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TRUE METABOLIZABLE ENERGY OF GLYCERIN IN ADULT ROOSTERS: PAVING THE WAY FOR SUSTAINABLE POULTRY NUTRITION IN SOUTHEAST ASIA

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Abstract

With rising global demand for sustainable feed alternatives, glycerin, a biodiesel byproduct, holds significant promise as an energy-rich ingredient. This study determined the true metabolizable energy (TME) and nutrient retention of glycerin in adult roosters, assessing its viability as an energy source for poultry diets, particularly in Southeast Asia. A rooster bioassay involving 12 Single Comb White Leghorn roosters, force-fed glycerin or fasted, was used to quantify metabolizable energy and digestibility coefficients. Glycerin, with a gross energy of 6,137 kcal/kg, exhibited high digestibility, particularly for ether extract (95.02%) and carbohydrates (91.37%). The calculated TME was 5,779.42 kcal/kg and TMEn 5,779.15 kcal/kg, indicating comparable energy levels to those of conventional poultry feed ingredients despite its low nitrogen content. These findings affirm glycerin's potential as a highly digestible, resource-efficient alternative for sustainable feed formulation. Its strategic inclusion can enhance the resilience and cost-effectiveness of poultry production systems in the ASEAN region, contributing to waste valorization and food security. The study provides crucial data to optimize feed formulations and promote environmentally friendly practices.

Keywords: Glycerin, True Metabolizable Energy, Poultry Nutrition, Adult Roosters, Sustainable Feed

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Introduction

Poultry production plays a critical role in food security and rural livelihoods across the ASEAN region. With rising demand for sustainable and cost-effective feed ingredients, alternative energy sources such as glycerin, a byproduct of biodiesel production, have gained attention for their potential nutritional value. Previous studies have investigated glycerin's metabolizable energy in poultry; however, research in the ASEAN context remains limited, and variations in glycerin composition across production processes are often overlooked. This study aims to address these gaps by determining the Apparent Metabolizable Energy (AME and AMEn) and True Metabolizable Energy (TME and TMEn) of glycerin in adult roosters, providing practical insights for feed formulation, cost reduction, and sustainable poultry nutrition.

The rising production of biodiesel in many countries has generated a growing surplus of crude glycerin, a byproduct whose disposal poses both environmental and economic concerns. Consequently, interest has increased in repurposing glycerin as an alternative energy source for poultry feed, particularly in regions facing feed-ingredient shortages. In Southeast Asia, where traditional feed ingredients such as corn and soybean meal are often expensive or in short supply, glycerin offers a promising, resource-efficient alternative. Recent investigations support this potential: for example, glycerin inclusion in broiler diets did not impair performance or nutrient digestibility at moderate inclusion levels (Carvalho et al., 2024), and earlier studies demonstrated that raw glycerin from the biodiesel industry can effectively replace a portion of dietary energy in broilers (Melo, 2015). Given the variability in glycerin composition across sources and production processes, accurate assessment of its true metabolizable energy (TME) and nutrient retention is essential before widespread adoption in poultry production systems. Therefore, evaluating glycerin via standardized methods such as the adult rooster bioassay can generate reliable data to support sustainable feed formulation for poultry farms across ASEAN.

Glycerin, a three-carbon alcohol, is a significant by-product of biodiesel production from vegetable oils or animal fats, accounting for approximately 10% of the input feedstock by weight (Dasari et al., 2005). With its high purity and energetic potential, gross energy approximately 4,100 kcal/kg (Thompson & He, 2006), glycerin has been increasingly explored as a valuable ingredient across multiple sectors, including pharmaceuticals, cosmetics, and animal nutrition.

In poultry nutrition, glycerin has garnered interest as a potential energy source due to its high metabolizable energy and digestibility. Previous studies have reported that glycerin supplementation at levels of 5-10% in broiler diets can improve growth performance and carcass characteristics (Simon et al., 1996; Barteczko & Kaminski, 1999). Furthermore, glycerin's sweet taste and low molecular weight facilitate intestinal absorption, with more than 97% absorption efficiency in laying hens (Bartelt & Schneider, 2002). Once absorbed, glycerin can be metabolized via glycolysis or converted to glucose through gluconeogenesis, contributing significantly to cellular energy production (Robergs & Griffin, 1998; Krebs & Lund, 1996).

Despite its potential, published data on the true metabolizable energy (TME) and corrected TME (TMEn) of glycerin in poultry remain limited. While apparent metabolizable energy (AME) values have been estimated in various poultry species such as 3,434 kcal/kg in broilers (Dozier et al., 2008), 3,805 kcal/kg in laying hens (Lammers et al., 2008; Min et al., 2010), and 4,200 kcal/kg in turkeys (Rosebrough et al., 1980a, 1980b) data generated using precision-fed assays in roosters are scarce.

Given the increasing availability of glycerin as a feed ingredient and the lack of robust TME-based evaluations in poultry, the present study was conducted to determine the AME, AMEn, TME, TMEn, and the coefficient of total tract apparent retention (CTTAR) values of glycerin using adult roosters in a bioassay setting. By determining the AME, AMEn, TME, and TMEn values for glycerin, this study aims to provide essential data to guide the formulation of cost-effective, nutritionally balanced poultry diets. These findings are expected to enhance feed efficiency, reduce reliance on conventional energy sources, and promote the sustainable utilization of biodiesel byproducts, thereby supporting environmentally friendly and economically viable poultry production in the ASEAN region.

Literature Reviews

Glycerin as a Byproduct and Its Nutritional Potential

Glycerin, a trihydroxy sugar alcohol, is a significant by-product of biodiesel production, contributing approximately 10% of the weight of the vegetable oil or animal fat used in the transesterification process (Dasari et al., 2005). Historically recognized for its utility in food, cosmetics, and pharmaceuticals, glycerin has recently garnered attention as a valuable energy source in animal nutrition due to its high gross energy value of approximately 4,100 kcal/kg (Thompson & He, 2006). Its potential as an energy source in poultry feed makes it a candidate for sustainable, cost-effective diet formulations.

Inclusion Levels and Effects in Poultry Diets

Numerous studies have evaluated the inclusion of glycerin in poultry diets. For broilers, supplementation at levels between 5% and 10% has shown improvements in feed conversion and lean carcass yield (Simon et al., 1996; Barteczko & Kaminski, 1999). However, higher inclusion levels, such as 10-15%, have been associated with metabolic alterations, including increased blood cholesterol and triglyceride levels (Barteczko & Kaminski, 1999). Cerrate et al. (2006) reported feed flow issues and reduced weight gain at 10% inclusion levels in broilers. These findings highlight the importance of determining safe and optimal inclusion rates to avoid adverse effects.

Absorption and Metabolism of Glycerin in Poultry

Glycerin is rapidly and efficiently absorbed in the gastrointestinal tract, with absorption rates exceeding 97% in laying hens (Bartelt & Schneider, 2002). Once absorbed, glycerin enters metabolic pathways either via glycolysis or gluconeogenesis, serving as a substrate for ATP production. Up to 60% of absorbed glycerin is utilized for energy metabolism under basal conditions (Robergs & Griffin, 1998), and its metabolism yields 22 moles of ATP per mole, indicating a high energy potential (Krebs & Lund, 1996). This metabolic efficiency underscores its suitability as a partial substitute for conventional energy sources such as oils or corn.

Metabolizable Energy Values of Glycerin

Studies on the metabolizable energy (AMEn) of glycerin have reported AMEn values ranging from 3,434 to 3,805 kcal/kg in broilers and laying hens (Dozier et al., 2008; Min et al., 2010; Lammers et al., 2008). In turkeys, an AME value of 4,200 kcal/kg has been reported (Rosebrough et al., 1980a, 1980b). Despite this, there remains limited data on the metabolizable energy of glycerin as a single ingredient in poultry diets. Controlled studies, such as the present work evaluating true metabolizable energy using rooster assays, are necessary to provide more precise data for feed formulation.

Comparative Insights and Regional Context

Comparisons with swine nutrition suggest similar energy utilization efficiencies, supporting glycerin's viability as a partial substitute for conventional energy sources in monogastric animals (Bartelt & Schneider, 2002; Lammers et al., 2007). Recent studies in the ASEAN region have examined glycerin as an alternative feed ingredient in poultry diets; however, results vary due to differences in glycerin source, purity, and experimental methods. By critically evaluating these conflicting findings, this review highlights existing knowledge gaps and underscores the need for standardized assessment of glycerin's true metabolizable energy and nutrient retention in local contexts.

Research Methodology

Experimental Design and Animal Management

This study utilized a rooster bioassay to evaluate the metabolizable energy values of glycerin. A total of 12 adult Single Comb White Leghorn roosters (29 weeks old, average body weight 2,442 g) were housed individually in stainless steel cages (50 × 40 × 50 cm) in a temperature-controlled room (25.2 ± 1.3 °C). Each cage was equipped with an excreta collection tray, a nipple waterer, and a feeder. The birds underwent a 7-day acclimation period during which they were fed a standard commercial diet and had ad libitum access to water supplemented with a vitamin-electrolyte premix (Permasol-500, Choong Ang Biotech Co., Ltd., Korea).

Adult roosters were selected for this study based on the Sibbald (1986) precision-fed rooster bioassay, a widely accepted method for determining True Metabolizable Energy (TME) of feed ingredients. This model allows for standardized assessment of energy utilization and nutrient retention, minimizing variability due to age, growth stage, or production type. The results obtained from adult roosters can be extrapolated to other poultry species, providing a reliable basis for feed formulation and practical application in poultry nutrition.

The Animal Care and Use Committee of Kangwon National University, Republic of Korea, approved all experimental protocols.

Glycerin Preparation and Force-Feeding Procedure

Glycerin was sourced from a local supplier in South Korea and stored at 4 °C until used. "The glycerin used in this study was obtained as a byproduct of palm oil biodiesel production. The sample's chemical composition was analyzed and is presented in Table 1, including pH, dry matter, gross energy, nitrogen, crude protein, ether extract, ash, organic matter, and total carbohydrates. The glycerin purity was confirmed to be high, with low residual nitrogen and protein content. Providing these details ensures consistency in experimental conditions and enables accurate interpretation of True Metabolizable Energy (TME) and nutrient retention data.

A feeding level of 15 g glycerin per rooster was selected following standardized protocols for TME determination in adult roosters. This dose provides measurable energy intake and nutrient excretion without exceeding the birds' metabolic capacity, enabling accurate calculation of metabolizable energy values. The selection is consistent with previous studies using the precision-fed rooster bioassay (e.g., Sibbald, 1986), enabling meaningful comparisons across studies. Following a 24-hour fasting period, six roosters were randomly selected and force-fed 15 g of glycerin via a stainless-steel funnel (34.5 cm stem, 0.9 cm outer diameter, 0.7 cm inner diameter). The remaining six roosters were kept fasted to quantify endogenous energy and nitrogen losses. Excreta from both groups were quantitatively collected for 48 hours, then freeze-dried and processed for chemical analysis.

Chemical Analysis

All feed and excreta samples were ground and analyzed in triplicate for proximate composition following AOAC International (2005) guidelines. Dry matter was determined after oven drying at 105 °C for 5 hours. Ash content was analyzed by ignition at 550 °C in a muffle furnace for 5 hours. Ether extract was measured using a Soxtest analyzer (Raypa®), and gross energy was assessed using an oxygen bomb calorimeter (Model 1261, Parr Corp., USA). Nitrogen content was determined by a Kjeldahl analyzer (Model 2300, Kjeltec, Foss Tecator, Sweden), and crude protein (CP) was calculated using the standard conversion factor (N × 6.25) as shown in Table 1.

Energy and Nutrient Utilization Calculations

The apparent metabolizable energy (AME), nitrogen-corrected AME (AMEn), true metabolizable energy (TME), and nitrogen-corrected TME (TMEn) were calculated using equations described by Sibbald (1986):

- AME (kcal/kg) = (EI - EO)/FI

- AMEn (kcal/kg) = AME - (8.22 × ANR/FI)
- TME (kcal/kg) = AME + (FEL/FI)
- TMEn (kcal/kg) = TME - (8.22 × ANR/FI) - (8.22 × FNL/FI)

Where:

- EI is energy intake,
- EO is energy output,
- FI is feed intake (g),
- FEL is fasting energy loss,
- FNL is fasting nitrogen loss,
- ANR is apparent nitrogen retention.

The coefficient of total tract apparent retention (CTTAR) for each nutrient was calculated using the formula:

CTTAR (%) = (Nutrient intake - Nutrient excretion) / Nutrient intake × 100

Organic matter (OM) was calculated by subtracting ash from the dry matter content as per Kawauchi et al. (2011). Total carbohydrate content was estimated by the difference method: CHO (%) = DM − (protein + fat + ash).

Table 1 Analyzed nutrient composition of glycerin and excreta of both fasted and fed-roosters

Nutrients	Excreta of both		Glycerin
	Fed glycerin	Fasted roosters	
pH			7.25
Dry matter (%)	89.28	96.36	82.21
Gross energy (Kcal/kg)	5182.38	3940.68	6137.16
Nitrogen (%)	7.55	13.33	0.02
Crude protein (%)	47.18	83.33	0.12
Ether extract (%)	2.31	0.97	20.79
Ash (%)	35.63	36.83	1.97
Organic matter (%)	64.37	63.17	98.03
Total CHO (%)	4.16	NA	59.34

Note: NA = Not available

Statistical Analysis

All data were analyzed using a completely randomized design. Descriptive statistics (mean ± standard deviation) were calculated for all measured variables. Differences in nutrient retention and True Metabolizable Energy (TME) values were evaluated using one-way analysis of variance (ANOVA), and significant differences between means were determined using Tukey's post hoc test. Significance was declared at $p < 0.05$. All statistical analyses were performed using [software name, version], ensuring accuracy and reproducibility of the results.

Results and Discussion

The analyzed nutrient composition of glycerin and excreta from both fasted and glycerin-fed roosters is presented in Table 1. The glycerin sample contained 82.21% dry matter, 6,137 kcal/kg of gross energy, 0.02% nitrogen, 0.12% crude protein, 20.79% ether extract, 1.97% ash, 98.03% organic matter, and 59.34% total carbohydrate. Notably, glycerin exhibited a high ether extract and carbohydrate content but was extremely low in nitrogen (Figure 1), consistent with values reported by Bartelt & Schneider (2002) and Min et al. (2010).

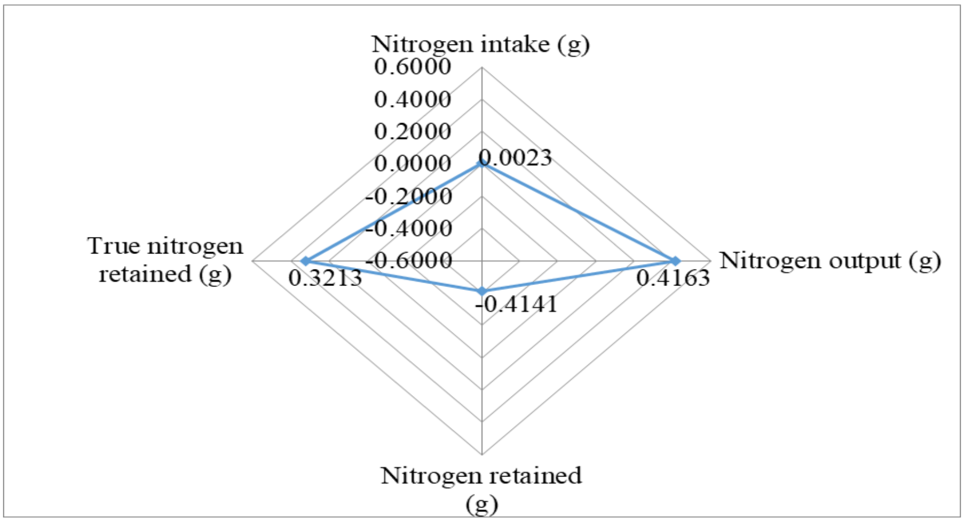

Figure 1 Nitrogen balance in rooster forced-fed glycerin.

During the 48-hour excreta collection, fasted roosters voided an average of 6.14 ± 0.94 g of dry matter, resulting in a fasting energy loss of 23.24 ± 3.55 kcal. The roosters that were force-fed glycerin excreted approximately 44.72% of the ingested dry matter. Furthermore, energy, fat, organic matter, and total carbohydrate excretion represented 37.77%, 4.98%, 29.37%, and 8.63% of their respective intakes, indicating high digestibility of fat and carbohydrate fractions, 95.02% and 91.37%, respectively (Table 2, Figure 2). These findings support earlier work suggesting glycerin is a highly digestible energy source in monogastrics (Cerrate et al., 2006; Dozier et al., 2008).

Table 2 Total Nutrient Intake, Excretion, and Digestibility of Glycerin by Adult Roosters

Nutrient	Total Intake	Total Excretion	Digestibility (%)
Dry matter (g)	12.33	5.52	55.28
Gross energy (kcal)	75.67	28.61	62.20
Nitrogen (g)	0.002	0.42	—
Crude protein (g)	0.01	2.60	—
Ether extract (g)	2.56	0.13	95.02
Ash (g)	0.24	1.97	—
Organic matter (g)	12.09	3.55	70.63
Total carbohydrate (g)	9.51	0.82	91.37

Note: Values are means from six roosters force-fed glycerin. Digestibility was calculated using the total tract apparent retention method.

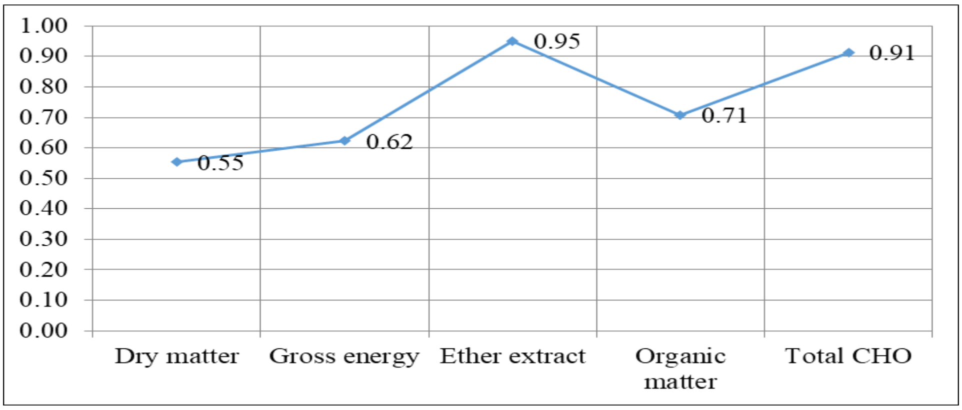

Figure 2 Coefficient of total tract apparent retentions (CTTARs) values of glycerin

The apparent nitrogen retention (ANR) was negative (-0.41 g), attributable to the extremely low nitrogen content in glycerin, as also observed by Min et al. (2010). Despite this, the calculated apparent metabolizable energy (AME) and true metabolizable energy (TME) values were high, at 3,817.07 and 5,779.42 kcal/kg, respectively. The nitrogen-corrected AMEn and TMEn values were 3,817.34 and 5,779.15 kcal/kg, representing approximately 62.20% and 94.17% of the gross energy content (Figure 3). This demonstrates that glycerin provides a substantial energy contribution when corrected for nitrogen, aligning with previous findings by Lammers et al. (2008) and Dozier et al. (2008).

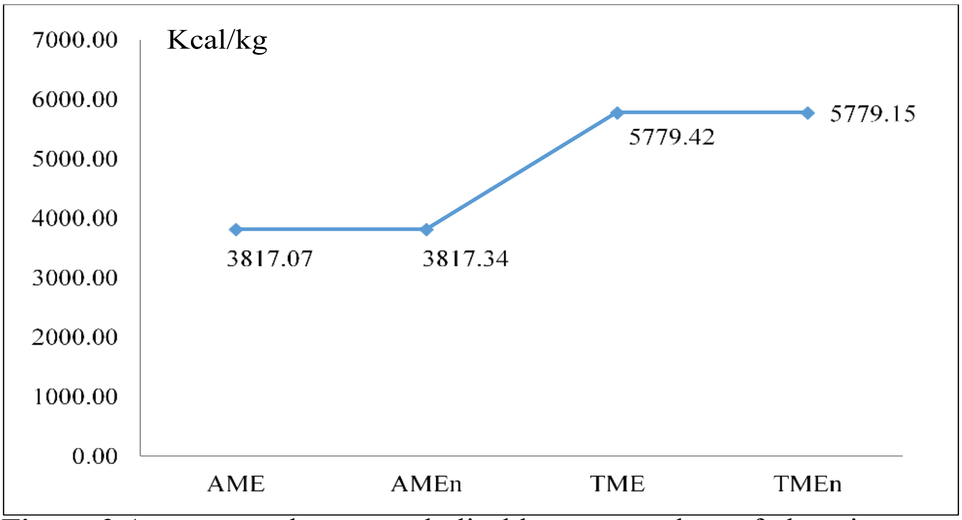

Figure 3 Apparent and true metabolizable energy values of glycerin

The efficient absorption of glycerin may be explained by its small molecular weight and by passive diffusion. Intestinal absorption of glycerin has been shown to exceed 97% in poultry (Bartelt & Schneider, 2002), and similar rates (70-89%) were reported in rats (Guyton, 1991). Once absorbed, glycerin is rapidly converted to glycerol-3-phosphate and enters metabolic pathways such as gluconeogenesis and glycolysis, yielding up to 22 moles of ATP/mol (Robergs & Griffin, 1998; Brisson et al., 2001).

When compared with other familiar energy sources, glycerin's AMEn value is approximately 10-12% higher than that of corn and grain sorghum, and about 40% and 36% higher than that of poultry oil and corn oil, respectively (National Research Council, 1994). The similarity in glycerin energy values between poultry and swine has been previously highlighted by Bartelt & Schneider (2002) and Lammers et al. (2007), suggesting a consistent pattern across monogastric species.

Previous research supports the inclusion of 5-10% glycerin in poultry diets, which improves growth performance, feed conversion ratio, and energy utilization (Simon et al., 1996; Cerrate et al., 2006; Min et al., 2010). However, excessive inclusion (e.g., 15% or more) may impair feed flow and reduce feed intake (Cerrate et al., 2006).

Glycerin is rapidly absorbed in the poultry gastrointestinal tract due to its small, water-soluble molecular structure, entering glycolytic and gluconeogenic pathways to supply energy (Dozier et al., 2008; National Research Council, 1994). Its effectiveness as an energy source is strongly influenced by purity, as residual methanol, salts, soaps, or free fatty acids from biodiesel by-products can reduce True Metabolizable Energy (TME) and potentially impair gut health (Carvalho et al., 2024). High-purity glycerin (> 95% glycerol) provides consistent TME values and can be safely incorporated into poultry diets. Additionally, glycerin may undergo partial fermentation by gut microbiota, producing short-chain fatty acids that contribute to energy supply, although these in vivo effects require further investigation. Therefore, thorough characterization of glycerin batches is crucial for optimizing feed formulation, ensuring nutritional value, and maintaining bird health.

In addition to nutritional value, the economic feasibility of incorporating glycerin into poultry diets is an important consideration. Glycerin, as a byproduct of biodiesel production, is often less expensive than conventional energy sources such as corn or wheat. A comparative cost analysis suggests that using glycerin can reduce feed costs while maintaining adequate energy supply for poultry. This presents a practical opportunity for ASEAN poultry producers, as feed costs account for a significant portion of production expenses. Incorporating glycerin not only contributes to sustainable waste utilization but may also enhance the economic efficiency of poultry production in the region.

This study demonstrates that glycerin alone provides substantial metabolizable energy for poultry; however, its inclusion must be strictly controlled. Doses exceeding 15 g/bird caused fatal diarrhea in adult roosters, establishing a clear safety threshold. For single-ingredient use, we recommend limiting glycerin to 5-10 g per bird per day to ensure safe use. While mixing with low-nutrient ingredients like rice husk could be explored in future studies to dilute glycerin concentration and reduce risk, the present results specifically apply to glycerin as a single feed ingredient. These findings offer practical guidance for sustainable energy supplementation in poultry production within the ASEAN region.

Conclusion

The present study demonstrated that glycerin possesses considerable potential as an energy-rich feed ingredient for poultry. The apparent metabolizable energy (AME) and true metabolizable energy (TME) values of glycerin were 3,817.07 kcal/kg and 5,779.42 kcal/kg, respectively, with nitrogen-corrected values (AMEn and TMEn) of 3,817.34 kcal/kg and 5,779.15 kcal/kg. The high digestibility of ether extract (95.02%) and total carbohydrates (91.37%) indicates that adult roosters efficiently utilize glycerin's energy-contributing components. Despite its negligible nitrogen content, which led to a negative apparent nitrogen retention, glycerin was effectively absorbed and metabolized, as reflected by the high CTTAR values, particularly for fat and carbohydrates.

Comparative analysis suggests that the energy values of glycerin in poultry are closely aligned with those reported for swine, supporting its suitability as an alternative to conventional energy sources such as corn and oil. Given its favorable digestibility profile, palatability, and metabolic assimilation, glycerin may serve as a viable partial replacement for traditional energy ingredients in poultry diets. Future studies should further explore its effects on performance parameters, carcass yield, and metabolic responses in different classes of poultry under commercial feeding conditions.

In conclusion, this study demonstrates that glycerin can serve as a valuable energy source in poultry diets, with high true metabolizable energy (TME) and digestibility values. However, the findings should be interpreted with consideration of the study's limitations, including sample size and variability in glycerin composition. Glycerin can serve as a high-energy single-ingredient feed for adult roosters, but its inclusion must not exceed 15 g/bird due to severe health risks. Safe use is recommended at 5-10 g/bird per day. These results provide actionable guidance for sustainable and careful energy supplementation in poultry production, with relevance to the ASEAN region. In the ASEAN context, these results provide practical guidance for poultry producers seeking sustainable, cost-effective feed alternatives. The study supports optimized feed formulation, efficient nutrient utilization, and the sustainable use of biodiesel byproducts. Future research should evaluate glycerin inclusion across different poultry types and production systems to maximize regional applicability. The key beneficiaries of this research are smallholder poultry farmers, who can use glycerin as a cost-effective energy source, and biodiesel-producing regions, where glycerin byproducts can be sustainably valorized in poultry feed.

Implication

The present study provides clear evidence that glycerin is a highly digestible and energy-rich ingredient for poultry, with its true metabolizable energy (TMEn) values approaching 94.17% of its gross energy content. The high digestibility of ether extract and total carbohydrates (95.02% and 91.37%, respectively) further emphasizes its nutritional potential. Notably, despite its low nitrogen content, glycerin can contribute meaningfully to the overall energy supply in poultry diets. These findings are consistent with earlier reports showing that glycerin has a high metabolizable energy (ME) value in poultry, ranging from 3,434 to 3,805 kcal/kg (Dozier et al., 2008; Min et al., 2010; Lammers et al., 2008).

Due to its small molecular size and passive diffusion absorption mechanism, glycerin is rapidly and efficiently absorbed in the gastrointestinal tract of poultry, with intestinal absorption rates above 97% (Bartelt & Schneider, 2002). This property makes glycerin a suitable alternative to traditional energy sources such as corn oil, with comparative AMEn values that are 10-12% higher than those of corn and grain sorghum (National Research Council, 1994). In addition, once absorbed, glycerin can serve as a precursor for glucose via gluconeogenesis or be directly oxidized to yield ATP (Robergs & Griffin, 1998; Brisson et al., 2001).

While glycerin is efficiently utilized at moderate levels in broiler diets, caution must be exercised at higher inclusion rates due to potential reductions in feed intake and flowability issues (Cerrate et al., 2006). This study supports the use of glycerin as an energy-dense ingredient, particularly in environments with limited access to traditional energy sources. Furthermore, the findings align with previous reports indicating that glycerin has comparable energy utilization in pigs (Lammers et al., 2007; Bartelt & Schneider, 2002), highlighting its broader applicability in monogastric animal nutrition.

Future research should investigate the impact of glycerin supplementation on carcass traits, metabolic profiles, and long-term performance outcomes in poultry. Additionally, economic evaluations are warranted to assess the feasibility of incorporating glycerin at various dietary levels across commercial poultry production systems.

Contribution to Practical Applications

This study provides valuable insights into the energy utilization efficiency of glycerin when used as a dietary ingredient in poultry nutrition. The determination of apparent metabolizable energy (AME), nitrogen-corrected AME (AMEn), true metabolizable energy (TME), and nitrogen-corrected TME (TMEn) using a standardized rooster bioassay offers reliable reference values for formulating poultry feeds. Notably, the TMEn value of glycerin (5,779.15 kcal/kg) indicates that it is a highly efficient energy source, comparable to those used in swine diets and substantially higher than those of conventional cereal-based ingredients.

Furthermore, the high digestibility of ether extract (95.02%) and total carbohydrate (91.37%) suggests that glycerin can be effectively absorbed and metabolized by adult roosters. These findings support the inclusion of glycerin as a viable alternative to traditional energy sources such as corn oil or animal fats, particularly in regions where biodiesel by-products are readily available.

By quantifying the nutrient digestibility and metabolizable energy values of glycerin as a single ingredient, this study establishes foundational data critical for optimizing poultry feed formulations. The results have direct implications for feed manufacturers and poultry producers seeking cost-effective, sustainable, and efficient energy alternatives in commercial feed production.

Limitations and Future Research

This study has several limitations that should be considered when interpreting the results. Variability in True Metabolizable Energy (TME) and nutrient retention may arise from differences in glycerin composition resulting from the biodiesel production process, as well as from individual metabolic differences among the roosters. Additionally, the study used a relatively small sample size and a single glycerin source, which may limit the generalizability of the findings. Future research should involve larger sample sizes, multiple glycerin sources, and potentially different poultry breeds to validate and extend these results, enhancing their applicability to practical feed formulation and sustainable poultry production.

Despite providing valuable insights into the metabolizable energy value of glycerin using a rooster bioassay, this study is subject to certain limitations. The experiment was conducted with a relatively small sample size (n = 12), which may constrain the generalizability of the findings. Additionally, only adult roosters were used, which may not fully represent the metabolic responses of broilers, layers, or other poultry species at different physiological stages.

The study was also limited to a single source and inclusion level of glycerin (15 g/bird). Variability in glycerin composition due to different production processes (e.g., biodiesel sources and refining stages) was not accounted for. Furthermore, the study focused solely on energy utilization and did not assess other important performance indices such as feed intake, growth performance, carcass traits, or blood biochemical parameters.

Future research should explore the effects of varying inclusion levels and glycerin sources in poultry diets across different species and age groups. Additional studies are warranted to evaluate the long-term impacts of glycerin supplementation on animal health, nutrient metabolism, and production efficiency. Investigations into the interactions between glycerin and other dietary components, as well as their impact on the gut microbiota and nutrient absorption, would further enhance our understanding of its nutritional value. Moreover, extending the assessment to encompass environmental and economic sustainability metrics would support broader adoption of glycerin as an alternative energy source in animal nutrition.

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