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Original Research Article

Effect of dietary chitosan on the feed efficiency and weight performance of high fat diet induced hyperlipidemia in male wistar rat

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ABSTRACT

Background: This study was carried out to determine the growth performance of rats fed graded levels of chitosan supplemented high fat diet.
Methods: Thirty male wistar rats weighing between 70 g and 90 g were purchased and randomly allotted into three (3) treatment groups with graded levels of chitosan in high fat diet (1%, 3% and 5%) and three (3) control groups namely: normal diet, high fat diet (HFD) and normal diet +5% chitosan. The feed intakes as well as weight change of the experimental rats were monitored for six (6) weeks.
Results: The results obtained showed that the highest level of feed intake and feed efficiency were recorded for animals in group fed 5% level of chitosan supplementation when compared to other treatment groups. Similar result was observed for the weight change (as there was significant reduction in the weight gain with increase chitosan supplementation in HFD) which can be attributed to the efficient utilization of feed consumption.
Conclusions: It can be concluded that dietary chitosan prevents excess weight gain in hyperlipidemia and improves the overall nutritional attributes of the experimental diets by improving their feed efficiencies as compared to the control.

Keywords: Chitosan, Feed intake, Feed efficiency, Weight performance, Hyperlipidemia

INTRODUCTION

Chitosan (the deacetylated derivative of chitin) is one of the abundant, renewable, nontoxic and biodegradable carbohydrate polymers available in the exoskeletons of shellfish and insects.[1] Chitosan has received much attention as a functional biopolymer for diverse applications, especially in pharmaceutics, food (as a source of crude fiber), and cosmetics.[2] Chitosan, a non-toxic natural polymer, has been used as an absorption enhancer for poorly absorbable drugs. It has a hypolipidemic effect, an immunomodulating function, and a hypoglycemic effect, and it reduces body weight, and improves serum urea, creatinine and cholesterol levels in patients with chronic renal failure.[3-8] There is some support that chitosan is more effective than placebo in the short-term treatment of obesity and hypercholesterolemia.[9,10]

Hyperlipidemia is a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters,

phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein along with reduced high-density lipoprotein level.[11] Hyperlipidemia is a condition of excess fatty substances called lipids, largely cholesterol and triglycerides, in the blood. It is also called hyperlipoproteinemia because these excess lipids travel in the blood attached to proteins.[11] There are different types of Hyperlipidemia depending on which lipid levels are high in the blood.[12] Diet and nutrients in the diet have significant impact on physiological well-being and pathological development. For instance, human studies have shown that high-fat diets (HFDs) can cause over weight or obesity, with varied degree due to different genetic background. HFD is one of those nutritional conditions that accounts for the largest incidence of metabolic syndromes and these metabolic syndromes include obesity and related metabolic disorders such as non-alcoholic fatty liver disease (NAFLD), diabetes, cardiovascular diseases and so on. It is the most common form of dyslipidemia (which includes any abnormal lipid levels).

Among the numerous functions and role played by chitosan, the impact of this polymer on weight performance and its palatability is yet to be assessed. Thus, it is expedient to assess the feed intake and weight performance in male wistar rats fed with different levels of chitosan diet inclusion.

METHODS

Experimental animals

Thirty (30) male albino rats, healthy with no sign of injury, weighing between 70-90 g, obtained from the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta were used for the study. The rats were allowed to acclimatize for three weeks before the commencement of the experiment, in the animal house of the Department of Biochemistry, Federal University of Agriculture, Abeokuta. The animals were allowed free access to water and rat grower mash for three weeks for acclimatization. The study was conducted in accordance to the US guideline as contained in the National Institute of Health guide for the care and use of laboratory animals NIH publication No. 18-23, 1985.

Crab shell collection and grinding

Crabs (Scylla serrata) were bought from Ojoo market (coastal ragion), Lagos State. The crabs were killed by boiling in hot water for 20 minutes, after which the shells (exoskeleton) were separated from crabs manually. The crab shells were dried and grinded to powder by a local grinding machine at Oluwo-keesin, Abeokuta, Ogun state.

Chitosan extraction

Chitosan extraction was carried-out by following the processes described by Burrow et al.[13] The process involved three steps namely deproteination, demineralization and deacetylation.

Deproteination

A sample (75 g) of the powdered exoskeleton was accurately weighed and then divided into three equal parts and placed in three different 250 ml beakers.

Each of the samples was treated with 100 ml of 4% w/v NaOH solution and boiled for 1 hour in order to dissolve the proteins and sugar. The mixture was allowed to cool and the supernatant was decanted off. The sediment was dried on cardboard paper for 30 min at room temperature.

Demineralization

The resulting powder was demineralized with 135 ml of 1% w/v HCl and was left overnight. This was done to remove the calcium carbonate content of the sample. The demineralized crab shell was then treated for 1 hour with 50 ml of 2% w/v NaOH to decompose the albumin into water soluble amino acid which was drained off. The sample was washed with deionized water and then air-dried.

De-acetylation (conversion of chitin to chitosan)

The chitin was converted to chitosan by de-acetylation process. This was carried out by adding 100 ml of 50% w/v NaOH to each sample and then heating at 100°C for 2 hours in a water bath. The samples were then removed and cooled for 30 min at room temperature. Afterward, each sample was washed continuously with 50% w/v NaOH and filtered to retain the solid matter which is chitosan. Each sample of the chitosan was placed in a 250 ml beaker and air-dried for 3 h and then oven-dried at 120°C for 24 h to obtain dry chitosan (a creamy white powder). The chitosan obtained was washed with distilled water and then dried.

Grouping of animals/duration of animal feeding

Thirty male wistar rats were randomly allotted into three (3) treatment groups with graded levels of chitosan in high fat diet (1%, 3% and 5%) and three (3) control groups namely: normal diet, High fat diet and normal diet + 5% chitosan. The feeding was carried out for 6 weeks (Mid-August to October 2018). The feed intakes as well as weight change of the experimental rats were monitored for the same six (6) weeks.

Proximate analysis

The proximate analysis of the samples for moisture, ash, fiber and fat were performed using the method of AOAC.[14] The nitrogen was determined by micro-Kjeldahl method as described by Pearson.[15] The percentage nitrogen was converted to crude protein by multiplying 6.25. All determinations were performed in triplicates.

Determination of feed intake

The average feed intake (AFI) in the various supplemented groups and the control group for 6 weeks was calculated by summing up the Feed intake for the eight weeks divided by six to (g/week).[16]

$$\text{Feed intake (g/week)} = \frac{\text{average feed intake of six weeks (g)}}{6}$$

Determination of weight performance (weight change)

The weekly weight change was evaluated by subtracting the initial average weight of the animals (IAW) from the final average weight of the animal (FAW) on weekly basis

$$Average\ weekly\ weight\ change = FAW - IAM$$

The average cumulative weight change (ACWC) was evaluated by summing up the result obtained from (FAW-IAW).

Determination of feed efficiency

The weight changes by the animal in respective groups were summed up on weekly basis to the end of the study (6 weeks). The weekly feed intakes were calculated for the supplemented and control groups and the feed efficiency is evaluated as:

$$\text{Feed efficiency} = \frac{\text{Weight gain (g/rat)}}{\text{Feed intake (g/rat)}}$$

Statistical analysis

The statistical significance between the controls and other groups of experimental animals were determined by one-way analysis of variance (ANOVA) followed by Bonferroni t-test for multiple comparisons. The results are presented as mean±SD at confidence interval of 95% ($p \leq 0.05$).

RESULTS

The result obtained on the feed intake of wistar rats fed with different levels of chitosan diet supplementation as seen in table 1 which shows that there is a significant increase in the group fed with 1%, 3% and 5% chitosan supplemented diet (500±7.20, 525±16.41 and 545±12.26) when compared to the group fed with the High fat diet (480±9.12), while there was a significant increase in feed intake of corresponding groups with increasing supplementation of chitosan in High fat diet between the groups fed with 1%, 3% chitosan and 5% chitosan (500±7.20, 525±16.41 and 545±12.26). The high fat diet group however gave the lowest intake by the animal (480±9.12).

The result obtained on the cumulative weight gain in consumption of chitosan (g/week) in male wistar rats for 6 weeks seen in Table 2 shows that there is a significant decrease in weight gain in group fed with 1%, 3% and 5% chitosan supplemented diet when compared to group fed with the High fat diet. Also, increasing supplementation of chitosan in High fat diet decreases the weight gain, thus, 5% chitosan gave the lowest weight gain while group fed with only HFD gave the highest weight gain.

Table 1: Assessments of the weekly feed intake in male wistar rats fed with different levels of chitosan inclusion.

Treatment	Weekly feed intake (g/week)
Normal diet	645±21.45^{d}
HFD	480±9.12^{a}
Normal diet and 5% Chitosan	550±21.57^{c}
HFD and 1% chitosan	500±7.20^{a,b}
HFD and 3% chitosan	525±16.41^{b}
HFD and 5% chitosan	545±12.26^{c}

Values with different superscript down the column are significantly different ($p<0.05$).

Table 2: Cumulative weight gain in consumption of chitosan (g/week) in male wistar rats for 6 weeks.

Treatment	Cumulative weight gain
Normal diet	42.331±1.76^{b}
HFD	60.05±3.44^{c}
Normal diet and 5% Chitosan	27.52±1.09^{a}
HFD and 1% Chitosan	26.61±1.06^{a}
HFD and 3% Chitosan	46.42±1.59^{b}
HFD and 5% Chitosan	40.78±1.61^{b}

Values with different superscript (a, b, c, d) down the column are significantly different ($p<0.05$).

Feed efficiency

The result obtained showed that animals fed with the High fat diet gave the highest efficiency and (0.625±0.075), while the group fed with 1%, 3% and 5% chitosan showed an increasing significant different in feed efficiency (0.266±0.015, 0.442±0.029 and 0.374±0.021). The groups fed with the Normal diet and 5% chitosan however showed the lowest feed efficiency (0.25±0.013).

The quantitative constituent of proximate analysis carried out on the different levels of chitosan diet supplementation in the experimental feed.

The quantitative constituent of proximate analysis carried out on the different levels of chitosan diet supplementation in the experimental diet as demonstrated in Table 6, shows that the percentages of Lipids decreases with increasing level of supplementation. Tentatively, the

crude fiber and ash content increases with increasing level of supplementation. The high fat diet fed and 5% chitosan fed group gave the lowest moisture content.

Table 3: The feed efficiency (gain/feed) of wistar rats fed with chitosan diet inclusion.

Treatment	Body weight gain (g/rat)	Feed intake (g/rats)	Feed efficiency
Normal diet	42.33±1.76^{b}	129±2.32^{c}	0.328±0.043^{b}
HFD	60±3.44^{c}	96±1.98^{a}	0.625±0.075^{d}
Normal diet and 5% chitosan	27.5±1.09^{a}	110±2.37^{b}	0.25±0.013^{a}
HFD and 1% chitosan	26.6±1.06^{a}	100±1.87^{ab}	0.266±0.015^{a}
HFD and 3% chitosan	46.4±1.49^{b}	105±2.43^{b}	0.442±0.029^{c}
HFD and 5% chitosan	40.8±1.61^{b}	109±2.67^{b}	0.374±0.021^{b}

Values with different superscript (a, b, c, d) down the column are significantly different ($p<0.05$).

Table 4: Proximate analysis of experimental feeds.

Groups	Moisture	Protein	Lipid	Ash	Crude fiber	Carbohydrates
NF	2.29±0.03^{e}	17.43±0.26^{cd}	7.47±0.33^{a}	5.18±0.29^{a}	8.14±0.37^{a}	59.50±0.54^{e}
HFD	1.99±0.01^{b}	17.92±0.10^{d}	30.39±0.35^{d}	8.59±0.02^{b}	8.75±0.14^{a}	32.37±0.33^{a}
NF and 5%	2.40±0.03^{d}	17.02±0.33^{c}	7.13±0.18^{a}	8.84±0.10^{b}	14.99±0.31^{c}	49.62±0.60^{d}
HFD and 1%	2.19±0.03^{c}	15.82±0.42^{b}	24.52±0.31^{c}	9.22±0.00^{bc}	9.93±0.22^{b}	36.40±0.14^{b}
HFD and 3%	1.99±0.01^{b}	15.47±0.09^{b}	22.03±0.36^{b}	9.95±0.03^{c}	14.30±0.13^{c}	36.26±0.10^{b}
HFD and 5%	1.82±0.04^{a}	14.65±0.21^{a}	15.12±0.19^{b}	9.98±0.57^{c}	17.79±0.13^{d}	40.76±1.09^{c}

Values with different superscript (a, b, c, d) down the column are significantly different ($p<0.05$).

DISCUSSION

The utilization of chitosan added to a standard High fat diet for male wistar rats gave some substantial results on the feed intake, feed efficiency, weight gain and cumulative growth performance as seen in this research work. There was an appreciable increase in the feed intake of the rats fed with chitosan supplementation as compared to the high fat diet fed group, this can be attributed to the fact that dietary chitosan possess a substantial amount of minerals and crude fibre which plays a role in stimulating the appetite of the rats.[2] This is in agreement with Khambualai et al., 2008 stating that dietary chitosan improves feed intake resulting in increased feed efficiency.[17] Also, the wistar rats on a high fat diet showed a significantly high weight gain as compared to the normal control which can be attributed to the high lipid content in their diet which could result in hyperlipidemia and obesity.[5] The chitosan supplemented diet suppressed weight gain in the groups fed with 1%, 3% and 5% to a significantly low level and this is attributed to the binding of the polycationic chitosan to the anionic lipids in the diet and also the high crude fiber content of the chitosan resulting in the elimination of the lipids contained in the diet hereby lowering the risk of hyperlipidemia.[18] This is also in agreement with Zhang et al stating the hypolipidemic activities of chitosan.[19] The feed efficiency of the chitosan fed group was discovered to be parallel with that of the normal diet as showed a significant difference when compared to the high fat diet fed groups. The 5% chitosan fed group showed no significant difference with the control which further buttress the fact that chitosan act as a Hypolipidemic agent by preventing the accumulation of fats in the body hereby preventing excess weight gain.[9]

CONCLUSION

It can be concluded that dietary chitosan prevents hyperlipidemia and improves the overall nutritional attributes of the experimental diets by improving their feed efficiencies as compared to the control.

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