

Effect of Dietary Supplementation of *Asparagus Racemosus* Essential Oil on Some Haemato-Biochemical Constituents and Oxidative Stress Indices of Gaddi Rams

Alagbe John Olujimi

Department of Food Hygiene and Control (Meat hygiene), Faculty of Veterinary Medicine, Benha University, Benha 13511, Egypt.

Corresponding author: Alagbe John Olujimi, Department of Food Hygiene and Control (Meat hygiene), Faculty of Veterinary Medicine, Benha University, Benha 13511, Egypt.

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Abstract:

This experiment was designed to determine the effect of dietary supplementation of *Asparagus racemosus* essential oil on some haemato-biochemical constituents and oxidative stress indices of Gaddi rams. Twenty-four male Gaddi rams with a mean body weight of 32.00 ± 1.8 kg and an average age of 7 months were used in a completely randomized design for a 60-day feeding period preceded by a 14-day quarantine period. Experimental treatments included: treatment 1 (control) – basal diet without essential oil; treatment 2 and treatment 3 – basal diet supplemented with 200 mg and 400 mg *Asparagus racemosus* essential oil per kg DM feed daily. Fresh water was supplied ad libitum and all other management practices were strictly observed. Results obtained revealed that *Asparagus racemosus* essential oil contained several bioactive compounds with therapeutic properties. *Asparagus racemosus* essential oil supplementation enhanced the level of pack cell volume, haemoglobin, red blood cell, lymphocytes, eosinophils and monocytes ($p < 0.05$). The serum levels of total protein, albumin, globulin, glucose, calcium, phosphorus, potassium, sodium, chloride and bicarbonate concentrations ($p < 0.05$). Conversely, concentrations of creatinine, urea, alanine amino transferase and aspartate transaminase were not affected ($p > 0.05$). *Asparagus racemosus* essential oil supplementation decreased malondialdehyde concentrations in the blood and increased the levels ($P < 0.05$) of catalase, superoxide dismutase and glutathione peroxidase. It was concluded that *Asparagus racemosus* essential oil dietary supplementation up to 400 mg per kg DM feed did not pose any negative effect on the health status of animals.

Key words: asparagus racemosus; essential oil; haematology; serum; oxidative stress

Introduction

Medicinal plants have the capability to synthesize secondary metabolites or phytochemicals which confers them the ability to perform several pharmacological activities [1]. These compounds have been generally regarded as safe, efficient, capable of reducing environmental pollution and have no withdrawal period [2,3]. Essential oils are plant extracts obtained through extraction with organic solvents and subsequent distillation. These extracts are a combination of different molecules because the way they are extracted will carry a general group of chemicals with specific characteristics. Essential oils have also been considered as safe alternative to antibiotics because they do not cause antimicrobial resistance nor do they create toxic residues in animal products [4]. Among the potential essential oils with therapeutic relevance are those from *Asparagus racemosus*.

Asparagus racemosus is a shrub with a tuberous root belonging to the family Asparagaceae. The genus *Asparagus* includes about 300 species widely distributed globally. The plant is found at low altitudes in shade and in tropical climates including Africa, Europe, Asia including India

[5,6]. Results from previous investigation into the chemical composition of *Asparagus racemosus* leaf, stem and root showed that the investigated samples contain phytochemicals such as polyphenols, saponins, tannins, steroids, alkaloids and flavonoids which have antiplatelet, cytotoxicity, angiogenic, antitumor, antineoplastic, antiviral, sedative, muscle relaxant, cytotoxic, antiviral, insecticidal, cardioprotective, analgesic, inhibition of lipid peroxidation, anti-inflammatory, fungicidal, antiprotozoal, antimalarial, and antirheumatic, Antifertility, antinociceptive, antidiarrhoea, antifungal, immuno-stimulatory, anti-tumor, hepato-protective, amongst others [7]. Chemical analysis of *Asparagus racemosus* revealed that the leaf has several nutritional benefits and it is loaded with vitamins such as vitamin A, B1, B2, C, E, Mg, P, Ca, Fe, and folic acid [8]. It's essential oil also contains important amino acid, asparagine, arginine, tyrosine and valine [9]. *Asparagus racemosus* leaf decoction is used to treat diarrhea, hypertension, gastro intestinal problems, malaria, skin infection, asthmas, cough, bronchitis, and expectorant [10]. Essential oil from the leaves have

also been confirmed to inhibit the activities of some pathogenic organisms [11].

It has been well-documented that dietary supplementation of essential oils could improve animal performance by increasing digestive enzyme secretion, lowering the number of harmful bacteria in the digestive tract, modulating intestinal morphology functions, and positively affecting productivity, blood metabolites, and immunological and antioxidant status of ruminants. [12,13]. Supplementation of thyme oil in the diet of sheep has been reported to confer positive immune effects such as increase in lymphocyte proliferation rate, phagocytic rate as well as increase in immunoglobulins in the blood of goats [14].

This research is timely because the demand for natural antioxidants in animal feed is increasing due to their health benefits against oxidative stress and several diseases. A timely evaluation of the bioactive compounds in *Asparagus racemosus* will further give insight their therapeutic properties and also use it to address the high rate of antimicrobial resistance and promote food safety.

Materials and methods

Study Area

The study was carried out at the Sheep and Goat section, Ganhi College of Agriculture located at Rajasthan, India. All animals used in the experiment were cared for according to applicable recommendations of Indian Society of Animal Production. All laboratory analysis was carried out at Sumitra Research Institute, Gujarat India using standard procedures according to Association of Analytical Chemist.

Animals, experimental design, and sampling

Twenty-four male Gaddi rams with a mean body weight of 32.00 ± 1.8 kg and an average age of 7 months were used in a completely randomized design for a 60-day feeding period preceded by a 14-day quarantine period. During the adaptation period, goats were vaccinated against *Peste des Petits* and dewormed using Wormzap Albendazole®. Animals were weighted at the end of the quarantine period using digital sensitive scale and stratified based on their weights into three dietary groups with 8 replicates each. Goats were housed in individual pens measuring (2m²/goat) equipped with feeders and drinkers. Experimental treatments included: treatment 1 (control) – basal diet without essential oil; treatment 2 and treatment 3 – basal diet supplemented with 200 mg and 400 mg *Asparagus racemosus* essential oil per kg DM feed daily. The basal diet was formulated to meet the standard nutrient requirements of goats according to Nutritional Research Council recommendations [15]. Before the morning feeding, 200 g total diet DM was mixed with *Asparagus racemosus* essential oil in each treatment to ensure the consumption of their full dose. Animals were fed thrice daily between at 07:00, 12:00 and 16:00 h and had unrestricted access to clean water. Cleaning of the pens and washing of drinkers was done daily. During the experimental period, goats were weighed individually weekly. The amount of both offered and refusal feed were recorded every day to determine their feed intake. Composition of *Asparagus racemosus* essential oil and experimental diet is presented in Table 1 and 2 respectively.

Asparagus racemosus oil extraction and Gas chromatography-mass spectrometry analysis

Fresh leaves of *Asparagus racemosus* were sourced from the premises of Gandhi College of Agriculture, Rajasthan in India. The collected leaves were taken to the Taxonomy Department, Sumitra Research Institute, Rajasthan for identification before it was assigned an authentication number – SRI-00T/2024. The leaves were rinsed in a plastic of water to remove dirt's, spread on a plastic sieve for 20 minutes to allow water drain and air dried for eight days. Dried samples were later pulverized into powder using a multi-purpose electric blender before extraction. Extraction of oil was done using steam distillation technique with Clevenger apparatus were recently published by [16]. The oil obtained was stored in the refrigerator at a temperature of 4 °C prior to laboratory analysis.

Analysis of *Asparagus racemosus* oil was performed on an Agilent Model 7890A Gas Chromatography interfaced to an Agilent 7000 GC/MS Triple Quad. The equipment carrier gas was helium which was maintained at a pressure, temperature and average velocity of 1.500 psi, 300 °C and 43.11 cm/sec respectively to maintain accuracy according to the manufacturer's recommendation in the GC. The MS was operated at an ionization voltage of 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C. Components identification The components of the essential oil were identified on the basis of their retention indices. Identification confirmation of reference compounds from the Library of National Institute of Standard and Technology database.

Collection and analysis of blood constituents

On the last day of the experiment, 10 mL of blood was collected from the jugular vein of four animals per treatment into two bottles (5 mL each). Samples for haematology were collected into bottles with anticoagulant while those for serum were put into plain bottles. Collected samples were placed in an ice pack before transporting them to the laboratory. Haematological parameters were analyzed using Mission® HA-360 3 – automatic haemo-analyzer (Netherlands) which uses cyanide free reagent. White blood cell count is determined through impedance method, red blood cell (sheath flow impedance method), haemoglobin count (colorimetric method) and the machine is adjusted to a temperature of 15 – 30 °C and humidity of 50 – 80 % for proper precision once the samples were arranged in the collection chamber.

Serum parameters were analyzed using SKLA-HB1-POC auto-chemistry analyzer (Taiwan). Kit is adjusted to a sample volume of 200 µL, temperature / humidity of 10 to 32 °C/ 50 to 90 % to ensure accuracy in the results.

Determination of oxidative stress indices

Malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase were analyzed using the samples for haematological studies. Fully automatic chemiluminescence immuno-analyzer (YTE-CF10, Netherlands) adjusted to a reaction temperature of 37°C, relative humidity (30 to 65 %) and a measuring time of up to 20 minutes for 12 simultaneous samples.

Statistical Analysis

All data collected were subjected to a one-way analysis of variance (ANOVA). Means showing significant differences were separated using Duncan's Multiple Range Test. The SPSS (version 25) statistical package was used for all statistical analysis.

S/N	Compounds	% Concentration	R.T (min)
1	Octadecanoic acid	0.10	6.00
2	2-methyloctacosane	0.16	6.24
3	Ethyl ricinoleate	0.08	7.08
4	Hexahydrofarnesyl acetone	0.11	7.37
5	Hexadecanoic acid	0.17	7.55
6	Tritetracontane	0.02	8.01
7	1-Butene,4-iodo	0.04	8.85
8	1,2,3-Propanetriol,1- acetate	0.19	8.97
9	Phytol	28.40	9.11
10	Squalene	2.19	10.10
11	1-Octanol, 2-butyl	0.01	10.37
12	Oleic acid	0.08	10.89
13	Trans-9-Octadecenoic acid, pentyl ester	0.13	11.51
14	9-Tetradecenal	0.02	11.90
15	Octadecanoic acid	0.18	12.33
16	Chloromethyl 7-chlorododecanoate	0.02	12.91
17	Stigmasterol	0.09	13.00
18	Ethyl ricinoleate	0.25	13.26
19	Linoleic acid	0.16	13.79
20	Butyl tetracontyl ether	0.02	14.02
21	Ethyl ricinoleate	0.06	15.02
22	2-Dimethylsilyloxytetradecane	8.71	15.28
23	2-Furanone,3,4- dihydroxytetrahydro	0.04	15.81
24	Cyclopentanedione	10.41	16.09
25	IsosorbideDinitrate	9.96	17.11
26	Ethyliso-allocholate	0.15	18.05
27	4H-Pyran-4-one,2,3- dihydro-3,5-dihydroxy-6-methyl 2(3H)-	10.90	18.39
28	9-Eicosyne	8.21	18.55
29	Phenol,4,4'-(3- ethenyl-1-propene-1,3-diyl)bis-(E)	12.04	18.92
30	7- Dehydrosiosgenin3- acetate	0.05	19.07
31	3-Deoxy-d-mannoic lactone	0.17	19.55
32	Quinoline,8- hydrazine	0.10	20.40
	Total	93.22	

Table 1: Chemical composition of *Asparagus racemosus* oil

Components	Quantity (g/kg DM)
Maize (890 g/kg CP)	300.0
Corn barn	260.0
Cowpea husk	200.0
Groundnut cake (400 g/kg CP)	135.0
Soya bean meal (480 g/kg CP)	100.0
Growers Mineral/Vitamin Premix	2.00
Limestone	2.00
Salt	1.00
Total	1000.0
Chemical composition (g/kg dry matter - DM)	
Dry matter	940.0
Organic matter	930.0
Crude protein	157.0
Ether extract	30.00
Ash	70.00
Non-structural carbohydrate	350.00
Acid detergent fibre	220.00
Acid detergent lignin	300.00
Neutral detergent fibre	371.00
Cellulose	190.7
Hemicellulose	15.00
Energy (Kcal/kg)	2600.8s

Table 2: Ingredient and chemical composition of basal diet (g/kg of DM)

.5 kg Grower's premix contained: 15,000 I.U. vitamin A; 8000 mg vitamin B1; 3000 I.U. vitamin D3; 60.0 mg vitamin E; 15 mg Choline; 0.96 mg Cobalt; 2.00 mg I; 50 manganese Mn; 0.50 mg Selenium; 250 mg.

Constituents	T1	T2	T3	SEM
Pack cell volume (%)	29.74 ^b	32.08 ^a	33.12 ^a	2.69
		9.03 ^a	9.11 ^a	0.18
Red blood cell ($\times 10^6/L$)	6.96 ^b	10.40 ^a	10.95 ^a	0.25
White blood cell ($\times 10^3/L$)	5.73 ^b	7.19 ^a	7.63 ^a	0.16
Lymphocytes (%)	50.09 ^b	67.99 ^a	68.05 ^a	4.08

Monocytes (%)	1.25 ^b	2.10 ^a	2.18 ^a	0.01
Eosinophils (%)	3.91 ^b	4.03 ^a	4.15 ^a	0.02

Table 3: Haematological parameters of Gaddi rams fed diet supplemented with *Asparagus racemosus* oil

^{a,b}: Means within a row with different superscripts are significantly different ($p < 0.05$); T1: basal diet without oil; T2 and T3: basal diet with supplemented with 200 mg and 400 mg *Asparagus racemosus* essential oil per kg DM feed daily

Constituents	T1	T2	T3	SEM
Total protein (g/dL)	4.01 ^b	5.18 ^a	5.36 ^a	0.02
Albumin (g/dL)	2.06 ^b	2.98 ^a	3.20 ^a	0.01
Globulin (g/dL)	1.95 ^b	2.20 ^a	2.16 ^a	0.01
Urea (mg/dL)	6.62	6.51	6.48	0.15
Creatinine (mg/dL)	4.02	4.00	3.98	0.10
Glucose (mg/dL)	50.90 ^b	65.17 ^a	66.02 ^a	3.07
Cholesterol (mg/dL)	55.02 ^a	47.62 ^b	46.61 ^b	2.05

Table 4: Serum biochemical indices of Gaddi rams fed diet supplemented with *Asparagus racemosus* oil

^{a,b}: Means within a row with different superscripts are significantly different ($p < 0.05$); T1: basal diet without oil; T2 and T3: basal diet with supplemented with 200 mg and 400 mg *Asparagus racemosus* essential oil per kg DM feed daily

Constituents	T1	T2	T3	SEM
ALT (U/L)	62.19	65.80	64.19	2.63
AST (U/L)	30.67	31.22	31.83	1.85
Calcium (mmol/L)	1.82 ^b	2.96 ^a	2.98 ^a	0.02
Phosphorus (mmol/L)	1.00 ^b	1.68 ^a	1.70 ^a	0.10
Potassium (mmol/L)	2.40 ^b	3.27 ^a	3.30 ^a	0.11
Sodium (mmol/L)	118.5 ^b	131.9 ^a	135.6 ^a	8.94
Chloride (mmol/L)	100.6 ^b	118.7 ^a	121.1 ^a	7.06
Bicarbonate (mmol/L)	61.55 ^b	90.31 ^a	90.88 ^a	3.77

Table 5: Serum enzymes and ions of Gaddi rams fed diet supplemented with *Asparagus racemosus* oil

^{a,b}: Means within a row with different superscripts are significantly different ($p < 0.05$); ALT: alanine amino transferase; AST: aspartate transaminase; T1: basal diet without oil; T2 and T3: basal diet with supplemented with 200 mg and 400 mg *Asparagus racemosus* essential oil per kg DM feed daily

Parameters	T1	T2	T3	SEM
Superoxide dismutase (ng/mL)	20.85 ^b	33.86 ^a	34.07 ^a	2.30
Glutathione peroxidase (ng/mL)	19.05 ^b	25.06 ^a	25.14 ^a	1.69
Malondialdehyde (ng/mL)	106.5 ^a	98.05 ^b	97.12 ^b	6.32
Catalase (μ mol/L)	40.08 ^b	54.18 ^a	55.02 ^a	4.11

Table 6: Oxidative stress parameters of Gaddi rams fed diet supplemented with *Asparagus racemosus* oil

^{a,b}: Means within a row with different superscripts are significantly different ($p < 0.05$); T1: basal diet without oil; T2 and T3: basal diet with supplemented with 200 mg and 400 mg *Asparagus racemosus* essential oil per kg DM feed daily

Results and Discussion

Asparagus racemosus oil GC-MS analysis (Table 2) showed the total of 32 bioactive compounds representing 93.22 %. The major constituents identified were; Phytol (28.40 %), Phenol,4,4'-(3- ethenyl-1-propene-1,3-diyl) bis-, (E) (12.04 %), 4H-Pyran-4-one,2,3- dihydro-3,5-dihydroxy-6-methyl 2(3H)- (10.90 %), Cyclopentanedione (10.41 %), IsosorbideDinitrate (9.96 %), 2-Dimethylsilyloxytetradecane (8.71 %) and 9-Eicosyne (8.21 %) while Chloromethyl 7-chlorododecanoate (0.02 %), Butyl tetratriacontyl ether (0.02 %), Tritetracontane (0.02 %) and 1-Octanol, 2-butyl (0.01 %) were amongst the minor compounds. The result obtained in this study is in agreement with earlier reports on the root extract of *Asparagus racemosus* [18, 19] with respect to the presence of Phytol, 2-Furanone,3,4- dihydroxytetrahydro and 2-Dimethylsilyloxytetradecane. However, all compounds identified in the sample has several pharmacological activities including, antimicrobial [20], anti-inflammatory, hypercholesterolemic [21], nematocide, anticancer, anti-tumor [22], hepato-protective [23], Insectifuge, anti-staminic, analgesics [24], 5 alpha reductase inhibitor, anti-eczemic, anti-androgenic, anti-coronary [25], anti-arthritis, antioxidant, anti-

androgenic, hemolytic, dermatitigenic, anti-helminthic [26,27], immunomodulatory [27], antidiarrheal, antifungal, antiviral amongst others.

Haematological parameters of Gaddi rams fed diet supplemented with *Asparagus racemosus* oil (Table 3). Pack cell volume, haemoglobin and red blood cell values were lower in treatment 1 {(29.74 %, 8.92 g/dL and 6.96 ($\times 10^6$ /L)} than treatment 2 {(32.08 %, 10.40 g/dL, 9.03 ($\times 10^6$ /L)} and treatment 3 {(33.12 %, 10.95 g/dL, 9.11 ($\times 10^6$ /L)} ($p < 0.05$). Pack cell volume recorded in this study was within (29.00 – 36.00 %) cited by [28]. Haemoglobin and red blood cell values were within 6.90 – 16.00 g/dL and 5.80 – 15.00 ($\times 10^6$ /L) reported by [29]. This outcome suggests that *Asparagus racemosus* oil dietary supplementation could enhance the production of iron to make haemoglobin which allows the efficient transportation of oxygen to the lungs and other parts of the body [30]. White blood cell {(5.73 – 7.63 ($\times 10^3$ /L)}, lymphocytes {(50.09 – 68.05 %)}, monocytes {(1.25 – 2.18 %)} and eosinophils {(3.91 – 4.15 %)} in treatment 2 and 3 were similar ($p > 0.05$) but significantly higher than those in treatment 1 ($p < 0.05$). This result suggests that *Asparagus racemosus* oil has antioxidant capacity thus increasing the phagocytic rate as well as the proliferation of lymphocytes and immunoglobulin to prevent infection

in the body of animals [28]. White blood cell, lymphocytes, monocytes and eosinophil counts were within the reference interval cited by [29]. [30] recorded a lower white blood cell count of $\{(4.91 - 6.12 (\times 10^3/L))\}$ in calves fed different levels of thyme and cinnamon essential oil. This variation could be as a result of influence of bioactive compounds, extraction method, differences in dosage used as well as duration of experiment [31].

Table 4 reveals the serum biochemical indices of Gaddi rams fed diet supplemented with *Asparagus racemosus* oil. Total protein (4.01 – 5.36 g/dL), albumin (2.06 – 3.20 g/dL) and globulin values (1.95 – 2.16 g/dL) were influenced by the treatment ($p < 0.05$). Total protein value obtained in this study is in agreement with the normal range (4.00 – 8.25 g/dL) reported by [32]. This result suggests that dietary protein in the experimental diet was sufficient for growth and development of animals [25]. Urea and creatinine values which ranged from 6.62 to 6.48 mg/dL and 3.98 to 4.02 mg/dL were not affected ($p > 0.05$) by the treatment. However, values recorded in this experiment were within the normal level [urea: 3.00 – 8.00 mg/dL] and [creatinine: 2.30 – 5.06 mg/dL] reported by [33]. This result is a clear sign that the kidneys of the animals were in good state indicating that supplementing *Asparagus racemosus* oil at 400 mg/kg in the diet of sheep was not detrimental to their body. Glucose and cholesterol values were consistent with the findings of [27] who recorded a normal range of 43.19 – 78.00 mg/dL and 50.60 – 110.0 mg/dL respectively. Cholesterol level declines as the level of *Asparagus racemosus* oil increases across the treatment indicating that it possesses hypo-lipidemic properties [26].

Serum enzymes and ions of Gaddi rams fed diet supplemented with *Asparagus racemosus* oil (Table 5). Calcium (1.82 – 2.92 mmol/L), phosphorus (1.00 – 1.70 mmol/L), potassium (2.40 – 3.30 mmol/L), sodium (118.5 – 135.6 mmol/L), chloride (100.6 – 121.1 mmol/L) and bicarbonate (61.55 – 90.88 mmol/L) follow similar pattern and were higher in treatment 3 and 2 than in treatment 1 ($p < 0.05$). Values obtained in this study were consistent with the findings of [34]. This outcome suggests sufficient concentrations of serum minerals needed to maintain fluid and pH balance to ensure proper functioning of the cells and nerves of animals [24]. Alanine amino transferase and aspartate transaminase values [(62.19 – 64.19 U/L; 30.67 – 31.83 U/L)] were not significantly affected ($p > 0.05$) by the treatment. However, values obtained were in agreement with the normal range reported by [33, 37]. This result indicates the absence of hepatic disorder in the body of animals [25, 36].

Oxidative stress parameters of Gaddi rams fed diet supplemented with *Asparagus racemosus* oil (Table 6). Superoxide dismutase, glutathione peroxidase and catalase values were lower in treatment 1 [(20.85 ng/mL; 19.05 ng/mL and 40.08 (μmol/L)] than in treatment 2 [(33.86 ng/mL; 25.06 ng/mL and 54.18 (μmol/L)] and [(34.07 ng/mL; 25.14 ng/mL and 55.02 (μmol/L)] ($p < 0.05$). Conversely, malondialdehyde values were higher in treatment 1 (106.5 ng/mL) than in treatment 2 (98.05 ng/mL) and 3 (97.12 ng/mL) ($p < 0.05$). This result suggests that supplementing *Asparagus racemosus* oil in the diet of animals is capable of neutralizing malondialdehyde by scavenging free radicals capable of causing disease and increased production of superoxide dismutase and glutathione peroxidase due to the presence of bioactive compounds (Table 1). The result obtained is in agreement with the report of [38] when rosemary oil was supplemented in the diet of fattening lambs.

Conclusion

In conclusion, *Asparagus racemosus* oil contains several bioactive compounds with therapeutic properties. Supplementing *Asparagus racemosus* oil up to 400 mg per kg DM feed daily improved phygocytic rate, blood constituents and scavenge the activities of free radicals thus helping animal to maintain a stable health status and resistance against disease.

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