# Effect of Different Feeding Ratios of Whole Crop Barley Silage on the Embryo Production in Hanwoo Donors

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## ABSTRACT

The purpose of this study was to determine the effect of different feeding ratios of whole crop barley silage on the embryo production in Hanwoo donors. All donors were basically fed 2.5 kg concentrate daily. Donors were divided into three groups according to the different feeding of forage; hay 70% and rice straw 30% (control, n = 21), whole crop barley silage 80% and rice straw 20% (T1, n = 25), and whole crop barley silage 60% and rice straw 40% (T2, n = 23) fed based on TDN 6.70/ BW 500 kg. All Hanwoo donors received a CIDR together with injections of 1 mg estradiol benzoate and 50 mg progesterone ($P_4$, Day 0). Four days later, they were superovulated with 28 mg FSH twice daily IM in decreasing doses over 4 days. Then donors received 2 doses of $PGF_2\alpha$ (25 and 15 mg) with the 5th and 6th injections of FSH on Day 6. CIDR were withdrawn at the 6th FSH injection and the donors received 100 $\mu$g GnRH 36 h after the second $PGF_2\alpha$ injection. The donors were artificially inseminated twice, at 8 and 24 h after GnRH, and embryos were recovered 7 or 8 days after the 1st insemination. The flush rate of the donors following positive superovulation responses did not differ among groups (76.2～96.0%, $p$>0.05). The number of corpus luteum (CL) at embryo recovery also did not differ among groups (10.6～14.0, $p$>0.05). Furthermore, the mean numbers of total ova (9.4, 10.5 and 12.0) and transferable embryos (5.3, 12.0 and 6.5) did not significantly differ among the control, T1 and T2 groups, respectively ($p$>0.05). However, mean concentrations of serum $P_4$ of the T1 (64.2 ng/ml) and T2 groups (55.7 ng/ml) were higher than that of control group (43.3 ng/ml, $p$<0.01), while serum cholesterol concentrations in the control (105.8 mg/dl) and T2 groups (96.9 ± mg/dl) were significantly lower than in the T1 group (121.1 mg/dl, $p$<0.05). Conclusively, whole crop barley silage can be fed a good substitute for hay forage for Hanwoo donors. Furthermore the ratios of whole crop barley silage 60% and rice straw 40% might be more worthful for embryo production.

(Key words : Hanwoo, donor, superovulation, embryo, whole crop barley silage)

## INTRODUCTION

Worldwide, bovine embryo transfer has been used for genetic improvement or multiplication using the superovulation treatment and embryo transfer technology (Jung *et al*., 1986; Christensen, 1991; Ruvuna *et al*., 1992; Hasler, 2003; Son *et al*., 2006). Successful superovulation responses and subsequently collection of more transferable embryos from donor animals must be critically important to improve embryo production, which has been closely associated with nutritional management of the donor animals (Yaakub *et al*., 1999; Dawuda *et al*., 2002; Rhoads *et al*., 2006).

Stroud and Hasler (2006) reported that the donor cow managed in good quality grassland had higher superovulation responses and produced good quality embryos than the donor cows fed in poor quality grassland, hay or silage. Feeding of domestic whole crop barley silage could replace as a cattle feed instead of assorted feed and imported forage (Youn *et al*., 1991; Kim *et al*., 2003). Cho *et al*. (2000) reported that feeding of whole crop barley silage had a higher gain in body weight than feeding of rice straw in castrated Hanwoo. Whereas, Son *et al*. (2009) suggested that the feeding of whole crop barley silage alone without supplement of other forage was not efficient for embryo production in Hanwoo donors.

Recently, whole crop barley silage is increasingly used as a good forage substitute for concentrate or imported hay in

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Hanwoo farms. Therefore, this study was to determine the effect of different feeding ratios of whole crop barley silage on the embryo production in Hanwoo donors.

## MATERIAL AND METHODS

### 1. Experimental Animals

Forty seven Hanwoo cows with an average body weight of 495 kg (325～639 kg) and age of 4.8～14.3 years raising at the Animal Genetic Resources Station, National Institute of Animal Science were used for this study.

### 2. Nutritional Management of Donor Animals

All donors were basically fed 2.5 kg concentrate daily. Donors were divided into three groups according to the different feeding of forage; hay (mixing of orchard grass, perennial ryegrass, tall fescue, timothy and kentucky bluegrass) 70% and rice straw 30% (control, n = 21), whole crop barley silage 80% and rice straw 20% (T1, n = 25), and whole crop barley silage 60% and rice straw 40% (T2, n = 23) fed based on TDN 6.70/ BW 500 kg.

### 3. Superovulatory Treatment and Artificial Insemination

All Hanwoo donors, at random stages of the estrous cycle, received a CIDR® (InterAg, New Zealand) together with injections of 1 mg estradiol benzoate (SY Esrone, Samyang, Seoul, Korea) and 50 mg progesterone ($P_4$, SY Ovaron, Samyang, Day 0). Four days later, they were superovulated with 28 mg Antorin®R・10 (Kawasaki Pharm, Japan) twice daily IM in decreasing doses over 4 days (5, 5, 4, 4, 3, 3, 2 and 2 mg). Then donors were received 2 doses (25 and 15 mg) of $PGF_{2}\alpha$ (Lutalyse™, Pharmacia & Upjohn) with the 5th and 6th injections of FSH on Day 6. CIDR were withdrawn at the 6th FSH injection and the donors received 100 $\mu$g Gonadorelin (GnRH, Fertagyl®, Intervet, European Union) 36 h after the second $PGF_{2}\alpha$ injection. The donors were artificially inseminated using 2 straws of frozen-thawed semen twice, at 8 and 24 h after Gonadorelin, and embryos were recovered 7 or 8 days after the 1st insemination.

### 4. Collection and Evaluation of Embryos

Embryos were recovered 7 or 8 days after the 1st insemination by flushing the uterus with flushing solution (BioLife™ Advantage Embryo Collection Medium, Agtech, Inc., USA) using 3 way Foley catheter (IMV Technologies) and collected at 14-84× under the stereomicroscope (Olympus, Japan). Embryos were evaluated according to the Manual of the International Embryo Transfer Society (Stringfellow and Seidel, 1998). Codes 1 (excellent or good), 2 (fair) and 3 (poor) were classified as transferable embryos, but code 4 (dead or degenerating) as non-transferable one.

### 5. Ultrasound Scanning

The ovaries of each cow were examined at embryo recovery by ultrasonography (Sonoace 600 with a 5.0 MHz linear array transducer; Medison Co., Ltd., Seoul, Korea). Examination involved counting the number of CL and large follicles ($\geq$ 10 mm in diameters).

### 6. Analyses of Serum Steroid Hormones and Metabolites

Blood samples were collected from the jugular vein just before embryo collection in donor cows. Ten ml of whole blood was centrifuged at 3,000 rpm for 10 min at 4℃ in a refrigerated centrifuge (Union 5KR, Hanil Co., Ltd, Seoul, Korea) to separate serum. The serum was stored at −20℃ until used. $P_4$ and estradiol ($E_2$) concentrations were read on Wallac 1420D Delfia fluorometer (Wallac, Turku, Finland) with use of $P_4$ and $E_2$ kits (Delfia Progesterone kit, Inc, USA). Blood urea nitrogen (BUN), cholesterol and glucose levels were analyzed on the automated Thermo Scientific Konelab 20XT analyzer (Thermo, Finland) using Thermo lab reagents.

### 7. Statistical Analyses

Statistical analyses were performed using SAS program (SAS, 1999). The proportion of donors flushed vs. treated were compared by chi-square analysis among groups. The mean number of CL, large follicles, transferable embryos, degenerate embryos, unfertilized ova, and total ova at embryo recovery among the control, T1 and T2 groups were analyzed by ANOVA. Mean concentrations of steroid hormones ($P_4$ and $E_2$), and serum metabolites (BUN, cholesterol and glucose) among groups were also analyzed by ANOVA.

## RESULTS AND DISCUSSION

The superovulatory responses according to the different feeding ratios of whole crop barley silage in Hanwoo donors were shown in Table 1. The flush rate of the donors following positive superovulation responses were 76.2, 96.0 and 91.3% for the control, T1 and T2 groups, respectively ($p$>0.05). The

Table 1. Superovulatory responses according to the different feeding ratios of whole crop barley silage in Hanwoo donors

| Treatments | No. donors treated | No. donors flushed (%) | No. CL at embryo recovery | No. large follicles at embryo recovery |
|---|---|---|---|---|
| Control | 21 | 16 (76.2) | 10.6 ± 1.0 | 1.9 ± 0.6 |
| T1 | 25 | 24 (96.0) | 11.8 ± 1.4 | 1.6 ± 0.5 |
| T2 | 23 | 21 (91.3) | 14.0 ± 2.0 | 2.2 ± 0.6 |

There were no significant differences among groups ($p>0.05$).

number of CL counted by ultrasonography at embryo recovery were 10.6 ± 1.0, 11.8 ± 1.4, and 14.0 ± 2.0 for the control, T1 and T2 groups, respectively ($p>0.05$). Un-ovulated large follicles (≥ 10 mm in diameters) also counted by ultrasonography at embryo recovery were 1.9 ± 0.6, 1.6 ± 0.5 and 2.2 ± 0.6 for the control, T1 and T2 groups, respectively ($p>0.05$).

Son *et al*. (2006) previously reported that the flush rate was 73.7～75.0% among Hanwoo donors superovulated during mid-cycle after estrus detection or in CIDR-treated at any stage of the estrous cycle. The same group demonstrated that the number of CL at embryo recovery was 15.9～16.7 following superovulation treatment in CIDR-treated Hanwoo donors (Son *et al*., 2007). The outcome of embryo recovery according to the different feeding ratios of whole crop barley silage in Hanwoo donors were shown in Table 2. The mean number of total ova (9.4 ± 1.2, 10.5 ± 1.5 and 12.0 ± 2.1) and transferable embryos (5.3 ± 0.9, 5.0 ± 1.0 and 6.5 ± 1.9) did not statistically differ among the control, T1 and T2 groups, respectively ($p>0.05$). Rhoads *et al*. (2006) reported that there were no differences on embryo quality among dairy donors with high plasma urea nitrogen (PUN) level (24.4 ± 1.0 mg/dl) or moderate PUN level (15.5 ± 0.7 mg/dl). However, they demonstrated that transfer of embryos collected from donors with high PUN level resulted in a lower pregnancy rate than the transfer of embryos collected from donors with moderate PUN level. Moreover, Sinclair *et al*. (2000) demonstrated that feeding of high plasma ammonia-generating diets in heifers increased ammonia level in follicular fluid. These results indicate that the ratios of different diets for donor cows affect the subsequent embryo survival or embryo transfer outcome.

Table 2. The outcome of embryo recovery according to the different feeding ratios of whole crop barley silage in Hanwoo donors

| Treatments | No. recovered | | | |
|---|---|---|---|---|
| | Transferable embryos | Degenerate embryos | Unfertilized ova | Total ova |
| Control | 5.3 ± 0.9 | 1.3 ± 0.6 | 2.9 ± 0.7 | 9.4 ± 1.2 |
| T1 | 5.0 ± 1.0 | 1.3 ± 0.6 | 4.1 ± 1.2 | 10.5 ± 1.5 |
| T2 | 6.5 ± 1.9 | 0.8 ± 0.3 | 4.4 ± 1.2 | 12.0 ± 2.1 |

There were no significant differences among groups ($p>0.05$).

In addition, Stroud and Hasler (2006) emphasized suitable husbandry and management practices on success of embryo production and transfer. In their study, the number of transferable embryos was more in a farm which has been in operation for 25 years maintaining pasture consisting primarily of native glasses (9.8) than in the other farm which has been in operation for only 3 years with much less experience (4.5). Son *et al*. (2007) reported that CIDR-treated Hanwoo donors kept on pasture produced 4 transferable embryos following superovulation with FSH. In addition, Son *et al*. (2009) demonstrated that the feeding of whole crop barley silage alone without supplement of other forage as a substitute for hay tend to decrease the number of transferable embryos after 1st superovulation (6.4 vs. 3.9) and 2nd superovulation treatments (4.2 vs. 1.2). In this study, however, the embryo recovery results following superovulation treatment of the Hanwoo donors fed two different feeding ratios of whole crop barley silage were comparable to that of Hanwoo donors fed imported hay.

Serum concentrations of $P_4$ and $E_2$ in donor cows were shown in Table 3. Concentrations of $P_4$ in the control, T1 and T2 was 43.3 ± 6.8, 64.2 ± 4.6 and 55.7 ± 6.6 ng/ml, respectively. The $P_4$ concentrations were significantly higher in the T1 and T2 groups than control group ($p<0.01$). Concentrations of $E_2$ in the control, T1 and T2 groups were 39.3 ± 3.1, 47.9 ± 5.3 and 40.9 ± 3.8 pg/ml respectively. It did not statistically differ

Table 3. Serum concentrations of $P_4$ and $E_2$ according to the different feeding ratios of whole crop barley silage in Hanwoo donors

| Treatments | $P_4$ concentration (ng/ml) | $E_2$ concentration (pg/ml) |
|---|---|---|
| Control | 43.3 ± 6.8[a] | 39.3 ± 3.1 |
| T1 | 64.2 ± 4.6[b] | 47.9 ± 5.3 |
| T2 | 55.7 ± 6.6[b] | 40.9 ± 3.8 |

[a,b] Values with different superscripts differ significantly ($p<0.01$).

among the control, T1 and T2 groups, respectively ($p$>0.05). Goto *et al.* (1987) reported that the number of transferable embryos was recovered highly when the blood concentration of $P_4$ was high level just before the superovulation using FSH hormone in donor cows. Furthermore, high concentration of $P_4$ at the time recovery of embryos was induced by plenty of CL, recovered embryos and transferable embryos. Bellows *et al.* (1991) also reported that the recovered number of normal and grade 1 embryos was increased when the blood concentration of $P_4$ was shown high level after superovulation in donor cows. Thus, the higher blood concentration of $P_4$ in donor cows could be related to the better responses of superovulation and more recovery of transferable embryos.

Serum metabolites of BUN, cholesterol and glucose according to the different feeding ratios of whole crop barley silage just before embryo recovery of Hanwoo donors were shown in Table 4. The BUN concentration (9.7 ± 0.7, 12.9 ± 3.0 and 15.7 ± 4.3 mg/dl) and glucose (66.2 ± 5.4, 68.0 ± 3.6 and 67.4 ± 5.2 mg/dl) did not differ among the control, T1 and T2 groups, respectively ($p$>0.05). But the cholesterol concentrations were significantly ($p$<0.05) lower in the control (105.8 ± 6.0 mg/dl) and T2 groups (96.9 ± 8.4 mg/dl) than T1 group (121.1 ± 6.8 mg/dl). Many researchers reported previously that donor cows having the higher blood concentrations of cholesterol produced more transferable embryos (Kweon *et al.,* 1987; Balakrishnan *et al.,* 1993; Leroy *et al.,* 2005). In the present study, however, the results related to the cholesterol concentration in Hanwoo donors did differ from the previous reports. Green *et al.* (2005) demonstrated that the blood concentration of glucose had no effect on the embryo survival and development, whereas glucose concentration collected in oviduct had an effect on the conception and development of embryo in dairy cattle. On the other hand, Chorfi *et al.* (2007) reported that the blood concentrations of glucose, cholersterol and BUN had no effect on the recovery of transferable embryos.

Table 4. Concentrations of serum metabolites according to the different feeding ratios of whole crop barley silage in Hanwoo donors

| Treatments | BUN (mg/dl) | Cholesterol (mg/dl) | Glucose (mg/dl) |
|---|---|---|---|
| Control | 9.7 ± 0.7 | 105.8 ± 6.0$^a$ | 66.2 ± 5.4 |
| T1 | 12.9 ± 3.0 | 121.1 ± 6.8$^b$ | 68.0 ± 3.6 |
| T2 | 15.7 ± 4.3 | 96.9 ± 8.4$^a$ | 67.4 ± 5.2 |

$^{a,b}$ Values with different superscripts differ significantly ($p$<0.05).

In conclusion, the results of this study represent that whole crop barley silage can be fed a good substitute for hay forage in Hanwoo donors. Furthermore, the ratios of whole crop barley silage 60% and rice straw 40% might be more worthful for embryo production.

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