

Effects of Supplementing Different Levels of Stinging Nettle Leaf Meal on the Growth Performance of Starter Broilers

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Abstract: There is a growing concern by consumers of broiler chickens in Kenya over the injudicious use of antibiotic growth promoters such as oxytetracyclines which has led high tissue residues and consecutively, resistance to the drugs in both livestock and humans. This problem has elicited increased research towards natural alternatives. The current research was thus conducted to determine the optimal dietary inclusion levels of stinging nettle (*Urtica dioica* L.) leaf meal (NLM) that result to improvements in feed intake (FI), growth rate (GR) and feed conversion efficiency (FCE) of Cobb 500 starter broiler chickens. Four isonitrogenous (20% crude protein (CP)) and isocaloric (3200 Kcal/kg) diets were formulated; Diet 1 (Control, NLM 0%), Diet 2 (NLM 1%), Diet 3 (NLM 1.5%) and Diet 4 (NLM 2%). Proximate analysis was undertaken for all experimental diets. A total of 48 unsexed chicks were weighed and randomly allocated the experimental diets with 4 replicates of 3 chicks each for 17 days. The FI and body weight gain (BWG) were weighed and recorded daily and weekly respectively. The GR and FCE were also calculated. Results showed that birds supplemented with NLM at 1% had significant mean (162.03g) for FI. Birds supplemented with NLM at 1.5% in the diet had the highest BWG (1930.50g) and GR (113.56g). However, 2% NLM supplemented birds showed the highest FCE (7.98). From the study, it was concluded that supplementing the diets of starter broiler chickens with NLM at 1.5% resulted to the highest BWG and GR.

Key words: broilers, stinging nettle leaf meal, growth rate, feed conversion.

I. Introduction

Antibiotics are used in broiler production for improving growth performance, feed utilization and controlling gastrointestinal pathogens (Langata et al., 2019). This ensures the birds achieve maximum market weights within the shortest time possible usually thirty-five days. However, the recent rise in consumer health consciousness have resulted to an increase in demand for healthy, naturally produced broiler meat (Booth et al., 2021). Further, the application of antibiotics as growth promoters in the production of broilers have been banned in many countries (Ebeid et al., 2021). Numerous research works have revealed high residue concentrations of antibiotics in tissues such as meat and bones of the broilers (Odore et al., 2015; Ferdous et al., 2020; Monir et al., 2021; Mohammadzadeh et al., 2021). Antibiotic residues increase resistance to medications hence difficulties in preventing, treating and controlling of clinical cases in humans and livestock (He et al., 2020).

In order to overcome this problem, more research is geared towards use of phytobiotics in broiler production (Arain et al., 2022). Phytobiotics are a class of natural growth promoters that can be obtained from spices, herbs or other plants (Khan et al., 2022). They are incorporated in livestock feed to enhance productivity through the improvement of feed intake, stimulation of digestive secretions, nutrient digestibility and absorption, immune stimulation, among other benefits (Raissy et al., 2022); thus are potential candidates for safer and natural alternatives for growth boosters (Pliego et al., 2020; Oluwafemi et al., 2020).

Additionally, phytobiotics are reported to have high levels of active biomolecules including essential oils, that confers them with among others, antibacterial, growth promoting, lipid lowering, antioxidant and pharmacological activities (Arain et al., 2022). In Kenya, plant based phytobiotics are indispensable as they are locally available and cheap as a source of healthy leafy vegetables and traditional medicine (Kamau et al., 2016; Odhiambo et al., 2011). The bioactive content and function of phytobiotics are influenced by among other factors the different agro ecological conditions, variety and strain of the plants, processing methods, and growing seasons (Kačániová, 2020).

Besides, phytobiotics have been reported to contain phytonutrients such as thymol and carvacrol which exhibit beneficial physiological properties such as antibacterial, growth promoting, antioxidant and lipid lowering activities (Raissy et al., 2022). This helps to reduce local inflammation in the small intestines leading to improved integrity of the intestinal mucosa which facilitates increased secretion of digestive juices, nutrient digestion and absorption (Alghirani et al., 2021).

Stinging nettle leaves are phytobiotics which are gaining research interest in chicken production as suitable replacement alternatives for growth promoting antibiotics. They have been reported to contain thymol and carvacrol which are essential oils with great antibacterial action (Hashemi et al., 2018). Yin et al. (2017) reported that supplementing thymol and carvacrol to broiler chicks was found to favour the proliferation of *Lactobacillus* spp. bacteria and decreased the colonization of the ileum by pathogenic *Clostridium perfringens*. The leaves are also rich in flavonoids notably quercetin, rutin and kaempferol (Ilhan et al., 2019). These compounds have also been shown to exhibit antibacterial action against common pathogens such as *Escherichia coli* (Ketema and Worku, 2020). Other beneficial compounds present in the nettle leaves include tannins and phenols (Al-Sal., 2020). However, there is insufficient and scanty documented information on the incorporation of stinging nettle leaf meal in the diets of starter broiler chickens in Kenya. The current research was thus conducted to ascertain the optimal dietary inclusion levels of NLM that will result to improvements in performance of starter broiler chickens.

II. Materials and Methods

2.1 Study site

This experiment was conducted at Chuka University, situated in Tharaka-Nithi County, Kenya. It is located 186 kilometres from Nairobi city along Nairobi-Meru highway, between Longitudes 37 18’37” and 37 28’33” East and Latitude 00 07’23” and 00 26’19” South. The area receives a yearly rainfall average of 1373 mm and temperatures ranging from 16.0°C to 24.0°C.

2.2 Animals and housing

Forty-eight mixed sex day old broiler chicks (Cobb 500 strain) were bought from Kenchic Limited Meru depot, Kenya. The chicks had been pre-vaccinated against Mareks disease.

The chicks were raised in a deep litter system. Sixteen experimental pens measuring 2 feet by 2 feet and a height of 1.5 feet were constructed using timber and chicken wire net. In every pen, wood shavings (used as litter) was spread on the pen floor at a depth of four inches. Prior to the experiment, the house, waterers and feeders were cleaned then thoroughly disinfected with Noro cleanse® (contains the active ingredients Glutaraldehyde 15% w/v and Coco-benzyl-dimethyl-ammonium chloride 10% w/v).

Infrared brooding bulbs were installed for heating and fluorescent tubes for lighting. Before arrival of the day old broiler chicks, the pens were pre-heated for six hours. Pen floor temperatures of 30 °C to 32 °C was maintained during the initial seven days then steadily lowered by 5 °C weekly. Room temperatures were monitored using a clinical thermometer. A lighting schedule of 24 hours was maintained. Biosecurity measures involving use of proper protective clothing were observed; and there was a foot bath containing the disinfectant Noro cleanse® at the entrance of the research house.

2.3 Experimental diets

Stinging nettle leaves were sourced from Ol Joro Orok constituency in Nyandarua county. The upper four leaves were picked since they were rich in essential oils and flavonoids. They were shade dried to a constant weight, ground and sieved using a mesh size of 1000 microns. Four isonitrogenous (20% CP) and isocaloric (3200 Kcal/kg) diets were formulated; Diet 1 (Control, NLM 0%), Diet 2 (NLM 1%), Diet 3 (NLM 1.5%) and Diet 4 (NLM 2%) (Table 1). The feed ingredients maize grains, soybeans and wheat were ground using a mesh size of 1500 microns. These ingredients were manually mixed using a shovel till thoroughly and evenly mixed. The amino acids methionine, tryptophan and lysine were added in order to balance off any deficiency. The feed was formulated to meet the nutrient requirements for broiler chicks (NRC, 1994). The ingredients were balanced using excel software.

Table 1: Ingredients composition (%) of experimental diets for broiler chicks supplemented with NLM at 0%, 1%, 1.5% and 2%

Ingredient	NLM 0%	NLM 1%	NLM 1.5%	NLM 2%
Maize meal	49.25	49.21	46.49	46.26
Soybean meal	25.26	24.51	24.2	24.00
Sunflower meal	2.67	2.50	2.55	2.50
Fish meal (<i>Rastrineobola argentea</i>)	5.00	5.00	5.00	5.00
MCP	0.93	0.89	0.88	0.87
Wheat	7.49	7.50	10.00	10.00
Vegetable oil	5.00	5.00	5.00	5.00
Ground limestone	0.30	0.30	0.30	0.30
Calcium carbonate	1.20	1.19	1.18	1.17
Common salt	0.10	0.10	0.10	0.10

Mineral premix	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50
NLM	-	1.00	1.50	2.00
DL-methionine	0.50	0.50	0.50	0.50
L-tryptophan	0.20	0.20	0.20	0.20
L-lysine	1.10	1.10	1.10	1.10
Total	100	100	100	100
Calculated Composition				
Crude protein	19.75	20.00	20.11	20.23
Crude fibre	3.27	5.24	5.27	5.29
ME (Kcal/Kg)	3176	3185	3194	3205

Diet code: NLM 0%, control with 0% stinging nettle leaf meal dietary inclusion level; NLM 1%, 1% stinging nettle leaf meal dietary inclusion level; NLM 1.5%, 1.5% stinging nettle leaf meal dietary inclusion level; NLM 2%, 2% stinging nettle leaf meal dietary inclusion level.

Abbreviations: MCP: monocalcium phosphate; ME: metabolizable energy (Kcal/Kg)

2.4 Laboratory analysis

The proximate analysis of ingredients and diets were carried out as described by the AOAC (2000). Moisture contents were obtained after drying in an oven at 110 °C for 24 hours. The loss in weight was the moisture content and what was left was the dry matter (DM) of the sample. The crude protein was determined using the Kjeldahl method. Ash was determined by heating the samples in a muffle furnace set at 550 °C for 4 hours. Ether extraction was carried out through the soxhlet extraction method. Nitrogen free extracts (NFEs) were estimated by subtracting the total moisture, crude protein, ether extracts, ash and crude fibre from 100.

2.5 Feeding

On arrival, the chicks' individual BW were taken (average weight per chick was 37.32g) then randomly placed into the experimental pens. Using a completely randomized design (CRD), each dietary treatment had twelve chicks (n = 12). The chicks were allowed a total of 3 days for adaptation to experimental diets and conditions before commencement of the experiment.

The chicks were offered feeds and water in an unrestricted approach. They were all put on the control diet for the first three days. On the fourth day, their BW were taken and recorded. Experimental diets were then offered to the respective groups of birds. Drinkers containing water mixed with multivitamins, glucose and liquid paraffin were also placed in each pen. The treated water was offered for the entire adaptation period. Newcastle disease and infectious bronchitis vaccine was administered on the seventh day through drinking water with multivitamins being administered after every vaccination. Every morning, leftover feeds from each pen were weighed and recorded. Fresh feeds were then weighed and offered to the birds. Waterers were also cleaned and fresh water offered. Weight taking and recording was done weekly at 0800hours.

2.6 Measurement of performance parameters

The BW of all three birds per pen were taken on days 7, 14 and 21. Average GR were calculated as the difference of final BW in grams and initial BW in grams divided by the length of the experiment (17 days). Data on FI was collected for a period of 20 days. On day 21, which was the last day of the experiment, final leftover feeds from day 20 was weighed and recorded in order to get the FI of day 20. The final BW of the birds were also taken and recorded. The mean GR was computed as the difference of mean final BW in grams and mean initial mean body weight in grams divided by the days of the experiment (17 days). The FCR was determined as the weight of all the feeds ingested (in grams) during the entire experiment divided by the weight gain in grams (final weight minus starting weight).

2.7 Data analysis

Statistical analysis system (SAS version 9.4) software (SAS Institute, Cary NC) was used to analyse the data. Tukey pairwise comparison was conducted to compare variations among treatments whereas ANOVA indicated differences at probability values ($\alpha = 0.05$). The following linear model for a CRD was utilised:

$$y_{ij} = \mu + t_i + \epsilon_{ij}$$

Where;

y_{ij} - is the response variables due to the dietary treatments (FI, GR and FCE).

μ - is the overall population mean of the starter broiler chickens.

t_i - is the effects (FI, GR and FCE) due to the NLM offered to the starter broiler chickens.

ϵ_{ij} - is the random error effect.

2.8 Ethical considerations

Ethical approval was done by the Chuka University Institutional Ethics Review Committee of application approval number NACOSTI/NBC/AC-0812. Research license was sought from NACOSTI license number NACOSTI/P/21/13883.

III. Results

3.1 Ingredients and diets proximate composition

Proximate analysis carried out as indicated in Table 2. The additive had the highest CP, ash, CF and energy content of 23.25%, 21.97%, 6.41% and 3400 Kcal/Kg respectively; as compared to the experimental diets and the control diet.

Table 2: Proximate Analysis of NLM and Experimental Diets on a DM Basis (%)

Treatments	DM	CP	Ash	CF	EE	NFE	Energy
NLM	87.54	23.25	21.97	6.41	3.60	44.77	3400.00
Control	90.40	19.75	5.33	3.27	6.40	65.25	3176.05
NLM 1%	90.05	20.00	19.76	5.24	7.15	47.85	3185.00
NLM 1.5%	89.00	20.11	19.70	5.27	7.12	47.80	3194.00
NLM 2%	90.01	20.23	19.73	5.29	7.16	47.59	3205.00

Diet code: NLM, stinging nettle leaf meal; Basal diet (control containing 0% NLM), the formulated research feed with 0% stinging nettle leaf meal dietary inclusion level; NLM 1%, 1% stinging nettle leaf meal dietary inclusion level; NLM 1.5%, 1.5% stinging nettle leaf meal dietary inclusion level; NLM 2%, 2% stinging nettle leaf meal dietary inclusion level.

Abbreviations: DM: Dry Matter; CP: Crude Protein; CF: Crude Fibre; EE: Ether Extract; NFE: Nitrogen Free Extract; ME: metabolizable energy

The estimated metabolizable energy content expressed as Kcal/Kg

3.2 Performance of broiler chicks fed diets supplemented with NLM

Table 3 shows the results of the feeding trial.

Table 3: Performance of Starter Broiler Chicks Supplemented with NLM at 0%, 1%, 1.5% and 2% (g)

Parameter	Treatments				±SE	p-value
	NLM 0%	NLM 1%	NLM 1.5%	NLM 2%		
IW	180.30	185.00	183.00	182.80	1.41	0.0001
FW	1722.50 ^c	1893.00 ^b	2113.50 ^a	1687.00 ^d	2.42	0.0001
GR	90.72 ^c	100.47 ^b	113.56 ^a	88.48 ^d	0.42	0.0001
FI	148.93 ^c	162.03 ^a	155.29 ^b	150.04 ^c	8.86	0.0001
FCR	7.73 ^b	7.59 ^c	6.44 ^d	7.98 ^a	0.02	0.0001

Data is represented as means ± SE.

Means not sharing a letter are different significantly at 95% confidence interval ($p < .05$).

Diet codes: NLM, stinging nettle leaf meal; Basal diet (control containing 0% NLM), the formulated research feed with 0% stinging nettle leaf meal dietary inclusion level; NLM 1%, 1% stinging nettle leaf meal dietary inclusion level; NLM 1.5%, 1.5% stinging nettle leaf meal dietary inclusion level; NLM 2%, 2% stinging nettle leaf meal dietary inclusion level.

Abbreviations: IW: Initial Weight; FW: Final Weight; FI: Feed Intake; GR: Growth Rate; FCE: Feed Conversion Efficiency; SE: Standard Error of the Mean

3.2.1 Feed Intake

Tukey pairwise mean comparison was performed to compare treatment means. The birds supplemented with NLM at 1% inclusion levels in their diets ($p < 0.05$) exhibited significantly higher mean FI of 162.03g, in comparison to the other groups. The birds in the control group (NLM 0%) had the lowest mean FI of 148.93g. It was also observed that as the levels of NLM supplementation increased from 1% to 2%, the FI decreased significantly (Figure 1).

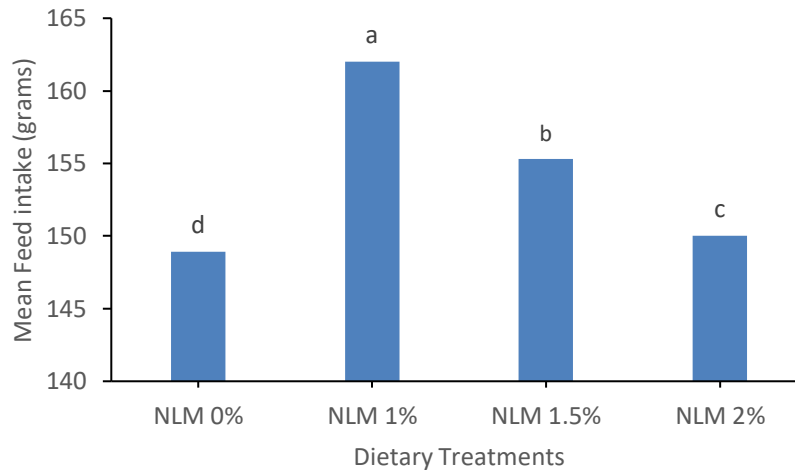


Figure 1. The Mean Feed Intake of Broiler Chicks Fed Different Levels of NLM

3.2.2 Growth Rate

The GR was significantly higher (113.56g) in birds supplemented with NLM at 1.5% inclusion levels. The control group (NLM 0%) had better GR (90.72g) compared to the group fed NLM at 2% level (88.48g) as shown in figure 2 below.

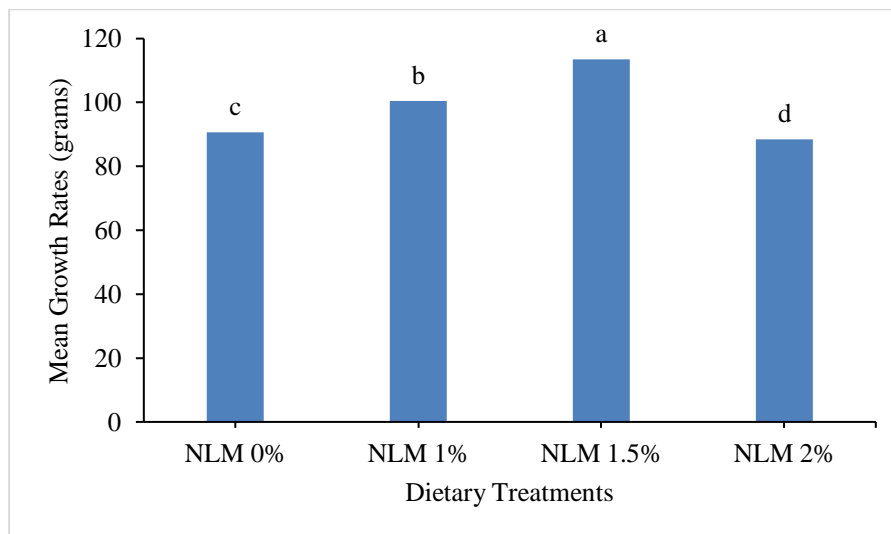


Figure 2. The Mean Growth Rate of Broiler Chicks Fed Different Levels of NLM

3.2.3 Feed Conversion Efficiency

The FCE for the entire experimental period showed that higher levels of NLM (2%) resulted to higher FCE (7.98). The control group (NLM 0%) performed better (7.73) than NLM at 1% (7.59) and 1.5% (6.44) as indicated in figure 3.

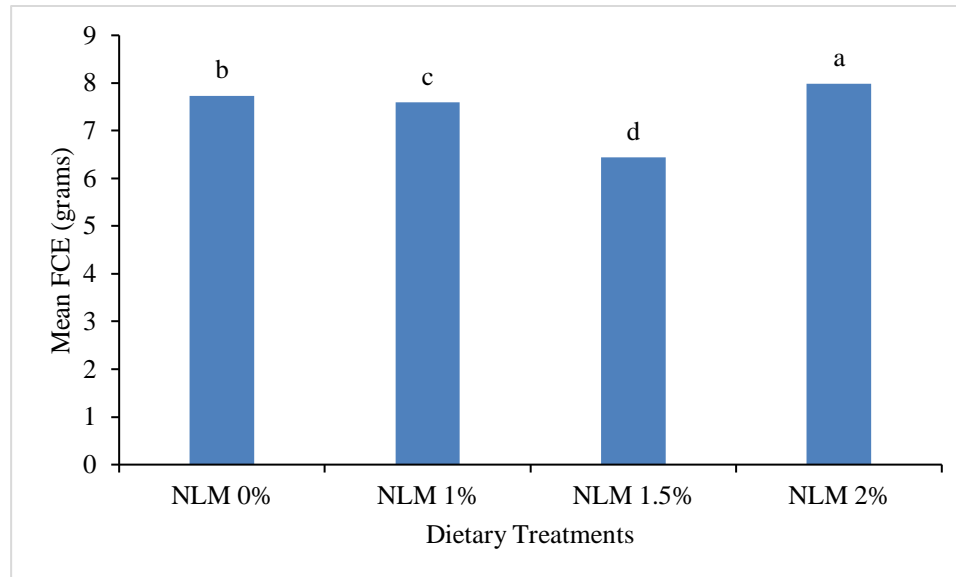


Figure 3. The Mean Feed Conversion Efficiency of Broiler Chicks Fed Different Levels of NLM

IV. Discussion

4.1 Ingredients and diets proximate composition

In the present study, analysed NLM was found to contain a CP of 23.25%. This shows that NLM is a rich source of proteins that can be utilised as a feed ingredient for starter broiler diets (Andualem et al., 2015). The results also compare to 16.08% to 26.89% as reported in studies by Rafajlovska et al. (2013) and Alemayehu et al. (2016). Adequate proteins are required by broiler chickens to support their rapid muscle deposition in order to reach market weight early (Maynard et al., 2022).

However, other studies have reported higher dietary CP of 33.8% (Adhikari et al., 2016). Excessive dietary proteins in the diets of broiler chickens have been described to increase faecal excretion of nitrogenous wastes like ammonia and nitrates leading to environmental pollution especially of soils and water ways such as streams and rivers (Musigwa et al., 2020; Chrystal et al., 2020). The CP of the experimental diets was within the NRC, (1994) recommendations for starter broiler chickens. The differences in the contents of dietary CP may be attributed to the varying agronomic conditions, variety and strain of the plants, postharvest handling and processing conditions (Milosevic et al., 2021).

From the study, the amounts of minerals in NLM (21.97%) and NLM diets was found to be quite high (average 19.73%). Similar studies by Bhusal et al. (2022) and Rutto et al. (2013) have reported that raw stinging nettle plant was rich in minerals including calcium, zinc potassium and iron. These elements have been documented to be especially important for broiler chicks as they have a rapidly growing skeletal and muscle tissues (Zafari and Nazari, 2022).

The analysed crude fibre content of the basal diet was 3.27%. Chickens require moderate amounts of crude fibre for proper growth (Tejeda and Kim, 2021). In their study, Adibmoradi et al. (2016) reported an improvement in nutrient absorption of broiler chicks fed on moderate dietary crude fibre inclusion level of 1.5%. This was attributed to positive effects on the morphology of jejunum section of the small intestines which resulted to better nutrient absorption. The dietary energy level was satisfactory in the growing of the chicks as it was within the NRC, (1994) recommended value of 3200 Kcal/Kg. Excessive dietary energy intake has been documented to have undesirable effects on broilers. A study by Meloche et al. (2018) reported significant increase in the thigh intramuscular and abdominal fat of broilers supplemented with excessive dietary energy. Hence, the diet was satisfactory for the growth of the birds.

4.2 Performance of broiler chicks fed diets supplemented with NLM

In the present study, the low feed intake of birds fed on NLM at 2% (150.04g) may be attributed probably to the existence of antinutrients such as phytates, saponins and alkaloids (Shonte et al., 2020). Additionally, high levels of the plant in all the diets may have resulted to high intake of crude fibre. A study by Jiménez-Moreno et al. (2019) reported reduced nutrient digestibility of broiler chicks fed high dietary levels of crude fibre (5%). Ingestion of large amounts of crude fibre has been reported to have laxative effects on the gut leading to faster transit of nutrients resulting to poor retention and hence low digestibility (Alshelmani et al., 2021). Also, high levels of crude fibre in the diet decreases energy density thereby increasing feed intake (Bekele et al., 2020).

Additionally, there was low FI of chicks within the first few days of the experiment. This is attributed to the fact that young chicks have an immature digestive system hence low secretion of digestive enzymes and small sizes of absorptive organs with limited surface area which limits nutrient absorption (Ravindran and Abdollahi, 2021). But as the bird grows, and the digestive system matures, it is able to ingest and utilize more feed. However, low absorption rates of the ingested amounts of the phytobiotics may also be due to the lack of specific enzymes of digestion by the birds to fully break down the phytonutrients to bring about the desired growth (Kikusato, 2021). However, Safamehr et al. (2012) reported no significant effects of supplementing stinging nettle meal ($p>0.05$) on the FI of broilers.

In determination of GR, the outcomes of the current study were consistent with prior studies which reported improved GR of broiler chickens supplemented with stinging nettle leaves in their diets. Safamehr et al. (2012) reported improved growth rate of broiler chicks supplemented with 1% of stinging nettle meal. A study by Mehboob et al. (2022) to explore the effects of different levels of stinging nettle leaf meal on the performance of broiler chickens reported an improved growth rates of birds supplemented with 2% of the meal on a dry matter basis resulting to a high dressing percentage. This was attributed to the high amount of phytonutrients such as flavonoids notably quercetin, rutin and kaempferol (Ilhan et al., 2019). Ketema and Worku, (2020) reported that these biomolecules have high levels of antibacterial activity that help in reducing disease causing microbes in the gut resulting to increased nutrient digestibility and utilisation.

In contrast, the current study also reported a reduced GR at higher levels of NLM supplementation (2%). This was in agreement with the work by Nasiri et al. (2011) who reported non-significant effects from supplementing increasing amounts of stinging nettle meal to starter and grower broilers. This was possibly due to the existence of elevated concentrations of secondary metabolites such as phytates, saponins and alkaloids that limited nutrient digestibility and use (Shonte et al., 2020).

The low FCR exhibited by NLM at 1.5% (6.44) was comparable to an investigation by Nasiri et al. (2011) who supplemented starter and grower broiler chickens with 1.5% stinging nettle meal in the basal diet. They described non-significant results of the additive on FCE and weight gain of the birds. These results can be explained by the fact that low levels of dietary inclusion of the herb resulted to low intake of bioactive compounds such as flavonoids hence low FCE. Additionally, a significantly higher FCE was observed at inclusion levels of 2% (7.98). This was comparable to an investigation by Keshavarz et al. (2014) on the effects of stinging nettle powder on the growth performance of broilers. They reported improved FCE with a dietary supplementation of 10g/kg. This was probably due to the increased amounts of flavonoids and essential oils such as thymol and carvacrol which have high antibacterial activity which promotes growth (Hashemi et al., 2018; Yin et al., 2017). However, reduced GR and FCE of the birds may be attributed to the differences in metabolism within the strain (de Souza Vilela et al., 2021).

4.3 Significance of the research findings in future production of broiler chickens

Use of antibiotics as growth promoters in broiler chicken production have been facing increased resistance and consequently, ban in many countries across the globe (Ebeid et al., 2021). Further, the increase in literacy levels and advent of technology especially the use of smart phones, there has been a recent rise in consumer health consciousness (Booth et al., 2021). This have resulted to an increase in demand for healthy, naturally produced broiler meat (Bagno et al., 2023). From the study, supplementing of NLM at 1.5% inclusion levels in the diets of starter broilers have resulted to improvements in the FI, GR and FCE thus the leaves of stinging nettle plant have shown the potential of being used as a replacement for growth promoting antibiotics in the production of broiler chickens. This will result to production of safe broiler chickens, improved health of broiler consumers, reduced environmental contamination due to reduced antibiotic use and consequently increased farmer profits.

V. Conclusion

From the results of the research, it can be concluded that: NLM can be supplemented in the diets of starter broiler chickens at 1.5% inclusion levels to improve on the FI, GR and FCE hence can successfully be used as a replacement for growth promoting antibiotics to promote productivity and health of starter broiler chickens.

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