International Journal of Advancing Research, Innovation and Entrepreneurship

Vol 1(1), DOI: 10.82036/g6yw3p17, April 2025 *ISSN:2977-9332*

Growth Performance and Haemato-Biochemical Indices of Broiler Chickens Fed Diets Supplemented with Different Levels of Sunflower (*Helianthus annuus*) Seed Oil

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ABSTRACT
This research was conducted to evaluate the growth performance and haemato-biochemical indices of broiler chicks fed diets supplemented with different amounts of sunflower (*Helianthus annuus*) seed oil. A total of 210 1-day-old broiler chicks (Arbo Acre) were randomly allotted into seven groups in a complete randomized design (CRD). There were three replicates, each containing ten birds. Basal diet was formulated to meet the nutrient requirements of broiler chicks. Birds in treatment 1 were given a basal diet with 1.2 g/kg of neomycin, whereas birds in treatments 2, 3, 4, 5, 6 and 7 were given basal diet mixed with 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mL of sunflower seed oil (SSO), respectively. Feed and clean water were given freely for the duration of 28 days. At day 28, 5 mL of blood sample were collected from three (3) birds per replicate for haematological and serum biochemical assay. All data generated were subjected to analysis of variance (ANOVA). Growth performance result showed significant ($P<0.05$) differences in final live weight, average weight gain and feed conversion ratio. It was observed that birds fed diets containing higher levels (0.7, 0.8, 0.9 and 1.0 mL) of SSO performed significantly better than those fed other diets. All haematological and serum parameters measured were influenced ($P<0.05$) by the treatment. However, values were within the established range for healthy birds. It was concluded that SSO can be included up to 1.0 mL in the diet of broiler chickens without compromising their health status and growth performance.

Keywords: Antibiotics, Broilers, Performance, Phytochemicals, Sunflower oil.

I INTRODUCTION

World Health Organization (2007) Technical Report on protein requirements in human nutrition states that the average requirement is 0·8 g protein/kg body weight per day; however many developing countries protein intake falls significantly short of this value. This gap in protein requirements can be closed by rearing species of animals with short generation interval, high prolific and rapid growth (Cheeke, 2005). Poultry have the above characteristics and can convert feed into food products quickly and efficiently. Among the poultry species reared, broiler chicken is the most widely consumed organic meat and predicted to have increased consumer demand and production because of its good source of protein (FAO, 2018). The use of antibiotics have been the most successful way to control diseases and reduce mortality in poultry production. However, the increased use of antibiotics, especially as a growth promoter for livestock, has led to a significant risk of antibiotic-resistant bacteria and its residual effects in animals' products constitute an adverse effect to consumers (Lee *et al*., 2004). Due to these detrimental impact (antibiotic-resistant bacteria, antibiotic residues in animal products and the danger it posed to human health), the European Union in 2006 ban the use of antibiotics in the poultry industry and suggested alternatives known as natural growth promoters. Among the potential alternatives used to replace antibiotics are prebiotics, probiotics and most recently essential oils (Zhaikai *et al*., 2015) from plant extracts. Essential oils are aromatic, oily liquids generated from different parts of the plants, such as leaves, barks, flowers and seeds. They can be extracted from plant materials using different extraction methods such as solvent extraction and mechanical extraction using a screw. Among the most commonly used are cold pressing (also known as expression) and steam distillation which is the most popular method and has been employed extensively, particularly in large-scale production (Di Leo Lira *et al*., 2009). Essential oils contain secondary metabolites that have therapeutic properties required for the suppression of pathogenic bacteria, scavenging free radicals, reducing oxidative stress, improving animal growth, boosting the immune system and improving efficient nutrient utilization (Huang *et al*., 2010). Phytochemicals have also been reported to be safe and effective without causing any negative effects on animals once administered in right doses and concentrations (Oluwafemi *et al.*, 2021). One of the potential plants loaded with these secondary metabolites is sunflower (*Helianthus annuus*) oil seed. This research was designed to evaluate the effect of sunflower (*Helianthus annuus*) seed oil as a substitute for antibiotic feed additives on the growth performance and blood profile of broiler chickens.

Vol 1(1), DOI: 10.82036/g6yw3p17, April 2025 ISSN:2977-9332

2 METHODOLOGY

Description of experimental location

This trial was carried out at the Poultry unit of Animal Science Department, Faculty of Agriculture, University of Abuja Teaching and Research Farm, Main Campus, along airport Road, Gwagwalada, Abuja, Nigeria. Gwagwalada is the headquarters of the Gwagwalada Area Council located between latitudes 08^0 57^1 and 09^0 37^0N and longitude of 70^0 20^l and 07^0 51^0E (Balogun, 2001).

Collection, extraction and analysis of phyto-constituents in sunflower oil

Sunflower (*Helianthus annuus*) seeds was purchased from local market in Gwagwalada and its environs in Abuja. The seed was authenticated at Department of Biological Science, University of Abuja, Nigeria with voucher specimen reference number BS/4ABJB. Extraction of the oil was carried out according to the method recently published by Agubosi *et al*. (2022), Briefly, Claveger apparatus with connected to a steam distillator as well as a condenser. 300 g of the seeds were placed in a round bottom flask and heated at 60^0C for 35 minutes, evaporated steam is captured in the condenser into the beaker before it was separated in a separatory funnel to obtain sunflower essential oil. Analysis of phyto-constituents was carried out using Stran® gas chromatography and mass spectrometry (Model HF/09GJ, China), adjusted at different optical densities according to the manufacturers' recommendation.

Experimental Animals, design and their Management

A total of two hundred and ten one-day old (210) Arbor Acre broiler chicks were obtained from a reliable commercial hatchery in Ibadan. The birds were randomly grouped into seven dietary treatments, each with three replications of 10 chicks in a Complete Randomized Design (CRD). The birds were reared in a battery cage with a pen dimension of 0.94 square meters. Supplemental heat and light were provided throughout the brooding period using lanterns, electricity and charcoal stoves. Routine vaccination (Gumboro and Lasota) were administered. Fresh feed and clean water were given freely for the duration of the 28-day trial. All other management procedures were done in line with the prescribed techniques outlined by the Nigerian Institute of Animal Science.

Experimental Diet

A broiler starter diet containing 23.50 % crude protein and 2897 Kcal/Kg of metabolizable energy was formulated as presented in Table I. Birds on Diet 1 were given the basal diet and synthetic antibiotic (Neomycin) at 1.2g/kg while birds on Diets 2, 3, 4, 5, 6 and 7 were fed the basal diet mixed with sunflower oil at 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mL/kg of feed, respectively. The diet was formulated to meet the nutrient requirements of broiler starter. The experimental layout is shown below:

Treatment 1: Basal diet + Neomycin at 1.2g/kg of feed

Treatment 2: Basal diet + 0.5 mL/kg of feed *Helianthus annuus oil*

Treatment 3: Basal diet + 0.6 mL/kg of feed *Helianthus annuus oil*

Treatment 4: Basal diet + 0.7 mL/kg of feed *Helianthus annuus oil*

Treatment 5: Basal diet + 0.8 mL/kg of feed *Helianthus annuus oil*

Treatment 6: Basal diet + 0.9 mL/kg of feed *Helianthus annuus oil*

Treatment 7: Basal diet + 1.0 mL/kg of feed *Helianthus annuus oil*

Data Collection

Initial weight (IW): Upon arrival, the weights of the day-old chicks were weighed and recorded. **Final Weight (FW):** The weights of the broiler chickens were weighed at the conclusion of the experiment. **Average Weight Gain (AWG) (g/bird):** This was calculated as final weight gain (g) minus initial weight (g). **Average Daily Weight Gain (ADWG)** per bird was calculated as final body weight minus the initial body weight divided by the total days of the experiment. F**eed intake (FI) (g/bird):** This was estimated as the difference between the feed supplied and left over feed while average daily feed intake was calculated as total feed intake divided by the total days of the experiment. **Feed Conversion Ratio (FCR):** This was calculated as feed intake (g) divided by weight gain (g). Mortality was recorded as it occurs.

Proximate Analysis

Proximate composition of the experimental diet was determined using near infra-red automated feed analyzer Fois® (DS-2001, Netherlands). Specific reagents were added according to the manufacturers' recommendation.

Blood collection and evaluation

At the end of the study, nine birds were randomly selected per treatment (three per replicate) for haematological and serum biochemical indices. 5 mL of blood was collected from each bird, 2 mL for haematology and 3mL for serum biochemical parameters. Blood samples for haematology was collected into bottles with anticoagulant while those for serum were collected into bottles free from anticoagulant and placed in an ice pack to maintain the quality of the samples. Haematological parameters were analyzed using Systmex automated blood analyzer (Model SF/009G, China) while those for serum analysis was done using Labrom auto analyzer (model GKL/Y002, China).

Vol 1(1), DOI: 10.82036/g6yw3p17, April 2025 *ISSN:2977-9332*

Statistical Analysis

Data generated on growth performance was subjected to analysis of variance (ANOVA) using SAS statistical package, SAS (2000). Duncan multiple range test (Duncan, 1955) was used to separate the means.. Significant was declared at P≤0.05.

TABLE I: COMPOSITION OF EXPERIMENTAL DIET FOR BROILER STARTER

INGREDIENTS (KG)	**LEVELS OF SUNFLOWER SEED OIL (ML)**						
	0.0	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
MAIZE	57.15	57.15	57.15	57.15	57.15	57.15	57.15
GROUNDNUT CAKE	11.50	11.50	11.50	11.50	11.50	11.50	11.50
SOYA BEAN CAKE	25.00	25.00	25.00	25.00	25.00	25.00	25.00
FISH MEAL LOCAL	2.00	2.00	2.00	2.00	2.00	2.00	2.00
BONE MEAL	3.20	3.20	3.20	3.20	3.20	3.20	3.20
SALT	0.30	0.30	0.30	0.30	0.30	0.30	0.30
*VITAMIN PREMIX	0.25	0.25	0.25	0.25	0.25	0.25	0.25
LYSINE	0.25	0.25	0.25	0.25	0.25	0.25	0.25
METHIONINE	0.35	0.35	0.35	0.35	0.35	0.35	0.35
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
PROXIMATE ANALYSIS							
NUTRIENTS	**0.0**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**
CRUDE PROTEIN (%)	23.50	23.50	23.50	23.50	23.50	23.50	23.50
METABOLIZABLE ENERGY (KCAL/KG)	2897	2897	2897	2897	2897	2897	2897
CRUDE FIBRE (%)	4.05	4.05	4.05	4.05	4.05	4.05	4.05
ETHER EXTRACT (%)	4.69	4.69	4.69	4.69	4.69	4.69	4.69
LYSINE (%)	1.27	1.27	1.27	1.27	1.27	1.27	1.27
METHIONINE (%)	0.56	0.56	0.56	0.56	0.56	0.56	0.56
CALCIUM (%)	1.10	1.10	1.10	1.10	1.10	1.10	1.10
PHOSPHORUS (%)	0.73	0.73	0.73	0.73	0.73	0.73	0.73

***BIOMIX PREMIX SUPPLIED PER KG OF DIET: VIT. A, 10,000IU; VIT. D_3 2000IU; VIT. E 23MG; VIT. K 2MG; VIT. B_1 1.8MG;VIT. B_2 5.5MG; NIACIN 27.5MG; PANTOTHENIC ACID, 7.5MG; VIT. B_{12} 0.015MG; FOLIC ACID 0.75MG; BIOTIN 0.06MG; CHOLINE CHLORIDE 300MG; COBALT 0.2MG; COPPER 3MG; IODINE 1MG; IRON 20MG; MANGANESE 40MG; SELENIUM 0.2MG; ZINC 30MG; ANTIOXIDANT 1.25MG.**

3 RESULTS AND DISCUSSION

The composition of phyto-constituents in sunflower seed oil is displayed in Table II. The most prominent compounds were phenolic compounds (561.0 mg/mL), flavonoids (310.06 mg/mL), followed by terpenoids (105.10 mg/mL), tannins (100.8 mg/mL), alkaloids (81.86 mg/mL), steroids (44.49 mg/mL) and saponins (31.51 mg/mL). According to Ojediran *et al*. (2024a), the presence of these compounds suggests that sunflower seed oil has a wide range of therapeutic benefits, including anti-inflammatory, antioxidant, antiviral, anti-cancer, cytotoxic, antimicrobial, immunological stimulant, anti-fungal and gastro-protective capabilities. The phyto-constituents concentration found in this investigation are not consistent with those reported by Karangwa *et al*. (2015). The discrepancy in results could be attributed to the plants age, geographical location, processing methods and other variables (Ojediran *et al*., 2024b). The presence of high phenolic compounds in this study indicates that sunflower seed oil has the potential to perform antioxidant, antimicrobial, anti-inflammatory and gastro-protective roles in animals (John, 2024a). Flavonoids and terpenoids have been reported to scavenge the activities of free radicals, protect against cardiovascular disease and inhibit the activities of some pathogenic organisms (John, 2024b). Saponins are appetite stimulators and can play the role of an anti-diarrhea (John, 2024c). According to John (2024c), alkaloids and tannins are potent analgesics and antimicrobials, respectively.

TABLE II: COMPOSITION OF PHYTO-CONSTITUENTS IN SUNFLOWER SEED OIL

Constituents	**Composition (mg/mL)**
Alkaloids	81.86
Tannins	100.8
Phenols	561.0
Terpenoids	105.10
Steroids	44.49
Saponins	31.51
Flavonoids	310.06

Table III shows the growth performance of broiler chicks fed different amounts of sunflower seed oil. The amount of sunflower seed oil added to the broiler chicks' diets significantly (P<0.05) affected their final body weight (FBW), average weight gain (WG) and feed conversion ratio (FCR). Nevertheless, there was no significant (P>0.05) difference in the initial body weight and feed intake (FI) of birds fed the experimental diets. Broiler chickens fed 0.7, 0.8,

Vol 1(1), DOI: 10.82036/g6yw3p17, April 2025 *ISSN:2977-9332*

0.9 and 1.0 mL of sunflower seed oil had similar final body weights which is significantly higher than the final body weight of birds fed 0.5, 0.6 mL of sunflower seed oil and the control diet. The higher final body weight observed among birds on 0.7, 0.8, 0.9 and 1.0 mL of sunflower seed oil could be traced to the presence of phyto-constituents in the test ingredient. Phyto-constituents have the ability to modulate the activities of intestinal enzymes, thereby reducing the retention time of feed and rapid absorption of nutrients in birds (Alagbe and Oluwafemi, 2019). The average weight gain of broiler chicks was significantly impacted by the different amounts of sunflower seed oil they were fed. The average weight gain was found to follow the same pattern as the final body weight gain. The pattern was such that as the level of sunflower seed oil inclusion increases, the average weight gain also increases. This result agrees with the reports of Zhang *et al. (*2014) who reported increased body weight as essential oil is increased in the diet as a growth promoter. Birds fed a diet containing 1.00 mL/kg feed had a similar feed conversion ratio with those fed 0.9 mL/ kg feed but significantly better than those fed other dietary treatments. This result aligns with the reports of Shittu *et al.* (2024), who observed an improved feed conversion ratio in broiler chicks fed diet supplemented with phytogenics.

TABLE III: GROWTH PERFORMANCE OF BROILER CHICKS FED VARYING LEVELS OF SUNFLOWER SEED OIL

Parameters	**0.00**	**0.50**	**0.60**	**0.70**	**0.80**	**0.90**	**1.00**	**SEM**
IBW (g)	39.20	40.00	40.08	41.00	41.10	40.70	40.80	0.26
FBW (g)	905.78^{b}	950.60^{b}	980.55^{b}	1008.8^{a}	1017.53^{a}	1078.4^{a}	1130.78^{a}	28.69
WG (g)	866.58^{b}	910.60^{b}	939.75^{b}	967.80^{a}	976.43^{a}	1037.7^{a}	1089.98^{a}	28.52
ADWG (g)	30.95^{b}	32.52^{b}	33.56^{b}	34.56^{a}	34.87^{a}	37.06^{a}	38.93^{a}	1.02
FI (g)	1566.7	1534.6	1564.33	1548.67	1596	1591	1599.6	9.42
ADFI (g)	55.95	54.81	55.87	55.31	57.00	56.82	57.13	0.34
FCR	1.8^{e}	1.69^{d}	1.67^{cd}	1.60^{bc}	1.64^{cd}	1.53^{ab}	1.47^{a}	0.04

IBW: initial body weight; FBW: final body weight; WG: weight gain; ADWG: average daily weight gain; FI: feed intake; ADFI: average daily feed intake; FCR: feed conversion ratio; SEM: standard error of means; LOS: Level of significant ($P<0.05$) difference; T1: basal diet + 0% SFO; T2: basal diet + 0.5 mls SFO;T3: basal diet + 0.6 mls SFO; T4: basal diet + 0.7mls SFO; T5: basal diet + 0.8 mls SFO; T6: basal diet + 0.9 mls SFO; T7: basal diet + 1.0mls SFO.

Haematological parameters of broiler chicks fed different amounts of sunflower seed oil are presented in Table IV. Packed cell volume (PCV), haemoglobin, white blood cell, red blood cell (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were influenced ($p<0.05$) by the treatment. The PCV, haemoglobin (Hb) and RBC values were within the normal ranges (21.00−36.00 %), (6.50−15.00 g/dL) and (1.20−3.50 x 10^{12}/L) cited by Bounous and Stedman (2000); Shittu *et al.* (2024). Result obtained suggests that sufficient oxygen is in the cells of birds, thereby giving room for nutrients in the body (John, 2024c). Low red blood cell and packed cell volume is an indication of iron and vitamin B12 deficiency in the blood (John, 2024a). Muritala *et al.* (2022) reported that low haemoglobin is recorded in birds on hepatic damage and low protein intake. Mean corpuscular haemoglobin, MCV and MCHC values were within the normal ranges (30.00−55.00 pg), (23.00−45.00 fl) and (60.00−120.00 g/dL) cited by Benerjee (2004). MCV is an important indicator used in determining the cell size of erythrocytes and the ability of poultry birds to withstand prolonged oxygen starvation (Mitruka and Rawnsley, 1977; RAR, 2009). Elevated MCH and MCHC is could be a sign of liver damage in birds (Olafadehan *et al*., 2021). The values of WBC were within the normal range of 9.20−25.00 ($\times10^{9}$/L) reported by Islam *et al.* (2004) for healthy birds. The white blood cells play an essential role in the production of antibodies to prevent diseases.

The values of total protein were within the normal range for birds as seen in Figure IV, which is 2.5 to 5.5 g/dL, as cited by Shittu *et al.* (2024). Albumin and globulin values reported in this study were within the reference range of 1.5 - 3.0 g/dL and 2.00 - 4.20 g/dL reported by Café *et al.* (2012). The total protein, albumin and globulin values suggest that the animals were not malnourished (John, 2024e). The values of aspartate transferase and alanine phosphatase (51.45 - 65.94 U/L) and 80.81 - 90.54 U/L observed in this study were within values 48.00 - 75.00 U/L and 80.00 - 140.0 U/L reported by Muritala *et al.* (2022). The result obtained suggests the absence of renal or hepato-cellular damage in birds (John, 2024d). This also indicates that supplementation up to 1.0 mls poses no detrimental effect on the health of birds (Olafadehan *et al*., 2021).

Vol 1(1), DOI: 10.82036/g6yw3p17, April 2025 *ISSN:2977-9332*

TABLE IV: HAEMATOLOGICAL PARAMETERS OF BROILER CHICKENS FED GRADED LEVELS OF SUNFLOWER OIL

TREATMENT	% LEVEL OF SUNFLOWER INCLUSION							SEM
Parameter	**0.0**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1.0**	
PCV (%)	26.46d	27.05bc	26.46d	26.72cd	27.39b	28.45a	28.40a	0.23
RBC (x 1012/L)	2.48c	2.37c	2.93b	3.45a	2.95b	2.97b	3.07b	0.08
Hb (g/dL)	9.47d	9.19d	9.49cd	9.85c	10.43b	10.5b	11.23a	0.18
WBC (×109/L)	19.33b	20.72b	20.86b	20.88b	20.62b	21.53a	20.75b	0.19
MCV (g/dL)	25.64d	29.11c	32.75b	32.75b	31.77b	34.55a	34.73a	0.78
MCH (pg)	48.11d	49.00d	54.35b	54.43b	50.99c	55.79a	54.38b	0.64
MCHC (fl)	68.38c	75.38b	74.52b	75.58b	79.12a	79.45a	78.12a	0.93

[a-f]Means in the same row with different superscript are significantly different ($P<0.05$); *RBC: red blood cell, Hb: haemoglobin, PCV: packed cell volume, WBC: white blood cell, MCV; mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration.*

TABLE IV: SERUM INDICES OF BROILER CHICKENS FED GRADED LEVELS OF SUNFLOWER SEED OIL

TREATMENT	% LEVEL OF SUNFLOWER INCLUSION							SEM
Parameter	0.0	0.5	0.6	0.7	0.8	0.9	1.0	
Total protein (g/dL)	3.95^{d}	4.01^{c}	4.08^{b}	4.05^{b}	3.98^{cd}	4.07^{b}	4.18^{a}	0.02
Albumin (g/dL)	1.92^{c}	1.95^{c}	2.09^{a}	2.01^{b}	1.96^{c}	2.01^{b}	2.08^{a}	0.02
Globulin (g/dL)	2.03^{bc}	2.06^{ab}	1.99^{c}	2.04^{b}	2.02^{bc}	2.06^{ab}	2.10^{a}	0.02
ALT (U/L)	51.45^{b}	63.63^{a}	63.94^{a}	64.04^{a}	65.84^{a}	64.80^{a}	65.94^{a}	1.17
ALP (U/L)	90.54^{a}	90.07^{a}	86.48^{b}	87.76^{b}	80.81^{c}	80.67^{c}	85.99^{b}	1.32

[a-d] Means in the same row with different superscript are significantly different (P<0.05); SEM= Standard Error of Mean. TP=Total protein; ALB=Albumin; GLO=Globulin; AST= Aspartate Transferase; ALT= Alanine phosphatase

4 CONCLUSION

Sunflower seed oil contains secondary metabolites that can improve food safety without having any negative effects when used continuously. Adding sunflower seed oil to broiler chicks' diet at a rate of 1.0 mL/kg basal diet improved weight gain and helped the birds to stay healthy by scavenging free radicals and suppressing the activity of microorganisms. The use of medicinal plant extracts within the recommended levels is strongly recommended in order to ensure food safety, increase the production of healthy poultry products to meet the growing demand worldwide, and also decrease the high cases of antibiotic resistance diagnosed in humans and animals.

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