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Production responses of Isa Brown laying birds to supplementary dietary levels of *Plumeria rubra* pod meal

Ahaotu EO[1]*, Ezeafulukwe CF[2], Oko EC[3], Marvellous SO[1], Okeke AW[1], Ahaotu VC[4], Patricio R[5] and Mbachu MU[6]

[1]Department of Animal Production and Health Technology, Imo State Polytechnic, Umuagwo, Nigeria
[2]Department of Fisheries and Aquaculture Technology, Federal University of Technology Owerri, Imo State, Nigeria
[3]Department of Agricultural Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria
[4]Department of Microbiology, Gregory University, Uturu, Abia State, Nigeria
[5]Universidad Católica de Temuco, Facultad de Recursos Naturales, Departamento de Ciencias Biológicas y Químicas, Chile
[6]Department of Agricultural Technology, Akanu Ibiam Federal Polytechnic, Unwana – Afikpo, Ebonyi State, Nigeria

Corresponding author: emmaocy@gmail.com

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ABSTRACT

Aim: The study was aimed to investigate the effect of dietary *Plumeria rubra* pod meal (PRPM) as a natural alternative to antibiotic on the growth performance, egg laying and carcass yield of Isa Brown Layer birds.
Method and materials: A total of sixty points of lay birds were randomly weighed and assigned to four dietary treatments having 15 birds in each. Each dietary treatment had three replicates of 5 birds and was reared in floor pens. Birds were distributed in 4 different inclusion levels of PRPM in diets; 0.0%, 10.0%, 15.0% and 20.0% respectively. The birds were fed the experimental diets and water was provided *ad-libitum* throughout the experimental period.
Results: The results showed that significant ($P<0.05$) effect on final body weight and weight gain in dietary supplementation of 15% PRPM as compared to that of control group. Better feed conversion (1.53) was observed at 15% PRPM group. Different levels of PRPM exhibit significant influence on egg laying performance of layers. There was no significant difference ($P>0.05$) observed among the average live weight, thigh meat, wing meat and drumstick meat. Lower mortality was found on diet supplemented with PRPM.
Conclusion: It was concluded that 15% PRPM in layer diets can be used as natural feed additive for enhancing growth performance and egg lay in layer production.

Keywords: Egg Laying and Carcass Yield, Feed Conversion, Growth Performance, Isa Brown Layers, *Plumeria rubra* Pod meal.

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Introduction

Unprecedented upward movement of feed prices which accounts to 70% of cost of layer production (Ogba *et al.,* 2020) has forced nutritionists to formulate/provide economical diet and to ensure optimum performance. Use of chemical feed additives as growth promoters to save feed and production costs by increasing feed efficiency has got criticism due to adverse effect on consumers. Natural feed additives particularly herbal growth promoters are generally liver tonics which optimize hepatic functions of birds (Scheideler *et al*., 2005). It helps in better feeding, synthesis of amino acids and minimize aflatoxin effects.

Although poultry are efficient converters of feed to body weight gain or egg production, they cannot digest 15-25% of the feedstuffs they consume because of anti-nutritional factors that may hinder digestion and/or remain indigestible to the endogenous enzymes present within the bird (Paloheimo *et al*., 2010). Anti-nutritional factors present in corn and soybean meal can interfere with the bird's feed utilization and may affect health and production. The anti-nutrients in corn include non-starch polysaccharides, phytic acid, enzyme inhibitors, and resistant starches. Soybean meal contains protease inhibitors, non-starch polysaccharides, lectins, phytic acid, saponins, phytoestrogens, anti-vitamins, and allergens (Barletta, 2010 and Cowieson, 2005). Therefore, it is important to understand the effects feed additives have on energy metabolism of poultry and how that energy is being utilized for maintenance, production, or storage.

Feed additives are ingredients added to poultry diets to enhance production efficiency, improve health and reduce morbidity (Cheng *et al.,* 2005). Feed additives are added to diets for reasons other than to supply nutrients to the animals for example antibiotics added at sub-therapeutic level in order to improve feed utilization by lowering the population of some unwanted microbes can be considered as feed additives (Gunawardana *et al*., 2009). The economic benefit of feed additives is typically to lower production cost as a result of an improvement in poultry efficiency. Phytogenic feed additives are plant extracts derived from herbs or spices, which have the potentiality to improve feed intake and digestion(Windisch *et al.* 2008) and to maintain micro biota balance in the gut (Mountzouris *et al.* 2007) so beneficially affect animal production and health. Feed additives are typically used in small quantities and are classified into both organic and inorganic in poultry industry. The organic feed additives are products derived from plants which are used in feeding animals to improve their performance (Harms and Russell, 2004). The inorganic feed additives are agrochemicals such as antibiotics. Other feed additives used in poultry diets include antioxidants, emulsifiers, binders, pH control agents and enzymes (Valkonen *et al.,* 2008).

Conventional synthetic feed additives such as antibiotic growth promoters, antioxidants, anti-parasitic agents and anti-fungal agents have been used in poultry feed for decades. However, they created multiple complications, such as traceability in animal products and resistance to antibiotics in the consumer, which became public health issues (Ao *et al*., 2011). On these grounds, the use of all kinds of antibiotic growth promoters was banned in animal feed in Europe (Wu *et al*., 2005). Revolutions in animal feed production gave rise to the idea of phytogenic feed additives (Ucan *et al.,* 2001). Plants and their metabolites, known as bioactive compounds, play a key role because of their feed additive attributes. These bioactive compounds, such as carotenoids, flavonoids, and essential oils, help to maintain animal health and productivity, and to produce safe and healthy chicken eggs (Francis *et al*., 2001). The primary mode of action of these active ingredients is inhibition of pathogenic microbes and endotoxins in the gut and enhanced pancreatic activity, resulting in better nutrient metabolism and utilization (Murugesan and Persia, 2013).

Among the plants, *Plumeria rubra* is one of the best choices as it meets all the necessary parameters of a phytogenic feed additive (Zaheer *et al.,* 2010). *Plumeria rubra* is widely distributed in the tropical and subtropical areas of the world (Marvellous and Okeke, 2021). Based on potential nutrient and bioactive compounds, *Plumeria rubra* is a versatile tree and is given considerable importance in poultry feed and human consumption (Devprakash *et al.,* 2012). Its pods are rich in bioactive compounds, especially carotenoids (Β-carotene), flavonoids (quercetin), polyphenols, vitamins and nutrients (Ahaotu *et al*., 2019a). *Plumeria rubra* could be used as feed additive based on its bioactive compounds, which might add value to eggs and have positive impacts on animal health and performance (Egwaikhide *et al.,* 2009). Β-carotene and quercetin in *Plumeria rubra* ranges from 2.7 to 3.10 mg/100 g and 80 to 150 mg/100 g of dried pods, respectively (Ashayerizadeh *et al*., 2009). When added to the feed, these bioactive, along with phytochemicals, enrich eggs and have positive effects on the health and well-being of birds. Due of its higher protein concentration (22-25%) and high profile of essential amino acids, Moringa pods can be used as a protein source in animal feed (Abd-El-Motaal *et al*., 2008).

Growth promoters are the substances that are added to a nutritionally balanced diet which provoke response towards the exploitation of maximum genetic potential of the host, in terms of growth as well as improvement in feed conversion efficiency. There are different types of growth promoters which are used to exploit the layer industry like antibiotics, probiotics (bio-growth promoters), prebiotics, exogenous enzymes, antioxidants and Coccidiostat. (Abd-EIslam *et al.,* 2013). Many other novel growth promoters include herbs and certain other nutritional substances (Abudabos, 2012). In India, the use of growth promoters has been accepted in the layer industry and they are usually included in the feed in very small quantities. Many antibiotics are used in animal and poultry feeds as growth promoters to improve the health and well-being of animals and as a prophylactic agent for promoting growth. The withdrawal of these antibiotic growth promoters not only affect or reduce productive performance, but also increases morbidity and mortality in poorly maintained flocks (Acamovic and Brooker, 2005). Research has been conducted in an intensified manner in the last two decades for developing antibiotic alternative for maintaining health as well as performance of animals.

Probiotics and prebiotics; acidifiers as well as extracts of plants; Nutraceuticals for example; copper as well as zinc are the alternatives of antibiotics. At the same time the potential of antimicrobial peptides; clay minerals; antibodies from chicken egg yolk, essential oils, medium chain fatty acids (from eucalyptus oil), rare earth elements; as well as enzymes (recombinant) have been tested for their ability of replacing antibiotics as dietary feed additives (Ahmad *et al.,* 2005). The cost factor and the possibility of evolution of antibiotic resistant microbes has made it necessary for the feed industry to use alternatives to antibiotics, such as probiotics, prebiotics, organic acids to maintain good production and health of poultry as well as livestock.

Plumeria belongs to the *Apocynaceae* family and is native to the New World. The plants from this genus are widely cultivated in the tropical and subtropical regions throughout the world. They are recognized as excellent ornamental plants and often seen in the graveyards (Lim, 2014). *Plumeria* plants are famous for their attractiveness and fragrant flowers. The essential oils from the flowers are used for perfumery and aromatherapy purposes. The decoction of the bark and roots of *P. rubra* is traditionally used to treat asthma, ease constipation, promote menstruation and reduce fever. The latex is used to soothe irritation (Das *et al.,* 2013).

Objectives are to evaluate the addition of *Plumeria rubra* as a feed additive to correct apparent metabolisable energy of laying hens to be fed with diets differing in energy concentration for a period of 8 weeks and to assess the inclusion of *Plumeria rubra* as a feed additive to improve performance and egg laying index of Isa brown layer birds.

The very high cost of poultry feed has limited the expansion of the poultry industry as this has forced many poultry producers to folding. This has further drawn the already low intake of animal protein by Nigerians. Feed additive is however expensive as a feed ingredients and this therefore increased the cost of poultry production. In order to prevent this trend, efforts are being directed towards the use of some unconventional and cheaper feed ingredients as additives with locally available, cheaper and quality feed ingredients. The use of *Plumeria rubra* pod meal to replace feed additives in layer diets is a possible way of reducing feed cost and producing cheaper eggs and meat for the populace.

The productivity of Nigerian livestock is well below their genetic potential mainly due to poor nutrition and inadequate quality feed. The high cost and poor quality of finished feed in the recent past have caused serious economic losses in poultry in Nigeria (Gupta *et al*., 2007). Effort to improve this situation according to (Ahaotu *et al.,* 2019b), include harnessing the potentials of good quality and relatively inexpensive feed ingredients as replacements of the more expensive feed ingredients.

Frangipani Plant

Plumeria rubra L. is the most commonly grown *Plumeria* species in Florida and in many warm climates. Its native range is from central Mexico to Colombia. The tree grows from 15 to 25 feet tall. The flowering period of *P. rubra* is from March to July. *Plumeria rubra* commonly known as Frangipani (temple tree) is well known for its intense fragrance and medicinal values (Baghel *et al*, 2010). The flowers, root bark and leaves of *Plumeria rubra* are known for their therapeutic uses. Decoction of bark is used as purgative and anti-herpetic. Root-bark is used as abortifacient, remedy for gonorrhea and venereal sores (Ahaotu *et al.,* 2019a). In Yucatan, latex is used for toothache. Decoction of flowers in Mexico is used for diabetes (Li *et al*., 2003). *Plumeria rubra* is used in ulcer, inflammation, bronchitis, fever (Baghel *et al.,* 2010), diarrhoea (Basavaraju *et al*, 2009), rheumatism, cancer (Kuete and Efferth, 2011), snake bite (ethno-veterinary) Deshmukh *et al*, 2011), infusion or extract from leaves is used for respiratory disorder (asthma) (Patil *et al*, 2008), A poultice of heated leaves is beneficial for swellings.

Plumeria rubra are among the most fragrant of tropical flowering plants. These small trees are grown primarily for their aroma in almost all tropical and subtropical areas of the world (Ahaotu *et al*., 2020). They have rather thick, waxy branches that exude a milky white sap when cut or broken. The milky exudates can cause allergic reactions in some people. The growth habit is usually open, but some are awkwardly shaped. Others grow into a distinct candelabrum form. As the tree ages, the canopy thickens. Technically, leaves are alternately arranged, but are so tightly clustered at the end of branches that they appear as whorls. The terminal cymes are 6 to 10 inches in diameter, and are made up of 20 or more blossoms. The flowers are waxy and salver-form. Salver-form is defined as a flower with slender tube corolla that abruptly expands into a flat limb.

Feed Additives for use in Poultry Diets

Feed additives are ingredients added to poultry diets to enhance production efficiency, improve health and reduce morbidity (Cheng *et al.,* 2005). Feed additives are added to diets for reasons other than to supply nutrients to the animals for example antibiotics added at sub-therapeutic level in order to improve feed utilization by lowering the population of some unwanted microbes can be considered as feed additives (Gunawardana *et al*., 2009). The economic benefit of feed additives is typically to lower production cost as a result of an improvement in poultry efficiency. Phytogenic feed additives are plant extracts derived from herbs or spices, which have the potentiality to improve feed intake and digestion (Windisch *et al.* 2008) and to maintain micro biota balance in the gut (Mountzouris *et al.* 2007) so beneficially affect animal production and health. Feed additives are typically used in small quantities and are classified into both organic and inorganic in poultry industry. The organic feed additives are products derived from plants which are used in feeding animals to improve their performance (Harms and Russell, 2004). The inorganic feed additives are agrochemicals such as antibiotics. Other feed additives used in poultry diets include antioxidants, emulsifiers, binders, pH control agents and enzymes (Valkonen *et al.,* 2008).

Conventional synthetic feed additives such as antibiotic growth promoters, antioxidants, anti-parasitic agents and anti-fungal agents have been used in poultry feed for decades. However, they created multiple complications, such as traceability in animal products and resistance to antibiotics in the consumer, which became public health issues (Ao *et al*., 2011). On these grounds, the use of all kinds of antibiotic growth promoters was banned in animal feed in Europe (Wu *et al.,* 2005). Revolutions in animal feed production gave rise to the idea of phytogenic feed additives (Ucan *et al.,* 2001). Plants and their metabolites, known as bioactive compounds, play a key role because of their feed additive attributes. These bioactive compounds, such as carotenoids, flavonoids, and essential oils, help to maintain animal health and productivity, and to produce safe and healthy chicken eggs (Francis *et al*., 2001). The primary mode of action of these active ingredients is inhibition of pathogenic microbes and endotoxins in the gut and enhanced pancreatic activity, resulting in better nutrient metabolism and utilization (Murugesan and Persia, 2013).

Varieties of different feed additives are commonly used in poultry diets, or can be used when the need arises.

Antioxidants

Fat is often added to poultry diets to increase the energy content, this is especially the case in high density broiler diets. In addition, if fish meal is used, it is high in fat. To help keep feed from going bad, which is common in high fat products, antioxidants are commonly added to the diet (Ahmad *et al*., 2006). Compounds with antioxidant properties include ethoxyquin, butylhydroxytoluene (BHT), butylhydroxyanisole (BHA), vitamin C, and vitamin E.

Free-flowing agents

It is important that the feed flow easily so that it does not cake in the feeders. Free-flowing agents are substances added to the diet to make sure the feeds do not pack down. Free-flowing agents typically have fine particulate structures while not reacting to the other ingredients in the feed. A common free-flowing agent is hydrated sodium aluminosilicate.

Pelleting additives

Pelleting of feeds has been shown to improve feed efficiency for some poultry species. Most broiler feeds are pelleted. It is important to make sure that the feed ingredients can be packed together in bite-sized pellets without a lot of fines. A variety of pelleting additives are available.

Sensory additives

Traditional sensory additives include substances affecting food odour and palatability, and colourings. Phytogenic additives are commonly used as colourings in laying hens to affect the egg yolk colour. Laying hens cannot synthesize egg yolkpigments; yet, the egg yolk colour is one of the main indicators of egg quality affecting consumer's preference (Englmaierova *et al*. 2014). Pigments of the egg yolk, xanthophylls are dependent onfat soluble pigments that are present in the feed (Yildirim *et al.* 2013). The source of these pigments may be natural or synthetic colourings (ethyl ester of β-apo-8′-carotenoic acid and canthaxanthin known as Carophyll Yellow and Carophyll Red) (Englmaierova *et al.* 2014) that are more economical but also potentially dangerous to human health (the maximum dose of canthaxanthin should not exceed 8 mg/kg; at higher doses, minute crystal formation may occur in the retina by a reversible deposition process (Breithaupt, 2007).

Natural colourings are preferred; the most frequently used ones include carotenoids, the source of which are carrot, *Chlorella* algae, marigold (*Tagetes erecta* L.), or lutein, however, natural carotenoids are unstable and their use is also limited by their price (Englmaierova *et al.* 2014). The effect of 1 and 2% (10 and 20 g) feed supplementation with biomass of *Chlorella* grown through heterotrophic fermentation on the concentration oftotal and individual carotenoids in egg yolk werestudied by Kotrbacek *et al*. (2013). In this study, asignificant increase ($P <$ 0.01) in the deposition oftotal carotenoids in egg yolk was found (by 46% and119%). The deposition of carotenoids significantly ($P < 0.01$) increased the colour characteristics, but Kotrbacek et al. (2013) also found a significantly decreased egg yolk weight in the group with 2%supplementation of *Chlorella*, which was probably related to lower feed consumption in these hens.

However, in Isa Brown hens fed diets supplemented with the algae *Chlorella* (12.5 mg/kg) and lutein (250 mg/kg) Englmaierova *et al*. (2013) found significantly increased egg weight ($P < 0.001$),shell weight ($P < 0.001$), and shell thickness (P =0.017), decreased yolk/albumen ratio (P = 0.035)of the egg, and *Chlorella* supplementation also significantly ($P < 0.001$) increased yolk colour. Well known carotenoids include also astaxanthin, which is commonly added to fish feed toprovide for a more attractive meat colour. Besides, this substance also has strong antioxidant effects(10× more effective than vitamin E); it is also a subject of recent studies for its effect against reactive oxygen species (ROS) **(**Gomez *et al*. 2013),or for its Neuro-protective effect in subarachnoid hemorrhage (Zhang *et al*. 2014).

Egg yolk colour was also studied by Nobakht and Moghaddam (2013). The authors reported that the addition of 1.5% and 2% costmary **(***Tanacetum balsamita***)** to the diet of laying hens has a positive effect not only on the egg yolk colour, but also on the overall production performance, and blood indicators (lower triacylglycerol and cholesterol levels). In contrast, no effect on the yolk colour and viscosity was demonstrated using *Beta vulgaris* L. ssp. *esculenta* var. *rubra* (Kopriva *et al.* 2014) or using a Korean ginseng extract (*Panax ginseng*) (Yildirim *et al.* 2013).

Technological additives

Phytogenic additives are newly studied also interms of decreasing the production of harmful gasesin pigs. Alam *et al*. (2013) have concluded in their *in vitro* study that adding 0.1% various additives (red ginseng barn powder, persimmon leaf powder, ginkgo leaf powder, and oregano lippia seed oil extract) during anaerobic incubation of swine faecal slurries for a period of 12 to 24 hours led in all groups to a significant decrease ($P < 0.05$) in propionate production, to higher production of volatile fatty acids ($P < 0.05$), and, with the exception of the group with the addition of oregano, to higher pH. In the group with the addition of oregano, they noted in contrast a decrease of pH and different bacterial population (uncultured bacterium clone PF_{6641} and *Streptococcus lutetiensis* and the dangerous gases ammonia-nitrogen (NH_3N) and hydrogen sulfide (H_2S) were not detected in this group; compared to control and other additives tested, they noted lower amounts of "odorous compounds", and recommended the use of this additive in the grower category of pigs.

When using a *Quillaja saponaria* extract, Veit *et al*. (2011) noted by 38% and 32% lower ammonia concentrations in stables of pigs with the addition of 100 ppm of the phytogenic feed additive AROMEX® ME Plus and of 150 ppm of the phytogenic feed additive FRESTA® F Plus, respectively.

Lower ileum ammonia concentration in broiler chickens fed essential oil (125 ppm including the essential oil derived from oregano, anise, and citruspeel) was noted by Hong *et al.* (2012).Various phytogenic substances or mixtures have the potential to reduce CH4 emissions from ruminants (Flachowsky and Lebzien 2012). Methane is the second most important greenhouse gas, and a large part of it originates in animal production.

Dong *et al.* (2010) found in an *in vitro* study after sampling the goat rumen fluid, that the addition of an *Artemisiae annuae* extract and a herbal mixture (60 g/kg diet; *Dryopteris crassirhizoma Nakai, Astragalus membranaceus, Crataegus pinnatifida, Mentha haplocalyx*) significantly (P < 0.05) lowered the methane production. Gunal *et al.* (2013) found decreased (P < 0.05) ammonia N concentrations using *in vitro*24 hour batch culture of rumen fluid with a 55 : 45forage : concentrate diet supplemented with a Siberianfir needle oil, citronella oil, rosemary oil, sage oil, white thyme oil or clove oil at three doses(125, 250, and 500 mg/l), with the exception for the highest dose of white thyme oil.

Cieslak *et al.*(2014) also achieved in their *in vitro* study a loweringof methane production by 29% compared tothe control group when using *Saponaria officinalis*s aponins, without any effect on rumen fermentation. However, Jayanegara *et al.* (2014) report that the methane mitigating properties of saponins in the rumen are level- and source-dependent; and they noted in their study the effectiveness of saponins-rich sources in mitigating methane in the order: yucca >tea > quillaja. Hristov *et al.* (2013) recorded when administering dietary supplementation of *Origanum vulgare* L. leaf material at the dose of 250–750 g/day, a decrease in rumen methane production in dairy cows within 8 h after feeding; however, the effect over a 24-hour feeding cycle was not determined in their study. When using anise oil, cedar wood oil, cinnamon oil, eucalyptus oil, and tea tree oil at concentrations of 125, 250, and500 mg/l, Gunal *et al*. (2014) noted in an *in vitro* study only moderate effects on rumen fermentation, but higher ammonia-N concentration in cultures incubated with essential oils regardless of the dose level.

Gunal *et al.* (2014) reported that it is unlikely that these moderate *in vitro* effects would correspond to any substantial impact on Ruminal fermentation *in vivo*. After characterization of the substances, *in vitro* studies should only be the first step to identify substances with a CH_4 reduction potential. Feeding studies, especially long term studies, aimed to consider animal health and welfare, adaptation of rumen microbes, efficiency of additives over long feeding periods, animal performance, safety of the additive to the animals, consumer and environment, quality of animal products and CH_4emissions, are essential prerequisites for the use of phytogenic feed additives in ruminant feeding practices (Flachowsky and Lebzien 2012).

Feeding enzymes

Many of feed ingredients available for use in poultry diets have anti-nutritional factors which limit their use. Feed enzymes have been developed that break down these anti-nutritional factors increasing the potential of many 'alternative grains'. The use of feed enzymes is quite common in Europe where wheat and barley are often used instead of corn.

Mold inhibitors or mycotoxins binders

Cereals are subject to mold growth, which can happen in the field, during post-harvest handling, storage and processing. Even if the mold is removed, the mycotoxins they produce will remain and can be very toxic to poultry (Aidara-Kane, 2012). Many feeds contain a mold inhibitor or a mycotoxins binder to prevent the mycotoxins from being absorbed through the gut and into the blood stream. Common mycotoxins binders are Mycosorb, Mycofix, ProSid, Mycoad and Toxisorb.

Coccidiostat

Coccidiosis is a problem in many species of poultry raised on the floor. The protozoa that cause coccidiosis are found everywhere. A low level of coccidia in the digestive tract is not a problem, but high levels can result in poor feed efficiency, poor health and ultimately death. Coccidiostat are sometimes added to feed to keep the coccidia at low levels, especially early in bird growth, to allow them to develop resistance (Bampidis *et al.,* 2005). They do not treat the condition, but help in preventing it. Common Coccidiostat include amprolium (Aprol, Corid), decoquinate (Deccox), diclazuril (Clinacox), halofuginone (Stenorol), lasalocid sodium (Avatec, Bovatec), Monessen (Coban), robenidine (Robenz) and salinomycin (Bio-Cox, Sacox).

Antibiotics

The digestive tracts of all animals contain a diverse population of microbes. Some are classified as 'good' and are necessary for maintaining gut health. There are also 'bad' bacteria which, when present at high levels can adversely affect gut health, and can ultimately result in damage to the intestines (Akinleye *et al.,* 2008). This is referred to as necrotic enteritis. Low-level or sub-therapeutic levels, of antibiotics can be added to feed to keep the 'bad' bacteria in check. Bacitracin is a commonly added antibiotic.

If a disease situation it may be necessary to add therapeutic levels of antibiotics to the feed, although for most treatments the antibiotics are added in the feed. A sick bird with typically drink but may not eat. By adding the antibiotics in the water you assure that the birds are being sufficiently treated.

Antibiotic alternatives

The desires to reduce use of antibiotics in animal production, alternatives have been developed. The best way to keep the 'good' bacteria at higher levels than the 'bad' is to add the nutrients which promote the growth of the good. These are referred to as probiotics, since they encourage the growth of some bacteria. Providing nutrients, such as mannan oligosaccharides (MOS) that promote good bacteria will help to maintain gut health (Patricio*et al*., 2020 and Al-Ghazzewi and Tester 2012).

Using *Plumeria rubra* pod meal as a feed additive in layer diets would go a long way in reducing the cost of production in poultry enterprise thereby making animal protein more available to the populace and also improving the profitability of the poultry farmers. Also findings from the research would represent a significant contribution, scientifically.

Materials and Methods

Preparation of Frangipani Pod Meal

Plumeria rubra pods was collected from the botanic garden of forestry department, Imo State Polytechnic Umuagwo and stored in polythene bags after shade drying and grinding for further analysis and addition to feed (Fig. 1). The pods were stripped off, washed, allowed to drain and spread in a well-ventilated room to dry for five days. Shade- dried *Plumeria rubra* pods was milled into powder using a blender (National Mx-795N), sieved with a muslin cloth and stored for used.

The pod meal was analysed for chemical composition in the Department of Science Laboratory Technology, Imo State Polytechnic Umuagwo, according to standard procedures (AOAC, 2005). Selenium and bioactive compounds (B-carotene and quercetin) will be analysed with an atomic absorption spectrophotometer and high-performance liquid chromatography (HPLC) respectively.

Preparation of Crude Extract

The pods collected was dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the pods was defatted with n-hexane and allowed to dry. The product thus obtained was then extracted with methanol in a Soxhlet apparatus. The solvent was completely removed under reduced pressure and a semi-solid mass was obtained.

Experimental Animals

Sixty (24 weeks old) Isa brown layer birds will be assigned to four treatments and three replicates with five birds each in a completely randomized design. Four levels substitution levels (0, 5, 10, and 15g PRFM /kg) will be added to the four diets.

Data Collection

Cholesterol estimation in the egg yolk samples were performed by the standard method using a UV-visible spectrophotometer (AOAC, 2005). Equal quantities of acetone and egg yolk will be taken and shaken vigorously for two minutes. After centrifugation, the supernatant was removed. This procedure will be repeated three times and the pooled supernatant will be evaporated to remove acetone and kept for cholesterol analysis.

A daily feed allowance of 100g per bird was offered. Feed offered, feed refused, feed intake and mortality was recorded daily and tabulated cumulatively for FCR every week. Daily egg production was recorded from each experimental unit separately to calculate various parameters, including egg weight, feed per dozen eggs and feed per kg eggs. Egg shape index, surface area, volume, shell thickness, yolk index and Haugh unit score was measured at the start of the experiment and then every two weeks throughout the experimental period. Three eggs were picked at random from each unit and subjected to egg circumference measurement.

Bird handling and collection of samples was carried out. Blood samples were taken with sterile syringes containing anticoagulant from the wing web on days 28 and 42 of the experiment and stored. Blood samples were also be centrifuged and the serum was separated and stored for analysis of parameters, including glucose, serum glutamic-pyruvic transaminase (SGPT), alanine transaminase (ALT), creatinine, and cholesterol, with protocols for every parameter using a commercial kit from Merck Microlab-300. The serum samples were analysed for antibody titers against Newcastle Disease by haemagglutination (HA) and haemagglutination inhibition (HI) techniques.

Data collected was be analysed through one-way ANOVA (Steel *et al*., 1997) using PROC GLM in SAS software (SAS Inc. 9.4). Significant means will be separated through Duncan's multiple range tests (Gordon and Gordon, 2004

Egg yolk and feed samples were analysed to estimate moisture, crude protein, crude fibre, ether extract, and ash (AOAC, 2005). Ash samples will be used for mineral analysis. The wet digestion procedure will be used for selenium analysis (AOAC, 2005).

HPLC techniques were used to estimate carotenoids (B-carotene) and flavonoids (quercetin) in the yolk samples (Gilman and Watson, 2016). Yolk samples was analysed for cholesterol level on a UV-visible spectrophotometric with the method described by AOAC (2005).

Quantification of carotenoids was done with the HPLC technique and yolk samples were prepared as described by Kuigoua *et al*. (2010). In an HPLC tube 1 g yolk sample was weighed with 8 ml methanol and 2 ml 1N HCL. The yolk sample was vortexed for five minutes and this process was repeated three times. The sample was centrifuged at 4000 rpm for 15 minutes. The supernatant was removed and dried in a water bath.

Egg yolk samples were analysed for quercetin levels using the HPLC technique with slight modifications to the standard method (Ogunwande *et al*., 2015). For sample preparation, a measured 1 g weight of egg yolk was taken in HPLC tubes and acidified methanol was added. After lowering the temperature, the sample was centrifuged at 1500 g and 5000 rpm for 15 minutes and repeated three times. The supernatant was filtered and shifted to HPLC vials for quercetin analysis.

Table 1.Chemical Composition of *Plumeria rubra* Pod Meal

Parameters	%
Moisture (%)	87.5
Carbon (%)	43.2
Hydrogen (%)	6.49
Nitrogen (%)	3.50
a-Cellulose (%)	38.38
Lignin (%)	6.24
Ash (%)	2.92
Polyphenol (%)	3.86
Hydrocarbon (%)	0.26
Crude protein (%)	15.62
Crude Fiber (%)	9.5
Calcium (%)	3.3

Table 2. Composition of the Experimental Diets (Layers 24-28 weeks)

Ingredients	Treatments			
	T_1 0	T_2 5	T_3 10	T_4 20
Maize	40.0	40.0	40.0	40.0
Wheat Offal	20.0	15.0	10.0	0.00
Palm Kernel Meal	7.0	7.0	7.0	7.0
Porzyme SF	0.10	0.10	0.10	0.10
Fish Meal	9.0	9.0	9.0	9.0
Soya bean Meal	16.5	16.5	16.5	16.5
Bone Meal	6.5	6.5	6.5	6.5
Lysine	0.25	0.25	0.25	0.25
Methionine	0.15	0.15	0.15	0.15
Common Salt	0.25	0.25	0.25	0.25
Layers Premix	0.25	0.25	0.25	0.25
Total (Kg)	100	100	100	100
Plumeria rubra Pod Meal	0.0	5.0	10.0	20.0
Crude Protein (%)	17.9	18.3	18.2	17.9
Crude Fiber (%)	2.45	2.47	3.01	3.70
Ether Extract(%)	4.00	4.50	5.00	5.45
Ash Content (%)	11.0	12.0	12.0	12.50
Moisture Content (%)	13.00	13.00	12.50	13.00
ME (Kcal/Kg)	2665.0	2676.2	2677.9	2647.3
Calcium	3.7	3.7	3.7	3.6
Phosphorus	0.7	0.9	0.7	0.7

Layer Vitamin/mineral premix containing the following per kg. Vitamin A 10,000000I.U; Vitamin D_3 2,000000IU; Vitamin E 10,000IU; Vitamin K 2,000mg; Thiamine 1,500mg; Riboflavin B 4,000mg; Pyridoxine B_6 1,500mg; Anti-oxidant 125g; Niacin 15,000mg; Vitamin B_{12}, 10mg; Pantothenic acids 5,000mg; Biotin 50mg; Choline chloride 400g, manganese 80g; Zinc, 20g; 1ron, 50g; copper, 20g; Iodine 1.5g; Selenium 200mg; Cobalt 200mgr; Folic acid 500mg; Vitamin C 100mg.

Results and Discussion

Comparatively, birds on T_2 PRPM diet recorded a significant ($p<0.05$) higher percentage hen day production of 81.33. Values between birds fed control diet and those on diets with T_3 and T_4 PRPM were however not significant ($p>0.05$). The lower values observed among pullets on the control diet and those eating higher levels of PRPM could be attributed to the reduced feed intake, resulting in relatively lower nutrient intake. This translates to lower nutrient availability for egg production (Yan-Qing *et al*., 2012 and NRC, 1994). The birds thus reduced egg production because the only available nutrients would also be required for

Other physiological needs of the birds. Hong *et al*. (2012) reported an average percentage hen day production of 94.34, 76.38 and 55.32, respectively whereas Hong *et al*., (2012), reported 55.32 and 35 – 82.1 as the percentages hen day production. Values reported by these authors were comparable with those obtained in this experiment.

Fig 1: *Plumeria rubra* pods

Result of feed efficiency (kg feed/kg egg) revealed that birds on T_3 PRPM diet were more efficient in converting its feed to unit weight of egg followed by group fed T_2 PRPM. The least feed efficiency was recorded by birds fed the control diet. The variation in feed efficiency was however not significant (P >0.05) among the groups. Ravindran, (2005) reported feed conversion ratio/kg egg mass of 2.33 and 2.61 for pullets and spent layers, respectively; while Ucan *et al.* (2001) gave a range of 1.90 to 2.06 as feed efficiency (kg feed kg-1 egg) for laying hens fed diet containing sprouted malted sorghum. Values reported by these authors were lower than those observed in this experiment.

The effect of treatment on average egg weight was significant (P <0.05). Egg weight increased as the inclusion level of PRPM in the diets increased. Bird eating T_4 PRPM diet had the highest average egg weight of 58.42g, while the least egg weight of 54.99g was observed in birds fed the control diet. Egg weight is a function of so many factors, notably; quality and quantity of feed, strain of the birds, stage of lay and management system. In the instant case, it seems there is a factor that increases egg weight as level of PRPM in the diets increased. It appears that the level of lysine increased with increasing level of PRPM in the diets. There is evidence (Mueller *et al.,* 2015), that each 0.1 unit of extra lysine increased egg weight by 1.16g.

Variation in the shell weight was not significantly (P>0.05) influenced by increasing levels of PRPM in the diets. Values obtained in this experiment were similar to 6.27 – 6.54g reported by Nasir and Grashon, (2010), but higher than the range (4.84 – 6.32g) and 5.8g reported by Nelson *et al*., (2007). Differences in the shell weight may be attributed to the age of the birds and the weight of eggs. A number of studies have shown that egg shell weight increases as the bird grows older (Kopriva *et al.,* 2014).

Table 3. Laying Performance and Egg Quality Evaluation of Pullets fed Diets Containing *Plumeria rubra* Pod Meal

Parameters	**Replacement levels (%)**				
	T_1	**T_2**	**T_3**	**T_4**	**SEM**
Hen day Egg production (%)	61.67^{b}	81.33^{a}	62.25^{b}	70.34^{b}	± 2.09
Feed efficiency (kg feed/kg egg)	2.65	2.91	3.32	3.33	± 0.21
Egg weight (g)	54.99^{b}	57.07^{ab}	57.53^{a}	58.42^{a}	± 0.50
Shell weight (g)	6.71	7.07	6.52	6.58	± 0.08
Mortality	0.00	0.00	0.00	0.00	0.00

Means with different superscripts within the same row are significantly (p<0.05) different SEM- standard error of mean.

Table 4. Effect of dietary supplemental PRPM preparations on growth performance of Isa Brown Laying Hens from Point of Lay to 4 Weeks.

Parameter ± SE*	**Treatment****			
	T_1	**T_2**	**T_3**	**T_4**
Body weight gain ± SE (g)				
24 weeks (POL)	$1276{\pm}5.3^{ab*}$	$1331{\pm}5.6^{a}$	$1257{\pm}5.7^{b}$	1264±6.7
25-28th week	$1548{\pm}7.7^{c}$	$1432{\pm}4.6^{ab}$	$1474{\pm}5.1^{ab}$	$1457{\pm}11.7^{b}$
Weekly Feed intake ± SE (g/bird)				
24 weeks (POL)	$676{\pm}0.0^{a}$	$775{\pm}0.0^{a}$	$641{\pm}0.0^{a}$	$763{\pm}0.0^{a}$
25-28th week	$1901{\pm}12.6^{a}$	$2046{\pm}5.8^{a}$	$2052{\pm}8.9^{a}$	$1983{\pm}11.5^{a}$
FCR ± SE				
24 weeks (POL)	$2.47{\pm}0.6^{ab}$	$2.36{\pm}0.5^{b}$	$2.53{\pm}0.6^{ab}$	$2.96{\pm}0.8^{ab}$
25-28th week	$2.95{\pm}0.6^{a}$	$2.04{\pm}0.6^{b}$	$2.35{\pm}0.3^{b}$	$2.19{\pm}0.6^{b}$

* Means within the same row having different letters are significantly different (P<0.05).
** Control T_1 = 0% (PRLM), T_2 = 5% PRLM, T_3 = 10% PRLM, T_4 = 20 PRLM. Point of Lay (POL)

The effects of *Plumeria rubra* pod meal as feed additives on body weight gain, feed consumption and feed conversion ratio of Isa brown laying birds from point of lay (24th week) through the 28th week periods are presented in Table 3. There were significant differences recorded in the body weight gain and FCR during the experimental periods. Significant differences in feed intake of the hens were observed. The diets supplemented with *Plumeria rubra* pod meal feed additives significantly improved the body weight gain and FCR of the birds during the experimental periods compared to the control.

Plumeria rubra pod meal feed additives improved the FCR of the laying birds. Several research findings reported that herbal extracts could increase the layer performance by improving live weight gain and FCR of laying birds (Kotrbacek *et al.,* 2013). The laying birds fed the control diets recorded the lowest FCR and the highest body weight gain during the experimental period even though it was not statistically different from the birds fed with *Plumeria rubra* pod mealbased feed additives except at 5% PRPM level. This might be due to the digestion stimulatory and the gastro-protective effects as reported by Abdulla *et al*. (2010), for the herbal components *Andrographis paniculata* and *Phylanthus niruri* in the commercial herbal products. Furthermore, the body weight gains of hens were increased with the increasing percentages of *Plumeria rubra* pod meal during the entire experimental periods. Moreover, numerically lower FCR was recorded in the laying birds when the percentage of *Plumeria rubra* pod meal in the diet was increased.

Conclusion

The addition of the phytogenic feed additives (*Plumeria rubra* pod meal at 10%) had positive effects on various indices of performance and hen day egg criteria of Isa brown laying hens**.**

Dietary levels of *Plumeria rubra* pod meal favours increase in the egg weight and shell weight in layers. It is concluded that the table eggs of commercial layers may be enriched with PRPM by dietary supplementation of 10% grams of *Plumeria rubra* pod meal without any adverse effects on the physiology and production performance of layer birds. Hence, the *Plumeria rubra* pod meal - enriched eggs may be utilized especially in the mid-day nutritious meals of government schemes. Such implementation will not only improve the health status of children as well as the pregnant women, but also helps in the sustainability of the poultry industry in our country.

From the results it can be concluded that dietary inclusion of *Plumeria rubra* pod meal irrespective of various levels had not shown any positive impact of performance of layer birds in terms of body weight, gain in body weight and feed intake. However, the values for feed conversion efficiency, performance index and net profit per kg layer bird showed positive impact in group of birds' supplementation with *Plumeria rubra* pod meal at 10.0 g/kg feed. Therefore, on the basis of results obtained during experiment, dietary supplementation of *Plumeria rubra* pod meal at rate of 10.0 g/kg feed can be advocated.

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