An assessment of meat quality attributes of Broiler chicken fed with *Samanea saman* meal

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Research Article

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Abstract

The study examined the meat quality attributes of broiler chicken fed with unfermented and *Aspergillus niger* fermented *Samanea saman* whole pod meal. The study was conducted with 360 broiler chickens for 5 weeks. *Samanea saman* whole pod meal was fermented with a fungus *Aspergillus niger* for 10 days and included at levels of 0. 60, 90, 120 and 150g kg^{-1}in diets to substitute soyabean meal. Five experimental diets were formulated: a control diet which had 0kg of Samanea saman meal and four other diets containing FFSSWP meal at various levels. The experiment commenced after brooding the broiler chickens for 21 days by randomly allotting 18 birds each into five treatments with four replications in a complete randomized design (CRD).The experimental diets and water were provided ad-libitum to the broiler chickens throughout the feeding trial. At 8weeks of age the birds were slaughtered and studies done with the carcass. Following meat qualities were assessed pH, cooking loss, water holding capacity, meat cholesterol, The results indicated that feeding of broiler chickens with the fermented whole pod meal showed no antioxidant or antimicrobial activity in the meat. Also feeding of the broiler chicken with the fermented whole pod meal reduced the total cholesterol content of the meat by 40–65%. Therefore it can be concluded that the FFSSWPM is a valuable feed ingredient to be included at 60 and 150 g kg^{-1} of broiler chickens diets without any harmful effects on meat quality attributes but lowered the total cholesterol content in the chicken meat. Farmers should be encourage to use fungus fermented *Samanea saman* and also further studies should be conduced on how to include *Samanea saman* meal into the feed diet of broiler chicken.

Introduction

Poultry meat is a functional food that provides bioactive substances such as linoleic acid, vitamins, antioxidants and a balanced *n*-6 to *n*-3 PUFA ratio good for human health[1, 2] With the recent consumer lifestyle demand for low fat meat with high unsaturated fatty acids, low sodium and cholesterol levels, poultry meat is the best option. Poultry production has become one of the most sustainable businesses that can make a country achieve an economic independence. In Ghana, the feed cost account for 80% of the cost of broiler production [3] and this is a major challenge to the poultry industry due to the high prices of these conventional feedstuff such as soyabean meal and fish meal. These conventional feedstuffs are mostly of protein source, expensive and sometimes scarce thus they can be substituted with legumes such as *Samanea saman* whole pods. *Samanea saman* tree bears the pods and it is abundant and widely distributed throughout the tropics. The whole pods are readily available, not expensive and most often goes to waste as they drop and litter the environment. *Samanea saman*whole pods are highly nutritious in terms of cabohydrates, fat, fibre and protein contents, edible and eaten by humans and livestock both domesticated and wildlife [4].

The Samanea saman, has some properties that has effect on the meat quality of broilers. The leaves, bark, root, seeds and pods of the rain tree are also used in traditional medicine to treat various ailments such as intestinal ailment, stomachache and diarrhea [5].

Some of these properties help in reducing the harmful effect of the meat of animal that fed on it. The study therefore aimed at assessing meat quality attributes of the broiler chicken fed with the *Samanea saman* both fermented and unfermented meal.

Materials and methods

The study was conducted at the Poultry Section of the Department of Animal Science of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi for a period of 8 weeks using fungus (*Aspergillus niger*) – various levels of fermented *Samanea saman* whole pod meal. The whole pods were milled with a hammer mill to produce the meal.

A quantity of 10ml of *Aspergillis niger* spore suspension was used to inoculate 100g of the sterilized *S. saman* whole pods which had been moistened with 20ml distilled water. This was put in a container covered and kept in a room for 10 days after, which it was oven dried at 70^{o}C for 48 hours and milled with an electric grinder into a powder. At day 10, the sample was oven dried at 70 ^{o}C for 48 hours and milled with an electric grinder, stored and used for the various chemical analyses.

Experimental Diets

Five experimental diets were formulated: a control diet with 0% of the *Samanea saman* meal (no FFSSWPM) and four other diets containing FFSSWPM incorporated at levels of 60 g, 90 g, 120 g and 150 g kg^{-1} diet to substitute soyabean meal. The compositions of the experimental broiler diets and their calculated chemical compositions are presented in Table 1.

Table 1
Composition of the Experimental Broiler Diets

		Fungus Fermented *Samanea Saman* Whole Pod Meal (FFSSWPM)			
Ingredeints (gkg^{-1})	**Control (T1)**	**T2 (60)**	**T3(90)**	**T4(120)**	**T5(150)**
Maize	583.00	560.00	543.20	529.00	514.50
Soyabean meal	290.00	230.00	200.00	170.00	140.00
FFSSWPM	0.00	60.00	90.00	120.00	150.00
Fish meal	20.00	45.00	59.80	74.00	88.50
Wheat bran	80.00	80.00	80.00	80.00	80.00
Oyster shell	10.00	10.00	10.00	10.00	10.00
Dicalcium phosphate	10.00	10.00	10.00	10.00	10.00
Vitamin premix	3.00	3.00	3.00	3.00	3.00
Salt	4.00	4.00	4.00	4.00	4.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated Composition ($g\ kg^{-1}$ DM)					
Crude protein	22.41	22.22	22.22	22.21	22.21
Phosphorus	0.66	0.67	0.67	0.67	0.68
Calcium	1.01	1.04	1.04	1.05	1.06
Lysine	1.95	2.03	2.06	2.08	2.11
Methionine	0.62	0.65	0.66	0.67	0.68
Cystine	0.56	0.58	0.59	0.60	0.60
ME (Kcal/kg)	2801.25	2777.50	2777.50	2776.25	2776.25

*Vitamin premix supplied (kg^{-1}diet); 10,000 IU Vit A; 2000 IU Vit D3; 10 IU Vit E; 3mg Vit K; 2.5mg Riboflvin; 0.05mg Cobalamin; 5mg Panthothenic acid; 12.5mg Niacin; 175mg Choline; 0.5mg Folic acid; 2.8mg Manganese; 0.5mg Iron; 2.5mg Zinc; 625mg Cobalt.

Experimental Design

The study made use of four hundred (400) unsexed one-day old Cobb 500 commercial strains of broiler chickens obtained from a commercial hatchery (Asamoah and Yamoah farms). The experimental diet

was fed to the chicks after 21days of brooding. The feed and water were provided *ad-libitum* the feeding trial was done after 21 days of age The study adopted a completely randomized design (CRD) and the birds were maintained in a deep litter pen for 35 days during the experimental period. During the period routine and periodic management practices such as vaccination (against Gumboro, Newcastle disease), drug administration (Coccidiostat, antibacterial) and maintenance of cleanliness within and outside the poultry pens were carried out.

Data Collection

The following physicochemical properties of the meat samples of the birds namely, water holding capacity, pH and cooking loss were assessed following [6, 7] procedure.

Water Holding Capacity (WHC)

The breast muscle was used to assess water-holding capacity of the experimental birds because it consists of only white glycolytic type IIB fibre, which brings about uniformity in the different samples used. Exactly 10 g each of the meat samples was weighed and wrapped in a tissue of 0.2 g and each was placed in a centrifuge tube. The initial weights of each centrifuge tubes as well as the weights of the centrifuge and its contents were recorded. The tubes and their contents were placed in the centrifuge machine (Heraeus Biofuge Primo Centrfuge, Jos. Hansen and Soehne GmbH Germany) and spinned at a speed of 560 rpm for 10 minutes. The tubes were then weighed after the spinning, the tissue removed and the tubes reweighed. These were done in triplicates and the percentage WHC of each sample treatment was then calculated as

$$\% \, WHC = \frac{(W_3 - W_4) - W_2}{W_1} X \, 100$$

Where:

w_1 = weight of meat w_2= weight of tissue w_3= weight of tube, meat and tissue

w_4 = weight of tube and meat less tissue after spinning

pH Value of Chicken Meat

The pH of the chicken breast was measured using a pH meter fitted with glass electrode (FC200, H19024C, Hanna Instruments, Singapore). The pH was measured by the probe method by inserting a thin electrode directly into the breast muscle of each chicken fed the various treatment diets after incision of the muscle at a minimum depth of 1 cm. This was done when the ultimate pH in the chicken meat had been reached, that is after 24 hours of slaughtering the chicken. The parameters were measured in in triplicates at 0 (immediately after deboning) and 24 hours post mortem.

Cooking Loss

A total of 10 g each of the breasts of chickens fed on the various dietary treatments was weighed, put in sealed bags and cooked at 100^{o}C for 10 minutes. The samples were in triplicates and the percentage cooking loss was calculated as:

$$Cooking\ loss\ (\%) = \frac{Wt\ of\ sample\ before\ cooking - Wt\ of\ sample\ after\ cooking}{Wt\ of\ sample\ before\ cooking} x100$$

Proximate Analysis of the Chicken Meat

Proximate composition of the ground chicken meat from the breast portion was evaluated by the methods described by [8] for moisture, crude protein, fat, and ash. The analyses were made in triplicates for all the treatments. The method for measurements of moisture in meat was oven drying. Ground muscle or meat (about 4 g) was dried in a conventional oven (air drying) at 100–102°C for 16–18 hours and then the residue was weighed. The protein was analysed using the Kjeldahl method. This method involves 2 phases namely, a catalysed mineralization of nitrogen by heating in concentrated sulphuric acid and an alkaline treatment followed by a distillation and measurement of thefree ammonia produced.2 g of the meat sample was used. Rapid solvent extraction for measurement of the total fat content was used, which involved the Soxhlet apparatus, approved by the [8] for meat analysis. 10 g of ground and homogenized meat was used.

Meat Cholesterol and Saponification

Cholesterol content of breast muscles from each treatment were determined using High Performance Liquid Chromatography (HPLC) as described by [9] and [10]. Saponification and cholesterol measurement was done by using high performance liquid chromatography (HPLC) were the two steps used in the determination of the cholesterol content in the meat as described by [9] and [10].

Saponification

In using the [11] method, 2 g of each sample was saponified with 4 ml of 50% potassium hydroxide and 6 ml of 95% ethanol and this was heated for complete solubilization at 40^{o}C and heated again for 10 minutes at 60^{o}C. 5 ml of water was then added to the mixture and the samples cooled. The non saponifiable portion was extracted using 10 ml of hexane (three times), after which the aliquots of hexane extract of about 3 ml were dried under nitrogen flow.

Cholesterol Measurement

At the end of the saponification process, the high performance liquid chromatography (HPLC) method was used in the measurement of the cholesterol contents. The extract was dissolved in 3 ml of

acetonitrile-isopropanol solution at a ratio of 70:30 v/v and 1 ml was injected into the HPLC using the method of [10] The HPLC apparatus is made up of a SHIMADZU system which includes a ternary solvent delicery system (LAD 10), a Rheodyne 20 ml loop injector with column temperature of 30^{O}C, an ultra violet detector and software (CLAS-VP 10) for processing data. Also included was a Lichrospher (5RP18 150 x 4.6 mm) analytical column, a holder with guard column (Chrompack, Netherlands) as well as a mobile phase with a flow rate of 1 ml/minute consisting of acetonitrile and isopropanol in a ratio 70:30 v/v. The cholesterol identification was performed by chromatography of which the result was processed at 210 nm and also by comparing sample retention time with the standard retention times (Sigma and Polyscinec, U.S.A. C 8667). Internal standardization (0.504 mg of 6 ketocholestanol, Sigma and Polyscience, U.S.A. K1250) was used to quantify each sample after the saponification.

Microbial Load

The total viable bacterial count was determined on frozen stored meat samples at intervals of 7 and 14 days as described by [12]. The nutrient agar was used to determine the overall bacterial load of meat, and meat products based on the number living microorganisms in a sample. Chicken meat sample of 10 g was homogenized with 90 ml of sterile peptone water (1 g/l) in a laboratory homogenizer (AM-5 Ace homogenizer, Nihonseiki,) and serial dilutions were prepared, then 0.1 ml of each dilution was spread with a bent sterile glass rod on duplicate plates of pre-poured and dried standard plate count agar (Nissui Pharmaceutical, Japan). After 48-h incubation at 25°C, colonies were counted and results were expressed as $\log_{10}$ cfu/g of chicken sample. The chicken skin is known to have a large number of pathogenic bacteria. Therefore, further test involving selective plate count was conducted to determine if the bacteria were harmful or harmless. This was done using selective bacterial culture media such as nutrient agar, Cetrimine agar, Bismoth agar, Maconky agar and Sabouraud Dextrose agar was used as indicator bacteria as it contains chemical additives which suppress the growth of bacteria except organisms detected. The Enterobacteriaceae for example, *E.coli* and *Salmonella* are usually used as indicator bacteria. The number of these Enterobacteriaceae ideally should not exceed 100 per cm. Microbiological safety of the chicken meat including *Pseudomonas*, *Salmonella*, *Escherichia coli* and fungus levels were evaluated.

Results

Meat quality attributes of the experimental birds

The physical characteristics of the meat of broilers fed the various dietary treatments are presented in Table 2. The result failed to indicate significant differences ($P > 0.05$) in the following: opening or initial pH, ultimate pH, water holding capacity and cooking loss among the dietary treatments. The initial or opening pH of the chicken meat ranged from 6.17–6.33. The ultimate pH of the chicken meat of birds fed on the dietary treatments were within the range of 5.3–5.7. The water holding capacity of meat for the 60 g (T2) FFSSWPM was higher than the other treatments, which had similar values however there

were no significant difference (P > 0.05) among the treatments. The cooking loss was low among the treatment diets.

The cholesterol level in the chicken meat decreases with increasing level of FFSSWPM at the level of 90g FFSSWPM chicken meat registered the least cholesterol level. The total cholesterol level in the chicken meat was high in the chicken fed on the control diet (without FFSSWPM inclusion) with 0.20 mmol/l as compared to those birds fed on the FFSSWPM- containing diets with values of 0.14 (60g), 0.07 (90g), 0.12 (120 g) and 0.12 mmol/l (150g).

Table 2

Meat quality attributes of meat of experimental Birds Diets (g kg^{-1})

Parameters	Control (T1)	60 (T2)	90 (T3)	120 (T4)	150 (T5)	SEM	P-value
pH at 0 hours (initial)	6.33	6.20	6.17	6.20	6.17	0.09	0.67
pH at 24 hours (ultimate)	5.43	5.33	5.59	5.46	5.47	0.06	0.12
Water holding capacity (%)	0.68	1.55	0.93	0.85	0.70	0.55	0.79
Cooking loss (%)	20.2	20.40	19.40	22.80	19.30	10.36	1.00
Total cholesterol (mmol/l)	0.20	0.14	0.07	0.12	0.12	-	-

Proximate analysis of the meat of experimental Birds

The proximate composition of the meat of broiler chicken fed the various experimental diets is presented in Table 3. The results revealed that meat of birds fed on the 150 g FFSSWPM and the control diets (T1) had more moisture, followed by the 90 g (T3) FFSSWPM and the 120 g (T4) FFSSWPM and lastly the 60 g (T2) FFSSWPM. Meat of the birds fed with the highest inclusion level of FFSSWPM (150 g) had the lowest crude protein, ether extract and ash contents but high nitrogen free extract (NFE). The ash content of the chicken meat decreased as the FFSSWPM inclusion level increased with the 60 g FFSSWPM having the highest value.

Table 3
Proximate composition of chicken meat from birds fed the experimental diets Diets (g kg^{-1})

Parameter (%)	Control(T1)	60 (T2)	90 (T3)	120(T4)	150 (T5)
Moisture	70.00	67.50	68.60	67.80	70.00
Ash	2.79	3.68	1.63	1.37	1.21
Ether extract	5.00	5.81	4.85	7.81	3.46
Crude protein	19.25	22.31	20.56	21.00	15.13
NFE	2.96	0.70	4.36	2.02	10.20

Microbial Load in Chicken Meat

The total microbial load of the chicken meat increased as the meat stayed longer from Day seven (7) to Day fourteen (14) for the dietary treatments Table 4 The growth of the microorganisms on the 14th day became too many and exceeded the maximum microbial load count of 300 cfu/g. Also the result showed that the FFSSWPM inclusion in the diets did not have any effect on the microbial load counts compared to the control, as their values were statistically similar when treated on the same dilution factor. The specific microorganism load counts for *E. coli, Salmonella*and fungus also presented same trend as those of the total load counts except that of the *Pseudomonas*, which had few counts. On the seventh day of storage, it was observed that meat of broilers on the 120 g (T4) FFSSWPM treatment had the highest total microbial counts with a value of 6.324 Log10 cfu/g followed by the 150 g (T5) FFSSWPM with a value of 6.318 Log10 cfu/g and the load counts reduced as the FFSSWPM inclusion level in the diet fed to the birds decreased. In comparing the test organisms it was observed that the T4 also led in the *E. coli* and *Pseudomonas* counts. The increase in time of storage produced significant proliferations in total count, *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa* and fungus on the 14th day, irrespective of the treatment conditions.

Table 4

Microbial Counts of the meat of the experimental Birds Stored at 4^{o}C from Day 7 to 14

Days	Treatments Log10 cfu/g	Total Load	*E.coli*	*Salmonella*	*Pseudomonas*	Fungus
7	T1	6.209	5.954	5.973	4.699	6.107
	T2	6.199	5.973	5.919	5.176	6.064
	T3	6.297	6.049	6.053	ND	6.041
	T4	6.324	6.097	6.049	5.439	6.149
	T5	6.318	6.021	6.164	5.653	6.190
14	T1	TNTC	6.283	6.326	5.544	6.281
	T2	TNTC	6.307	6.348	5.462	6.316
	T3	TNTC	6.332	6.358	5.243	6.281
	T4	TNTC	6.477	6.474	5.863	6.299
	T5	TNTC	6.462	6.476	5.756	6.415

TNTC = Too numerous to count Cfu/g = Colony forming unit/gram ND = Not detected

Discussion

pH and Physical Characteristics and Total Cholesterol of Meat the experimental birds

There was no significant difference (P > 0.05) in the initial pH, ultimate pH, water holding capacity and cooking loss among the dietary treatments (Table 2). The ultimate pH of the birds fed on the dietary treatments was within the range of 5.3–5.7 but was lower than that reported by [13] The water holding capacity for the 60 g (T2) FFSSWPM was higher than the other treatments which had similar values however, they did not differ significantly. This high water holding capacity will result in higher yield upon cooking, makes the meat juicier upon eating and highly nutritious because most of the nutrients are retained in the meat during cooking.

The cooking loss was low among the treatments and this is a good quality as increased cooking loss leads to reduced juiciness and less palatable meat. The cooking loss was similar indicating that the test ingredients did not affect the meat negatively.

The cholesterol level in the chicken meat decreased as the inclusion level of the FFSSWPM increased with the 90 g FFSSWPM chicken meat having the least cholesterol level. The total cholesterol level in the chicken meat was high 0.20 mmol/l in those fed the control diet as compared to birds fed on the FFSSWPM- containing diets.The differences in the cholesterol content of the chicken meat can be

attributed to the variations in absorption and biosynthesis of cholesterol, lipoprotein metabolism, diet, muscle fiber type distribution, subcutaneous and intramuscular fat and body weight of the animal [14, 15] **Kumar and Rani** [16] indicated that the cholesterol balance in the chicken meat is influenced by the crude protein content and unsaturated fatty acid profile. Poultry meat without the skin contains less cholesterol and saturated fats but the breast meat has the least cholesterol content. Cholesterol is a blood lipid, which is synthesised by the liver for the formation of bile salts for fat digestion and used by adrenal gland for hormones production. **Jaturasitha et al**.[17] acknowledged that chicken meat is a healthier food as compared to red meat because it contains low fat and cholesterol. The result indicated that the inclusion of the FFSSWPM probably helped to reduce the level of cholesterol in the meat, which most consumers will prefer because of health reasons.

Proximate Analysis of Meat of Broiler Chickens fed the Experimental Diets

The result revealed that meat of birds fed on the 150g FFSSWPM and the control diet had high moisture content than the other treatments (Table 3). The differences observed in the protein content of the chicken meat were probably due to the birds fed with different protein content in the treatment diets. According to [18] the nutrition, muscle function, age, breed and gender of the animal are factors that bring about disparities in the protein content. The result obtained from this study is in agreement with that of [19] who reported moisture content in a range of 75.51–72.50, 21.80–19.50% protein, 5.71–1.97% ether extract and ash content of 1.54–1.37% for broiler meat of chicken fed on different barley based diets. Nonetheless, [20] also reported the protein content of breast muscle-plus-skin to range from 21.9 to 23.5%, fat content of 3.9 to 8.45%, ash content of 1.02 to 1.2% and moisture content of 68.9 to 72.3%. Onibi, (2006) established that the nutritional value of meat is affected by how the animal is reared, produced and prepared. **Kumar and Rani** [16] also observed that the chemical composition of breast meat depends on the type of dietfed to the animal.

Microbial Counts of Chicken Meat

Meat though low in fat and carbohydrate is a suitable protein source. Due to the constitute elements of meat, it tends to attract and support the growth of pathogenic microorganisms which cause spoilage and food related diseases [21, 22]. Poultry meat is nutritious and has a favourable pH making them suitable medium for microbial growth [23]. Different types of microorganisms are attracted and grow on meat and meat products. The growth and activities of this microorganism depends on several factors including, storage temperature, texture, PH of the meat and meat products [24, 25]. Healthy muscles do not contain microorganism however, the slaughtering process, environmental conditions at the abattoir, slaughtering and transportation process as well conditions at the sales point do influence the growth of microorganism on meat and meat products [26, 27, 28].

The result presented in Table 4 indicated that the microbial load increased with storage time. Report from [29] states that a microbial load above 10^7 cfu/g or 10000000 log10 cfu/gis an indication of spoilage. The total microbial load of the chicken meat increased as the meat stayed longer from day

seven (7) to fourteen days (14) among the dietary treatments. The result showed that the FFSSWPM inclusion in the diets of the broiler did not have any effect on the microbial load of the meat compared to the control as their values are statistically similar when treated on the same dilution factor (cfu/g).

The total viable counts had similar values among the treatments. The increase in time of storage produced significant proliferations (uncountable number of organisms) in total viable count on the 14th day despite the treatment conditions. The total viable counts obtained in this study on the seventh day were higher than what was obtained by [30] that is 5.25 log10 cfu g-1 from chicken breast. In comparing the test organisms it was observed that the T4 had the highest *E. coli* and *Pseudomonas* counts while T5 had the highest *Salmonella*, and Fungus counts. The high presence of these harmful food poisoning bacteria (*Salmonella, E. coli* and *Pseudomonas*) in the chicken meat is not advisable to be eaten as it can bring serious problem to consumers.

This high prevalence of *Salmonella*and *E. coli* in the chicken meat on the fourteenth day was attributed to poor hygiene and the technique used in opening the abdomen such as the technique of hand evisceration practiced with infrequent hand washing probably introduced this bacteria [31, 32]. **EFSA [33] and** [21] reported that *Salmonella* is mostly isolated from the slaughtering environments and gastrointestinal tracts of animals especially poultry. The presence of *Pseudomonas,Salmonella,E. coli* and fungus in the chicken meat can be attributed to contaminations from the environment hence proper hygienic measures should be followed in slaughter houses.

Conclusions and recommendations

Feeding of broiler chickens with the fermented whole pod meal showed no antioxidant or antimicrobial activity in the meat. Also feeding of the broiler chicken with the fermented whole pod meal reduced the total cholesterol content of the meat by 40–65%. Therefore it can be concluded that the FFSSWPM is a valuable feed ingredient to be included at 60 and 150 g kg^{-1} of broiler chickens diets without any harmful effects on the meat quality attributes but lowered the total cholesterol content in the chicken meat. On the basis of the findings and conclusions it is recommended that 150 g/kg FFSSWPM diet should be used to reduce the total cholesterol content in the chicken meat. Different concentrations of the ethanolic whole pod extract should be put in the drinking water of broilers or other poultry to determine its antioxidant and antimicrobial potentials on the meat and meat product.

Declarations

1. Funding

There was no funding for the study

2. Ethics approval

The Kwame Nkrumah University of Science and Technology Ethics Committee approved the right to conduct this research and certify that the study was conducted in accordance of standard operating procedures of Research Ethics. Thus, this study was carried out in accordance with the university research ethics standards and guidelines.

3. Clinical trial number:

The study is not a clinical trial hence, clinical number not applicable

4. Consent to Participate

Not Applicable

5. Consent to Publish declarations from co-authors:

Not applicable.

6. Author contribution declaration

The author conceive the idea, developed the topic, design and developed the methodology undertook the study, collected data, analyze the data and develop the manuscript.

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8. Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

9. Plant reproducibility -

For this study reproducibility is not applicable

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