

# Response of Blood Eosinophils in Lactating Crossbred Cows Fed with Bamboo Leaves as a Replacement to Pasture Hay in Central Ethiopia

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## Abstract

The objective of this experiment was to evaluate the effect of feeding bamboo leaf alone or in mixture with hay as basal diet on physiological and Blood eosinophil values in high grade crossbred milking cows. Five milking cows were grouped randomly into five treatments consisting of hay and bamboo leaves at the proportion of 100:0 (T1), 75:25 (T2), 50:50 (T3), 25:75 (T4) and 0:100% (T5) respectively. A prospective study was undertaken to study the effects of bamboo leaves on the response of eosinophils in experimental crossbred cows. In this experiment, mean rectal temperature of crossbred milking cows that had fed 25% (T2), 50% (T3) and 75% (T4) inclusion of bamboo leaves replacement over cows that received the control diet (T1) was  $39.06 \pm 0.64$ ,  $38.02 \pm 0.70$  and  $39.04 \pm 0.62$  respectively. Similarly, mean blood eosinophil responses ( $55.33 \pm 3.90$ ), mean packed cell volume indices ( $23.75 \pm 0.66$ ) and milk yield ( $7.9 \pm 0.25$ ) had dropped significantly ( $p < 0.05$ ) in crossbred cows that were fed with bamboo leaf replacement of the pasture hay at the ratio of 100% (T5). Mean Milk yield ( $8.3 \pm 0.23$ ), mean packed cell volume indices ( $30.5 \pm 2.10$ ) and mean blood eosinophils ( $118.75 \pm 9.71$ ) were significantly ( $P < 0.05$ ) increased for crossbred cows that had fed bamboo leaf at the ratio of 25% (T2) and 50% (T3) as compared to T1 values. Change of body weight of cows that were fed with 75% (T4) was significantly declined and lost in 100% (T5) which was negatively correlated with complete substitution ( $p < 5\%$ ). The Eosinophil index of milking cows was generally greater in T2 and T3 values. Outputs of the experiment showed that Bamboo leaf supplementation had linearly improved blood eosinophil responses significantly ( $p < 5\%$ ) at 25 percent and 50 percent inclusion levels over crossbred cows that had received the control diet (T1). Based on the result of this experiment, blood eosinophils of crossbred cows were effective and responsive when bamboo leaves had replaced the pasture hay at the ratio of 25% (T2) and 50% (T3) levels. On the other hand, bamboo leaf supplementation at the rate of 75% (T4) and 100% (T5) inclusion levels had resulted in poor performances of blood eosinophils and depressed milk yield that had adversely induced profuse diarrhea, weight loss, gait disorders, convulsions, toxic and other adverse effects in the experimental lactating cows. In conclusion, Bamboo leaves had better blood response values as compared to pasture hay in this experiment and hence can be a good substitute to hay at the rate of 25% and 50% in the long dry season when conventional roughages are in short supply without any adverse health effect on the cows.

**Keywords:** Bamboo • Blood • Crossbreds • Eosinophils • Response

## Introduction

Livestock production sector contributes about 17%-25.3% of the national gross domestic product, 39%-49% of agricultural outputs and 50% of smallholder income in Ethiopia. Despite its tremendous potential, the contribution of livestock sector for the livelihoods of smallholders and development of the country is low and this was hindered by the quality and quantity of animal feeds which adversely affect the livestock performances [1]. To mitigate these problems, innovations to find out new alternatives and locally available materials

as a potential source of feed is important. The use of bamboo species that are distributed in the low land and highlands of Ethiopia can be used as potential sources of animal diet [2]. The low land bamboo species in Benishangul Gumuz area covers 64% of bamboo species in the country and a recent conducted study showed on average 11.7 tons of leaf biomass on dry matter basis can be harvested from bamboo species.

Bamboo leaves have medicinal values which contains active ingredients such as flavonoids, polyphenols, and active polysaccharides. The combined properties of these macromolecules

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lead to multiple biological effects such as dietary supplements. These ingredients have anti-inflammatory properties, enhance immunity and treat various ailments. Fragmented reports indicated that bamboo leaf extract has potential properties that can reduce cholesterol concentration of hyperlipidemia and improve liver function [3]. Other authors also reported that bamboo leaf extracts reduce the adhesion of vascular epithelial factors, increase vascular mobility, and reduce atherosclerosis. Bamboo leaves have high content of tannin substances which is able to reduce worms [4]. Therefore, tannins are potential strategic alternatives for the control of nematode worms in ruminants and blood eosinophils are also responsive against ruminant worms. Generally, Bamboo leaf has pharmacological, antibacterial, antioxidant, and antitumor agents. Bamboo leaf as parasitic agent was evaluated and effective on mortality of haemonchus contortus and its morphometric parts which was previously reported. Similarly reported bamboo can kill nematode worms such as Haemonchus contortus. Dietary components have measurable effects on blood constituents. Animal blood components are influenced by quantity, quality and the level of anti-nutritional elements or factors present in the feed.

Diets containing low protein quality could lead to poor transportation of oxygen. Reduction in the concentration of packed cell volume is due to the presence of toxic factors such as hemagglutinin and low protein intake which has adverse effect on the blood formation [5]. These medicinal properties of bamboo leaves could reflect the physiological and hematological traits of lactating cows which is highly correlated with milk yield. The effect of substitution of dry Bamboo leaves at different proportion with concentrate mix on ruminant particularly sheep was reported. Limited or no research has been conducted and explored so far on the blood eosinophil responses in bamboo leaf supplemented in lactating cows in Ethiopia. Therefore, this study was conducted to practically evaluate responses of blood eosinophils and physiological values in bamboo leaves supplemented high-grade crossbred lactating cows as a replacement diet in Central Ethiopia.

## Materials and Methods

### Prophylactic treatment of high grade crossbred cows

On station experiment was conducted at Holetta Research Center, livestock research farm on five mid-lactating 75 percent Boran × Fresian crossbred cows of different parities. All milking cows were prophylactically treated with Ox tetracycline (20% LA) and therapeutically treated with broad spectrum Tetraclozan 3400 mg per os and immunized against common diseases in subcutaneous route before the start of the experiment [6-12]. The weight of all milking cows was taken and milk yield was recorded on daily basis. Experimental crossbred cows were separately given hay and bamboo fodder inclusion. In this treatment set up, high-grade crossbred cows were monitored for 140 days for the experiment and the occurrence of events were treated therapeutically for any adverse effects.

### Experimental protocols and data measurements

A prospective study design was used with purposive sampling of milking cows based on their parities and lactation phases. Five 75%

Boran × Fresian crossbred cows were randomly blocked in 5 × 5 Single Latin Square design composed of five dietary treatments, five parity and five periods with each period categorized into fourteen days of adaptation and fourteen days of clinical data measurement periods. The total experimental period was 140 days for these studies. Cows were then randomly allotted to one of the five dietary treatments that contained bamboo leaves at 25%, 50%, 75% and 100% level of supplementation. The cardinal physiological variables such as Pulse rate (RT/beats/min), Respiratory rate (RR/breaths/min), Rectal temperature (RT/°c) were measured to evaluate the long term effects of Bamboo leaves on the health values of crossbred cows during the entire periods of experiment. Control cows were fed with reference diet while five experimental cows were supplemented with bamboo leaves at the rate of 25% (T2), 50% (T3), 75% (T4) and 100 percent complete substitution (T5) (Table 1).

| Treatment categories | Treatment feeds              |                   | Supplements feeding level (kg/L of milk/day) | Prophylaxis (Prophylactic drug and Multivitamin (MVT)) |
|----------------------|------------------------------|-------------------|--|--|
|                      | Natural pasture hay (NPH, %) | Bamboo leaves (%) |  |  |
| Treatment 1          | 100                          | 0                 | 0.5  | 20% LA+MVT 5-10 ml, IM                                 |
| Treatment 2          | 75                           | 25                | 0.5  | 20% LA+MVT 5-10 ml, IM                                 |
| Treatment 3          | 50                           | 50                | 0.5  | 20% LA+MVT 5-10 ml, IM                                 |
| Treatment 4          | 25                           | 75                | 0.5  | 20% LA+MVT 5-10 ml, IM                                 |
| Treatment 5          | 0                            | 100               | 0.5  | 20% LA+MVT 5-10 ml, IM                                 |

**Table 1.** Set up of experimental treatments and prophylaxis.

### Blood collection and determination of Eosinophil indices

Blood samples were collected by using 10ml vials that has anticoagulant from the Jugular vein of milking cows. This was done by following the procedure of Jain (1993) and samples were subjected to standard laboratory techniques to perform packed cell volume as described [13-18]. Samples were centrifuged at 1000 rpm for 20 min at 4°C. A haematological eosinophils profile within normal physiological limits reflects a good health status and is highly correlated with milk production.

### Statistical analysis

Data generated from blood and physiological parameters were subjected to analysis of variance by stata, version 13 and procedures of Statistical Analysis System, version 9.3 (SAS, 9.3). Differences in mean PCV mean cardinal values and hematological eosinophils indices count measured between milking groups were analyzed by two-way repeated measures of analysis. The variation was set at P <5% significance level.

## Results and Discussion

### Physiological values in bamboo leaves supplemented crossbred cows

Cows treated with combination of basal diet and different levels of bamboo leaves inclusions showed slightly better responses of physiological indices. Mean rectal temperatures (RT) of high grade crossbred milking cows that had received 25 (T2), 50 (T3) and 75 (T4) percent inclusion levels of bamboo leaves replacement over cows receiving the control diet (T1) was  $39.06 \pm 0.64$ ,  $38.02 \pm 0.70$  and  $39.04 \pm 0.62$  respectively. Crossbred cows supplemented with 25 percent of bamboo leaves replaced the pasture hay had slightly higher pulse rates (PR) and respiratory rates (RR) while cows supplemented with 50 and 75 percent of bamboo leaves substitution of the pasture hay had fluctuated and influenced the rhythms (T2)(Table 2).

| Treatments | Particulars of dietary Treatments (M ± SD) |              |              |
|------------|--|--------------|--------------|
|            | RT (°c)                                    | PR (beats/m) | RR (beats/m) |
| T1         | 38.80 ± 0.37                               | 74.80 ± 0.30 | 16.4 ± 0.20  |
| T2         | 39.06 ± 0.64                               | 78.2 ± 0.26  | 18.6 ± 0.14  |
| T3         | 38.02 ± 0.70                               | 68.06 ± 0.42 | 16.34 ± 0.24 |
| T4         | 39.04 ± 0.62                               | 76.24 ± 0.2  | 22.6 ± 0.62  |
| T5         | 39.04 ± 0.32                               | 72.42 ± 0.28 | 22.42 ± 1.20 |

**Table 2.** Responses of Physiological values in bamboo leaves supplemented cows.

### Correlation of milk yield, DWG and Eosinophil indices in bamboo leaf supplemented cows

In this experiment, the mean blood eosinophils, mean PCV indices and milk yield had dropped significantly ( $p < 0.05$ ) in high grade crossbred milking cows that were fed with complete bamboo fodder replacement of the natural pasture hay at the rate of 100 percent (T5) as compared to T1. Mean Milk yield, mean packed cell volume indices and mean blood eosinophils responses were significantly ( $P < 0.05$ ) increased for crossbred cows that had received bamboo fodder at the rate of 25 (T2) and 50 (T3) percent level of replacement for natural pasture as a basal diet. It has been also noted that the body weight of experimental cows that were fed with 75 and 100 percent bamboo leaf substitution of the natural pasture hay was significantly declined and lost ( $p < 5\%$ ) and this was negatively correlated with complete substitution. The current result showed that 25 and 50 percent (T2 and T3) level supplementation of bamboo leaf in the basal diet had improved Eosinophil responses of lactating high grade crossbred cows. Eosinophil and PCV responses were significantly different ( $P < 5\%$ ) in the order of  $T2 > T3 > T4 > T1 > T5$  in this experiment. On the other hand, when bamboo leaf had completely replaced the basal diet (100 percent substitution), mean blood packed cell volume responses, milk yield, blood eosinophil responses and daily weight gain of high grade crossbred cows had significantly ( $p < 0.05$ ) dropped as compared to cows that received pasture hay as control diet. Therefore, 25 and 50 percent supplementation of bamboo leaf as a feed additive has a certain economic and health values. In this experiment, a significant decrease in mean PCV and Eosinophil responses were observed in Bamboo inclusion fed groups at the rate of 75 and 100 percent level replacement of the pasture

hay compared with basal diets and this could be due to the presence of pancytopenia that is anemia, leukopenia, and thrombocytopenia associated with subclinical parasitic infections of cattle. A relative deficiency of blood cells occurs initially due to hemodilution and further exacerbated by hemolytic anemia [19]. Hemolysis could be caused by damage of erythrocytes by the lashing action of the organisms. An increase in body temperature also increases the rate of immunochemical reactions thereby initiating lipid peroxidation of erythrocytes [20]. This finding showed that as sharp decline in PCV occurred during the last experiment due to pyrexia. Furthermore, living and dead infection can produce various forms of active chemical substances, which can elicit blood erythrocyte damage (Table 3).

| Particulars | T1            | T2            | T3            | T4           | T5            | P-value |
|-------------|---------------|---------------|---------------|--------------|---------------|---------|
| Milk yield  | 8.1 ± 0.17    | 8.3 ± 0.23    | 8.6 ± 0.27    | 8.4 ± 0.21   | 7.9 ± 0.25    | 0.001   |
| PCV         | 29.58 ± 1.26  | 30.5 ± 2.10   | 40.58 ± 3.75  | 29.38 ± 1.57 | 23.75 ± 0.66  | 0.013   |
| Eosinophils | 61 ± 3.0      | 118.75 ± 9.71 | 105.17 ± 6.49 | 64 ± 1.85    | 55.33 ± 3.90  | 0.017   |
| weight gain | 516.8 ± 111.2 | 457.1 ± 152.1 | 407.1 ± 82.6  | 379.1 ± 21.9 | -180.9 ± 30.0 | 0.0012  |

**Table 3.** Correlation of mean milk yield weight gain, packed cell volume and blood eosinophils responses in bamboo leaf supplemented cross bred cows.

Cows that consumed basal diet and supplemented with bamboo leaves at the rate of 25 percent produced high concentration of blood eosinophils. The mean blood eosinophil profile was  $118.75 \pm 9.71$ ,  $75.17 \pm 6.49$ ,  $64 \pm 1.85$ ,  $61 \pm 3.0$  in T2, T3, T4 and T5 treatment groups respectively. Bamboo leaf substitution at the rate of 75 of T4 and 100 percent of T5 in lactating high grade crossbred cows significantly ( $P < 0.05$ ) decreased mean blood eosinophil counts while a marginal increase was observed with 25 and 50 percent substitution. The declined haematological responses of crossbred cows on diets T4 and T5 reflects as a decline in the production of defensive mechanisms which naturally predisposes cows to various physiological stresses due to infection and weight changes. The elevated responses of eosinophil indices in this experiment indicated immunological responses of cows to foreign challenges and antimicrobial properties of bamboo fodder. In significant ( $P > 0.05$ ) reduction in values of blood eosinophil among diets possibly explained the inclusion levels of the test diets that were tolerant and did not have inverse effect in the blood response of lactating cows. High grade cows that were fed with bamboo fodder at the rate of 25 and 50 percent level replacement of the pasture hay had induced higher eosinophilia responses over cows receiving the control diet. This could be associated with the role of eosinophils in attacking worm infection which is provided by the significant correlations that exist between blood and tissue eosinophilic responses of cows. The lower counts of eosinophil responses observed at T4 and T5 treated experimental cows may be attributed to the immunosuppression. Hematology refers to the numerical and morphological study of the cellular elements of the blood, as well as the usage of these results in the diagnosis and monitoring of disease. Hematological parameters are good markers of the physiological status of animals while White

blood cell counts are a rough indication of immune status of an animal. In this experiment, blood eosinophil responses in cows that fed with 100 percent Bamboo leaves was lower and this signified that bamboo fodder provided to cows alone had a depressing effect on the immune responses of lactating cows [21]. Bamboo leaves have a high tannin content and this high amount of tannins are able to play a significant role in binding proteins and turning nematode walls into inactivity and killing them. Tannins commonly found in plants (leguminous) is condensed tannins. Condensed tannins are effective against gastrointestinal parasites and this can affect hatching and growth of infective larvae. The condensed tannin also has the ability to bind proteins, and make nematode worms walls to be inactive and subsequently kill them. Indirectly, tannins may bind plant proteins in the rumen to prevent microbial degradation. Furthermore, this will increase the flow of proteins into the duodenum. In addition, condensed tannins in bamboo leaves may have different effects on ruminants when consumed on the growth of adult worms and larvae. Increased protein nutrition will decrease parasite infections by increasing host immunity which is directly correlated with the effect of eosinophils in cows. Such finding was related to tannin content in bamboo leaves. Natural ingredients that have anthelmintic properties were active ingredients of tannins, saponins, flavonoids, and alkaloids. Plants containing 5% tannin extract can reduce contamination of larvae and can be used as anthelmintic [22]. Bamboo leaves can be an alternative to herbal anthelmintic as a substitute for commercial anthelmintic, as it has been ruminants like goats. Cows that had fed bamboo fodder inclusion at the rate of 75%-100% was vulnerable to pathophysiological disorders such as profuse diarrhea, weight loss, convulsion and gait disorders (Table 4).

| Treatments | Eosinophil responses | P value |
|------------|----------------------|---------|
| T1         | 55.33 ± 3.90         | 0.001   |
| T2         | 118.75 ± 9.71        | 0.016   |
| T3         | 75.17 ± 6.49         | 0.03    |
| T4         | 64 ± 1.85            | 0.017   |
| T5         | 61 ± 3.0             | 0.008   |

**Table 4.** Response of Eosinophil indices.

Blood eosinophil responses in crossbred cows that had received bamboo fodder inclusion at the rate of 25 and 50 percent level replacement of pasture hay was increased by 63.4 unit of coefficient (T2) and 19.83 unit of coefficient (T3) respectively over cows that had provided the control diet (T1). The model P value (0.001) indicated that there was a strong association between eosinophil responses of cows that had received the experimental diet [23]. Cows that had supplemented with 75 (T4) and 100 percent (T5) level of bamboo fodder inclusion produced low units of coefficients of eosinophil responses as compared to crossbred cows that received the control diet (T1) and this response is statistically in significant ( $P > 0.05$ ) (Table 5).

| Blood Eosinophil responses | Coefficients | Std Err  | t    | P>t   | [95% Conf. Interval] |
|----------------------------|--------------|----------|------|-------|----------------------|
| Treatments                 |              |          |      |       |                      |
| 2                          | 63.41667     | 8.873919 | 7.15 | 0.001 | 81.31263             |

|      |          |          |      |       |          |
|------|----------|----------|------|-------|----------|
| 3    | 19.83333 | 8.873919 | 2.24 | 0.031 | 37.7293  |
| 4    | 8.666667 | 9.921344 | 0.87 | 0.387 | 28.67496 |
| 5    | 5.666667 | 12.54962 | 0.45 | 0.654 | 30.97538 |
| Cons | 15.33333 | 6.274809 | 8.82 | 0     | 17.98769 |

**Table 5.** Responses of blood eosinophilia in bamboo leaves supplemented high-grade crossbred lactating cows.

## Conclusion

Bamboo leaf extract contains active ingredients such as flavonoids, polyphenols, and active polysaccharides, which possess anti-inflammatory, antioxidant and lipid-lowering effects. Results of the current experiment showed that high-grade crossbred lactating Cows treated with combination of basal diet and different levels of bamboo leaves inclusions showed slight improvement of physiological indices over the control diets. Mean rectal temperatures (RT) of high grade crossbred lactating cows that had received 25 (T2), 50 (T3) and 75 (T4) percent inclusion of bamboo leaves replacement over cows receiving the control diet (T1) was  $39.06 \pm 0.64$ ,  $38.02 \pm 0.70$  and  $39.04 \pm 0.62$  respectively. In this experiment, the mean blood eosinophils and lymphocyte responses, mean PCV indices and milk yield had dropped significantly ( $p < 0.05$ ) in high grade crossbred lactating cows that were fed with complete bamboo fodder replacement of the natural pasture hay at the rate of 100 percent (T5) as compared to cows receiving the control diet (T1). Mean Milk yield, mean packed cell volume indices and mean blood eosinophils were significantly ( $P < 0.05$ ) increased for crossbred cows that had received bamboo fodder at the rate of 25 (T2) and 50 (T3) percent level of replacement for natural pasture as a basal diet over cows maintained on the control diet (T1). A significant ( $P < 0.05$ ) increased in blood neutrophil response was observed with Bamboo leaf substitution at the rate of 25 and 50 percent while substitution at the rate of 75 and 100 percent led to a marginal decrease of blood neutrophils. It has been also noted that the body weight of experimental cows that were fed with 75 and 100 percent bamboo leaf substitution of the natural pasture hay was significantly declined and lost ( $p < 5\%$ ) and this was negatively correlated with absolute substitution.

The result of the present study showed that 25 to 50 percent bamboo leaves as dietary supplementation could improve blood packed cell volume responses and eosinophil concentration in high grade lactating crossbred cows. Bamboo fodder can help small and medium-scale farmers to overcome shortages of good quality feeds and therefore sustain and improve their livestock productivity. Based on the result of this experiment, Responses in terms blood indices and milk yield were effective when bamboo leaves has replaced the natural pasture hay at the rate of 25 and 50 percent (T2 and T3) levels and this proportion is non-toxic to lactating crossbred cows. On the other hand, bamboo fodder supplementation at the rate of 75% (T4) and 100% (T5) inclusion level had resulted in poor performances, depressed milk yield there by induced profuse diarrhea, weight loss, gait disorders, convulsions, toxic and adverse effects in the experimental lactating cows. The use of determined levels of bamboo fodder in the diet of lactating high-grade crossbred cows at mid lactation in this experiment have demonstrated that

bamboo is a potential source of energy and protein that would aid in filling feed shortage gaps without any adverse health effect on the cows. Therefore, appropriate level of bamboo leaves can be utilized as alternative basal diets for lactating crossbred cows and thus, livestock rearing communities can use this recommended values to alleviate the nutritional and health challenges during the long term dry seasons. However, biologically active ingredients that have medicinal properties and the potential effect of tannins in the Bamboo fodder needs further research study.

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