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Effects of Diets Supplemented with Green Tea By-Product on Growth Performance and Hematological Parameters in Calves

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Abstract: The study aimed to explore the effect of Green Tea by-product (GTB) on body weight and hematological parameters in calves of the Sylhet region in Bangladesh. Four replicate groups were attributed to each treatment with three calves per replicate. Each group numbered as T_1, T_2, T_3 and T_0. All groups were served with standard calf feed and fresh drinking water *ad libitum*. The control group T_0 was fed with normal calves feed. Calves of group T_1, T_2 and T_3 were maintained as treated group where group T_1 was fed with 0.5% GTB with normal calves feed, group T_2 was treated with 1% GTB and group T_3 was treated with 2% of GTB, respectively. The body weight of calves was taken on day 0 of the experiment and again on day 30 and day 60 to compare with the initial body weight. Blood samples were collected at day 30 and day 60 of treatment for hematological and biochemical experiments. Body weight was significantly ($p<0.05$) increased in treated groups in comparison to the control group. GTB improved Hb concentration and Total Protein (TP) in treatment group T_3 which was significantly ($p<0.05$) higher than that of control group T_0. Effects of green tea by-product on RBC, WBC and glucose concentration results insignificant ($p<0.05$) enhance in the treated groups compared to the control group. Cholesterol, albumin and BUN

concentration were decreased significantly ($p<0.05$) in treated groups (T_1, T_2 and T_3). Our experimental data suggested that GTB has a momentous effect on body weight gain and physiological characteristics.

INTRODUCTION

The livestock industry has a significant contribution to the national economy of Bangladesh. As a subordinate occupation, many farmers rear calf to supplement their livelihood. It can play a vital role in improving farmers living status by increasing their income and can contribute substantially to the national economy. But the availability of calf feed is a major drawback in this sector.

In Eastern Asia, Green tea is a famous beverage that is prepared from the leaves of the Tea plant (*Camellia sinensis*)[1]. To meet the demand for this popular drink numerous beverage companies prepare ready-made tea drinks, for which an abundant amount of by-product is produced from the tea leaf. This green tea by-product contains almost 20-30% Crude Protein (CP)[2,3] which can be an important protein source for the animal if it is used as animal feed. Green tea holds catechin which is known for its antioxidant properties[1, 4–6]. Epigallocatechin-3-gallate is the principal catechin subsist in green tea which is regarded as accountable for many health benefits attributed to green tea[7–10].

Green tea has several other components such as tannins, amino acids, caffeine and vitamins [11] which helps to increase feed efficiency and enhance body weight in animals[12–15]. Saponins, another salutary component of green tea which helps to reduce the level of serum cholesterol[16, 17], act as a natural pesticide[18] and have a suppressive effect against protozoa[19, 20].

Malnutrition and anemia is a very common problem for farm animals in our country characterized by weakness, weight loss and poor health condition of animals and thus, causes loss of production. Supplementation of green tea by-product, especially during early lactation will improve lactation performance which will improve the livelihood of the farmer family and children will get better nutrition. Tea is cultivated, especially in hilly zones in the eastern part of Bangladesh mainly in 4 districts (Sylhet, Moulvibazer, Habigong and Chittagong) because of its significant demand in the internal market. Green tea by-products such as factory tea waste, decaffeinated tea waste and tea plant by-products are available in Bangladesh but there are no more works about it. Therefore, the current study was carried out to determine the effects of GTB supplemented with diets on growth performance and hematological parameters in calves.

MATERIALS AND METHODS

Study area: This study was performed in calves of the Sylhet district which is a North-East part of Bangladesh and situated between 24°32'North latitude and 91°52'East longitudes. In Sylhet district, average maximum temperature is 23°C and the minimum is 7°C, the yearly average rainfall is 3.334 millimeters and seventy percent humidity. All the tests for this study were performed in the Laboratory of Medicine, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh.

Animals and dietary treatments: Information concerning health history, identification, age, sex and breed of calves were recorded. Uniform basal diets were provided for all calves (Table 1). The number of calves and the housing system was also recorded to obtain more information about the predisposing factors involved in worm infestations. Three replicate groups were allocated to each treatment with three calves per replicate. The calves (36) were alienated into four groups: one control group (Group T_0, n = 3) and three trial groups, Group T_1 (n = 3), Group T_2 (n = 3), Group T_3 (n = 3). The initial body weight of the three trial groups and one control group is 41.75 kg. GTB was collected from different tea processing companies in the Sylhet region and dried under sunlight for three days. Then the GTB was mix with the normal diet of the calves. This was used as the crude leaf extract for this study. This tea by-product was applied in three trial groups daily. Group T_1 was treated with 0.5%, group T_2 was treated with 1% and group T_3 was treated with 2% green tea by-product. Group T_0 named as the control group was not given any tea by-product supplement. This procedure continued for 60 days from the beginning of the experiment.

Body weight measurement: Calves were given a period of 2 weeks to accustom to the pen environment and routine feeding before the initiation of the actual feeding. After the beginning of the feeding trial, experimental diets were given two times a day at 09:00 and 16:00 (3% of body weight). The temperature of the room was sustained at 24°C and lighting and other management were carried out under general practices. All the animals were handled following the guidelines for the care and use of animals in the research of the ethical committee of Sylhet Agricultural University, Bangladesh. The body weights of the animals were measured with the space of 30 days. Hematological Parameters: Blood samples (5 microliters) were taken from the jugular vein of the calves of treated and the control group in a vial containing Ethylene Diamine Tetra-Acetic acid (EDTA) as the anticoagulant (Cat. No. 366643; Becton Dickinson, Franklin Lakes, NJ, USA) at day 0 of treatment period to determine the effects of tea by-product on the following hematological parameters; Hemoglobin (Hb) content, Different Leukocyte Count (DLC), Total Erythrocyte Count (TEC),

Table 1: Ingredients and chemical composition of calve's starter diet on Dry Matter (DM) basis

Ingredient composition (% of DM)	Diets
Alfalfa hay	8.00
Corn grain, ground	43.90
Barley grain, ground	9.20
Soybean meal	29.40
Extruded soybean	4.70
Fat supplement[1]	0.50
Calcium carbonate	1.70
Sodium bicarbonate	1.10
Mono-calcium phosphate	0.50
Vitamin and mineral mixture[2]	0.50
Salt	0.50
Chemical composition (% of DM)	
DM (%)	89.20
CP	12.30
Non fibrous carbohydrate[3]	53.10
NDF	14.70
ADF	6.30
Ether-extract	4.30
Ash	4.30
Calcium[3]	0.98
Phosphorus[3]	0.52
Chromium (mg/kg of DM)	0.95
Metabolizable energy[3] (Mcal/kg of DM))	3.10
Net energy for maintenance[3] (Mcal/kg of DM))	2.32
Net energy for growth[3] (Mcal/kg of DM))	1.76

[1]Palmac® 80-16 (IOI Oleochemical Industries Sdn Bhd, Prai, Malaysia). Product contained: 2% C12:0, 5% C14:0, 80% C16:0, 2% C18:0, 9% C18:1 and 3% C18:2; [2]Contained per kilogram of supplement: 975 000 IU of vitamin A, 750 000 IU of vitamin D, 1800 IU of vitamin E, 143 g of Zn, 76 g of Mn, 48.6 g of Cu, 19.5 g of Se, 18.4 g of Fe, 8 g of Ca and 1.3 g of Co; [3]Calculated from national Research Council (2001)

Albumin, Glucose, Blood Urea Nitrogen (BUN), Cholesterol, Total Protein (TP). An auto-analyzer (COBAS MIRA; Roche, Mannheim, Germany) was used to analyze the concentrations of plasma glucose, albumin, total cholesterol and total protein and blood urea nitrogen.

Statistical analysis: All the data were subjected to ANOVA using the PROC GLM of the Statistical Analysis System (Version 9.1; SAS Ins., Cary, NC, USA). Pens were employed as the experimental unit for growth performance parameters, whereas individual calves served as the experimental units for blood parameter analyses. Orthogonal polynomials were performed to determine the linear and quadratic effects of increasing levels of the GTB in diets on growth performance and blood metabolites. The results are reported as the least-squares means with the respective standard error of the mean (SEM).

Ethical consideration: All animals were handled with standard procedure according to the Ethical committee of Sylhet Agricultural University, Sylhet, Bangladesh. All the samples were collected by ensuring minimum disturbance to animals.

RESULTS AND DISCUSSION

Effects of Tea By-Product (TB) on body weight gain: The growth performance of calves fed diets supplemented with GTB is summarized in Table 2. Significant changes in body weight were recorded in experimentally treated calf at an age of 30 and 60 days. From the data recorded on day 30, it was observed that the entire treated group except T_1 had significant ($p<0.05$) higher weight than control group (Table 2). Eventually, the result showed that the body weight of the entire treated group was significantly ($p<0.01$) higher than the control group. Among the treated groups, group T_3 has higher body weights than those of other treated groups. GTB supplementation in diet enhanced the body weight in calves from 0-30 days and 30-60-day experimental period ($p<0.01$).

Table 2: Body weight (Mean±SD) of different groups (n = 4) after treating with GTB

Treatment	Body weight		
Groups/	Day 0	Day 30	Day 60
Group T_1	41.43±0.55^{d}	37.33±0.49^{d}	45.57±0.21^{ab}
Group T_2	42.93±0.49^{a}	45.57±0.21^{b}	45.00±0.61^{b}
Group T_3	41.97±0.15^{b}	47.53±0.42^{a}	46.63±0.35^{a}
Group T_0	40.70±0.46^{c}	41.07±0.30^{c}	43.23±1.20^{c}
Level of significance	**	**	**
P values	0.0000	0.0000	0.0023

Values with different letter in a column differ significantly ($p<0.01$)

Table 3: RBC count (Mean±SD) of different groups (n = 4) after treating with GTB

Groups/	RBC(Million/ml)		
Treatment	Day 0	Day 30	Day 60
Group T_1	6.53±0.51^{bc}	5.80±0.0^{b}	6.00±0.75^{bc}
Group T_2	7.40±0.46^{b}	7.47±0.38^{a}	7.80±0.89^{ab}
Group T_3	8.47±0.67^{a}	7.53±0.55^{a}	9.23±2.25^{a}
Group T_0	5.80±0.36^{c}	6.20±0.20^{b}	5.00±0.66^{c}
Level of significance	**	**	**
p-values	0.0012	0.0009	0.0175

Values with different letter in a column differ significantly ($p<0.01$)

Effect on hematological parameters: The Total Erythrocyte Count (TEC) was demonstrated in Table 3. TEC was increased in the treatment group T_2 and T_3 but decreased in the control group and T_1 group after sixty days. The GTB has a salutary effect on RBC count in calf treated with 1 and 2% green tea by-product in their diet (Table 3).

The total number of White Blood cells (WBC) was demonstrated in Table 4 showing increased WBC in both treatment group and control group but WBC count was significantly higher in treatment group T_2 and T_3 than control group after sixty days of the experiment.

In Table 5, Haemoglobin (Hb) concentration was presented. After finishing the experiment, it was observed that Haemoglobin (Hb) concentration increased only in the treatment group T_3 and which is significantly higher than the control group T_0.

The Total Protein (TP) concentration was demonstrated in Table 6. After 60 days TP concentration decreased in the control group and all the treatment group except T_3.

Table 4: WBC (Mean±SD) of different groups (n = 4) after treating with GTB

Groups/ Treatment	WBC(Million/mL) Day 0	Day 30	Day 60
Group T_1	11.47±0.35^{b}	12.37±0.31^{c}	13.33±1.31^{ab}
Group T_2	13.80±0.10^{a}	14.23±0.25^{a}	15.07±0.29^{a}
Group T_3	13.87±0.06^{a}	13.73±0.29^{b}	14.53±0.70^{a}
Group T_0	11.10±0.10^{c}	13.60±0.10^{ab}	12.27±1.01^{b}
Level of significance	**	**	*
p-values	0.0000	0.0001	0.0217

Values with different letter in a column differ significantly (p<0.01)

Table 5: Hb (Mean±SD) of different groups (n = 4) after treating with GTB

Groups/ Treatment	Hb (gm dL^{-1}) Day 0	Day 30	Day 60
Group A	6.80±0.0^{bc}	6.47±0.55^{b}	6.57±0.21^{bc}
Group B	7.40±0.50^{a}	7.37±0.47^{a}	7.27±0.21^{b}
Group C	7.67±0.51^{ab}	7.47±0.35^{a}	8.13±0.55^{a}
Group D	6.73±0.57^{c}	6.47±0.40^{b}	6.37±0.46^{c}
Level of significance	*	*	**
p-values	0.0230	0.0403	0.0021

Values with different letter in a column differ significantly (p<0.01)

Table 6: TP (Mean±SD) of different groups (n = 4) after treating with GTB

Groups/ Treatment	TP Day 0	Day 30	Day 60
Group A	7.40±0.46^{a}	8.33±0.15^{a}	7.00±0.30^{b}
Group B	8.00±1.08^{a}	7.20±0.10^{c}	7.20±0.26^{b}
Group C	7.53±0.55^{a}	7.73±0.21^{b}	7.87±0.15^{a}
Group D	8.17±0.15^{a}	8.23±0.12^{a}	7.73±0.12^{a}
Level of significance	NS	**	**
p-values	-	0.0001	0.0037

Values with different letter in a column differ significantly (p<0.01), NS = Not significant

The cholesterol concentration was showed in Table 7. In our study, we found the effect of GTB treatment on cholesterol concentration was not clear as cholesterol concentration decreased in all four groups.

The glucose concentration was demonstrated in Table 8. In our experiment, we found glucose concentration decreased in the T_2 group and it was significantly lower than other treatment groups and control group after 60 days.

After finishing the experiment on day 60, we found that albumin concentration (Table 9) was decreased in the treatment groups T_1, T_2 and control group T_0. Calf diet containing 2% GTB (T_3 group) has a significant effect on the enhancement of albumin concentration.

In Table 10, BUN concentration was demonstrated. In our experiment BUN concentration decreased in all the treated groups and the control group (Table 10) as the day progresses.

The results of this study elicit that dietary GTB supplementation is availed to enhance the overall average weight gain in calves. Compatible with our findings another study reported an increased average daily gain in

Table 7: Cholesterol (Mean±SD) of different groups (n = 4) after treating with GTB

Groups/ Treatment	Cholesterol Day 0	Day 30	Day 60
Group A	69.00±2.65^{bc}	68.67±2.52^{ab}	54.67±0.58^{c}
Group B	70.33±2.89^{b}	65.33±7.02^{a}	57.67±0.58^{b}
Group C	60.67±2.08^{a}	58.33±5.13^{a}	55.33±1.53^{a}
Group D	68.00±2.00^{c}	63.00±7.00^{b}	60.00±1.00^{b}
Level of significance	**	*	**
p-values	0.0025	0.0420	0.0000

Values with different letter in a column differ significantly (p<0.01; p<0.05)

Table 8: Glucose (Mean±SD) of different groups (n = 4) after treating with GTB

Groups/ Treatment	Glucose Day 0	Day 30	Day 60
Group A	48.00±3.00^{a}	49.33±1.53^{c}	55.00±1.73^{a}
Group B	50.67±3.51^{a}	55.67±1.15^{b}	48.00±2.65^{b}
Group C	56.00±5.00^{a}	59.00±1.00^{a}	60.00±4.36^{a}
Group D	49.00±10.44^{a}	42.67±0.58^{d}	59.00±2.65^{a}
Level of significance	NS	**	**
p-values	-	0.0000	0.0046

Values with different letter in a column differ significantly (p<0.01), NS = Not significant

Table 9: Albumin (Mean±SD) of different groups (n = 4) after treating with GTB

Groups/ Treatment	Albumin Day 0	Day 30	Day 60
Group A	1.57±0.06^{a}	1.77±0.06^{a}	1.33±0.15^{bc}
Group B	1.53±0.06^{a}	1.47±0.06^{b}	1.26±0.12^{c}
Group C	1.47±0.06^{a}	1.87±0.12^{a}	1.83±0.06^{a}
Group D	1.57±0.06^{a}	1.77±0.06^{a}	1.53±0.15^{b}
Level of significance	NS	**	**
p-values	0.1927	0.0011	0.0023

Values with different letter in a column differ significantly (p<0.01), NS = Not significant

Table 10: BUN (Mean±SD) of different groups (n = 4) after treating with GTB

Groups/ Treatment	BUN Day 0	Day 30	Day 60
Group A	14.37±0.15^{a}	12.00±0.52^{a}	11.07±0.49^{b}
Group B	13.27±0.76^{b}	11.90±0.56^{a}	10.40±0.26^{b}
Group C	12.30±0.79^{b}	9.83±0.84^{b}	10.90±0.92^{b}
Group D	14.97±0.31^{a}	11.50±0.26^{a}	14.53±0.58^{a}
Level of significance	**	**	**
p-values	0.0021	0.0061	0.0001

Values with different letter in a column differ significantly (p<0.01), NS = Not significant

goats supplemented with green TC (tea catechins) components[21]. The findings of another study were in agreement with our results which reported that a diet supplemented with 2.0% GTB has increased the weight gain of nishing pigs[13]. For the micro-organisms of rumen and intestine higher concentration of tea catechin in GTB may act as a growth promoter which results in high nutrient digestion and may be responsible for the augmentation of weight gain and feed intake[21].

Whereas, other studies reported that various levels of green tea by-products supplemented with basal diets reduced the body weight gain in rats and broilers and this may be because of elevated tannin levels in green tea[22, 23]. According to Westerterp-Plantenga[24], GTB can be used for maintenance and gain of body weight, due to the presence of phenolic compounds in it. Our study showed that the number of RBC in treated groups increased except for group T_1 where the average number of RBC decreased after the end of the experiment which might be due to some physiological and environmental factors. Elkirdasy *et al*.[25] reported that in diabetic rabbit treated with tea and/or ginger results in enhancement of the number of RBC which could be because of the reduced lipid peroxide level in RBC membrane resulting in a diminished susceptibility of RBC to hemolysis, those findings are analogous with the findings of T_2 and T_3 groups of this study.

Here it is observed that the total WBC count was also increased in the treatment groups and WBC count in T_2 and T_3 was significantly higher than the control group which is very much closure to the findings of Kapetanovic *et al*.[26] where mild to remarkable enhancement in WBC, neutrophil, monocyte, platelet and platelet crit (percentage volume of platelets) values were reported in dogs treated with PPE (Polyphenon E®).

A significant increase of Hb concentration in T_3 in comparison to T_0 may be due to the effect of GTB on hemopoietic organs. Because GTB acts as a cofactor for methionine synthase and L-methylmalonyl-CoA mutase where methionine synthase catalyzes the conversion of homocysteine to methionine[27]. According to Sachdev and Jothipriya[28], hemoglobin level declines because of the constant consumption of green tea which is similar to the findings of the T_1 and T_2 group of the present study.

The protein concentration of GTB (CP-22-35%) may also positively influence the feed intake and weight gain of broiler chicks[3] which is in accordance with our present study findings. But another study reported over the long-period there was no additive effect of green tea supplement with a high-protein diet on weight maintenance[29].

According to the result, the blood cholesterol concentration was decreased through treatment with GTB, where fiber of the GTB may affect the cholesterol components because fiber is an indigestible feed component affecting cholesterol metabolism and concentration of cholesterol in the blood[30]. Cholesterol level of rats serum was abated as fiber content in the diet was improved, reported in another study by Tsai *et al*.[31] which satisfies the criteria. Similar findings were found in laying hens reported by Menge *et al*.[32]. Using ensiled green tea waste in the diet list of lactating cows results in significant suppression of serum cholesterol contents[2] which supports the present study findings. Crespy and Williamson[33] stated that green tea or its polyphenols in diet reduce plasma total cholesterol by inhibiting the intestinal lipid metabolism which is very much closure to the current study findings.

Suppression of hyperglycemia and prevention of glycogen accumulation in the proximal tubules usually occur due to the beneficial effect of Green Tea (GT) extract on long-term diabetic nephropathy. The therapeutic property of Green tea seems conducive to the improvement of kidney nephropathy through the improvement of serum and urine parameters significantly[34].

Abatement of Albumin concentration in the treatment group with the exception in T_3 does not support the findings described by Shekarforoush *et al*.[35] where it is stated that a significant improvement was seen in total protein and albumin level of serum in GT receivers compared with thioacetamide group.

Our present study found a significantly lower BUN concentration in the treatment group than the control group. In malaria-infected people, GT extract has potent antioxidant and anti-inflammatory characteristics to abate BUN and creatinine where polyphenols of green tea are useful supplements[36] that support the present study findings.

CONCLUSION

The current study suggests that GTB supplementation has a significant effect on growth performance and certain hemato-biochemical parameters in goats which indicate the beneficiary effect of GTB in the calves. Therefore awareness should be increased among people to use GTB as a dietary supplement in a specific ratio. However, further study is required to observe any pernicious effect regarding histopathology and biochemistry before making any conclusion about the salutary effect of GTB in the calf.

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