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Research Article

Overview on Antioxidant and Oxidative Stress Markers after Garlic Oil Supplement in Suckling Buffalo Calves

Hamada Mahmoud YOUSIF [1 (*)] Ahmed Mohamed EL MAHDY [1] Marwa Fouad HASSAN [1] Mogda Kamel MANSOUR [1]

[1] Agricultural Research Center (ARC), Animal Health Research Institute (AHRI), Department of Biochemistry, Toxicology and Feed Deficiency, Postal code 12618, Dokki, Giza, EGYPT

[(*)] **Corresponding author:** Hamada Mahmoud YOUSIF

Tel: +20 122 3074010

E-maill: dr.hamadayousif@gmail.com

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Abstract

Calves during the suckling period have serious attention and induce immense bearing on early maturity and production. This work aimed to scrutinize the affections of garlic oil supplements on antioxidants, oxidative stress, and immune response in suckling buffalo calves. To achieve this aim we used twenty suckling buffalo calves, and divided them into two groups. The first group (n=10) did not receive any supplement, and the second group (n=10) received garlic oil as a supplement 5 mL/calf/day orally for 7 days. Whole blood and serum samples were collected on the 1st, 3rd, 5th, and 7th day of supplement from all calves. Our data showed that the supplemented garlic oil significantly increase lysated blood and serum SOD, CAT, GSH, and TAC levels, while a gradual decrease in MDA and non-significant changes in NO as indicators of oxidative stress, associated with a significant increase in serum vitamins A and E, selenium, iron, and zinc. Total protein, albumin, globulin, γ1, γ2, α1, α2 were gradually and significantly increased in comparison with the non-supplemented control group. In conclusion, garlic oil supplement have promotives effects on enzymatic and non-enzymatic antioxidant system, immunity, nutrient utilization required for immunity modulation and regulation in calves.

Keywords: Antioxidant, Buffalo calves, Electrophoresis, Garlic oil, Oxidative stress, Vitamin

Introduction

Calves were considered a fundamental stage for livestock farming. As calf rearing is essential to the continued existence of the dairy industry, it is also important to obtain and conserve good quality genetic material. Calf rearing depends on good hygiene management and good nutrition [1]. For calves to grow well, it is very important to get good nutrients, probiotics and feed supplements. Supplementing with rumen modulators, liver tonics, and immunomodulators at a young age helps boost immunity and prevent disease [2,3].

Most of the substances required for a calf feed to achieve promising results have been found in herbs. The beneficial effects of herbs on farm animals include activation of feed intake, stimulate the immune system, as well as antibacterial, anthelmintic, antiviral, anti-inflammatory activity, and antioxidant effects. Herbs also meet the animal's nutritional needs, stimulate the endocrine system and the metabolism of intermediate nutrients [4]. Garlic contains many nutrients and active substances such as organo-sulphur compounds, proteins, free amino acids, vitamins, trace elements, enzymes, flavonoids, phenols and organo-selenium compounds [5,6].

Garlic supplementation through feed has many favorable experimental and clinical benefits, which include stimulation of immune function, improved detoxification of foreign substances, and restoration of physical strength and resistance to various stresses. Allicin is the main bioactive component of garlic and may be responsible for some effects of garlic [7]. The flavonoids contained in garlic oil interact with radical stabilizing of reactive oxygen species (ROS). The radicals were inactivated by the strong reactivity of the hydroxyl group on the flavonoids [8,9]. The antioxidant properties of garlic oil and its constituent diallyl disulfide have been reported. They owe their antioxidant effect to organo-sulphur compounds [10]. Feed additives containing garlic provided calves with higher

serum total protein and globulin levels, as well as improved liver function with better digestibility of nutrient leading to good calf performance [11].

Therefore, this study was designed to scrutinize the effects of garlic oil supplements on antioxidants, oxidative stress, immune response, and performance in suckling buffalo calves.

Material and Methods

Ethical Statement

This protocol was approved by the Research Committee of the Animal Health Research Institute and authorized by The Institutional Animal Care and Use Committee (ARC-IACUC)/Agricultural Research Center (ARC/AHRI/43/23).

Experimental Design

This work was established on a private farm of buffalo. Twenty suckling buffalo calves aged (15-21) days and weighted (42±5) kg, were used and divided into two groups (n=10). The first group (G1) calves did not receive any supplement, used as a control group. The second group (G2) calves received garlic oil supplements in a dose of 5 mL/calf/day orally after 1 h post morning breastfeeding for 7 days continuously. All calves received breastfeeding twice daily with a little of roughage, concentrate diets and water ad-libitum.

Sampling

A sample of the garlic oil was sent to lab for gas chromatographic analysis. Blood samples were collected from the jugular vein of all calves on the 1st, 3rd, 5th, and 7th days of garlic oil supplement. Each blood sample was collected in two tubes, the first tube with EDTA as anticoagulant for whole blood obtaining and used to prepare the RBCs lysate, and the second one was collected without anticoagulant for serum obtaining. Serum samples were obtained by centrifuging the blood samples at 3000 rpm for 5 min. The clear sera were transferred into clean dry eppendorf tubes and stored at -20°C till biochemical analysis.

Chemicals and Materials

Garlic oil; GARLIC OIL HUILE DE AIL, manufactured by El Captain Company (CAP Pharm) for extracting natural oils, plants and cosmotics.

Drabkin′s reagent; Contains sodium bicarbonate, potassium ferricyanide, and potassium cyanide, Catalog Number D5941, SIGMA-ALDRICH, St. Louis, MO 63103, USA.

Gas Chromatography Examination of Garlic Oil

The chemical composition of the samples was determined using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a TG–5MS direct capillary column (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was first maintained at 60°C, then increased by 5°C/min to 250°C for 2 min then increased to 300 at 30°C/min. The injector temperature was maintained at 270°C. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 4 min and 1 µL of diluted samples was injected automatically using auto sampler AS3000 coupled to the GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and transfer line were set at 200°C and 280°C respectively. The components were identified by comparing their mass spectra with those of WILEY 09 and NIST14 mass spectral database, according to the method identified by Kareem et al.[12].

Hematology and Blood Redox Parameter Analysis

Whole blood samples were used for detection of RBCs antioxidant parameters. Preparation of lysate 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 the hemoglobin levels [13]. Catalase activity (CAT), malondialdehyde (MDA) as indicator of lipid peroxidation and reduced glutathione (GSH) in lysated RBCs were determined according to Aebi [14], Okhawa et al.[15] and Pleban et al.[16], respectively. Superoxide dismutase (SOD) were estimated according to Nishikimi et al.[17].

Serum Biochemical Analysis

Serum zinc (Zn), iron (Fe) and selenium (Se) levels were detected by using atomic absorption Spectrophotometer (A7942, SensAA-Dual, GBC Scientific Equipment, Braeside, Victoria, Australia), retinol (vitamin A) and α-tocopherol (vitamin E) concentrations in serum determination were performed according to the method described by Roberts et al.[18]. Total antioxidant capacity (TAC) were estimated by using Bio-diagnostic kit CAT. No. TA2513 and nitric oxide (NO) was determined by kits CAT. No. NO 25 33.

Total Protein and Electrophoretic Protein Estimation

Total protein and electrophoretic pattern were estimated according to Sonnenwirth et al.[19] and Davis [20], respectively, and calculated according to SynGene S. No. 17292*14518 sme*mpcs.

Statistical Analysis

Statistical analysis of data using an Excel T.test to test the significance difference between the two groups. Results are presented as means ± SE, those considered statistically significant at $P<0.05$, highly significant at $P<0.01$, and very highly significant at $P<0.001$.

Results

The gas chromatographic analysis of garlic oil results *(Table 1; Fig. 1)*, revealed a constituent of fatty acids such as Hexadecanoic Acid (Palmitic Acid), 9,12-Octadecadienoic acid, Erucic Acid (13-Docosenoic Acid) and Cis-13-Octadecenoic Acid (Oleic Acid). Organosulphur compounds such as Di-2-Benzothiazole Disulfane, 2-Aminoethanethiol Hydrogen Sulfate, 3-Allyl-2-Methylthio-4-Phenylthiophene. Flavonoids and phenolic derivatives such as 03027205002 Flavone 4'-OH, 5-OH, 7-Di-O-Glucoside, 4H-1-Benzopyran-4-One, 2-(3,4-Dimethoxyphenyl)-3,5-Dihydroxy-7-Methoxy, 4H-1-Benzopyran-4-One, 2-(3,4-Dihydroxyphenyl)-6,8- Di-Á-D-Glucopyranosyl-5,7- Dihydroxy.

The results of antioxidants and oxidative stress in lysated blood and serum in calves received garlic oil supplement *(Table 2)* revealed a gradual increase of SOD, CAT, and

Table 1. *Gas chromatography-mass spectrometry (GC-MS) analysis of garlic oil*

RT	Compound Name	Molecular Formula	Molecular Weight
4.03	Di-2-Benzothiazole Disulfane	C14H8N2S4	332
7.06	5à-Androstan-16-one, cyclic ethylene mercaptole	C21H34S2	350
9.51	2-Aminoethanethiol Hydrogen Sulfate (Ester)	C2H7NO3S2	157
11.14	Ethanimidothioic Acid, 2-(Dimethylamino)-N-[[(Meth Ylamino)Carbonyl]Oxy]-2-O Xo-, Methyl Ester	C7H13N3O3S	219
13.66	2-Myristynoyl pantetheine	C25H44N2O5S	484
15.76	tert-Hexadecanethiol	C16H34S	258
18.23	3-Methyl-4-(Phenylthio)-2-Prop-2-Eny L-2,5-Dihydrothiophene 1,1-Dioxide	C14H16O2S2	280
21.75	2-Methylthio-3,4-Dihydrona Phtho[2,1-C]Thiophene	C13H12S2	232
24.46	3-Benzylidene-2-(P-Methylp Henyl)Sulfonylamino-1-Me Thylindoline	C23H20N2O2S	388
25.8	3-Allyl-2-Methylthio-4-Phenylthiophene	C14H14S2	246
26.52	Hexadecanoic Acid (Palmitic Acid)	C16H32O2	256
28.26	1-Methoxy-3-Pentyl-6,6a,7,8-Tetrahydro-6,6-Dimethyl-9 H-Dibenzo[B,D]Pyran-9-One (Pyrethrin 1)	C21H28O3	328
	Hexadecanoic Acid, Trimethylsilyl Ester (Palmitic Acid, Tms Derivative)	C19H40O2Si	328
29.74	11,14-Eicosadienoic Acid, Methyl Ester	C21H38O2	322
	9,12-Octadecadienoic Acid (Z,Z)-	C18H32O2	280
	Z-(13,14-Epoxy) Tetradec-11-En-1-Ol Acetate	C16H28O3	268
	1,2-15,16-Diepoxyhexadecane	C16H30O2	254
31.23	Cis-13-Octadecenoic Acid (Oleic Acid),(9-Octadecenoic Acid, (E),(Trans-13-Octadecenoic Acid),(Cis-Vaccenic Acid)	C18H34O2	282
33.07	Octadecanoic Acid	C18H36O2	284
35.0	Cis-11-Eicosenoic Acid	C20H38O2	310
	14-Á-H-Pregna	C21H36	288
	22-Tricosenoic Acid	C23H44O2	352
38.05	Tetrapentacontane, 1,54-Dibromo-(1,54-Dibromotetrapentacon Tane)	C54H108Br2	914
	17-Pentatriacontene	C35H70	490
	Heptacosane	C27H56	380
40.52	Erucic Acid (13-Docosenoic Acid)	C22H42O2	338
42.83	4H-1-Benzopyran-4-One, 2-(3,4-Dihydroxyphenyl)-6,8- Di-Á-D-Glucopyranosyl-5,7- Dihydroxy	C27H30O16	610
44.84	03027205002 Flavone 4'-Oh,5-Oh,7-Di-O-Glucoside	C27H30O15	594
	9,12-Octadecadienoic Acid (Z,Z)-, 2,3-Bis[(Trimethylsilyl)Oxy-Propyl Ester	C27H54O4Si2	498
	9,12,15-Octadecatrienoic Acid, 2,3-Bis[(Trimethylsilyl)Oxy-Propyl Ester, (Z,Z,Z)-	C27H52O4Si2	496
	Rhodopsin (.PSI.,.PSI.-Carotene, 1,2-Dihydro-1-Hydroxy)	C40H58O	554
45.11	4h-1-Benzopyran-4-One, 2-(3,4-Dimethoxyphenyl)-3,5- Dihydroxy-7-Methoxy	C18H16O7	344

RT: *Retention Time*

Table 2. *Results of antioxidant and oxidative stress, vitamins, and trace elements in suckling calves supplied by garlic oil (G2) compared with the control group (G1)*

Parameters	Time	Groups		
		G1	G2	P Value
SOD (U/g Hb)	1st day	5.214±0.216	5.234±0.204	0.884
	3rd day	5.376±0.201	5.602±0.237	0.143
	5th day	5.588±0.209	6.216±0.21 **	0.0015
	7th day	5.754±0.216	6.658±0.209 ***	0.00015
CAT (U/g Hb)	1st day	26.866±2.36	27.34±2.22	0.752
	3rd day	26.766±2.29	29.206±2.2	0.124
	5th day	26.584±2.275	30.858±1.678 **	0.0096
	7th day	26.078±1.843	33.174±1.613 ***	0.00019
TAC (mU/L)	1st day	1.144±0.136	1.184±0.141	0.66
	3rd day	1.216±0.144	1.392±0.138	0.084
	5th day	1.332±0.132	1.708±0.141 **	0.0025
	7th day	1.618±0.135	2.074±0.133 ***	0.00066
GSH (mmol/g Hb)	1st day	35.692±2.092	35.812±2.071	0.93
	3rd day	35.538±2.147	36.056±2.118	0.711
	5th day	35.502±2.074	37.266±2.092	0.217
	7th day	35.492±2.058	38.78±2.02 *	0.034
MDA (nmol/g Hb)	1st day	8.798±1.189	8.758±1.15	0.958
	3rd day	8.902±1.261	7.778±1.117	0.174
	5th day	9.052±1.282	6.938±0.824 *	0.015
	7th day	9.12±1.28	6.26±0.605 **	0.002
Nitric Oxide (NO) (nmol/L)	1st day	33.068±3.144	32.97±3.278	0.963
	3rd day	34.488±3.03	33.788±3.233	0.733
	5th day	35.08±3.011	34.482±3.223	0.7695
	7th day	36.142±3.023	35.228±3.083	0.6486
Vitamin E (µg/dL)	1st day	89.052±4.336	91.628±5.085	0.4138
	3rd day	91.528±4.904	105.34±6.016 **	0.0041
	5th day	96.17±5.938	119.64±7.062 ***	0.00046
	7th day	102.34±4.8	146.14±8.543 ***	0.0000085
Vitamin A (µg/dL)	1st day	54.824±3.894	56.036±3.449	0.61648
	3rd day	56.672±3.462	69.214±3.986 ***	0.000718
	5th day	61.316±3.933	82.928±4.903 ***	0.000058
	7th day	67.26±4.145	104.44±6.117 ***	0.0000035
Selenium (Se) (µg/dL)	1st day	6.322±0.334	6.448±0.368	0.58636
	3rd day	6.474±0.395	7.232±0.416 *	0.01832
	5th day	6.698±0.402	8.106±0.476 ***	0.000981
	7th day	7.052±0.368	9.762±0.565 ***	0.0000186
Iron (Fe) (µg/dL)	1st day	160.8±11.3	161.4±10.57	0.933
	3rd day	163.4±10.69	170.6±10.31	0.31
	5th day	172.4±10.67	181.8±10.31	0.194
	7th day	182.4±10.88	198.6±10.5 *	0.043
Zinc (Zn) (µg/dL)	1st day	156.14±10.97	157.28±10.04	0.868
	3rd day	159.32±10.48	166.68±10.1	0.291
	5th day	168.94±10.46	178.82±10.57	0.176
	7th day	179.48±10.71	196.5±10.37 *	0.034

** Superscript means significant at P<0.05; ** Superscript means highly significant at P<0.01; *** Superscript means very highly significant at P<0.001*

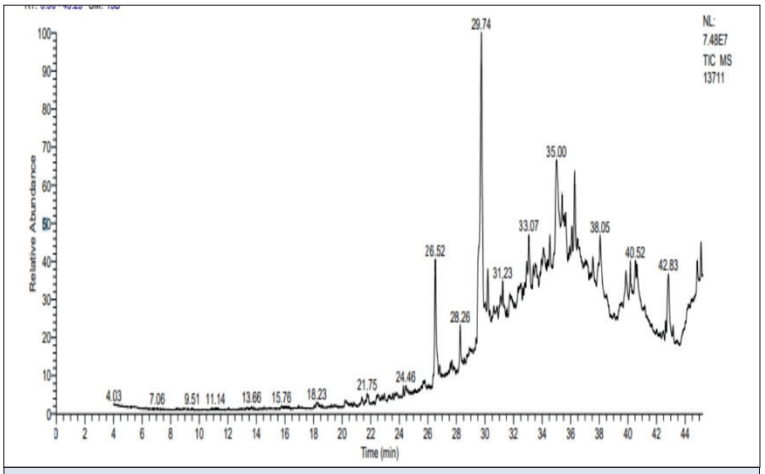

Fig 1. Curve of gas chromatography-mass spectrometry (GC-MS) analysis of garlic oil

Table 3. *Results of total protein, albumin, and globulin and protein electrophoresis in suckling calves supplied by garlic oil (G2) compared with the control group (G1)*

Parameters	Time	Groups		
		G1	G2	P Value
Total Protein (g/dL)	1st day	5.952±0.212	5.984±0.218	0.82
	3rd day	6.078±0.228	6.27±0.204	0.198
	5th day	6.224±0.231	6.604±0.212 *	0.027
	7th day	6.352±0.216	7.09±0.233 ***	0.00083
Albumin (g/dL)	1st day	1.982±0.071	1.975±0.072	0.875
	3rd day	2.006±0.075	2.075±0.067	0.162
	5th day	2.085±0.077	2.16±0.069	0.147
	7th day	2.128±0.072	2.34±0.077 **	0.002
Globulin (g/dL)	1st day	3.97±0.142	4.009±0.146	0.677
	3rd day	4.072±0.153	4.195±0.136	0.218
	5th day	4.139±0.153	4.444±0.142 *	0.011
	7th day	4.224±0.144	4.75±0.156 ***	0.0005
Alpha 1 (α1) (g/dL)	1st day	0.655±0.023	0.646±0.024	0.584
	3rd day	0.638±0.024	0.677±0.022 *	0.028
	5th day	0.672±0.025	0.726±0.023 **	0.007
	7th day	0.654±0.022	0.723±0.024 **	0.0015
Alpha 2 (α2) (g/dL)	1st day	0.548±0.02	0.562±0.02	0.272
	3rd day	0.577±0.022	0.589±0.019	0.382
	5th day	0.56±0.021	0.627±0.02 ***	0.0008
	7th day	0.597±0.02	0.681±0.022 ***	0.0003
Beta 1 (β1) (g/dL)	1st day	0.625±0.022	0.652±0.024	0.098
	3rd day	0.669±0.025	0.69±0.022	0.198
	5th day	0.685±0.025	0.66±0.02	0.14
	7th day	0.692±0.024	0.702±0.023	0.536
Beta 2 (β2) (g/dL)	1st day	0.565±0.02	0.562±0.02	0.825
	3rd day	0.577±0.022	0.577±0.019	0.966
	5th day	0.573±0.021	0.594±0.019	0.127
	7th day	0.597±0.02	0.61±0.02	0.351
Gamma 1 (γ1) (g/dL)	1st day	1.071±0.038	1.065±0.039	0.805
	3rd day	1.064±0.04	1.129±0.037 *	0.03
	5th day	1.12±0.042	1.242±0.04 **	0.002
	7th day	1.131±0.038	1.347±0.044 ***	0.00003
Gamma 2 (γ2) (g/dL)	1st day	0.506±0.018	0.521±0.019	0.245
	3rd day	0.547±0.021	0.533±0.017	0.275
	5th day	0.529±0.02	0.594±0.019 ***	0.0007
	7th day	0.553±0.019	0.688±0.023 ***	0.000007

** Superscript means significant at P<0.05; ** Superscript means highly significant at P<0.01; *** Superscript means very highly significant at P<0.001*

TAC from the 3rd day of supplement with a significant (P<0.01) increase on the 5th day and became significantly increase (P<0.001) on the 7th day. Also, there was a significant increase (P<0.05) of GSH on the 7th day with a gradual decrease of MDA until became significantly (P<0.05) decreased on the 5th day and the 7th day. There was a non-significant changes in nitric oxide (NO) in comparison with the control non-supplemented group.

Serum vitamin results *(Table 2)* revealed a significant increase (P<0.01) of vitamin E on the 3rd day with a gradual significant increase (P<0.001) from the 5th day of the supplement. Also, there was a gradual significant increase (P<0.001) of vitamin A from the 3rd day of the calves group that received garlic oil compared to the control non-supplemented group.

Trace element results in the serum of calves received garlic oil supplement *(Table 2)* revealed a gradual significant (P<0.05) increase of selenium (Se) from the 3rd day and extended gradually to be more significant (P<0.001) from the 5th day of supplement. Also, there was a gradual increase in iron (Fe) and zinc (Zn) levels till became a significant increase (P<0.05) on the 7th day of the supplement compared to the control non-supplemented group.

The results of serum total protein and its fractionations in calves received garlic oil supplement *(Table 3)* revealed a significant increase (P<0.05) on the 5th day and (P<0.001) on the 7th day of total protein and globulin, and a significant increase (P<0.01) on the 7th day of serum albumin level. On the other hand, there was a significant increase (P<0.05) of both gamma 1 (γ1) and alpha 1 (α1) globulin on the 3rd day and a gradual significant increase (P<0.01) from the 5th day. Both gamma 2 (γ2) and alpha 2 (α2) globulin were gradually significantly increased (P<0.001) from the 5th day of supplement, with non-significant changes of both beta 1 (β1) and beta 2 (β2) globulin compared to control non-supplemented group.

Discussion

The result of garlic oil gas chromatographically analysis that contains fatty acids, organosulphur compounds, flavonoids, phenolic derivatives and antioxidants bio-active compound were in accordance with the results recorded in previous studies [21,22]. Active components of garlic oil, diallyl sulphide (DAS) and diallyl disulphide (DADS), have been found to protect and treat oxidative damage [23]. Hexadecanoic acid constituents in garlic oil has immunomodulation effects [24]. Organosulphur compounds 2-Aminoethanethiol Hydrogen Sulfate (Ester), DAS, DADS and diallyl trisulfide which modulate the activity of several metabolizing enzymes that activate cytochrome or detoxify glutathione S-transferases and inhibit the formation of DNA adducts in several target tissues [25].

The antioxidant results of garlic oil-supplemented calves revealed an increase of SOD, CAT, TAC, and GSH, while decreased the oxidative stress marker MDA following the results recorded by Chen et al.[26] in rats and Alagawany et al.[27] in rabbits. These results also resemble the result recorded by Asghari et al.[4] by using herbal essential oil in suckling calves. These results may be attributed to the ability of garlic oil to improve antioxidant enzyme activity [28]. In the present study, the decreased MDA level in RBCs by garlic oil may be originated from the elevation of SOD, CAT, TAC, and GSH and/or its act as a radical scavenger. Garlic functions as an antioxidant by several mechanisms, and one of the best defense mechanisms against oxidative damage is enhanced by the antioxidant bioactive compound constituting in garlic. Garlic oil has a role as an antioxidant activator and decreases oxidative stress [29,30]. Another mechanism for the antioxidant activity of garlic is the reaction with free radicals, flavonoids constituents of garlic oil may prevent free radicals injury by a direct scavenging of free radicals. Flavonoids interact with the radical stabilizing of ROS. Radicals were inactivated by the high reactivity of the hydroxyl group on flavonoids [8,9]. Also, the activities of organosulphur compounds diallyl sulfide (DAS), and diallyl disulfide (DADS) constituted in garlic oil protect and treat oxidative damage [23].

The result of elevated serum levels of vitamins A and E with trace elements of selenium, iron, and zinc of garlic oil-supplemented calves may be attributed to the constituent of garlic oil to some active compounds, like that organosulphur compounds, enzymes, sterols, steroids, and organo-selenium compounds [5], also garlic containing many nutrient components such as protein, free amino acids, vitamins, and trace elements [6]. Garlic physiological effects are provided by organosulphur-containing compounds as well as flavonoids, minerals (Ca, Fe, K, Mg, Na, Zn), and vitamins (A, E, C, and B complex) [31].

The elevation of serum total protein, albumin, and globulin after garlic oil supplement in suckling calves was in agreement with Hassan and Abdel-Raheem [11] and Duvvu et al.[32], and similar results were recorded by Alagawany et al.[27] after garlic dietary supplement in growing rabbit, and El-Nomeary et al.[33] since supply essential garlic oil in the rabbit. These results provided the improvement of the metabolic and general health status of calves and the role of garlic in improving nutrient digestibility and protein utilization [11,32,34]. Garlic and its bioactive compounds activate protein utilization [35]. The albumin concentration involves the transport of several exogenous chemical compounds and endogenous metabolites and regulates osmotic pressure, whereas globulin is an important part of

the immune system [36]. Increased globulin levels indicated improved immune response. These results may be referred to the organosulphur compounds constitute in garlic and their immunomodulation effects [24,35]. This increase in globulin may be related to the elevation of gamma globulin in those following El-Nomeary et al.[33] in rabbits. These results provided the role of garlic oil supplements in improving the immunity and utilization of nutrients responsible for immunity modulation and regulation [5,27].

In conclusion, garlic oil supplement in suckling buffalo calves have a promotives effects on antioxidants, they can directly and rapidly scavenge free radicals and/or block their formation by increasing endogenous antioxidant and serum vitamins level. Garlic oil constitutes a bioactive compound that provided physiological effects, increasing immunity and helping in the utilization of nutrients responsible for immunity modulation and regulation, and by extension improving general health status of calves. So we recommend garlic oil be used as a supplement for suckling buffalo calves to increase the productivity of large animal farms.

Availability of Data and Materials

The data sets analyzed during the current study are available from the corresponding author (H. M. Yousif) on reasonable request.

Funding Support

There was no funding support.

Ethical Statement

This protocol was approved by the Research Committee of the Animal Health Research Institute and authorized by The Institutional Animal Care and Use Committee (ARC-IACUC)/ Agricultural Research Center (ARC/AHRI/43/23).

Conflict of Interest

No potential conflict of interest was reported by the authors. Authors only are responsible for the content and writing of the paper.

Author Contributions

H. M. Yousif designed the research and collect the samples. M. K. Mansour, H. M. Yousif, A. M. El Mahdy and M. F. Hassan performed the experimental duties of this study and analyzed the data. M. K. Mansour and H. M. Yousif did the statistical analyses. All authors participate in writing and approved the final version of the manuscript.

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