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GREEN AND RIPE NONI (*Morinda citrifolia L.)* FRUIT POWDER AS FEED ADDITIVE FOR BROILER CHICKENS

by

Priya Pritika Lal

A thesis submitted in fulfillment of the
requirements for the degree of
Master of Agriculture

School of Agriculture and Food Technology
Faculty of Business and Economics
The University of the South Pacific

September 2020

DECLARATION OF ORIGINALITY

Statement by Author

I, Priya Pritika Lal, declare that this thesis is my own work and literature cited has been duly acknowledged.

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ABSTRACT

The gradual ban of chemical additives in poultry diets due to consumer health concerns, has directed research interest in alternative plant sources. There are reports of the use of Noni *(Morinda citrifolia)* fruit powder as phyto additive in poultry diets but information on the effect of stage of fruit maturity is scanty. This study was conducted to compare the performance of broiler chickens fed diets supplemented with green and ripe Noni fruit powder. A control diet and 4 other diets containing two levels (0.25 and 0.5%) green and ripe Noni powder each were formulated to meet the requirements of broiler chickens at two phases (starter and finisher). One hundred and fifty, 7-d old Cobb 500 broiler chicks were used for the experiment. Each diet was fed to five replicates of six birds in a completely randomized design. The starter diets were fed from 8 – 21 d and the finisher from 22 – 42d. Data were collected on growth performance (feed intake, weight gain and feed conversion ratio), nutrient digestibility, carcass and organ measurements. Results showed reduced feed intake (FI) on the 0.5% green powder during the starter phase ($P<0.05$). Body weight gain (BWG) and feed conversion ratio (FCR) were not affected by dietary treatment ($P>0.05$). None of the performance parameters was affected ($P>0.05$) during the finisher phase and in the overall growth (8 - 42 d). Digestibility of dry matter and ether extract was not affected ($P>0.05$) by dietary treatment. Nitrogen retention was depressed ($P<0.05$) on 0.25% green powder compared to the control and the ripe powder based diets. The relative weight of carcass was reduced ($P<0.05$) on 0.5% ripe powder. Breast weight was increased($P<0.05$) on 0.25% ripe and 0.5% green powder compared to the control, 0.25% green and 0.5% ripe powder fed groups. Thigh weight was reduced ($P<0.05$) on 0.25% ripe and 0.5% green powder diets. The weight of drumstick was not affected ($P>0.05$) by the diets. In conclusion, at these (0.25 and 0.5%) levels of inclusions, the beneficial effect of Noni fruit powder in broiler chickens diets is more noticeable on carcass traits than growth performance. There is need for more studies on blood biochemical indices and gut health to explain better the effect of the green and ripe Noni powder on poultry.

LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
DM	Dry matter
FAO	Food and Agricultural Organization
SPSS	Statistical Package for Social Sciences

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CHAPTER ONE: INTRODUCTION

1.1 Background information

Feed additives have been included in the diet to improve the productivity of poultry. Many antimicrobial (antibiotic growth promoters, coccidiostats) and antioxidants (ethoxyquin, BHA and BHT) are included in the feeds to improve bird health and product quality. Antibiotics may however, result in dis-functionality of valuable gut microbiota and build resistance among microbial pathogens in poultry. Presence of antibiotic residues in poultry meat has also been reported in many studies as one of the likely causes of antibacterial resistance in human consumers (Yamashita *et al*., 2009; Muaz, 2018). Many countries of the European Union including, Sweden and Denmark have already banned the use of antibiotics for preventive, as well as growth-promoting purposes (Akhtar, 2018). Farmers are trained to monitor the withdrawal period, prohibit the use of antibiotics as growth promoters, and adopt the feed directives of the US Food and Drug Administration as important measures in mitigating the emergence of antibiotic resistance in bacteria related to poultry production (Akhtar, 2018).

In 2006, European Union prohibited the use of antibiotics as growth promoters (Sunder *et al.,* 2015). Other substitutes to antibiotics including probiotics, prebiotics and use of medicinal plant extracts have now become an important area of research in poultry production due to increasing public health concerns in the use of antimicrobials and commercial antioxidants in animal feeds (Yamashita *et al*., 2009). The use of plant products (several herbs and shrubs) as phyto additives (PA) in poultry feed has increased (Mirzaei-Aghsaghali, 2012; Mirzaei and Venkatesh, 2012; Eevuri and Putturu, 2013) and research into new plants products with PA potential in poultry feed is anticipated.

Several researchers have reported on the use of medicinal plant extracts in poultry for growth and immunity (Mishra *et al*., 2008; Narimani-Rad *et al*., 2011). *Morinda citrifolia* generally known as Noni has a rich history in Ayurveda and grows widely throughout the coastal regions of many countries including the Andaman and Nicobar group of Islands. In Asian countries, Noni fruit and leaves are important traditional foods (West *et al*., 2006). Reports suggest that the fruit has been used as a feed

supplement for livestock and poultry (Sunder *et al*., 2011). Noni is known for its antibacterial, anti-coccidial, anti-stress properties (Yahia, 2011) but has little food value in most regions because of its poor taste. Currently, researches on the use of Noni plant parts in poultry feeding are limited. There is need to more research into maximum utilization of Noni products in poultry diets.

In view of changes in the composition of plant products with stage of maturity, this study was designed with the objective to compare the efficacy of the green and ripe *Morinda citrifolia* fruit powder in broiler chicken.

1.2 Problems Statement

Several biological compounds identified in different plant parts of *M. citrifolia* (Yang *et al*., 2007) have antimicrobial, cholesterol reducing, immune enhancing and digestibility enhancing effects (Raj, 1975; Earle, 2001; Wang *et al*., 2002; Brown, 2012; Saminathan *et al*., 2013; Assi *et al*., 2015). Despite these attributes however, the use of *M. citrifolia* in livestock feeding is limited due to its strong smell and poor acceptability by most animals Diarra *et al*., 2019). Because chickens pick their feed mainly by seeing and feeling rather than scent or taste, *M. citrifolia* could be an alternative PA in poultry diets on account of their antibacterial, anticoccidial, anti-stress properties and is readily available in the Pacific Island countries. These activities in Noni coupled with the low competition as food for humans justifies its use in place of chemical additives in poultry diets.

1.3 Hypothesis

a. Noni fruit powder supplementation will improve the performance of broiler chickens.
b. The stage of maturity will not affect the utilisation of the fruit as PA by broiler chickens.

1.4 Objectives

To investigate the efficacy of feeding green and ripe Noni fruit powder to broiler chickens on:

i) Growth performances (feed intake, weight gain and feed conversion ratio)
ii) Carcass measurements; and
iii) Nutrient digestibility

CHAPTER TWO: LITERATURE REVIEW

2.1 Description of Noni (*Morinda citrifolia*) plant

The genus *Morinda* belongs to the family; Rubiaceae and includes approximately 80 species, including *Morinda citrifolia* Linn. It is popularly called Noni or Indian Mulberry. *Morinda citrifolia* is a shrub, 3-10 m tall, with abundant wide elliptical leaves (5-17 cm length, 10-40 cm width) with small tubular white flowers grouped together and inserted on the peduncle. The petioles leave ring-like marks on the stalks and the corolla is greenish white (Morton, 1992; Elkins, 1998; Dixon *et. al*., 1999; Ross, 2001; Cardon, 2003). The Noni fruit (3-10 cm length, 3-6 cm width) is oval and fleshy with an embossed appearance. It is slightly wrinkly, semi-translucent, and ranges in color from green to yellow, to almost white at the time of picking. It is covered with small reddish-brown buds containing the seeds. The ripe fruit exhales a strong butyric acid-like rancid smell (Morton, 1992; Dixon *et al*., 1999). The pulp is juicy and bitter, light dull yellow or whitish, gelatinous when the fruit is ripe; numerous hard triangular reddish-brown pits are found, each containing four seeds (3-5 mm) (Dittmar, 1993). The fruit can grow in size up to 12 cm or more and has a lumpy surface covered by polygonal-shaped section. The seeds, which are triangular shaped and reddish brown, have an air sac attached at one end, which makes the seeds buoyant. The mature Noni fruit has a foul taste and odor. Noni is identifiable by its straight trunk, large, bright green and elliptical leaves, white tubular flowers and its distinctive, ovoid, "grenade-like" yellow fruit.

Figure1. *Morinda citrifolia* plant with fruit

2.2 Statistics of Noni production in Fiji

Noni is found all over the country either cultivated or in wild state. Jacks of Fiji are one of the best sellers of Noni products to the Chinese customers. According to Frezco Beverages Limited (2019), Noni export has exceeded their sales expectation. The company manufactures several products from Noni fruits including skin care products – moisturizers, shampoo, conditioner, juice, capsules, soap and tea. These products are sold locally and in International Markets Philippines, North and South America, Europe (Narayan, 2017). Herbex, a major buyer of Noni in Fiji, exports organic Noni pulp to China, Canada, Australia, Taiwan, Korea, and South Africa (Lal, 2012). Noni export increased from about 519 tonnes to 1,091 tonnes with corresponding export values of FJ$ 8,812,616 and 6,100,314 in 2014 and 2017 respectively (Fiji Bureau of Statistics, 2018).

2.3 Chemical composition of Noni fruit

About 160 phytochemical compounds have been identified in the Noni plant, the major ones being phenolic compounds, organic acids and alkaloids (Wang and Su, 2001). Of the phenolic compounds, the most important reported are anthraquinones (damnacanthal, morindone and morindin), aucubin, asperuloside and scopoletin (Wang and Su, 2001). The main organic acids in Noni are caproic and caprylic acids (Dittmar, 1993). Xeronine is the principal alkaloid reported in Noni (Heinicke, 1985). Several factors including the plant part and age affect the chemical composition of Noni products (Diarra *et al.,* 2019). The complete physico-chemical composition of the fruit has not yet been reported and only partial information is available on Noni juice (Table 2). The fruit contains 90% water and the main components of the dry matter appear to be soluble solids, dietary fibers and proteins Table 2. The fruit protein content is surprisingly high, representing 11.3% of the juice dry matter, and the main amino acids are aspartic acid, glutamic acid and isoleucine. Minerals account for 8.4% of the dry matter, and are mainly potassium, sulfur, calcium and phosphorus; traces of selenium have been reported in the juice (Chunhieng, 2003). Vitamins, mainly ascorbic acid (Morton, 1992; Shovic and Whistler, 2001), and pro-vitamin A (Dixon *et al*., 1999) have been reported in the fruit.

Phenolic compounds have been found to be the major group of functional micronutrients in Noni juice (Morton, 1992; Dittmar, 1993; Dixon *et al*., 1999; Wang and Su, 2001). Damnacanthal is an anthraquinone with important functional properties (mainly anti-carcinogenic) (Solomon, 1999). Scopoletin is a coumarin that was identified in 1993 at the University of Hawaii and found to have analgesic properties as well as a significant ability to control serotonin levels in the body (Levand and Larson, 2009). Other researchers have shown that scopoletin may also have anti-microbial (Duncan *et al*., 1998) and anti-hypertensive effects (Solomon, 1999). Different Hawaiian teams (Heinicke, 1985; Solomon, 1999) reported the presence of a novel component, proxeronine, in the Noni, which is the precursor of xeronine, an alkaloid believed to combine with human proteins and improve their functionality (Chunhieng *et al.*, 2005). About 51 volatile compounds including organic acids (mainly octanoic and hexanoic acids), alcohols (3-methyl-3-buten-1-ol), esters (methyl octanoate, methyl decanoate), ketones (2-heptanone), and lactones [(E)-6-dodecenoglactone] have been identified in the ripe Noni fruit (Farine *et al*., 1996, Sang *et al*., 2001).

Table 1. Some phyto-chemical characteristics of *Morinda citrifolia*

Constituents	Chunhieng (2003)	Shovic and Whisler (2001)	European Commission (2002)
pH value	3.72	-	3.6
Dry matter (%)	9.8 ± 0.4%	-	10 - 11%
Total soluble solids	8	-	-
Protein content	2.5 %	0.4 g/100g	0.2 - 0.5%
Lipid 0.15%	0.30 g/100g	0.1- 0.2%	
Glucose	11.9 ± 0.2g/l	-	3.0 - 4.0%
Fructose	8.2 ± 0.2g/l	-	3.0 - 4.0%
Potassium	3900 mg/l	188 mg/100g	30 - 150 mg/100g
Sodium	214 mg/l	21 mg/100g	15 - 40 mg/100g
Magnesium	14 mg/l	14.5 mg/100g	3 - 12 mg/100g
Calcium	28 mg/l	41.7 mg/100g	20 - 25 mg/100g
Vitamin C	-	155 mg/100g	3 - 25 mg/100g

2.4 Uses of Noni products

All the plant parts are used in the treatment of various diseases and disorders. The fruit has a wide range of therapeutic potentials such as anti-bacterial (Henry, 1928), anti-viral (Krishnakumar, 2015), anti-tumor (Maiden, 1889), anti-helminthes, hypertensive, anti-inflammatory and immune enhancing effects (Krishnakumar, 2015). The roots are used to synthesize red dye while the leaves, bark, and fruits are used to produce facial creams, soaps, toothpaste, lotions, tea powder and various other products (Levand and Larson, 2009; Diarra *et al*., 2019). Abbott (1992) reported that Noni has been used as drink, food, medicine and dye. In the past decade the global popularity of Noni has increase dramatically (Dixon *et al*., 1999; Clatchey, 2012).

In some South-East Asian countries, the fruits are used to treat diabetes, swollen spleen, and liver diseases and cough (Palu *et al.,* 2006; West *et al*., 2018). Some of the constituents of the wood of *Morinda citrifolia* have been found to exhibit anti-cancer properties (Anekpankul *et al*., 2007). Traditional Tahitian healers believe the Noni plant to be useful for a wide range of maladies, and Noni juice consumers throughout the world have similar perceptions (Chunhieng *et al*., 2005). Carrillo-Lopez and Elhadi (2011) found that Noni juice may provide protection against tobacco smoke-induced DNA damage, blood lipid and homo-cysteine elevation as well as systemic inflammation. Human intervention studies (West *et al.,* 2018) also indicated that Noni juice may improve joint health, increase physical endurance, increase immune activity, inhibit glycation of proteins, aid weight management, help maintain bone health in women, help maintain normal blood pressure and improve gum health. Further, investigation showed a notable antioxidant activity in Noni juice, more than other fruit juices which served as trial placebos. It is this antioxidant effect and its interaction with the immune system and inflammation pathways that may account for many of the observed health benefits of Noni juice (Windisch and Kroismayr, 2007; Anantharaj *et al*., 2017).

Geographical factors like and variations in processing methods are known to produce commercial Noni juice products with divergent phytochemical and nutrient compositions. Therefore, different sources of Noni products may have different toxicological and pharmacological profiles. Anantharaj (2017) found that Noni fruit

extract could alter blood lipid profile by reducing LDL cholesterol and increasing HDL: LDL ratio. Several reports (Goh *et al*., 1995; Hadijah *et al*., 2008; Mandukhail *et al*., 2010; Anantharaj, 2017; Etsuyankpa, 2017), attributed the lipid and cholesterol reducing activities of Noni to flavonoids, alkaloids and glycosides (mainly citrifolin) present in the fruit/seed, leaves and roots (Mandukhail *et al*., 2010).

2.4.1 Pharmaceutical and nutraceuticals uses of Noni

Noni juice is a globally popular health beverage originating in the Tropics. Traditional Tahitian healers believe the Noni plant to be useful for a wide range of maladies, and Noni juice consumers throughout the world have similar perceptions. Nevertheless, human clinical trials are necessary for a precise understanding of what the health benefits of Noni juice are.

M. citrifolia is also known for its antimicrobial properties. Atkinson (1956) attributed the antibacterial effect of Noni to the presence of acubin, L-asperuloside, alizarin and anthraquinone. Reports (Bushnell *et al*., 1950; Tabrah and Eveleth, 1966; Leach *et al*., 1988; Locher *et al*., 1995) suggested that these compounds are responsible for antibacterial activity against *Pseudomanus aeruginosa, Proteus morgaii*, *Bacillus. subtilis*, *S. aureus*, *E. coli*, *Shigella* and *Salmonella* as well as treatment of skin infection, cold fever and other bacterial infection. Duncan *et al*. (1998), showed that scopoletin, a compound available in the Noni is responsible for antibacterial activity against *E. coli* and control bacterial infections, cancer and even death. Umezawa (1992) demonstrated that anti HIV activity in the Noni is due to the presence of a compound (1-methoxy2-formyl-3-hydroxyanthraquinone) that suppressed the cytopatheic effect of HIV infected cells (Locher *et al*., 1995). Broad spectrum antibacterial activity of various solvent extracts of *M. ctirifolia* was reported against Gram positive and Gram negative microorganisms (Jayaraman *et al*., 2008; Wei *et al*., 2008; Selvam *et al*., 2009; Kumar *et al*., 2010; Usha *et al*., 2010; Sunder *et al*., 2012; West *et al*., 2012). The report (Saludes *et al.,* 2002) of the use of Noni against tuberculosis showed that, bactericidal activity of the Noni leaf extracts was 89 % compared to 97 % with rifampicin. Also the antifungal activity of *M. citrifolia* was reported (Banerjee *et al*., 2006) to inhibit the growth of *C. albicans* in-vitro and the same extract showed inhibitory activity against *Apergillus nidulans* spores. Sunder *et*

al. (2012) found that methanol, ethanol, ethyl acetate, chloroform and acetone in extracts of leaf, stem bark, fruit and seed of *M. citrifolia* had broad spectrum antibacterial and antifungal activity in – vitro.

Krishnakumar (2015), reported that, the Polynesians utilized the whole Noni plant for preparation of herbal remedies: the root is used as a cathartic and febrifuge and applied externally to relieve pain and gout, the leaves are considered as tonic and are applied on wounds and on knees to treat gout. The charred leaves made in to a decoction with mustard are a favorite domestic remedy for infantile diarrhoea (Krishnakumar, 2015). Noni fruit juice is widely used for treatment of spongy gums, sore throat, dysentery, leucorrhoea and sapraemia. The fruit juice is useful as medicine for different kinds of illnesses: arthritis, diabetes, high blood pressure, muscle aches and pains, gastro ulcers, menstrual difficulties, head ache, heart diseases, mental depression, poor digestion, atherosclerosis, blood vessel problems, cancer, AIDS and drug addiction (Palu *et al.,* 2006). According to these authors, Noni-based nutritional supplementation together with exercise, gives a positive affect body composition without any side effect and are recommended to be used in combination for combating weight gain.

2.4.2 Antioxidant

It was believed that the Noni fruit juice contained significant levels of antioxidants. This has been proven scientifically by the analysis of Tahitian Noni Juice (TNJ). In a study to measure how the TNJ scavenged super oxide anion radicals (SAR) and quenched lipid peroxides (LPO) by Tettrazollium Nitroblue assay and LMB assay. Auerbach (1992) and Wang and Su (2001) examined super oxide anion radicals scavenging activity in vitro by Tettrazolium nitroblue (TNB) assay. The super oxide anion radicals scavenging activity of TNJ was then compared to that of three known antioxidants; vitamins C, grape seed powder, and pyncogenol at the daily dose per serving level recommended by US RDA's or manufacturer's recommendations. Under the experimental conditions the SAR scavenging activity of TNJ was shown to be 2.8 times that of vitamin C, 1.4 times that of pyncogenol and 1.1 times that of grape seed powder. Therefore TNJ has a great potential to scavenge reactive oxygen free radicals (Wang and Su, 2001). The flavonoids of Noni are mainly present as catechins,

epicatechins and quercetin (Kampkotter *et al*., 2007). Catechins improve poultry performance mainly, through their anti-oxidative (Kim *et al*., 2014) and anti-coccidial (Khan, 2014) properties.

2.4.3 Food uses

Morinda citrifolia fruit has long history of use as a food in tropical regions throughout the world. Documentation of the consumption of the fruit as a food source proceeds the twentieth century (Singh *et al*., 2007). A publication (Seemann and Flora, 1866) in London explained that *M. citrifolia* fruit was consumed as a food in the Fiji Islands. Later publications described the use of this fruit throughout the Pacific Islands, Southeast Asia, Australia and India (Malik *et al*., 2009). In Samoa, Noni fruit was common fare and in Burma it was cooked in curries or eaten raw with salt. Merrill (1943) described *M. Citrifolia*, L. as an edible plant in a technical manual of edible and poisonous plants of the Pacific Islands, in which the leaves and fruits were used as emergency food. Abbott (1992) reported that Noni had been used as food, drink, medicine and dye. The tribes i.e. Nicobarese are known to have consumed this fruit raw with salt as well as cooked as vegetable (Singh *et al*., 2007). Noni fruit and leaves have a history of food use among Pacific Islanders as well as in Southern and Southeast Asia. Although the fruit is edible, its flavor has been described as akin to bad cheese (Wang *et al*., 2002). Despite this, Rarotongans ate the fruit often and the Burmese used it to prepare curries (Cheeseman, 2009). Australian Aborigines ate Noni fruit during the cool-dry season from May to August in the Northern Territory of Australia (Maiden, 1889) . Noni leaves were eaten both in raw and cooked form in Java and Thailand (Ochse, 1977). In Tahiti, fish were wrapped in the leaves as a part of baking to impart an appealing flavor to the cooked fish (Henry, 1928).

2.5 Use of Noni products in poultry nutrition

Several Noni plant products (fruits, roots, bark and leaves) have become popular in animal nutrition (Brooks *et al*., 2009; Sunder and Kundu, 2015; Sunder *et al*., 2015). Noni fruit is a medicinal plant with biological activities such as antimicrobial and antioxidant that could potentially be used as a feed additive in poultry diets. There are

few reports on the use of Noni as phyto additives in diets of broiler chickens and the Japanese quails.

Ponce *et al*. (2011) credited the increased total leucocytes and induce oxidative burst by neutrophils. Primarily to the immune modulatory property of Morinda. There is high content of quercetins, a bioactive ingredient in Noni which mediates the effects of heat stress in poultry (Flees *et al*., 2017). This report found no effects on feed intake and weight gain in heat stressed broiler birds when supplemented with 2 g dried Noni powder / kg diet. Supplementation improved the hepatic expression of heat-shock protein in a time-specific manner suggesting that Noni powder has the potential to relieve stress in poultry (Flees *et al.,* 2017). In a study, (Sunder *et al*., 2011b) reported that Noni fruit extract supplementation at 1.5 ml/bird/day improved weight gain, feed conversion ratio and dressing percentage in broiler chickens. Sunder *et al*. (2007) also observed improved growth and feed utilization of Japanese quails supplemented with 5 g Noni fruit extract/kg feed. Body weight gain and improved feed conversion ratio in Japanese quails were enhanced by replacing 5 g/kg maize with Noni fruit powder (Sunder *et al*., 2013a). In a report (Sunder *et al*., 2013b) found improvement in body weight gain and egg production in Japanese quails fed a mixture of concentrate with Noni fruit granules at 200 g/kg (w/w). Supplementation of the diet with Noni fruit or leaf extract at 5 g/kg diet lowered serum cholesterol in broiler chickens (Sunder *et al*., 2011a). Santoso *et al*. (2017) reported increased HDL/LDL ratio and reduced plasma triglycerides in broiler chickens supplemented with 5 g Noni fruit powder / kg diet. Addition of 3 g/kg Noni fruit powder to the diet was also reported to reduce plasma cholesterol and triglycerides below 50% (Fetina, 2010). Other constituents identified in Noni products, including beta-sitosterols (Ali *et al*., 2016) are known to have cholesterolemic activity (Wang *et al*., 2006).

The growth performance stimulation of *M. citrifolia* fruit was tested in Nicobari fowl; an indigenous poultry bird of Andaman and Nicobar Islands and India (Sunder *et al*., 2011b). The *Morinda* fed group was found to perform more than the control birds in terms of body weight gain, dressing percentage, egg production and feed efficiency. This suggested that several factors including plant part, level of concentration and class of birds might influence the response of poultry to dietary supplementation of *Morinda* products.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Experiment site

The feeding experiment was carried out at Janson's Poultry Farm, South Eastern Region of Viti Levu Island of Fiji. Noni plants are both cultivated by people for medicinal purpose and also grow wild in the area of the trial.

3.2 Preparation of Noni powder

Noni fruits (green and ripe) were plucked from the tree and chopped into pieces. The chopped fruits were then oven dried at 60degrees for 48 hours. The dried fruit was then ground to pass through a 2mm sieve to obtain Noni fruit powder. The powder from green and ripe fruits were labeled separately and stored until needed for diet formulation.

Fig 2: Fresh whole *Morinda citrifolia* fruit

Fig 3: chopped fruits

Fig 4: Chopped fruits in the oven

Fig 5: oven dried fruits

Fig 6: Grinding *Morinda citrifolia* fruit

Fig 7: Morinda fruit powder

3.3 Experimental diets

Five diets were formulated (Table 2 and 3) to meet the requirements for broiler chickens in the starter and finisher phases (Cobb, 2018). The control (basal) diet had no Noni fruit powder. The test diets contained two levels (0.25 and 0.5 %) green and ripe Noni fruit powder each. All the diets were prepared as mash.

Table 2: Ingredient and chemical compositions of broiler chickens diets (% as fed basis) in the starter phase

Ingredients	**Diets**				
	Control	Green powder		Ripe powder	
Maize	14.3	14.3	14.3	14.3	14.3
Crushed wheat	22.5	22.5	22.5	22.5	22.5
Wheat bran	7.1	7.1	7.1	7.1	7.1
Pea meal	37.1	37.1	37.1	37.1	37.1
Fish meal	9.3	9.3	9.3	9.3	9.3
Copra meal	7.3	7.3	7.3	7.3	7.3
Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.3
Enzyme	0.03	0.03	0.03	0.03	0.03
Coral Sand	5	5	5	5	5
Noni	0	0.25	0.5	0.25	0.5
Chemical Analysis (%)					
Crude protein	21	21.5	22	21.2	21
Crude fibre*	4.3	4.3	4.3	4.3	4.3
Crude fat	2.9	2.9	2.9	2.9	2.9
Phosphorus	1.0	1.1	0.8	0.9	1.1
Calcium	5.7	5.6	5.8	5.6	5.6
ME (MJ/kg)*	13.3	13.3	13.3	13.3	13.3

Table3: Ingredient and chemical compositions of broiler chickens diets (% as fed basis) in the finisher phase

Ingredients	**Diets**				
	Control	Green powder		Ripe powder	
Maize	18.7	18.7	18.7	18.7	18.7
Crushed wheat	27.8	27.8	27.8	27.8	27.8
Wheat bran	9.0	9.0	9.0	9.0	9.0
Pea meal	28.4	28.4	28.4	28.4	28.4
Fish meal	6	6	6	6	6
Copra meal	8.2	8.2	8.2	8.2	8.2
Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.2	0.2	0.2	0.2	0.2
Methionine	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.3
Enzyme	0.03	0.03	0.03	0.03	0.03
Limestone	5	5	5	5	5
Noni	0	0.25	0.5	0.25	0.5
Chemical Analysis (%)					
Crude protein	19.2	18.9	18.8	19.0	18.7
Crude fibre*	4.7	4.7	4.7	4.7	4.7
Crude fat	3.4	3.4	3.4	3.4	3.4
Phosphorus	0.8	0.8	0.8	0.7	1.0
Calcium	5.6	5.3	5.3	5.6	5.3
ME (MJ/kg)*	13.4	13.4	13.4	13.4	13.4

Note : ME: metabolisable energy.

3.4 Experimental birds and management

A total of 160 day old broiler chicks were purchased from Pacific Feed Ltd for the experiment. The chicks were brooded together for 7 days on commercial starter diet. The commercial diet contained 210, 40 and 50 g/kg crude protein, crude fibre fat and ash respectively and 12 MJ/kg metabolisable energy. From day 8, 150 chicks were weighed individually and allotted to 15 floor pens containing 10 birds of similar weight (247± 7.5g). Each of the five diets was fed to birds in 3 replicate pens in a completely randomized design. Feed and clean water were provided *ad libitum* throughout the experimental period of 35 days (starter 8 – 22 days; finisher 23 – 42 days). The birds received 24 h light during the first 10 days and 13 h light thereafter.

3.5 Data Collection

Data were collected on growth performance (feed consumption, weight gain and feed conversion ratio), carcass measurements and nutrient digestibility. Growth performance data were collected on weekly basis. Nutrient retention study was conducted at the age of 35 days and carcass measurement at the end of the experiment (42 days).

3.5.1 Growth performance data

Weighed quantities of feed were fed every morning at 7am and the left over feed was weighed to account for the quantity consumed at daily basis. Birds were weighed individually at the start and end of the experiment and weight gain calculated by difference. Feed conversion ratio (FCR) was calculated by dividing the feed consumed by the weight gained for each group and corrected for mortality.

3.5.2 Nutrient retention

At 35d, one bird was randomly selected per replicate (3 birds per treatment) and kept in the metabolism cages for seven days to measure apparent nutrient retention by total excreta collection method. The birds were allowed to adapt to the cage condition for the first three days followed by excreta collection. Feed intake was monitored through the last four days and all excreta were collected and air-dried. The dried

excreta from each replicate was pooled and ground to obtain homogenous samples. Excreta samples were analyzed and apparent nutrient retention calculated as:

$$\text{Apparent retention (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient in faecas}}{\text{Nutrient intake}} \text{ x } 100$$

3.5.3 Carcass recovery measurements

At the end of the experiment (42 d), one broiler weighing close to the mean of the pen was selected from each replicate (3 birds per treatment) for carcass measurements. The birds were fasted overnight but water was available *ad-libitum*. The birds were slaughtered early in the morning by cervical dislocation, bled, scalded in hot water (about 57 ºC for 2 minutes), plucked manually and eviscerated. Eviscerated birds were then dressed and dressing percentage was calculated as:

$$\text{Dressing \%} = \frac{\text{Weight of dressed chicken}}{\text{Live weight}} \text{ x } 100$$

Some carcass cut-up parts (thighs, drumstick and breast) were then separated, weighed and recorded. Data obtained were expressed as percentages of the live weight of the bird. The skin in the crease between the thigh and the body was cut to expose and dislocate the hip joint. The thighs were removed by cutting the tendons and ligaments around the joint. The thighs and drumsticks were separated by cutting at the stifle joint. The breast muscle was removed from the sternum using a scalpel blade.

3.6 Data analysis

3.6.1 Chemical Analysis

Noni powder, diets and excreta samples were analyzed for proximate composition, calcium and phosphorus content at the Fiji Agriculture Chemistry Laboratory, Koronivia Research Station of the Fiji Ministry of Agriculture.

3.6.1.1 Proximate analysis

3.6.1.1.1 Dry matter determination

Dry matter + Organic Matter were determined according to AOAC (1990) (ID 930.15/925.10/942.05). The sample collected was weighed and oven dried at 105 ^{o}C until constant weight was achieved. The dried sample weight was taken and the dry matter (DM) was calculated as:

$$\text{Dry matter (\%)} = \frac{\text{wet fecal} - \text{dry fecal}}{\text{wet fecal}} \text{ x } 100$$

3.6.1.1.2 Crude protein (CP) determination

Crude protein was determined using the Kjeldahl procedure (AOAC, 1990) (nitrogen-protein conversion = 6.25). The procedure consisted of 3 stages namely digestion, distillation and titration. For digestion, 2.0 g of the sample was placed into a digestion tube. Two (2) digestion tablets, which served as catalysts and 25 ml of concentrated sulphuric acid, were added. The tube was then placed on a digestion 25 block covered with the exhaustion cap and switched on. Digestion started with a low temperature to avoid rapid reaction, which would cause the sample to over boil. Digestion continued for about 2 h. The digested sample was allowed to cool at room temperature, and then diluted with distilled water to a volume of 100 ml. Distillation was carried out in a distillation machine. Fifty (50 ml) of NaOH was added to the digested sample which now consists of protein + H2SO4 to form (NH4)2SO4. This ammonium sulphate reacted with NaOH to give NH4OH + Na2SO4.

The distillation machine has a provision for water that is heated to give steam. The steam is passed through the digested sample + NaOH to carry over the NH4OH that is condensed into a receiver flask containing boric acid + indicators (bromocresol green + methyl red). The NH4OH in boric acid changed the initial reddish color of boric acid to green indicating the presence of a base (NH3). After distillation the NH3 in boric acid was then carried to a machine (burette) for titration with 0.1 ml HCl. Nitrogen was then obtained by calculation as follows:

$$\% \text{ N} = \frac{(14.01(\text{ml of titrant sample} - \text{ml of titrant of blank}) \text{x molarity of standard acid}}{(\text{g})\text{sample x } 10} \times 100$$

Nitrogen-protein conversion was taken at 6.25.

3.6.1.1.3 Ether extract determination

Fat extraction was done according to the method of Mojonnier (AOAC 954.02). Two (2) grams of the sample was placed in a thimble and the mouth closed with a piece of cotton wool. The thimble was placed into an extraction chamber of the Soxhlet system. The chamber was fixed onto an extraction flask (round bottom flask of 500 ml) in which 200 ml of petroleum ether was placed. The two units were fixed onto a heating mantle and a condenser placed on top of the unit. The heating mantle was turned on at a temperature of 60C. As the ether in the flask evaporates it follows the side arm and goes to the condenser of the Soxhlet where it condenses and comes down into the extraction chamber. As it drips on the sample in the thimble it extracts the fat and when the chamber is full, the siphon arm fills up also and when it cannot go up any more, it follows the bench and pours down in the flask. As the process is repeated (refluxing), the whole fat in the sample is removed and received into the flask. After about 5 hours, the ether coming down is received into a beaker leaving the flask with only extracted fat. The flask is then oven-dried at 100 C for 1 h, cooled in a desiccator and weighed. Ether extract is then calculated as follows:

$$\% \text{ EE} = \frac{\text{weight of dry flask} - \text{weight of empty flask}}{\text{weight of dry material}} \times 100$$

3.6.1.1.4 Nitrogen-free extract (NFE) and metabolizable energy (ME)

Nitrogen free extract, which represents the soluble carbohydrate, was calculated by difference of the other constituents from one hundred (100).

% NFE = 100 - % (CP + EE + CF + Ash + Moisture)

The metabolizable energy (ME) in Kcal/Kg was calculated according to Fisher and Boorman (1986) as: ME (Kcal/Kg) = 37× CP + 81× EE + 35.5 × NFE

Where:

CP = Crude Protein

NFE = Nitrogen-Free Extract

EE = Ether Extract.

3.6.1.1.5 Determination of Calcium and phosphorus

The sample was ignited at 550 °C to burn all organic material. The remaining mineral was digested in 6 M HCl and Ca determined using a spectrophotometric assay based on reaction of calcium with o-cresolpthalein complexone (CPC) in alkaline solution (Balthrop *et al*., 2011). Approximately 1 g of sample was weighed in a beaker and placed in cold Muffle furnace. The furnace was closed and the temperature was gradually raised to 550 °C for 90 minutes. The temperature was maintained for 16 hours to remove carbonaceous material and then the furnace was opened to allow cooling. 10 ml 6 M hydrochloric acid was added to each beaker and placed on a preheated hot plate (approximately 250 ˚C), covered the beakers with a glass plate, digested for 20 minutes. The beakers were removed from the hot plate and allowed to cool. The content was quantitatively transferred off the beakers to a 25 ml volumetric flask, made up to the smark with distilled water and mixed well. Calcium in the solutions was then measured using the test kit for calcium from Roche based on the CPC method.

Percent of calcium is calculated as: % Calcium = (C x V x DF) / (W x 10)

Where; C = concentration calcium in measure solution (mg/litre), V = volume of solution (in litre, i.e. 0.025 (L)), DF = dilution factor (1), W = weight of the sample (g), and 10 = factor to convert g/kg to %.

The sample for phosphorus determination was ashed following digestion in hydrochloric acid. Molybdovanadate reagent was added resulting in a characteristic yellow color after reacting with phosphorus, which is measured spectrophotometrically**.** Approximately 1 g of sample was weighed in a beaker and placed in cold Muffle furnace. The furnace was closed and the temperature was gradually raised to 550 °C for 90 minutes. The temperature was maintained for 16 hours to remove carbonaceous material and then the furnace was opened to allow cooling. 10 ml 6 M hydrochloric acid was added to each beaker and placed on a preheated hot plate (approximately 250 ˚C), covered the beakers with a glass plate, digested for 20 minutes. The beakers were removed from the hot plate and allowed to cool. The content was quantitatively transferred off the beakers to a 25 ml volumetric flask, made up to the mark with distilled water and mixed well. The solution was allowed to stand overnight to settle. Calcium in the solutions was then measured using

the test kit for calcium from Roche based on the CPC method. An aliquot of the solution was diluted with distilled water to obtain phosphorus content. 10 ml each of the diluted solution and standard solutions were transferred to separate test tubes. The test tubes with 10 ml water (blank) were taken and to each tube 10 ml molybdovanadate reagent was added. The mixture was left to stand for 10 minutes at 20 °C. The absorbance of the solution (6.2.2) at 430 nm was measured using a spectrophotometer against the blank.

Percentage of phosphorus was calculated as: % Phosphorus = (C x V x DF) / (W x 10) Where, C = concentration phosphorus in measured solution (mg/litre), V = volume of solution (in litres, i.e. 0.025 L), DF = dilution factor (normally, i.e. 1), W = weight of the sample (g), and 10 = factor to convert g/kg to %.

3.7 Statistical analysis

Growth, nutrient digestibility and carcass measurements data were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1980) using the Statistical Package for Social Sciences (IBM SPSS, version 25). Pen was the unit for body weight, while nutrient digestibility, carcass and organ measurements were taken on individual birds. Differences between treatment means were compared using the Least Significant Difference (LSD) and significant differences were reported at 5% probability.

CHAPTER FOUR: RESULTS

4.1 Growth performance

The growth performance results of the broiler chickens are presented in Tables 4, 5 and 6. During the starter phase (8-22 d; Table 4), feed intake weight gain and feed conversion ratio were not affected by dietary treatment ($P > 0.05$).

During the finisher phase (23-42d; Table 5) there were no significant treatment effects ($P>0.05$) on any of the growth performance parameters observed (feed intake, weight gain and feed: gain). There was a numerical but that statistical reduction in feed conversion ratio gain on the Noni powder supplemented groups.

The combined growth performances of the birds (8-42d; Table 6) showed a similar pattern to the starter and finisher phases. Feed intake, weight gain and feed conversion ratio were not statistically affected by the diet but feed: gain was numerically reduced with Noni powder supplementation.

Table 4. Growth performance of broiler chickens fed diets supplemented with Noni powder (8-22d)

Parameters	**Treatments**						
	Control (0.00%)	GP (0.25%)	RP (0.25%)	GP (0.5%)	RP (0.5%)	SEM	P
Feed Intake (kg)	23.78	22.26	23.61	22.58	23.06	0.425	0.124
Weight gain (kg)	6.81	5.94	6.34	6.26	6.29	0.467	0.777
Feed: gain	3.50	3.88	3.74	3.64	3.68	0.283	0.911

GP: green powder; RP: ripe powder; SEM: standard error of the mean; a, b superscripts: means in the row with different letters differ significantly ($P<0.05$).

Table 5. Growth performance of the broiler chickens fed diets supplemented with Noni powder (23 - 42d)

Parameters	Treatments						
	Control (0.00%)	GP (0.25%)	RP (0.25%)	GP (0.5%)	RP (0.5%)	SEM	P
Feed Intake (kg)	28.52	27.88	30.24	28.79	29.55	1.103	0.616
Weight gain (kg)	10.16	12.13	11.88	11.10	11.43	1.007	0.680
Feed: gain	2.89	2.33	2.55	2.62	2.59	0.192	0.422

GP: green powder; RP: ripe powder; SEM: standard error of the mean

Table 6. Growth performance of the broiler chickens fed Noni powder supplemented diets (8 -42d)

Parameters	Treatments						
	Control (0.00%)	GP (0.25%)	RP (0.25%)	GP (0.5%)	RP (0.5%)	SEM	P
Feed Intake (kg)	52.30	50.14	53.85	51.37	52.61	1.233	0.344
Weight gain (kg)	16.97	18.07	18.23	17.36	17.71	1.183	0.938
Feed: gain	3.10	2.84	2.96	2.97	2.97	0.140	0.775

GP: green powder; RP: ripe powder; SEM: standard error of the mean.

4.2 Nutrient digestibility

The results of nutrient digestibility of broiler chickens is presented in Table 7. Dry matter and ether extract digestibility was not affected ($P>0.05$) by the diet. Nitrogen retention was significantly ($P<0.05$) reduced on the 0.25% green powder diet compared to the control and ripe powder supplemented groups. Nitrogen retention did not differ between the green powder fed birds as well as among the control, the ripe powder and 0.5% green powder ($P>0.05$).

Table 7. Dry matter and ether extract digestibility and nitrogen retention of 42-d old broiler chickens fed Noni supplanted diets

Parameters (%)	Treatments						
	Control	GP 0.25	RP 0.25	GP 0.5	RP 0.5	SEM	P
DM	75.73	78.19	78.69	77.05	74.91	1.354	0.305
Ether extract	64.26	53.57	67.78	50.70	48.06	10.003	0.580
Nitrogen retention	62.63[a]	52.74[b]	63.81[a]	54.72[ab]	63.87[a]	2.798	0.042

GP: green powder; RP: ripe powder; SEM: standard error of the mean; a, b: means in the row with different letters differ significantly (P<0.05).

4.3 Carcass and some cut-up parts

From the results of carcass studies presented on Table 8, there were significant (P<0.05) dietary effects on the relative weights of carcass, breast and thighs. Carcass weight was markedly decreased (P<0.05) on diet containing 0.5% ripe powder. Breast weight increased (P<0.05) on treatments 0.25% ripe and 0.5% green powder compared to control, 0.25% green and 0.5% ripe powder. Thigh weight were reduced (P<0.05) on 0.25% ripe and 0.5% green powder compared to the control, 0.25% green powder and 0.5% ripe powder. Thigh weight did not differ significantly between the 0.25% ripe and 0.5% green powder. There was no difference in thigh weight among the 0.5% green powder and other diets (P>0.05). The yield of drumsticks was not affected by dietary treatments (P>0.05).

Table 8. Relative weight of carcass and cuts of broiler chickens fed Noni supplemented diets

Parameters (% live weight)	Treatments						
	Control	GP 0.25	RP 0.25	GP 0.5	RP 0.5	SEM	P
Carcass	71.57[a]	71.85[a]	71.95[a]	75.60[a]	68.61[b]	2.122	0.312
Breast	14.25[b]	16.47[b]	19.88[a]	18.08[a]	16.71[b]	0.858	0.011
Thigh	14.10[a]	13.09[a]	11.60[b]	12.60 [ab]	13.68[a]	0.454	0.022
Drumstick	10.74	11.02	10.81	10.37	10.48	0.397	0.789

GP: green powder; RP: ripe powder; SEM: standard error of the mean; a, b: means in the row with different letters differ significantly (P<0.05).

CHAPTER FIVE: DISCUSSION

5.1 Growth performance

The composition of Noni fruit is reported to change with the stage of maturity (Millonig *et al*., 2005; Motshakeri, *et al*., 2015). Motshakeri, *et al*. (2015) reported increased phenolic content of Noni fruit from the green to white hard and a decrease from the white hard to the ripe soft stages. Several constituents identified in Noni fruit, including anthraquinones, are known to exert antinutritional effects at high concentrations (Shalan *et al*., 2016). The report observed toxicity and high mortality in mice consuming 2 mg Noni fruit extract/ml and attributed this to the anthraquinone content. The lack of differences in growth performance among treatments during both stages of growth suggest that at these levels of inclusion, phenolic compounds in Noni fruit will not exert adverse effects on bird performance. Similar to these findings, Flees *et al*. (2017) also found no effects of 0.2% dietary dried Noni fruit powder on feed intake in heat stressed broiler chickens. Supplementation of the diet with Noni fruit extract at 1.5 ml/bird/day improved feed utilisation in broiler chickens compared to the control without supplemental juice (Sunder *et al*., 2011b). Improved feed intake and feed conversion ratio were observed in Japanese quails fed diets containing 15% Noni fruit granules as replacement for maize (Sunder *et al*., 2013a). Sunder *et al*. (2007) also observed improved feed utilization of Japanese quails receiving 0.5% Noni fruit extract in the diet. Although not analysed in the present study, it could be speculated that the concentration of toxic substances in the fruit reduces and the nutritive quality increases with maturity. Several factors including the stage of maturity, method of processing, the species and age of poultry and composition of the basal diets may affect the utilisation of Noni fruit products by poultry. Despite the reduced feed intake during the starter phase, weight gain and feed conversion ratio were not affected by dietary Noni fruit powder, probably due to similarity in nutrient concentration among the diets and lower digestive capacity during the starter phase. There was no effect of Noni powder inclusion on feed intake and growth performance during the finisher and overall growth phases suggesting age differences in its utilisation by poultry.

5.2 Nutrient digestibility

Nitrogen retention was depressed on the diet containing 0.25% green powder. This trend of digestibility could be attributed to changes in concentration with fruit maturity. Currently, there are limited reports on the effect of Noni products on nutrient digestibility. Improved feed utilisation of broiler chickens (Sunder *et al.,* 2011b) and Japanese quails (Sunder *et al*., 2013a) with Noni fruit extract supplementation may be the result of improved nutrient digestion. The findings of the present study suggest that at lower dietary concentrations, the ripe fruit is more effective in improving protein digestibility.

5.3 Dressing percentage and some carcass cut up parts

Upon digestion, a part of the nutrients absorbed is used for tissue synthesis. Despite the reduced protein digestibility on the 0.25% green powder, there was no difference in dressing percentage between this group and the control, 0.25% ripe and 0.5% green powder fed groups. The relative weights of carcass and breast were depressed on the 0.5% ripe powder despite the improved nitrogen retention. The trend could not be explained but may probably be due to differences in micro-nutrient intakes resulting from differences in feed intake in the starter phase. The possible increased in the concentration of phenolic compounds in the ripe fruit as earlier mentioned may also be speculated. The depressed thigh weight on the 0.25% and subsequent improvement on the 0.5% ripe powder could not be explained. Contrary to this findings however, Sunder *et al*. (2007) reported improved dressing percentage in broiler chickens fed 1.5 ml ripe Noni fruit extract per bird per day. Increased intake of essential nutrients in Noni fruit in the form of drink may be possible explanation for the improvement observed Sunder *et al*. (2007) compared to intake from the feed in this study, but this needs to be established.

CHAPTER SIX: SUMMARY CONCLUSION AND RECOMMENDATION

A six-week study was conducted to ascertain the effect of supplementing diets with green and ripe Noni fruit powder on the performance, nutrient digestibility and carcass measurements of broiler chickens. Five diets consisting of a control and 4 diets containing two levels (0.25 and 0.5%) green and ripe Noni powder were formulated to meet the requirements of broiler chickens. One hundred and fifty, 7-d old Cobb 500 broiler chicks were used for the experiment. Each of the diets was fed to 5 replicates of 6 birds in a completely randomized design. The starter diet was fed from 8 – 21 d and the finishers from 22 – 42 d. Data were collected on growth performance (feed intake, weight gain and feed conversion ratio), nutrient digestibility and carcass measurements. Results showed no effects ($P>0.05$) on growth parameters (feed intake, weight gain and feed conversion ratio) during the starter or finisher phases. None of the performance parameters was affected during the overall growth (8 - 42 d) phases ($P>0.05$). Digestibility of dry matter and ether extract was not affected by dietary treatment ($P>0.05$). Nitrogen retention was depressed ($P<0.05$) on 0.25% green powder compared to the control and the ripe powder based diets. The relative weight of carcass was reduced on 0.5% ripe powder ($P<0.05$). Breast weight was increased on 0.25% ripe and 0.5% green powder compared to the control, 0.25% green and 0.5% ripe powder fed groups ($P<0.05$). Thigh weight was reduced on 0.25% ripe and 0.5% green powder ($P<0.05$). The weight of drumstick was not affected by the diet ($P>0.05$).

In conclusion, at 0.25 and 0.5% levels of inclusion, the effect of Noni fruit powder in broiler chickens diets is noticeable on carcass traits than growth performance. Based on these findings, the following recommendations are made:

1. Analyse green and ripe fruits for nutritional and anti-nutritional properties;
2. More studies on blood biochemical indices and gut health will help explain better the effect of the green and ripe Noni powder on poultry.
3. More research into higher inclusion levels of the green and ripe powders in the diet.

LIMITATIONS OF THE STUDY

Many aspects in the initial research proposal, which would have helped in discerning the effect of green and ripe Noni fruits on poultry, could not be addressed due to lack of facilities in the study area. These include:

1. Chemical analysis

   The study proposed to analyse the powder for selected phyto-constituents (flavonoids, anthraquinones, terpenoids and ascorbic acid). Unfortunately, these could not be carried out due to lack of facilities in the study area neither could samples be shipped overseas.

2. Gut health

   The microbial count of caecal content was proposed but this was not done at the Koronovia laboratory due to lack of expertise at the time the experiment.

MANUSCRIPT SUBMITTED

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APPENDICES

Appendix 1: ANOVA for feed intake at starter phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.093[a]	4	1.273	2.354	.124
Intercept	7975.993	1	7975.993	14747.235	.000
Treatment	5.093	4	1.273	2.354	.124
Error	5.408	10	.541		
Total	7986.494	15			
Corrected Total	10.501	14			

a. R Squared = .485 (Adjusted R Squared = .279)

Appendix 2: ANOVA for weight gain at starter phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.152[a]	4	.288	.440	.777
Intercept	601.034	1	601.034	918.543	.000
Treatment	1.152	4	.288	.440	.777
Error	6.543	10	.654		
Total	608.729	15			
Corrected Total	7.695	14			

a. R Squared = .150 (Adjusted R Squared = -.190)

Appendix 3: ANOVA for feed conversion ratio at starter phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.228[a]	4	.057	.237	.911
Intercept	204.094	1	204.094	848.953	.000
Treatment	.228	4	.057	.237	.911
Error	2.404	10	.240		
Total	206.725	15			
Corrected Total	2.632	14			

a. R Squared = .086 (Adjusted R Squared = -.279)

Appendix 4: ANOVA for feed intake at finisher phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10.072[a]	4	2.518	.689	.616
Intercept	12611.520	1	12611.520	3453.079	.000
Treatment	10.072	4	2.518	.689	.616
Error	36.523	10	3.652		
Total	12658.115	15			
Corrected Total	46.595	14			

a. R Squared = .216 (Adjusted R Squared = -.097)

Appendix 5: ANOVA for weight gain at finisher phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.134[a]	4	1.783	.586	.680
Intercept	1928.707	1	1928.707	633.723	.000
Treatment	7.134	4	1.783	.586	.680
Error	30.435	10	3.043		
Total	1966.275	15			
Corrected Total	37.568	14			

a. R Squared = .190 (Adjusted R Squared = -.134)

Appendix 6: ANOVA for feed conversion ratio at finisher phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.474[a]	4	.119	1.068	.422
Intercept	101.192	1	101.192	911.586	.000
Treatment	.474	4	.119	1.068	.422
Error	1.110	10	.111		
Total	102.776	15			
Corrected Total	1.584	14			

a. R Squared = .299 (Adjusted R Squared = .019)

Appendix 7: ANOVA for feed intake combined

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	23.196[a]	4	5.799	1.271	.344
Intercept	40646.366	1	40646.366	8906.086	.000
Treatment	23.196	4	5.799	1.271	.344
Error	45.639	10	4.564		
Total	40715.201	15			
Corrected Total	68.835	14			

a. R Squared = .337 (Adjusted R Squared = .072)

Appendix 8: ANOVA for weight gain combined

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.182[a]	4	.796	.190	.938
Intercept	4682.727	1	4682.727	1115.948	.000
Treatment	3.182	4	.796	.190	.938
Error	41.962	10	4.196		
Total	4727.871	15			
Corrected Total	45.144	14			

a. R Squared = .070 (Adjusted R Squared = -.301)

Appendix 9: ANOVA for feed conversion ration combined

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.104[a]	4	.026	.444	.775
Intercept	132.017	1	132.017	2246.200	.000
Treatment	.104	4	.026	.444	.775
Error	.588	10	.059		
Total	132.709	15			
Corrected Total	.692	14			

a. R Squared = .151 (Adjusted R Squared = -.189)

Appendix10: ANOVA for dressing percentage

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	73.886[a]	4	18.472	1.368	.312
Intercept	77582.981	1	77582.981	5743.892	.000
Treatment	73.886	4	18.472	1.368	.312
Error	135.070	10	13.507		
Total	77791.938	15			
Corrected Total	208.957	14			

a. R Squared = .354 (Adjusted R Squared = .095)

Appendix 11: ANOVA for breast muscle weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	52.159[a]	4	13.040	5.898	.011
Intercept	4374.871	1	4374.871	1978.929	.000
Treatment	52.159	4	13.040	5.898	.011
Error	22.107	10	2.211		
Total	4449.138	15			
Corrected Total	74.266	14			

a. R Squared = .702 (Adjusted R Squared = .583)

Appendix 12: ANOVA for thigh weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.490[a]	4	2.873	4.638	.022
Intercept	2540.463	1	2540.463	4101.358	.000
Treatment	11.490	4	2.873	4.638	.022
Error	6.194	10	.619		
Total	2558.148	15			
Corrected Total	17.685	14			

a. R Squared = .650 (Adjusted R Squared = .510)

Appendix 13: ANOVA for drumstick weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.802[a]	4	.200	.423	.789
Intercept	1712.645	1	1712.645	3614.802	.000
Treatment	.802	4	.200	.423	.789
Error	4.738	10	.474		
Total	1718.185	15			
Corrected Total	5.540	14			

a. R Squared = .145 (Adjusted R Squared = -.197)

Appendix 14: ANOVA for dry matter digestibility

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	30.619[a]	4	7.655	1.392	.305
Intercept	88739.528	1	88739.528	16142.932	.000
Treatment	30.619	4	7.655	1.392	.305
Error	54.971	10	5.497		
Total	88825.118	15			
Corrected Total	85.590	14			

a. R Squared = .358 (Adjusted R Squared = .101)

Appendix 15: ANOVA for crude protein digestibility

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	347.735[a]	4	86.934	3.701	.042
Intercept	53198.993	1	53198.993	2265.027	.000
Treatment	347.735	4	86.934	3.701	.042
Error	234.871	10	23.487		
Total	53781.599	15			
Corrected Total	582.606	14			

a. R Squared = .597 (Adjusted R Squared = .436)

Appendix 16: ANOVA for ether extract digestibility

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	900.427[a]	4	225.107	.750	.580
Intercept	48524.328	1	48524.328	161.636	.000
Treatment	900.427	4	225.107	.750	.580
Error	3002.076	10	300.208		
Total	52426.832	15			
Corrected Total	3902.504	14			

a. R Squared = .231 (Adjusted R Squared = -.077)