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

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Abstract: A study was conducted with 48 Hanwoo calves from birth to 90 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 claves were supplied pellet feeds *ad libitum*. They were allowed to suck mother's milk and timothy grass hay were supplied after 1 month and multi nutrient block. Water was supplied *ad libitum* through the water tub. The significantly highest ($p<0.05$) BW and ADG were found in antibiotic fed calves than other groups. There were almost similar values observed in control and illite fed calves. Significant lowest ($p<0.05$) weight was observed in propolis fed calves. Highest feed intake was recorded in control group followed by antibiotic, illite and propolis group. Illite group seems to be suitable among the feed additives groups but the daily weight gain was statistically lowest ($p<0.05$). Blood profiles didn't show any effects after addition of additives except significant reduction of A/G ratio. So considering all the factors both the natural resources, lllite and Propolis didn't show potential for production of Hanwoo calf at pre-weaning

Key words: Neomycine, propolis, illite, additives, growth, feed intake, Hanwoo calves

INTRODUCTION

The Hanwoo beef cattle is considered one of the most important native livestock resources in Korea. The meat is considered by Koreans to be of exceptional quality and thus expansive than that of exotic species. The total number of Hanwoo reached 2.25 million head nationwide at the end of June, 2008 (Lee, 2008) raising by almost 178,721 farms. The farm size of this beef cattle varies from small (<20 cattle) to several hundreds of cattle in one farm. The overall production is increasing compared to the past years so the demand of feed supply is also increasing. 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. Propolis or bee glue is collected by bee which is rich in polyphenols, flavonoids, phenolic acids, cafeic acid and their related esters. Some studies 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). Illite is a non-expanding, clay-sized, mineral mixture. This natural clay contains Mg, Ca, K, Mn, Zn, P, Fe, Al, Si, Co, Se, Co, Mo as well as other minor elements. The name was derived from its type location in Illinois, USA in 1937. It is a layered silicate and structurally quite similar to muscovite or sericite with slightly more silicon, magnesium, iron and water and slightly less tetrahedral aluminium and interlayer potassium. According to Mitchell (1993), Illite is used in food supplement with claimed benefits that range from bowel function to reduction of heavy metals in the blood.

Now a days, many farms are using antibiotics as growth promoter for improving economic and effective animal production (Wierup, 2000). The antibiotic supplementation improve growth rate and feed efficiency by 2-16% as well as having disease control effects (Zimmerman, 1986). In fact the residual effect of these antibiotics goes to some extent in the final products (meat, milk and egg). These products are finally consumed by human and there might be a chance to arise various types of health hazards or diseases. So production of safe and hazard free animal products for human consumption hypothetically seems to be beneficial by using natural resources as feed additives which provides natural antibiotic properties. Based on the current global demand the lab is concerned to use some medicinal plants and natural resources as feed additives to find out suitable and profitable feed formula for the farm animals. However in this study, we have used two natural resources as feed additives namely illite and propolis which have the natural antibiotic properties. These additives may have

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potentiality to improve growth performance, feed efficiency and optimum feed intake of the animals. Considering the above circumstances, this study was conducted on Korean native claves to produce a suitable calf's feed formula using illite and propolis feed additives in comparison to neomycin.

MATERIALS AND METHODS

Animals and arrangement of calves: A total of 48 newborn Korean native beef calves (Hanwoo) were used in this experiment in a commercial farm. The calves were arranged in four different dietary feed additives groups maintaining similar number, age, body weight and sex ratio (6:6). The calves were fed experimental diets from calving to pre-weaning age (90 days). They were reared with their mother in the breeding pens. Each group contains 12 calves and was identified by ear tags containing calves number and farm number. Normally right ear containing tag indicates calves number and its mother's number. The left ear tag refers farm number with bar code number.

Feeding management: The calves were supplied commercially produced pellet feed in their pens. The diet containing ingredients and nutrient contents are shown in Table 1 and 2. During this period they were supplied pellet feed as concentrate and after 1 month they were also supplied imported roughage, timothy grass hay. Besides their concentrate and roughage feed they were allowed to suck milk from their mother from calving to weaning stage (3 months). So most part of their nutritional share come from this undetermined source during this pre-weaning period. For mineral and vitamin supplement the animals are supplied multi nutrient block. Water was supplied *ad libitum* through the water channel.

Addition of additives in diet: Propolis powder (0.05%) was mixed with concentrate feed thoroughly and used in the respective groups calves. The illite powder (fine 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.

Record keeping

Feed intake: Daily feed supplied and calculated by left over count.

Growth rate: Every month body weight checked and growth rate were calculated by deducting birth weight.

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

Ingredients	(Percentage as fed basis)
Corn, 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 kg^{-1} of diet: vit. A, 6,000 IU; vit. D_3, 800 IU; vit. E, 20 IU; vit. K_3, 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 kg^{-1} 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 (%)

Nutrients	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	3.91
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	2.62
NFE†	58.71	59.07	58.70	57.55	48.39

†NFE, Nitrogen Free Extract

Blood parameters: Red blood cell, hemoglobin, red cell distribution width, white blood cell, lymphocyte (%), monocyte (%), granulocyte (%), albumin, globulin and immunoglobulins (IgG, IgA and IgM).

Environmental management: The experiment was conducted during winter season so the environmental temperature and day length become reduced. For preventing cold weather shock in the experimental calves pen were provided electric bulb for heating. Moreover the outside of the shed area were covered with polyethelene paper.

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). Probability level, $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Table 3 shows that all the feed additives groups calves birth weight were similar which are statistically indifferent ($p>0.05$). Average body weight and daily weight gain (64.32 and 0.71 kg^{-1} calf, respectively) in

Table 3: The effect of different feed additives on body weight (kg) of Korean native calves at pre-weaning age

Treatments	Control	Antibiotic (110 ppm)	Propolis 0.05%	Illite 2%
Birth weight (kg h^{-1})	22.98±0.58	23.56±0.75	23.11±0.92	22.66±1.80
Body weight (kg h^{-1})	78.56±0.61^{b}	87.62±0.67^{a}	72.18±0.58^{c}	78.00±0.83^{b}
BWG (kg $head^{-1}$)†	55.56±0.31^{b}	64.32±0.25^{a}	49.18±0.25^{c}	55.00±0.32^{b}
ADG (kg $head^{-1}$)‡	0.62±0.06^{b}	0.71±0.07^{a}	0.61±0.06^{b}	0.63±0.05^{b}
ADFI (kg/head)§				
Concentrate	0.58	0.53	0.39	0.48
Roughage	0.20	0.21	0.16	0.17
Total	0.78	0.74	0.55	0.66
FE (Gain:Feed)¶	0.79:1	0.95:1	1.10:1	0.95:1

^{a,b,c}Values with different superscripts within rows are significantly different, $p<0.05$, †BWG = Body Weight Gain, ‡ADG = Average Daily Gain, §ADFI = Average Daily Feed Intake, ¶FE = Feed Efficiency

Table 4: The effect of different feed additives on haematological indices in Hanwoo calves at three months of age

Blood profiles	Control	Antibiotic	Propolis	Illite
Red blood cell (10^{6} mm^{-3})	14.92±1.640	13.30±0.78	13.52±1.25	14.77±0.39
Hemoglobin (g L^{-1})	133.00±15.75	121.25±5.58	119.25±8.81	120.50±3.52
Red cell distribution width (%)	28.40±0.360	28.05±1.30	27.77±0.57	28.40±1.53
Platelet (%)	0.59±0.030	0.57±0.15	0.62±0.15	0.86±0.22
White blood cell (10^{3} mm^{-3})	15.06±5.980	10.05±1.85	11.28±0.95	10.32±0.50
Lymphocyte (%)	41.62±9.830	32.37±4.85	34.50±1.80	43.82±7.30
Monocyte (%)	4.82±1.320	6.77±1.06	5.12±1.19	4.82±1.69
Granulocyte (%)	53.55±9.130	60.85±5.05	60.40±2.71	51.35±8.24

Mean±SE, values in the same rows are not significant different ($p>0.05$)

antibiotic fed calves were found significantly highest compared to other groups at 90 days of age. In beef cattle production weight gain as well as daily weight gain is very important factor in economic point of view. Feeding is a complex activity which includes such actions as the search for feed, recognition of feed and movement towards it, sensory appraisal of feed, the initiation of eating and ingestion. In the alimentary tract the feed is digested and the nutrients are then absorbed and metabolized. All these movements and processes can influence feed intake on a short term basis. In current study reveals that higher feed intake does not lead to higher growth always because control (antibiotic free) calves average daily feed intake was highest (0.78 g^{-1} calf) and ADG was 0.62 kg which was lower than antibiotic fed calves.

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. Among the feed additives group illite and propolis intake calves showed better feed efficiency than others' though their average daily weight gain were lowest rank among other groups (Table 3). The overall ADG of calves in current study was found 0.64 kg^{-1} calf which is almost similar with the findings of Kwon *et al.* (2007). They recorded ADG of Hanwoo calves was 0.66 kg at pre-weaning age. Cho *et al.* (2000a, b) also found ADG of Holstein calves 0.61 g^{-1} calf but they found ADG of Hanwoo calves lowest (0.45 g^{-1} calf) in another study. The highest body weight and body weight gain were found in antibiotic (neomycin) fed group calves than other groups. There were almost similar weight gains were observed in control and illite fed calves and lowest in propolis group calves. The value of ADG 0.63 kg) in Hanwoo calves is lower than other researchers.

Kang *et al.* (2001) used 2% illite and recoded ADG is 0.74 kg at 90 days of age. They opined that illite is a sort of clay minerals increased the growth rate and feed efficiency of early weaned calves for growing period. McDonald *et al.* (1995) stated that the amount of feed consumed by an animal in a certain period of time is an important factor. 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. This statement is not appropriate to all extent for example with some breeds of bacon pigs, excessive intake of feed lead to very fat carcass which are unacceptable to the consumer therefore economically undesirable. This result of better feed efficiency agrees with the findings of Denli *et al.* (2005), they used propolis in Quail diets and found better feed efficiency. In this study, propolis and illite fed calves less DM intake tended to lower body weight of calves. Similar opinion expressed by Ackgoz *et al.* (2005), they said propolis could not be recommended as a feed additive in broiler production. In the study, we found the daily concentrate (calf starter) intake in illite fed Hanwoo calves is 0.48 kg^{-1} calf at pre-weaning age.

Whereas Cho *et al.* (2000a, b) found the average daily intake of calf starter was 0.12 kg^{-1} calf in illite fed Hanwoo calves which are almost 4 times lower than the present study. They used 5% illite with basal diet as additives but we used 2% illite as additives with basal diet. Illite fed calves showed similar feed efficiency like antibiotic group (0.95:1) which was recorded higher than control but in case of Average Daily Gain (ADG) statistically ($p<0.05$) lower value was recorded in illite compared to antibiotic. Highest feed intake was recorded in antibiotic free group followed by antibiotic, illite and propolis group. The pattern of roughages intake slightly varied with total feed (Leukemia Society of America, 1994). There were no significant differences observed in lymphocyte count among the additives groups (Table 4). Monocytes defend

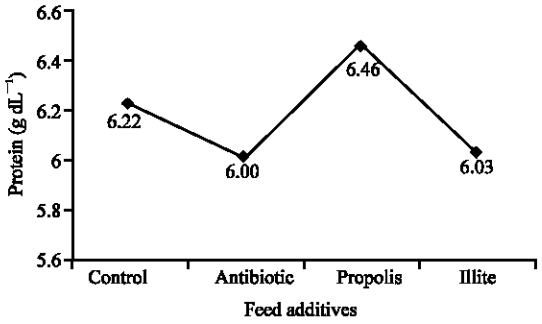

Fig. 1: Effect of feed additives on plasma protein of Hanwoo calves. Values are not significantly different at p>0.05

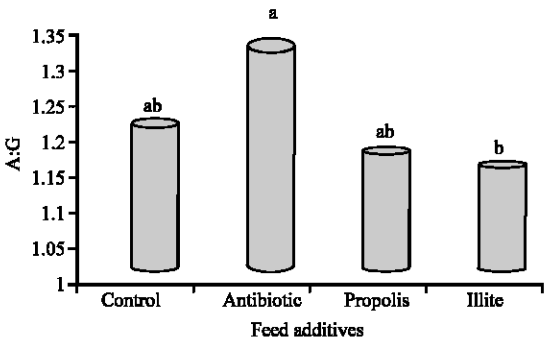

Fig. 2: Effect of feed additives on plasma albumin globulin ratio of Hanwoo calves. Data with same superscripts are not significantly different at p>0.05

the body against bacterial infection. The percent of monocytes were found highest in antibiotic group compared to other groups. Granulocytes percent was found in propolis fed calves 60.40 which is similar with antibiotic fed calves and higher than other additives groups though there were no significant difference (p>0.05) observed. They combat infection by rapidly increasing in number, engulfing and destroying foreign substances. They then die and in turn are ingested by monocytes. Once an infection is under control, production of neutrophils returns to the original preinfection count and steady state. Eosinophils and basophils also play infection fighting roles (Leukemia Society of America, 1994). In Fig. 1, plasma protein level is shown, where illite fed calves possessed almost similar value with antibiotic calves (6.00 and 6.03 g dL^{-1}) with a highest value in propolis fed calves (6.46 g dL^{-1}).

The blood plasma protein A/G ratio found significantly highest in antibiotic group compared to illite group (p>0.05), although there were no statistical difference observed with propolis and control groups (Fig. 2). The amount of immunoglobulins (IgG, IgA and Ig M) in propolis group calves found highest but intake.

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

Items	Control	Antibiotic	Propolis	Illite
Albumin (g dL^{-1})	3.39±0.07	3.41±0.04	3.45±0.14	3.20±0.07
Globulin (g dL^{-1})	2.83±0.09	2.59±0.13	3.01±0.26	2.83±0.08
A/G ratio (g dL^{-1})	1.20±0.04^{ab}	1.33±0.05^{a}	1.16±0.06^{ab}	1.14±0.05^{b}
IgG (mg dL^{-1})	770.77±142.86	684.53±37.89	691.58±162.54	623.27±139.78
IgA (mg dL^{-1})	76.46±14.28	124.47±50.51	191.14±89.93	121.29±56.58
IgM (mg dL^{-1})	26.50±4.58	32.81±7.27	44.40±6.08	32.52±4.73

Mean±SE, ^{a,b} Values with different superscripts within row are significantly different, p<0.05, Ig = Immunoglobulins (IgA, IgG and IgM)

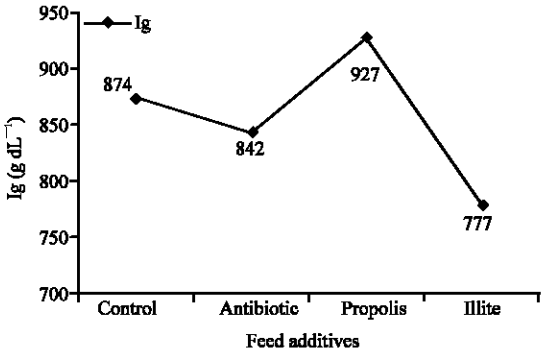

Fig. 3: Effect of feed additives on serum immunoglobulins (Ig) of Hanwoo calves. Values are not significantly different at p>0.05

Neomycin and control groups calves roughage intake were almost similar, same trend were found in between illite and propolis group. A shortage of red blood cells occurs anemia can cause weakness, dizziness, shortness of breath, headaches and irritability statistically insignificant (p>0.05) than other claves (Table 5). In Fig. 3, the pattern of total immunoglobulins in blood at three months of age seems to be highest in propolis group (927 mg dL^{-1}) compared to illite (777 mg dL^{-1}) and other two groups (842 and 874 mg dL^{-1} for neomycine and control respectively but the value were statistically similar (p>0.05).

The values of albumin and glubulin are almost similar with the values of other early stage ruminants such as buffalo calves (Kumar and Dass, 2006) in lamb (Harton, 1992) and in dairy calves (Belibasakis and Tsirgogianni, 1996) though the: G ratio was found higher in present study. The neonatal calf does not possess a well response immune system (Yaghoubi *et al*., 2008).

As a result the young calf may not effectively manage combating the external etressors during the early stages of growth (Franklin *et al*., 2003). During the first few days of birth, serum IgG would tipically reflect the efficacy of acquiring passive immunity by the adequate and timely consumption of colostrums (Quigley *et al*., 1995). Importantly, the IgG is the major component of humoral immunity comprising >70% of body's immunoglobulin pool. In the study agrees with this statement, the overall Ig in current study was calculated around 80%.

CONCLUSION

Considering all the factors the natural resources, ilite and propolis didn't show potential compared to antibiotic group for Korean native calf production during the pre-weaning age although propolis showed positive response to some extent. The reason might be minimum level of supplement in to the basal diet. Further studies are needed to conduct with different doses of the feed additives to draw a rigid conclusion.

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