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Effects of garlic (Allium sativum) and ginger (Zingiber officinale) powder and their combination on antioxidant and hematological response in sheep

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Abstract

Garlic and ginger as herbs exhibit strong antioxidant and immunological properties, but their combination may have a complementary role on each other. This study was done to assess the complementary effect of supplementing garlic and ginger powder on the antioxidant status and physiological response of sheep. Sixteen (16) mixed breed rams were maintained on four experimental diets in a completely randomized design for 84 days. The four diets were Control (no garlic or ginger powder), GaP (garlic powder 2.5 g/kg diet), GiP (ginger powder 2.5 g/kg diet) GGP (garlic powder 1.25 g/kg diet + ginger powder 1.25 g/kg diet). A one-way analysis of variance as contained in the GLM of SPSS (version 23) was used to analyze data on hematological parameters, serum indices, serum mineral concentration, and oxidative stress biomarkers of the sheep. Findings from the results revealed a reduction ($p<0.05$) in red blood cell total protein and serum concentrations of sodium and calcium in the herbal groups. The reduction ($p<0.05$) in the concentration of manganese, iron, and zinc in the ginger powder group was enhanced ($p<0.05$) by its combination with garlic powder. Albumin, superoxide dismutase and malondialdehyde concentrations as markers of oxidative stress were enhanced ($p<0.05$) in the herbal combination group compared to only the garlic group. Nitric oxide concentration further reduced in the herb combination group compared to feeding garlic or ginger alone. The combination of garlic and ginger powder gave optimum immunological and anti-oxidant responses than feeding only garlic or ginger. The combination of 1.25 g/kg diet of garlic and ginger each would achieve an overall better anti-oxidant and immunological performance in sheep than feeding 2.5 g/kg diet of garlic or ginger singly.

Keywords: Sheep, garlic, ginger, combination, antioxidant, physiology

Introduction

Livestock production has continued to face challenges arising from global warming and management systems leading to oxidative stress and reduced immune response in animals. Feeding strategies that will improve the antioxidant and immune response of the animals are, therefore, advocated. These strategies must also involve the use of natural products.

As a result of its oil which is said to exert a protective effect on DNA, ginger may be known to exhibit great antioxidant activity as demonstrated in cell culture (Chaiyakunapruk et al. 2006). Ginger prevents lipid peroxidation and also inhibits or breaks its chain (Afzal et al. 2011). Garlic, on the other hand, is reported to enhance serum levels of catalase and glutathione peroxidase thereby making it strong antioxidants (Wojdylo et al. 2007). Garlic organo-sulfur compounds may be able to prevent depletion of glutathione (Torok et al. 1994) and also exert antioxidant activities as protective compounds against free radical damage (Chung 2006). The use of these two herbs in the diet of ruminants may be a useful strategy in enhancing antioxidant capacity and serum indices of the animals.

Ginger and garlic have been extensively used in the diet of ruminants with improved serum, hematological and mineral indices of the animals (Abdulmumin et al. 2014; Muhammad et al. 2016; Ikyume et al. 2017; Al-Azazi et al. 2018). While reports of the use of ginger and garlic have shown promising results, with each having comparative advantages, not much has been done to harness the complementary effect of the two herbs to achieve the possibility of achieving an improved physiological and antioxidant capacity of sheep. Thus, we predicted
that the supplementation of garlic or ginger powder alone or together might positively affect the physiology and antioxidant capacity of sheep. This research, therefore, seeks to unveil the comparative effect of ginger and garlic used singly or in combination with oxidative stress biomarkers, blood minerals, hematological, and serum parameters.

Materials and methods

Experimental site

The experiment was carried out at the Animal Science Teaching and Research Farm of the Federal University of Agriculture, Makurdi. The site lies between latitude 7° 44' 1.50'' N and longitude 8° 31' 17.00'' E

Collection and preparation of plant bulbs material

The garlic and ginger bulbs were purchased from the local market in fresh condition. The bulbs were oven-dried at a temperature of 70°C for three days. After drying, the plant materials were ground with the help of an electric grinding machine.

Experimental animals management, diet, and design

Sixteen (16) mixed breed (Yankasa and West African Dwarf breeds) yearling rams with average weight of 15.43±2.29 used in this study were divided into four groups and assigned to four (4) treatment groups with each animal serving as a replicate. The rams were purchased from Lafia town, Nasarawa State, Nigeria. They were quarantined for four weeks. During the quarantine period, oxytetracycline LA (1 ml/10 kg) was administered to the animals for prophylactic treatment against bacterial disease. They were also treated for ecto and endoparasite using Ivermectin® (1 ml/10 kg). The four experimental diets (Table 1) were Control (no garlic and/or ginger powder), GaP (Garlic inclusion: 2.5 g/kg diet inclusion), GiP (Ginger inclusion: 2.5 g/kg diet) GGP (garlic: 1.25 g/kg diet + ginger: 1.25 g/kg diet). The dosage of ginger adopted for this experiment was as recommended by Muhammad et al. (2016). Levels of garlic powder adopted were to reflect the amount of ginger used in the study. The experiment was laid out as a completely randomized design and lasted for 84 days. All animal management protocols were approved by the Department of Animal Production, Federal University of Agriculture, Makurdi, Nigeria (CAS/ANP/2017/2018/10).

Table 1 Gross composition of experimental diets

	[1]Treatment Diets			
Ingredient	Control	GaP	GiP	GGP
Maize offal	45	45	45	45
Palm kernel cake	30	30	30	30
Soybean meal	10	10	10	10
Rice offal	10	10	10	10
Bone meal	3.5	3.5	3.5	3.5
Mineral premix	0.5	0.5	0.5	0.5
Salt	1	1	1	1
Garlic	-	0.25	-	0.125
Ginger	-	-	0.25	0.125
Total	100	100	100	100
[2]Determined analysis				
Dry matter	84.09	85.01	85.71	85.34
Crude protein	16.58	17.18	16.65	16.98
Ether extract	17.20	18.00	17.63	17.89
Ash	6.69	6.97	6.68	6.83
ADF	38.96	36.76	37.31	36.98
NDF	47.23	45.43	46.52	46.12

[1] Control (no garlic and/or ginger powder), GaP (Garlic inclusion: 2.5 g/kg diet inclusion), GiP (Ginger inclusion: 2.5 g/kg diet) GGP (garlic: 1.25 g/kg diet + ginger: 1.25 g/kg diet).
[2]ADF (acid detergent fiber), NDF (Neutral detergent fiber)

Data collection

Blood samples were collected from all animals per treatment after 84 days of feeding garlic and/or ginger powder for determination of hematological parameters (PCV, WBC, RBD differentials), serum indices (total protein, globulin, AST, ALT, glucose, total cholesterol, HDL and LDL), serum minerals (Na, Ca, Mn, Cu, Fe, and Zn) and serum oxidative stress biomarkers (albumin, bilirubin, uric acid, SOD, glutathione peroxidase, MDA, and nitric oxide). Blood samples for hematological indices were emptied into sample bottles containing ethylene tetra acetic acid (EDTA), to prevent blood clotting while samples for serum analysis were collected into bottles without EDTA to allow for clothing to harvest the serum.

Hematological parameters

Blood parameters such as RBC, WBC, and lymphocyte counts were evaluated utilizing an automatic blood analyzer (ADVIA 120, Bayer, USA). Packed cell volume (PCV) and hemoglobin (Hb) were determined using the microhaematocrit method and cyanmethemoglobin method, respectively.

Serum indices

The serum total protein concentration was determined using Biuret reagent as described by King and Wooton (1965).

Plasma glucose concentration was determined by the enzymatic colorimetric method using a kit (Spinreact, S.A., Spain). The amounts of total Cholesterol, AST, ALT, triglyceride, HDL, LDL were spectrophotometrically determined on Olympus Au 400 system autoanalyzer.

Serum minerals

The blood sample was centrifuged at 3000 rpm for 15 minutes (SPEC23) to obtain serum. Serum was thereafter mixed with 1 ml of concentrated HNO_3 and 0.5 ml of H_2O_2 in the propylene tubes. The mixture was maintained at 60°C for 2 hours using a flame photometer (FP640) to allow for the digestion of samples. The digests obtained were diluted by adding 2.5 ml of distilled water, the sample solutions were then centrifuged at 200 rpm for 5 minutes and subsequently analyzed using AAS210 VGP to obtain concentrations of trace elements (Zn, Cu, Fe, Na, Ca and Mn).

Oxidative stress biomarkers

Bilirubin was assayed as described by Nedredal et al. (2009) and Beppu et al. (2009). Glutathione peroxidase (GSHPx) was spectrophotometrically analyzed as described by Chen et al. (2014). Malondialdehyde (MDA) was assayed as described by Davison (2011). Superoxide dismutase (SOD) activity in blood serum was determined by the method of Janknegt et al. (2007). The nitric oxide (NO) level was spectrophotometrically analyzed as described by Zeng et al. (2011). Uric acid was analyzed as described by Whidden et al. (2009). Serum albumin concentration was determined as described by Maier et al. (2006) and Shin et al. (2009).

Results and discussion

Results

Haematological parameters of sheep fed garlic or ginger and their combination

The result of hematological parameters of sheep fed diet containing garlic or ginger powder and their combination is shown in table 2. Red blood cells (RBC) decreased ($p<0.05$) in the entire herb groups with the highest ($p<0.05$) RBC found in control while all herb groups had comparatively least ($p<0.05$) RBC. Basophil count was comparatively higher ($p<0.05$) in the control and ginger group, but reduced ($p<0.05$) in the garlic and herbal combination group with comparatively least ($p<0.05$) basophil count. All other hematological parameters measured were not affected ($p>0.05$) by the feeding of garlic or ginger and their combination.

Table 2 Haematological parameters of sheep fed diet containing garlic and ginger powder

[1]Parameter	[2]Treatment diets				[3]SEM
	Control	GaP	GiP	GGP	
Packed cell volume (%)	33.33	27.33	31.00	29.50	1.03
Haemoglobin (g/dl)	11.10	9.90	10.33	9.80	0.36
Red blood cells	8.83[a]	7.60[b]	6.97[b]	6.85[b]	0.28
White blood cells	6.47	6.00	5.87	6.20	0.15
Mean corpuscular volume (fl)	40.80	38.93	44.50	43.10	0.97
Mean corpuscular haemoglobin (pg)	13.63	13.00	15.27	14.30	0.39
MCHC (g/dl)	33.27	33.33	33.27	33.20	0.03
Lymphocyte (%)	66.00	65.33	63.00	67.00	0.82
Neutrophil (%)	26.67	29.00	31.67	29.50	0.96
Eosinophil (%)	3.00	1.67	3.33	1.50	0.41
Basophil (%)	0.67[a]	0.00[b]	1.00[a]	0.00[b]	0.16
Monocyte (%)	3.67	3.33	4.67	2.00	0.49

. [a,b]Means with different superscript along the row are significant ($p<0.05$)
[1]MCHC (Mean corpuscular haemoglobin concentration)
[2] Control (no garlic and/or ginger powder), GaP (Garlic inclusion: 2.5 g/kg diet inclusion), GiP (Ginger inclusion: 2.5 g/kg diet) GGP (garlic: 1.25 g/kg diet + ginger: 1.25 g/kg diet).
[3]SEM (standard error of the mean)

Serum indices of sheep fed garlic or ginger and their combination

Table 3 represents the selected serum indices of sheep fed diet containing garlic or ginger powder and their combination. Total protein decreased ($p<0.05$) in entire herbal groups with the control group having the highest ($p<0.05$) total protein concentration. Least ($p<0.05$) comparative total protein concentration was observed in the herb groups. All other serum indices measured were not affected ($p>0.05$) by the feeding of garlic or ginger powder and their combination.

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Table 3 Selected serum indices of sheep fed diet containing garlic and ginger powder

[1]Parameter	[2]Treatment diets				[3]SEM
	Control	GaP	GiP	GGP	
Total protein (g/dl)	5.23[a]	4.53[b]	4.23[b]	4.25[b]	0.15
Globulin (g/dl)	2.00	1.57	0.77	0.35	0.21
AST (U/L)	105.53	98.80	101.20	103.65	1.45
ALT (U/L)	36.90	37.47	38.50	33.65	0.98
Glucose (mg/dl)	46.03	46.03	47.70	48.90	0.91
Total Cholesterol (mg/dl)	93.20	84.70	89.10	92.45	2.37
HDL (mg/dl)	17.47	15.87	15.27	17.20	0.92
LDL (mg/dl)	57.50	50.63	55.73	59.25	1.92

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[a,b]Means with different superscript along the row are significant ($p<0.05$)
[1]AST (Aspartate transaminase), ALT(Alanine transaminase), HDL (High density lipoprotein), LDL (Low density lipoprotein)
[2] Control (no garlic and/or ginger powder), GaP (Garlic inclusion: 2.5 g/kg diet inclusion), GiP (Ginger inclusion: 2.5 g/kg diet) GGP (garlic: 1.25 g/kg diet + ginger: 1.25 g/kg diet).
[3]SEM (standard error of the mean)

Serum mineral concentration of sheep fed diet containing garlic or ginger and their combination

The concentration of serum trace minerals of sheep fed diet containing garlic or ginger and their combination is presented in Table 4. Sodium and calcium concentration decreased ($p<0.05$) in the entire herb groups. Highest ($p<0.05$) sodium was found in the control group while the least ($p<0.05$) was observed in the ginger group. The concentration of calcium was highest ($p<0.05$) in the control group with comparative least ($p<.05$) values in the herb groups. Manganese concentration was decreased ($p<0.05$) in the ginger group with the least concentration. Highest ($p<0.05$) comparative concentration of manganese was observed in control, garlic, and herb combination groups. Iron concentration decreased ($p<0.05$) in the ginger and herb combination group. The control and garlic group had a comparatively higher ($p<0.05$) concentration of iron while the least ($p<0.05$) was observed in the ginger group. Zinc concentration only decreased ($p<0.05$) in the ginger group with comparatively highest ($p<0.05$) values observed in the control, garlic, and herbal combination groups.

Table 4 Trace mineral concentration in serum of sheep fed diet containing garlic and ginger powder

	[1]Treatment diets				
Parameter	Control	GaP	GiP	GGP	[2]SEM
Sodium (µg/L)	0.31[a]	0.27[b]	0.23[c]	0.27[b]	0.01
Calcium (µg/L)	0.42[a]	0.33[b]	0.30[b]	0.35[b]	0.02
Manganese (µg/L)	0.76[a]	0.72[ab]	0.64[b]	0.67[ab]	0.02
Iron (µg/L)	1.61[a]	1.45[a]	0.67[c]	1.00[b]	0.13
Copper (µg/L)	0.75	0.67	0.70	0.68	0.02
Zinc (µg/L)	0.07[a]	0.06[a]	0.05[b]	0.06[a]	0.002

. [a,b,c]Means with different superscript along the row are significant ($p<0.05$)
[1] Control (no garlic and/or ginger powder), GaP (Garlic inclusion: 2.5 g/kg diet inclusion), GiP (Ginger inclusion: 2.5 g/kg diet) GGP (garlic: 1.25 g/kg diet + ginger: 1.25 g/kg diet).
[3]SEM (standard error of the mean)

Oxidative stress biomarkers of sheep fed diet containing garlic or ginger and their combination

Result of oxidative stress biomarkers of sheep fed diet containing garlic or ginger powder and their combination is shown in Table 5. Albumin concentration decreased ($p<0.05$) in the garlic group with comparative higher ($p<0.05$) albumin values in control, ginger, and herb combination groups. Superoxide dismutase (SOD) increased ($p<0.05$) in all the herb groups compared to the control group. Comparative highest ($p<0.05$) SOD was observed in the ginger and herb combination group, while the least ($p<0.05$) was found in the control group. Malondialdehyde (MDA) decreased ($p<0.05$)

in all herb groups with the highest ($p<0.05$) MDA in the control group. Ginger and herb combination groups had comparatively least ($P<0.05$) MDA concentration. There was an increase ($p<0.05$) in nitric oxide (NO) in the herb groups with herb combination group having the highest ($p<0.05$) NO while the least was in the control group. Bilirubin, uric acid, and glutathione peroxidase concentrations were unchanged ($p>0.05$) in all the treatment groups.

Table 5 Oxidative stress biomarkers of sheep fed diet containing garlic and ginger powder

[1]Parameter	[2]Treatment diets				[3]SEM
	Control	GaP	GiP	GGP	
Albumin (g/dl)	3.23[ab]	2.97[b]	3.47[ab]	3.90[a]	0.13
Bilirubin (mg/dl)	0.41	0.40	0.42	0.46	0.02
Uric acid (mg/dl)	5.27	5.80	5.50	4.90	0.33
SOD (U/L)	968.98[c]	993.39[b]	1010.50[a]	1001.46[ab]	4.93
Glutathione peroxidase (U/L)	191.32	194.43	192.96	193.20	0.67
MDA (U/L $\times 10^{-09}$)	0.65[a]	0.56[b]	0.52[c]	0.54[bc]	0.02
Nitric oxide (μM)	70.13[a]	59.63[b]	62.99[b]	51.02[c]	2.16

. [a,b,c]Means with different superscript along the row are significant ($p<0.05$)
[1] SOD (Mean corpuscular haemoglobin concentration), MDA (Malondialdehyde)
[2] Control (no garlic and/or ginger powder), GaP (Garlic inclusion: 2.5 g/kg diet inclusion), GiP (Ginger inclusion: 2.5 g/kg diet) GGP (garlic: 1.25 g/kg diet + ginger: 1.25 g/kg diet).
[3]SEM (standard error of the mean)

Discussion

Red blood cells are involved in the transport of oxygen and carbon dioxide in the body (Isaac et al. 2013). The decrease in the values of the red blood cells (RBCs) in all the herb groups was without an attendant decrease in PCV and Hb concentrations, as such a situation of hypoxia which stimulates the production of RBCs is not envisaged, and the herbs may not be toxic to the red blood cells. The administration of various doses of garlic and ginger extracts to the animals significantly elevated the red blood cell count with an attendant decrease in PCV and Hb in rats and was suggested that garlic and ginger components may compete with hemoglobin in the red blood cell for oxygen, resulting in hypoxia, which then stimulates red blood cell production (Tende et al. 2014**)**. Saastamoinen et al. (2019) have reported an increased risk of lower hematologic values in the horse on garlic for a prolonged period of 84 days. Packed cell volume of grazing lambs fed garlic extract also decreased (Amin et al. 2014). On the contrary, Hb, PCV, WBC, lymphocytes, and RBC values in cows increased at the addition of ginger roots to their diet (Al-dain and Jarjeis 2015). Also, Hendawy et al. (2019) reported increased RBC values in ewes on ginger powder for a prolonged period of five months. However, the RBC values recorded in all the treatment groups were within the range of values reported for sheep in the semi-arid environmental zone of northern Nigeria (Njidda et al. 2014). Decreased basophil in garlic and herb combination group may also be explained by the possibility of garlic to increasing the risk of lower hematological values under prolonged feeding. The antimicrobial activity of allicin has been demonstrated by modulation of the cytokines activating macrophages that controlled the parasitic infection (Arreola et al. 2015). Garlic powder may have lowered the activity of these cytokines which can trigger activation of basophils in non-sensitized individuals. It is important to carry out a controlled experiment in sheep to ascertain the veracity of ginger and garlic or their combination to lower hematological values after feeding for a prolonged period.

Reduced total protein in the herbal groups is at variance with the report for grazing lambs on garlic extract and cow on ginger roots (Amin et al. 2014; Al-dain and Jarjeis 2015). The values obtained in the herbal groups were below the range values (55-94 g/L) reported for sheep of northern Nigeria (Njidda et al. 2014). The action of garlic and ginger to reduce serum total protein in sheep may be associated with their anti-inflammatory actions in the body. An increased total protein in the blood is associated with inflammatory disorders. Contrary to reports of reduced cholesterol and glucose in lamb and cow on garlic and ginger respectively (Amin et al. 2014; Al-dain and Jarjeis 2015), the total cholesterol and glucose levels of sheep in the current study were unchanged. The form of garlic used and the species of animals, respectively would have accounted for the differences between this report and that of others mentioned. The result of Redoy et al. (2020) supports the current result of serum glucose in sheep on herbal diets. Also, an increase in globulin and ALT in cow and lamb on ginger and garlic respectively (Amin et al. 2014; Al-dain and Jarjeis 2015) is not consistent with this current study as those values remained unchanged when compared to control. This also may be as a result of species differences and the form of garlic used by the different researchers. Like the current study, cholesterol, AST, and ALT values were not affected by the ginger root in the diet of the cow (Al-dain and Jarjeis 2015).

Trace minerals are important for various functions of the body. The variations observed in the various serum components of the trace minerals as affected by either garlic or ginger was complemented by the combination of the two. Contrary to report that blood minerals were not affected in dairy cow fed ginger roots (Al-dain and Jarjeis 2015), sodium, manganese, iron, and zinc were affected by feeding ginger in this current study. The differences in the effect of ginger on blood minerals levels between this study and that of Al-dain and Jarjeis (2015) may be species differences. However, such effect of ginger to reduce the blood levels of sodium, manganese, iron and zinc compared to control group was complemented in the herb combination group. Such a complementary effect will make the combination of ginger and garlic more ideal than the use of ginger singly to improve the serum minerals. On the contrary, the effect of garlic to reduce the levels of sodium and calcium in the blood compared to control could not be complemented in the herb combination group.

Malondialdehyde (MDA) is a by-product of lipid peroxidation indicative of free radicals, Nitric oxide (NO), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) are antioxidant enzymes that quench free radicals and are measured as biomarkers of oxidative stress. Other biomarkers of oxidative stress could include non- enzymes such as bilirubin, albumin, and uric acid. Albumin is exclusively synthesized by the liver, and a reduction in liver function will mean lower levels of albumin. The complementary effect between garlic and ginger as can be seen in the better concentration of albumin in the herbal combination group is an indication of a better function of the liver when the herbs are combined than when fed singly. The action of ginger powder to increase SOD and reduce MDA in the blood was more, compared to garlic, but the combination of the herbs complimented the effect of garlic alone. Ginger or garlic consumption has also been reported to decrease lipid peroxidation (Ahmed et al. 2008; Ma et al. 2013), normalized the activities of superoxide dismutase as well as glutathione peroxidase, in rats (Ahmed et al. 2008). Allicin in garlic also increased SOD activity (Ma et al. 2013). The presence of Gingerol and allicin in ginger and garlic respectively may be responsible for the strong antioxidant properties exhibited in this research. This is because they may be involved in several bioactivities (Ahmed et al. 2008; Chan et al. 2013) including anti-inflammatory, anthelmintic, antioxidant, anticoccidial, growth promoters and antimicrobial activities. The result of nitric oxide indicated that herbal combinations gave optimum NO reduction which is indicative that their combination may further reduce oxidative stress in the sheep than if they were fed singly. Gingerol, a component of ginger was reported to dose-dependently inhibit NO production and reduce iNOS in lipopolysaccharide (LPS)-stimulated mouse macrophages (Ippoushi et al. 2003; Uz et al. 2009) while allicin, a component of garlic is reported to increase the release of NO (Ma et al. 2018).

Conclusion

1. The feeding of garlic and ginger powder and their combination; and garlic powder lowered the red blood cells and basophils, respectively. However the values obtained were within the range of values reported for sheep in the semi-arid environmental zone of northern Nigeria.
2. Total protein levels of sheep were reduced compared to control as a result of feeding of both garlic and ginger and their combination.
3. Some blood minerals levels were reduced when feeding ginger powder alone compared to control, however such effect was improved when the herb was fed in combination with garlic powder.
4. The combination of garlic and ginger powder had a complementary effect in improving the antioxidant capacity of the sheep.
5. The combination of garlic and ginger powder gave optimum immunological and anti-oxidant responses than feeding only garlic or ginger. Garlic and ginger powder may be combined at 1.25 g/kg diet each to achieve an overall better anti-oxidant and immunological performance in sheep than feeding 2.5 g/kg diet of garlic or ginger singly.

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