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NUTRACEUTICAL EFFECTS OF SNOT APPLE POWDER ON TRIIODOTHYRONINE, OXIDATIVE STRESS MARKERS, HAEMATOLOGY AND GROWTH OF BROILER CHICKENS

Olatunji Abubakar JIMOH[1]*, Unity Daniel OSAYANDE[2], Simeon Olugbenga AYODELE[1], Uchechi Gift Daureen IHEJIRIKA[3]

[1]*The Federal Polytechnic Ado-Ekiti, Department of Agricultural Technology, Ekiti State, Nigeria*

[2]*Edo State College of Agriculture and Natural Resources, Department of Agricultural Technology, Iguoriakhi, Edo State, Nigeria*

[3]*Kingsley Ozumba Mbadiwe University Ideato, Department of Animal and Environmental Biology, Imo State, Nigeria*

Abstract: This study investigated the effects of incorporating snot apple powder with or without probiotics on performance, hematological indices, serum protein profile, oxidative markers, and triiodothyronine levels in chickens. The treatments included a control (0% snot apple powder) and varying levels of snot apple powder (1%, 2%, and 3%), all supplemented with probiotics at a rate of 0.5%. Over a 42-day trial period, parameters such as feed intake, weight gain, feed conversion ratio (FCR), mortality, and blood samples for hematological and serum analyses were collected. Growth indices revealed significant variations ($P<0.05$) among treatments, with birds in T3 exhibiting the highest final body weight, followed by T1, while T4 recorded the lowest. Weight gain and feed intake were also significantly influenced by treatment, with T3 demonstrating superior performance in weight gain and T1 in feed intake. The feed conversion ratio was notably efficient in birds on T3 and T4 compared to T1. The serum protein profile indicated higher total protein and globulin levels in probiotic-treated groups (T2, T3, and T4) compared to T1, whereas albumin and uric acid varied significantly among treatments. Enhanced total antioxidant capacity in T2 and higher superoxide dismutase activity was observed in T2, T3, and T4. Triiodothyronine levels differed significantly among treatments, with T1 and T3 showing higher values compared to T2 and T4. Incorporating snot apple powder and probiotics in broiler diets positively impacted growth performance, health markers, and antioxidant capacity, suggesting potential benefits for poultry nutrition and health management strategies.

Keywords: Antioxidant enzymes, Feed efficiency, Immune response, *Azanza garckeana*, Thyroid function

***Corresponding author:** Department of Agricultural Technology, The Federal Polytechnic Ado-Ekiti, Ekiti State, Nigeria
E mail: abubakarjimoh2011@gmail.com (A. O. JIMOH)
Olatunji Abubakar JIMOH https://orcid.org/0000-0001-8204-5816
Unity Daniel OSAYANDE https://orcid.org/0000-0002-8525-264X
Simeon Olugbenga AYODELE https://orcid.org/0000-0003-2913-6123
Uchechi Gift Daureen IHEJIRIKA https://orcid.org/0009-0002-2004-6965

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1. Introduction

The poultry industry is continually exploring new strategies to enhance production efficiency and poultry health, while meeting consumer demands for nutritious products. Recently, nutraceuticals, including plant-derived compounds and probiotics, have gained attention for their potential benefits in poultry diets. Snot apple, known for its antioxidant, antimicrobial, anti-inflammatory, and anticancer properties due to bioactive compounds like acetogenins, flavonoids, and phenolics, has been studied for its health-promoting effects (Jung et al., 2010; Li et al., 2015; Ferdous et al., 2019; Feng et al., 2023). These compounds can modulate physiological processes such as immune function, oxidative stress, and metabolism (Ryszard, 2007; Tungmunnithum et al., 2018), making snot apple a promising addition to poultry feed. Probiotics, beneficial live microorganisms, enhance poultry health by improving gut microbiota and boosting immune responses (Beski and Al-Sardary, 2015; Kikusato, 2021). They prevent pathogen colonization, enhance nutrient absorption, and reduce intestinal inflammation, improving broilers' growth efficiency (Rocha et al., 2022). The combined use of snot apple powder and probiotics may offer synergistic benefits, promoting health and performance through complementary mechanisms. Snot apple's antioxidant and immunomodulatory properties (Alozieuwa et al., 2022) may reduce oxidative stress, improve immune function, and enhance nutrient metabolism in broilers. Despite increasing interest, research on the combined effects of snot apple powder and probiotics on triiodothyronine (T3) levels, oxidative stress markers, hematological parameters, and growth performance in broilers is limited. Triiodothyronine, a crucial thyroid hormone, regulates poultry's metabolism, growth, and development (Jiang et al., 2020). However, Snot apple fruit includes nutrients, minerals, and phytochemical substances such as flavonoids, steroids, triterpenes, saponins, phenols, and tannins that are helpful to human and animal health (Maroyi and Cheikh-Youssef, 2017), Probiotics also shown to improve immune functions by interacting with various immune cells and having a favorable impact on the composition of intestinal microbiota (Azcárate-Peril et al., 2011; Adel et al., 2017; Umair et al., 2022). Thus, probiotics are known to have immunomodulatory and health-promoting characteristics (Ashraf et al., 2017; Peng et al., 2022) revealing how they influence broilers' physiological and metabolic processes. Evaluating growth performance metrics like body weight gain, feed intake, and feed conversion ratio will provide practical insights into the economic viability of snot apples and probiotic supplementation in broiler diets (Ravindran and Abdollahi, 2021). Integrating snot apple powder and probiotics into broiler diets appears to be a promising strategy for boosting poultry health, productivity, and sustainability. Their combined effects on metabolism, immune function, and gut health could significantly enhance growth performance, oxidative balance, and overall well-being. Thus, comprehensive research is essential to optimize their use in commercial broiler production and advance sustainable poultry farming practices. The present study is distinguished from others by its focus on the combined effects of snot apple powder and probiotics on various critical parameters in broilers, including growth performance, oxidative stress markers, hematological indices, serum protein profiles, and triiodothyronine (T3) levels. While previous studies have investigated the individual benefits of plant-derived compounds and probiotics, this research uniquely explores their potential synergistic impact in poultry nutrition. Additionally, the study delves into the under-researched area of how these dietary components influence thyroid function (via T3 levels) and antioxidant capacity, providing insights into their mechanisms of action and practical implications for improving poultry health and production efficiency. This comprehensive approach addresses gaps in existing research, offering new perspectives for sustainable poultry farming practices.

2. Materials and Methods

This study was carried out in Teaching and Research Farm, The Federal Polytechnic Ado-Ekiti, Ekiti State in the Southwest of Nigeria. The sample size was calculated at the level of significance of 5% and power of study at 80% as described by Charan and Biswas (2013) and the G Power tool (Faul et al., 2009).

2.1 Preparation of Test Ingredient

Ripe Snot apple (*Azanza garckeana*) fruits were procured at a local market in Tula, Northern Nigeria. The identification of the plants was confirmed by Mr Ayodele S.O. (a Principal Technologist) in the Department of Agricultural Technology, Federal Polytechnic Ado Ekiti. They were air-dried and ground to powder and designated as Snot apple powder. Phytochemical analysis and proximate composition of the powder were carried out using Association of Analytical Chemistry Standard Procedures 1990 and presented in Table 1 and 2.

A proprietary probiotic (Natupro®) was procured from a reputable veterinary store in Ibadan, Oyo State. It contains 150 CFUx10^6/g each of *Bacillus subtilis*, *Bacillus licheniformis*, *Bacilus amyloliquefaciens* 390 and *Bacillus amyloliquefaciens* 700.

2.2 Experimental Animals and Management

200-a-day-old arbor-acre broiler chickens were randomly allotted to four treatments in a completely randomized design. The birds were allotted 10 replicates, 5 birds per replicate. The probiotics was administered to all four treatments at a uniform rate of 0.5% Naturpro (as recommended) and Snot apple meal were incorporated 0%, 1%, 2% and 3%, respectively into T_1, T_2, T_3 and T_4, and were thoroughly mixed in the feed shown in Table 3 and 4. Standard experimental starter and finisher rations were formulated as shown in Table 1 and 2, respectively. Birds were fed *ad libitum* and fresh water was offered daily throughout a 42-day trial. The vaccination program as recommended by the hatchery was followed and no medication was offered throughout the study.

2.3 Data Collection

The feed intake and weight changes were monitored throughout the study to evaluate their growth in a 42-day trial. Mortality was recorded, and feed conversion was computed per replicate.

2.3.1 Performance evaluation

Records of feed intake, weight gain, and mortality were taken weekly. The feed conversion ratio (FCR) was obtained by calculation. Total Feed Intake (g) = Total Feed supplied (g) - Total feed left over (g), Average feed intake (g/bird) = Total Feed Intake/Number of birds, Total weight gain = Final weight – Initial weight, Feed conversion ratio = Total Feed intake (g)/Total weight gain (g), %Mortality = (Number of dead birds/ Total number of stocked birds) ×100.

2.3.2 Blood sample collection

At the end of the feeding trial, blood samples were collected from 3 birds per replicate into the plain samples and heparinized bottles for serum and hematology, respectively. Hematology samples were assayed using standard procedures. For serum, blood samples in plain tubes were centrifuged and serum was obtained using standard procedures and stored at -20^{o}C until analysis. The serum was assayed for total protein, albumin, globulin and Uric acid carried out using Fortress diagnostics commercial assay kits and its procedures.

Determination of serum total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase activities and lipid peroxidation were assays as outlined in Jimoh (2019).

Serum triiodothyronine was assayed using enzyme linked immunosorbent assay, with commercial ELISA kits and its protocol. Triiodothyronine (T3) ELISA Catalog No. T3225T (Calbiotech Inc., 1935 Cordell Ct., El Cajon, CA 92020)

2.4 Data Analysis

All data were subjected to one way analysis of variance and significant means were separated using Duncan multiple range test SAS (version 2003).

3. Results

The growth indices of chicken fed snot apple powder with/or without probiotics is presented in Table 5. The final body weight of chicken was significantly ($p<0.05$) highest in T3 (2604.58 g) followed by T1 (2545.42 g), and the significantly ($P<0.05$) least values was obtained in birds on T4 (2383.21 g). Weight gain (g/bird/day) was significantly ($p<0.05$) higher in T3 (61.09) and T2 (59.69) compared to T4 (55.81). Feed intake (g/bird/day) was significantly ($P<0.05$) higher in T1 (78.74) compared to those on T4 (64.32). Feed conversion ratio (FCR) was ($P<0.05$) better in birds on T3 (1.19) and T4 (1.17) compared to those T1 (1.32). The hematological indices of chickens fed snot apple powder with/without probiotics are presented in Table 6. The Packed cell volume, Haemoglobin, erythrocytes, leukocytes, thrombocytes and lymphocytes of birds on the different treatments were statistically ($P>0.05$) similar. The Heterophils of birds on T1 differed significantly ($P<0.05$) from T2 and T3. Monocytes of birds on T2 were significantly ($P<0.05$) higher than those on other treatment. Eosinophils of birds on T3 were significantly ($P<0.05$) higher than other treatment. There were no significant ($P<0.05$) differences between mean obtained for basophils. The Heterophil: Lymphocyte ratio of birds on T2 and T3 were higher ($P<0.05$) than those from T1. The serum protein profile of chickens fed snot apple powder with/without probiotics is presented in Table 7. The serum protein of birds fed snot apple powder with probiotics (T2, T3, and T4) were higher than those on T1. Chickens on T2, T3, and T4 had significantly ($P<0.05$) higher globulin levels compared to those T1. Albumin values of birds on T1 were significantly ($P<0.05$) lower than those T2, but birds on T3, and T4 share statistically similar values with other treatment. The values obtained for Uric Acid in birds on T1 were least and was significantly ($P<0.05$) lower than those on T2, T3, and T4. The oxidative markers in chickens fed snot apple powder with/without probiotics are presented in Table 8. The total antioxidant capacity showed that birds administered 1% snot apple meal (T2) exhibited the highest total antioxidant capacity (14.34 mM) and significantly ($P<0.05$) least was found in those on T1. The lipid peroxidase activity was highest in T2 and T3 compared to those in T1 and T4. Birds on T2, T3 and T4 had higher ($P<0.05$) SOD activity when compared to those on T1. Birds on control had the highest catalase activity (148.63 u/ml mins), while birds on T2, T3, and T4 had significantly ($P>0.05$) similar catalase activity. The estimated values of glutathione peroxidase (GPx) showed that T2 had the highest GPx activity (36.01 U/L), but significantly ($P>0.05$) similar values were obtained amongst birds on T1, T3, and T4. Triiodothyronine of chickens fed snot apple powder with/without probiotics is presented in Figure 1. Chickens on T1 and T3 had significantly ($P<0.05$) higher triiodothyronine than birds on T2 and T4.

Table 1. Proximate and phytochemical analysis of snot apple powder

	Snot Apple
Dry Matter (%)	2.64
Crude Protein (%)	25.59
Ash (%)	7.57
Ether Extract (%)	13.60
Crude Fiber (%)	3.00
Nitrogen Free Extract (%)	50.32
Alkaloid (%)	1.13
Terpenoid (%)	0.90
Tannin (%)	1.19
Flavonoid (%)	5.56
Phytate (%)	0.22
Phenol (%)	10.17
Saponin (%)	1.17

Table 2. Phytochemical screening of snot apple powder

Secondary metabolites	Test	Inference
Alkaloid	Wagner's test	+ve
Tannin	Braymer's test	++ve
Glycoside	Keller's test	-ve
Saponin	Frothing test	++ve
Flavonoid	Alkaine reagent test	++ve
Terpenoid	Salkowski test	+ve
Phenol	Ferric chloride test	+++ve
Anthraquinone	Carbon tetrachloride test	+ve

-= absent, += trace, ++= moderate, +++= abundant.

Table 3. Gross composition of broiler starter ration

Feedstuff	T1	T2	T3	T4
Maize	51.25	50.2	49.2	48.2
Soyabean Meal (42% CP)	41.5	41.5	41.5	41.5
Fishmeal (65% CP)	3	3	3	3
Bonemeal	2	2	2	2
Limestone	0.5	0.5	0.5	0.5
Premix	0.25	0.25	0.25	0.25
Methionine	0.5	0.5	0.5	0.5
Lysine	0.2	0.2	0.2	0.2
Salt	0.3	0.3	0.3	0.3
Vegetable oil	0.5	0.5	0.5	0.5
Probiotics	0	0.05	0.05	0.05
Snot Apple Powder	0	1	2	3
TOTAL	100	100	100	100
Calculated Nutrient analysis				
Dry Matter %	85.21	84.29	83.41	82.53
Crude Protein (%)	24.72	24.61	24.51	24.41
Metabolizable Energy (kcal/kg)	2966.23	2930.17	2895.83	2861.49
Ether Extract %	3.64	3.6	3.56	3.52
Crude Fibre (%)	3.75	3.73	3.71	3.69
Lysine (%)	1.61	1.61	1.61	1.61
Methionine (%)	0.89	0.89	0.89	0.89
Calcium (%)	1.19	1.19	1.19	1.19
Available Phosphorus (%)	0.69	0.68	0.68	0.68

* Premix composition per kg diet: Vit A:400000 IU, Vit D:80000 IU, Vit E:40000 ng, Vit k3:800 mg, Vit B1:1000MG, Vit B2:6000 mg, Vit B6:500 mg, VitB12:25 mg, Niacin:6000 mg, Panthothenic acid:2000 mg, Folic acid: 200 mg, Biotin:8 mg, Manganese: 300000 g, Iron:8000 mg, Zinc:20000 g, Cobalt:80 mg, Iodine:400 mg, Selenium:40 mg, Choline:800000 g.

Table 4. Gross composition of broiler finisher ration

Feedstuff	T1	T2	T3	T4
Maize	58.25	57.2	56.2	55.2
Soyabean Meal (42% CP)	31.5	31.5	31.5	31.5
Rice Offal	3	3	3	3
Fishmeal (65% CP)	3	3	3	3
Bonemeal	2	2	2	2
Limestone	0.5	0.5	0.5	0.5
Premix	0.25	0.25	0.25	0.25
Methionine	0.5	0.5	0.5	0.5
Lysine	0.2	0.2	0.2	0.2
Salt	0.3	0.3	0.3	0.3
Vegetable oil	0.5	0.5	0.5	0.5
Probiotics	0	0.05	0.05	0.05
Snot Apple Powder	0	1	2	3
Total	100	100	100	100
Calculated Nutrient analysis				
Dry Matter %	85.07	84.15	83.27	82.39
Crude Protein (%)	21.58	21.47	21.37	21.27
Metabolizable Energy (kcal/kg)	3022.41	2986.35	2952.01	2917.67
Ether Extract %	3.94	3.9	3.86	3.82
Crude Fibre (%)	3.62	3.6	3.58	3.56
Lysine (%)	1.37	1.36	1.36	1.36
Methionine (%)	0.85	0.85	0.85	0.85
Calcium (%)	1.17	1.17	1.17	1.17
Available Phosphorus (%)	0.65	0.64	0.64	0.64

* Premix composition per kg diet: Vit A:400000 IU, Vit D:80000 IU, Vit E:40000 ng, Vit k3:800 mg, Vit B1:1000MG, Vit B2:6000 mg, Vit B6:500 mg, VitB12:25 mg, Niacin:6000 mg, Panthothenic acid:2000 mg, Folic acid: 200 mg, Biotin:8 mg, Manganese: 300000 g, Iron:8000 mg, Zinc:20000 g, Cobalt:80 mg, Iodine:400 mg, Selenium:40 mg, Choline:800000 g.

Table 5. Growth indices of chickens fed probiotics and snot apple inclusive diets

	T1	T2	T3	T4	SEM
Initial Weight (g)	38.54	37.80	38.66	39.03	1.30
Final weight (g)	2545.42^{b}	2461.39^{bc}	2604.58^{a}	2383.21^{c}	41.76
Weight gain (g/b/d)	59.69^{a}	57.70^{ab}	61.09^{a}	55.81^{b}	0.99
Feed Intake (g/b/d)	78.74^{a}	71.29^{ab}	72.75^{ab}	64.32^{b}	2.28
Feed conversion ratio	1.32^{a}	1.23^{ab}	1.19^{b}	1.17^{b}	0.03

a,b= means along the same row with different superscripts, differ significantly ($P<0.05$), SEM= standard error of mean.

Table 6. Haematological indices of chickens fed probiotics and snot apple powder inclusive diets

	T1	T2	T3	T4	SEM
Packed cell volume (%)	30.00	27.33	29.33	29.67	0.92
Haemoglobin (g/dL)	9.43	8.63	9.43	9.60	0.31
Erythrocytes (x 10^{6}/L)	3.02	2.70	2.75	3.28	0.15
Leukocytes (x10^{8}/L)	15.38	14.37	17.12	15.18	0.48
Thrombocytes (x10^{7}/L)	1.27	1.23	1.29	1.28	0.02
Lymphocyte (%)	63.33	59.67	60.33	63.33	1.29
Heterophils (%)	29.67^{b}	33.33^{a}	33.00^{a}	31.67^{ab}	1.26
Monocyte (%)	3.00^{b}	4.00^{a}	3.00^{b}	2.33^{b}	0.34
Eosinophils (%)	3.33^{b}	3.00^{b}	5.00^{a}	2.67^{b}	0.44
Basophils (%)	0.67	0.00	0.67	0.00	0.14
Heterophil: Lymphocyte Ratio	0.47^{b}	0.56^{a}	0.55^{a}	0.50^{ab}	0.01

a,b= means along the same row with different superscripts, differ significantly ($P<0.05$), SEM= standard error of mean.

Table 7. Protein of chickens fed probiotics and snot apple powder inclusive diets

	T1	T2	T3	T4	SEM
Protein (g/dL)	27.82^{b}	36.30^{a}	33.82^{a}	33.95^{a}	1.14
Globulin (g/dl)	1.48^{b}	1.92^{a}	1.91^{a}	1.93^{a}	0.08
Albumin (g/dL)	1.30^{b}	1.71^{a}	1.47^{ab}	1.46^{ab}	0.05
Uric Acid (mg/dl)	11.68^{b}	18.53^{a}	17.59^{a}	18.82^{a}	1.34

a,b= means along the same row with different superscripts, differ significantly ($P<0.05$), SEM= standard error of mean.

Table 8. Oxidative markers of chickens fed probiotics and snot apple powder inclusive diets

	T1	T2	T3	T4	SEM
Total antioxidant capacity (mM)	7.37^{b}	14.34^{a}	9.79^{ab}	11.72^{ab}	1.04
Lipid Peroxidation (MDA uM)	0.87^{b}	1.00^{a}	0.92^{a}	0.86^{b}	0.03
Superoxide dismutase (U/mL)	1.16^{b}	2.83^{a}	2.59^{a}	2.78^{a}	0.29
Catalase (u/ml mins)	148.63^{a}	106.98^{b}	102.75^{b}	103.08^{b}	10.86
Glutathione peroxidase (U/L)	25.13^{b}	36.01^{a}	28.88^{b}	24.38^{b}	2.39

a,b= means along the same row with different superscripts, differ significantly ($P<0.05$), SEM= standard error of mean.

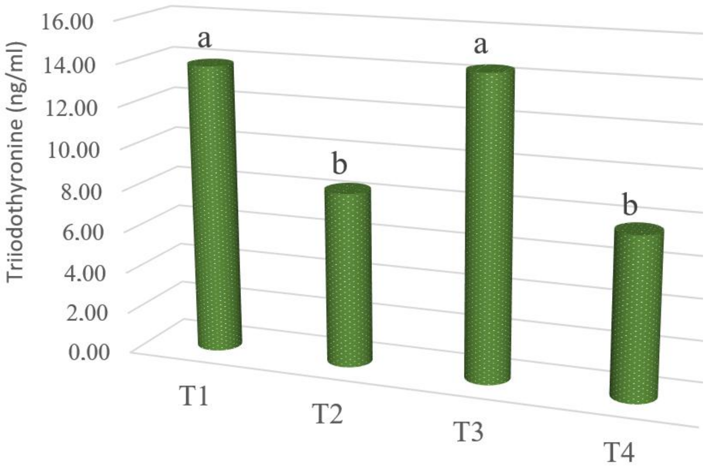

Figure 1. Triiodothyronine of chickens fed probiotics and snot apple powder inclusive diets. a,b= means values with different superscripts, differ significantly ($P<0.05$).

4. Discussion

In this study, notable distinctions emerged in weight gain, feed intake, and FCR, where T_3 displayed the highest final body weight, indicating its potential for maximizing overall growth, followed closely by T_1 and T_2, with T_4 exhibiting the lowest final body weight. The results obtained in this study concurred with those obtained by Jimoh et al. (2022a) recording birds fed on moringa and mistletoe supplements had higher performance indices than birds without supplementation during heat stress condition. However, other factors may influence final body weight beyond diet alone. The birds on 1% (T_2) and 2% (T_3) inclusion levels outperformed those on 3% (T_4) in weight gain, showcasing the positive impact of their respective diets on growth rates. Although weight gain indices were lower than that reported for fennel seed powder supplementation in broiler chicken (Al-Sagan et al., 2020). It is crucial to note that feed intake provides insights into dietary consumption patterns, suggesting potential differences in palatability or nutrient utilization between these diets (Rocha et al., 2022). The FCR is a key indicator of feed efficiency, where lower values denote better utilization of feed for growth (Feng et al., 2023). Here T_3, T_4, and T_2 with probiotics demonstrated significantly better FCRs than T_1 without probiotics administration, indicating that their diets were more efficiently converted into body mass. This suggests that the inclusion of probiotics and phytobiotics in T_3, T_4, and T_2 may have positively influenced nutrient absorption and metabolism, leading to improved feed efficiency. The observed variations in weight gain, feed intake, and FCR seem to be an integral part of dietary interventions on growth performance and these are key areas that commercial production needs to leverage on so as to meet demands in the broiler meat production enterprise (Ilaboya et al., 2024). Birds on 2% snot apple powder had higher final body weight compared to those on 0% and 3%. This suggests that moderate inclusion of snot apple powder enhances body weight in broiler chickens. In summary, weight gain of birds on 1% snot apple powder showed significantly higher weight gain compared to 3%, indicating that higher concentrations of snot apple powder (3%) may not be as effective for weight gain. Birds on 0% snot apple powder had significantly higher feed intake compared to 3%, possibly due to differences in palatability or digestibility influenced by the snot apple powder. Birds on 2% and 3% snot apple powder exhibited better FCR values compared to 0%, indicating improved feed efficiency with snot apple powder supplementation. The improved feed conversion ratio (FCR) and growth performance in birds supplemented with 1-2% snot apple powder (T2 and T3) may be attributed to the bioactive compounds in snot apple, such as flavonoids and phenolics. These compounds are known to enhance nutrient absorption and gut health by modulating intestinal morphology and enzyme activity. Probiotics contribute by stabilizing gut microbiota, reducing harmful pathogens, and enhancing nutrient utilization, which synergistically improve feed efficiency when combined with snot apple powder.

The variations across treatment groups (T_1 to T_4) suggest potential differences in blood volume or red blood cell mass in chickens subjected to different dietary interventions. Similarly, variations in Hb concentration and erythrocyte count further reveal the oxygen-carrying capacity of the blood. Leukocyte counts were in line with values obtained for broiler chickens (Besksi and Sardary, 2015) and quail (Ufele and Ebenebe 2017) and are crucial for assessing immune function, with variations indicating potential differences in immune responses among treatment groups. Higher leukocyte counts may suggest enhanced immune activity or a response to stressors, while lower counts could indicate a less robust immune response (Berghof et al., 2018). Higher lymphocyte percentages in this study may indicate a stronger immune response or better overall health status. Variations in percentages of heterophils, monocytes, eosinophils, and basophils suggest potential immune system modulation by dietary interventions, with birds on probiotic treatment potentially promoting specific types of immune responses over others (Lee et al., 2012). The heterophil:lymphocyte ratio serves as an indicator of stress and immune function balance. The observed variations in this ratio across treatment groups suggest differential impacts on stress levels and immune function among the dietary interventions. Overall, the hematological parameters (packed cell volume, hemoglobin, erythrocytes, leukocytes, thrombocytes, lymphocytes) did not significantly differ among treatments, suggesting that neither snot apple powder nor probiotics had adverse effects on blood parameters.

The result of this study indicates that birds in treatments with snot apple powder and probiotics (T2, T3, T4) had higher total protein levels compared to T1, indicating better protein utilization and potentially enhanced growth. This finding corroborates the results obtained by Jimoh et al. (2018) who reported mistletoe inclusion significantly ($P<0.05$) influenced serum total protein of pullets across the treatment, Pullets fed diets with 6% inclusion of MLM had significantly ($p<0.05$) highest serum total protein. Globulin levels were significantly higher in T2, T3, and T4 compared to T1, which suggests improved immune response and overall health status. Albumin levels varied but generally favored treatments with snot apple powder. This finding disagrees with Jimoh et al. (2018) whose result revealed that serum globulin and albumin of laying pullets were not influenced by mistletoe leaf meal. Birds on T1 had significantly lower uric acid levels compared to T2, T3, and T4, indicating potential differences in metabolic processes influenced by the additives. The observed variations in protein profile parameters highlight the diverse effects of probiotics and phytobiotics supplementation on chicken physiology and metabolism (de Vries et al., 2022). T_2 emerges as a promising treatment with enhanced total protein and globulin levels, suggesting improved protein metabolism and immune function. T_3 and T_4 also show positive effects on protein profile parameters, indicating potential benefits for overall health and performance. Chickens on T_2 exhibited the highest globulin level, suggesting its diet may enhance immune function or stimulate the production of immune-related proteins, while values obtained were lower than that reported by Tóthová et al. (2019) in broiler chicken and in grower turkey (Szabó et al., 2005). The elevated serum protein and globulin levels in T2, T3, and T4 could indicate enhanced protein metabolism and immune function due to the immunomodulatory effects of both snot apple powder and probiotics. These effects may be mediated by increased production of immune-related proteins and improved nitrogen metabolism. Probiotics could stimulate immune cells in the gut-associated lymphoid tissue (GALT), while the phytochemicals in snot apple may act as immunostimulants, enhancing lymphocyte proliferation and activity. The intermediate uric acid levels in T_3 and T_4 suggest their diets may impacts on nitrogen metabolism differently compared to T_1 (without probiotics) and T_2.

Birds on 1% snot apple powder exhibited the highest TAC, indicating superior antioxidant defense in these birds, which could contribute to better health and stress resilience. Higher lipid peroxidase activity in 1% and 2% suggests potential oxidative stress, although within manageable levels. Oxidative stress refers to any situation where there is a serious imbalance between the production of free radicals (FR) or reactive oxygen species (ROS), called the oxidative load, and the antioxidant defense system (Jimoh, 2022b). Birds on T2, T3, and T4 showed higher SOD activity compared to T1, indicating enhanced antioxidant enzymatic activity. Catalase activity was similar among treated groups, possibly indicating balanced oxidative status. Birds on T2 had the highest GPx activity, further supporting snot apple powder's antioxidative benefits. The significantly higher TAC observed in birds fed with 1% snot apple meal (T_2) suggests that this dietary intervention enhances the birds' antioxidant capacity. Similarly, T_3 and T_4 also exhibit elevated TAC levels compared to the control group without probiotics treatment and values obtained were higher than that obtained for Astathanxin supplementation in broilers in Asia (Hosseindoust et al., 2020), and the result obtained by Jimoh et al. (2020; 2022a) who recorded that Birds on phytogenic supplements tend to have lower lipid peroxidation and better antioxidant profile than birds on control treatment during heat stress conditions, Serum lipid peroxidation of birds on treatments phytogenic supplements were statistically ($P>0.05$) similar and significantly ($P<0.05$) lower than birds on treatment control indicating the potential benefits of probiotics and phytobiotics supplementation in bolstering antioxidant defenses. However, T_1 and T_4 exhibited lower lipid peroxidation levels, indicating potential protective effects against lipid oxidation conferred by their respective diets. SOD is a critical antioxidant enzyme that catalyzes the conversion of superoxide radicals into less harmful molecules, thus playing a crucial role in reactive oxygen species (ROS) detoxification (Xue et al., 2018; Jafari et al., 2021). The significantly higher SOD activity observed in T_2 suggests that this diet enhances the birds' ability to neutralize superoxide radicals. Similarly, T_3 and T_4 also exhibit elevated SOD activities compared to the control group (without probiotics), indicating the potential antioxidant effects of probiotics and phytobiotics supplementation (Jung et al., 2010; Kim et al., 2016). Catalase is another important antioxidant enzyme responsible for breaking down hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage (Wang et al., 2022). The lower catalase activity observed in birds fed with probiotics and phytobiotics-enriched diets (T_2, T_3, and T_4) compared to the control group (T_1) suggests that these dietary interventions may modulate catalase activity to maintain cellular redox balance. The significantly higher GPx activity observed in T_2 and T_3 indicates that this diet enhances the birds' ability to detoxify peroxides (Daun and Akesson, 2004), suggesting the potential benefits of probiotics and phytobiotics supplementation in enhancing GPx-mediated antioxidant defenses. The higher total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity observed in birds fed snot apple powder and probiotics likely result from the antioxidant properties of the bioactive compounds in snot apple, such as tannins and saponins, which neutralize reactive oxygen species (ROS). Probiotics may enhance the production of endogenous antioxidants by supporting gut health and reducing systemic inflammation, further bolstering oxidative defenses.

In this study, chickens fed 2% treatment levels seem to have the most active thyroid hormone and knowing fully well the role this hormone plays in regulating metabolism, growth, and development. The values obtained were within the expected range for healthy broiler chickens (Jiang et al., 2020). Birds on 0% and 2% snot apples had significantly higher T3 levels compared to T2 and T4, suggesting differential effects on thyroid function possibly influenced by the dietary additives. In broiler chickens, thyroid hormones play a crucial role in regulating metabolism, growth, and development (Hyeong-Kyu and Rexford, 2023). The result in this study partially agrees with Jimoh et al. (2023) that triiodothyronine of birds fed phyllanthus and mistletoe were significantly ($P<0.05$) higher than birds on basal diet. The increased triiodothyronine (T3) levels observed in birds on 0% and 2% snot apple powder diets suggest that the additives may influence thyroid function. This could be linked to improved energy metabolism and growth, as thyroid hormones are critical regulators of these processes. The bioactive compounds in snot apple may modulate thyroid hormone synthesis or activity through antioxidant or anti-inflammatory pathways.

However, 1-3% inclusions of Snot apple meal seem essential for proper growth, feed efficiency, and overall health in broilers. Any significant deviations from these levels could indicate underlying health issues, stress, or nutritional imbalances that may impact the birds' performance and well-being. Anwar et al (2018) reported the need to monitor thyroid hormone levels in broiler chickens as it is important for optimizing production outcomes and ensuring the welfare of the birds.

5. Conclusion

This study demonstrates that incorporating snot apple powder, particularly at 1% and 2% levels, along with probiotics in broiler chicken diets, positively influences growth performance, serum protein profiles, antioxidant status, and potentially thyroid function. Specifically, 2% snot apple powder showed optimal performance outcomes, while maintaining favorable hematological parameters and oxidative balance. These findings suggest that snot apple powder can be a beneficial dietary supplement in poultry nutrition, enhancing both performance and health parameters. To refine dietary recommendations for commercial application and fully understand the benefits of snot apple powder in poultry nutrition, further research should explore the mechanisms of action, optimal dosages, species-specific effects, interactions with feed components, long-term impacts on health and productivity, environmental and economic implications, and consumer acceptance to refine the use of snot apple powder in poultry diets.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	A.O.J.	U.D.O.	S.O.A.	U.G.D.I.
C	50	50		
D	100			
S	100			
DCP			50	50
DAI			50	50
L			80	20
W	40		60	
CR	60		20	20
SR	60		20	20
PM	20	80		
FA	20	50	10	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal as noted on the journal's author guidelines page have been adhered to and the institutional ethics committee for care and use of animal for research approved the study (approval date: November 01, 2023, protocol code: AP/REC/2023/0864). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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