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Original article

TRIALS OF DIARRHOEA TREATMENT IN BUFFALO CALVES BY GARLIC POWDER COMPARED TO TRADITIONAL TREATMENT

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Summary

Yousif, H. M., A. M. El Mahdy, R. A. A. Rezk, E. A. M. El-Kelesh & M. K. Mansour, 2024. Trials of diarrhoea treatment in buffalo calves by garlic powder compared to traditional treatment. *Bulg. J. Vet. Med.* (online first).

Diarrhoea in veal calves causes serious problems, so more effective treatments and faster responses are required. This work aimed to investigate the efficacy of garlic powder in treatment of diarrhoea compared to the traditional treatment in buffalo calves. Ten apparently healthy buffalo calves were used as a control group (G1), ten diarrhoeic buffalo calves were treated with garlic powder (G2) and ten diarrhoeic buffalo calves were treated with nitazoxanide and colitrim (G3). Whole blood and serum samples were obtained on day 0, and 3rd, 5th, and 7th days post-treatment. The clinical picture of diarrhoeic calves showed white to yellowish, greenish profuse watery diarrhoea, and inability to stand with dermatological changes. The bacteriological examination isolated *E. coli* from anal swabs, and *Cryptosporidium* was detected by faecal parasitological examination. Blood lysate and serum biochemical analyses revealed significantly decreased SOD, CAT, TAC and GSH with significantly increased MDA, associated with decreased total protein, albumin, globulin, vitamin E, selenium, Ca, iCa, Ph, Mg, Cu, Fe, Na, Cl, and significantly increased LDH, CK, lipase, amylase and potassium levels of diarrhoeic calves on day 0 compared to G1. Most of biochemical parameters were completely recovered in G2 (treated with garlic powder) from the 5th day of treatment and some parameters were more improved compared to G1. Calves in G3 that received traditional treatment recovered on the 7th day of treatment but the values of some parameters were less improved than those in G2. Therefore, garlic powder is a very effective treatment of diarrhoeic buffalo calves, improving antioxidants, immunity, enzyme activity and trace elements.

Key words: buffalo calves, *Cryptosporidium*, diarrhoea, garlic powder, serum biochemistry

INTRODUCTION

Diarrhoea is considered one of the most common illnesses of young calves and cause of calves' deaths mainly in the first three weeks of life (Birhan *et al.,* 2019). Calf diarrhoea may result from numerous non-infectious (environmental conditions,

inadequate nutrition and management defects) or infectious causes such as bacteria, viruses and protozoa (El-Seadawy *et al.,* 2020). Many of enteric pathogens as viruses (rotavirus and coronavirus), bacteria (*E. coli* and *Salmonella*), and protozoa (*Cryptosporidium* and *Giardia*) are intertwined in developing diarrhoea alone or in combination (Delgado-González *et al.,* 2019; Mullusew *et al.,* 2020). *Cryptosporidium* is amongst the most common cause of diarrhoea in newly born and young calves (Elkelesh *et al.,* 2023).

Diarrhoeic calves show fluid and electrolyte loss, rapid dehydration, and acidosis which may result in death (Radostits *et al.,* 2007). Electrolyte loss, acidosis, and hypovolemia resulting from diarrhoea may lead to kidney failure and heart block due to hyperkalemia (Taylor *et al.,* 2017). Elevated liver enzymes, amylase and lipase, with decreased total protein, albumin, sodium, potassium, and chloride were recorded in diarrhoeic calves (Saleh *et al.,* 2022).

Calf diarrhoea treatment depends mainly on causative agents. Administration of antimicrobials with oral anti-inflammatory rehydration therapy solutions, parenteral vitamins may be necessary for calves with chronic diarrhoea. There is no evidence supporting the use of motility modifiers, immune stimulants, intestinal protectants or probiotic substances in diarrhoeic calves' treatment. Using trimethoprim/sulfonamide administration is permitted to treat calves with *E. coli* diarrhoea (Constable, 2009).

Garlic has been used as a therapeutic agent worldwide since ancient times. Garlic physiological effects are due to organosulphur containing compounds as well as polyphenols (flavonoids), minerals (Ca, Fe, I, K, Mg, Na, Zn) and vitamins (A, E, C, and B complex) (Kovarovič *et al.,* 2019). Garlic contains many nutritional components such as carbohydrates, organosulphur compounds, proteins, free amino acids, vitamins and trace elements (Zhang *et al.,* 2018). Previous studies reported that garlic has anti-parasitic, anti-protozoal and antiviral effects (Ankri & Mirelman, 1999). The biological functions of garlic include antioxidant, cardiovascular protective, anti-inflammatory, immunomodulatory, and antibacterial properties. Those actions are mediated through the organosulphur compounds: S-allyl cysteine (SAC) has antioxidant and anti-inflammatory effects , and diallyl disulfide (DADS) is the main breakdown product of allicin and contributes to garlic anti-inflammatory and cholesterol-lowering effects (Lee *et al.,* 2012). Antioxidants in garlic extract prevent oxidative damage through the suppression of damage risk to vital molecules and prevent the disease occurrence and progression (Gutteridge, 1993).

Multiple therapeutic substances could be used for treatment of cryptosporidiosis in ruminants. Calf diarrhoea was treated by nitazoxanide within 3 or 4 days of initiation (Rossignol *et al.,* 2001). Garlic has a promising effect in treatment of diarrhoeic calves and was recommended to be used in the animal diet on a daily basis to give a protective effects against parasites. This means that garlic and nitazoxanide treatments were effective not only in eliminating oocyte counts, but also in making the animal health better (Abdel Megeed *et al.,* 2015).

This study aimed to overview the efficacy of garlic powder for treatment of diarrhoea in buffalo calves compared to the traditional treatment.

MATERIALS AND METHODS

Ethical approval

This study protocol was approved by the Research Committee of the Animal Health Research Institute (AHRI) and authorised by The Institutional Animal Care and Use Committee (ARC-IACUC)/Agricultural Research Center (ARC/AHRI/44/23).

Experimental design

The work was designed in a private buffalo farm. All calves in the farm received Bovine Rota-Coronavirus Vaccine immediately after birth. Thirty buffalo calves aged 2–4 months were used and divided into three groups. The 1st group of calves (G1, N=10) were apparently healthy and kept as a control group. The 2nd group (G2, N=10) diarrhoeic calves were orally treated by garlic powder at a dose of 150 mg/kg body weight (dissolved in 30 mL water) for 7 days continuously. The 3rd group (G3, N=10) diarrhoeic calves were treated by the combination of nitazoxanide at a dose of 50 mg/10 kg body weight orally for 5 days + sulphadiazine/ trimethoprim at a dose of 1 mL/15 kg BW slowly intravenously once daily for 5 days. All diarrhoeic calves received mineral element solution with drinking water at a dose of 2 mL/L drinking water during the period of treatment.

Chemicals and medications

Garlic powder was obtained from Imtenan herbal products. Nanazoxid® (nitazoxanide 100 mg/5 mL) antiseptic antiprotozoal, was manufactured by Utopia Pharmaceuticals. Colitrim®, each 1 mL containing sulphadiazine sodium 200 mg and trimethoprim 40 mg, was manufactured by PHARMA SWEDE Co. Calf-Guard® (Bovine Rota-Coronavirus Vaccine, Modified Live Virus), was manufactured by Zoetis Company.

Sampling

Garlic powder sample was sent to the laboratory for chemical composition analysis, performed according to AOAC (2005). Faecal samples were collected from calves with profuse watery diarrhoea in clean dry plastic bags, as well as from healthy calves and sent to the lab for parasitological examination. Anal swabs were collected from the diarrhoeic calves, preserved in ice box and send to the lab for bacteriological examination.

Two blood samples were collected from the jugular vein of all calves on treatment days 0, 3rd, 5th, and 7th. One of the blood samples was collected in tubes with anticoagulant, and the other sample: in tubes without anticoagulant for obtaining serum. Sera were obtained by centrifuging the blood samples at 5,000 rpm for 5 min. Clear sera were transferred into clean dry Eppendorf tubes and stored at –20 °C until biochemical analysis.

Parasitological examination

The procedure of Modified Ziehl-Neelsen (MZN) staining included three steps: carbol fuchsin, decolorisation, and counterstaining. A floated material of concentrated feacal smear from each sample was transferred to a glass slide and allowed to dry at room temperature. Following fixation with methanol (2 minutes), the slides were flooded with basic carbol-fuchsin for 5 minutes. Then the slides were washed with distilled water, and decolorised with 3% acid alcohol until red colour disappearance (45–60 s), and rinsed in tap water. Counter-staining with 0.5% malachite green was done for one minute, and then rinsed with tap water and air dried. The smear was examined microscopically for

the presence of oocytes using 40× and 100× oil immersion objective. *Cryptosporidium* oocytes appeared in red/pink colour versus the green background (Elkelesh *et al.,* 2023).

Bacteriological examination

The anal swab samples were prepared, cultured and identified according to Quinn *et al.* (2011). The prepared samples were incubated aerobically at 37 °C for 24–48 hours, then inoculated in the culture media. A loopful from the initial suspension was subcultured on both Xylose Lysine Deoxycholate (XLD) agar and MacConkey's agar (Oxoid, CM0115) and incubated aerobically at 37 °C for 24–48 h. The suspected colonies were isolated on nutrient slope agar (Lab M, LAB008) and incubated at 37 °C for 24–48 h for further identification (appearance on incubated plates, colony morphology, Gram staining and different biochemical tests).

Blood analyses

For lysate preparation and assays of antioxidant parameters, RBCs were separated from plasma by centrifugation, washed three times with saline and lysed. The lysate was mixed with an equal volume of Drabkin's reagent to determine haemoglobin levels (González-Arostegui *et al.,* 2022). Catalase activity (CAT), lipid peroxidation as malonaldehyde (MDA) and reduced glutathione (GSH) in lysed RBCs were determined according to Aebi (1984), Okhawa *et al.* (1979) and Beutler *et al.* (1963), respectively. Superoxide dismutase (SOD) was assayed according to Nishikimi *et al.* (1972). The obtained results were calculated as U/g Hg, U/g Hg, mmol/g Hg and nmol/g Hg, respectively, for SOD, CAT activities, GSH and MDA levels.

A commercial kit Cat. No. TA2513 was used for total antioxidant capacity (TAC) estimation according to Koracevic *et al.* (2001). Kits of Greiner Diagnostic GmbH, were used for lipase and amylase determination in serum. A special kit (BIODIAGNOSTIC Company, Egypt) was used for determination of lactate dehydrogenase (LDH) and creatine kinase (CK), according to the manufacturer instructions. Selenium (Se) level was determined by atomic absorption spectrophotometry. Elabscience colorimetric assay kits were used for detection of calcium (Ca), ionised calcium (iCa), inorganic phosphorus (Ph), sodium (Na), potassium (K), chloride (Cl), copper (Cu), magnesium (Mg). Vitamin E (α-tocopherol) concentrations were assayed by the method described by Roberts *et al.* (2018).

The serum total protein and electrophoretic protein pattern were estimated according to Sonnenwirth *et al.* (1980) and Davis (1964), respectively and calculated according to SynGene S. No. 17292*14518 sme*mpcs.

Statistical analysis

The data were statistically analysed using two-way (ANOVA) using SPSS 22 software to test both the treatment and time. The results were presented as means ± SEM and considered statistically significant at $P<0.05$.

RESULTS

The chemical analysis of garlic powder revealed protein 12.5%, moisture 10%, fibre 2%, fat 4.5%, ash 3.8%, organosulphur compounds 2.27%, Ca 1%, and Mg, Na, K, Fe, Zn and Cu of 7.4, 35.7, 80.6, 2.33, 1.7 and 0.51 mg/100 g respectively; Vitamin A 70 IU/g, vitamin E 0.5 mg/100

Fig. 1. Clinical picture of diarrhoeic buffalo calves: Greenish profuse watery diarrhoea (A); white to yellowish diarrhoea (B); soiling of the perineum with diarrhoeic faeces (C); a diarrhoeic buffalo calf unable to stand with dermatological changes (D).

g, vitamins B6, B1, and B2 of 0.93, 0.43, and 0.09 mg/100 g respectively.

The clinical picture of diarrhoeic calves showed white to yellowish and greenish profuse watery diarrhoea, soiling of the perineum and inability to get stand with some dermatological changes (Fig. 1). Microscopic faecal examination showed that *Cryptosporidium* oocytes isolated from the faeces of naturally infected calves were fully stained by modified Ziehl-Neelsen technique. The oocytes appeared as acid fast (red-pink) spherical to ovoid in shape against a green background (Fig. 2). *E. coli* was isolated after bacteriological examination of anal swabs, with large, flat, yellow colonies on Xylose Lysine Deoxycholate (XLD) agar and pink to dark pink colonies surrounded by dark pink area of PPT bile salt on MacConkey's agar.

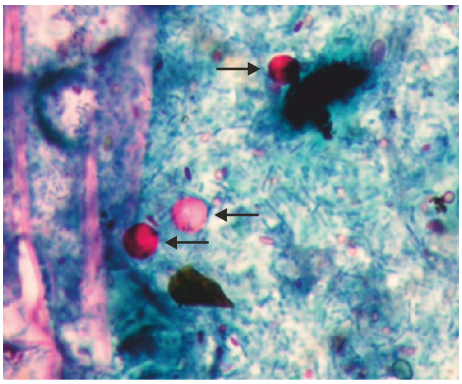

Fig. 2. Microscopical examination of a faecal sample. *Cryptosporidium* spp. oocytes in faecal smear are stained by modified Ziehl-Neelsen (1000×).

The analysis of blood lysate and serum antioxidants (Table 1) revealed significantly ($P<0.05$) decreased SOD, CAT, TAC, and GSH associated with significantly ($P<0.05$) increased MDA of the diarrhoeic calves on day zero compared to control healthy calves. The calves treated with garlic powder (G2) showed a gradual increase of SOD and TAC with non-significant differences on the 5th day compared to control healthy group (G1) and significant ($P<0.05$) increase on the 7th day compared to G1. In the same time, they were significantly ($P<0.05$) increased from the 3rd to 7th day in comparison with calves that received the traditional treatment (G3). The calves in G2 showed non-significant changes of CAT and GSH from the 3rd day compared to G1, and significant ($P<0.05$) increase on the 3rd and 5th day only compared to G3. On the other hand, there was a gradual decrease in MDA level of G2 with non-significant differences from the 5th day compared to G1, and significant reduction ($P<0.05$) on the 3rd and 5th day compared to G3 only.

Serum enzymes results (Table 1) of diarrhoeic calves on day 0 revealed a significant ($P<0.05$) increase in amylase, lipase, LDH and CK compared to control healthy calves. The calves treated with garlic powder (G2) showed a gradual decrease in all enzymes until insignificant differences from the 5th day for amylase and CK levels and on the 7th day for lipase and LDH in comparison with control healthy group (G1). On the other hand, calves in G2 showed significantly ($P<0.05$) decreased amylase and lipase levels all over the period of treatment with a significant ($P<0.05$) decrease on the 5th day only of LDH and CK levels in comparison with calves receiving the traditional treatment (G3).

Serum vitamin E and selenium (Se) (Table 1) of diarrhoeic calves on day 0 were significantly ($P<0.05$) decreased compared to control healthy calves. Calves in G2 showed non-significant differences in vitamin E level on the 3rd and 5th day and considerable ($P<0.05$) increase on the 7th day, associated with non-significantly different selenium level on the 5th day and a significantly ($P<0.05$) increased one on the 7th day compared to G1. However, compared to G3 calves, both parameters of G2 calves showed a substantial ($P<0.05$) increase over the entire treatment period.

The results of serum minerals and trace elements (Table 2) of diarrhoeic calves on day 0 revealed significantly ($P<0.05$) decreased levels of calcium (Ca), ionised calcium (iCa), phosphorus (Ph), magnesium (Mg), copper (Cu), iron (Fe), sodium (Na), chloride (Cl) and significantly ($P<0.05$) increased potassium (K) compared to control healthy calves. There was a complete recovery of all minerals and trace elements in calves treated with garlic powder (G2). Compared to the control healthy group (G1), the differences became insignificant from the 5th day onward. However, the recovery in diarrhoeic calves that received traditional treatment (G3) lasted to the 7th day of the experiment for all parameters.

The results of total protein and protein electrophoretic patterns (Table 3) of diarrhoeic calves on day 0 revealed significantly ($P<0.05$) decreased total protein, albumin, globulin, $\alpha1$, $\beta2$, $\gamma1$, $\gamma2$ globulin and significantly ($P<0.05$) higher $\alpha2$ and $\beta1$ globulin compared to control healthy calves. In relation to control healthy calves (G1), diarrhoeic calves treated with garlic powder (G2) showed a gradual increase in total protein and albumin with inconsistent differences on the 5th day and

Table 1. Results of serum antioxidants, enzymes, vitamin E and selenium. Data are expressed as mean±SE of 10 samples

Parameters	Time	Groups		
		Control healthy group (G1)	Treated with garlic powder (G2)	Treated with Nitazoxa-nide+Colitrim (G3)
SOD (U/g Hb)	0 day	5.382±0.354 ^{a,1}	4.234±0.367 ^{b,1}	4.252±0.351 ^{b,1}
	3rd day	5.354±0.368 ^{a,1}	4.904±0.334 ^{b,2}	4.536±0.346 ^{c,1,2}
	5th day	5.408±0.341 ^{a,1}	5.432±0.304 ^{a,3}	4.912±0.343 ^{b,2}
	7th day	5.452±0.355 ^{b,1}	6.422±0.313 ^{a,4}	5.438±0.327 ^{b,3}
CAT (U/g Hg)	0 day	29.254±1.875 ^{a,1}	22.108±1.809 ^{b,1}	22.246±1.789 ^{b,1}
	3rd day	29.174±1.86 ^{a,1}	29.03±1.935 ^{a,2}	24.104±1.899 ^{b,1}
	5th day	29.818±1.804 ^{a,1}	30.474±1.913 ^{a,2}	27.114±1.819 ^{b,2}
	7th day	30.066±1.704 ^{a,1}	31.068±1.936 ^{a,2}	30.074±1.805 ^{a,3}
TAC (mU/L)	0 day	1.806±0.121 ^{a,1}	1.062±0.128 ^{b,1}	1.068±0.132 ^{b,1}
	3rd day	1.798±0.124 ^{a,1}	1.594±0.124 ^{b,2}	1.394±0.129 ^{c,2}
	5th day	1.818±0.112 ^{a,b,1}	1.922±0.103 ^{a,3}	1.682±0.113 ^{b,3}
	7th day	1.866±0.116 ^{b,1}	2.452±0.167 ^{a,4}	1.874±0.118 ^{b,3}
GSH (mmol/g Hg)	0 day	35.866±1.973 ^{a,1}	23.936±2.702 ^{b,1}	24.798±2.462 ^{b,1}
	3rd day	36.016±1.972 ^{a,1}	35.396±2.572 ^{a,2}	26.446±2.912 ^{b,1}
	5th day	35.898±2.056 ^{a,1}	36.788±2.247 ^{a,2}	30.964±2.334 ^{b,2}
	7th day	35.932±2.01 ^{a,1}	37.352±2.373 ^{a,2}	36.134±1.832 ^{a,3}
MDA (nmol/g Hg)	0 day	6.28±0.48 ^{b,1}	10.156±0.769 ^{a,1}	10.036±0.665 ^{a,1}
	3rd day	6.314±0.457 ^{c,1}	8.04±0.66 ^{b,2}	9.49±0.43 ^{a,1}
	5th day	6.296±0.434 ^{b,1}	6.304±0.476 ^{b,3}	7.774±0.582 ^{a,2}
	7th day	6.33±0.413 ^{a,1}	6.276±0.46 ^{a,3}	6.354±0.495 ^{a,3}
Amylase (U/L)	0 day	460.4±36.7 ^{b,1}	647.6±62.4 ^{a,1}	623.6±60.4 ^{a,1}
	3rd day	469±34.04 ^{b,1}	564.8±55.48 ^{a,2}	604.4±55.9 ^{a,1,2}
	5th day	478.6±30.24 ^{b,1}	514.4±44.39 ^{b,2,3}	587.2±54.6 ^{a,1,2}
	7th day	479.4±31.8 ^{b,1}	484±30.29 ^{b,3}	549.6±39.4 ^{a,2}
Lipase (U/L)	0 day	1.762±0.164 ^{b,1}	4.9±0.715 ^{a,1}	4.868±0.704 ^{a,1}
	3rd day	1.758±0.153 ^{c,1}	3.694±0.433 ^{b,2}	4.42±0.705 ^{a,1}
	5th day	1.798±0.196 ^{c,1}	2.594±0.198 ^{b,3}	3.726±0.382 ^{a,2}
	7th day	1.858±0.206 ^{b,1}	1.902±0.201 ^{b,4}	2.704±0.342 ^{a,3}
LDH (U/L)	0 day	877.48±49.35 ^{b,1}	1175.7±73.7 ^{a,1}	1167.2±68.3 ^{a,1}
	3rd day	888.16±48.92 ^{b,1}	1094.5±56.5 ^{a,2}	1138.9±71.5 ^{a,1}
	5th day	895.64±48.5 ^{c,1}	973.88±47.23 ^{b,3}	1045.2±48.1 ^{a,2}
	7th day	905.66±48.55 ^{a,1}	914.74±43.6 ^{a,3}	944.98±44.7 ^{a,3}
CK (mg/dL)	0 day	241.07±15.67 ^{b,1}	374.54±28.13 ^{a,1}	373.18±27.48 ^{a,1}
	3rd day	242.93±16.76 ^{b,1}	315.61±22.37 ^{a,2}	343.58±23.93 ^{a,2}
	5th day	244.94±15.42 ^{b,1}	256.27±14.87 ^{b,3}	314.3±19.42 ^{a,3}
	7th day	245.2±17.45 ^{a,1}	244.86±14.46 ^{a,3}	256.03±16.71 ^{a,4}
Vitamin E (µg/dL)	0 day	118.16±8.46 ^{a,1}	61.76±7.14 ^{b,1}	61.97±7.07 ^{b,1}
	3rd day	118.51±8.55 ^{a,1}	116.32±6.83 ^{a,2}	77.31±7.78 ^{b,2}
	5th day	119.88±7.59 ^{a,1}	125.5±7.92 ^{a,2}	98.51±8.19 ^{b,3}
	7th day	121.78±7.28 ^{b,1}	140.22±7.67 ^{a,3}	115.76±7.37 ^{b,4}
Selenium (Se) (µg/dL)	0 day	8.341±0.349 ^{a,1}	6.098±0.411 ^{b,1}	6.165±0.435 ^{b,1}
	3rd day	8.335±0.346 ^{a,1}	7.743±0.392 ^{b,2}	6.782±0.407 ^{c,2}
	5th day	8.364±0.316 ^{a,1}	8.599±0.315 ^{a,3}	7.416±0.362 ^{b,3}
	7th day	8.396±0.304 ^{b,1}	9.622±0.313 ^{a,4}	8.189±0.342 ^{b,4}

^{a,b,c} Significant difference between groups at the same time interval at $P<0.05$; ^{1,2,3} Significant difference among times of treatment within the same group at $P<0.05$.

significant (P<0.05) increase on the 7th day. Globulin concentrations were insignificantly different on the 3rd and 5th day and became significantly (P<0.05) higher on the 7th day. There was a significant (P<0.05) increase in total protein, albumin and globulin all over the period of treatment compared to diarrhoeic

Table 2. Results of serum minerals and trace elements. Data are expressed as mean ± SE of 10 samples

Parameters	Time	Groups		
		Control healthy group (G1)	Treated with garlic powder (G2)	Treated with Nitazoxanide+Colitrim (G3)
Calcium (Ca) (mmol/L)	0 day	2.575±0.17 ^{a,1}	2.16±0.162 ^{b,1}	2.18±0.16 ^{b,1}
	3rd day	2.55±0.175 ^{a,1}	2.33±0.165 ^{b,1}	2.25±0.17 ^{b,1}
	5th day	2.58±0.162 ^{a,1}	2.565±0.13 ^{a,2}	2.35±0.127 ^{b,1}
	7th day	2.585±0.16 ^{a,1}	2.6±0.135 ^{a,2}	2.575±0.137 ^{a,2}
Ionised calcium (Ca^{++}) (mmol/L)	0 day	1.244±0.09 ^{a,1}	1.056±0.086 ^{b,1}	1.068 ±0.082 ^{b,1}
	3rd day	1.256±0.088 ^{a,1}	1.17±0.085 ^{a,b,2}	1.13±0.085 ^{b,1,2}
	5th day	1.28±0.087 ^{a,b,1}	1.296±0.074 ^{a,3}	1.18±0.072 ^{b,2}
	7th day	1.306±0.085 ^{a,1}	1.326±0.069 ^{a,3}	1.302±0.07 ^{a,3}
Phosphorus (Ph) (mmol/L)	0 day	2.039±0.168 ^{a,1}	1.597±0.19 ^{b,1}	1.606±0.152 ^{b,1}
	3rd day	2.026±0.19 ^{a,1}	1.761±0.187 ^{b,1,2}	1.697±0.152 ^{b,1}
	5th day	2.035±0.187 ^{a,1}	1.945±0.177 ^{a,b,2,3}	1.797±0.145 ^{b,1}
	7th day	2.068±0.174 ^{a,1}	2.09±0.152 ^{a,3}	2.061±0.155 ^{a,2}
Magnesium (Mg) (mmol/L)	0 day	1.413±0.092 ^{a,1}	0.99±0.1 ^{b,1}	0.996±0.103 ^{b,1}
	3rd day	1.41±0.097 ^{a,1}	1.153±0.092 ^{b,2}	1.093±0.1 ^{b,1,2}
	5th day	1.421±0.088 ^{a,1}	1.298±0.102 ^{a,b,3}	1.202±0.092 ^{b,2}
	7th day	1.426±0.084 ^{a,1}	1.434±0.095 ^{a,3}	1.396±0.086 ^{a,3}
Copper (Cu) (µmol/L)	0 day	19.76±1.32 ^{a,1}	16.6±1.24 ^{b,1}	16.76±1.21 ^{b,1}
	3rd day	19.73±1.35 ^{a,1}	18.05±1.27 ^{b,1}	17.4±1.31 ^{b,1}
	5th day	19.86±1.29 ^{a,1}	19.83±1.02 ^{a,2}	18.02±1.07 ^{b,1,2}
	7th day	19.94±1.27 ^{a,1}	20.55±0.99 ^{a,2}	19.32±1.05 ^{a,2}
Iron (Fe) (µmol/L)	0 day	327.34±21.67 ^{a,1}	251.06±19.88 ^{b,1}	252.85±20.41 ^{b,1}
	3rd day	326.98±22.74 ^{a,1}	281.86±22.03 ^{b,2}	263.95±22.74 ^{b,1,2}
	5th day	328.41±22.03 ^{a,1}	324.12±18.98 ^{a,3}	291.53±19.88 ^{b,2}
	7th day	328.06±22.56 ^{a,1}	337.37±19.34 ^{a,3}	329.13±19.16 ^{a,3}
Sodium (Na) (mmol/L)	0 day	138.2±5.9 ^{a,1}	115.4±7.6 ^{b,1}	115.8±7.5 ^{b,1}
	3rd day	137.4±5.7 ^{a,1}	125±5.6 ^{b,2}	118±6.5 ^{b,1}
	5th day	138.4±5.2 ^{a,1}	133.6±6.1 ^{a,3}	120±6.1 ^{b,1}
	7th day	139.5±5.6 ^{a,1}	142.2±6.2 ^{a,3}	134.2±5.5 ^{a,2}
Potassium (K) (mmol/L)	0 day	4.37±0.33 ^{b,1}	6.23±0.38 ^{a,1}	6.21±0.38 ^{a,1}
	3rd day	4.4±0.35 ^{c,1}	5.66±0.39 ^{b,2}	6.08±0.35 ^{a,1,2}
	5th day	4.39±0.33 ^{b,1}	4.73±0.31 ^{b,3}	5.73±0.36 ^{a,2}
	7th day	4.36±0.34 ^{a,1}	4.4±0.29 ^{a,3}	4.69±0.31 ^{a,3}
Chloride (Cl) (mmol/L)	0 day	95.06±6.22 ^{a,1}	77.87±6.81 ^{b,1}	78.84±6.54 ^{b,1}
	3rd day	94.54±6.51 ^{a,1}	85.93±6.09 ^{b,1}	81.38±5.92 ^{b,1}
	5th day	95.34±5.99 ^{a,1}	94.72±5.3 ^{a,2}	85.39±5.83 ^{b,1}
	7th day	96.02±6.22 ^{a,1}	98.01±5.67 ^{a,2}	93.85±5.68 ^{a,2}

^{a,b,c} Significant difference between groups at the same time interval at P<0.05; ^{1,2,3} Significant difference among times of treatment within the same group at P<0.05.

Table 3. Results of serum total protein, albumin, globulin and protein fractions. Data are expressed as mean ± SE of 10 samples

Parameters	Time	Groups		
		Control healthy group (G1)	Treated with garlic powder (G2)	Treated with Nitazoxanide+Colitrim (G3)
Total protein (g/L)	0 day	66.36±2.78 ^{a,1}	53.08±2.15 ^{b,1}	53.36±2.03 ^{b,1}
	3rd day	66.76±2.83 ^{a,1}	63.24±2.41 ^{b,2}	58.26±2.32 ^{c,2}
	5th day	66.9±2.95 ^{a,1}	68.74±2.22 ^{a,3}	62.24±2.19 ^{b,3}
	7th day	67.74±2.74 ^{b,1}	72.76±2.13 ^{a,4}	67.26±2.2 ^{b,4}
Albumin (g/L)	0 day	22.89±0.96 ^{a,1}	13.85±0.56 ^{b,1}	13.93±0.53 ^{b,1}
	3rd day	22.7±0.96 ^{a,1}	18.66±0.71 ^{b,2}	16.6±0.66 ^{c,2}
	5th day	22.41±0.99 ^{a,1}	22.75±0.74 ^{a,3}	18.92±0.66 ^{b,3}
	7th day	22.69±0.92 ^{b,1}	24.59±0.72 ^{a,4}	21.46±0.7 ^{c,4}
Globulin (g/L)	0 day	43.47±1.82 ^{a,1}	39.23±1.59 ^{a,1}	39.43±1.5 ^{b,1}
	3rd day	44.06±1.87 ^{a,1}	44.58±1.7 ^{a,1}	41.66±1.66 ^{b,2}
	5th day	44.49±1.96 ^{a,b,1}	45.99±1.49 ^{a,1}	43.32±1.52 ^{b,2}
	7th day	45.05±1.82 ^{b,1}	48.17±1.41 ^{a,2}	45.8±1.5 ^{b,3}
Alpha 1 (α1) (g/L)	0 day	6.3±0.26 ^{a,1}	5.2±0.21 ^{b,1}	5.23±0.2 ^{b,1}
	3rd day	6.21±0.25 ^{a,1}	6.01±0.23 ^{a,2}	5.53±0.22 ^{b,2}
	5th day	6.15±0.27 ^{a,b,1}	6.39±0.21 ^{a,3}	5.98±0.21 ^{b,3}
	7th day	6.37±0.26 ^{a,1}	6.55±0.19 ^{a,3}	6.32±0.21 ^{a,4}
Alpha 2 (α2) (g/L)	0 day	6.97±0.29 ^{b,1}	8.81±0.36 ^{a,1}	8.86±0.34 ^{a,1}
	3rd day	7.21±0.31 ^{b,1}	8.85±0.34 ^{a,1}	8.74±0.35 ^{a,1}
	5th day	7.36±0.32 ^{b,1}	7.56±0.24 ^{b,2}	8.09±0.28 ^{a,2}
	7th day	7.38±0.3 ^{b,1}	7.64±0.22 ^{a,b,2}	7.94±0.26 ^{a,2}
Beta 1 (β1) (g/L)	0 day	7.3±0.31 ^{b,1}	8.28±0.34 ^{a,1}	8.32±0.32 ^{a,1}
	3rd day	7.14±0.3 ^{b,1}	8.03±0.31 ^{a,1}	8.27±0.33 ^{a,1}
	5th day	7.23±0.32 ^{b,1}	7.15±0.23 ^{b,2}	7.78±0.27 ^{a,2}
	7th day	6.98±0.28 ^{b,1}	7.35±0.22 ^{a,b,2}	7.67±0.25 ^{a,2}
Beta 2 (β2) (g/L)	0 day	5.31±0.22 ^{a,1}	4.51±0.18 ^{b,1}	4.54±0.17 ^{b,1}
	3rd day	5.67±0.24 ^{a,2}	5.57±0.21 ^{a,2}	5.13±0.2 ^{b,2}
	5th day	6.02±0.27 ^{a,3}	6.19±0.2 ^{a,3}	5.6±0.2 ^{b,3}
	7th day	6.37±0.26 ^{a,4}	6.4±0.19 ^{a,3}	5.72±0.19 ^{b,3}
Gamma 1 (γ1) (g/L)	0 day	11.94±0.5 ^{a,1}	8.28±0.34 ^{b,1}	8.32±0.32 ^{b,1}
	3rd day	12.08±0.51 ^{a,1}	11.07±0.42 ^{b,2}	9.32±0.37 ^{c,2}
	5th day	12.04±0.53 ^{b,1}	12.72±0.41 ^{a,3}	10.58±0.37 ^{c,3}
	7th day	12.06±0.49 ^{b,1}	13.39±0.39 ^{a,4}	12.11±0.4 ^{b,4}
Gamma 2 (γ2) (g/L)	0 day	5.64±0.24 ^{a,1}	4.14±0.17 ^{b,1}	4.16±0.16 ^{b,1}
	3rd day	5.74±0.24 ^{a,1}	5.06±0.19 ^{b,2}	4.66±0.21 ^{c,2}
	5th day	5.69±0.25 ^{b,1}	5.98±0.21 ^{a,3}	5.29±0.19 ^{c,3}
	7th day	5.89±0.24 ^{b,1}	6.84±0.2 ^{a,4}	6.05±0.2 ^{b,4}

^{a,b,c} Significant difference between groups at the same time interval at $P<0.05$; ^{1,2,3} Significant difference among times of treatment within the same group at $P<0.05$.

calves that received traditional treatment (G3). On the other hand, the electrophoretic patterns of calves in G2 showed a significantly ($P<0.05$) increased γ1 and γ2 globulins on the 5th and 7th day, associated with a gradual decrease of both α2 and β1 globulins. The differences became insignificant from the 5th day whereas both α1 and β2 showed irrelevant differences from the 3rd day compared to G1.

DISCUSSION

The results from chemical analysis of garlic powder were consistent with the results recorded by Tosin *et al.* (2017). Garlic contains many nutrients as protein, vitamins and trace elements (Zhang *et al.,* 2018), minerals (Ca, Fe, I, K, Mg, Na, Zn) and vitamins (A, E, C, and B complex) (Kovarovič *et al.,* 2019).

The clinical picture of diarrhoeic calves: white to yellowish and greenish profuse watery diarrhoea, soiling of the perineum and inability to get up with dermatological changes was in accordance with clinical signs recorded in diarrhoeic buffalo calves (El-Seadawy *et al.,* 2020), bovine calves (Saleh *et al.,* 2022) and Friesian calves (Shehta *et al.,* 2022).

Cryptosporidium oocytes were isolated from the faeces of naturally infected calves in accordance with data of Elkelesh *et al.* (2023). *E. coli* was isolated in bacteriological examination of diarrhoeic calves (Shehta *et al.,* 2022). Calf diarrhoea may be caused by mixed *Cryptosporidium* and *E. coli* infection (Delgado-González *et al.,* 2019; Mullusew *et al.,* 2020).

The decreased SOD, CAT, TAC, and GSH associated with increased MDA in diarrhoeic calves were in agreement with data of Kumar *et al.* (2018); Mahran *et al.* (2020) and Ramadan *et al.* (2021). Decreased TAC with increased MDA was recorded in diarrheoic calves (Ebrahim & Abdullaziz, 2023). Intestinal inflammation due to infection may result in release of inflammatory mediators as cytokines that activate polymorphonuclear cells and macrophages in the ileum. These phagocytes are considered a major source of oxidants and reactive oxygen metabolites (ROM) in the intestine (Argenzio & Rhoads, 1997). The production of these ROM correlated to increased MDA concentrations during the infection. Peroxidation of cell membrane lipid layer may be related to the free radicals – an important feature of cellular injury of intestine. Oxidative damage induced by reactive oxygen species (ROS) may result in many pathological conditions of gastrointestinal tract (GIT) including enteritis (Metwaly *et al.,* 2015). *Cryptosporidium* infestation has an adverse effect manifested with increased ROM and lipid peroxidation in infected buffalo calves, which may lead to tissue damage (Mahran *et al.,* 2020). The improvement of antioxidants' levels and oxidative stress in diarrhoeic calves treated with garlic powder compared to both the control group and traditionally treated diarrhoeic calves may be attributed to the anti-oxidative effects of garlic, as its components might scavenge and block the production of superoxide radicals (Ide & Lau, 2001). Garlic may increase cellular activity of enzymatic antioxidants such as GSH and SOD (Colín-González *et al.,* 2012). It plays a role of antioxidant activator and decreases oxidative stress acting as defense mechanism against oxidative damage, which is enhanced by its antioxidant bioactive compounds (Kovarovič *et al.,* 2019). Garlic has anti-parasitic, antibacterial and antiprotozoal effects. The biological functions of garlic, including antioxidant and immunomodulatory ones, are mediated through the organosulphur compounds such as S-allyl cysteine (SAC) with antioxidant and anti-inflammatory effects, and diallyl disulfide (DADS): the main breakdown product of allicin, contributing to garlic's anti-inflammatory effects (Lee *et al.,* 2012).

The elevated activities of enzymes lipase, amylase and LDH were in accordance with the results recorded in previous studies (Kumar *et al.,* 2018; Saleh *et al.,* 2022). Those results may be referred

to GIT inflammation and damage of intestinal mucosa, progressive inflammation and release of the intestinal fraction of the enzyme in the circulation (Saleh *et al.,* 2022). Significantly increased LDH activities in diarrhoeic buffalo calves indicate the presence of cellular damage in tissues of major organs like liver, heart and musculature of buffalo calves (Kumar *et al.,* 2018). Elevated creatine kinase (CK) serum levels were similar to results recorded by El-Seadawy *et al.* (2020). CK enzyme plays a vital role in energy homeostasis of tissue cells and ensures a constant ATP level in the cells, so it is useful for evaluating the disorders involving damage to the myocardium and skeletal muscle. So, its elevation in diarrhoeic calves indicates fatigue, metabolic disorders and skeletal muscle degeneration (Minkaet & Ayo, 2010). The rapidly improved serum enzymes in G2 treated with garlic powder compared to animals in G3 that received traditional treatment may be due to containing organosulphur compounds, which help preventing the oxidative damage by reduction of injury risk to vital molecules and prevention of disease progression (Gutteridge, 1993).

The decreased concentrations of vitamin E, Se, Ca, Ph, Mg, Cu, Fe, Na and Cl with increased serum K levels in diarrhoeic buffalo calves are in accordance with the results reported by Ghanem *et al.* (2012). Decreased Mg, Cu, Fe, Na and Cl with increased K levels were recorded in diarrhoeic newly born calves (Singh *et al.,* 2022). Decreased serum Na and Cl with elevated K levels were observed in diarrhoeic calves (El-Seadawy *et al.,* 2020). Decreased serum Zn and Cu were demonstrated in buffalo calves infected by coccidiosis (Ramadan *et al.,* 2021). Low Ca level may be attributed to persistent diarrhoea and dehydration with loss of Ca in faeces, whereas decreased Ph – to greater electrolyte loss than water loss (El-Dessouky & El-Masry, 2005). Low serum Cl and Na may be due to the excessive loss of Cl and Na ions following increased intestinal secretion and excessive water losses in diarrhoeic faeces, leading to dehydration and impaired cell membrane permeability. Hyperkalemia results from increased potassium retention through the kidneys and also to its movement from intracellular to extracellular fluid (Seifi *et al.,* 2006).

Lower Mg, Cu, Zn, Fe and vitamin E levels may be explained with decreased absorption of digested nutrients through the intestine and excessive losses in watery diarrhoeic faeces (Khan *et al.,* 2009). Low serum Fe in diarrhoeic calves may be related to the presence of pathogenic bacteria that use several strategies to adsorb iron to multiply in infected cells, while haemolytic bacterial cytotoxins damage host cells, leading to ferritin release, thus iron is carried out by secreted bacterial siderophores that specifically bind Fe^{3+} and transport it into the cytoplasm resulting to iron reduction in serum of infected calves. However, decreased zinc level due to losses of this electrolyte in diarrhoeic faeces, increases zinc requirements for the immune system and also the utilisation of its tissue stores for synthesis of antioxidant enzymes (Ranjan *et al.,* 2006). Low level of vitamin E may be correlated to stress condition that increases its uptake in response to oxidative stress, as vitamin E acts as a scavenger antioxidant, neutralising the free radicals and preventing lipid oxidation (Franchini *et al.,* 1991). The rapid recovery in G2 treated by garlic powder in comparison to G3 calves that were treated traditionally may be attributed to the anti-inflammatory effects of garlic organosulphur constitutes (Poojary

et al., 2017), able to cure the intestinal inflammation and prevent intestinal loss of minerals, trace elements and vitamins. Also, garlic contains many nutrient components, minerals (Ca, Fe, I, K, Mg, Na, Zn) and vitamins (A, E, C, and B complex) (Zhang *et al.,* 2018; Kovarovič *et al.*, 2019), able to replenish the losses of nutrients, minerals and vitamins.

Decreased serum levels of total protein, albumin, and globulin in diarrhoeic calves are comparable to those reported by Abdel Megeed *et al.* (2015) and Choi *et al.* (2021). Also, the lower levels of total protein and albumin are consistent with the results recorded by Galbat *et al.* (2015); El-Seadawy *et al.* (2020) and Saleh *et al.* (2022). The decreased serum total protein and albumin may be due to the excretion of those nutrients in the intestinal lumen associated with diarrhoea-induced disturbance of nutrient absorption or water loss (Choi *et al.,* 2021). Also, it could be attributed to stress of diarrhoea which may affect the hepatic parenchyma leading to disturbance in protein synthesis (Galbat *et al.,* 2015). Significantly decreased globulin level in diarrhoeic calves may be due to the marked depression in gamma globulin fractions. The reduction of γ-globulin in diarrhoeic calves may be consequent to the shift from γ-globulin to α2-globulins. As such, lowered γ-globulin may be responsible for lower immune response when calves need protection from various infections (Choi *et al.,* 2021). The rapid recovery of total protein, albumin, globulin and globulin fractions in G2 calves that received garlic powder compared to G3 (traditional treatment) might be attributed to the role of garlic as a hepato-protectant through inhibition of lipid peroxidation, oxidative stress, and inflammation (Lee *et al.,* 2018). Garlic contains many bioactive compounds that help in improving the immune system. Garlic polysaccharides have an immunomodulatory effect that may be due to the degradation of fructan constituents during processing (Li *et al.,* 2017).

CONCLUSION

According to the results of this work, diarrhoea in buffalo calves is a very serious problem and requires rapid reaction to avoid loss of animals. Diarrhoea in buffalo calves may be caused by mixed infection of *Cryptosporidium* and *E. coli*. Diarrhoeic buffalo calves suffer from decreased antioxidants with increased oxidative stress level, hypovitaminosis, decreased concentrations of minerals and trace elements, hyperkalemia, hypoproteinaemia, hypoalbuminaemia, hypoglobulinaemia, and increased activity of LDH, CK, lipase, and amylase. Compared to the traditional treatment, garlic powder ensured a rapid very effective treatment of diarrhoeic buffalo calves, along with improving antioxidant status, immunity, enzyme activity and trace elements.

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