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Effect of Garlic Supplementation on the Blood Biochemical Profile of Murrah Buffalo Calves

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A B S T R A C T

The present study was carried out to investigate the effect of different levels of garlic supplementation on the blood biochemical profile of Murrah buffalo calves. In a CRD model, 18 murrah buffalo calves in the age group of 4-5 months were randomly divided into three groups (T_0, T_1 and T_2) with six calves in each group. The T_0 group served as the control whereas the T_1 and T_2 groups were supplemented with garlic powder at the dose rate of 250 and 300 mg per kg body weight, respectively for a period of 90 days. The results of the study revealed a significant (P<0.01) increase in the total protein, albumin, globulin and HDL-cholesterol levels and a significant (P<0.01) decrease in the blood glucose level, serum cholesterol level and SGOT levels in the garlic supplemented calves compared with the control group calves. In addition to this, no significant difference was observed between the garlic supplemented group calves and control group calves with respect to serum calcium, serum phosphorus and blood urinary nitrogen (BUN) values. Thus, by this study we can conclude that dietary supplementation of garlic improved the total protein, serum albumin, serum globulin and HDL-cholesterol values and decreased the serum glucose, serum cholesterol and SGOT values in the murrah buffalo calves.

Introduction

Blood biochemical parameters are major indices of the physiological, pathological and nutritional status of an animal and a slight change in the values when compared to the normal values could be used to interpret the metabolic state as well as the health status of the animals (Mitruka and Rawnshey, 1977; Babatunde *et al.*, 1992; Lamorde, 1996; Ramprabhu *et al.*, 2010). Adulugba and Joshua *et al.*, (1994) have opinion that it is often difficult to asses the correct health status of an animal without recourse to its blood examination. The serum biochemical profile of animals is influenced by the quantity and quality feed supplements being offered and also by the level of anti-nutritional factors present in the feed (Oyawoye and Ogunkunle, 1998; Akinmutimi, 2004).

In recent years, dairy farmers worldwide are incorporating feed additives in the animals' diet to enhance the performance and health status of the calves. With the ban on the use of antibiotics by the European Commission, the livestock scientists have shifted their attention towards herbal feed additives/ herbal growth promoters (Jayasena and Jo, 2013). Most of the herbal feed additives belong to the class of isoprene derivatives, flavonoides and glucosinolates which act as natural antibiotics or anti-oxidants (Rhodes, 1996 or Hirasa and Takemasa, 1998). In farm animals, herbal growth promoters serve as appetizers, digestive stimulants, antibacterial, antiviral, antihelmintic, anti-inflammatory and also possess immuno-stimulation properties (Platel *et al.*, 2002; Wenk, 2003).

Garlic (*Allium sativum*) has been a subject of considerable interest as a medicinal and therapeutic agent globally since ancient times. It was used as a remedy for intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of ageing and many other ailments. The main pharmacological effects of garlic are attributed to 'allicin', an organosulphur compound that exhibits antibacterial (Lanzotti, 2006; Toghyani *et al.*, 2011), antifungal, antiparasitic, antiviral (Ankri and Mirelman, 1999), antioxidant (Banerjee *et al.*, 2003; Lee *et al.*, 2009), hypocholesterolemic (Gupta and Porter, 2001), hypoglycemic and hypotensive action.

The hypoglycemic property of garlic was reported by Kumar and Reddy (1999) in rats and Singh *et al.*, (2017) in broilers. Further the hypolipidemic property of garlic was demonstrated by Omojola *et al.*, (2009) in pigs and Prasad *et al.*, (2009) in broilers. Also, Garlic has the capacity to increase the total protein, albumin and globulin concentrations as evident by the findings of Hassan *et al.*, (2013) in growing buffalo calves.

However, there is lack of convincing information on the effect of garlic supplementation on the blood biochemical profile in murrah buffalo calves. Hence it is against this background that the present study was conducted to evaluate the effect of varying levels of garlic powder supplementation on the blood biochemical profile of murrah buffalo calves.

Materials and Methods

Experimental location

The present experiment was carried out at Buffalo Research Station, Venkataramannagudem, West Godavari, Andhra Pradesh. This research station is located at 16.49´ N latitude and 81.30´E and 18metres above the mean sea level.

Experimental animals and experimental design

The present study was carried out on 18 Murrah buffalo calves of 4-5 months age, which were randomly allotted into 3 groups (T_0, T_1 and T_2) of 6 animals in each group based on their body weight and sex in Completely Randomized Design.

The calves in the T_0 group served as control and the calves in the T_1 and T_2 group were supplemented with garlic powder at the dose rate of 250 and 300 mg per kg body weight, respectively in their concentrate feed. The experiment was conducted for a period of 90 days.

Housing and feeding management

All the experimental calves were housed individually in a well-ventilated shed with a provision for individual feeding and water facilities.

The calves were fed with chopped green fodder (Hybrid Napier) and concentrate mixture (calf grower) as the basal diet as per their body requirements.

Preparation of garlic powder

Garlic bulbs were purchased from the local market and were dried under the shade for a period of 15 days.

After drying, the outer husks were removed and the bulbs were ground to fine powder by using electrical mixer.

Health management

All the experimental calves were dewormed 10 days prior to the beginning of the experiment with Fenbendazole at the dose rate of 10 mg per kg body weight and were vaccinated with Foot and Mouth disease vaccine during the second month of the experimental trial.

Blood collection

The blood samples were collected acseptically from the jugular vein of the calves. About 3 ml of blood is collected from each calf for serum biochemical analysis into clean vaccutainers.

This whole blood is allowed to clot by keeping it in slant position at 45° angle for 30 minutes and then on centrifuging it at 4500 rpm for 10 minutes, clear serum is obtained. This serum is used for estimating the biochemical profile of the experimental calves.

Biochemical analysis

The serum biochemical profile of the experimental calves was assessed at every fortnightly interval. Glucose, total protein, serum albumin, serum globulin, serum calcium, serum phosphorus, serum total cholesterol, High density lipoprotein (HDL) cholesterol, Blood urinary nitrogen (BUN) and Serum glutamic-oxoloacetic transaminase (SGOT) were estimated using ERBA kits and by using 'Spectrophotometer' (MULTISKAN GO, Thermoscientific, Japan).

Analysis of data

The data was analysed for significant differences among the groups for biochemical parameters using ANOVA and Post-hoc tests as implemented in SPSS version 16.0 (SPSS, 2008).

Results and Discussion

The results of the current study revealed a significant ($P<0.01$) decrease in the blood glucose levels in garlic supplemented calves compared with the control group (Table 1). This hypoglycaemic effect might be attributed to an increase in the pancreatic secretion of insulin from the beta cells (Jain and Vyas., 1975; Sodimu *et al.,* 1984). Banerjee and Maulik (2002) revealed that allyl propyl disulphide-one of the active ingredients of garlic, may lower glucose by competing with insulin which results in increased free insulin. This increased insulin secretion causes an increase in the nitric oxide production and significantly decrease the inflammatory cytokines, glycosylated haemoglobin, post prandial blood glucose and fasting blood glucose level (Kumar *et al.*, 2013). Vazquez-Prieto *et al.,* (2011) and Ademiluyi *et al.,* (2013) have opinion as S-allyl cysteine sulfoxide present in garlic is responsible for its hypoglycaemic activity.

These results were supported by the findings of Kumar and Reddy (1999) in mice and Singh *et al.,* (2017) in broilers. Contrary to the present findings, increase in the serum glucose concentration on garlic supplementation was reported by Pirmohammadi *et al.,* (2014) in pre partum mahabadi goats and Kholif *et al.,* (2012) in lactating goats. This increase in the serum glucose concentration might be due to enhancement of gluconeogenesis process by the garlic in the diet. However, Balamurugan *et al.,* (2014) in crossbred calves and Chaves *et al.,* (2008) in sheep did not notice any significant effect in the serum glucose levels on garlic supplementation in the diet.

Significant (P<0.01) improvement in the serum total protein, albumin and globulin values was noticed in garlic supplemented calves over the control group calves (Table 2, 3 and 4 respectively). The increase in the total protein level with relative increase in the albumin and globulin level may be attributed to the organosulphur compounds present in garlic and to their hepato-protective action (Ajayi *et al.,* 2009).

These results were supported by the findings of Hassan *et al.,* (2013) and Alagawany *et al.,* (2016) in growing buffalo calves and rabbits respectively. Contrary to the present findings, garlic supplementation resulted in no significant improvement in the serum total protein, serum albumin and serum globulin levels in crossbred calves (Balamurugan *et al.,* 2014), grazing lambs (Amin *et al.,* 2014) and pigs (Chen *et al.,* 2008 and Yan *et al.,* 2011).

The results showed a significant (P<0.01) decrease in the serum cholesterol levels in garlic supplemented calves as compared with the control group (Table 5). This might be due to the inhibition of hepatic cholesterol synthesis. Garlic has hypocholesterolaemic action which is exhibited by depressing the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl-CoA (HMG CoA) reductase (Qureshi *et al.,* 1983, Mahmoud *et al.,* 2010). This reduction in the serum cholesterol suggests the ability of garlic to lower some of the risk factors associated with the development of cardiovascular diseases in animals (Ademola *et al.,* 2009).

Pirmohammadi *et al.,* (2014) reported that garlic supplementation in the diet resulted in a decrease in the serum cholesterol level in mahabadi goats. Similar findings were observed by Omojola *et al.,* (2009) and Yan *et al.,* (2011) in weanling pigs and Rahimi *et al.,* (2011) in broilers. However, garlic supplementation resulted in no significant decrease in the serum cholesterol levels in sheep (Anassori *et al.,* 2015) and broilers (Carrijo *et al.,* 2005).

The HDL-cholesterol levels were significantly (P<0.01) increased in the garlic supplemented calves as compared with the non-supplemented calves (Table 6). This increase might be due to the hypocholesterolaemic mechanism and the hypolipidemic action of garlic powder. 'Allicin' present in garlic combines with the –SH (sulphadryl) group that is important in activation of Acetyl CoA which is essential for the biosynthesis of cholesterol (Puvaca *et al.,* 2014). The current results are in agreement with the findings of Mirhadi *et al.,* 1991 in rabbits, Rahimi *et al.,* (2011) in broilers and Faisal Irshad *et al.,* (2017) in male albino rats. Contrary to the present findings, decrease in the HDL-cholesterol levels on garlic supplementation was reported by Azza*et al.,* (2012) in Zaraibi goats. However, no significant effect on the HDL-cholesterol levels on garlic supplementation was observed by Canogullari *et al.,* (2009) and Ao. X *et al.,* (2011) in laying hens and broilers, respectively.

Table.1 The Mean ± SE and analysis of variance of blood glucose levels of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (mg/dl)	T_1 group (mg/dl)	T_2 group (mg/dl)
0 day	85.68 ± 3.87	92.33 ± 4.47	94.56 ± 5.58
90th day **	113.39 ± 5.55[a]	71.62 ± 5.89 [b]	73.82 ± 5.88[b]

Means with similar superscript doesn't differ significantly
** indicate P value < 0.01

Table.2 The Mean ± SE and analysis of variance of total protein values of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (g/dl)	T_1 group (g/dl)	T_2 group (g/dl)
0th day	4.63 ± 0.14	4.74 ± 0.26	4.97 ± 0.10
90th day **	7.28 ± 0.06 [a]	10.62 ± 0.10 [b]	11.53 ± 0.06 [c]

Means with similar superscript doesn't differ significantly
** indicate P value < 0.01

Table.3 The Mean ± SE and analysis of variance of albumin levels of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (g/dl)	T_1 group (g/dl)	T_2 group (g/dl)
0 day	2.31 ± 0.15	2.28 ± 0.07	2.09 ± 0.06
90th day **	3.68 ± 0.07 [a]	4.81 ± 0.04 [b]	5.01 ± 0.05 [c]

Means with similar superscript doesn't differ significantly
** indicate P value < 0.01

Table.4 The Mean ± SE and analysis of variance of globulin levels of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (g/dl)	T_1 group (g/dl)	T_2 group (g/dl)
0 day	2.32 ± 0.13	2.45 ± 0.23	2.88 ± 0.12
90th day **	3.60 ± 0.10 [a]	5.80 ± 0.09 [b]	6.53 ± 0.06 [c]

Means with similar superscript doesn't differ significantly
** indicate P value < 0.01

Table.5 The Mean ± SE and analysis of variance of serum cholesterol levels of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (mg/dl)	T_1 group (mg/dl)	T_2 group (mg/dl)
0 day	87.36 ± 5.52	92.44 ± 4.98	89.73 ± 6.07
90th day **	121.56 ± 4.57[a]	72.25 ± 3.98 [b]	69.39 ± 3.24 [b]

Means with similar superscript doesn't differ significantly
** indicate P value < 0.01

Table.6 The Mean ± SE and analysis of variance of HDL-cholesterol levels of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (mg/dl)	T_1 group (mg/dl)	T_2 group (mg/dl)
0 day	41.12 ± 2.31	42.44 ± 1.92	40.27 ± 3.22
90th day **	124.44 ± 2.37[a]	177.97 ± 2.53 [b]	186.54 ± 0.91 [c]

Means with similar superscript doesn't differ significantly
** indicate P value < 0.01

Table.7 The Mean ± SE and analysis of variance of BUN values of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (mg/dl)	T_1 group (mg/dl)	T_2 group (mg/dl)
0 day	28.91 ± 2.86	24.54 ± 1.36	25.14 ± 0.84
90th day	41.80 ± 5.33	43.09 ± 4.32	35.04 ± 5.28

Table.8 The Mean ± SE and analysis of variance of SGOT values of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (I.U)	T_1 group (I.U)	T_2 group (I.U)
0 day	106.32 ± 5.38	109.45 ± 5.98	104.28 ± 6.11
90th day **	119.56 ± 5.86 [a]	76.69 ± 5.32 [b]	74.45 ± 5.79 [b]

Means with similar superscript doesn't differ significantly
** indicate P value < 0.01

Table.9 The Mean ± SE and analysis of variance of serum calcium levels of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (mg/dl)	T_1 group (mg/dl)	T_2 group (mg/dl)
0 day	8.64 ± 0.36	8.28 ± 0.23	8.97 ± 0.48
90th day	12.08 ± 0.54	12.11 ± 0.50	12.36 ± 0.56

Table.10 The Mean ± SE and analysis of variance of serum phosphorus levels of murrah buffalo calves supplemented with different levels of garlic in the diet

Time Interval	T_0 group (mg/dl)	T_1 group (mg/dl)	T_2 group (mg/dl)
0 day	4.32 ± 0.22	4.27 ± 0.28	4.39 ± 0.35
90th day	6.47 ± 0.31	6.49 ± 0.31	6.54 ± 0.32

The results of the current study showed there exist no significant difference in the BUN values between the garlic supplemented calves and non-supplemented calves (Table 7). The current results are supported by the findings of Amin *et al.,* (2014) in grazing lambs, Anassori *et al.,* (2015) in sheep and Balamurugan *et al.,* (2014) in crossbred calves.

Contrary to the present findings, Onu and Aja (2011) in rabbits, reported a significant (P<0.01) increase in the BUN value on garlic supplementation in the diet. Likewise, a significant (P<0.01) decrease in the BUN value on garlic supplementation was reported by Ghalehkandi *et al.,* (2012) in rats.

Garlic supplementation resulted in a significant (P<0.01) decrease in the SGOT levels in the garlic treated calves as compared with the control group calves (Table 8). Serum glutamic-oxoloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) enzymes are a relatively specific indicator of acute liver cell damage and pathological manifestation of liver dysfunction. They are released into the blood stream only when the liver is damaged. The organosulphur compounds present in garlic decrease the SGOT and SGPT levels, thus, indirectly protecting the liver from any damage and maintaining its integrity and function.

Alagawany *et al.,* (2016) in rabbits, also reported a decrease in the SGOT levels on garlic supplementation in the diet. However, no positive influence of garlic supplementation on the SGOT levels was observed by El-katcha *et al.,* (2016) and Onyimonyi *et al.,* (2013) in fattening lambs and pigs, respectively.

No significant difference was observed in the serum calcium and phosphorus level in garlic treated calves over the control group calves (Table 9 and 10, respectively). This might be due to the inability of garlic to exert its effect on the calcium and phosphorus regulation mechanisms in the body. Similar results were observed by El-katcha *et al.,* (2016), Pirmohammadi *et al.,* (2014) and Zakeri *et al.,* (2014) in fattening lambs, pre-partum goats and lactating goats, respectively.

Based on the above results, it can be concluded that dietary supplementation of garlic powder either at the dose rate of 250 mg or 300 mg per kg body weight has a significant effect on the blood biochemical profile in murrah buffalo calves.

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