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https://doi.org/10.5109/1564090

出版情報：九州大学大学院農学研究院紀要. 61 (1), pp.109-114, 2016-02-29. Faculty of Agriculture, Kyushu University

J. Fac. Agr., Kyushu Univ., **61** (1), 109–114 (2016)

Effects of Dietary Zilpaterol Hydrochloride on Growth Performance, Carcass Characteristics, and Serum Compositions of Hanwoo Bulls

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(*Received November 10, 2015 and accepted November 19, 2015*)

This study was conducted to determine the effects of zilpaterol hydrochloride (ZH) on the growth performance and carcass characteristics of Hanwoo bulls. Twenty Hanwoo bulls (653 ± 22.1) were randomly assigned by body weight (BW) to determine the effects of feeding zilpaterol hydrochloride (Ten bulls with 8.3 mg/kg ZH and ten bulls without ZH) on the ultrasound measurement, carcass traits, and serum compositions. Treatments comprised diets with and without ZH supplemented for 20 days prior to harvest with three days withdrawal period. Treatment of ZH led to an increase either in the final BW or average daily gain (ADG) in Hanwoo bulls ($P<0.05$). There was no treatment effect on the back fat thickness, ribeye area, and marbling score ($P>0.05$). ZH treatment tended to increase at dressing percentage ($P=0.068$). There was a high positive correlation ($R^2=0.9226$) at treatment but relatively low positive correlation ($R^2=0.7156$) at control between ultrasound longissimus muscle area and ribeye area. The treatment of ZH affects the serum triglyceride and non–esterified fatty acid ($P<0.05$). Moreover, we demonstrated that the treatment of ZH did not have a negative effect on the serum level of GOT or GPT of Hanwoo bulls. We concluded that the treatment of ZH has little impact on the carcass trait of Hanwoo bulls regardless of the positive effect on BW and ADG. However, dietary ZH increased lipid related serum metabolites of Hanwoo bulls at 14th day.

Key words: Zilpaterol hydrochloride, Hanwoo bulls, carcass, NEFA

INTRODUCTION

Hanwoo bulls have been genetically selected since the last few decades not only for enhancing meat quality but also for improving growth performances. The typical carcass weight of Hanwoo cattle increased to an average of 100 kg compared with a decade before, and the marbling score, a major economic trait in Korean markets, also increased since the last ten years (KAPE, 2012). Although there was an improvement of meat quality in the Hanwoo industry, the yield grade neither increased nor decreased in last ten years. It is possible that the genetic variation should be limited to the yield grade of Hanwoo cattles. However, Hanwoo cattles have a low carcass weight compared with genetically resembled Japanese Wagyu cattle. This fact indicated that there was potential ability to improve the yield traits of Hanwoo beef cattle. Regardless of the genetic traits of Hanwoo beef cattle, the yield trait or meat quality of beef cattle was affected by environments, such as diets, ages, or breed types (Crouse *et al*., 1989; Lunt *et al*., 2005). Feedlot program for increasing the meat quality has been used in high corn–based diet for long fattening periods. This system typically increased the backfat thickness during fattening periods and then finally decreased the yield grades of beef carcass (Lunt *et al*., 2005). Zilpaterol hydrochloride commercially used in US and South America was approved by the US Food and Drug Administration since 2006. Zilpaterol hydrochloride treatment for 20 or 30 days increased the BW, feed efficiency, and carcass leanness of beef cattle (Montgomery *et al*., 2009). According to recent reports, ZH treatment for 20 days with two day withdrawals increased not only the yield traits but also the growth performance of Hanwoo steers (Choi *et al*., 2013). Although there were not many studies conducted with ZH treatment in Hanwoo cattle, limited data showed the effects of ZH in the high quality diet program. Hanwoo bulls relatively high yield grade but low quality grade compare to same BW of Hanwoo steers. The physiological difference between Hanwoo steers and bulls may have diverse response of ZH treatments. Therefore, this study demonstrates the treatment of ZH on the growth performance, carcass characteristics, and serum compositions of Hanwoo bulls.

METHODS AND MATERIALS

Animals and Management

All experimental procedures were conducted according to the official protocols for animal care in National Institute of Animal Science, and were performed at the Korean Ministry for Food, Agriculture, Forestry and Fisheries Safety System. The procedures were approved by the respective institutional animal care and use committee. Five head of 24–month old Hanwoo bulls were used to feed in 4 pens, respectively and 2 pens were used for treatment. Hanwoo bulls were treated with ZH (8.3 mg/kg) for 20 days and with 3 day withdrawal peri-

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ods. The diet was used to achieve final fattening concentrate with rice straw for 23 days. The diet formulation and chemical compositions are provided in Tables 1 and 2. Chemical compositions of diet were analyzed by the AOAC method with 2 kg of samples from each diets (A.O.A.C., 1995). Hanwoo bulls were evenly fed twice a day (8:00, 16:00) and rice straw was fed *ad libitum*. The treatment group fed 20 g of additives with ZH (8.3 mg/kg) and control group was fed 20 g of additive itself. Bulls were consuming the final concentrated diet at the onset of the trial. The live BW of each bull was measured on d 0 and d 20 on ZH treatment. Ultrasound measurement was conducted for evaluating fat deposition and back fat thickness either before or after treatment of ZH. Ultrasound measurements were recorded at 0 and 20th days. Ultrasound measurements were taken by an experienced operator using a B–mode real–time ultrasound device (HS–2000, FHK Co. Ltd, Japan) with an 18 cm, 3.5 MHz linear probe. Scanning was made by positioning the probe vertically from dorsal to ventral line parallel to the ribs between 13th thoracic vertebra and first lumbar vertebrae on the left side. Then ultrasound images were collected to the Korean cattle Synthesis System (Ver. 1.0, 2010). Collected images were analyzed by trained technicians of the National Agriculture Cooperative Federation (NACF) to estimate measures of backfat thickness (UBF), longissimus muscle area (ULMA) and marbling score (UMS). Each bull was fitted with individual identification tags that were recorded by a beef traceability system.

Table 1. Composition of concentrate diet in feeding trial

Ingredients	%, DM
Steam–flaked corn	35.00
Wheat	5.00
Tapioca	5.00
Corn gluten feed	20.00
Wheat bran	9.20
h	1.50
Palm kernel meal	5.00
Coconut meal	6.00
Rapeseed meal	3.65
Soybean hulls	3.00
Beat pulp	0.40
Wheat flour	0.40
Cane molasses	4.00
Salt	0.50
Calcium carbonate Limestone	1.25
Vitamin premix[1]	0.05
Vitamin premix[2]	0.05
Total	100.00

[1] per kg : vitamin A, 8,000,000 IU ; vitamin D3, 2,000,000 IU ; vitamin E, 7,500 IU ; Antioxidant, 1,000 mg.
[2] per kg : Fe, 40,000 mg ; Cu, 10,000 mg ; Zn, 90,000 mg ; Mn, 54,000 mg ; Mg, 6,000 mg ; Co, 300 mg ; I, 1,800 mg ; Se, 900 mg

Table 2. Chemical composition of diets used in this experiment (DM basis, %)

Item	Concentrate	Rice straw	ZH Mixture[7]
DM[1]	90.52	91.43	90.00
CP[2]	14.08	4.39	14.00
EE[3]	4.80	2.36	1.60
CA[4]	9.41	13.07	–
NDF[5]	28.05	70.21	–
ADF[6]	11.10	38.13	18.00

[1] DM : Dry matter
[2] CP : Crude protein
[3] EE : Ether extract
[4] CA : Crude ash
[5] NDF : Neutral detergent fiber
[6] ADF : Acid detergent fiber.
[7] ZH mixture : Amaferm (BioZyme Incorporated) with ZH

Carcass evaluation

Carcasses were chilled for 24 h at 4°C and ribbed at between the 12th and 13th rib. Cold carcass weight, meat quality, and yield grade were evaluated by the Korean grading standard documented in the Korean Ministry for Food, Agriculture, Forestry and Fisheries. Carcasses were evaluated for marbling score, backfat thickness (BFT), longissimus dorsi (LD) area, meat color, fat color, and quality grade at the 13th rib area. Yield grade and dressing percentage were calculated from the carcass data.

Serum analysis

Blood samples (10 mL/hd) were collected from the Hanwoo bulls into serum vacutainer (BD Vacutainer serum, USA) at 0, 14, and 20 days, respectively. Blood was drawn every in the morning before the feeding. After storage at refrigeration for 24 hours, blood was centrifuged with 3,000 RPM at 15min and the serum stored at – 70°C until serum analysis. Serum albumin (ALB), cholesterol (CHO), triglyceride (TG), glucose (GLU), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), total protein (TP), inorganic phosphorus (IP), and non–esterified fatty acids (NEFA) concentrations were analyzed by automatic serum analyzer (Hitachi 7020, Japan).

Statistical analysis

Data were analyzed as a randomized complete block design with as mean ± SEM. Variation analysis and significant differences in this experiment was analyzed by using PROC MIXED procedure of SAS (Statistical Analysis System software version 9.2) system. Serum data were analyzed as a randomized complete block design with a 2 × 4 factorial arrangement of treatment with repeated measures. Day 0 was included as a covariate tin the model for analysis of serum metabolites. The effect of group was tested to ensure that there was no group by treatment interactions. Treatment means were analyzed and separated ($P<0.05$) using the LSD procedure of SAS.

RESULTS AND DISCUSSIONS

Performance and Ultrasound measurement

The initial BW did not differ between the control and ZH supplemental bulls ($P>0.05$; Table 3). However, the treatment with ZH led to either an increased final BW or an average daily gain (ADG; $P<0.05$). Although there was a variation between individual bulls, the treatment of ZH increased live BW during 20 days without change in dry matter intakes. Ultrasound measurement was conducted to evaluate the effect of ZH on ultrasound backfat thickness (UBF), longissimus muscle area (ULMA), and marbling score (UMS) before and after the ZH supplement (Table 4). There was no initial difference between the control and treatment groups on UBF, ULMA, and UMS of bulls ($P<0.05$). The levels of UBF and ULMA were numerically increased after 20 days ZH treatments but there was no significance between the treatments. A previous study indicated that ZH treatment on the finishing diet for 20 days increased by approximately 9 kg of BW compared with the control diets as well as the increased feed efficiency (Elam *et al*., 2009; Vasconcelos *et al*., 2008). Similarly, other studies indicated that the Holstein steers increased ADG at the treatment of ZH in the final feedlot system (Beckett *et al*., 2009). The mode of action of β–adrenergic agonist leads to the activation of the β–receptor in skeletal muscle and the increase in the rate of lean growth in beef cattle. ZH has a high affinity for the β–adrenergic receptor located in the plasma membrane of the bovine skeletal muscle and adipose tissue (Beermann 2002; Verhoeckx *et al*., 2005). Although several studies reported the treatment of ZH in Angus, Holstein, and crossbred steers, it has not been reported to treat ZH in Hanwoo bulls. The treatment of ZH increased not only the final BW but also the ADG compared with the control groups (Table 3). The increases equate to an average of 34 kg heavier BW in ZH–fed Hanwoo bulls. US feedlot managers who want to typically increase the growth rate of feedlot cattle not only treated ZH but also implanted steroid hormones, such as estradiol–17β or trenbolone acetate. Synergistic treatment of the β–agonist and steroid hormone could maximize ADG and BW in crossbreed cattles in US. However, Hanwoo bulls could not be treated with steroid hormones but may have a greater effect on carcass traits compared with treating with steroid hormone (Baxa *et al*., 2010). Because previous studies show that the control and treatment groups were evaluated with carcass characteristics after slaughter, it is hard to evaluate the individual effect of ZH. This study observed the effect of ZH of individual Hanwoo bulls before and after ZH treatments. Ultrasound marbling score for reducing individual variation of LD muscle of Hanwoo bulls were compared with

Table 3. Body weight and average daily gain of Hanwoo bulls treated with ZH

Item	Control	Treatment	SE	P–value
Initial BW[1] (kg)	647.6	657.4	7.4	0.7668
Final BW (kg)	677.2[b]	691.6[a]	8.7	0.0201
ADG[2] (kg/d, 0–20 days)	1.13[b]	1.71[a]	0.31	0.0120
Feed Intake (kg)				
Concentrate	8.88	7.99	1.08	0.7855
Rice Staw	0.31	0.47	0.16	0.6734
DMI	8.08	7.45	0.95	0.7625

[1] BW : body weight.
[2] ADG : average daily gain.
[a,b] Means with different superscripts in the same line differ significantly ($P<0.05$).

Table 4. Ultrasound measurement of Hanwoo bulls treated with ZH

Item		Control	Treatment	SE	P–value
UBF[1]	0 day	2.20	2.60	0.58	0.6195
	20 day	2.30	2.80	0.66	0.5680
ULMA[2]	0 day	82.80	82.20	1.32	0.8985
	20 day	86.4	87.40	2.06	0.8182
UMS[3]	0 day	11.20	11.60	0.20	0.3972
	20 day	11.20	11.80	0.37	0.1950

[1] UBF : Ultrasound measurement of Backfat Thickness
[2] ULMA : Ultrasound measurement of Longissimus Muscle Area
[3] UMS : Ultrasound measurement of Marbling Score .

the initial and final scores. The result represented that there were no significantly differenced UMS between the control and ZH treatment. Furthermore, 20 days of treatment would not affect the carcass marbling score in Hanwoo bulls. There was no difference not only in UBF but also in UFA on ZH treatments (Table 4). There was a high positive correlation (R^2=0.9226) in treatment but relatively low positive correlation (R^2=0.7156) in control between UFMA and ribeye area (REA; Fig. 1). Although there were large variations caused by the low number of individuals, prediction with ultrasound measurement of longissimus muscle had a relatively high correlation in Hanwoo bulls. This data shows the similar result to a previous study that the genetic analysis of ULMA and REA were highly correlated in the Hanwoo steer population (Perkins *et al.*, 1992; Hwang *et al.*, 2014). However,

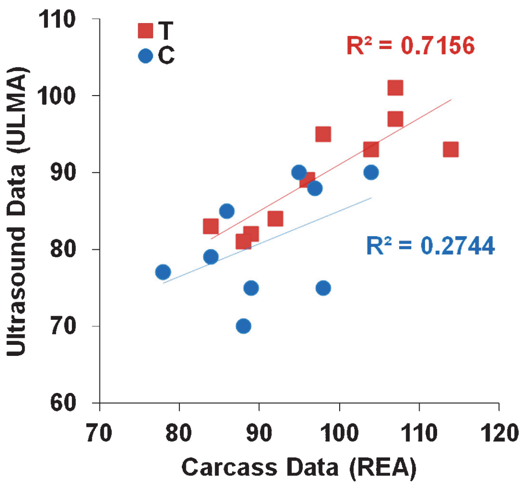

Fig. 1. Relationship between ultrasound measurement of longissimus muscle and carcass ribeye area in Hanwoo bulls. The red line is regression one representing ZH treatments and the blue line is regression one representing non–treatments.

standardized ultrasound measurement for long fed–high quality steers or heifers may reduce the accuracy on the ultrasound measurement of short–fed bulls.

Carcass characteristics

The measurements of carcass characteristics were summarized in Table 5. The carcass weight of the control and ZH treatment were 347 and 363 kg, respectively. Although there was no significant difference in either BFT or REA, ZH treatment tended to increase at the dressing percentage (P=0.07). There was no difference in the meat color, fat color, texture, maturity, and marbling score of ZH treated Hanwoo bulls. A previous study indicated that the treatment of ZH efficiently increased the carcass weight (15 kg) compared with increased the live weight (9 kg) of crossbreed steers (Vasconcelos *et al.*, 2008). In addition to the previous result, the dressing percentage presented in the traits of yield grade was increased by more than 1.5–2.0% compared with the control (Delmore *et al.*, 2010). ZH–fed Hanwoo bulls were increased by more than 15.6 kg compared with the control groups. Therefore, our data was consistent with previous reported studies. From the mode of action of the β–agonist, ZH treatment accumulated more protein and water in the skeletal muscle, which not only increased the muscle mass but also decreased the adipose tissue development (Mersmann, 1998). ZH treatment for 30 or 40 days not only dramatically increased the yield grade but also decreased the marbling score in Holstein steers (Beckett *et al.*, 2009). However, Choi *et al.* (2013) reported that ZH was effective in increasing BFT, REA, and carcass weight but was not effective in increasing the marbling score of Hanwoo steers. Our data indicated that ZH treatment for 20 days did not affect the marbling score but increased BW and ADG of Hanwoo bulls. This result demonstrated that Hanwoo bulls contained less marbling fat compared with steers, which did not affect the quality grades of the carcass. There was no previous

Table 5. Carcass characteristics of Hanwoo bulls treated with ZH

Item	Control	Treatment	SE	P–value
Yield traits				
Carcass weight (kg)	347.20	362.80	12.62	0.3945
Dressing Percentage (%)	55.95	57.70	0.67	0.0679
Back fat thickness (mm)	1.80	2.80	0.37	0.1869
Rib eye area (cm^2)	93.20	94.40	3.68	0.8178
Yield index	74.15	73.23	0.38	0.1755
Yield grade (A:B:C,%)	100:0:0	100:0:0		
Quality traits				
Marbling score	1.00	1.00	0.00	–
Meat color	5.20	5.60	0.20	0.2415
Fat color	3.00	3.00	0.00	–
Texture	21.20	21.20	0.20	–
Maturity	2.00	2.00	0.00	–
Quality grade (1:2:3,%)	0:0:100	0:0:100		–

Table 6. Serum parameters of Hanwoo bulls treated with ZH

Item	0 day		7 day		14 day		20 day		SE	Day	Trt	D × T
	C[11]	T[12]	C	T	C	T	C	T				
ALB[1]	3.16	3.31	3.12	3.27	3.20	3.43	2.96	3.03	0.11	0.0435	0.0671	0.9204
GLU[2]	99.3	88.6	101.1	79.8	91.2	82.0	84.2	69.8	7.65	0.1496	0.0122	0.8618
CHO[3]	146.5	155.3	160.1	171.6	185.3	197.2	155.6	175.3	8.85	0.0003	0.0416	0.9354
TG[4]	14.7	24.3	11.3	12.0	10.6	16.9	15.4	18.4	1.90	0.0005	0.0005	0.1075
BUN[5]	16.12	17.79	14.80	14.93	15.14	15.19	13.94	16.21	0.71	0.0143	0.0426	0.3000
TP[6]	7.25	6.88	7.52	7.02	7.76	7.56	7.06	6.63	0.22	0.0030	0.0171	0.9137
GOT[7]	153.0	148.5	128.4	170.7	170.5	143.3	131.6	117.1	19.0	0.3373	0.9424	0.2916
GPT[8]	24.7	23.7	23.3	22.6	28.3	24.8	24.3	21.0	1.39	0.0261	0.0337	0.6376
IP[9]	7.74	6.95	7.30	6.82	7.56	7.40	7.47	6.68	0.29	0.3809	0.0078	0.6493
NEFA[10]	168.2	168.4	231.1	251.0	265.0	218.0	77.6	93.3	27.7	0.0001	0.8396	0.6061

[1] ALB: albumin (g/dL)
[2] GLU: glucose (mg/dL)
[3] CHO: cholesterol (mg/dL)
[4] TG: triglyceride (mg/dL)
[5] BUN: Blood Urea Nitrogen (mg/dL)
[6] TP: total protein (g/dL)
[7] GOT: glutamic oxaloacetic transaminase (IU/L)
[8] GPT: glutamic pyruvic transaminase (IU/L)
[9] IP: inorganic phosphate (g/dL)
[10] NEFA: non-esterified fatty acid (μEq/L)
[11] C: control
[12] T: treatment

ZH study for bull performance in feedlot system. According to our result of Hanwoo bull study, ZH treatment may be attenuated by the difference of hormonal system. Dietary ZH had continuously presented lower REA in heifer at the previous studies (Rathmann *et al.*, 2012; Johnson *et al.*, 2014). Previous heifer and steer studies indicated that the castration of beef cattle may ameliorate the activity of ZH treatment. Therefore, performance of dietary ZH had consistently increased ZH treatment but had diverse activity between hormonal systems.

Serum metabolites

The effect of ZH in the serum metabolites are shown in Table 6. The serum was collected from d 0, d14, and d 20 after ZH administration and we analyzed the serum albumin, cholesterol (CHO), triglyceride (TG), glucose (GLU), GOT, blood urea nitrogen (BUN), total protein (TP), and non–esterified fatty acid (NEFA) compositions. The treatment of ZH affected the serum TG and NEFA (P<0.05). Serum TG composition of ZH treatment tended to decrease at 20 days compared with zero days. The serum NEFA level was dramatically increased at 14 days and decreased at 20 days. Although ZH treatment did not affect the serum BUN and TP, the serum BUN and TP decreased in the non–treated ZH bulls during 20 days (P<0.05). Serum GOT, GPT, GLU, and CHO were not changed during the days of ZH treatment.

Serum metabolites have been indicated as a marker to study nutritional physiology for evaluating the metabolic regulation of beef cattle (Choi *et al.*, 2009; Vernon. 1992). This study analyzed the serum metabolites, which regulated the physiological change under ZH treatment. The diet of the β–agonist was not only up–regulated protein synthesis but also induced muscle hypertrophy in the body. On the contrary, the treatment of the β–agonist down–regulated lipogenesis and activated lipolysis of accumulated triglycerides (Mersmann 1998). Our data was consistent with the mode of action in previous reported studies. Hanwoo beef cattles on the final fattening not only reduced protein synthesis, which not only induced the serum BUN and TP levels but also induced lipid accumulation, which increased the serum lipogenic metabolites (Lee *et al.*, 2013). Our serum data indicated that the treatment of Hanwoo bulls with ZH decreased the serum BUN and TP levels and this result revealed that ZH treatment may induce lean tissue proteins but reduced serum level of BUN in Hanwoo bulls. Similar to our results for ZH, previous study indicated that the treatment of ZH decreased serum urea–N concentrations in finishing beef steers (Parr *et al.*, 2014). This paper also indicated that the level of NEFA was increased at 10 days after ZH treatment. Other β–agonist such as cimaterol (Byrem *et al.*, 1996) or clenbuterol (Eisemann *et al.*, 1988) has resulted in increased serum NEFA levels in steers. Our data also demonstrated that feeding ZH was increased NEFA level at first 14days but reduced at 20th day in Hanwoo bulls. Serum NEFA level increased by lipolysis of peripheral triglyceride was increased after treatment of ZH and this result indicated that ZH activated lipolysis of Hanwoo bulls (Beever, 2006). Serum GOT level regulated by the damage of liver tissues were typically 48 U in the cattles or pigs (Do *et al*., 1990). Hanwoo steers had typically high serum GOT level and this result may reveal the long–fed system for producing high quality beef (Lee *et al*., 2013). According to the serum level of GOT or GPT, the treatment of ZH did not have a negative effect on the liver function of Hanwoo bulls. Our results indicated that ZH treatment in Hanwoo bulls up–regulated lipolysis and down–regulated proteolysis metabolism.

This study was conducted to evaluate the effect of ZH on the performance, carcass characteristics, and serum metabolites in Hanwoo bulls. Twenty Hanwoo bulls were randomly assigned by BW to determine the effects of feeding zilpaterol hydrochloride (ten bulls with 8.3 mg/kg ZH and ten bulls without ZH) on the growth performance, carcass traits, and changes in blood compositions. There was no difference in BFT and marbling score between the treatment and control. This data revealed that ZH did not affect the carcass characteristics and performance at final fattening of Hanwoo bulls. However, ZH treatment regulated several serum metabolites levels, which were related to protein or lipid metabolism. Therefore, our data indicated that ZH treatment affected physiological regulation in Hanwoo bulls.

ACKNOWLEDGMENT

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ009414)" Rural Development Administration, Republic of Korea.

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