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Effect of Different Feed Additives on Growth Performance and Blood Profiles of Korean Hanwoo Calves*

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ABSTRACT : This experiment was conducted on 60 Hanwoo calves comprising five feed additive groups, with 12 calves in each group, to determine the effects of additives at pre- and post-weaning on growth performance and blood profile. The groups were control, antibiotic (Neomycin 110 ppm), illite (2%), fermented green tea probiotics (FGTP, 0.5%), and mixed additives (FGTP 0.25%, illite 1% and licorice 0.1%). The calves were offered experimental pellet feeds *ad libitum* and after one month were supplied with imported timothy hay. They moved freely within the group and suckled their mother's milk during the pre-weaning stage (birth to 3 months) and were separated from their dam during the post-weaning stage (4-5 months). During the pre-weaning stage, the highest average daily gain (ADG) was recorded in the antibiotic- and mixed additive-fed groups followed by FGTP, control and illite groups. In the post-weaning stage, significantly higher total weight gain and ADG were recorded in both the FGTP and mixed additive groups compared to the other groups ($p<0.05$). Feed efficiency of mixed additive- and illite-fed calves were almost similar with antibiotic-fed calves compared to the other two groups, but the ADG was lowest in illite-fed calves during the pre-weaning stage. In contrast, post-weaning calves fed FGTP and mixed additives showed better feed efficiency. The values of hematological indices, differential leukocyte count, blood proteins and immunoglobulin among the additive-fed calves were not significantly different ($p>0.05$), although hemoglobin and hematocrit values were lower in FGTP compared to control, but similar in mixed additive and antibiotic groups. These results indicate no detrimental effects of feed additives on the blood profile of calves at both pre- and post-weaning age. Serum albumin in post-weaning calves of all feed additive groups were similar but significantly lower ($p<0.05$) than in the control group. Post-weaning, IgM was significantly lower ($p<0.05$) in illite-fed calves compared to other treatment groups, but there was no difference at pre-weaning. Considering all factors, the mixed feed additives and FGTP can be the replacement feed formula for antibiotic for Hanwoo beef calf production, especially when used post- weaning. (**Key Words :** Neomycin, Illite, Feed Additives, Growth Performance, Blood Profile, Hanwoo Calf)

INTRODUCTION

Most beef cows on an adequate plane of nutrition will produce enough milk during the first 90 days of lactation to satisfy the nutritional requirements of the growing calf. After this time, milk production may be insufficient to meet the nutrient requirement of the calf due to its rapid growth. The rapid growth must then be maintained with additional nutrients (Hamilton, 2002). The meat of the Hanwoo is considered by Koreans to be of exceptional quality and thus its price is several times higher than other beef from abroad. This factor has contributed to the need to maintain a stable population of the breed. The animals have a yellow-brown to tan color (Cho, 2006) and their body weight at 30 months of age is about 600 kg (Lee, 2008). 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). Overall production is increasing compared to past years, so the demand for feed supply has also been increased. Many farms are using antibiotics as growth promoters for improving economic and effective animal production (Wierup, 2000). Neomycin is approved for use in cattle and calves (Talley, 1991). It is generally believed to be absorbed very slightly when given orally. However, some data indicate that young calves may absorb neomycin to a greater extent (Gilbertson et al., 1991). Antibiotic supplementation in animal feed can result in bacterial resistance in human pathogens through consumption of animal products, and this is a major public health concern worldwide (Benko et al., 2008). A ban on antibiotics as feed additives in animal nutrition has been in

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place since 1986 in Sweden and since 1999 in Switzerland. The European Union has banned the use of several antibiotics in animal feeds (Bowman, 1998). With the restriction or ban of dietary antimicrobial agents, we must explore new ways to improve and protect the health status of farm animals, to guarantee animal performance and to increase nutrient availability. So to produce food which is safe for human consumption, it is highly desirable to use medicinal plants or herbs and natural resources having natural antibiotic properties. Some examples of medicinal plants are green tea, artemisia, acanthopanax, amongst others (Yang et al., 2003; Kwon et al., 2005). In addition to human consumption of green tea (*Camellia sinensis*), low grade product has been used as an ingredient of feed for broilers (Kaneko et al., 2001; Cao, 2005) and calves (Ishihara et al., 2001). Licorice (*Curcuma aromatica*), also known as gamcho, is the major constituent of curcuminoids, and is reported to be a natural antioxidant with inhibitory effects on cytotoxicity and cancer (Govindarajan, 1980; Soudamini and Kuttan, 1988). According to Michaell (1993), illite is used in food supplements, with the benefit of developing bowel function to reduce the levels of heavy metals in the blood. However, it is very susceptible to alteration by grinding (Yang et al., 2005). These products and herbs can be used for animal feed additives to produce safe and hazard-free animal products. Probiotics, with medicinal plants, are being suggested as effective alternatives to antibiotics (Fuller, 1989; Mohan et al., 1996). The interactions between nutrition and immunity are diverse and particularly important for animal well-being and efficiency of production. Estimates of nutrient requirements for the development, maintenance and activation of the immune system have been calculated from the weight of leukocytes and protective proteins as well as from their rates of nutrient use (Klasing, 1988; Klasing and Leshchinsky, 1999; Klasing and Calvert, 2000). Newborn calves have very low concentrations of blood immunoglobulins, but they have the ability to absorb IgG and other immunoglobulins from colostrum.

Currently, our laboratory has been conducting some research using feed additives like probiotic organisms, green tea, licorice (gamcho) root powder, and illite. These natural additives have pseudo-antibiotic properties to improve feed efficiency, growth performance, immune response, and sound health of animals. Considering the above circumstances, this study was conducted on Korean beef calves at both pre- and post-weaning age to produce a suitable feed formula for calves.

MATERIALS AND METHODS

Experimental animal

Sixty newborn Hanwoo calves were used for this experiment. They were arranged in five different feed additive groups of similar number, age, body weight and sex ratio. The calves were fed the experimental diets from calving to pre-weaning age (90 days) and then for 60 days post-weaning (91-150 days of age).

Arrangement of calves

The calves were reared in different groups with their mother in the breeding pens during the pre- weaning stage and then separated from their dam post-weaning. In both pre- and post-weaning stages, the calves were fed group-wise and there was no access of the dam during the pre-weaning stage to the calf pen when feeds were given. All calves were identified by ear tags containing calf number and farm number; the right ear tag indicated calf number and its mother's number and the left ear tag referred to the farm number (bar code number).

Feeding and health management

The calves were supplied with a commercially produced pellet feed in their pens. The ingredients and nutrient content of these pellets are shown in Table 1. The calves were supplied pellet feed as concentrate and, after one month, they were also supplied with imported timothy grass hay. Besides their concentrate and roughage feed, they were

Table 1. Feed ingredients and composition of basal diet for pre- and post-weaning Hanwoo calves

Ingredients	%, 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
Composition	% of DM
Calcium	0.80
Phosphorus	1.00
TDN- Total digestible nutrient	66.00

Vitamin. premix provided per kg 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 B_6, 2 mg; vit B_{12}, 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.

only allowed to suckle milk from calving to weaning age (90 days), so their nutrition was provided mostly from this undetermined source during the pre- weaning period. The animals were also supplied with a multi-nutrient block to provide necessary minerals and vitamins. Water was supplied *ad libitum* through the water channel. A combined vaccine, Elite 4-HS (Boehringer Ingelheim GmbH, Germany) was administered (5 ml per calf) intramuscularly to the post-weaning calves at 90 days as a first dose and at 105 days of age as a booster dose. This is a mixed type vaccine which protects against 5 different bovine diseases (IBR-Infectious bovine rhinotracheitis, BVD-Bovine viral diarrhea, PI3-Bovine respiratory parainfluenza-3, BRSV-Bovine respiratory syncytial virus, and a respiratory disease occurred by *Haemophilus somnus*). The experiment was conducted during winter, so the environmental temperature and day length was progressively reduced. Electric bulbs were provided to the calves' pen for heating and to prevent cold weather shock. Moreover, the outside of the shed area was covered with polyethylene paper.

Addition of feed additives in the calves' diet

The antibiotic, Neomycin (110 ppm) was added in the feed during the manufacturing process. Control calves were fed pellet feed without any antibiotic or other feed additives. Illite (2%), fermented green tea probiotics, FGTP (0.5%), and mixed feed additives (FGTP 0.25%, illite 1% and licorice 0.1%) were used for making feeds for the respective groups. The dietary feed additive levels were adjusted with the basal concentrate feed to ensure all diets were similar in their chemical composition, as determined by analysis using AOAC methods (Table 2). Fermented green tea probiotics used in this experiment contained four different types of microflora, their identity, population size and chemical composition were calculated by AOAC methods (1994) and are shown in Table 3. All the other feed additives were mixed thoroughly with antibiotic-free (control) feed just before feeding to calves.

Record keeping

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

Growth rate : measured every month, and growth rate was calculated by deducting birth weight at pre- weaning stage and 90 days weight was considered as initial body weight (IBW) for calculation of post- weaning growth rate.

Blood analysis : A veterinary hematology analyzer, ProCOUNT^{TM}, was used for analyzing the blood profile. The machine is made in Austria by DIATRON Messtechnik Ges.m.b.H. After 90 days, blood (10 ml) from the jugular vein of 4 calves in each feeding group and the following analyses were undertaken.

Red blood cell (RBC) : RBC, Hemoglobin (Hb), Hematocrit (HCT), Mean cell volume (MCV), Red cell distribution width (RCDW), Mean cell hemoglobin (MCH), Mean cell hemoglobin concentration (MCHC) and Red cell distribution width (RDWs). White Blood Cell (WBC): WBC, Lymphocyte (LYM %), Monocytes (MONO), Granulocytes (GRA), Platelets (PLT). Blood: Serum Albumin, Globulin and A/G ratio, Immunoglobulin: IgG IgA, IgM from Serum.

Table 3. Microflora population and chemical composition of fermented green tea probiotics used for pre- and post-weaning calves

Items	Contents
Number of microflora in FGTP [1]	
Lactobacillus acidophilus (KCTC 3111)	4.2×10^{7} cfu/g
Lactobacillus plantarum (KCTC 3104)	5.8×10^{6} cfu/g
Bacillus subtilis (KCTC 3239)	2.6×10^{7} cfu/g
Saccharomycetes cerevisiae (KCTC 7915)	6.2×10^{9} cfu/g
Chemical composition [2]	
Moisture (%)	12.40
Crude protein (%)	19.20
Crude fat (%)	2.92
Crude fiber (%)	11.08
Crude ash (%)	10.63

[1] FGTP=Fermented green tea probiotics.
[2] Calculated chemical composition.
KCTC = Korean collection for type culture.

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.

Table 2. Nutrient composition of basal diet with feed additives for pre- and post-weaning Hanwoo calves

Nutrient (%)	Control	Antibiotic	Illite	FGTP	Mixed additives	Timothy hay
Moisture	12.29	12.19	12.05	12.26	12.06	12.00
Crude ash	6.10	6.20	7.92	6.16	7.63	3.91
Crude fat	2.57	2.44	2.52	2.58	2.54	1.64
Crude fiber	5.71	5.34	5.62	5.74	5.74	31.44
Crude protein	14.62	14.77	14.33	14.64	14.37	2.62
NFE	58.71	59.07	57.55	58.63	58.66	48.39

FGTP = Fermented green tea probiotics, NFE = Nitrogen free extract.

Table 4. Effect of different feed additives on weight gain, feed intake and feed efficiency of Hanwoo calves at pre- and post-weaning age

Treatment	Control	Antibiotic	Illite	FGTP	Mixed additives
Pre-weaning calves					
ABW (kg/calf)	23.00±0.71	23.33±0.49	23.00±1.13	23.70±1.01	23.20±1.06
FBW (kg/calf)	78.54±3.91	87.58±3.90	78.00±4.21	83.80±4.72	86.90±4.06
WG (kg/calf)	55.54±4.08	64.25±3.87	55.00±3.74	60.10±5.08	63.70±4.63
ADG (kg/calf)	0.62±0.04	0.71±0.04	0.61±0.04	0.67±0.05	0.71±0.05
ADFI(kg/calf)	0.78	0.73	0.65	0.81	0.76
FE (gain/feed)	0.79:1	0.97:1	0.94:1	0.83:1	0.93:1
Post-weaning calves					
IBW (kg/calf)	78.54±3.91	87.58±3.90	78.00±4.21	83.80±4.72	86.90±4.06
FBW (kg/calf)	148.27±6.80^{b}	160.33±7.61^{ab}	149.10±6.32^{b}	169.80±7.23^{ab}	172.00±7.17^{a}
WG (kg/calf)	69.72±3.50^{b}	72.75±4.34^{b}	71.10±3.66^{b}	86.00±3.62^{a}	85.10±4.74^{a}
ADG (kg/calf)	1.16±0.05^{b}	1.21±0.07^{b}	1.18±0.06^{b}	1.43±0.06^{a}	1.42±0.07^{a}
ADFI(kg/calf)	5.15	5.09	5.01	4.60	4.25
FE (gain/feed)	0.23:1	0.24:1	0.24:1	0.31:1	0.33:1

FGTP = Fermented green tea probiotics.
ABW = Average birth weight, FBW = Final body weight, WG = Weight gain, ADG = Average daily gain, ADFI = Average daily feed intake, FE = Feed efficiency, IBW = Initial body weight.
Mean values (±standard error) in the same row with different superscripts are significantly different ($p<0.05$).

RESULTS AND DISCUSSION

Body weight, feed intake and feed efficiency

Body weights are commonly used for monitoring nutritional status and growth of animals (Chimonyo et al., 2000; Ndlovu et al., 2007). As shown in Table 4, calves in all the feed additive groups had similar birth weights. At the end of the pre-weaning stage (90 days), there was no difference ($p>0.05$) in body weight, total weight gain or average daily live weight gain (ADG) between the different treatment groups. However, by the end of the post-weaning stage (day 150), calves fed mixed feed additives and FGTP showed significantly higher body weight gain and ADG compared to the other treatments ($p<0.05$). In beef cattle production, total weight gains as well as daily weight gain are very important factors from an economic point of view.

The patterns of group average daily feed intake (ADFI) of pre- and post-weaning calves are shown in Table 4. The trend of ADFI seems to be higher in the FGTP group (0.81 kg/calf) compared to control (0.78 kg/calf), mixed (0.76 kg/calf) and the other two groups during the pre-weaning stage, although ADG did not show a higher value but was statistically similar to other groups ($p>0.05$). During the post-weaning stage, a higher ADG was recorded in FGTP and mixed additive groups compared to the control, antibiotic and illite groups ($p<0.05$). The pattern of roughage intake varied with total feed intake among the calves and concentrate intake was about 3 to 4 times higher than roughage intake during both the pre- and post-weaning periods (Figure 1 and 2). During the post- weaning stage of this study, each additional unit of forage intake tended to improve calf gain by 0.02 kg/d. It was observed that in post-

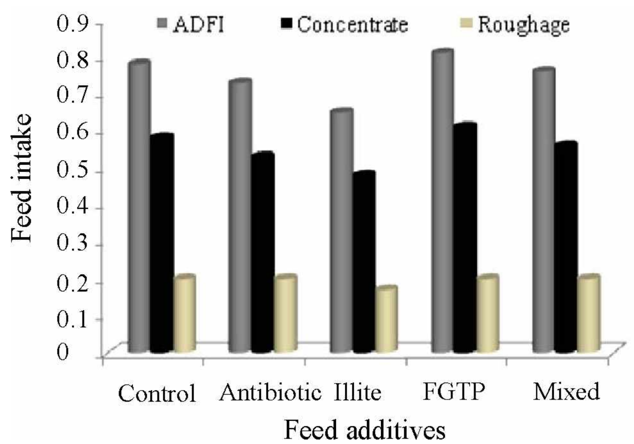

Figure 1. Feed intake (kg/calf/d) pattern of calves in different feed additives at pre weaning age.

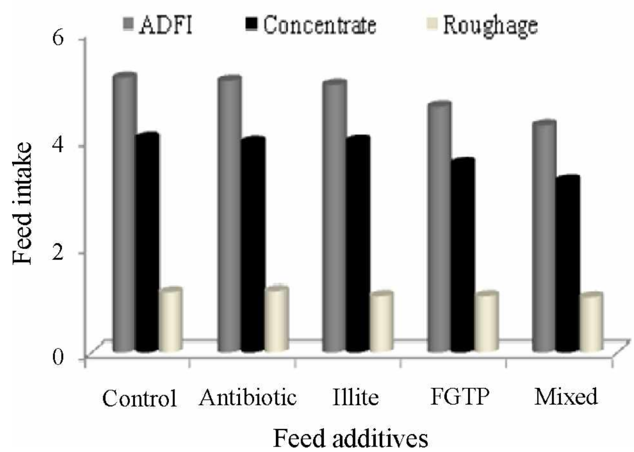

Figure 2. Feed intake (kg/calf/d) pattern of calves in different feed additives at post weaning age.

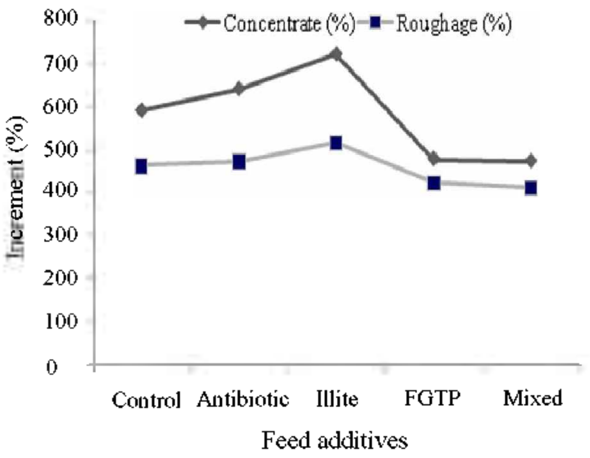

Figure 3. Feed intake increment in post weaning age compared to pre weaning among different groups.

weaning calves increment (%) of concentrate and roughage intake occurred sharply in all groups compared with pre-weaning calves and the highest increment (%) was found in illite fed calves compared to other groups (Figure 3). The reason for increasing feed intake might be that post-weaning calves were raised excluding their dam and, due to ongoing development of the rumen, nutritional demand also goes up. McDonald et al. (1995) stated that an important factor which was essential to consider was the amount of feed consumed by an animal in a certain period of time. The optimum amount of feed consumed each day, the greater will be the opportunity for increasing daily production. An increase in production obtained by higher feed intake 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 always appropriate. According to the findings of feed efficiency, it seems that mixed additive and illite groups were similar to the antibiotic-fed group compared to the other two groups, but daily weight gain was lowest in the illite group at pre-weaning age. Post-weaning calves fed green tea and probiotics (FGTP and mixed) showed better feed efficiency than other groups. The positive trend of green tea in pre-weaning calves supports other research (Lee, 2005) and even other studies in broilers (Yang et al., 2003). The improved feed efficiency of calves fed probiotics in our study also agrees with other researchers (Schwab et al., 1980; Christen et al., 1995). They concluded that when calves were fed a *Lactobacillus* fermentation product, there was a trend towards improved starter intake. Another report (Bechman et al., 1977) also noted improved feed efficiency when *L. acidophilus* was included in the diet of young dairy calves. Our current study suggests that efficiency of feed containing probiotic bacteria was higher than for other additives and enhanced ADG in calves during the post-weaning period compared to other groups.

Red blood cells (RBC) and its components and platelets

The haematological indices among the different feed additive groups in the pre- and post-weaning stages are shown in Table 5. No significant differences were observed among the parameters except for haemoglobin, hematocrit and platelet percentage ($p<0.05$). RBC contain hemoglobin, an iron-rich protein which picks up oxygen as the blood passes through the lungs, and transports and releases it to organs and tissues throughout the body. RBC are produced in the bone marrow, and are continuously being formed and broken down. They live for approximately 120 days in the circulatory system and are eventually removed by the spleen. A shortage of these red cells, a condition known as anemia, can cause weakness, dizziness, shortness of breath, headaches and irritability (LSA, 1994). Mixed feed additive- and illite-fed calves showed statistically similar hemoglobin and hematocrit concentrations to antibiotic-fed calves, with a lower value in FGTP groups. These haematological values among different feed additive groups did not affect blood profiles of the calves at both pre- and post-weaning age. The percentage of blood volume composed of red blood cells (the "hematocrit") was similar among the feed additive groups compared to green tea probiotic group. Anemia is defined by Benjamin (1978) as a decrease below normal in RBC, Hb, and PCV. There were no significant differences in MCV, MCH, MCHC, RDWs and MPV among the feed additive groups. Significantly highest ($p<0.05$) platelet percentage was observed in FGTP compared to other groups, and the mixed additive group possessed a higher value in comparison to the other three groups. Platelets, or thrombocytes, are tiny disc-shaped cells which help prevent abnormal or excessive bleeding by forming clots. A deficiency of platelets can cause bleeding of the mucous membranes or other tissues, such as the skin. 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

White blood cells (WBC) play a major role in defending the body against disease-producing bacteria, viruses and fungi. There are three main types, lymphocyte, monocyte and granulocyte, each of which perform a specific function. T and B cells of lymphocytes are very important in regulating the immune response. T cells attack virus-infected and malignant cells, whereas B cells produce and release antibodies, or protein substances, which bind to infectious agents and help in preventing them from damaging the body. A deficiency in any type of normal white blood cell may result in an increased susceptibility to infections. There were no significant differences observed in WBC count among the additive groups (Table 6).

Table 5. Effect of different feed additives on haematological indices of Hanwoo calves at pre- and post- weaning age

Blood profiles	Control	Antibiotic	Illite	FGTP	Mixed additives
Pre-weaning calves					
RBC (10^6/mm^3)	14.92±1.64	13.30±0.78	14.77±0.39	13.22±0.49	13.92±0.50
HGB (g/L)	133.00±15.75^a	121.25±5.58^{ab}	120.50±3.52^{ab}	112.00±3.31^b	122.00±2.34^{ab}
HCT (%)	40.90±4.69^a	35.05±1.54^{ab}	36.20±1.19^{ab}	32.92±0.99^b	37.27±0.91^{ab}
MCV (fl)	27.50±1.25	26.50±1.25	24.25±0.62	24.75±0.47	27.00±1.00
MCH (pg)	8.90±0.52	9.07±0.41	8.12±0.12	8.45±0.31	8.75±0.34
MCHC (g/L)	324.50±3.79	345.00±2.44	332.75±7.54	339.25±3.66	327.00±5.58
RDWs (%)	28.40±0.36	28.05±1.30	28.40±1.53	27.05±0.88	29.17±0.67
PLT (%)	0.59±0.03^b	0.57±0.15^b	0.86±0.22^a	0.74±0.03^{ab}	0.74±0.07^{ab}
MPV (fl)	5.13±0.04	5.15±0.31	5.10±0.23	5.22±0.11	5.02±0.04
Post-weaning calves					
RBC (10^6/mm^3)	12.25±0.41	12.22±0.62	12.67±0.94	11.15±0.51	12.05±0.74
HGB (g/L)	132.50±1.65^a	123.25±1.03^{ab}	126.50±10.41^{ab}	109.00±3.80^b	123.25±6.25^{ab}
HCT (%)	37.65±1.06^a	34.97±1.28^{ab}	35.87±2.12^{ab}	31.75±1.25^b	35.40±1.31^{ab}
MCV (fl)	30.50±1.19	28.50±1.25	28.50±0.50	28.50±1.32	29.75±1.93
MCH (pg)	10.82±0.50	10.10±0.51	9.92±0.30	9.80±0.65	10.27±0.59
MCHC (g/L)	351.75±11.12	353.25±9.93	351.00±12.34	343.00±10.20	347.25±7.82
RDWs (%)	28.75±0.70	27.95±1.61	30.22±0.39	27.52±0.80	29.15±0.59
PLT (%)	0.39±0.04^{ab}	0.29±0.05^b	0.44±0.02^{ab}	0.59±0.18^a	0.51±0.03^{ab}
MPV (fl)	5.25±0.06	5.27±0.42	5.30±0.07	5.35±0.20	5.30±0.13

FGTP = Fermented green tea probiotics, RBC = Erythrocytes count, HGB = Hemoglobin, HCT = Hematocrit, MCV = Mean cell volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, RDWs = Red cell distribution width, PCT = Platelet percent, PDWs = Platelet distribution width and MPV= Mean platelet volume.

$^{a, b}$ Means (±standard error) in the same row with different superscripts are significantly different ($p<0.05$).

Monocytes defend the body against bacterial infection. The percentage of monocytes was highest in antibiotic and FGTP groups compared to other groups in the post-weaning stage. Granulocyte percentage was 58.25 in mixed additive-fed calves, which was similar to antibiotic-fed calves and higher than other additive groups, although no statistically significant difference ($p>0.05$) was found among the groups (Table 6). Illite-fed calves possessed higher WBC count than other feed additive groups which agreed with the findings of Cho et al. (2000), though they observed a higher value in Holstein calves. They found that WBC in Hanwoo calves at the same age was 8.64 (10^3/mm^3); this difference might be due to the different level of illite used. In the current study 2% illite was used as a feed additive but Cho

Table 6. Effect of different feed additives on WBC and differential leukocyte counts in Hanwoo calves at pre- and post-weaning age

Blood parameters	Control	Antibiotic	Illite	FGTP	Mixed additives
Pre-weaning calves					
WBC (10^3/mm^3)	15.06±5.98	10.05±1.85	10.32±0.50	8.76±1.38	9.40±0.63
LYM (10^3/mm^3)	4.56±0.23	3.28±0.75	4.53±0.79	3.70±0.22	3.58±0.77
LYM (%)	41.62±9.83	32.37±4.85	43.82±7.30	44.37±5.21	37.85±3.78
MONO (10^3/mm^3)	0.81±0.43	0.65±0.12	0.49±0.16	0.50±0.07	0.35±0.15
MONO (%)	4.82±1.32	6.77±1.06	4.82±1.69	6.32±1.33	3.92±1.83
GRA (10^3/mm^3)	9.70±5.66	6.16±1.41	5.34±0.91	4.55±1.25	5.49±0.82
GRA (%)	53.55±9.13	60.85±5.05	51.35±8.24	49.32±6.14	58.25±8.31
Post-weaning calves					
WBC (10^3/mm^3)	11.46±1.56	10.85±0.43	12.75±1.04	9.93±1.01	11.34±1.19
LYM (10^3/mm^3)	4.49±1.09	3.72±0.89	4.77±0.92	2.67±0.29	4.42±0.63
LYM (%)	38.42±5.93	35.32±10.05	37.20±5.68	27.45±3.90	39.35±4.92
MONO (10^3/mm^3)	0.41±0.19	0.40±0.22	0.67±0.19	0.69±0.33	0.83±0.23
MONO (%)	3.60±1.74	4.02±2.41	5.67±1.87	6.27±2.40	7.65±2.15
GRA (10^3/mm^3)	6.51±0.87	6.76±1.54	7.35±1.21	6.58±0.75	6.13±0.97
GRA (%)	58.02±7.08	60.65±12.41	57.12±6.79	66.27±4.23	52.92±5.14

Values are mean±standard error.

FGTP = Fermented green tea probiotics, WBC = White blood cell count, LYM = Lymphocyte count, LYM (%) = Lymphocyte percent, MONO = Monocytes count, MONO (%) = Monocyte percent, GRA = Granulocytes count and GRA (%) = Granulocyte percent.

et al. (2000) used 5% illite. Our findings on the range of monocytes in calves were similar to those of Cho et al. (2000). Granulocyte percentage of post-weaning calves in the FGTP group showed the highest value (66.27%) followed by antibiotic (60.65%), control (58.02%), illite (57.12%) and mixed additive groups (52.92%), though there were no statistical differences among the groups. The feed additive effect on WBC and its components indirectly prove that there was no negative effect on blood of pre- and post-weaning calves, similar to the control or antibiotic group.

Blood proteins and immunoglobulins

Serum albumin is a very sensitive and early nutritional indicator of protein status (Agenas et al., 2006) because its turnover is only 16 days. Deficiency of protein impairs both humoral and cell mediated immunity, thus predisposing an animal to diseases (Titgemeyer and Loest, 2001). In Table 7, blood proteins, namely albumin and globulin, in the plasma of the Hanwoo calves were analyzed at the end of the experiment. No statistically significant differences were observed among the feed additive groups at pre-weaning age, but in the post-weaning stage all the feed additive group calves showed statistically similar levels and varied from control calves ($p<0.05$). Antibiotic group calves possessed a similar albumin value (3.41 g/dl) to mixed feed additive calves at pre-weaning age. Mixed additives contained medicinal plant, green tea with probiotic bacteria, natural clay mineral illite and licorice root powder. The values of albumin and globulin are similar to those of other early stage ruminants such as buffalo calves (Kumar and Dass, 2006), lambs (Harton, 1992) and dairy calves (Belibasakis and Tsirgogianni, 1996) though the A:G ratio was higher in the present study. It is well recognized that immunoglobulins are absorbed from the intestine for only a short period after birth and the efficiency of absorption is dependent on ensuring that the calf receives adequate colostrum in the immediate post-partum period (Earley and Fallon, 1998). Immunoglobulins are proteins that bind to and help in eliminating foreign agents from the body such as bacteria and viruses. In the present study (Table 7), IgG and IgA values showed no significant difference among the feed additives at both pre- and post-weaning age, though the highest IgA value was recorded on mixed additives (176.14 mg/ml) followed by 124.47, 121.29, 87.70 and 76.46 mg/dl in antibiotic, illite, fermented green tea probiotic and control groups, respectively. IgM concentrations of pre-weaning calves fed fermented green tea probiotics (FGTP and mixed) showed higher values followed by antibiotic and other treatments. In the post-weaning stage, serum IgM concentration in illite-fed calves was significantly lower than in other groups ($p<0.05$). 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 colostrum absorption in the newborn calf (Terosky, 1995).

In conclusion, based on the findings among the feed additives investigated, mixed feed additive and fermented green tea probiotic (FGTP) may be suitable alternatives to antibiotic for Hanwoo beef calves, especially post-weaning. Further research is required to establish a more rigid recommendation.

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Table 7. Effect of different feed additives on blood proteins and immunoglobulins in Hanwoo calves at pre- and post-weaning age

Blood proteins	Control	Antibiotic	Illite	FGTP	Mixed additives
Pre-weaning calves					
Albumin (g/dl)	3.39±0.07	3.41±0.04	3.20±0.07	3.33±0.11	3.41±0.02
Globulin (g/dl)	2.83±0.09	2.59±0.13	2.83±0.08	2.87±0.13	2.73±0.09
A/G ratio	1.20±0.04	1.33±0.05	1.14±0.05	1.17±0.08	1.26±0.05
IgG (mg/dl)	770.77±143	684.53±38	623.27±140	899.60±65	552.41±147
IgA (mg/dl)	76.46±14	124.47±50	121.29±57	87.70±10	176.14±55
IgM (mg/dl)	26.50±4.58	32.81±7.00	32.52±4.73	49.19±13	46.21±5.15
Post-weaning calves					
Albumin (g/dl)	3.48±0.05^a	3.20±0.12^b	3.25±0.04^b	3.19±0.02^b	3.28±0.01^b
Globulin (g/dl)	2.92±0.12	3.08±0.08	3.26±0.43	3.04±0.14	3.03±0.10
A/G ratio	1.19±0.03	1.04±0.06	1.04±0.12	1.05±0.05	1.09±0.04
IgG (mg/dl)	777.60±38	677.72±125	932.36±22	757.94±130	727.74±158
IgA (mg/dl)	73.40±12.65	109.67±61.06	70.76±13.41	178.94±74.47	211.95±66.36
IgM (mg/dl)	59.14±7.83^a	54.61±10.57^a	22.82±3.82^b	53.93±5.07^a	46.62±11.59^a

FGTP = Fermented green tea probiotics, IgG = Immunoglobulin G, IgA = Immunoglobulin A, IgM = Immunoglobulin M.

Mean (±standard error) values in the same row with different superscripts are significantly different $p<0.05$).

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