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Effect of garlic (*Allium sativum*) and onion (*Allium cepa* L.) extract chitosan nanoparticles on antioxidant enzymes activities and relative weight of visceral organs of rainbow rooster chicken

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Poultry meat is susceptible to oxidation but increased antioxidant enzyme increases its availability in muscle during processing and storage. In livestock, synthetic antioxidant has some side effects; plant polyphenols can enhance the level of antioxidant enzymes but they are in-active in the gut of chicken and therefore, nanotechnology can be of importance in augmenting the stability of polyphenols. In the study, seventy two rainbow rooster chickens were treated with nanoparticles- prepared from Chitosan with Aqueous Garlic and Onion (CHIAGO), chitosan with total phenol and ajoene rich extract (CHITPA), and chitosan solution (CHISOLN) of 5 to 10%, with 0.5 g and 1 g Fosbac (antibiotic) administered orally twice a week for a period of 8 weeks. One chicken from each of the group and a control group were sacrificed on weekly basis with the muscles and visceral organs removed for analysis. The weight of visceral organs, catalase (CAT) enzyme activities of thigh and breast were analyzed from 1st to 8th weeks and 2, 2-Diphenyl-1-picryl hydroxyl (DPPH) inhibition of thigh and breast muscles for 1st, 4th and 8th weeks. Relative weight of the heart, liver and spleen did not change when compared with the control (p>0.05) but it increased in the gizzard (p<0.05). Catalase increased in thigh and breast muscles (p>0.05) but without increase in the erythrocytes and liver (p>0.05). DPPH inhibition increased with CHIAGO 10%, CHITPA 10% in week 4 and week 8 in the breast and thigh muscle. Garlic and onion extract chitosan nanoparticles can act as natural antioxidant compound.

Key words: Chitosan, garlic and onion extract, visceral organ, meat, antioxidant enzyme activities.

INTRODUCTION

Broilers are like other domestic birds believed to be susceptible particularly to oxidative stress, likely due to

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the genetic selection towards large breast muscles, increased total weight, and faster growth rate (Sihvo et al., 2013). Broiler meat has been traditionally recognized as highly sensitive to oxidative process due to high degree of unsaturated muscle lipids (Min et al., 2008). Excess free radicals resulting from natural metabolism can damage important biological activities.

Lipid oxidation in meat and fish-products leads to rancidity, off-flavor and many harmful substances (Sen and Mandal, 2017). Oxidation increases with high intake of oxidizable lipids peroxidation, deterioration of sensitive poly unsaturated fatty acids and low intake of antioxidant nutrients (Morrissey et al., 1998; Smet et al., 2008). Oxidative reaction happening in muscle after postmortem causes deterioration of meat quality during storage. High levels of unsaturated fatty acids in poultry meat have high levels of poly unsaturated fatty Acids (PUFA) which makes the poultry meat more susceptible to oxidation process than beef or pork; so, poultry diet has to be supplemented with antioxidant for optimal growth performance, reproduction and meat quality (Delles et al., 2014). The need in natural antioxidant in poultry industry in recent years has been on the increase due to synthetic antioxidants (butylated hydroxyanisole, butylated hydroxytoluene), and their use in stimulating the occurrence of various chronic diseases in humans, animals and birds which prohibited their use.

The alternative to synthetic antioxidants are natural ones which are safer, cheaper, and can prevent oxidative reactions in products during storage; they do not cause metabolic diseases in animals and birds (Caleja et al., 2017). One of the approaches to increase the oxidative stability of meat is to add antioxidant during the period of feeding or directly during processing (Rojas and Brewer, 2007). Polyphenol compounds, especially, flavonoids, have received an important attention because of their antioxidant activities *in vitro* systems. However, it has been shown that flavonoid compounds are poorly absorbed in the gut and their concentrations in target tissues are too low to perform an effective antioxidant defenses (Surai, 2013). Additionally, nanoparticles are studied as nutrition supplements of diets for improvement of broilers health and performance since they are able to carry nutrients directly to the cell (Elkloub et al., 2015). Recorded nanoparticles, such as silver, increased the weight of small intestine and liver but had no effect on heart, gizzard, and proventriculus of broilers (Felehgari et al., 2013). Silver nanoparticles reported to have a negative effect on liver weight relative to live body weight of broilers (Andi et al., 2011).

The antioxidant activities of components of onion and chitosan have been reported (Lampe, 1999; Swiatkiewicz et al., 2015). Onion and garlic possess well defined antioxidant activity (Stajner and Varga, 2003) corollary with the presence of efficient antioxidant enzymatic system such as superoxide dismutase, catalase and glutathione- S- transferase as well as antioxidant from radical scavengers in erythrocytes (Rai et al., 2009). Garlic powder showed no effect on the relative liver, gizzard and heart (Issa and Abo, 2012) with unaffected spleen when broilers were treated with herbal treatments of garlic, thymus and coneflower (Rahimi et al., 2011).White Mini broilers relative weight of various organs, such as liver, spleen, bursa of fabricius etc. remained unaffected by the onion dietary treatments (An et al., 2015), with a report (Aditya et al., 2017) indicating the relative weight of liver, heart, spleen etc. were not affected by the dietary onion extract. Jakubcova et al. (2014) reported owning to higher concentration of garlic extract in feed ratio, the antioxidant status of chicken has increased; in addition, garlic effective antioxidant activity *in vivo* and *in vitro* has been reported (Jackson et al., 2002). The weight of visceral organs did not show changes when broilers were fed on low percentage of dietary chitosan (Khambualai et al., 2008). Chitosan was reported to improve plasma antioxidant enzymes in broiler chickens (Osho and Adeola, 2020). The current study investigated the effect of garlic and onion extract chitosan nanoparticles on antioxidant enzymes activities and relative weight of visceral organs of rainbow rooster indigenous chicken**.**

MATERIALS AND METHODS

Study location

The rearing of the experimental chicken, visceral organs weight measurement and sampling of meat (thigh and breast muscles) after sacrificing the chicken took place in Jomo Kenyatta University of Agriculture and Technology (JKUAT) at Safari Animal Research Facility; whilst the nanoparticles preparation, and the antioxidant enzymes analysis were done at Pan African University of Science Technology and Innovations (PAUSTI)- Molecular Biology and Biotechnology Laboratory in (JKUAT)-Kenya.

Preparation of aqueous onion and garlic, total phenol and ajoene rich extracts

Onion (*Allium cepa* L.) and garlic (*Allium sativum*) were bought from a market in Juja of Kiambu County. Onion (red *Creole orallium*) and garlic (softneck) were identified by the Botany Department at Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya. The aqueous extract of garlic preparation was done according to the method described by Huzaifa et al. (2014) with modification. 50 g of peeled bulbs of garlic was chopped, ground, mixed with 500 ml distilled water, stored for 24 h. It was then filtered the following day using filter paper (Whatman No. 1) and finally stored at 4°C for use. The ajoene rich extract was prepared according to Viswanathan et al. (2014) with modification. 50 g of peeled garlic bulbs was chopped and homogenously blended with 500 ml of cold distilled water, well stirred, filtered with cotton cloth. Then it was transferred in a new flask containing ethyl acetate, stirred and allowed to stabilize for separation of Ajoene rich extract. The upper layer was pipetted into a filter paper (Whatman No. 1) to remove all the water and collection of semi aqueous substance which is the ajoene rich extract. Hence, ethyl acetate was evaporated and the extracted product was stored at 4°C for use. Preparation of aqueous extract of onion was done according to (Oyebode and Fajilade, 2014) with some modification. The onion bulbs together with the outer part

were cleaned with ethanol to remove dirt, and chopped into smaller and thin slices; then they were air dried for 2 weeks and 50 g was weighed. 500 ml of distilled water was added and heated at 72°C for 3 h and allowed to cool at room temperature. The extract was then filtered with filter paper (Whatman No. 1), and then stored at 4°C ready for use. Preparation of total phenol extract was done according to Mujic et al. (2009) with some modification. 50 g of chopped, air dried onion bulb was soaked in 500 ml of 70% ethanol. It was left over night and filtered the following day by filter paper (Whatman No. 1); and the ethanol solvent was completely removed by rotary evaporator under vacuum at a temperature of 55°C and the extract was stored at 4°C for use.

Determination of total phenolic and flavonoids content from garlic (softneck) and onion (red *Creole orallium*)

The amount of total phenolic content was determined by the Folin-Ciocalteu method as described by Ainsworth and Gillespie (2007) with modification. 5gm of dried samples of garlic and onion were weighed into separate 250 ml conical flask and about 100 ml ethanol was added. The flask was closed safely using parafilm and covered with aluminum foil. Then, the samples were placed in a shaker and shaken for about 3 h and the extract was kept in darkness for 72 h for further extraction and filtration using filter paper (Whatman No. 4). The extract was topped to 50ml using ethanol; hence, centrifuged for 10 min at 25 degrees at 150 rpm. 1ml was taken from the supernatant and filtered using 0.45 µm micro filter for total phenolic content determination. Then it was placed in a test tube of 2 ml folin ciocateu 10%; 4 ml of 0.7M sodium carbonate was added and vortexed again, then left for 2 h to develop color. It was read in the Uv-vis at 765nm using gallic acid as a standard. Aluminum chloride colorimetric method was used for determination of flavonoids (Jagadish et al., 2009). In 10 ml volumetric flask 4 mL of distilled water and 1 ml of the filtered plant extract were added. After 3 min, 0.3 mL of 5 % sodium nitrite solution was added and after 3 min again, 0.3 ml of 10% aluminum chloride was added. After 5 min, 2 ml of 1 M sodium hydroxide was added and the volume made up to 10 mL with water. Absorbance was measured at 415 nm using UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The amount of total flavonoids was calculated from calibration curve of standard prepared from quercetin (Figures 1 and 2 and Table 3).

The preparation of chitosan solution, chitosan nanoparticles and characterization

Chitosan solution was prepared as described by Rasaee et al. (2016) with some modifications. The low molecular weight chitosan was purchased from Sigma Anderia; 2 g (w/v) was used with 0.5% (v/v) acetic acid and the pH of the solution was raised to 5 with 1N NaOH under magnetic stirring for 24 h and topped to a volume of 200ml with distilled water, and stored at 4°C till application. Preparation of chitosan nanoparticles was done through ionic gelation interaction between positive and negative charged compounds as described by Rasaee et al. (2016) with some modifications. Aqueous chitosan was prepared by mixing 40 ml of chitosan solution with 10 ml of garlic and onion aqueous extract (CHIAGO) mixture as treatment 1, and a mixture of 10 ml of total phenol and homogenous ajoene rich extract with 40 ml chitosan solution (CHITPA) as treatment 2. The mixtures were stirred for 10 min at 60°C, centrifuged at 200 rpm and allowed to rest at room temperature for 30 min to form an opalescent solution. The nanoparticles prepared from aqueous garlic and onion, total phenol and ajoene rich extracts were characterized by their sensitivity to pH to confirm the formation of the nanoparticles, and Fourier

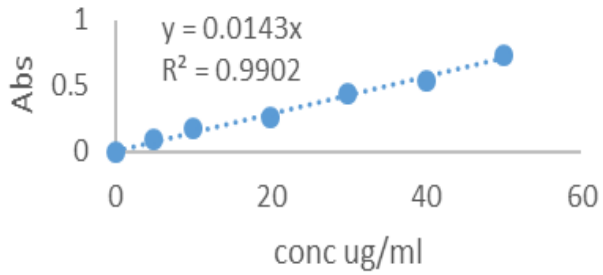

Figure 1. Standard curve for total phenolic content.

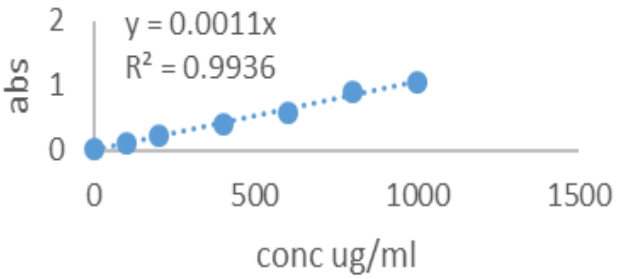

Figure 2. Standard curve for total flavonoid content.

Transform Infrared Spectroscopy (FTIR) to identify the functional groups responsible for the formation of the chitosan nanoparticles of chitosan with the extracts of garlic and onion. Field Emission Scanning Electron Microscopy (SEM) analysis was done to confirm the morphology and size of the nanoparticles prepared (Tables 4 and 5).

Chicken husbandry, treatment and samples collection

Seventy-two chickens were selected from a mixed population of indigenous Rainbow Rooster Chickens aged 8 weeks which were procured from Kukuchic Company Ltd in Nairobi. The chickens were reared on wooden cages in Jomo Kenyatta University of Agriculture and Technology (JKUAT) at Safari Animal Research Facility for a period of 8 weeks. Nine chickens were placed in one treatment with three chickens per cage and three replicates. The chickens were treated with four treatments at 5% and 10% of CHISOLN, CHIAGO, CHITPA and 0.5 g and 1 g of Fosbac orally administered 2 times a week for a period of 8 weeks with the control group placed on water and feed. Food was provided *ad libitum* for both control and treatment groups; the chickens were fed on Kienyeji grower mash produced from a commercial feed company (First Animal Feed) (Tables 1 and 2). The chickens were acclimatized for two weeks followed by oral administration of the treatments for a period of 8 weeks. One chicken from each group and control were sacrificed by method of cervical dislocation according to Laudadio et al. (2012). One chicken from the four treatments with different concentrations and a control was sampled for determination of relative weight of visceral and antioxidant enzymes activities. 72 chickens (9 each week) were analyzed from 1st to 8th weeks relative weight of visceral organs and CAT activity of meat (thigh and breast muscles) stored at -20°C and 27 samples (9 each week) was analyzed for DPPH inhibition of meat (thigh and breast muscles) for 1st week, 4th week and 8th week of the treatment.

Table 1. Basic diet composition.

Feed ingredient	Quantity(kg)
Maize	50
Maize bran	400
Wheat bran	250
Wheat pollard	171
Fish meal	30
Sunflower seed cake	30
Cotton seed cake	20
Stock lime	30
Bone meal	11
Calcium phosphate	2
Methionine	0.5
Coccidiostat	0.5
Grower pre-mix	2
Red salt	3
Total	1000

Table 2. Chemical composition.

Dry matter (%)	92
Crude fiber (%)	11
Metabolisable energy (kcal/kg)	2435.904
Crude protein (%)	14
Calcium (%)	3.75
Total phosphorus (%)	0.34

Table 3. Total phenolic and flavonoid content in softneck garlic and red *Creole orallium* onion.

Item	Solvent used for extraction	TPC (mg QAE/100 g of extract)	TFC (mg QCE/ 100 g of extract)
Onion	70% Ethanol	46.791±1.486	151.828 ± 7.195
Garlic	70% Ethanol	81.606±2.698	260.913 ± 17.113

GAE gallic acid equivalent; QCE quercetin equivalent; TFC total flavonoid content; TPC total phenol content.

Table 4. Composition of chitosan nanoparticles encapsulated with total phenols and ajoene rich extract (CHITPA).

Chitosan nanoparticles prepared	Total phenolic and flavonoid content / 5 ml of onion extract	Ajoene rich extract in/ 5 ml	40 ml	50 ml
(CHITPA)	0.2339 mg TPC and 0.7591 mg TFC	0.5 mg	Chitosan solution	Chitosan nanoparticles

Table 5. Composition of chitosan nanoparticles encapsulated with aqueous of garlic and onion (CHIAGO).

Chitosan nanoparticles prepared	Aqueous of garlic	Aqueous of onion	40 ml	50 ml
(CHIAGO)	5 ml	5 ml	Chitosan solution	Chitosan nanoparticles

Feed composition

Determination of catalase antioxidant activities in meat muscle and blood

The catalase assay was determined by Jenway model 68 spectrophotometer at 240 nm by measuring the disappearance of H_2O_2 with bubble formation; it was characterized by a decrease in absorbance at 240 nm three times for each 30 s according to a modified version of a method described by Aebi (1984) and Babiker et al. (2016). 1 cm quartz cuvette was used with 5g sample of meat muscle mixed with 25 ml of 50 mM phosphate buffer (pH 7.0 at 25°C) using a homogenizer for 15s at 13,500 rpm. The mixture was then centrifuged at 1,800×*g* and 2°C for 15 min. The supernatant of the mixture was taken and filtered through a filter paper (Whatman' No. 1); then, 1 ml of filtered supernatant was mixed with 2 ml of 10 mM H_2O_2. The decrease in absorbance at 240 nm was recorded every 30 s for 1 min. The CAT activity was expressed for meat muscle, liver and erythrocytes as unit/g and unit/ ml of samples, respectively. Measuring CAT absorbance in blood erythrocytes; blood samples of 4 ml of blood was collected from the brachial vein of each chicken and placed into 5 ml aseptic EDTA tubes. The blood samples were centrifuged immediately at 1,370 rpm and 4°C for 10 min; the plasma was separated and erythrocyte was lysed with distilled water (1: 1 v/v). It was inverted vigorously, and centrifuged at 4,020 rmp and 4°C for 15 min. The erythrocytes lysate was collected for the measurement of CAT by adding 1ml of 10 mM H_2O_2, 1ml PBS and 0.1 ml of the sample. In measuring CAT in liver, the liver organ was washed thoroughly in ice-cold physiological saline and weighed. Homogenate of 10% was prepared as described by Patlolla et al. (2009) with modifications; 2.5g liver was put in 25 ml of 0.05 M phosphate buffer (pH 7.4) containing 0.1 mM EDTA; the sample was homogenized, followed by sonication and centrifugation at 4,000 rpm and 4°C for 10 min. The supernatant was decanted and centrifuged at 16000 rpm for 30 min at 4°C. The supernatant fraction was obtained and called "homogenate; it was used to measure CAT by adding 2ml of 10 mM H_2O_2, and 0.1 ml of the sample. The extension coefficient of 40 M-1 cm-1 for H_2O_2 at 240 nm was used to calculate catalase enzyme activities as per Babiker et al. (2016).

$$\text{Catalase activity (umole/ ml)} = \frac{\text{Decrease in absorbance of hydrogen peroxide at 240 nm}}{\text{Molar extension coefficient of hydrogen peroxide at 240 nm}}$$

One unit of catalase activity is defined as the amount of activity required to convert 1µmole/ ml (umole/ ml) of hydrogen perioxide to water and oxygen per minute at 25°C.

Determination of free radical scavenging activity of thigh and breast muscle

Radical scavenging activities of the chicken's thigh and breast muscle sample stored at -20^{o}C with 2, 2-Diphenyl-1-picryl hydroxyl (DPPH) free radical (Sigma-Aldrich) were determined by UV spectrophotometer at 517 nm according to the method of Molyneux (2004). One gram of fresh meat stored at -20°C was weighted and socked into a 100 ml Falcon tubes; 25 ml methanol was added.

The sample in Falcon tube was covered with aluminum foil. The sample was placed in a shaker and stirred for about 3 h and then kept in darkness to extract for 72 h. The sample was filtered through filter paper (Whatman No. 4), and 1:1 serial dilution was made by picking 1 ml out of 25 and 1 ml of methanol as first concentration (40 mg/ml); the dilution continued 5times and the following concentrations of the extracts were prepared, 40, 20, 10, 5 and2.5 mg/ml in methanol (Analytical grade). Vitamin C was used as the antioxidant standard at the same concentration as the extract, that is 1 g into 25 ml of methanol and 5 dilution of 1:1. One ml (1 ml) of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 mL of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Methanol was used to zero the spectrophotometer and the absorbance was read at 517 nm after 5 min in UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan); the radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition of DPPH} = \{(A_B - A_A)/A_B\} \times 100$$

Where A_B is the absorption of blank sample and A_A is the absorption of tested extract solution. The results were expressed as percentage inhibition of DPPH.

Statistical analysis

Graphpad Statistical Package version 7.1 was used to draw graphs and the data analyzed statistically using Tukey mean differences in Statistical Analysis System (SAS) statistical software version 9.1; statistical significant difference was considered at $p<0.05$.

RESULTS

Determination of active components from garlic and onion and the composition of garlic and onion chitosan nanoparticles prepared

Effect of Garlic and onion chitosan nanoparticles on body weight and relative weight of visceral organs

Compared to the control, all treatments had increase significantly ($p=0.0001$) with a decrease observed in CHIAGO 10% treatment in the chicken body weight (Figure 3). Treatment with 0.5 g Fosbac, 5% CHIAGO and CHSOLN 10% significantly increased the relative weight of the gizzard (Figure 6) in comparison to the control ($p<0.05$). The relative weight of the heart (Figure 4), liver (Figure 5) and the spleen (Figure 7) were not significantly different from the control group ($p>0.05$).

Catalase enzyme activity in erythrocytes, liver, thigh and breast muscle

The CAT activity in thigh muscle was significantly increased by all the treatments ($p=0.0027$) (Figure 8). In the breast muscles CAT activity was significantly ($p=0.0108$) increased by all the treatments (Figure 9). CAT activity in erythrocytes (Figure 10) and liver (Figure 11) have no significant changes ($p>0.05$).

Free radical scavenging activity of thigh and breast muscle of Rainbow rooster chicken

Vitamins C (Vit C) showed high DPPH inhibition%. CHIAGO 10%, CHITPA 10% showed higher inhibition% in week 4 and week 8 compared to the other treatments;

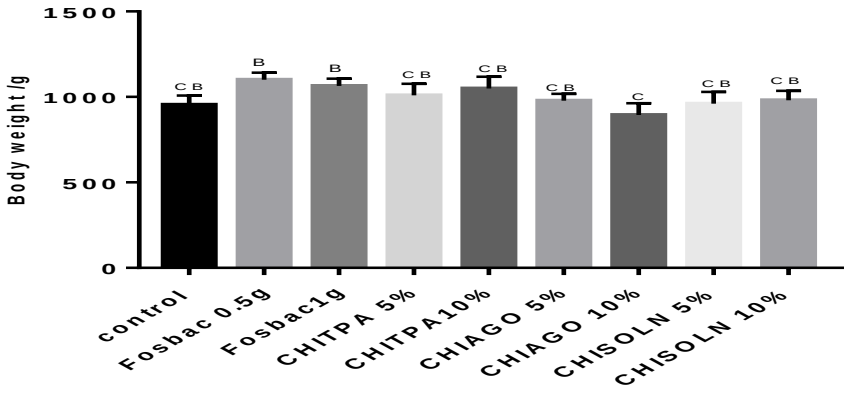

Figure 3. Mean body weight of chicken (P value = 0.0001).

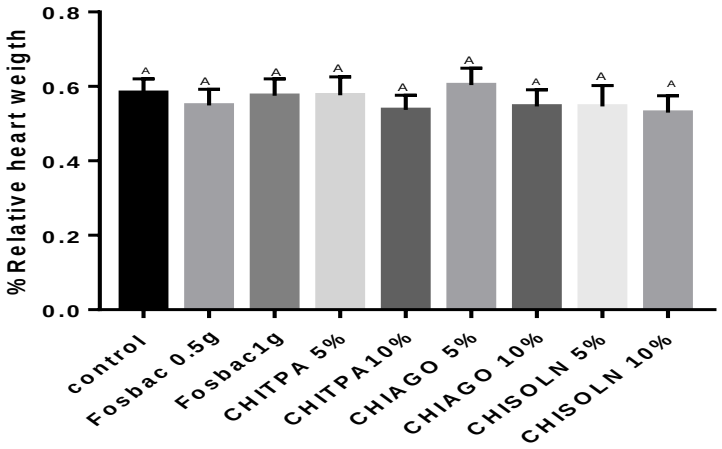

Figure 4. Mean % relative heart weight of chicken (P value = 0.1812).

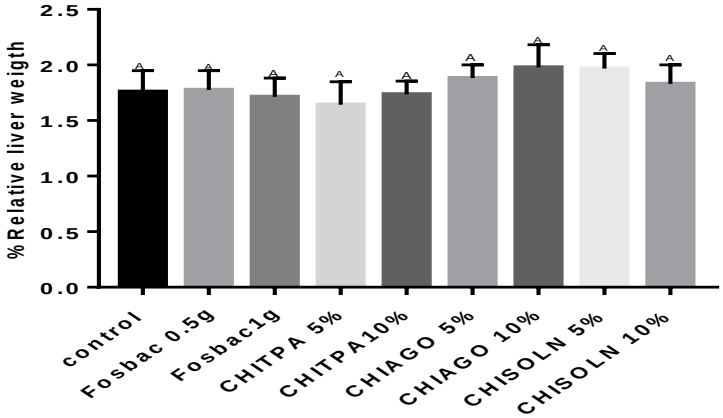

Figure 5. Mean % relative liver weight of chicken (P value = 0.7315).

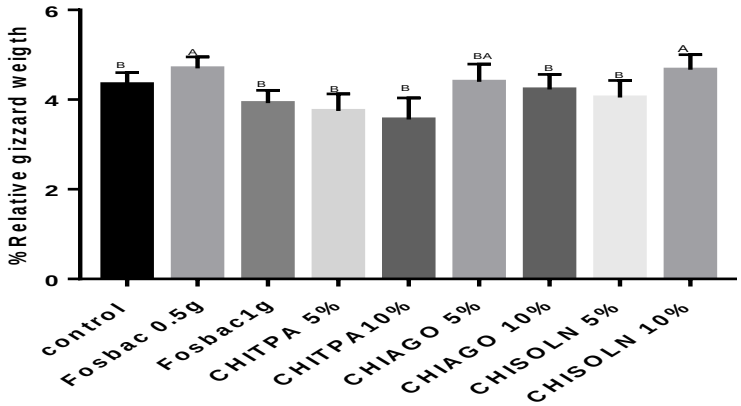

Figure 6. Mean % relative gizzard weight of chicken (P value = 0.0002).

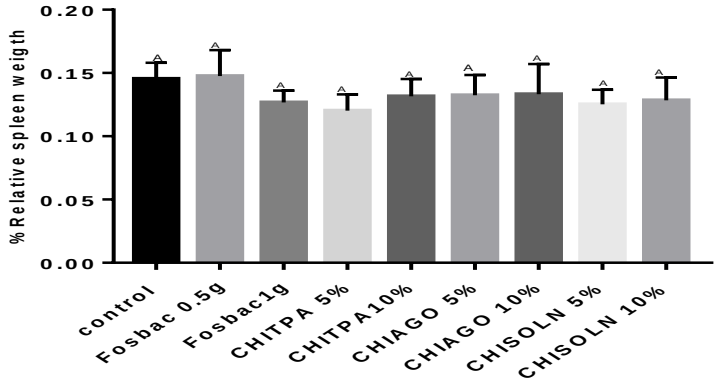

Figure 7. Mean % relative spleen weight of chicken (P value = 0.3545). Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract. Means with the different letters are significant different at (*P* 0.05). Standard error of the mean (SEM) (n=8).

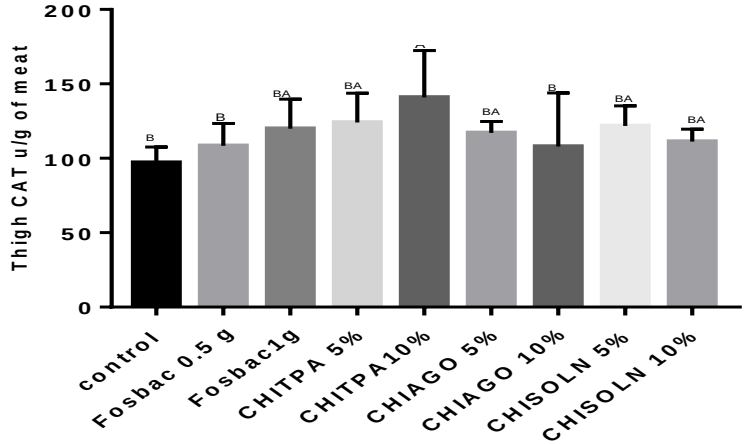

Figure 8. Mean CAT activities of chicken thigh (P value = 0.0027).

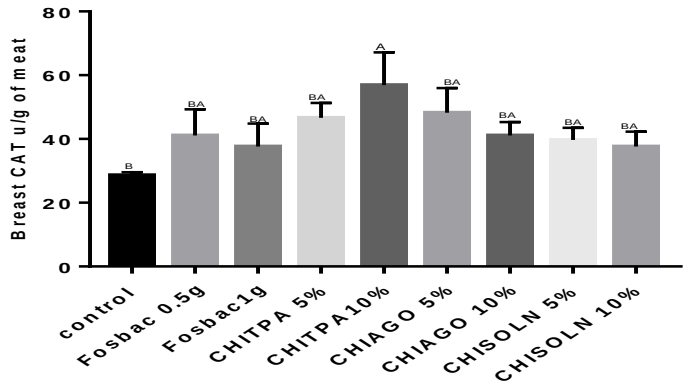

Figure 9. Mean CAT activities of chicken breast (P value = 0.0108).

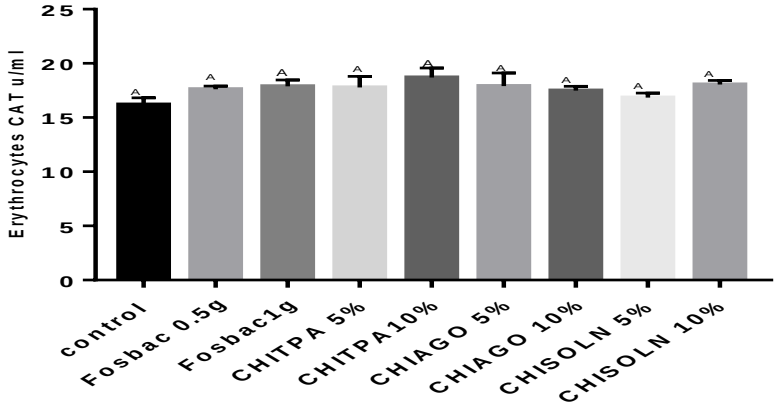

Figure 10. Mean CAT activities of chicken erythrocytes (P value = 0.3526). Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract. Means with the different letters are significant different at (P 0.05). Standard error of the mean (SEM) (n=8).

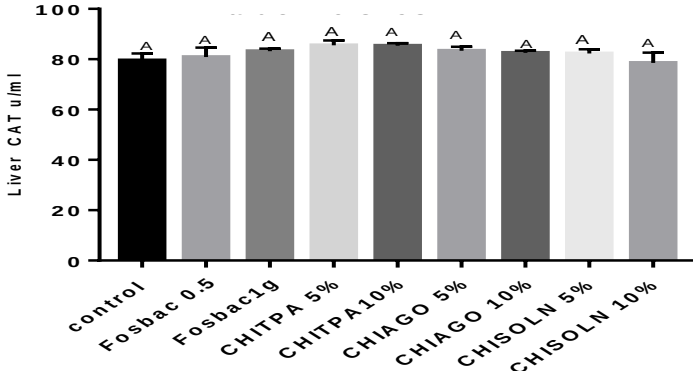

Figure 11. Mean CAT activities of chicken liver (P value = 0.3198) Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract. Means with the different letters are significant different at (P 0.05). Standard error of the mean (SEM) (n=8).

in week 1, CHIAGO 5%, CHIAGO 10% had higher scavenging activities in thigh, in breast, the treatments, 1 g of Fosbac and CHITPA 5% had a higher activity when compared with other treatment and the control chicken (Figures 12 to 17).

DISCUSSION

Garlic and onion extract chitosan nanoparticles effect on relative weight of visceral organs

The relative weight of heart, liver and spleen from chicken in the treatment group did not change significantly when compared with those in the control group ($p>0.05$). The result is compariable with reports of Issa and Abo (2012), An et al. (2015) and Khambualai et al. (2008) and also with agreement with the findings of of Pourmahmoud et al. (2013) who reported no effect on the internal organs when thyme extract was supplemented in broilers. Similarly, Abo et al. (2016) revealed that herbal supplementation had no significant effects on some visceral organs. There is a significant increase ($p<0.05$) in gizzard for a few treatments correlate with the findings of Aguzey and Gao (2018) who reported that feeding broilers on mash diets have positive effect on gizzard development than feeding pelleted diets by increasing the relative weight. The result also corrlates with research finding on Hawthorn plant extract having content of flavoniods; when added to the drinking water of broilers it reduces the proportion of the body attributed to abdominal fat, liver and heart (Ahmadipour et al., 2017) and reduces relative liver weight and abdominal fat (Ahmadipour et al., 2018).

Catalase enzyme activity in erythrocytes, liver, thigh and breast meat muscle

Catalase enzyme activities in erythrocytes and liver did not differ significantly ($p>0.05$) when compared with the control chicken. CAT activity though statistically indicated no significant differences in the levels of all treatment were slightly higher than the control in erythrocytes and liver except CHISOLN10% that had the same amount as the control. It correlates with the findings of Kelussia odoratissima medicinal plant extract with flavonoids and polyphenols; when fed to broilers it significantly suppresses hepatic lipogenesis by downregulating key hepatic lipogenic enzyme genes and boosts antioxidant capacity by up-regulating hepatic antioxidative genes SOD1 , catalase in the liver (Ahmadipour et al., 2018). It is likely an indication of catalase as a very important enzyme for protection of cells from the toxic effects of H_2O_2 and radical oxygen species such as superoxide, hydroxyl radical reactive oxygen species generated during metabolism attacks cell components such as DNA, protein and lipid membrane. Sometimes lethal damages may occur in the cells, and those potentially injured are neutralized by antioxidant enzymes such as catalases,

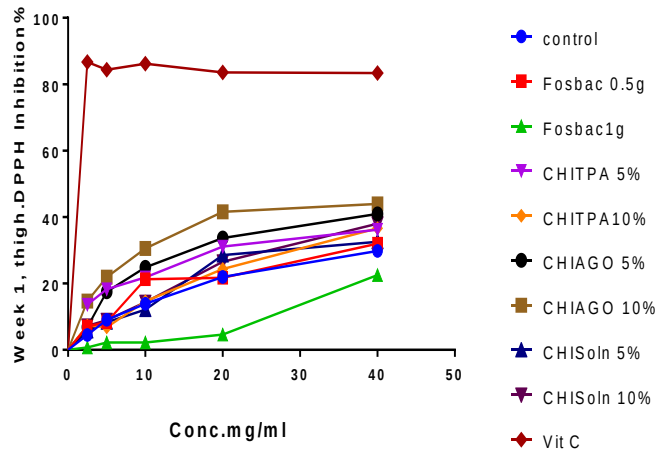

Figure 12. Week1; Conc. DPPH inhibition % of Thigh.

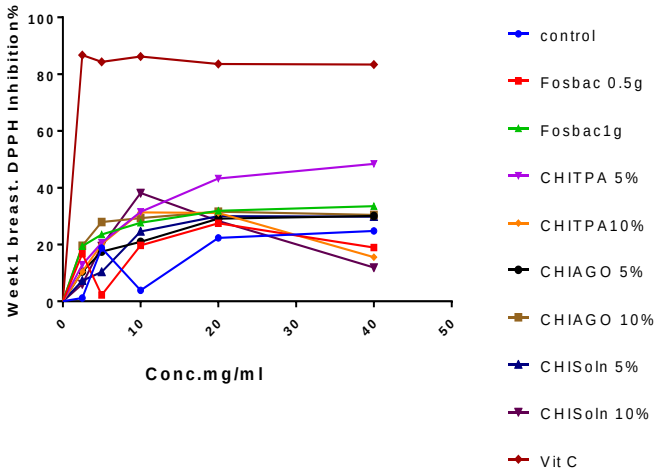

Figure 13. Week1; Conc. DPPH inhibition % of breast.

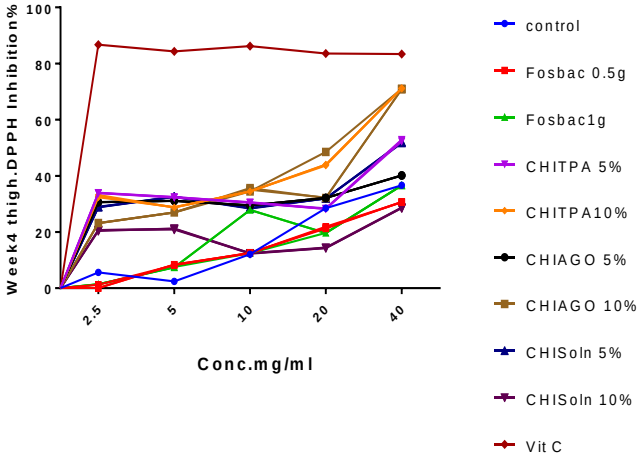

Figure 14. Week4; Conc. DPPH inhibition % of thigh.

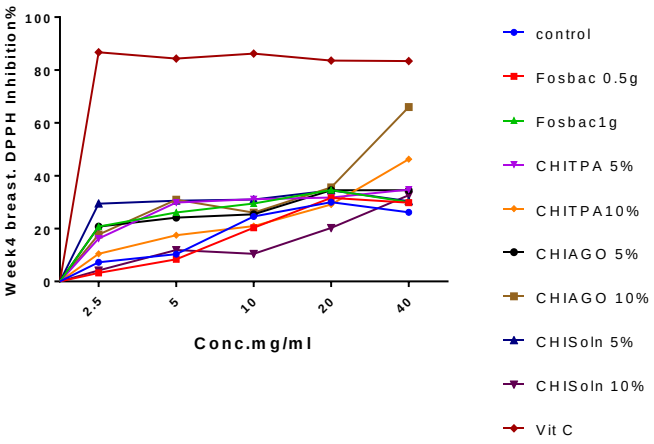

Figure 15. Week4; Conc. DPPH inhibition % of breast.

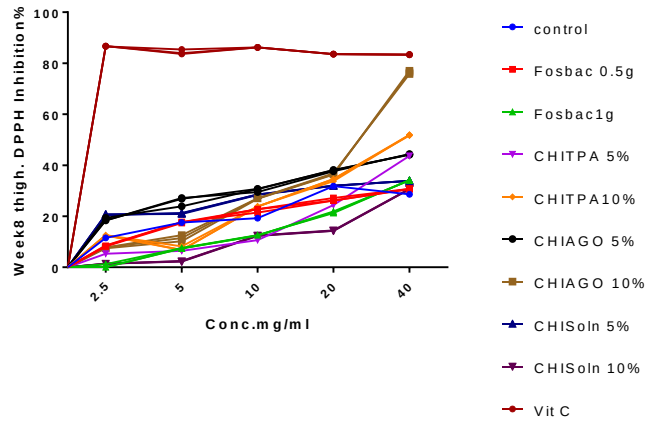

Figure 16. Week8; Conc. DPPH inhibition % of thigh.
Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract, Vit C (Vitamins C).

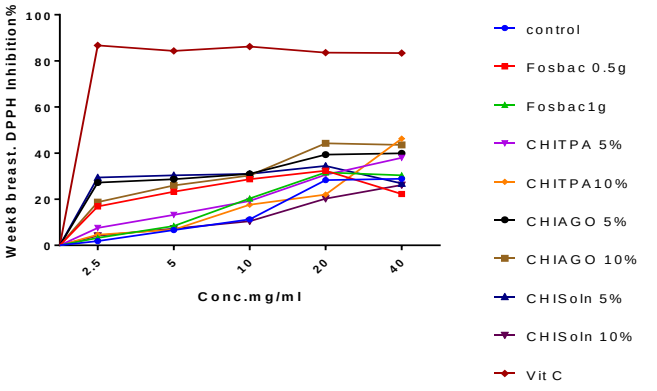

Figure 17. Week 8 Conc. DPPH inhibition % of breast. Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract, Vit C (Vitamins C).

superoxide dismutase and peroxidases (Aydemir and Kuru, 2003). Chitosan used as feed additive for poultry has an effect on antioxidant properties as reported by Swiatkiewicz et al. (2015). Plant extracts with polyphenols can reduce serum levels of triglycerides and cholesterol as well as abdomnal fat deposition (Ahmadipour et al., 2015). The muscle fibres are categorized into two different metabolic types, oxidative (red) or glycolytic (white), based on the chemical composition and enzyme activities (Warris, 2000).

In this study, thigh and breast muscle antioxidant enzymes activities showed a difference when compared with the control chicken (p<0.05). The result is in agreement with Saleh et al. (2018) who indicated that broiler thigh meat may be enriched successfully with long chain polyunstaturate fatty acids n-3 and its antioxidant potential and functional quality characteristics may be improved by dietary supplymentation.

Renerre et al. (1996) and Muhlisin et al. (2016) indicated that oxidative muscle exhibits higher antioxidant enzyme activities than glycolytic muscles and Lee et al. (1996) reported a higher antioxidant enzyme activities in thigh meat of turkey than breast meat. The activities of antioxidant differ in meat of different animal species (Pradhan et al., 2000; Hernandez et al., 2004). Dellees et al. (2014) reported that the dietary antioxidants can minimize the oxidative instability of proteins and lipids, and the protection may be linked to improved cellular antioxidant enyzymes activities. Catalase and Glutathione peroxidase (GSH-Px) are considered the major peroxide-removing enzymes located in cytosol, whilst superoxide dismutase (SOD) plays a role in the protection against damage resulting from superoxide anion radicals (Chen et al., 2012). SOD and catalase are coupled enzymes in which SOD scavenges superoxide anions by forming hydrogen peroxide, and catalase safely decomposes hydrogen peroxide to water and oxygen; GSH-Px can decompose both hydrogen peroxide and lipoperoxides formed during lipid oxidation (Gatellier et al., 2004; Terevinto et al., 2010).

Free radical scavenging activity in thigh and breast meat

DPPH assay is a rapid, simple and inexpensive, and stable free radical which is widely used to determine antioxidant activities of different biological system. In this study, thigh and breast meat muscles scavenging activities to DPPH was found to be higher in thigh than breast meat of rainbow rooster chicken. There is an increase in antioxidant activities both for thigh and breast at (p<0.05) of the treated groups in comparison to the control groups of chicken. Thigh meat expresses high DPPH inhibition percentage than the breast meat and it showed the inhibition percentage increases as the concentration of the samples increases and the duration

of the treatments increases. In the 1st week, thigh with the treatments; CHIAGO 5%, CHIAGO 10% had higher antioxidant scavenging activities. In breast, the treatments; 1 g of Fosbac and CHITPA 5% had a higher activity. In week 4 and week 8; CHIAGO 10%, CHITPA 10% gave high DPPH inhibition percentage both in breast and thigh meat. The result was in comparison with Saleh et al. (2018), who indicated broiler thigh meat may be enriched successfully with long chain polyunstaturated fatty acids n-3 and its antioxidant potential and fuctional quality characteristics may be improved by supplementing the diet. Polyphenols are natural antioxidant that showed antioxidant and animicrobial activities (Lorenzo et al., 2014) and it can prevent lipid oxidation by preventing chain inhibition by scavening initiating radicals, breaking chain reaction, decomposing peroxides, decreasing localized oxygen concentration and binding chain intiating catalyst such as metal ions (Juntachote et al., 2006).

Conclusion

Chitosan nanoparticles of garlic and onion extract do not significantly affect the relative weight of the heart, liver, spleen and gizzard of rainbow rooster chicken. Catalase antioxidant enzyme activities increases with CHIAGO and CHITPA treatments on thigh and breast meat. Thigh and breast meat free radical scavenging increases with increase in the sample concentration and the duration of application of the treatments with CHIAGO 10% and CHITPA 10% showed an increase in the meat muscle antioxidant enzyme activities. Chitosan nanoparticles of garlic and onion extract prepared can be used as a natural antioxidant supplement in rainbow rooster chicken. Further research on the application of the prepared products on the type of breeds and duration of application is advisable.

CONFLICT OF INTERESTS

The authors have declared no any conflict of interest.

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