Effect of aqueous extract of fresh sweet orange peel on growth performance and non-specific immune response of broiler chickens

D.J. Ibigbami[1,*], O.J. Odutayo[1], O.Y. Ayo-Ajasa[1], B.O. Ibigbami[2], A.O. Fafiolu[2] and O.M. Sogunle[1]

[1] Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State 111101, Nigeria

[2] Livestock Science and Sustainable Environment, Center of Excellence for Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Ogun State 111101, Nigeria

* Corresponding author: ibigbamidj@funaab.edu.ng

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ABSTRACT

Background and Objectives: One of the many approaches proposed to enhance immune response is to supplement the diet with natural products like sweet orange peel extract, which is said to increase the production of antibodies, improving the immune system indirectly through their antiviral, antioxidant, and antibacterial effects. This study investigated the effect of aqueous extract of fresh sweet orange (*Citrus sinensis*) peel (AEFSOP) on the performance and non-specific immune response of broiler chickens.

Methodology: Three hundred (300) unsexed day-old (Cobb500) broiler chicks were used in the experiment made up of three treatment groups of AEFSOP (0, 50, and 100 mL/L) containing 100 birds per treatment with 20 birds replicated 5 times in each treatment group in a completely randomized design. Fresh sweet oranges were procured and peeled to produce the aqueous extract. The peels were soaked in water for 12 hours (200 g to 1 liter of water), and the extract was infused into the drinking water of the birds three consecutive days a week. The birds were fed *ad libitum* throughout the experiment which lasted for 42 days. Data were collected on performance traits, including feed intake, water intake, weight gain, feed conversion ratio, and survivability. In addition, a non-specific immune response was determined at day 21.

Main Results: Results showed no significant ($P > 0.05$) differences in performance. Statistically similar values for final weight in birds administered AEFSOP at 0, 50, and 100 mL/L (1,665.20 ± 52.39, 1,680.00 ± 123.00, and 1,635 ± 112.91 g, respectively; $P = 0.777$). Albeit, the values obtained in the birds administered with the aqueous solution had a better performance numerically, though not statistically. Similarly, values recorded for feed conversion ratio (1.94 ± 0.07, 1.92 ± 0.15, and 1.97 ± 0.14, respectively; $P = 0.841$) were also non-significant ($P > 0.05$). There was an increasing trend with increasing levels of administration from 0, 50 to 100 mL/L in non-specific immune response (0.33 ± 0.11, 0.69 ± 0.18, and 0.69 ± 0.18 mm, respectively; $P = 0.843$).

Conclusions: The study concluded that administration of AEFSOP at 50 or 100 mL/L tended to enhance the non-specific immune response in broiler chickens.

Keywords: Infusion, immune, orange, growth, non-specific

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INTRODUCTION

The utilization of antibiotics has been practiced for more than a decade in animal production as a growth promotant, especially for poultry production, owing to the need to increase meat production to support the needs of the ever-growing population. Antibiotics inhibit the negative health implications of pathogenic intestinal bacteria on poultry birds, affecting their productive performance. However, antibiotics use in poultry production results in significant negative health impacts on the consumers due to the fact that the synthetic products accumulate in tissues leading to antibiotic resistance in humans and finally causing therapeutic failure (Levy and Marshall, 2004). Several countries of the world have prohibited or restricted the use of antibiotics in animal production, and there is the question of finding suitable alternatives with similar growth-promoting traits of synthetic antibiotics.

One of the many approaches proposed to enhance the performance of birds is the use of phytogenics which are natural, less toxic, and residue-free, as well as the possession of nutritional and health benefits. Phytogenics are a wide variety of herbs, spices, and products derived thereof, which contain mainly essential oils (Hashemi and Davoodi, 2010). Phytogenics have been found to stimulate appetite and palatability and enhance digestion, which results in improved growth. They are also known to have medicinal, antimicrobial, anticoccidial, fungicidal, and anti-oxidative properties (Jamroz *et al.,* 2006; Applegate *et al.,* 2010). One of such phytogenics, which is an agro-industrial waste as well as scarcely utilized in poultry production, is sweet orange peel aqueous extract.

Sweet orange (*Citrus sinensis*) peels contain high concentrations of phenols, especially flavonoids (Manthey, 2004), and significant amounts of beta-carotene (Ghazi, 1999) and vitamin C (Rinzler, 1990). Sweet orange peel also contains ethyl acetate extracts, which have been shown to inhibit the growth of gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus,* and *Listeria monocytogenes*), yeasts, and molds (Chanthaphon *et al.*, 2008; Pourhossein *et al.*, 2015).

Despite the significant potential of orange peel as a natural dietary supplement for broiler chickens, aimed at enhancing both growth performance and immune response, up until now, few studies have discussed the extraction of the bioactive compounds from orange peels (Abdoun *et al.*, 2022). Several studies have reported the presence of bioactive compounds in orange peel, including flavonoids, terpenoids, and phenolic compounds, which have the potential to boost immune response (Saini *et al.*, 2022). Hesperidin belongs to a class of flavonoids known as citrus bioflavonoids, which are abundant in citrus fruits such as oranges. Several studies have demonstrated the beneficial effects of hesperidin on broiler chicken performance and immune response. Flavonoids and especially their subgroup flavanones, which contain hesperidin and naringin, which are well known for their antioxidant properties, are health-promoting molecules with multifunctional biological activities. They have been shown to attenuate inflammation to quench active oxygen species and their intake appears to be inversely related to the risk of cardiovascular disease and several forms of cancer (Erlund, 2004). Furthermore, hesperidin has been reported to exhibit immunomodulatory properties (Chen *et al.*, 2018; Li *et al.*, 2018; Han *et al.*, 2022). Hesperidin's ability to improve growth and enhance non-specific immune response makes it a promising candidate for supplementation in broiler chicken diets, contributing to overall health and performance.

Additionally, limited studies have investigated the direct impact of orange peel supplementation on the immune response of broiler chickens, with the need to explore the potential immunomodulatory properties it may offer (Han *et al.*, 2022). Research has indicated that orange peel supplementation improved the immune response and decreased broiler chickens' susceptibility to diseases, suggesting the potential of the orange peel as a natural stimulant of the immune system (Pourhossein *et al.*, 2019). Therefore, it is imperative to investigate the influence of orange peel supplementation on the growth and immune system of broiler chickens, alongside developing convenient extraction techniques to harness its potential for sustainable poultry production. This research will contribute to the broader understanding of natural feed additives and their role in enhancing broiler health and performance, thus paving the way for improved strategies in the poultry industry.

Pourhossein *et al.* (2015) reported that dietary sweet orange peel extract supplementation improved the immune response and disease resistance in broiler chicken production, indicating that it can constitute a useful additive in broiler feeding. Ebrahimi *et al.* (2013) found that adding dried sweet orange peel into the diet of broiler chickens in the proportion of 1.5% of feed promoted feed intake and weight gain in the period between 1 and 21 days of age.

The hypothesis of this research is that fresh sweet orange peel can improve the growth performance and non-specific immune response of broiler chickens due to a powerful flavonoid called hesperidin contained in sweet orange peels. Hence, the objective of the study is to determine the effect of the aqueous extract of fresh sweet orange peel on the growth performance of broiler chickens in terms of their body weight, feed intake, and feed conversion ratio and to investigate the effect of the aqueous extract of fresh sweet orange peel on non-specific immune response by measuring the production of phytohaemagglutinin using the PHA test along with other methods.

MATERIALS AND METHODS

Experimental Site

This study was carried out at the Poultry Unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria which is within the rainforest vegetation zone of South-western Nigeria on latitude 7° 13’ 49.46” N, longitude 3° 26’ 11.98” E and altitude of 98 m above sea level (Google Earth, 2019). The climate is humid, with a mean precipitation of 1,003 mm per annum. Annual mean temperature and humidity ranges from 31.9 to 34.8°C and 79.7 to 90.1%, respectively. The study protocol was approved and conducted in accordance with the Animal Ethics Committee guidelines of the Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB, 2018).

Sourcing, Processing, and Administration of Test Ingredient

Fresh sweet oranges with weights ranging from 160–200 g were procured from a local market in Abeokuta and peeled with the aid of a sharp knife. The peels obtained from the citrus fruits constitute between 16% and 17% of the total weight of the fruits. To produce the aqueous extract, 200 g of fresh sweet orange peels were soaked in 1 L of water at room temperature for 12 hours. The peels were removed, and the water was sieved. The extract was immediately administered into the drinking water of the birds starting from the first week of the experiment, three consecutive days a week, throughout the 6 weeks of the experiment, at concentrations of 0, 50, and 100 mL/L. A fresh batch of extract was made every week.

Experimental Birds and Management

A total of three hundred (300) one-day-old unsexed (Cobb500) broiler chicks were purchased from Zartech Farms, Ibadan. On arrival, they were given water containing glucose as an anti-stress before feeding. The chicks were brooded for two weeks in a controlled brooding room. They were randomly distributed into three treatments of five replicates with ten birds per replicate. The birds were housed in a deep litter pen with wood shavings spread on the cemented floor to a depth of 2–5 cm. The stocking density of the pen was 0.1 m^2/bird. Daily routine management was carried out, such as supplying clean water and feed, observing for sick birds, checking for mortalities, and maintaining appropriate record keeping.

Experimental Diet

Three different diets were compounded to meet the nutritional needs of broiler chickens at 0–7, 8–21, and 22–42 days, respectively. Feed and water were supplied to the birds *ad libitum*. Table 1 shows the ingredient and nutrient composition of experimental diets.

Table 1 Ingredient and nutrient composition (%) of experimental diets

Parameter	Pre-starter (0–7 days)	Starter (8–21 days)	Finisher (22–42 days)
Ingredient			
Maize	57.00	58.60	62.50
Soybean meal	37.00	36.10	31.50
Vegetable oil	1.87	1.65	2.20
Limestone	1.00	1.00	1.10
Dicalcium phosphate	1.75	1.75	1.75
Salt (NaCl)	0.35	0.35	0.35
Lysine	0.40	0.10	0.12
Methionine	0.20	0.20	0.20
Threonine	0.18	0.00	0.00
Premix*	0.25	0.25	0.28
Total	100.00	100.00	100.00
Calculated analysis			
ME (kcal/kg)	2,995.50	2,991.50	3,047.75
Crude protein	22.51	21.89	20.07
Lysine	1.52	1.26	1.15
Methionine	0.55	0.51	0.44
Threonine	0.98	0.78	0.72
Tryptophan	0.23	0.23	0.21
Valine	0.89	0.88	0.80
Arginine	1.37	1.35	1.21
Calcium	1.00	1.00	0.99
P available	0.45	0.45	0.41

Note: *Trace mineral premix 0.1%, vitamin premix 0.1%, B-complex 0.02%, choline 0.05%, and salt 0.3%. Trace mineral premix supplied mg/kg diet: Mg 300, Mn 55, I 0.4, Fe 56, Zn 30, and Cu 4. The vitamin premix supplied per kg diet: vitamin A 8,250 IU, vitamin D_3 1,200 IU, vitamin K 1 mg, vitamin E 40 IU, vitamin B1 2 mg, vitamin B2 4 mg, vitamin B12 10 mcg, niacin 60 mg, pantothenic acid 10 mg, and choline 500 mg. ME = metabolizable energy.

Experimental Design

The experiment was laid out in a completely randomized design involving 3 levels of aqueous extract of fresh sweet orange peel (AEFSOP) inclusions: 0, 50, and 100 mL/L of water.

Data Collection

Phytochemical determination

A 50 mL sample of AEFSOP was taken to the Veterinary Physiology and Biochemistry Department, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta to test for the phytochemical constituents of the extract.

Phytochemical screening was performed using standard procedures according to Trease and Evans (1989) and Sofowora (1993).

Growth performance characteristics

Data on feed intake, weekly body weight changes, feed conversion ratio, and survivability were recorded throughout the experimental period. Feed intake was recorded weekly on a replicate basis. Left-over feed was subtracted from the total feed given to the birds to determine feed intake.

$$\text{Average feed intake} = \frac{\text{Feed given} - \text{Feed left over}}{\text{Number of birds}}$$

For body weight gain, birds were weighed per replicate at the start of the experiment and subsequently on a weekly basis. Weight gain was obtained by calculating the difference between the final body weight and the previous body weight.

$$\text{Average weight gain} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Number of birds}}$$

The feed conversion ratio (FCR) was calculated as the ratio of feed intake per gram body weight gain.

$$\text{FCR} = \frac{\text{Average feed intake (g/d)}}{\text{Average body weight gain (g/d)}}$$

Survivability

The total number of live broilers at the end of the experiment was recorded and expressed as a percentage (%) of the total number of birds alive at the start of the experiment.

$$\text{Survivability (\%)} = \frac{\text{Number of live birds}}{\text{Total number of birds}} \times 100$$

Non-specific immune response

The reagent was phosphate buffer saline (PBS), which contained 8.0 g of sodium chloride, 0.20 g of potassium chloride, 0.20 g of potassium dihydrogen phosphate, 1.44 g of disodium hydrogen phosphate, and 1 L of distilled water at pH 7.2.

The local immune response to phytohaemagglutinin type P (PHA-P) was studied using the method of Corrier and DeLoach (1990). At 21 days old, 0.1 mL (concentration 1 mg/mL) of PHA-P was injected at the 3rd and 4th inter-digital space of the left foot. The right foot served as a control and was injected with 0.1 mL of PBS. The foot web index was calculated as the difference between the swelling in the right and left feet before and after 24 hours of injection and expressed as millimeters. The foot web/pad index was calculated as follows: (R2 – R1) – (L2 – L1)
where R2 is the thickness of the right foot web after 24 hours of injection, R1 is the thickness of the right foot web before injection, L2 is the thickness of the left foot web after 24 hours of injection, and L1 is the thickness of the left foot web before injection.

Statistical Analysis

All data collected were subjected to a one-way analysis of variance using Minitab 17. Significant ($P < 0.05$) differences among means were separated using the Tukey test.

RESULTS AND DISCUSSION

The performance indices of broiler chickens on AEFSOP used in this trial showed that there were no significant ($P > 0.05$) differences among means i.e. AEFSOP had no significant effect on all the performance indices measured as shown in Table 2. It could be inferred that any of the inclusion levels investigated in this study may be utilized in broiler production to bring about similar growth performance indices in the birds. However, a numerical increase existed among the treatment with respect to the final weight in birds at 50 mL/L (1,680.00 ± 123.00 g/bird) followed by control (1,665.20 ± 52.39 g/bird), and least value was recorded in 100 mL/L (1,635 ± 112.91 g/bird). This suggests that the concentration of the bioactive components (tannin, phytate, flavonoid, saponin, and cyanogen) in AEFSOP was not sufficient to stimulate a significant growth rate in the experimental birds, as depicted in Table 3. A similar outcome was reported in studies conducted by Jang *et al.* (2007), Agu *et al.* (2010), and Erdogan *et al.* (2010), who did not record significant effects of different phytogenic compounds on broiler chicken growth performance. The AEFSOP did not affect weight gain, water intake, and feed conversion ratio. This study corroborates the report of Agu *et al.* (2010), who observed no significant differences in water intake in birds given sweet orange peel. Statistically similar values ($P > 0.05$) were obtained for feed conversion ratio in birds administered AEFSOP at 0, 50, and 100 mL/L (1.94 ± 0.07, 1.92 ± 0.15, and 1.97 ± 0.14, respectively). Jamroz *et al.* (2006) reported improved feed conversion ratio due to the addition of a plant extract containing cinnamaldehyde, carvacrol, and capsaicin to a maize or wheat and barley-based diet, respectively. In contrast, body weight was not affected by the treatment.

Table 2 Effect of aqueous extract of fresh sweet orange peel (AEFSOP) on growth performance of broiler chickens

Parameters	AEFSOP Inclusion levels			SEM	P-value
	0 mL/L	50 mL/L	100 mL/L		
Initial weight (g/bird)	45.98	45.28	45.00	0.40	0.240
Final weight (g/bird)	1,665.20	1,680.00	1,635.00	45.20	0.777
Daily weight gain (g/bird)	38.55	38.92	37.86	1.07	0.781
Daily feed intake (g/bird)	74.70	74.30	74.12	0.23	0.230
Water intake (mL/bird)	179.50	183.20	182.40	0.01	0.552
Feed: water ratio	0.41	0.40	0.41	0.00	0.750
Feed conversion ratio	1.94	1.92	1.97	0.06	0.841
Survivability (%)	90.00	100.00	90.00	1.63	0.619

Note: SEM = standard error of mean

Table 3 Phytochemical constituents of aqueous extract of fresh sweet orange peel (AEFSOP)

Constituent	Percentage (%)
Tannin	2.91
Saponin	13.26
Alkaloids	16.01
Flavonoids	6.84
Terpenoid	4.16

On the contrary, Ebrahimi *et al.* (2013) reported that the incorporation of dried sweet orange peel in the diet of broiler chickens in the proportion of 1.5% of feed enhanced feed consumption and weight gain at the starting phase while feeding 3% dried sweet orange peel in the same phase led to reduced feed intake and weight gain thereby increasing feed conversion ratio relative to other treatments. There was no nutritional disorder recorded, so the mortality recorded could be due to other challenges such as health and mishaps. The non-significant difference observed may be seen as an indication of the safety and adequacy of the test ingredient (Alu *et al.,* 2009)

The citrus peels are rich in nutrients and contain many phytochemicals with strong potential for use in drug production or as food supplements (Mathew *et al.*, 2012; Chede, 2013; Lawal *et al.*, 2013). Our results agree with the stated claims as a range of phytochemicals viz; alkaloids, terpenoids, tannins, flavonoids, and saponins that were detected in the orange peels. Oikeh *et al*. (2013) found very high levels of alkaloids, saponins, and tannins, and medium levels of flavonoids and terpenoids in comparison to the findings of this study. Non-specific immune response is the body's immediate defense mechanism against pathogens (Alberts *et al.*, 2002).

The method used in carrying out the non-specific immunity assay is a type of delayed-type hypersensitivity (DTH), which involves observation of the swelling that results from leukocyte recruitment to a site of inter-digital injection of an antigen in an immune animal. Therefore, it permits *in vivo* detection of cell-mediated responses to a wide variety of infectious agents. Another advantage is that the test can be performed relatively easily without expensive equipment. Because of these features, the assay is still used frequently (Hall *et al.,* 2003; Moriello *et al.,* 2003). There are some downsides to the method, however. It is tough to produce a quantitative narration of the results, and specifying which immune cells infiltrate the site of injection is often impractical. Also, the DTH test may only be valid the first time it is carried out in an individual animal (Pollock *et al.*, 2003).

The outcome of the study with respect to non-specific immune response revealed that AEFSOP inclusion groups demonstrated a numerically increased trend in local immune response (P > 0.05) at 50 and 100 mL/L (0.69 ± 0.18 mm) compared to the control (0.33 ± 0.11 mm) which indicated that AEFSOP benefitted the immune system of the broiler chickens. This is revealed in Figure 1. This is in agreement with the findings of Ragab and Hassan (2007) who revealed that *C. sinensis* peel enhanced the immune system activities of laying chickens due to their antioxidant properties. Flavonoids which are present in sweet orange peels have antioxidant, anti-inflammatory, anti-bacterial, and immune-stimulating effects (Harborne and Williams, 2000). Pourhossein *et al.* (2015) also found that *C. sinensis* peel extract improved the immune response of broiler chickens. Similarly, Nijveldt *et al*. (2001) stated that citrus fruit flavonoids have a synergistic effect on the immune system. It can be recommended that fresh sweet orange peels can support the growth performance of broiler chickens in terms of weight gain, feed conversion ratio, and local immune response. The existence of these phytochemicals may, therefore make the orange peel that no one pays attention to an untapped source of pharmacologically salient material. This study, therefore suggests that byproduct reuse from orange wastes offers twofold benefits. The industrial economics of the processing unit is likewise enhanced as useful derivatives are produced from the waste materials while considerably reducing environmental pollution (Ashok Kumar *et al.*, 2011). Additionally, higher inclusion levels than 100 mL/L AEFSOP should be used to further assess the possible growth-promoting effects of the aqueous extract on the growth performance of broiler chickens.

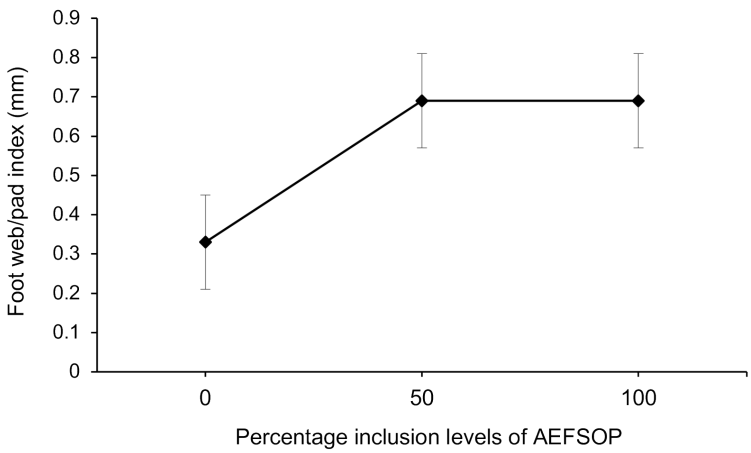

Figure 1 Effect of aqueous extract of fresh sweet orange peel (AEFSOP) on non-specific immune response of broiler chickens at 21 days

CONCLUSIONS

From the study, it can be concluded that the administration of AEFSOP up to 100 mL/L of water had no significant influence on the performance traits of broiler chickens. Also, the administration of AEFSOP at 50 and 100 mL/L tended to improve the non-specific immune response of broiler chickens.

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