

Growth Assessment of Broiler Chickens Given Bitter Leaves (*Vernonia amygdalina*) as Phyto-additive, Potentially Antimicrobial Agents of Lipids and Amino Acids

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ABSTRACT

This research was conducted to evaluate the growth of broiler chickens given bitter leaves (*Vernonia amygdalina*) in the drink as phytoadditive, potentially antimicrobial agents of lipids and amino acids. Amino acids of bitter leaves were analyzed by HPLC method, the fatty acid and fat content of bitter leaves were analyzed by A.O.A.C. Official Methods 2012. Antibacterial analysis of bitter leaves was prepared in three concentrations (2.5%, 5% and 10%), and analyzed with disc diffusion method. A total of 200 D.O.C. broiler chicks were treated under a standard broiler management program until 7 days of age. The experiment was conducted as 4x5 completely randomized arrangement. The experiment lasted 28 days from. Bitter leaves juice was blended and given to broilers through drinking water in four kinds of treatment: 0, 10, 20 and 30 ml/L drinking water. All chickens were fed diet contain 70% commercial feed, 27% broken corn and 3% coconut oil and given *ad libitum*. Results showed that the bitter leaves contain high linolenic, linoleic and palmitic fatty acid, and phenylalanine, serine, isoleucine, glycine and arginine amino acids. Bitter leaves showed the power of antibacterial activity for *S. aureus*. Bitter leaves as feed additives in drinking water of broiler caused feed intake and carcass percentage were non significantly different. Final weight, WG, SGR, GE, FCR were highly significantly increased. Abdominal fat was highly significantly decreased, and there was no effect on the gible. It can be concluded that bitter leaf can be used as phytoadditive in broiler diet.

Keywords: Antimicrobial, Bitter leaf, Broilers, Growth performance, Phytoadditive.

1. INTRODUCTION

The anti-microbial, anti-inflammatory, and anti-oxidative potential of herbal plants can affect poultry performance through improving digestive tract function. Herbs can also provide many functions in the poultry body system [1]. Antibiotic and antibacterial medications still used in poultry industry in several purposes that are therapeutic treatment, prevention or as traditional growth promoters [2]. Alternative phyto-genic additives improve a number of principal processes in the livestock's body. They are also applicable in the food industry thanks to their antibacterial properties [3].

The biological activity of free fatty acids has an important role in the host's defense against pathogenic microorganisms, and, it plays a role in inhibiting bacterial growth. There are researches on the fatty acid derivatives and their antimicrobial activity [4]. Lauric, linoleic, palmitic, stearic, linolenic, myristic and oleic acids are

fatty acids that have antibacterial and antifungal properties. Lauric acid is a saturated fatty acid with the most potential as a gram-positive antibacterial, while linoleic acid is an unsaturated fatty acid with the most potential as a gram-positive antibacterial. Oleic acid has potential antibacterial activity against *S. aureus* [5].

Antimicrobial peptide (AMP) a component of the plant defense system. Can be isolated from roots, seeds, flowers, stems, and leaves, and has activity against phytopathogens, as well as against pathogenic bacteria in humans [6]. Wang and Wang [7] and Hammami et al. [8] reported a study comparing the primary and tertiary structures of plant antimicrobial peptides showed that 33% of plant peptides had activity against bacteria, and these antibacterial peptides were composed of cysteine and/or glycine residues.

Plant AMP is considered a good drug because of its chemical properties combined with biological specificity

such as antibodies [9]. AMP exhibits broad-spectrum antibiotic activity against pathogenic bacteria, fungi, enveloped viruses, and parasites [10]; [11]. The gastrointestinal microbial community has the ability to release low molecular weight peptides with antimicrobial properties capable of triggering an immune response [12]. AMP with broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, and fungi, is an important defense barrier against pathogenic microorganisms [13].

Bitter leaves (*Vernonia amygdalina* Delile), is a small tree of Asteraceae family. It is called bitter leaf because of its abundant bitter taste [14]. *V. amygdalina* is drought tolerant although it grows better in humid environments [15]. It is used in tropical Africa for multiple purposes especially in culinary and traditional medicine for malaria, hepatitis, diarrhea, venereal disease, diabetes, digestive problems, skin disorders, coughs, constipation and in wounds treatments [16].

The administration of *V. amygdalina* up to 400 g/150 kg of feed affected the growth and measured hematological parameters, and prevented the occurrence of coccidiosis in broiler chickens. Bitter leaf can be used as an anticoccidial in broiler rations and does not interfere with the health status of poultry [17]. Giving bitter leaf increases gastro intestinal enzymes; hence, it could improve digestion and nutrients' assimilation. Inclusion 0.3% improved carcass quality parameters such as carcass weight, dressing percentage and has no deleterious effect on the internal organs of the birds and positively influenced the serum metabolites, thereby confirming that bitter leaf meal can reduce the risk factors of high cholesterol level [18]. The administration of 50 mL/L of bitter leaf water extract in drinking water did not have an adverse effect on performance, reducing total cholesterol, LDL and glucose in broiler blood plasma [19].

Traditionally, much attention has been directed justifiably to the role of essential amino acids and fatty acids in animal nutrition. Fatty acids are in natural fats and dietary oils and are known to have antibacterial and antifungal activities. However, there is no information about the antibacterial activity of bitter leaves, and this study was to determine the fatty acids and amino acids of bitter leaves, as well as their potential antibacterial activity, and the effect of bitter leaf juice on the growth performance, carcass, and body fat level of broiler chickens.

2. MATERIAL AND METHODS

2.1. Material

Bitter leaves that were used for analyzing of fatty acids and amino acids were dried, and powdered. For biological test, a total of 200 CP 707 broiler chicks were

used, and bitter leaves juice were used as treatment. Bitter leaves were blended to make juice. At this stage, after washing, the bitter leaves are cut into small pieces with scissors. Then add water with a ratio of 1:10. Then the juice is made by crushing the leaves in a blender. Then stored in a refrigerator at a temperature of 4 °C to keep the bioactive compounds from the juice.

2.2. Methods

2.2.1. General

Phase I of the research was in laboratory, for analysing of fatty acids, amino acids, and antibacterial test. The fatty acid and fat content were analysis by A.O.A.C. Official Methods 2012. Amino acids were analyzed by HPLC method, used Thermo Ultimate 3000 RS Fluorescence Detector.

Antibacterial test was done by disc diffusion method [20]. *S. aureus* and *E. coli* as bacterial test. Bitter leaves powder was used in three solution concentrations of 2.5%, 5%, and 10% using DMSO as solvent. Chloramphenicol (0.1%/disc) was used as the positive reference standard, and antibacterial activity was observed by measuring the zone of inhibition, and all inhibition tests were performed in triplicate.

Phase II, research was carried out experiment in cage. The birds were treated under a standard broiler management program until 7 d of age. The birds were randomly distributed into 20 pens. The experiment was conducted as 4 x 5 completely randomized arrangement each of the 4 treatment groups with 5 replication, 10 birds per cage. The experiment lasted 28 days from 8 to 35 d of age. Bitter leaves juice then given to broilers through drinking water in several kinds of treatment: R0 =drinking water without bitter leaves; R1= 10 ml of bitter leaves juice (BLJ)/liter of drinking water, R2= 20 ml of BLJ/liter of drinking water, and R3= 30 ml of BLJ/liter of drinking water. Bitter leaves juice began to be given to chickens aged 8 days. All chickens were fed kibble contain 70% commercial diet, 27% corn, 3% coconut oil and given *ad libitum*. Consumption of drinking water and feed is calculated every day. On the 35th day the chickens were weighed, then slaughtered, and the carcass weight was calculated after removing the feathers, head, legs and intestines. Gizzard, liver, heart and abdominal fat are weighed.

2.2.2. Statistic

The data were collected on body weight gain, feed intake and feed conversion ratio, carcass, edible organs and abdominal fat percentage, growth efficiency (GE) and specific growth rate (SGR %). Data were subjected to one-way analysis of variance, and then the differences between the treatment means were compared using

Duncan Multi Range Test. All statistical analysis was performed with IBM SPSS Statistics 24 software.

3. RESULTS AND DISCUSSION

The data of amino acids, fatty acids, and antibacterial activity of bitter leaves are shown in Table 1, Table 2 and Table 3. Result showed that bitter leaves contain amino acids phenylalanine, serine, isoleucine, glycine, arginine, valine, lysine, and fatty acids linolenic acid, linoleic acid, palmitic acid, cis-13,16-docosadienoic acid, oleic acid, stearic acid, lauric acid, palmitoleic acid, and fatty acid total was 38.55 % w/w, whereas fat content was 2.10 % w/w.

Table 1. Dominant amino acids of bitter leaves

Parameter	Bitter Leaves (ppm)
Phenylalanine	320.92
Serine	297.91
Isoleucine	138.65
Glycine	136.85
Arginine	111.43
Valine	27.58
Lysine	6.31
Parameter	Bitter Leaves (ppm)
Phenylalanine	320.92
Serine	297.91
Isoleucine	138.65

Table 2. Dominant fatty acid of bitter leaves

Parameter	Bitter Leaves (% w/w)
Fat Content	2.10
Lauric acid	0.46
Palmitic acid	7.72
Palmitoleic acid	0.43
Stearic acid	0.71
Oleic acid	0.93
Linoleic acid	10.11

Table 3. Dominant amino acids of bitter leaves

Parameter	%	Inhibition zone (mm)	
		<i>E. coli</i>	<i>S. aureus</i>
Bitter Leaf	2.5	6.00	6.00
	5	6.00	7.76
	10	6.00	9.05
Chloramphenicol (C+)	0.1	8.96	11.57
DMSO (C-)		0	0

The method of giving bitter leaves juice through drinking water to broilers resulted in the same feed

consumption, a highly significant increase in body weight, weight gain, feed conversion ratio, carcass weight, growth efficiency (GE), specific growth rate (SGR%), significantly decreased in abdominal fat percentage (47.46%), and have no effect in gible (Table 4).

Zone of inhibition of bitter leaves for *E. coli* showed the same response for three concentration (6 mm), lower than the positive control (8.96 mm), and, for *S. aureus* at 5 and 10% concentration of bitter leaves (9.05 mm) was high enough but still under the positive control (11.57 mm). Bitter leaves showed the highest value of inhibition zone at 10% concentration on *S. aureus* (9.05 mm); whereas the lowest inhibition zone was on *E. coli* (6 mm). This showed that antibacterial in bitter leaf is effective against Gram positive bacteria at a concentration of 10% while is resistant against Gram negative. This study recommended the use of bitter leaf up to 10% concentration which is resistant to *E. coli*. For inhibition of *S. aureus* that the higher the concentration, the larger the diameter of the inhibition zone formed.

The antimicrobial property of fatty acids is closely related to the fatty acids' structure and their ability as antimicrobial agents. Saturated fatty acids with shorter chain lengths are more effective against microorganisms, while monounsaturated and polyunsaturated fatty acids are more effective against microorganisms at longer chain lengths. [21]. A report by Anzaku et al. [22] mentioned that free fatty acids (FFAs) with chain lengths C8 to C18 were more effective as antibacterial agents against Gram-positive bacteria than Gram-negative bacteria. Clavijo and Flo'rez [23] reported that for the nutrients to be digested, cooperation between biochemical functions and the microbiota in the digestive tract of chickens is required. It is necessary to select beneficial microbiota which involved in the production and health aspects; as well as in the protection from pathogens, detoxification processes, and modulation of the immune system.

E. coli inhabited the intestinal tract as well as the external environment. In both poultry and humans, *E. coli* present in the lower gastrointestinal tract [24]. Yoon et al. [5] reported that all Gram-positive test bacteria sp. are susceptible to administration of 0.01 mM arachidonic acid. The time and concentration of *S. aureus* treatment affect the bactericidal activity of arachidonic acid. There was neither significant positive effect nor negative effect on broilers fed the various supplemental of bitter leaves [25]. This study was similar than that Osho et al. [26] reported that the bitter leaves extract 15 g/L water increased feed conversion ratio of broilers without affecting haematological profile. Bitter leaves possess considerable amounts of proximates, minerals, vitamins. In this study, abdominal fat significantly reduced because of bitter leaves. Owens et al. [27] reported that the medicinal properties of bitter leaf have been attributed to

Table 4. Effect of Bitter Leaves in drinking water on the growth performance

Variables	Treatments				SEM	pValue
	R0	R1	R2	R3		
Total FI (g/head)	1844.30	1877.40	1867.60	1887.80	7.24	.17
FI (g/head/day)	87.82	89.40	88.93	89.89	.345	.17
Initial wt, g	284.50	290.20	289.20	293.60	2.17	.55
Final wt, g	1243.40 ^a	1362.20 ^b	1360.40 ^b	1477.20 ^c	21.71	.000
WG, g	965.00 ^a	1062.20 ^b	1065.0 ^b	1183.60 ^c	20.32	.000
SGR (%)	7.05 ^a	7.33 ^b	7.35 ^b	7.69 ^c	.064	.000
GE	3.40 ^a	3.66 ^b	3.70 ^b	4.03 ^c	.064	.000
FCR	1.91 ^a	1.76 ^b	1.76 ^b	1.60 ^c	.29	.000
Carcass wt, g	912.60 ^a	987.20 ^a	990.80 ^a	1119.00 ^b	21.43	.001
Carcass (%)	73.09	73.02	73.27	74.57	21.43	.56
Liver (%)	2.98	2.47	2.89	2.57	.12	.36
Gizzard (%)	1.88	1.68	1.78	1.54	.58	.196
Heart (%)	0.64	0.67	0.50	0.59	.04	.45
Abdominal Fat (%)	3.75 ^a	3.08 ^b	2.46 ^c	1.97 ^d	.16	.000

FI= feed intake; WG = weight gain; SGR =specific growth rate; GE = growth efficiency

the biochemistry present in the plant. Certain chemicals from this plant have lipid-lowering properties, and that precise information about the proximate composition, phytochemical composition and micronutrients is the basis for understanding the mechanisms of this plant's anti-atherogenic potential.

4. CONCLUSION

Results showed that bitter leaves contain linoleic and palmitic fatty acid, and phenylalanine, serine, isoleucine, glycine and arginine amino acids in high percentage. Bitter leaves showed the power of antibacterial activity for *S. aureus*. Bitter leaves as feed additives in drinking water of broiler caused feed intake and carcass percentage were non significantly different. Final weight, WG, SGR, GE, FCR were highly significantly increased. Abdominal fat highly significantly decreased, and there were no effect on gible. So, bitter leaf was recommended as feed additives alternative in broiler diet.

AUTHORS' CONTRIBUTIONS

First and second author designed the project; first, second and third author performed the experiments; first and second author prepared the article.

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