

Propolis and Illite as Feed Additives on Performance and Blood Profiles of Post-Weaning Hanwoo Calves

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Abstract: A study was conducted with 48 Hanwoo post weaning calves from 91-120 days to know the effect of natural feed additives on growth, feed intake and blood profiles. The calves were arranged in four groups; control, antibiotic (Neomycine 110 ppm), propolis (0.05%) and illite (2%) having 12 calves (male female ratio 6:6) in each. The calves were supplied pellet feeds *ad libitum*. Multi nutrient block and timothy grass hay were supplied to the calves. Water was supplied *ad libitum* through the water tub. Significantly lowest ($p < 0.05$) initial BW was recorded at the beginning of post-weaning age in propolis group compared to others. The ADG at 5 months age were found statistically similar among the groups which indicates the effectiveness of the propolis. Higher feed intakes were recorded in control and propolis group followed by antibiotic and illite group. Illite and propolis group showed better compared to control diet in terms of ADG and feed efficiency. Addition of propolis and illite in the diet of post-weaning calves increased the percentage of platelets count ($p < 0.05$) keeping other blood component statistically unchanged. The serum albumin and globulin values also falls within the range of antibiotic group although lowest IgM was recorded in illite group. In conclusion, the natural resources, illite and propolis showed potential without any detrimental effect for production of Hanwoo post-weaning calves.

Key words: Neomycine, propolis, illite, growth, feed intake, blood profile, Hanwoo post weaning calves

INTRODUCTION

The Korean native cattle, Hanwoo is a pure breed maintained by the Korean national breeding system (Kee *et al.*, 2008). The phylogenetic tree based on mitochondrial DNA sequence homology shows that Hanwoo belongs to the humpless *Taurine* species, *Bos taurus* (Kikkawa *et al.*, 2003). Hanwoo meat is a popular brand in Korea. The loin eye muscle, *Longissimus dorsi* (LD) in particular has high economic value in the market. Therefore, efforts are on-going to improve its taste. Three major elements related to the eating quality of meat are tenderness, juiciness and flavor (Moloney *et al.*, 2001). According to the National Statistical Office (NSO) the number of Hanwoo reached 2.25 million head nationwide at the end of June, 2008 (Lee, 2008).

The overall production is increasing compared to the past years so the demand of feed supply has also been increased. Due to the less availability of cultivable land as mountain is about 70% in Korea, the dependency on imported feeds and forages are increasing. So the efficiency of feed utilization by feed additives is essentially needed. Imported meat took about 61% of

South Korean beef consumption in 2007 but this percentage is expected to fall to 59.4 in 2008 as domestic production ratio is expected to increase by 4.4% while imported meat is only expected to increase by 1.6% according to USDA projections (Herlihy, 2008). Many farms are using antibiotics as growth promoter for improving economic and effective animal production (Wierup, 2000). In Korea, many feed company and farms are using antibiotics as growth promoter and disease control for calves and young animals.

Antibiotic supplementation in animal feed results bacterial resistance in animal products and it's a major public health problem worldwide (Benko *et al.*, 2008). A ban of antibiotics as feed additives in animal nutrition was raised since 1986 in Sweden and since 1999 in Switzerland. The European Union banned the use of several antibiotics in animal feeds (Bouwman, 1998). With the restriction or ban of dietary antimicrobial agents, new ways must explore to improve and protect the health status of farm animals to guarantee animal performance and to increase nutrient availability. Rosen (1996) defined the pronutrients as microfeedingstuffs used orally in a relatively small amount to improve the intrinsic value of the nutrient mix

in an animal diet. Some research studies with propolis are focused on antibody synthesis (Toma *et al.*, 1981; Giurgea *et al.*, 1981; Konig, 1986; Hegazi *et al.*, 1995; Kong *et al.*, 2004) and has antibiotic as well as antimicrobial properties (Cushnie and Iamb, 2005; Yaghoubi *et al.*, 2007, 2008).

Illite is used in food supplement with claimed benefits that range from bowel function to reduction of heavy metals in the blood (Mitchell, 1993) and improves growth performance when used with green tea and licorice (mixed feed additives) in Hanwoo calves feed additives (Sarker *et al.*, 2010).

In this study there was a hypothesis to produce organic and natural animal products by using some feed additives like illite and propolis powder. These additives have potentiality to improve feed efficiency, growth, immune response and over all sound health of the animals. So this study was conducted on Korean beef calves to produce suitable feed formula using illite and propolis as feed additives at post-weaning age.

MATERIALS AND METHODS

Experimental animal: A total of forty eight beef calves of 3 months age were used in this experiment. The calves were arranged in four different feed additive groups maintaining number, age, body weight and sex ratio. The calves were fed with experimental diets for 2 months starting just after pre-weaning age (91 days) to 2 months (up to 150 days).

Arrangement of calves: The calves were reared separately from their mother in different group wise. Each group contains 12 calves and was identified by ear tags containing calves number and farm number: The right ear tag indicates calf number and its mother's number and the left ear tag refers farm number with bar code number.

Feeding management: The calves were supplied commercial pellet feed are shown in Table 1. The diets containing ingredients and nutrient contents were provided same as pre-weaning age. During this period, they were supplied pellet feed and imported timothy hay as roughage. Water was supplied *ad libitum* through the water channel.

Addition of feed additives in the calves diet: The antibiotic supplementation improves growth rate and feed efficiency by 2-16% as well as having disease control effects (Hayes, 1977; Zimmerman, 1986). Propolis powder (0.05%) was mixed with concentrate feed thoroughly and used in the respective groups calves. The illite powder (fine

Table 1: Feed ingredient used in the calf starter (basal concentrate diet) of Hanwoo calves during pre and post weaning age

Ingredients	(Percentage as-fed basis)
Com (ground)	22.37
Wheat bran	15.00
Wheat (ground)	12.00
Molasses	5.00
Palm meal	13.00
Coconut meal	11.50
Protein concentrate	9.90
Grape seed meal	3.50
Soybean meal	1.76
Rapeseed meal	1.00
Distillers dried grains	1.00
Salt	0.60
Limestone (1 mm)	1.87
Di Calcium Phosphate (DCP)	0.50
Calcium sulfate [†]	0.30
Mineral premix [‡]	0.35
Vitamin premix	0.35
TDN-Total Digestible Nutrient	66.00 (% of DM)

[†] Vit-min. premix provided per kg of diet: vit A, 6,000IU; vit D3, 800 IU; vit E, 20 IU; vit K₃, 2 mg; thiamin, 2 mg; riboflavin, 4 mg; vit B6, 2 mg; vit B12, 1 mg; pantothenic acid, 11 mg; niacin, 10 mg; biotin, 0.02 mg; [‡]Mineral premix provided per kg of diet: Cu, 21 mg; Fe, 100 mg; Zn, 60 mg; Mn, 90 mg; I, 1.0 mg; Co, 0.3 mg; Se, 0.3 mg

Table 2: Nutrient compositions in the calf's diet containing concentrate with feed additives and timothy hay (%)

Nutrient	Control	Antibiotic (110 ppm)	Propolis 0.05%	Illite 2%	Roughage (Timothy)
Moisture	12.29	12.19	12.29	12.05	12.00
Crude protein	14.62	14.77	14.63	14.33	2.62
Crude fat	2.57	2.44	2.57	2.52	1.64
Crude fiber	5.71	5.34	5.71	5.62	31.44
Crude ash	6.10	6.20	6.11	7.92	3.91
NFE [†]	58.71	59.07	58.70	57.55	48.39

[†]NFE, Nitrogen Free Extract

fraction <2 µm in size) was commercially available and used (2%) as additives. The antibiotic, neomycin (110 ppm) was added in the feed by the company during manufacturing process. In case of control group concentrate feed was used as basal diet to the calves. The chemical composition with (all treatment) or without (control) addition of feed additives are shown in Table 2.

Record keeping

Feed intake: Estimated from daily feed supplied and feed residues.

Growth rate: Measured at 4th month and 5th months of age of the calves body weight were checked and growth rate was calculated by deducting initial body weight, IBW (just after pre-weaning age).

Blood analysis: After 5 months age of the calves blood was collected from jugular vein from 4 calves in each feeding group and 10 mL of blood were collected for the analysis of blood namely Red Blood Cell (RBC); RBC, Hemoglobin (Hb), Red Cell Distribution Width (RCDW), Platelets: (PLT) of Blood, White Blood Cell (WBC), Leukocyte Yield (LY%), Monocyte (MONO%), Granulocyte (GRA%).

Albumin and globulin: Albumin, Globulin, A/G ratio from Serum, Immunoglobulin: IgG, IgA, IgM from Serum.

Statistical analysis: Data presented as mean±SE were analyzed by one way Analysis Of Variance (ANOVA) using the Compare Means procedure (SPSS 10.0 software for windows, SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Body weight, feed intake and feed efficiency: Table 3 shows that initial body weight of the calves among the feed additive groups were not statistically similar. The same group calves are considered because it was a planned experiment to know the effects of feed additives at pre weaning (3 months) and post weaning (2 months) experiment.

At the end of post-weaning age the feed additives (Propolis and Illite) group calves BWG and ADG were found higher compared to control and antibiotic group calves. Lower feed intake ($5.01 \text{ kg calf}^{-1}$) was recorded in illite fed group among the feed additives groups with higher ADG.

The pattern of roughages intake varied with total feed intake among the calves and concentrate intake was about 3-4 times higher than roughage. Roughage intake of the control and neomycin group calves were 1.14 and 1.15 kg day^{-1} , respectively and other two groups roughage intake were almost similar. During 3rd-5th month, each additional unit of forage intake tended to improve calf gains by 0.02 kg day^{-1} . It was observed that post weaning calves roughage intake increases rapidly possibly because of departing from their dam during this period. Moreover, during that time they had more

nutritional demand as the development of rumen were ongoing. Milk intake had a negative effect on forage intake which was in agreement with Wyatt *et al.* (1977). Davis *et al.* (1983), Marshall *et al.* (1976) and Bowden (1980) observed that calves compensated for lower dam milk production level by consuming more creep feed.

McDonald *et al.* (1995) stated that an important factor which essentially needed to consider the amount of feed is consumed by an animal in a certain period of time. The optimum amount of feed consumes each day; the greater will be the opportunity for increasing its daily production. An increase in production obtained by higher feed intakes is usually associated with an increase in overall efficiency of the production process, since maintenance costs are decreased proportionately as productivity rises. Control mechanisms for the feed intake of farm animals can be envisaged as operating at three levels. At the metabolic level, concentration of nutrients, metabolites or hormones may stimulate the nervous system to cause the animal to start or stop feeding. At the level of digestive system, the quantities of digesta may determine whether or not the animal ingests more feed. Finally, external influences such as climatic variables influence feed intake. In beef cattle production weight gain as well as daily weight gain is very important factor for economic point of view. According to the findings of feed efficiency it seems that addition of feed additives to calves diet showed higher feed efficiency like antibiotic compared to control group. It can be noted that both groups had natural feed additives propolis and illite those might be a reason for better result.

Red blood cells and its components and platelets: In Table 4, the highest value of RBC count was found in illite fed calves $12.68 (10^6 \text{ mm}^{-3})$ followed by control

Table 3: The effect of different feed additives on body weight of Korean native Hanwoo calves at post-weaning age depending on different feed additives

Treatments	Control	Antibiotic (110 ppm)	Propolis 0.05%	Illite 2%	Significance
Initial weight (kg calf ⁻¹)	78.50±3.57	87.58±3.90	72.17±3.06	78.00±3.47	*
Body weight (kg calf ⁻¹)	144.71±6.07	153.72±7.25	139.88±4.20	146.80±5.15	NS
BWG (kg calf ⁻¹)	66.21±3.00	66.13±3.95	67.71±2.90	68.80±2.93	NS
ADG (kg calf ⁻¹)	1.10±0.05	1.10±0.07	1.13±0.05	1.15±0.05	NS
ADFI (kg calf⁻¹)					
Concentrate	4.02	3.94	4.07	3.96	-
Roughage	1.14	1.15	1.09	1.05	-
Total	5.16	5.09	5.16	5.01	-

BWG-Body Weight Gain, ADG-Average Daily Gain, ADFI-Average Daily Feed Intake; *Values with different superscripts within columns are significantly different at $p < 0.05$

Table 4: The effect of different feed additives on haematological indices in Korean native Hanwoo calves at 3 months

Blood profiles	Control	Antibiotic (110 ppm)	Propolis 0.05%	Illite 2%	Significance
RBC (10^6 mm^{-3})	12.25±0.420	12.22±0.620	11.20±0.250	12.68±0.950	NS
HGB (g L ⁻¹)	132.50±1.660	123.25±1.030	116.50±4.290	126.50±10.41	NS
RDWs (%)	28.75±0.710	27.95±1.620	28.05±0.450	30.22±0.390	NS
PCT (%)	0.39±0.040 ^b	0.30±0.060 ^b	0.56±0.070 ^a	0.44±0.030 ^{ab}	*
WBC (10^3 mm^{-3})	11.46±1.560	10.85±0.430	11.89±1.940	12.75±1.050	NS
LYM (%)	38.42±5.940	35.32±10.05	43.28±2.790	37.20±5.680	NS
MON (%)	3.60±1.740	4.02±2.420	8.78±2.800	5.68±1.880	NS
GRA (%)	58.02±7.080	60.65±12.42	47.92±2.410	57.12±6.790	NS

Mean±SE, NS-Not Significant different ($p > 0.05$). Here, RBC-Erythrocytes count, HGB-Hemoglobin, RDWs-Red cell Distribution Width, PCT-Platelet percent, WBC-White Blood Cell count, LYM%-Lymphocyte percent, MON%-Monocyte percent and GRA%-Granulocyte percent

Table 5: The effect of different feed additives on blood proteins and immunoglobulins in Hanwoo calves at 3 months of age

Items	Control	Antibiotic (110 ppm)	Propolis 0.05%	Illite 2%
Albumin (g dL ⁻¹)	3.48±0.050 ^a	3.20±0.1200 ^b	3.32±0.0700 ^{ab}	3.26±0.0400 ^{ab}
Globulin (g dL ⁻¹)	2.92±0.130	3.09±0.0800	2.98±0.1400	3.26±0.4300
A/G ratio	1.20±0.04:1	1.04±0.06:1	1.12±0.05:1	1.04±0.12:1
IgG (mg dL ⁻¹)	777.61±38.30	677.72±124.83	613.03±173.49	932.37±21.900
IgA (mg dL ⁻¹)	73.40±12.65	109.67±61.060	38.53±7.7500	70.76±13.420
IgM (mg dL ⁻¹)	59.14±7.840 ^a	54.61±10.580 ^a	36.31±9.0900 ^{ab}	22.82±3.8200 ^b

Mean±SE, NS-Not Significant different (p>0.05), *Significant different -p<0.05, ^{a,b}Values with different superscripts within row are significantly different, p<0.05; Ig = Immunoglobulins (IgA, IgG and IgM)

12.25 (10⁶ mm⁻³), antibiotic 12.22 (10⁶ mm⁻³) and propolis 11.20 (10⁶ mm⁻³) but there were no significant differences observed in RBC among the groups. Red Blood Cells (RBC) are perhaps the most recognizable component of whole blood. RBC contains hemoglobin, a complex iron containing protein that carries oxygen throughout the body and gives blood its red colour. RBC produced in the bone marrow, they are continuously being formed and broken down. They live for approximately 120 days in the circulatory system and are eventually removed by the spleen. Hemoglobin concentration of the calves showed statistically similar with other groups. Anemia is defined by Benjamin (1978) as a decrease below normal in RBC, Hb and packed cell volume. There were no significant differences in red cell distribution width among the feed additive groups. Significantly highest (p<0.05) platelet percent was observed in propolis group (56%) compared to control (39%). Although illite fed calves possesses statistically similar value with control or with antibiotic but the value is higher (44%) than others. The primary function of platelets or thrombocytes is blood clotting. They are much smaller in size than other blood cells. They group together to form clumps or a plug in the hole of a vessel to stop bleeding.

White blood cells and its components: There were no significant differences observed in WBC count among the feed additives groups compared to control (Table 4). In this study, the amount of WBC in illite fed calves was noted 10.32 (10³ mm⁻³) which is more than the observation of Cho *et al.* (2000b). They found the amount of WBC in Hanwoo calves at same age was 8.64 (10³ mm⁻³) this difference might be due to the level of illite used differently. In current study 2% illite was used as feed additives but they used 5% illite in Hanwoo calves diet. The findings in blood lymphocytes and monocytes range of calves were similar with the findings of Cho *et al.* (2000a). Granulocytes percentage of calves in antibiotic showed higher value (60.85%) followed by propolis (60.40%), control (53.55%), illite (57.12%) and illite (51.35%), though there were no statistical difference among the groups observed.

Blood proteins and immunoglobulins: In Table 5, the albumin and globulin content of calves in blood serum among the feed additive groups were found similar with

antibiotic fed group though there is no statistically significant differences (p<0.05) were observed. Control group calves possessed higher albumin globulin ration (A:G ratio) than other calves. In case of immunoglobulins IgA and IgM concentrations of calves fed feed additives (propolis and illite) showed higher values compared to control diet fed calves. Almost similar IgG concentrations were recorded in propolis and antibiotic fed calves. The relationship between IgG concentration and serum protein is well documented (McEwan *et al.*, 1968; Nocek *et al.*, 1984; Perino *et al.*, 1993). Blood immunoglobulin concentration is related to the total serum protein concentration, the immune response and the colostrum absorption in the newborn calf (Terosky, 1995).

CONCLUSION

In this study, the natural resources, illite and propolis showed potential as feed additives without any detrimental effect for production of Hanwoo post-weaning calves.

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