for supplementation groups were 0.05% per kg for treatment 1 (T1) and 0.1% per kg of concentrate for treatment 2 (T2). The additive wascomposed of 85% of malate, 14% of eugenol and 1% of thymol (T.M. Semi Co., Ltd., Seoul, Korea). These ratios were based on our previous *in vitro* studies (Kim et al., 2009). Thirty six Hanwoo steers (average body weight, 564.5 ± 25.7 kg; average age, approximately 23 months) were allotted to 3 treatment groups and two pens per treatment (6 steers per pen). The experiment was carried out from 23 months to 30 months of age.

### 2. Experimental diets, feeding and management

Steers were fed the experimental diets twice daily at 08:00 and 18:00 h, and water was accessible freely through the automatic water provider. Each diet was fed to steers in an individual gate feeding system. Ingredients composition and chemical analysis of the experimental diets are shown in Table 1.

Dailyfeed intake was recorded by the difference between the supply and orts, and the difference of initial and final body weights was used for body weight gain on daily basis

Table 1. Ingredient composition and chemical analysis of the experimental diet[1)]

| Items | | |
|---|---|---|
| **Concentrate ingredients** | As-fed basis, % | |
| Ground corn | 47.80 | |
| Wheat | 41.00 | |
| Soybean meal | 5.00 | |
| Rapeseed meal | 2.00 | |
| Molasses | 2.00 | |
| Calcium phosphate | 1.50 | |
| Salt | 0.40 | |
| Vitamin-mineral additive[2)] | 0.20 | |
| Lasalocid | 0.11 | |
| **Chemical analysis** | Concentrate | Rice straw |
| Dry matter, % | 86.34 | 88.43 |
| | % of DM | |
| Crude protein | 15.36 | 4.37 |
| Ether extract | 4.97 | 1.83 |
| Crude fiber | 8.04 | 34.72 |
| Ash | 7.24 | 12.97 |
| Neutral detergent fiber | 24.00 | 49.20 |
| Acid detergent fiber | 7.94 | 31.97 |

[1)] Steers had free access to water and mineral blocks (Rincal block, Daehan New Pham, Seoul, Korea; provided following nutrients per kg: I, 150 mg; Mn, 200 mg; S, 4,000 mg; Co, 100 mg; Fe, 2,000 mg; Zn, 100 mg; Ni, 50 mg; Cu, 100 mg; Mg, 3,000 mg; Ca, 2,000 mg; Se, 40 ug; NaCl, 380 g) throughout the experiment.
[2)] Provided following nutrients per kg of additive (Grobic-DC, Bayer HealthCare, Leverkusen, Germany): Vit. A, 2,650,000 IU; Vit. $D_3$, 530,000 IU; Vit. E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg.

throughout the feeding trial. At the end of the feeding trial, all animals were slaughtered at commercial abattoir (National Agricultural Cooperative Federation, Seoul, Korea).

### 3. Chemical analyses

Experimental diets were dried by forced-air oven (at 60℃, 48h), ground by a Wiley mill (Thomas scientific, Model 4, U.S.A.) and analyzed for moisture, CP, EE, and ash according to the procedure of Association of Official Analytical Chemists (AOAC, 1990). The concentration of NDF corrected for residual ash was determined with heat-stable amylase and sodium sulphate according to the method of Van Soest et al. (1991), while the content of ADF corrected for residual ash was determined according to the procedure of AOAC (1990).

### 4. Blood collection and analytical methods

Blood samples were collected from the jugular vein before feeding in 10 mL vacuum tubes (BD Vacutainer, Becton & Dickinson, NJ, USA) without any anticoagulant. Blood samples were collected twice daily at the beginning and the end of the experiment. These samples were centrifuged (Hicen21, Herolab, Wiesloch, Germany) at 2,000 × g for 15 min at 4℃ to collect serum and stored at −70℃ until analysis.

Serum was analyzed for aspartate aminotransferase (AST) and gamma-glutamyltransferase (γ-GT) using an automatic blood analyzer (Express Plus, Ciba-Corning, CA, USA). Determination of AST concentration in the serum was based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method for L-aspartate amino-transferase (1986). AST catalyzes the transfer of amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate. Oxaloacetate is reduced to malate by malate dehydrogenase with the simultaneous oxidation of NADH to NAD. The reaction can be measured with the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD. Determination of γ-GT concentration in the serum was based on the method ofSzasz (1969), and Rosalki and Tarlow (1974). Briefly, γ-GT in the serum catalyzes the transfer of the glutamyl group from the substrate to glycylglycine forming glutamyl- glycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2-nitro-benzoate was measured at 405 nm.

### 5. Carcass evaluation

All steers at the end of feeding trial were slaughtered after 24 h fasting. Carcasses were chilled at 0 to 2℃ for 24 h and graded for quality and yield factors by trained personnel of Animal Products Grading Service in Seoul, Korea. Carcass weight, carcass yield, back-fat thickness and size of loin-eye area were assessed. Yield grade was classified with a scale of A, B, C or D. Quality grade was scored on a scale of $1^{++}$, $1^{+}$, 1, 2, and 3, which was mainly determined by marbling score but also by meat color, fat color and maturity. Marbling score was evaluated and scored on a scale of 1 to 7, where 1 is very abundant and 7 is traces. Fat color was scored on a scale of 1 to 7, where 1 is white and 7 is yellow. Meat color was scored on a scale of 1 to 7, where 1 is dark pink and 8 is dark red. Texture was scored on a scale of 1 to 3. Carcass grade was scored by combination of carcass yield grade and quality grade.

### 6. Statistical analyses

Data obtained from the analysis was subjected to statistical analysis using the GLM procedure of SAS version 9.1 (SAS, 2002) according to the following statistical model:

$$Y_{ijk} = \mu + A_i + T_j + (A \times T)_{ij} + e_{ijk}$$

where $Y_{ijk}$, $\mu$, $A_i$, $T_j$, $(A \times T)_{ij}$ and $e_{ijk}$ are the response, overall mean, mean effect of animal, mean effect of treatment, and interaction between animal and treatment and random residual error, respectively.

Duncan's multiple range test was used to interpret any significant differences among the mean values of the treatment. Statistical significance was set at $P<0.05$.

## RESULTS AND DISCUSSION

The effects of the mixture of eugenol, thymol and malate on growth performance and dry matter intake (DMI) in Hanwoo steers fed a high-concentrate diets are shown in Table 2. Final body weight and average daily gain (ADG) were significantly higher ($P<0.05$) in T2 than in control. However, the DMI was unaffected by the treatments.

The effects of the mixture of eugenol, thymol and malate on carcass characteristics in Hanwoo steers fed high-concentrate diets is shown in Table 3. Carcass weight was

Table 2. Effect of dietary additives on growth performance and feed intake in Hanwoo steers

| Items | Treatments[1)] | | | SEM[2)] |
|---|---|---|---|---|
| | Control | T1 | T2 | |
| Number of steers | 12 | 12 | 12 | |
| Initial age (months) | 23.40 | 23.86 | 23.18 | 0.18 |
| Final age (months) | 30.77 | 31.30 | 30.64 | 0.19 |
| Initial body weight, kg | 564.29 | 564.75 | 564.33 | 4.19 |
| Final body weight, kg | 738.67$^{b}$ | 759.36$^{ab}$ | 766.27$^{a}$ | 6.71 |
| Body weight gain, kg | 174.38$^{b}$ | 196.18$^{ab}$ | 198.95$^{a}$ | 4.58 |
| ADG, kg/day[3)] | 0.69$^{b}$ | 0.78$^{ab}$ | 0.79$^{a}$ | 0.02 |
| DMI, kg/day[3)] | | | | |
| Concentrate | 7.89 | 7.79 | 7.88 | 0.07 |
| Rice straw | 0.61 | 0.61 | 0.62 | 0.01 |

1) Control, No additives; T1, added with 0.05% of concentrate; T2, add 0.1% of concentrate.
2) SEM: standard error of means.
3) ADG: average daily gain; DMI: dry matter intake.
a,b Means with different superscripts in the same row are significantly different ($P<0.05$).

Table 3. Effect of dietary additives on carcass characteristics in Hanwoo steers

| Item[1)] | Treatments[2)] | | | SEM[3)] |
|---|---|---|---|---|
| | Control | T1 | T2 | |
| Carcass weight, kg | 434.42 | 453.73 | 452.64 | 5.98 |
| Loin-eye area, $cm^2$ | 87.92 | 87.91 | 90.91 | 1.57 |
| Back fat thickness, mm | 15.17 | 17.55 | 17.36 | 0.89 |
| Yield index, % | 62.93 | 60.98 | 61.51 | 0.59 |
| Yield grade (A:B:C), head | 3:2:7 | 1:2:8 | 0:5:6 | |
| Marbling score | 6.00 | 6.09 | 5.73 | 0.26 |
| Meat color | 4.92 | 4.64 | 4.64 | 0.08 |
| Fat color | 3.00 | 3.00 | 3.00 | 0.00 |
| Texture | 1.00 | 1.09 | 1.09 | 0.04 |
| Maturity | 2.25 | 2.27 | 2.45 | 0.08 |
| Quality grade ($1^{++}$:$1^{+}$:1:2:3), head | 3:4:5:0:0 | 2:5:3:1:0 | 1:6:4:0:0 | |
| Appearance rate of over 1 grade, % | 100 % | 90 % | 100 % | |

1) Grading ranges are 1 to 7 for marbling score, meat color score and fat color score with higher numbers for better quality. And 1 to 3 for texture score, maturity and grade with lower numbers for better quality.
2) Control, No additives; T1, add 0.05%/kg of concentrate; T2, add 0.1%/kg of concentrate.
3) SEM: standard error of means.

numerically higher in T1 and T2 than in control but there were no significant differences in loin-eye area and back fat thickness among the treatments. There are no previous reports investigating the effects of a mixture of thymol, eugenol and malate on animal performance and carcass characteristics in beef cattle. Furthermore, no other study is available to compare beef cattle performance when fed essential oils. Bampidis et al. (2005) evaluated performance and carcass characteristics of growing lambs fed dried oregano leaves, containing carvacrol and thymol. No differences in lamb performance or carcass characteristics were noted when dried oregano leaves were fed at levels of

0, 144, or 288 mg/kg of concentrate mixture.

Cardozo et al. (2006) reported that the combination of cinnamaldehyde (180 mg/d) plus eugenol (90 mg/d) reduced total DMI in beef cattle. The reduction in DMI was also observed in dairy cattle and might be related to palatability problems, suggesting that the product needs to be encapsulated to overcome this problem. Meanwhile, no data are available evaluating essential oils for beef cattle fed high-concentrate diets to determine the effect on ADG and feed efficiency. On the other hand, malate has been shown to improve ADG and feed efficiency in steers fed high-concentrate diets based on corn (Sanson and Stallcup, 1984). Therefore, the ineffective result in DMI and effective result in ADG can be explained by the presence of thymol and eugenol. In one study, evaluation of growth performance using essential oil compounds consisting of thymol, eugenol, vanillin and limonene showed that DMI and ADG were not affected by the addition of essential oil compounds mixture. However, the gain to DMI ratio was affected quadratically with a dose of 2 g/day maximizing feed efficiency (Benchaar et al., 2006).

In addition, Benchaar et al. (2007) reported that feeding a blend of essential oil to dairy cattle increased ruminal pH and ADF digestion, but had no effects on VFA and animal performance. In our previous study, eugenol and thymol showed negative effects on ruminal fermentation (Kim et al., 2009), but in the present study, the DMI was unaffected by the treatments and the ADG was significantly increased by 0.1% additives. It is considered that the negative effects on rumen fermentation by eugenol and thymol are not observed *in vivo*. However, data on ruminal VFA profiles are required to accurately determine the effect of mixed additives on carcass characteristics.

The effects of the mixture of eugenol, thymol and malate on serum AST and γ-GT values in Hanwoo steers fed high-concentrate diets is shown in Table 4. The serum AST and γ-GT levels were calculated as the difference of initial values (24 months of age) and final values (31 months of age). The difference in serum AST was significantly lower for T2 treatment than for other treatments, whereas no significant difference was seen in the serum γ-GT between the treatments. Changes in the concentration of blood components of ruminants have been used as indices of metabolic disturbance or toxicity (Puoli et al., 1992). The concentration of specific enzymes such as AST and γ-GT serves as indicators of tissue damage or metabolic disorders (Wee et al., 1988; Kramer, 1989; Puoli et al., 1992). Increased levels of serum γ-GT and AST indicate impaired liver function and cellular damage (Wee et al., 1988). High-concentrate diet in the finishing beef cattle would develop an acidic rumen condition. In addition, acid-induced rumenitis and damage of the ruminal wall are associated with a sudden change to high energy diets (Elam, 1976). Moreover, acid-induced rumenitis and damage of the ruminal wall predispose to ruminal abscesses by *Fusobacterium necrophorum*. These bacteria can gain entry into the blood and subsequently causes bacterial emboli in the portal circulation. Bacteria from the portal circulation are filtered by the liver, leading to infection and abscess (Nagaraja and Chengappa, 1998). Lechtenberg and Nagaraja (1991) detected that rectal temperature, leukocyte counts, fibrinogen, globulin, bilirubin, AST, γ-GT and sorbitol dehydrogenase concentrations increased when hepatic abscesses were induced experimentally in five steers by inoculating *Fusobacterium necrophorum* via ultra-sonography-guided, percutaneous catheterization of the portal vein.

Table 4. Effect of dietary additives on serum AST and γ-GT values in Hanwoo steers[1)]

| Item[2)] | Treatments[3)] | | | SEM[4)] |
|---|---|---|---|---|
| | Control | T1 | T2 | |
| Number of steers | 12 | 12 | 12 | |
| AST, u/l | 16.38[a] | 9.14[ab] | −0.38[b] | 0.45 |
| γ-GT, u/l | 4.75 | 4.25 | −4.82 | 1.96 |

[1)] Calculated as the difference of initial (24 months of age) values and final (31 months of age) values.
[2)] AST: aspartate aminotransferase; γ-GT: gamma glutamyltransferase.
[3)] Control, No additives; T1, add 0.05%/kg of concentrate; T2, add 0.1%/kg of concentrate.
[4)] SEM: standard error of means.
[a,b] Means with different superscripts in the same row are significantly different ($P<0.05$).

In the present study, increased concentrations of serum γ-GT and AST in the control group suggest that feeding high-concentrate diets to Hanwoo steers in the fattening period could increase the incidence of hepatic dysfunction. On the other hand, the decrease of γ-GT and AST enzymes in the T2 treatment suggests a protective effect of the mixture from the incidence of hepatic dysfunction associated with feeding a high-concentrate diet. It could be speculated that positive effects on ruminal pH improve liver function by the dietary additives because the resulting decrease in ruminal pH may increase the risk of acidosis (Nocek, 1997).

Although supplementation of experimental additives did not show a noticeable improvement on carcass characteristics in this study, it showed positive effects which increased feed efficiency and decreased AST concentration in Hanwoo finishing steers fed a high-concentrate diet. Therefore, it is considered that a mixture of thymol, engenol and malate could reduce the risk of acidosis in Hanwoo finishing cattle consuming a high-concentrate diet. However, further research is required using different mixing ratios and repeated efforts using various types of non-antibiotics to improve growth performance, beef quality and liver function in Hanwoo finishing steers fed a high-concentrate diet.

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