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Blood parameters and physiological responses on supplementation garlic powder (*Allium sativum*) in Sahiwal Calves

Zara Kaku Sorang, Raj Jyoti Deka, Dilip Kumar Baruah, Jakir Hussain, Gunaram Saikia, Arijit Shome and Purabi Kaushik

Abstract
The present experiment was conducted to investigate the effect of supplementing garlic powder (*Allium sativum*) on the performance of Sahiwal calves. Twelve healthy Sahiwal calves of both sexes of similar age and body weight were allocated to two experimental groups, control (T_0) and treatment (T_1) with six animals in each group. Calves of both groups were fed with a standard basal diet consisting of concentrate, green fodder, and paddy straw. While in the treatment group, garlic powder was supplemented @ 250 mg/kg body weight along with concentrate feed. The experiment was conducted as a feeding trial for eight fortnights and a digestibility trial thereafter for five days in the two experimental groups. Analysis of the variance of the data revealed that no significant ($p>0.05$) difference in respiration rate (breath per minute), pulse rate (beat/min), and rectal temperature (ºF) between control (T_0) and treatment (T_1) in respect of fortnightly and overall values. The difference observed was statistically significant ($p<0.01$) between the control group (T_0) and treatment group (T_1) for initial and final total protein level, hemoglobin level and RBC level. Further it can be concluded that the supplementation of garlic powder at dosage of 250 mg/kg body weight in the Sahiwal calf ration it has a positive impact on total protein level, hemoglobin level and RBC level, while does not had any impact on serum Blood glucose.

Keywords: *Allium sativum*, garlic, Sahiwal calves, physiological responses, hematological parameters

Introduction
To improve the performance of calves and their health status, dairy farmers in the country have started adding feed additives to their animals' diets. Feed additives are compounds that are added to feed to enhance its quality and effectiveness, which eventually improves the performance of the animal. A few examples of feed additives are antioxidants, acidifiers, antibiotics, vitamins, minerals, amino acids, toxins binders, and immunomodulators. The ban on the use of antibiotics in animal feed and due to increased awareness of the consumers about the health hazards of antibiotics as feed additives triggered a need for natural and safe feed additives such as organic acids, probiotics, prebiotics, and plant extracts (Jayasena and Jo, 2013) [16]. Livestock scientists and producers are now examining new feed additives that can benefit animal health and production performance.

To achieve the intended outcome, the feed of the calves has been supplemented with a variety of substances. A more recent development is the use of herbs. It is well-recognized that adding herbs to feed can help an animal's health. The majority of herbal feed additives fall under the category of isoprene derivatives, flavonoids, and glucosinolates, and many of these substances are thought to have antibacterial or antioxidant properties (Hirasa and Takemasa, 1998) [15]. Activating feed intake, immunological stimulation, antibacterial, coccidiostat, anthelmintic, antiviral, or anti-inflammatory activity, as well as antioxidant qualities, are some of the ways that herbs benefit farm animals. The endocrine system is stimulated by herbs, and they also help the animals' intermediate nutrition metabolism (Wenk, 2003) [29].

One of the most important essential bulb crops in India is garlic (*Allium sativum*). Allicin is a flavorless, fragrant, and water-soluble substance found in garlic bulbs. In India, it is typically consumed as a condiment. Enzymes, vitamin B, flavonoids, and other minerals are all present in garlic. Garlic is a top-notch source of protein and antioxidants. Garlic is the key element of the Mediterranean region and is utilized for food preparation fairly frequently in Asian,

African, and European nations. The garlic plant has been used for both food and medicine all across the world (Neeraj *et al*., 2014) [20]. It restores cholesterol to normal levels and lowers the risk of heart disease. It guards against a variety of malignancies. Garlic, therefore, provides a rich foundation of bioactive substances that satisfy the basic dietary needs of daily life. The addition of garlic to the diet has several positive *viz*. better foreign compound detoxifications, restoration of bodily potency, and resistance to various pressures (Amagase *et al.*, 2001) [1]. The steroidal glycosides prostaglandins, pectin, essential oil, adenosine, vitamins B1, B2, B6, C, and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, anthocyanins, flavonoids, phenolics, and significant amino acids are other components of whole garlic.

Materials and Methods

Location and period of study

The research was carried out at Sahiwal Cattle Farm, College of Veterinary Science Khanapara, Assam Agricultural University. The experiment was conducted for a period of 120 days from 14th July to 14th November 2022. The farm is in a plain area where the water source mainly depends on rainfall and bore wells. The farm is situated at 26.1213° N latitude, 91.8216° E. In Khanapara, during the entire year, the rain falls for 197.8 days and collects up to 1663 mm (65.47") of precipitation. The temperature here typically varies from 53°F to 89°F.

Experimental animal and design

Twelve healthy Sahiwal calves were selected randomly and divided into two groups *viz*. Control (To) and Treatment (T1) consist of 6 animals (5-6 months of age) in each group with equal number of both sexes (Table 1). The initial body weight of T_0 (81.81 kg) and T_1 (81.46 kg) did not differ significantly.

Table 1: Distribution of animals in different treatment groups

Groups	No. of animal	Treatment
Control (T_o)	6	Without feeding Garlic
Treatment (T_1)	6	Feeding Garlic @ 250 mg/kg body weight with concentrate mixture

Management and feeding of animals

All the experimental calves were housed in a close shed with a provision for individual feeding and watering facilities. The animals were allowed to exercise 1 hour daily morning and afternoon in the open paddock. All the calves were provided a mixer of green grasses (Para, Napier and Guinea grasses) and paddy straw as basal diet *ad libitum* twice daily. The standard concentrate ration consisting of DCP: 17.27% and TDN: 73.67% offered twice daily. The animals were fed individually from the manger tying with a rope. The feeding was done as per the feeding regimn of ICAR, 2013. Along with the feed, each calf of the treatment group (T_1) were supplemented with Garlic powder @ 250 mg/kg body weight in concentrate feed.

Haematological Parameters

Hematological parameters were studied every after 60 days i.e. on the 0 day, 60th day and 120th day. 2 ml blood samples were collected from the jugular vein of each animal into a vacutainer. The hematological parameters were analyzed with an Automatic hematology analyzer (MELET SCHLOESING lab., MS4e©) Department of Veterinary Biochemistry, College of Veterinary Science, Khanapara, Assam.

Serum biochemical profile

Serum biochemical profile parameters of the experimental calves were assessed at every 60 days interval i.e., on the 0 day, 60th day, and 120th day, respectively of the experimental trial. The blood samples were collected from the jugular vein of the animal. Glucose and total protein were estimated using the 'Spectrophotometer'. The serum biochemical analysis was done at the Department of Veterinary Biochemistry, College of Veterinary Sciences, Khanapara, Assam.

Statistical Analysis

Two-way analysis of variance with interaction was done to test the significance of the difference in the treatment means to arrive at a conclusion as per methods prescribed.

Results and Discussion

Physiological responses

Respiration rate, pulse rate and rectal temperature

The overall mean values of the respiration rate in the T_o and T_1 groups respectively were 28.61±0.21 and 28.79±0.14 beats/min, while average initial respiratory rate of both the groups were 27.0±1.46 and 28.8±0.70 beats/min. Average respiratory rate were recorded from 1st to 8th fortnight for control and treatment group as 28.6±0.42 And 28.6±0.49, 28.5±0.43 and 28.83±0.31, 29.17±0.31 and 29.17±0.31, 29.00±0.37 and 28.50±0.43, 28.67±0.49 and 29.17±0.31, 29.00±0.40 and 8.83±0.40, 29.17±0.31 and 28.17±0.48, and 28.34±0.34 and 29.00±0.26 beats/min. Analysis of variance of the data revealed no significant (P>0.05) difference in respiration rate (breath/min) between control (T_0) and treatment (T_1) in respect of fortnightly and overall values.

The overall mean values of the pulse rate in the T_0 and T_1 groups respectively were 72.20±0.07 and 72.20±0.07. In respect of period, average pulse rates were 72.33±0.21, 72.16±0.17, 72.33±0.21, 72.17±0.17, 72.17±0.31, 72.00±0.26, 72.17±0.31 and 72.33±0.21 in control (T_0) and 72.33±0.21, 72.16±0.17, 72.33±0.21, 72.17±0.17, 72.00±0.26, 72.17±0.17, 72.17±0.31, and 72.33±0.21 in treatment (T_1) groups respectively. Analysis of variance revealed no significant (p>0.05) difference in pulse rate (beat/min) between control (T_0) and treatment (T_1) groups in respect of fortnightly and overall values.

The overall mean values of the rectal temperature (°F) in the T_0 and T_1 groups respectively were 101.36±0.04 and 101.30±0.03 (°F). In respect of period, average rectal temperatures were 101.27±0.07, 101.27±0.07, 101.52±0.09, 101.85±0.10, 101.23±0.08, 101.40±0.1, 101.33±0.11, and 101.24±0.055, in control (T_0) and 101.27±0.07, 101.16±0.02, 101.36±0.07, 101.38±0.10, 101.13±0.01, 101.27±0.10, 101.3±0.14, and 101.52±0.09 in treatment (T_1) groups at initial, 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th fortnights respectively. Analysis of variance revealed no significant (p>0.05) difference in rectal temperatures (°F) between control (T_0) and treatment (T_1) groups in respect of fortnightly and overall values.

The present experimental findings were in agreement with the findings of Rokde (2007) [24] who did not find any significant differences in the rectal temperature in the probiotics-supplemented group of crossbred calves. Kobeisy *et al*. (2015) [18] studied the effect of sugarcane bagasse, silage treated with urea, live yeast and found no significant (P>0.05) effect on rectal temperature, respiration rate, and pulse rate.

Conte *et al.* (2018) [9] reported that the addition of feed additives, yeast, and plant extracts may have a beneficial effect on rumen metabolism and regulate ruminants' body temperatures. Similar findings were also reported by Shinde *et al.* (2019) [25] who did not find any significant differences in (p>0.05) pulse rate, respiration rate and rectal temperature in the probiotics-supplemented group. However, some workers find significant differences in rectal temperature, pulse rate, and respiration rate by supplementing molasses and probiotics. Purwar and Dang (2017) [22] and Campos *et al.* (2018) reported significant differences (p<0.01) in rectal temperature, respiration rate, and pulse rate.

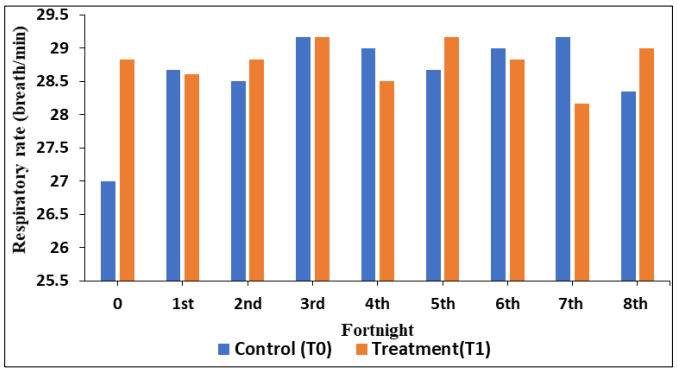

Fig 1: Average respiration rate (breath/min) of Sahiwal calves

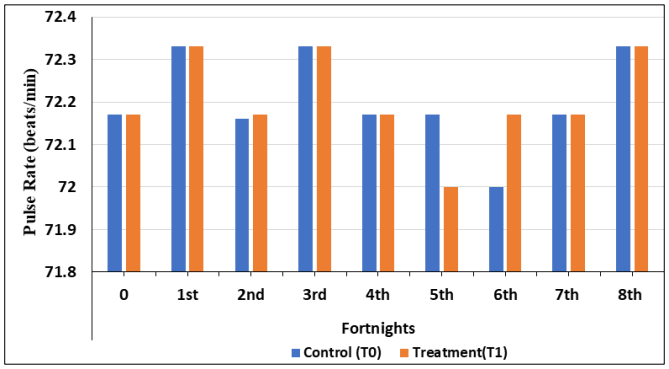

Fig 2: Average pulse rate (beats/min) of Sahiwal calves

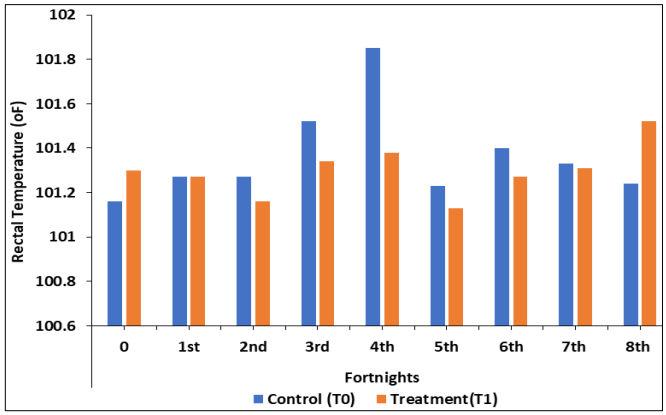

Fig 3: Average rectal temperature (^{0}F) of Sahiwal calves

Blood biochemical and hematological parameters

Serum Glucose (mg/dl)

The serum glucose level was estimated at the 60 days interval that is on 0 day, 60th day and 120th day. The average blood glucose level of Sahiwal calves of the control group (T_0) and treatment group (T_1) were 69.81±9.83 and 78.73±6.96, 37.03±2.42 and 52.55±7.98 and 31.23±1.74 and 38.94±5.60 mg/dl at 0 day, 60th day and 120th day respectively. No significant (p>0.05) differences was found between control (T_0)) and treatment (T_1) for serum glucose level irrespective of treatment, period and interaction between treatment and period.

The result of the current study is in agreement with the findings of Balamurgan *et al.* (2014) [4] observed a non-significant effect of garlic on the serum glucose level in crossbred calves. Chaves *et al.* (2008) [7] reported a similar finding in lambs and Anassorri *et al.* (2015) [3] in sheep.

Contrary to the present findings, Singh *et al.* (2017) [27] found a significant (p<0.05) decrease in serum glucose levels in broiler chicks fed 2% garlic in their diet. While Pirmohammadi *et al.* (2014) [21] reported a significant (P<0.05) increase in serum glucose concentration in pre-partum mahabadi goats fed raw garlic. Kholif *et al.* (2012) [17] observed a significant (p<0.05) improvement in serum glucose concentrations in lactating goats and Zakeri *et al.* (2014) [31] in dairy goats when garlic was supplemented in their diet.

Total Protein (gm/dl)

The serum serum total protein was estimated at the 60 days interval that is on 0 day, 60th day and 120th day. The average total protein level of Sahiwal calves of the control group (T_0) and treatment group (T_1) were 5.07±0.12 and 5.42±0.16 at 0 day, 6.85±0.40 and 7.24±0.41 60th day and 7.48±0.37 and 8.40±0.28 at 120th day.

The difference observed was statistically significant (P<0.05) between the control group (T_0) and treatment group (T_1) and highly significant (p<0.01) between periods and non-significant when there is interaction between treatment and period.

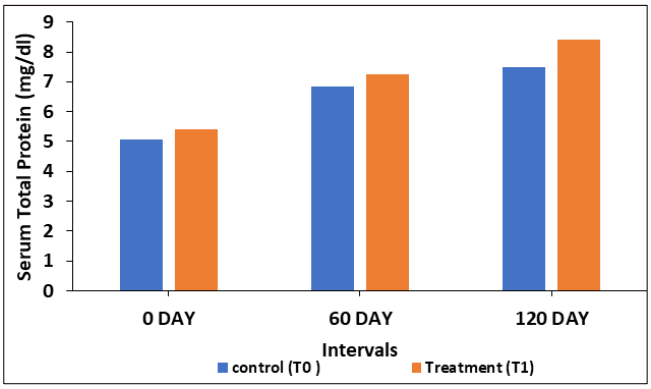

Fig 4: Average serum total protein (mg/dL) of Sahiwal calves

The findings of the current study are in agreement with that of Hasan *et al.* (2015) [14] and Duvvu *et al.* (2018) [10], who observed a significant (P<0.05) increase in total protein levels in growing buffalo calves fed garlic in the diet. Kholif *et al.* (2012) [17] found increased total protein levels in lactating goats.

In contrast to the present findings, Balamurugan *et al.* (2014) [4] found that garlic supplementation had no effect on total protein levels in crossbred calves. Amin *et al.* (2014) reported similar findings in grazing lambs, Anassori *et al.* (2015) [3] in sheep, and Pirmohammadi *et al.* (2014) [21] in pre-partum mahabadi goats. Chen *et al.* (2008) [8] and Yan *et al.* (2011) [10] also found that garlic supplementation in the diet had no effect on total protein levels in finishing pigs. Furthermore, Fadlalla *et al.* (2010) [12] in broilers in laying hens found no

significant difference in total protein levels when garlic was supplemented in the diet.

Hemoglobin (g/dL)

The hemoglobin was estimated at the 60 days interval that is on 0 day, 60th day and 120th day. Overall average hemoglobin in control and treatment groups were 8.54±0.33 and 10.74±0.40 g/dL respectively. The average hemoglobin level of Sahiwal calves of the control group (T_0) and treatment group (T_1) were 9.75±0.44 and 10.04±0.65 g/dL at 0 day, 8.42±0.05 and 10.62±0.10 g/dL at 60th day and 9.50±0.09 and 11.56±0.05 g/dL at 120th day.

The difference observed was statistically highly significant ($P<0.01$) between the control group (T_0) and treatment group (T_1) at 60th, 120th day and overall average.

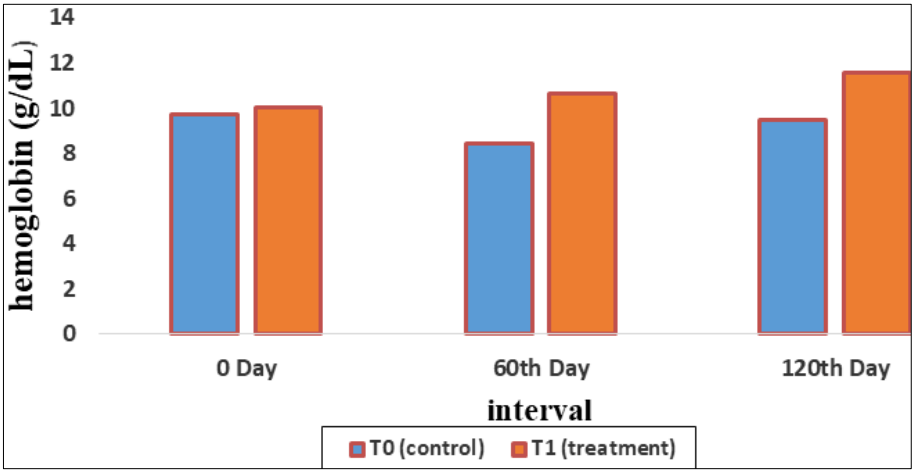

Fig 5: Average hemoglobin (g/dl) of Sahiwal calves

Garlic extract, according to Iranloye *et al.*, (2002) [32], is an active oxygen scavenger. It is thus possible that garlic components compete for oxygen with hemoglobin in RBCs, causing hypoxia and stimulating hemoglobin synthesis and RBC production.

The results of the current study are in support of the findings of Hasan *et al.* (2015) [14] and Shokrollahi *et al.* (2016) [26] noted a significant increase in the hemoglobin levels in Black bengal goats and new born goat kids respectively, on garlic supplementation in the diet. Duvvu *et al* (2018) [10] also found similar results with garlic powder supplementation on Murrah buffalo calves and crossbred female calves. Similar findings were reported by Toghyani *et al*. (2011) [28] ibroiler. However, Balamurugan *et al.* (2014) [4] observed no significant increase in the hemoglobin levels in crossbred jersey calves supplemented with garlic powder in the feed. Similar findings were also reported by Chen *et al.* (2008) [8] in finishing pigs in broiler birds.

RBC (m/mm^3)

The RBC was estimated at the 60 days interval that is on 0 day, 60th day and 120th day. Overall average hemoglobin in control group (T_0) and treatment group (T_1) were 8.33±0.23 and 9.94±0.04 m/mm^3 respectively. The average hemoglobin level of Sahiwal calves of the control group (T_0) and treatment group (T_1) were 7.16±0.04 and 8.15±0.02 m/mm^3 at 0 day, 8.36±0.05 and 10.14±0.03 m/mm^3 at 60th day and 9.46±0.03 and 11.53±0.02 m/mm^3 at 120th day. The difference observed was statistically highly significant ($P<0.01$) between the control group (T_0) and treatment group (T_1) at 60th, 120th day and overall average.

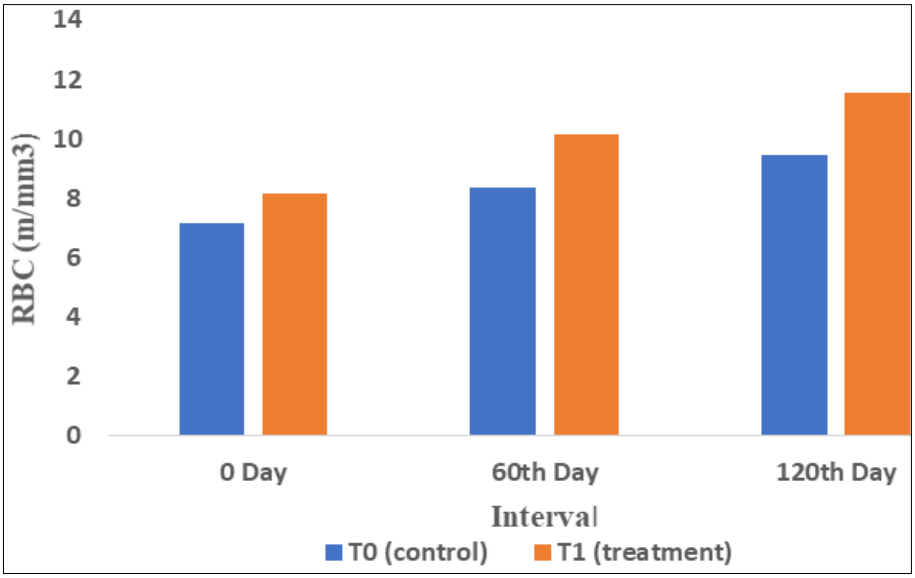

Fig 6: Average RBC (m/mm^3) of Sahiwal calves

Garlic extract, according to Iranloye *et al*., (2002) [32], is an active oxygen scavenger. It is thus possible that garlic components compete for oxygen with haemoglobin in RBCs, causing hypoxia and stimulating haemoglobin synthesis and RBC production. Garlic components boost the immune system and improve the function of organs involved in blood cell formation, such as the thymus, spleen, and bone marrow (Li *et al.*, 2002) [19]. As a result, garlic had blood-promoting effects, as evidenced by an increase in erythrocyte count.

In accordance with the current findings, Shokrollahi *et al*. (2016) [26] and Hasan *et al*. (2015) [14] found a significant ($P<0.01$) increase in RBC count in newborn goat kids and Black Bengal goats supplemented with garlic extract. Duvvu *et al.*, (2018) [10] also found similar results with garlic powder supplementation on Murrah buffalo calves. Toghyani *et al.* (2011) [28] found similar results in broilers.

In contrast to the current findings, Balamurugan *et al.* (2014) [4] found no significant improvement in RBC count in crossbred calves fed garlic powder. Amin *et al*. (2014) [2] discovered that garlic supplementation had no effect on RBC count in grazing lambs. This was supported by Elagib *et al.* (2013) [11] findings in broilers.

The current findings contradict those of Chen *et al*. (2008) [8] and Yan *et al.* (2011) [28], who found a reduction in erythrocytes in growing pigs fed a garlic-enriched diet.

Conclusion

From the above findings, it could be summarily concluded that supplementing garlic powder at a dosage of 250 mg per kg body weight were economic and it has a positive impact on total protein level, hemoglobin level and RBC level, while does not had any impact on serum Blood glucose. However, Garlic supplementation increased cost of feeding of calves, but, the cost of feeding per kg body weight gain was less than the control group.

Conflict of interest

There is no conflict between the authors regarding the preparation of the manuscript. All authors contributed to the research woks and members of the research advisory committee. All authors read and approved the final manuscript.

Ethics approval

The research was carried out according to the guidelines of Institutional Animal Ethics Committee of Assam Agricultural University, Khanapara, Guwahati-781022 as per the ethics application approval number 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/21-22/925 dated 20.08.2022.

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