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การศึกษาคุณสมบัติทางกายภาพ คุณสมบัติทางเคมี และพลังงานใช้ประโยชน์ได้แบบปรากฏของกลีเซอรอลในไก่เนื้อ

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บทคัดย่อ: การศึกษาองค์ประกอบทางเคมี กายภาพ และพลังงานที่ใช้ประโยชน์ได้แบบปรากฏของกลีเซอรอล โดยแบ่งการศึกษาออกเป็น 2 การทดลองหลักๆ ได้แก่ การทดลองที่ 1) ศึกษาองค์ประกอบทางเคมีและกายภาพของกลีเซอรอล โดยทำการสำรวจจากโรงงานผลิตไบโอดีเซลและกลีเซอรอลในระดับอุตสาหกรรมภาคเอกชนจำนวน 4 แห่ง และระดับรัฐวิสาหกิจชุมชนจำนวน 5 แห่ง โดยการทำการสุ่มตัวอย่างกลีเซอรอล บริสุทธิ์และกลีเซอรอลดิบจากแต่ละโรงงานๆ ละ 1 ลิตร เพื่อทำการวิเคราะห์สี ความหนืด การปนเปื้อน ปริมาณกลีเซอรอล ความชื้น และปริมาณเมทานอล พบว่ากลีเซอรอลที่ได้จากโรงงานระดับอุตสาหกรรมภาคเอกชนจะเป็นกลีเซอรอลบริสุทธิ์ แต่กลีเซอรอลที่ได้จากโรงงานระดับรัฐวิสาหกิจชุมชนจะเป็นกลีเซอรอลดิบ ซึ่งคุณภาพของกลีเซอรอลดิบมีความแปรผันไปตามคุณภาพของวัตถุดิบและกระบวนการผลิตที่ใช้ โดยมีสัดส่วนของกลีเซอรอล 28.63–45.01% สารปนเปื้อน (matter organic non glycerin, MONG) 29.76–59.89% และเมทานอล 3.86–20.18% แต่คุณสมบัติทั้งทางเคมีและทางกายภาพของกลีเซอรอลบริสุทธิ์จากโรงงานแต่ละแห่งใกล้เคียงกัน โดยมีสัดส่วนของกลีเซอรอลมากกว่า 99.00% และสัดส่วนของเมทานอลไม่เกิน 0.01% และการทดลองที่ 2) การศึกษาค่าพลังงานที่ใช้ประโยชน์ได้แบบปรากฏของกลีเซอรอล โดยเลือกศึกษาในกลีเซอรอลบริสุทธิ์ เนื่องจากมีปริมาณเมทานอลต่ำและไม่เป็นพิษต่อสัตว์ ในการศึกษาค่าพลังงานที่ใช้ประโยชน์ได้แบบปรากฏนี้ใช้วิธี substitution method ทำการศึกษาในไก่เนื้อสายพันธุ์ Ross 308 เพศผู้ จำนวน 128 ตัว แบ่งกลุ่มทดลองออกเป็น 2 กลุ่ม กลุ่มละ 8 ซ้ำ ซ้ำละ 8 ตัว เลี้ยงในกรงเมแทบอลิซึม โดยให้อาหารทดลองแบบผงตามกลุ่มทดลอง ประกอบด้วย สูตรที่ 1 อาหารควบคุมที่มีข้าวโพดและกากถั่วเหลืองเป็นองค์ประกอบ (corn soy basal diet) และสูตรที่ 2 อาหารควบคุม 90% + กลีเซอรอลบริสุทธิ์ 10% โดยใช้ Ce-lite™ ที่ระดับ 1.5% เป็นตัวบ่งชี้ค่าการย่อยได้ของอาหารทดลอง ให้ไก่กินอาหารทดลองเป็นเวลา 7 วัน (ช่วงปรับตัว 4 วัน และช่วงเก็บข้อมูล 3 วัน) ทำการเก็บมูลในช่วง 3 วันสุดท้ายของการทดลองเพื่อวิเคราะห์ค่าพลังงานรวมและปริมาณเถ้าที่ไม่ละลายในกรด (AIA) พบว่าค่าพลังงานใช้ประโยชน์ได้แบบปรากฏของกลีเซอรอลบริสุทธิ์ในไก่เนื้อมีค่า 3,712 kcal/kg ซึ่งมีค่าพลังงานที่ใช้ประโยชน์ได้แบบปรากฏใกล้เคียงกับข้าวโพด และมีค่าพลังงานที่ใช้ประโยชน์ได้แบบปรากฏประมาณครึ่งหนึ่งเมื่อเทียบกับน้ำมันถั่วเหลือง ดังนั้นกลีเซอรอลบริสุทธิ์จึงมีสามารถนำมาใช้เป็นวัตถุดิบพลังงานในอาหารสัตว์ปีกได้

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The Study of Physical, Chemical Properties, and Apparent Metabolizable Energy of Glycerol in Broiler

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Abstract: The study of physical properties, chemical elements, and apparent metabolizable energy (AME) of glycerol was conducted in two experiments. Experiment 1 was a survey of physical properties and chemical content of glycerol produced by 4 industrial and 5 community biodiesel factories operating in Thailand. Representative samples of refined and crude glycerol (1 liter) from each factory was collected and evaluated for color, viscosity, impurity, glycerol content, moisture content, and methanol content. Representative samples of crude glycerol from community biodiesel factories contain highly variable glycerol content (28.63 – 45.01%) and methanol content (3.86 – 20.18%), depending on the quality of raw materials and efficacy of methanol recovery. Glycerol content in crude glycerol is relatively low because of high contamination with matter organic non-glycerin (29.76 – 59.89%) and methanol content (3.86 – 20.18%). Representative samples of refined glycerol from the four industrial factories were consistent in terms of glycerol (>99%) and methanol content (< 0.01%). Experiment 2 measured the AME of refined glycerol for broiler chickens. The AME of a representative sample of refined glycerol was determined by using a substitution method. A total of 128 male Ross308 broilers 15 days old were assigned to two dietary treatments: (1) corn-soy basal diet and (2) 90% corn-soy basal diet plus 10% refined glycerol. Celite™ at a level of 1.5% was added to both diets as an indigestible marker. Experimental diets were offered to the birds for a total of 7 days. Excreta were collected for 3 consecutive days to evaluate the AME. The result show that the AME of refined glycerol for broiler chicken was 3,712 kcal/kg, which is similar to that of corn and approximately half of the AME for soybean oil. Therefore, refined glycerol can be used as an energy source in broiler chicken diets.

Keywords: Glycerol, Physical property, Chemical element, Apparent metabolizable energy

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Introduction

Depletion of fossil fuels has increased the need for alternative fuels (Ondul and Dizge, 2014). Biofuels have received increasing interest in the past decade because of environmental concerns. Biofuels can be classified into ethanol and biodiesel. Biodiesel is an alternative fuel obtained from vegetable or animal fats and oils. It is considered as a green fuel. Glycerol, a mono alkyl ester, is a by-product from biodiesel production. Commercially, biodiesel is produced by trans-esterification. The reaction can be catalyzed by either using homogeneous catalysts (acid or base) or heterogeneous catalysts (acid, base, or enzyme) (Helwani *et al*., 2009; Atadashi *et al*., 2010). During the reaction, a short-chain alcohol (methanol or ethanol) reacts with triacylglycerol and a catalyst to form glycerol and long-chain fatty acid esters. After the reaction, the final mixture comprises alkyl esters, residual alcohol, glycerol, and the catalyst, along with mono-, di-, and triacylglycerol, which are intermediate reaction products (Gomes *et al.*, 2010). From this reaction, crude glycerol is produced in a quantity approximating 10% of the biodiesel production. As the biodiesel industry is rapidly expanding, an excess of crude glycerol is being created. After crude glycerol undergoes purification, refined glycerol is obtained at 30-40% of the original volume. Normally, refined glycerol contains 99% glycerol and less than 0.01% methanol. When an animal ingests glycerol, it is absorbed in the manner of triacylglycerols (Lammers *et al*., 2008). Glycerol is a precursor to glyceraldehyde-3-phosphate, an intermediate in the lipogenesis and gluconeogenesis pathways, and yields energy through glycolysis and Kreb's cycle (Lin, 1977; Brisson *et al.*, 2001). At present, we are facing to the shortage of energy feed stuff. From the reason above, glycerol is potentially to be an alternative energy feed stuff. Many studies examining the effects of including refined or crude glycerol from biodiesel production in diets for broiler chickens (Simon *et al*., 1996; Cerrate *et al.*, 2006) and pigs (Kijora *et al.*, 1995) have shown that glycerol could be used as a source of dietary energy for livestock. Dozier *et al.* (2008) conducted three experiments and reported that the average apparent metabolizable energy (AME) content of refined glycerol was approximately 3,434 kcal/kg. Lammer *et al.* (2008) reported that the AME of crude glycerol in layer chickens was 3,805 ± 238 kcal/kg. Similarly, Bartelt and Schneider (2002) reported that the AMEs of glycerol in broiler and layer chickens were 3,993 and 3,929 kcal/kg, respectively. Consistent with the finding of Silva *et al. (*2012*)* who studied the

AME of crude glycerol in broilers and found that the AME of glycerol in broilers was 3,442 kcal/kg. Many studies indicated that glycerol is suitable for use as an energy feedstuff for poultry. Thus, this study aim to survey the type of glycerol produced in Thailand and evaluates the AME of glycerol for broiler.

Materials and Methods

The study of physical and chemical properties and AME of glycerol were divided into two experiments. Experiment 1 was a survey of physical properties and chemical elements of glycerol produced by industrial and community biodiesel factories. Experiment 2 measured the AME of refined glycerol for broiler chickens.

Experiment *1*: Survey of physical properties and chemical elements of glycerol produced by industrial and community biodiesel factories in Thailand.

The study of physical properties and chemical elements of glycerol started with a survey of industrial facilities producing refined glycerol and community biodiesel factories producing crude glycerol. The survey included four purified biodiesel factories in Thailand (Thai oleo Chemistry, Pathum Vegetable, oil Verasuwan and Absolute Energy). All of the community biodiesel factories are public factories, namely (1) biodiesel factory of Kung Kraben Bay Royal Development Study Center, Chantaburi province; (2) biodiesel factory of Huaysai Royal Development Center, Petchaburi province; (3) biodiesel factory of Kao Tao Royal Development Center, Prachuapkhirikhan province; (4) biodiesel factory of Development Study Center, Ratchaburi province; and (5) Naval Dockyard Department. Glycerol samples were collected from each industrial or community biodiesel factory to test color (Colorview spectrophotometer, Model: Benchtop Color Spectrophotometer, Premier Colorscan Instrument Pvt. Ltd., India), viscosity (Brookfield Model DV-II+Pro, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA), impurities [1754-2542 (ISO 2462-1973) method], glycerol content (USP 32-NF method), moisture (ASTM E 203-01 method), and methanol content (ASTM D 3695-95 method).

Experiment 2: Study of apparent metabolizable energy of refined glycerol for broiler chickens.

Animals and treatments

Refined glycerol from Absolute Energy was used for AME assay in broiler chickens. The AME of refined glycerol was measured using a substitution method (Nalle *et al.*, 2011). A total of 128 male Ross308 chickens 15 days old were randomly assigned to two dietary treatments (1) corn-soy basal diet and

(2) 90% corn-soy basal diet plus 10% refined glycerol (Table 1). Celite^{TM} at a level of 1.5% was added to both diets as an indigestible marker. Each treatment contained eight replications with eight birds per replication. All birds were placed together in metabolic cages equipped with one trough feeder, one trough drinker, and an aluminum tray underneath for excreta collection. Experimental diets were offered to the birds for a total of 7 days (4 days preliminary period and 3 days test period).

Sample collection and laboratory analysis

- **Excreta collection.** Excreta were collected at 24-hour intervals and pooled for three consecutive days to evaluate the AME of the experimental diets. Excreta collection was carried out from 19 to 21 days of age. Approximately 200 g of clean (free of feed and visible feather contaminants) representative samples of excreta were collected once daily at 8:00 a.m. from the aluminum collection trays placed under each pen. Excreta were preserved by adding H_2SO_4 to adjust the pH to 4 (acidic) and then stored at -20°C. Excreta from each replicant pen was pooled over the 3-day collection period and added cumulatively to polyethylene bags and stored at -20°C until analysis.

- **Sample analysis.** Prior to analysis, the frozen excreta samples were allowed to defrost overnight at room temperature. Approximate 200 g of representative excreta from each pen was then blended with 100 mL water to form a slurry. The excreta slurry was then dried overnight in a forced-air convection oven at 70°C. Dried excreta was then ground in a blender and stored in polyethylene storage bags at -20°C until removal for analysis (Sungwaraporn, 2004). The gross energy content of the feed and excreta were measured using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) (Bolin *et. al.*, 1952). Acid-insoluble ash recovery was performed using the method described by AOAC *(*2012*).*

The analytical data were used to calculate the AME according to Equation 1*:*

$$\text{AME/g feed} = \text{GE/g feed} - \text{E/g feces } (\text{Celite}^{TM} \text{ adjusted}), \quad ....(1)$$

where AME is measured per gram of feed on a dry matter basis *(*Hill and Anderson, 1960*).*

Table 1: Ingredient and nutrient composition of the basal diets

Ingredients (%)	
Corn	55.46
Rice bran	5.00
Soybean oil	1.93
Soybean meal 49%	21.04
Full fat soybean	12.50
L-lysine	0.144
DL-methionine	0.235
choline chloride (60%)	0.02
MDCP (P21)	1.65
$CaCO_3$	1.43
Salt	0.41
Premix[1]	0.25
Calculated analysis (%)	
ME (kcal/kg)	3,150
Crude protein	20.00
Ether extract	7.34
Crude fiber	3.21
Calcium	0.90
Available. Phosphorus	0.45
Lysine	1.13
Methionine	0.55
Met + Cys	0.82
Choline (mg/kg)	1.300
Sodium	0.18

[1]This premix provided the following microelements per kilogram: vitamin A, 4,800,000 IU; vitamin D3, 3,000 IU; vitamin E, 6,000 IU; vitamin K3, 0.600 g; vitamin B1, 0.600 g; vitamin B2, 2.200 g; vitamin B6, 0.800 g; vitamin B12, 0.400 g; folic acid, 0.200 g; nicotinic acid, 10,000 g; pantotenic acid, 4.800 g ; biotin, 0.048 g; Mn, 2,640 mg; Fe, 16.000 g; Zn, 24.000 g; Cu, 3,200 mg; I, 0.200 g, Se, 0.040 g; and Co, 0.040 g.

Results

Experiment 1: Survey of physical properties and chemical elements of glycerol produced by industrial and community biodiesel factories.

From the survey, we found that the industrial factories produce refined glycerol and community biodiesel factories produce crude glycerol. The qualities of refined glycerol from four industrial factories were similar in terms of glycerol content (>99%), methanol content (<0.01%), gross energy (3,843 – 4,119 kcal/kg), water content (0.1 – 0.48%), MONG (0.14 - 0.3%), and moisture content (5 – 6%). Glycerol content in crude glycerol is relatively low (28.63-45.01%) because of high MONG contamination (29.76 – 59.89%) and methanol content (3.86-20.18%), depending on the efficacy of methanol recovery.

Experiment 2: Study of apparent metabolizable energy of refined glycerol for broiler chickens.

Glycerol obtained from Absolute Energy Public Co. Ltd. (industrial factory) contained 4,109 kcal/kg of gross energy. In this study, the refined glycerol fed to broiler chickens was found to contain 3,712 kcal/kg AME. Thus, the AME of refined glycerol was approximately 90% of the gross energy.

Discussion

Experiment 1: Survey of physical properties and chemical elements of glycerol produced by industrial and community biodiesel factories.

Physical properties and chemical composition of all representative samples of refined glycerol were similar because every factory used palm oil as a raw material for biodiesel production and they had a similar methanol recovery process. The quality of crude glycerol, however, varied due to different types and qualities of raw materials, as well as different biodiesel production processes. Raw materials for biodiesel production in community biodiesel factories are mainly used cooking oil with high variation in chemical composition (e.g., free fatty acids) and contamination. The quality of used cooking oil affected the quality of the produced biodiesel and glycerol in term of glycerol content, methanol content, and MONG. Therefore, refined glycerol is more suitable for use as an energy feedstuff in poultry diets.

Experiment 2: Study of apparent metabolizable energy of glycerol for broiler chickens.

Representative samples of refined glycerol used in the present study contained 99.7% glycerol and yielded 3,712 kcal AME/kg. The result was in good agreement

Table 2 Physical and chemical composition of glycerol from industrial biodiesel factories and community biodiesel factories

Teat Item	Test Method	Thai oleo Chemistry	Pathum Vegetable oil	Verasuwan	Absolute Energy	Kung Kraben	Naval Dockyard	Khao Tao	Huaysai	Ratchaburi
Glycerin content (%wt)	USP 32	99.58	99.74	99.22	99.46	35.03	28.63	45.01	34.31	30.79
Methanol (%wt)	ASTM D 3695-95 (Reapproved 2001)	< 0.01	< 0.01	< 0.01	< 0.01	20.18	3.86	17.72	19.48	7.24
Water content (%wt)	ASTM E 203-01	0.117	0.095	0.484	0.317	2.627	2.234	2.874	9.387	4.924
MONG (%wt)	1754-2542(ISO 2462-1973)	0.3	0.16	0.14	0.17	37.16	59.89	29.76	32.92	51.12
Sulphate ash (%wt)	Ph. Eur. 5	0.003	<0.001	0.153	0.008	4.996	5.295	4.642	3.904	5.933
Mouisture (%wt)	Proximate analysis	5.15	4.99	6.07	5.21	20.53	28.12	43.85	19.78	11.90
Gross energy (kcal/kg)	Bomb calorimeter	3,842.69	4,118.45	4,057.71	4,109.24	6,753.45	6,781.52	6,087.02	6,642.31	7,152.36
Viscosity (centipoises)	Digital visxometer	843.2	823.2	802.4	821.5	111.9	22.7	117.8	107.5	665.2
Color	Color-view spectrtophotometer	Clear	Clear	Clear	Clear					
L						0.84	0.77	0.56	3.67	15.19
a						0.65	0.4	0.76	0.46	4.88
b						-0.05	-0.3	0.16	-0.41	13.12

with Dozier *et al.* (2008), who conducted three experiments for different ages of broiler chickens (4-11, 17-24, and 38-45 days) and reported that the AMEn (AME corrected for nitrogen) of refined glycerol for broilers was 3,621, 3,331, and 3,349 kcal/kg for the three age groups, respectively. The average AMEn content of glycerol across the three experiments was 3,434 kcal/kg, which was 95% of the gross energy content. The AME level of glycerol was related to the glycerol content. The AME of glycerol in the present experiment was higher than the AMEn (2,737 kcal/kg) reported by Nemeth *et al.* (2013) because the glycerol content was higher (99.7 vs. 86.80%). The AME of glycerol found in this experiment was very close to the gross energy value (90%), which indicated that glycerol was efficiently used as an energy source by the broiler chickens. Normally, animals can use glycerol for an energy source via glycolysis. Glycerol is converted to glyceraldehyde-3-phosphate and pyruvate before entering Kreb's cycle, which generates NADH and $FADH_2$ and feeds them into the oxidative phosphorylation (electron transport) pathway. The net result of these pathways is the oxidation of nutrients to produce usable chemical energy for animals in form of ATP (Lehninger *et al.*, 1993).

Conclusion

Glycerol obtained from community biodiesel production was crude, with a high variation in glycerol and methanol content as well as being high in impurities. In contrast, glycerol produced by industrial factories was more refined. It had high and consistent glycerol content with very low methanol levels. The AME of refined glycerol for broiler chickens was found to be 90% (3,712 kcal/kg) of the gross energy. Our findings indicated that refined glycerol could potentially be a good energy source for broiler feed.

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