



Phytochemical Content Analysis of 4 Marigold (*Tagetes Erecta*) Accessions in Bengkulu Province Which has the Potential as a Poultry Feed Supplement

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ABSTRACT

Feed is the main factor that determines the success of livestock farming, both in terms of quality and quantity of livestock production. Functional feed containing bioactive compounds can improve health, endurance, and livestock productivity. Marigold flowers (*Tagetes erecta* L.) are known to contain bioactive compounds such as flavonoids, terpenoids, and carotenoids that have potential pharmacological activities such as antioxidants, antimicrobials, and immunostimulants. Four marigold accessions (*Tagetes erecta*) were used, namely 1. WRRL (Orange crown with small size); 2. WRLB1 (Yellow crown with wide size) ; 3. WRLB2 (Orange crown with small upper part and wide lower part) ; 4. WRRM (Orange crown with wide size). Bioactive compounds were extracted using ethanol and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) method. The results of the analysis showed the presence of dominant compounds such as beta-Amyrin, Caprolactam, Vitamin E, and 9,12-Octadecadienoic acid which have the potential to have anti-inflammatory, antioxidant, and antibacterial activities. It is hoped that the results of this study can support the development of functional feed based on natural ingredients as an alternative to synthetic additives in the poultry farming industry that is more environmentally friendly and sustainable

INTRODUCTION

Feed in poultry is the main factor that determines the success of livestock farming, both in terms of quality and quantity of poultry production. The availability of good feed is not only intended to meet the nutritional needs of livestock such as protein, energy, vitamins, and minerals, but is also expected to provide additional effects that can improve health, endurance, and overall livestock productivity (Wina et al ., 2016). In modern livestock practices, the concept of feed is no longer just a source of nutrition, but has developed into functional feed , namely feed containing bioactive compounds that can provide additional health benefits for livestock (Hashemipour et al ., 2013).

The use of synthetic feed additives that are commonly applied so far, such as antibiotic growth promoters (AGP), in the long term is known to leave residues in livestock products such as meat, eggs, and milk , which have the potential to endanger consumer health and trigger antimicrobial resistance (Windisch et al ., 2008). This condition encourages researchers and livestock practitioners to look for alternative natural additives that are safer, more environmentally friendly, and more sustainable. One approach that is currently being widely developed is the use of plant-based phytogetic or phytoadditive materials, which contain bioactive compounds with diverse pharmacological activities (Wina et al ., 2016). Marigold flowers are one of the plants that have the potential as a source of natural phytoadditives, namely marigold flowers (*Tagetes erecta* L.). This plant has long been known to contain bioactive compounds including flavonoids, terpenoids, alkaloids, carotenoids, and thiophenes, each of which has biological activities such as antioxidants, antimicrobials, immunostimulants, anti-inflammatory, and antiparasitic (Sharangi & Acharya, 2009; Giri et al ., 2020). Marigolds with orange flower crowns have a protein content of 10.51% and carotenoids of 166.41 mg/kg (Rita, et al. 2024a). Several studies have shown that bioactive compounds in marigold flowers can improve poultry performance by suppressing the population of pathogenic microorganisms in the digestive tract, improving the balance of intestinal microflora, and increasing the efficiency of body metabolism (Hashemipour et al ., 2013; Ao & Kim, 2020).

Specific phytochemical profile information on marigold flower crowns, as well as their potential application as animal feed supplements, is still very limited. Therefore, identification of bioactive compounds through the Gas Chromatography-Mass Spectrometry (GC-MS) analysis method is a strategic step to describe the profile of volatile and semi-volatile compounds contained therein. This identification is important as a scientific basis in determining the active components that contribute to the biological activity of the plant (Bakkali et al ., 2008). Marigold flowers (*Tagetes erecta*) have good nutritional and phytochemical content as poultry feed additives (Rita., et al ., 2024b)

The results of this analysis are expected to not only provide comprehensive data on the composition of bioactive compounds in marigold flowers, but also become a basis for the development of functional feed supplement products for poultry based on natural ingredients that are safe, effective, and support the concept of healthy and sustainable farming. Based on this background, this study aims to identify bioactive compounds in the crown

of marigold flowers using the GC-MS method and evaluate the potential of these compounds as natural supplements in animal feed.

Based on the background description above, the formulation of the problem in this study is: 1). What are the types of bioactive compounds contained in marigold flower crowns (*Tagetes erecta* L.) based on the results of Gas Chromatography-Mass Spectrometry (GC-MS) analysis. 2). What is the potential of the identified bioactive compounds to be used as natural supplements in animal feed to support the health, performance, and productivity of poultry.

This study aims to 1). Identify the types of bioactive compounds contained in the crown of marigold flowers (*Tagetes erecta* L.) using the Gas Chromatography-Mass Spectrometry (GC-MS) method. 2). Evaluate the potential of these bioactive compounds in supporting the development of natural feed supplements that can improve the health, performance, and productivity of poultry.

LITERATURE REVIEW

Benefits of this study are 1). Providing scientific information on the profile of bioactive compounds of marigold flower crowns, especially volatile and semi-volatile compounds that have the potential as natural additives in poultry feed. 2). Becoming the basis for the development of safe, environmentally friendly, and sustainable plant-based functional feed supplements, as an alternative to the use of synthetic additives in the poultry industry. 3). Supporting efforts to improve the performance and health of poultry through the use of natural feed ingredients that have pharmacological activities such as antimicrobials, immunostimulants, and antioxidants.

METHODOLOGY

Time and Place

This study was conducted in March–April 2024. The sample extraction process and analysis of bioactive compounds using Gas Chromatography-Mass Spectrometry (GC-MS) were carried out at the DKI Jakarta Regional Health Laboratory. Four marigold flower samples were used, obtained from a survey in Bengkulu Province covering 7 districts/cities, namely Bengkulu City, Kepahiyang Regency, Rejang Lebong Regency, Central Bengkulu, Seluma, South Bengkulu and Kaur Regency.

Materials and Tools

The part of the plant used included the flower crown. The solvent used was 96% technical ethanol. The main tools included analytical scales, blenders, rotary evaporators, and GC-MS devices, Agilent Technologies type 7890B GC/5977A MSD.

Sample Preparation

Fresh marigold flowers were separated between the crown parts. The samples were dried using a sanggai machine at a temperature of 60-70°C for 48 hours until the water content reached $\pm 10\%$. After drying, the samples were crushed using a blender into a fine powder.

Extraction

A total of 100 grams of dry powder from each part of the marigold flower crown was extracted using 96% ethanol with a material to solvent ratio of 1:10 (w/v). The extraction process was carried out by maceration for 72 hours at room temperature with stirring every 12 hours. The maceration filtrate was then filtered using filter paper, and the solvent was evaporated using a rotary evaporator at 40°C until a thick extract was obtained.

GC-MS Analysis

The viscous extract from the rotary evaporator was analyzed using a GC-MS device with the following parameters: HP-5MS capillary column (30 m × 0.25 mm id × 0.25 µm film thickness), injector temperature of 250°C, and oven temperature program starting at 50°C (held for 2 minutes), then increased by 10°C per minute until it reached 280°C (held for 10 minutes). Helium was used as a carrier gas with a flow rate of 1 mL/min. Ionization was carried out using Electron Impact (EI) at an energy of 70 eV with a mass range of 50–550 m/z. Compound identification was carried out by matching the mass spectra of the analysis results to the NIST (National Institute of Standards and Technology) Library 2017 database.

Data Interpretation

The GC-MS analysis data in the form of chromatograms and lists of detected compounds are interpreted based on the percentage of peak areas and supported by literature related to their pharmacological activity, with the main focus on compounds that have the potential as antioxidants, antimicrobials, immunostimulants, and poultry health supporters.

RESULTS AND DISCUSSION

GC-MS Analysis Results

The results of Gas Chromatography-Mass Spectrometry (GC-MS) analysis of marigold flower crown extract showed the presence of various bioactive compounds that are suspected to play a role in biological activity. Based on the chromatogram results, a number of volatile and semi-volatile compounds were detected with varying peak area intensities. The main compounds identified include large groups such as sterols and triterpenoids, vitamin E (tocopherol), aliphatic hydrocarbons, aromatic derivatives, heterocyclic compounds (including thiophene, pyridone, pyridine, and benzoxepine), lactams, aliphatic ketones, alcohols, ethers, purines, piperazine derivatives, and phenol derivatives. These compounds are included in secondary metabolites or natural bioactive compounds that are commonly found in plant, animal, and microorganism extracts, and are often associated with pharmacological activity and functional benefits (Harborne, 1998; Dewick, 2002; Crozier et al., 2006). Marigold accession WRR1 contains 20 compounds, marigold accession WRLB2 contains 19 compounds, marigold accession WRRM contains 17 compounds, and marigold accession WRLB1 also contains 17 compounds as presented in Table 1.

Table 1. Compound Content of Marigold Flower Crown Extract (*Tagetes Erecta*)
With GC-MS analysis

Compound	Group	WRR M	WRLB 1	WRLB 2	WRR L
+/-Tetrahydro-3-furanmethanol	Furan Alcohol Derivatives	2.51		2.35	2.23
Phenol, 2,6-dimethoxy-	Phenol Derivatives	4.87			
n-Hexadecanoic acid	Fatty acid	4.20			
9,12-Octadecadienoic acid (Z,Z)-	Fatty acid	8.06	1.22	12.49	10.55
Octadecanoic acid	Fatty acid	4.55	7.12	4.08	2.54
Hexacosane	Aliphatic Hydrocarbons	6.78			
beta-D-Mannothiofuranoside, Sn-octyl-	Aliphatic Thiofuranosides	7.30			
Caprolactam	Cyclic Lactams	16.20			
Squalene	Triterpenoid	2.06	6.13	1.15	2.48
Hexadecane, 1-iodo-	Fatty Acid Ester	1.12			
beta-Tocopherol	Vitamin E / Tocopherol	1.52			1.17
gamma-Tocopherol	Vitamin E / Tocopherol	1.33	1.17		
Gamma-Tecoperol	Vitamin E / Tocopherol				1.07
Hentriacontane	Aliphatic Hydrocarbons	1.05			
Vitamin E	Vitamin E / Tocopherol	6.85	6.78	2.28	3.83
gamma-Sitosterol	Stereo	2.27	3.16	1.29	
beta-amyrin	Triterpenoid	18.67	14.47	8.04	11.19
alpha-amyrin	Triterpenoid	3.41	3.22	2.04	2.58
Benzoic acid, 4-hydroxy-3,5-dimethoxy-	Aromatic Acid Derivatives		1.44	1.29	1.51
Hexadecanoic acid, methyl ester	Fatty Acid Ester		1.30		
n-Hexadecanoic acid	Fatty acid		11.41	6.79	4.98
10,13-Octadecadienoic acid, methyl ester	Fatty Acid Ester		1.22		
11-Octadecenoid acid, methyl ester	Fatty Acid Ester		1.12		

Compound	Group	WRR M	WRLB 1	WRLB 2	WRR L
2,2';5',2''- Terthiophene	Heterocyclic Compounds (Thiophene)		4.23	1.14	1.10
Hexacosane	Long chain alkanes (2.85		
Octadecane	Aliphatic Hydrocarbons		1.88		
Eicosane	Aliphatic Hydrocarbons		1.78		
Tetradecanoic acid	Fatty acid			1.43	1.58
Tetradecane	Aliphatic Hydrocarbons			5.26	4.37
4-Fluoro-2-pyridone, N-acetyl-	Heterocyclic Compounds (Pyridone)			7.39	
5-Octyn-1-ol tetrahydrofuranol ether	Aliphatic Ether Alcohol			8.22	
Caprolactam	Cyclic Lactams			18.90	
5-Fluoro-2- hydroxypyridine	Heterocyclic Compounds (Pyridine)			7.61	2.63
Linoleylmethyl ketone	Aliphatic Ketone			1.56	1.36
4-Pyrrolidin-1-yl- benzene-1,3-diol	Aromatic Amine Compounds			1.06	
5-Hexen-2-one, 4- methyl-3-phenyl-	unsaturated aromatic ketone				3.47
7-tridecanone	Aliphatic Ketone				
4-(2-Hydroxyethyl) piperazine-2-one, TMS	Piperazine Derivatives				1.19
6H-Purine-6-one, 3- (dimethylamino)-1,7- dihydro	Purine Derivatives				1.17
4-Pyrrolidin-1-yl- benzene-1,3-diol	Aromatic aminophenols				1.06

Dominant compounds based on the highest area percentage in each accession: WRRL: Caprolactam (18.90 %) & 9,12-Octadecadienoic acid (10.55%). WRRM: beta-Amyrin (18.67 %) & Caprolactam (16.20%). WRLB1: beta-Amyrin (14.47 %) & Vitamin E (6.78%). WRLB2: 9.12 -Octadecadienoic acid (12.49%) & beta-Amyrin (8.04%). The dominant compound is a chemical compound that has the highest area percentage in the results of gas chromatography- mass spectrometry (GC-MS) analysis of a sample, which indicates that the compound is the main or most abundant component contained in the extract or fraction analyzed (Kamboj & Saluja, 2012; Olsson et al ., 2019). The area percentage represents the relative proportion of the compound compared to the total compounds detected in the sample (Sánchez et al ., 2014).

Table 2. Summary of Compounds Found in 4 Marigold Flower Accessions (Tagetes Erecta)

Compound	Group	WRR M	WRLB 1	WRLB 2	WRR L
+/-Tetrahydro-3-furanmethanol	Furan Alcohol Derivatives	2.51	-	2.35	2.23
Phenol, 2,6-dimethoxy-	Phenol Derivatives	4.87	-	-	-
n-Hexadecanoic acid	Fatty acid	4.20	11.41	6.79	4.98
9,12-Octadecadienoic acid (Z,Z)-	Fatty acid	8.06	1.22	12.49	10.55
Octadecanoic acid	Fatty acid	4.55	7.12	4.08	2.54
Hexacosane	Aliphatic Hydrocarbons	6.78	2.85	-	-
beta-D-Mannothiofuranoside, Sn-octyl-	Aliphatic Thiofuranosides	7.30	-	-	-
Caprolactam	Cyclic Lactams	16.20	-	-	18.90
Squalene	Triterpenoid	2.06	6.13	1.15	2.48
beta-Tocopherol	Vitamin E / Tocopherol	1.52	-	-	1.17
gamma-Tocopherol	Vitamin E / Tocopherol	1.33	1.17	-	1.07
Vitamin E	Vitamin E / Tocopherol	6.85	6.78	2.28	3.83
gamma-Sitosterol	Sterol	2.27	3.16	1.29	-
beta-amyrin	Triterpenoid	18.67	14.47	8.04	11.19
alpha-amyrin	Triterpenoid	3.41	3.22	2.04	2.58
Benzoic acid, 4-hydroxy-3,5-dimethoxy-	Aromatic Acid Derivatives	-	1.44	1.29	1.51

2,2';5',2''- Terthiophene	Heterocyclic Compounds (Thiophene)	-	4.23	1.14	1.10
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The results of GC-MS analysis of extracts of four marigold accessions showed variations in the composition of dominant compounds in each accession. The WRRL accession had the highest content of Caprolactam (18.90 %) and linoleic acid (10.55%). The main compounds found in the WRRM accession were beta-Amyrin (18.67 %) and Caprolactam (16.20%). The WRLB1 accession was also dominated by beta-Amyrin (14.47 %), accompanied by Vitamin E (6.78%). The WRLB2 accession showed a dominance of linoleic acid (9.12 - Octadecadienoic acid) of 12.49%, followed by beta-Amyrin (8.04%). These findings reflect the diversity of bioactive compounds between accessions that can affect the bioactivity characteristics of marigold flower extracts (Kumar et al ., 2020; Rohman *et al.* , 2019).

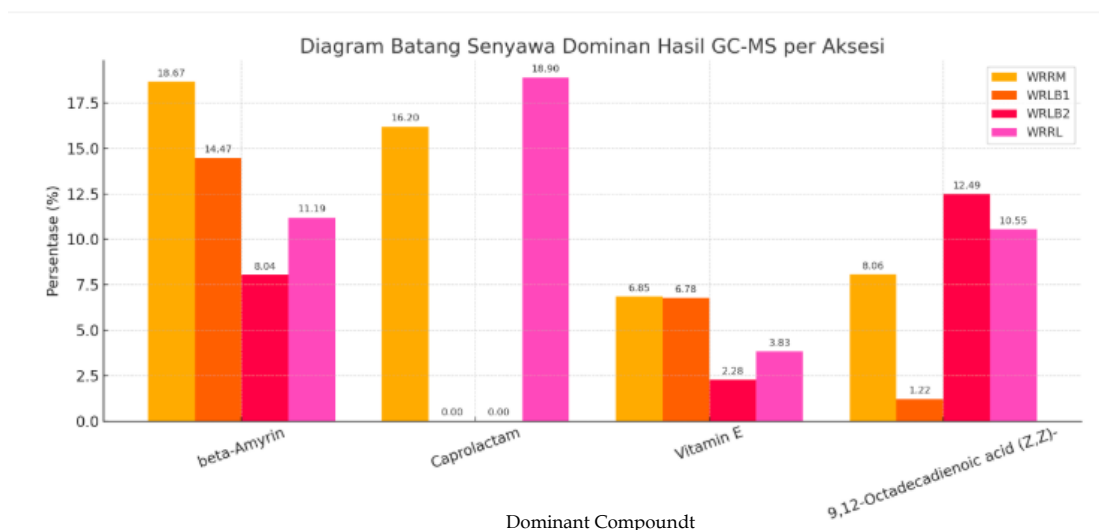


Figure 1. Bar Diagram of Dominant Compounds From GC-MS Results

In this study, each compound identified by GC-MS was plotted horizontally based on its relative concentration (%) for each accession: WRRM, WRLB1, WRLB2, and WRRL. The bar chart visualization shows the dominant compounds in each accession, where some compounds have a much higher area percentage, indicating that they are the main components in the extract of a particular accession. The dominance patterns of these compounds can be directly compared through the diagram, which helps researchers in identifying the main compounds that potentially contribute to bioactive activity. These results also provide a basis for further analysis of the relationship between dominant compound content and potential bioactivity (Zhao et al ., 2018; Li et al ., 2019).

WRRL accession is dominated by Caprolactam (18.90 %), a cyclic lactam compound, β -Amyrin (11.19%) from triterpenoids, and 9,12-Octadecadienoic acid (Z,Z) (10.55%) as unsaturated fatty acids. In addition, Vitamin E (3.83 %),

Tetradecane (4.37%), and aliphatic hydrocarbon compounds were also detected. Caprolactam, although better known in the polymer industry, has the potential to show certain biological activities (Kumar et al., 2020). β -Amyrin, a triterpenoid with anti-inflammatory, antimicrobial, and hepatoprotective activities, and 9,12-Octadecadienoic acid which has benefits as an antioxidant and in preventing cardiovascular disease, were also detected in significant concentrations (Jäger et al., 2009; Aziz et al., 2017). Vitamin E acts as an important natural antioxidant, while Tetradecane functions as an aliphatic hydrocarbon compound. The dominance of these bioactive compounds shows the great potential of WRRM in various applications, especially in the fields of health, antioxidant supplements, and the natural-based functional food industry. WRRM accessions show significant bioactivity potential, especially in antioxidant, anti-inflammatory, and antimicrobial properties, with Caprolactam (cyclic lactam) domination, followed by β -Amyrin (triterpenoid) and 9,12-Octadecadienoic acid (unsaturated fatty acid) (Kumar et al., 2020; Jäger et al., 2009; Aziz et al., 2017). The dominant presence of Caprolactam opens up opportunities for further research into its pharmacological benefits, especially in the health and therapeutic fields, although this compound is better known in the polymer industry. The composition of these compounds makes WRRM an attractive candidate for the development of natural product-based products in the healthy food, health supplement, and natural cosmetic industries that rely on antioxidant and anti-inflammatory properties (Brigelius-Flohé & Traber, 1999; Jäger et al., 2009). In general, all accessions showed similar patterns of compound dominance, with differences in the relative proportions of each compound.

WRRM accession is dominated by several main bioactive compounds with relatively high concentrations, including β -Amyrin (18.67%), Caprolactam (16.20%), 9,12-Octadecadienoic acid (*Z,Z*)- or linoleic acid (8.06%), Hexacosane (6.78%), and Vitamin E (Tocopherol) (6.85%). β -Amyrin, a triterpenoid compound, is a dominant compound known to have pharmacological activities such as anti-inflammatory, antibacterial, and anticancer (Jäger et al., 2009). Caprolactam, a cyclic lactam compound, was also found in significant amounts and showed certain bioactivity potential (Kumar et al., 2020). Linoleic acid has antioxidant and anti-inflammatory activities (Aziz et al., 2017), while Hexacosane is known to have antibacterial activity and as an insect repellent (El-Gengaihi et al., 2013). Vitamin E or Tocopherol plays an important role as an antioxidant in protecting cells from oxidative stress (Brigelius-Flohé & Traber, 1999). These results indicate that WRRM accession is rich in triterpenoid compounds, unsaturated fatty acids, and cyclic lactams that have the potential to have pharmacological benefits.

WRRM accession was shown to be rich in triterpenoid compounds (β -Amyrin), unsaturated fatty acids (9,12-Octadecadienoic acid), and cyclic lactams (Caprolactam) among other accessions studied. The composition of these bioactive compounds shows significant potential pharmacological benefits, such as anti-inflammatory and hepatoprotective activities of β -Amyrin (Jäger et al., 2009), antioxidant activity and reducing the risk of cardiovascular disease of linoleic acid (Aziz et al., 2017), and the potential bioactivity of Caprolactam in

medical applications (Kumar et al ., 2020). Based on these results, WRRM has the potential as a natural raw material for health products, antioxidant supplements, and additives in the healthy food industry. The composition of these compounds also provides an important basis for further exploration of the pharmacological activity, safety, and effectiveness of WRRM extracts in various natural ingredient-based applications.

Accession WRLB1 is dominated by Caprolactam compound (18.90 %), which is a cyclic lactam, followed by β -Amyrin (14.47%) and Squalene (6.13%), both of which are triterpenoid compounds, and n-Hexadecanoic acid (11.41%), a saturated fatty acid. These compounds show significant potential biological activities. Caprolactam has certain potential medical applications (Kumar et al ., 2020), while β -Amyrin and Squalene have anti-inflammatory, hepatoprotective, and immunomodulatory activities (Jäger et al ., 2009). n-Hexadecanoic acid is known to have antimicrobial, anti-inflammatory, and antioxidant activities (Garba & Samuel, 2011). In addition, Vitamin E (6.78 %), which functions as a natural antioxidant, was also detected in this extract (Brigelius-Flohé & Traber, 1999). The dominance of these compounds indicates that WRLB1 has the potential to be developed for applications in natural health products, antioxidant supplements, and additives in the healthy food industry. The presence of Caprolactam in high concentrations opens up opportunities for further research related to bioactive potential and its applications in natural product-based products.

Accession WRLB2 is dominated by the compound 9,12 - Octadecadienoic acid (Z,Z) (12.49%), which is an unsaturated fatty acid, β -Amyrin (8.04%) from the triterpenoid group, and Caprolactam (18.90%) as a cyclic lactam. In addition, Tetradecane (5.26 %) from the aliphatic hydrocarbon group, and Vitamin E (2.28%) or Tocopherol as a natural antioxidant were also detected. The compound 9,12 - Octadecadienoic acid is known to have antioxidant and anti-inflammatory activities and plays a role in cardiovascular health (Aziz et al ., 2017). Caprolactam, although better known in the polymer industry, has the potential to have bioactive activity (Kumar et al ., 2020), while β -Amyrin has benefits as an anti-inflammatory, hepatoprotective, and antimicrobial (Jäger et al ., 2009). Tetradecane and Vitamin E also contribute to the potential bioactivity, with Vitamin E functioning as an antioxidant that protects cells from free radicals (Brigelius-Flohé & Traber, 1999). The dominance of these bioactive compounds suggests that WRLB2 has great potential in the development of natural health products, antioxidant supplements, and applications in the natural-based functional food industry. Further research is needed to test its potential bioactivity and pharmacology. The compounds contained in marigold flowers and their roles as feed supplements for poultry are presented in Table 3.

Table 3. Phytochemical Compounds and Their Functions in Poultry

Compound	Main Functions	Uses in Chicken	Scientific References
9,12-Octadecadienoic acid (Linoleic Acid)	Essential fatty acids, energy metabolism	Increase body weight, improve meat quality	Kamaleldin & Shahidi (2002)
Vitamin E (alpha, beta, gamma-Tocopherol)	Antioxidant, immunostimulant	Reduce mortality, strengthen the immune system, increase carcass weight	Mane (2002)
Squalene	Antioxidant, hepatoprotective	Increases resistance to heat stress and infection	Kelly (1999)
gamma-Sitosterol	Anti-inflammatory, lowers cholesterol	Improves gut health, supports growth	Bouic (2001)
beta- & alpha-Amyrin	Anti-inflammatory, immunomodulator	Increase appetite and immune response	Chakraborty & Partners (2008)
Benzoic acid, 4-hydroxy-3,5-dimethoxy- (sinapic acid derivative)	Antioxidant, antimicrobial	Increase feed efficiency, suppress pathogenic microbes	Peng <i>et al.</i> (2010)

The improvement of broiler chicken production performance is not only determined by the content and quality of nutrition, but is also greatly influenced by the supplementation of bioactive compounds that support health and metabolic efficiency. Several natural compounds have been shown to have significant functional roles in the digestive, immune, and metabolic systems of chickens.

Linoleic acid (9,12 - Octadecadienoic acid) is one of the essential unsaturated fatty acids that plays a role in the formation of cell membranes and energy metabolism. Supplementation of linoleic acid in feed has been shown to increase final body weight and improve carcass quality of broiler chickens, especially in the form of more proportional body fat (Kamal-Eldin & Shahidi, 2002).

Vitamin E in the form of alpha-, beta-, and gamma-tocopherol, is known as a natural antioxidant that protects the body tissues of laying hens from oxidative stress and can increase ration efficiency, increase immunity and reduce mortality rates in chickens experiencing environmental stress (Surai, 2002).

Squalene, a triterpenoid compound found abundantly in fish liver oil and certain plants, exhibits antioxidant and immunoprotective activities.

Administration of squalene in certain amounts can help chickens survive temperature stress conditions and improve the response to vaccination (Kelly, 1999).

Gamma-sitosterol, a phytosterol with a chemical structure similar to cholesterol, is able to lower cholesterol levels in blood serum and support the health of the chicken's digestive tract. The anti-inflammatory effects of this compound are also useful in suppressing the risk of infection and increasing growth (Bouic, 2001).

Beta- and alpha-amyrin, two natural triterpenoids from plants, have a wide range of biological activities including anti-inflammatory and hepatoprotective effects. Amyrin supplementation in chicken feed can help increase appetite and accelerate weight gain by maintaining optimal liver function (Chakraborty & Mitra, 2008).

Benzoic acid derivatives (4-hydroxy-3,5-dimethoxy- or sinapic acid derivatives) can be used as natural feed additives due to their antioxidant and antimicrobial activities. These compounds help control pathogenic microbes in the digestive tract, improve intestinal health, and increase the efficiency of feed nutrient utilization (Peng et al., 2010).

Bioactive compounds detected in the 4 accessions studied include Caprolactam, β -Amyrin, Squalene, and 9,12-Octadecadienoic acid (linoleic acid), showing great potential as natural feed additives that can support the immune system, growth, and feed efficiency of broiler chickens. The use of these materials is an environmentally friendly alternative to synthetic antibiotics, in line with the global trend towards sustainable food production.

GC-MS analysis of these 4 accessions (WRRM, WRLB1, WRLB2, and WRRL) showed three dominant compound groups: cyclic lactams (Caprolactam), triterpenoids (such as β -Amyrin and Squalene), and unsaturated fatty acids (especially linoleic acid). Caprolactam was found in high concentrations, especially in WRRL and WRLB1 which have potential bioactivities including antimicrobial activity. β -Amyrin has anti-inflammatory, hepatoprotective, antibacterial, and anticancer effects. While Squalene supports the immune system which functions as an antioxidant. Linoleic acid plays a role in maintaining cardiovascular health which has anti-inflammatory and antioxidant properties.

Vitamin E (Tocopherol) was also detected in all accessions and acts as a natural antioxidant to protect cell membranes from oxidative stress. In addition, aliphatic hydrocarbon compounds such as Hexacosane and Tetradecane, although in smaller amounts, contribute to certain biological activities.

The results show that WRRL accession has a diverse composition of bioactive compounds that have the potential to be used for health products, antioxidant supplements, and additives in the poultry healthy feed industry. The diversity of these compounds also forms the basis for further research related to its bioactivity, toxicity, and clinical effectiveness.

CONCLUSION AND RECOMMENDATION

Provide some conclusions and the implementation of the research results. Marigold accession WRRL contains the most compounds, namely 20, WRLB2 19 compounds, while WRRM and WRLB1 17 compounds. Natural bioactive compounds obtained such as linoleic acid, vitamin E (tocopherol), squalene, gamma-sitosterol, beta- and alpha-amyrin, and benzoic acid derived from sinapic acid show great potential as a poultry feed supplement. Each compound has a biological function that supports broiler chicken performance, either by improving the immune system, feed efficiency, or controlling oxidative stress. The use of this natural supplement provides a safer and more sustainable alternative to the use of antibiotic growth promoters (AGPs), and can contribute to the quality of healthier and higher-value livestock products.

Marigold accessions have potential bioactive compounds that are used to increase poultry productivity. Further research is needed by utilizing bioactive compounds from marigold flowers, especially WRRL accessions as natural feed additives in poultry feed.

FUTHER STUDY

This research still has limitations so further research is needed on the topic of Marigold (*Tagetes erecta*) as a poultry feed ingredient in order to perfect this research and increase insight for readers.

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