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RESEARCH ARTICLE

GROWTH PERFORMANCE AND VILLI MORPHOLOGY OF BROILERCHICKENS IN RATION SUPPLEMENTED WITH DIGMAN (HYDRILLAVERTICILLATA) AND DRINKING WATER WITH KAMIAS (AVERRHOA BILIMBI) AND KATMON (DILLENIA PHILIPPINENSIS) EXTRACTS

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Abstract

This study evaluated the growth performance and villi morphology of Ross broiler chickens under five different treatments: Control (commercial feeds and water), Commercial feeds + 3% Dried Digman, Commercial feeds + 200ml Kamias Fruit Extract (diluted in 1L water), Commercial feeds + 90ml Katmon Fruit Extract (with water), and Doxycycline (13 to 15 days old). A total of 240 Ross broilers, with 12 birds per treatment group were used. Weekly data collection included body weight and feed intake, while jejunal villi were sampled at 12, 21, and 28 days for measurement.

Results indicated significant differences in villi morphology. At 5 days, Kamias Fruit Extract showed notable improvements in villi width and surface area compared to the Control and Dried Digman. By 14 days, Katmon Fruit Extract significantly outperformed the Control, Dried Digman, and Kamias Fruit Extract. At 21 days, Dried Digman and Katmon Fruit Extract demonstrated superior villi measurements compared to the Control and Kamias Fruit Extract. Notably, Katmon Fruit Extract exhibited the greatest enhancement in villi length and crypt depth ratio, particularly at 5 and 21 days.

In conclusion, all treatments showed potential as alternatives to commercial antibiotics, with Kamias Fruit Extract beneficial in early stages and Katmon Fruit Extract significantly improving villi morphology and absorption capabilities. Dried Digman also performed comparably to Doxycycline across the study period.

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Introduction:-

The fast increase in the global human population has driven up the demand for animal protein in human diets, resulting in intensive broiler chicken production to satisfy this need. According to the Philippine Statistics Authority, chicken demand has grown faster than that for other meats, largely due to its affordability, lower fat content, and the lack of cultural or religious barriers. However, in addition to rising demand, the greatest challenge in global animal husbandry is the complete elimination of antibiotics. Antibiotics have commonly been included in broiler feed to promote intestinal health, growth, and feed efficiency (Ayalew *et al* 2020). However, some countries have prohibited the use of antibiotic growth promoters (AGPs) due to concerns about their impact on human and animal health. (Paul, S.S *et al* 2021 and Koorakula, *et al* 2020). However, directly restricting antibiotic use in poultry will likely result in reduced production performance. (Rathnayake, D *et al* 2021). This highlights the need to pursue antibiotic-free production, encouraging researchers to find alternatives to antibiotics in broiler chicken farming.

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Recently, a variety of studies have focused on discovering functional feed additives that can provide benefits comparable to those of antibiotic growth promoters. (Qidong Zhu et al 2021).

The efficiently converting feed into its fundamental components for optimal nutrient absorption is essential for the production and welfare of both broilers and broiler breeders (Richard A. Bailey, 2019). Gut health is becoming an increasingly crucial focus in broiler chicken production. (Qidong Zhu et al 2021). It is a complex and dynamic area that integrates aspects of nutrition, microbiology, immunology, and physiology, playing a vital role in production outcomes. Compromised gut health can disrupt digestion and nutrient absorption, leading to poor feed conversion, economic losses, and increased vulnerability to diseases. Furthermore, recent shifts in regulations concerning antimicrobial use, along with evolving feed requirements and the emergence of more efficient poultry breeds, highlight the urgent need for a deeper understanding of gut function and overall gut health. (Richard A. Bailey, 2019).

Therefore, different studies relating to gut health and natural plants alternative for antibiotics on broiler chickens are taking into focus to know their potentials in the poultry industry. Water thyme (*Hydrilla verticillata*) is an invasive, rapidly growing submerged aquatic macrophyte located in Laguna de Bay. This monoecious plant features vibrant green leaves with serrated edges, arranged in whorls of 3 to 10 along its stems (Chaturangani, et al 2016), this aquatic plant confirmed to be an invasive species not only in Laguna Lake but also in different countries. It has an active ingredient of loliolide and thymidine (XIAO, Yu, 2007), also antioxidant activity was also been proven (Vashaith P Bhavsar et al 2016), and antimicrobial properties of ethanolic extracts derived from *Hydrilla verticillata* against *E. coli* and *C. albicans* bacterium. The alkaloids and flavonoids are also responsible for antioxidant and antimicrobial activity (Pandi Prabha S. et al 2015). Its major component is phytyl that constitutes a (70.29%) (Pandi Prabha and Rajkumar, 2015). The natural compound phytyl, after being converted into phytyl sulfate, was evaluated for its antibacterial properties. Phytyl demonstrated significant antibacterial activity, proving to be considerably more effective than both positive controls, streptomycin and ampicillin. (B. Pejic et al 2014). It also boasts a high concentration of various vitamins and minerals that are often difficult to obtain from plant sources. For instance, it contains 15% elemental calcium on a dry weight basis, which is bound to carbohydrates, proteins, and polyphenols. Additionally, it serves as a rich source of B vitamins and a variety of minerals such as chromium, selenium, boron, and silica, along with amino acids, essential fatty acids, enzymes, antioxidants, and other health-promoting compounds. Furthermore, it comprises approximately 50-60% complete protein and is particularly high in lysine. (Vashaith P Bhavsar, et al 2016).

Kamias (*Averrhoa bilimbi*) is a small tree that can reach heights of up to 15 meters, characterized by its sparsely arranged branches. Its compound leaves consist of 20 to 40 leaflets, each measuring between 5 to 10 centimeters in length. (De Lima VL et al 2001). *A. bilimbi* is primarily grown for its medicinal properties in various tropical and subtropical regions worldwide. Research indicates that this plant is commonly utilized in folk medicine for treating diabetes mellitus and hypertension, as well as serving as an antimicrobial agent. Chinju Merin Abraham highlights that the bioactive compounds in *A. bilimbi* make it a promising candidate for future research in the fight against drug-resistant bacteria, given its impressive antimicrobial properties. A comparative study on the fruit stages of kamias revealed that extracts from mature *A. bilimbi* fruits exhibited the largest inhibition zones of 12.3 mm and 9.7 mm against the Gram-positive bacteria *S. aureus* and *B. cereus*, respectively. Young fruit extracts demonstrated a maximum inhibition zone of 12 mm against *Salmonella* spp., followed closely by the mature stage at 11 mm and the ripe stage at 9.3 mm. Both young and mature extracts displayed significantly stronger antimicrobial activity compared to those from the ripe stage. (Seri Intan Mokhtar., et al 2016). *A. bilimbi* contains oxalic acid, a strong acid that may influence bacterial growth. The presence of oxalic acid in *Averrhoa* species can serve as a potent source of antioxidants and exhibit antimicrobial activity against *S. aureus*. (Sripanidkulchai B et al 2002), according to this study the Kamias extract has the potential to decrease the harmful bacteria in the broiler's gut.

Dillenia philippinensis, commonly referred to as katmon in various local Philippine languages, belongs to the Dilleniaceae family, which encompasses around 120 species. This particular species is endemic to the Philippine islands and is typically found in forests at low to medium elevations. (NRCS- US, D.A 2010), reaching heights of 6 to 15 meters, the katmon tree has a smooth or nearly smooth trunk. Its leaves are leathery and glossy, shaped ovate, elliptic, or oblong-ovate, measuring 12 to 25 centimeters long with coarsely toothed edges. The flowers are large, white, soft, and fleshy, measuring 6 to 8 centimeters in diameter, with substantial fleshy sepals that tightly encase the true fruit. The fruit of this tree is commonly used to add a sour flavor to Filipino dishes, such as sinigang, and is particularly abundant in areas like Real, Infanta, and General Nakar in Quezon. (Wagan A.D.M et al UPLB 2017). This tree is commonly planted as an ornamental species, used in urban greening, as a hedge, living fence, and

windbreak, and is also beneficial for riparian or wetland management. Additionally, it serves as a valuable timber tree in Southeast Asia. The wood is utilized for various purposes, including general construction, poles, interior works, furniture, boards and panels, veneers, plywood, and wooden articles. Moreover, a red dye can be extracted from the bark. Beyond its common applications, this crop holds significant potential in the medicinal field. Consolacion Ragasa et al. (2009) from De La Salle University concluded that the leaf extract exhibits notable antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *Trichophyton mentagrophytes*. Additionally, it has shown promising anti-cancer properties.

This study recognizes the needs for researching a potential and an alternative method on improving the performance of broiler chickens without compromising its gut health, also limiting or complete removal of commercial anti-biotic in their production process to eliminate the threat of anti-bacterial resistance that might affect the health of the humans.

Materials and Method:-

A total of 240 Ross Broiler chicks were distributed across five treatments using a completely randomized design (CRD). Each treatment was replicated four times, with 12 chicks assigned to each treatment. The treatments used were as follows:

T1: Pure commercial feeds & drinking water (control)

T2: Commercial feeds + 3% percent of Dried Digman (1 kilo of feeds + 30 grams)

T3: Commercial feeds and 20% of Kamias fruit extract + Drinking water (200ml + 1 liter drinking water)

T4: Commercial feeds and 9% Katmon fruit extract + Drinking water (90ml + Drinking water)

T5: Commercial feeds and plain water + Commercial anti-biotic (Doxycycline) utilize in rate of the manufacturer

Two samples per treatment were gathered at the age of 12 days, 21 days, and 28 days (harvesting stage). The jejunum cut for about 2 inches was harvested and put in a container with formalin, it was delivered to the laboratory for processing, measurement and analysis. The jejunum's villi and crypt tissue slides having 5 microns of thickness (cross section), were measured using Compound microscope E200 M1 4x/0.10 magnification scanner. Villi length was measured from the tip of the villi to the crypt opening, while crypt length was assessed from the crypt opening to the base. Villi width was determined by measuring the distance from the left edge to the right edge of the villi. A.A.Saki., et al 2012.

The collected data were organized and presented in both textual and statistical tables. They were analyzed using Analysis of Covariance (ANCOVA) for body weight, along with Analysis of Variance (ANOVA). Complete Randomized Design (CRD) single factorial experiment for all the remaining dependent variables.

Results and Discussion:-

Body weight

The body weight gain per week of all the broiler chicken from the initial body weight to final body weight shows no significant difference using at $p \leq 0.05$ from initial weight of the broiler chicken having mean value of 236.21 grms, 225.60 grms, 207.9 grms, 224.27 grms, for Control, Dried digman, Kamias, Katmon fruit extract and Doxycycline respectively, however the Katmon mean value (498.18 grms, 950.961 grms, and 1459.111 grms) continually shows higher value in comparison to other treatments mean value from week 1, week 2 up to final body weight, Dried Digman (1438.59 grms) and Doxycycline (1408.45) mean value at final body weight are also higher than control group (1390.01) and Kamias fruit extract (1352.02). This data is also shown in the study of Zi Xie et al. 2020; some of the treatments using antimicrobial peptides result in no significant difference in terms of body weight of yellow feather chickens but significant in most of the intestinal morphological values.

Feed Intake

The feed intake per week of the broiler chickens shows no significant effect at 7 days exposure with mean value of 4217 grms, 3959 grms, 3713 grms, 3975 grms, and 4027 grms for Control, Dried digman, Kamias fruit extract, Katmon fruit extract, and Doxycycline, also no significant difference at 14 days of exposure with mean value of 10906 grms, 10084 grms, 9537 grms, 9587 grms and 9600 grms, for Control, Dried digman, Kamias fruit extract, Katmon fruit extract, and Doxycycline respectively, however at 21 days of exposure the control group show significant mean value of feed intake in comparison to all the treatments, having mean value of 16820 grms for control and 15037 grms,

13921grms, 14974grms, and 14294grms for Dried digman, Kamias fruit extract, Katmon fruit extract, and Doxycycline respectively.

Feed Conversion Ratio

The analysis of variance shows the least mean value of FCR among the treatments per week; the result represents a no significant difference from the 1st week to the final week (week 3); however, the data from Katmon fruit extract performed to be the lowest FCR at the first 2 weeks of the experiment (\bar{x} 1.308 and \bar{x} 1.466); however, at the final week, Katmon and Doxycycline show the lowest mean values of \bar{x} 1.504 and \bar{x} 1.55, respectively; however, there is still no significant difference when compared to other treatments since their overall mean values of FCR are somewhat the same from the data of week 1 up to the final week.

Villi Length

The result of data analysis on broiler chickens' jejunum villi length in terms of days exposure, chickens exposed to dried Digman, Kamias fruit extract, and Katmon fruit extract data (\bar{x} 780.07 μ m, \bar{x} 793.72 μ m, \bar{x} 771.77 μ m) resulted in a higher mean value compared at the control group and Doxycycline group at 5 days exposure (\bar{x} 720.38 μ m, \bar{x} 720.88 μ m), however not statistically significant, this representation of data progressed till the 14 days of exposure and 21 days of exposure. This data also aligns with a study that evaluated the intestinal health of broiler chickens using antibacterial peptides. In this study, the control group showed no significant difference in villi length compared to the groups treated with antibacterial peptides, as determined by a least significant difference test with $p \leq 0.005$, indicating that the differences were not significant. (Zi Xieet al., 2020).

VilliWidth

The villi width data analysis for 5days exposure with the Kamias Fruit Extract (\bar{x} 211.34 μ m), show significant result in both Control group, and Doxycycline (\bar{x} 161.03 μ m,173.58 μ m), and the Dried Digman and Katmon fruit extract (\bar{x} 202.39 μ m, \bar{x} 200.97 μ m) shows no significant difference in all the treatments, at 14days of exposure the Katmon fruit extract (\bar{x} 225.24 μ m) shows significant difference in comparison to the Control group(\bar{x} 165.86 μ m), Dried digman (\bar{x} 181.15 μ m), and Kamias fruit extract (\bar{x} 170.26 μ m), and Doxycycline (\bar{x} 213.81 μ m) on the other hand shows no significant difference compared to Katmon fruit extract, and Dried digman, but significantly different from the Kamias fruit extract and control group. The table for 21 days of exposure shows that Katmon fruit extract (227.41 μ m) and dried Digman (227.35 μ m) show significant differences compared to the control group (164.03 μ m), and Kamias fruit extract (198.12 μ m) shows no significant difference in comparison to Doxycycline (220.18 μ m). Kamias fruit extract shows no significant difference in Doxycycline. All throughout this data the Katmon perform the highest, Dried digman and Doxycycline shows no significant difference from the start of data gathering, Kamias fruit extract on the other hand perform the highest at first 5days of exposure but declined upon continuity of the exposure, this may be caused by the Kamias extract capability of lowering the PH level on the broiler's gut due its organic acid components and a study suggested that lowering the PH level on broiler chickens gut can enhance the overall performance and villi absorption capability Dibner, J.J.,etal.(2002), however the effect of long exposure to the Kamias fruit extract may lower the PH level in a level that broiler can't tolerate. These differences in the villi width of jejunum in the broiler chicken can affect their overall performance by enhancing the absorption capability of the intestine and widening the surface area of the villi. (Ivana Prakaturet et al., 2019)

Villi Surface Area

The villi surface area analysis shows that at 5days of exposure the Kamias fruit extract (\bar{x} 22.91mm²) shows a significant difference on the control group (\bar{x} 18.91mm²) and Doxycycline (\bar{x} 19.63mm²).However, when compared to Dried digman (\bar{x} 22.15mm²) and Katmon fruit extract (\bar{x} 22.01mm²) it shows no significant difference. The 14days of exposure resulted in a significant difference for Katmon fruit extract (\bar{x} 26.16mm²) in comparison to the Control group (\bar{x} 22.26mm²), Dried digman (\bar{x} 22.30mm²), and Kamias fruit extract (\bar{x} 22.08mm²) but statistically not significant at Doxycycline treatment (\bar{x} 23.51mm²), all the treatments also show no statistical difference at the mean value of Doxycycline. The data for 21days of exposure resulted in a significant difference for Katmon fruit extract (\bar{x} 27.13mm²), Dried digman (\bar{x} 26.14mm²), and Doxycycline (\bar{x} 25.95mm²), when compared to the Control group (\bar{x} 22.54mm²) and Kamias fruit extract treatment(\bar{x} 23.48mm²).The data shown that indicates the higher value of surface area in jejunums' villi enhances the absorbative capability of the intestine, this data of villi surface area also manifested in the study of Mile R.D.,et.al 2006 which uses antibiotics as a growth promoters on broiler chickens. This data may suggest that the chemical composition of the treatment said on the related studies may alter the commercial antibiotics in terms of growth promoting and the development of overall surface area.

Villi Length to Crypt Depth Ratio

The villi length and crypt ratio show the difference of the treatments in different number of days the subjects have been exposed. In the first 5 days of exposure, the Katmon Fruit extract treatment (\bar{x} 7.25 μ m) shows a significant difference in comparison to Control group (\bar{x} 5.04 μ m) Doxycycline (\bar{x} 4.94 μ m), Kamias (\bar{x} 4.88 μ m) and Dried Digman (\bar{x} 4.47 μ m). The data on comparing the treatments in their 14 days of exposure resulted in no significant difference in all the treatments, Katmon fruit extract data present the highest mean value of 6.16 μ m followed by Kamias fruit extract 5.65 μ m, Doxycycline 5.49 μ m, Dried digman 4.80 μ m, and control performing the least for having mean value of 4.67 μ m. The data comparing the treatments for their 21 days of exposure resulted in a significant difference for Katmon fruit extract (\bar{x} 7.21 μ m) in comparison to the 4 treatments, namely Doxycycline (\bar{x} 5.38 μ m), Dried digman (\bar{x} 4.91 μ m), Control (\bar{x} 4.80 μ m), and Kamias fruit extract (\bar{x} 4.73 μ m). This data suggests that a higher ratio of villi length to crypt depth is associated with improved digestion and nutrient absorption. Additionally, this measurement technique is used to assess the impact of various diets on gut microanatomy and for histological quantification of intestinal responses to disease processes, Wilson, F.D., et al 2018, also the lower the value of the villi to crypt ratio indicates severe damage K. Matsuda, et al 2020. It is noticeable that Kamias fruit extract show lowest mean value at the end of the experiment this poor performance of Kamias compared to control group may be due to the presence of high acid level on kamias fruit extract since that it could possibly affect the overall intestinal villi of the broiler chicken by lowering the PH level in a significant manner as resulted in the study of Rouhollah N., et al 2010.

Table 1:-Results of tests in difference dependent Variables.

Dependent Variables	Days of Exposure	Control	3% Dried Digman	Kamias F.E	Katmon F.E	Doxycycline
Body Weight	1day	228.08	225	221.85	211.14	224.23
	7days	491.63	491.48	486.54	465.56	498.7
	14days	930.67	953.35	911.7	872.38	961.4
	21days	1404	1424.37	1399.03	1381.15	1439.62
	7days	4217	3959	3713	3975	4027
Feed Intake	14days	10906	10084	9537	9587	9600
	21days	16820 ^a	15037 ^b	13921 ^b	14974 ^b	14294 ^b
Feed Conversion Ratio	7days	1.22	1.26	1.24	1.31	1.25
	14days	1.39	1.46	1.4	1.47	1.4
	21days	1.66	1.61	1.6	1.57	1.55
Villi Length	5days	720.38	780.07	793.72	771.77	720.88
	14days	986.08	884.39	923.62	981.35	832.45
	21days	989.31	978.4	888.09	1033.51	987.39
Villi Width	5days	161.03 ^c	202.39 ^{ab}	211.34 ^a	200.97 ^{ab}	173.58 ^{bc}
	14days	165.86 ^c	181.15 ^{bc}	170.26 ^c	225.24 ^a	213.81 ^{ab}
	21days	164.03 ^c	227.35 ^a	198.12 ^b	227.41 ^a	220.18 ^{ab}
Villi Surface Area	5days	18.91 ^c	22.15 ^{ab}	22.91 ^a	22.01 ^{ab}	19.63 ^{bc}
	14days	22.26 ^b	22.30 ^b	22.08 ^b	26.16 ^a	23.51 ^{ab}
	21days	22.54 ^b	26.14 ^a	23.48 ^b	27.13 ^a	25.95 ^a
Villi Length: Crypt Depth Ratio	5days	5.04 ^b	4.47 ^b	4.88 ^b	7.25 ^a	4.94 ^b
	14days	4.67 ^a	4.8 ^a	5.65 ^a	6.15 ^a	5.49 ^a
	21days	4.80 ^b	4.91 ^b	4.73 ^b	7.21 ^a	5.38 ^b

In a column, mean value with the same letter is not significantly different at $P \leq 0.005$ by LSD,

Conclusion:-

The Katmon fruit extract and dried Digman can have the highest potential to alter the commercial antibiotics in terms of enhancing the overall villi length, villi width, surface area, and villi length to crypt depth ratio of the jejunum's microvilli. Since Katmon performs higher, if not equally, in comparison to doxycycline treatment, in terms of growth performance, Katmon fruit extract steadily shows a higher mean value all throughout the experiment. Dried digman shows no significant difference in comparison to doxycycline treatment in most of the

parameters, proving that it also has the potential to alter commercial antibiotics. Kamias also had the potential to greatly affect the jejunum's villi morphology. Kamias fruit extract presented a significant difference from villi width and surface area at the 5 days of exposure, meaning the effect of the Kamias fruit extract could depend on the duration of exposure since the mean value diminishes as it prolongs the exposure. Given this data, the villi morphology, namely villi length, width, surface area, and villi length to crypt depth ratio, is commonly used as an indicator for the damage accession against diseases and harmful microbes.

Recommendation:-

1. Broiler chicken farmers may utilize the Digman and Katmon fruit for it is widely available on the country and there was no price value for these crops, and Digman also considered pest in some country and areas in the Philippines.
2. When it comes to the overall jejunum morphology the results of the study shows that the Katmon significantly enhance the intestinal absorption of jejunum, making it a good alternative for the commercial antibiotic as a growth promotants, while the Dried digman can also consider since it shows no significant difference on the doxycycline treatment for the whole experiment.
3. Using Kamias extract maybe a good growth promotant however the exposure to the extract can be decrease.
4. Researchers may use Katmon in different inclusion, or powderize the Katmon and check its chemical constituents/ compositions, may be by using a powderized Katmon that can be dilute into the water in much more manipulated manner could eliminate the viscosity issue that limiting the inclusion ratio. They can also reconduct the experiment with having a higher sample size and to add a blood serum parameter to analyze the other aspects of the broiler's health.
5. Dried digman may also be a valuable inclusion for the Broiler feeds, replacing other raw materials, may also be used for pelletized feeds, crumbles and mash.

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