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Dry matter digestibility and metabolizable energy of crude glycerines originated from palm oil using fed rooster assay

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ABSTRACT. A study was conducted to determine the dry matter digestibility, gross energy (GE), the nitrogen-corrected apparent metabolizable energy (AMEn), and the nitrogen-corrected true metabolizable energy (TMEn) of two crude glycerine from two different sources. The first crude glycerine (CG1) was from a large scale biodiesel producer with high content of glycerol (89.49%) and low content of crude fat (1.73%), meanwhile the second crude glycerine (CG2) was from a medium scale biodiesel producer with lower content of glycerol than CG1 (38.36%) and high content of crude fat (23.63%). Fed rooster assay based on Sibbald (1976) was used in the experiment. The experimental feed consisted of ground corn and three levels of crude glycerine (0, 10, and 20%). Twenty four Hisex brown roosters were housed in metabolic cages. Roosters were force fed with 30 g experimental feed, after 24 hours of fasting. Excreta collection was performed for two days while the roosters were fasting again. The content values of GE, AMEn, and TMEn of CG1 were 4065.18, 2926.59, and 3068.73 kcal kg^{-1} and for CG2 were 5928.09, 4010.11, and 4054.52 kcal kg, respectively.

Keyword: large scale biodiesel producer, medium scale biodiesel producer, Sibbald method.

Digestibilidade da matéria seca e energia metabolizável da glicerina bruta originada a partir de óleo de palma usando ensaio de alimentação forçada com galos

RESUMO. O presente estudo foi realizado para determinar a digestibilidade da matéria seca, energia bruta (EB), energia metabolizável aparente corrigida pelo balanço de nitrogênio (EMAn) e energia metabolizável verdadeira corrigida pelo balanço de nitrogênio (EMVn) de glicerinas brutas de duas diferentes fontes. A primeira glicerina bruta (GB1) foi obtida de um produtor de biodiesel de grande escala contendo alto teor de glicerol (89,49%) e baixo teor de gordura bruta (1,73%), enquanto a segunda glicerina bruta (GB2) era de um produtor de biodiesel de média escala com menor conteúdo de glicerol do que GB1 (38,36%) e alto teor de gordura bruta (23,63%). O ensaio de alimentação forçada com galos baseado em Sibbald (1976) foi utilizado no experimento. A ração experimental foi composta de milho moído e três níveis de glicerina bruta (0, 10 e 20%). Vinte e quatro galos Hisex brown foram alojados em gaiolas metabólicas. Os galos foram alimentados a força com 30 g da ração experimental após jejum de 24 horas. A coleta de excretas foi realizada durante dois dias enquanto os galos permaneceram novamente em jejum. Os teores de EB, EMAn e EMVn de GB1 foram 4065,18, 2926,59 e 3068,73 kcal kg^{-1} para GB2 foram 5928,09, 4010,11 e 4054,52 kcal kg^{-1}, respectivamente.

Palavras chave: produtor de biodiesel de grande escala, produtor de biodiesel de média escala, método Sibbald.

Introduction

Since the demand of renewable fuels, biodiesel industry is rapidly promoting in the last decade. The main by-product, crude glycerine, derived from this biodiesel production has been increasing. Crude glycerine approximately contains 50 to 90 percent glycerol and 15 to 35 percent impurities, such as, methanol or ethanol, fatty acids, water, and some chemical compounds that obtained from transesterification process (Jung & Batal, 2011; Dozier et al., 2008). Nevertheless, it is broadly used in various purposes, for instance, fuel, lubricant, human foods, and animal diets (Dasari, 2007; Thompson & He, 2006)

Due to crude glycerine contains high gross energy approximately 3680 to 6742 kcal kg^{-1} (Jung & Batal, 2011), crude glycerine is used as a source of energy feedstuff for livestock in Brazil, European countries, and the United States. Results from the previous studies confirmed that crude glycerine can be mixed for about 5 to 10% in the diet for non-ruminants, and up to 10% for ruminants without any negative effect (Chanjula, Pakdeechanuan, & Wattanasit, 2015; Min, Yan, Liu, Coto, & Waldroup, 2010; Simon, Bergner, & Schwabe, 1996). Due to the wide range of gross energy content, nevertheless, it is important to determine the metabolizable energy of crude glycerine before using it as a feedstuff.

In Thailand, a second largest biodiesel producer in Asia, crude glycerine is an option to use in the livestock industry. In addition, results from the work of Sri-muang, Wattanachant, Ngampongsai, and Settapong (2015) which is determined the chemical content of crude glycerine from large and medium scale biodiesel producer in Thailand, concluded that the crude glycerines had similar chemical content like Dozier et al. (2008), although the glycerol content from medium scale is lower. Therefore, the objective of this study was to determine the dry matter digestibility and metabolizable energy of crude glycerine from two different sources of biodiesel producers in Southern Thailand. Information from this study could be useful as the references for applying crude glycerine as an energy feedstuff in poultry.

Material and methods

Bird and housing

A total of 24 adults Hisex brown roosters with average body weight 2.44 ± 0.16 kg were located randomly in the individual metabolic cage 55 x 35 x 60 cm^3 width, length, and height, respectively. The individual metabolic cages were inside an evaporative housing system. The roosters were provided with 16 hours light per day and the ambient temperature was maintained at 26 to 27°C throughout the study, relative humidity in the housing was 70 to 85%. This study by was developed according to the Institucional Board (IRP) unde the ethical principles for the use of animals for scientific purposes of Prince of Songkla University which was approved by the National Council of Thailand

Feeding treatment

The fed rooster assay based on Sibbald (1976) was used to determine the dry matter digestibility, AMEn and TMEn of the two sources of crude glycerine. Crude glycerines used in this experiment were obtained from large (CG1) and medium scale (CG2) plants located in the Southern Thailand. The nutrient content and characteristics are based on Sri-muang et al. (2015) which used the same crude glycerine sources (Table 1).

Table 1. Characteristics of the crude glycerines used in the experiment.

Item	CG1	CG2
Biodiesel feedstock	RBD palm oil[1]	Wasted vegetable oil and animal oil
Physical characteristics	Light yellow, transparent	Black, high turbidity
Glycerol (%)	89.49±0.01	38.36±0.12
Crude fat (%)	1.73±0.40	23.91±3.03
Crude protein (%)	0.06±0.04	0.16±0.02
Moisture (%)	10.46±2.42	23.63±2.88
Ash (%)	4.41±0.01	5.49±0.01

[1]Refined bleached deodorized.

Roosters were fasted for 24 hours for emptying the gastrointestinal tract before giving the experimental feed. The experimental feeds were using two crude glycerines with three different levels, 0, 10 and 20%, crude glycerine were mixed with ground corn. There were 6 groups of roosters each with 4 replications as follow;

K1 = Rooster fed with 30 g ground corn (basal)

K2 = Rooster fed with 27 g ground corn + 3 g CG1 (10%)

K3 = Rooster fed with 24 g ground corn + 6 g CG1 (20%)

K4 = Rooster fed with 27 g ground corn + 3 g CG2 (10%)

K5 = Rooster fed with 24 g ground corn + 6 g CG2 (20%)

K6 = Fasted rooster for calculating the endogenous losses

After 24 hours fasting, roosters were forced fed with 30 g experimental feed except the K6 group for calculating the endogenous losses. Following a period of 48 hours fasting while excreta were collected, the water was provided *ad libitum*. The collected excreta was weighed, ovened and grounded in a grinder to pass through a 14 mesh screen. Subsamples of crude glycerine, excreta, and feed samples were analysed according to procedures of the Association of Official Analytical Chemists International (AOAC, 2006). Crude glycerine, feed, and excreta were analysed for gross energy in an adiabatic bomb calorimeter (Gallenkamp, Germany). Excreta and feed were also analyzed for dry matter via ovenedll PL 105°C and nitrogen content via kjeldahl method.

Calculations and statistical analyses

The experimental feeds and crude glycerines were examined for determining the dry matter digestibility and the metabolizable energy based on the feed intake, excreta, analysis of dry matter, and gross energy. The dry matter digestibility value of experimental feed was calculated as follow:

DM digestibility (%) =

$$\left[\frac{(\text{FI (DM)} - \text{E feed (DM)} + \text{E endogenous(DM)})}{\text{FI (DM)}}\right] \text{x } 100\%$$

where:
DM digestibilty: Dry matter digestibility;
FI (DM): Feed intake in dry matter;
e feed (DM): Excreta from the bird with experimental feed in dry matter;
e endogenous (DM): Excreta from fasted bird in dry matter.

The nitrogen-corrected apparent metabolize energy (AMEn) value of experimental feed was calculated based on Dozier, Kerr, and Branton (2011) as follow :

AMEn =

$$\frac{[\text{GE feed} - (\text{GE excreta feed} + 8.22 \text{ Nitrogen feed})]}{\text{Feed consumption (g)}}$$

where:
AMEn: The nitrogen-corrected apparent metabolize energy;
GE feed: Gross energy of the experimental feed;
GE excreta feed: Gross energy of the excreta with experimental feed;

The nitrogen-corrected true metabolize energy (TMEn) of the experimental diets was calculated according to Parsons, Potter, and Bliss (1982):

TMEn =

$$\frac{\left[\begin{array}{c}\text{GE feed} - (\text{GE excreta feed} + 8.22 \text{ Nitrogen feed}) \\ + (\text{GE excreta endogenous} + 8.22 \text{ Nitrogen endogenous})\end{array}\right]}{\text{Feed consumption (g)}}$$

where:
TMEn: The nitrogen-corrected true metabolize energy;
GE feed: Gross energy of the experimental feed;
GE excreta feed: Gross energy of the excreta with experimental feed;
GE excreta endogenous: Gross energy of the excreta of fasted bird.

Based on the data of feed intake, excreta, dry matter, AMEn, and TMEn of the experimental diets, AMEn and TMEn of crude glycerines were calculated using the equation based on Matterson, Potter, and Stutz (1965) as follow:

Crude glycerine AMEn = AMEn basal feed +

$$\left[\frac{(\text{AMEn experimental feed} - \text{AMEn basal feed})}{\text{g of crude glycerine/g feed}}\right]$$

Crude glycerine TMEn = TMEn basal feed +

$$\left[\frac{(\text{TMEn experimental feed} - \text{TMEn basal feed})}{\text{g of crude glycerine/g feed}}\right]$$

The significant difference of digestibility and energy between crude glycerine and different level will be analyzed statistically by Completed Randomized Design (CRD) and further analysis (post-hoc) with tukey using computer software SPSS version 16.0 (2007).

Results and discussion

Crude glycerine used in this study had significant differences in its physical and chemical properties, CG1 was light yellow and transparent, CG2 was black and it has high turbidity. CG1 had a low content of crude fat (1.73%) and high content of glycerol (89.49%), CG2 had a high content of crude fat (23.91%) and lower content of glycerol than CG1 (38.36%).

Higher GE, AMEn, and TMEn value were found in the experimental feed with crude glycerine inclusion (Table 2). This was due to the crude glycerine has high energy and high digestibility value. The high digestibility can be examined in the amount of dry matter digestibility of experimental feed, which was increasing as the level of crude glycerine escalated. This finding was in agreement with Silveira et al. (2015) who reported that replacement of basal diet to crude glycerine increased the gross energy, digestible dry matter, digestible energy, and metabolizable energy in the experimental feed. High digestibility of crude glycerine is expected because of its main composition is glycerol. Glycerol has small molecular weight. It will be absorbed passively rather than forming a micelle that is compulsory to absorb the medium and long chain fatty acid (Mcmurry & Begley, 2005; Guyton, 1991)

AMEn and TMEn value of experimental feed with crude glycerine tended to decrease eventhough not statistically difference when the level of crude glycerine increased. This is due to the increased blood glycerol levels (Kerr, Shurson, Johnston, & Dozier III, 2011; Simon et al., 1996) after crude glycerine is absorbed, the complete renal reabsorption is averted and urine will increase due to glycerol excretion (Kijora, Bergner, Kupsch, & Hageman, 1995). Same result happened in the work of Lammers et al. (2008), using crude glycerine in finishing pig, it decreased the metabolizable energy as the crude glycerine inclusion increased from 0 to 20% in the diet.

Table 2. GE, AMEn, TMEn (kcal kg^{-1}) and DM digestibility (%) value of experimental feed.

Energy value		CG1		CG2	
	0%	10%	20%	10%	20%
GE	3956.45 ± 77.17[a]	4064.20 ± 599.51[ns]	3958.01 ± 482.75[ns]	4163.72 ± 29.98[a]	4070.60 ± 289.80[a]
AMEn	3594.70 ± 54.57[b]	3504.52 ± 464.38[ns]	3527.04 ± 143.52[ns]	3788.74 ± 13.56[b]	3618.50 ± 184.83[b]
TMEn	3631.39 ± 79.43[b]	3632.80 ± 468.89[ns]	3569.56 ± 141.59[ns]	3799.86 ± 34.68[b]	3679.94 ± 163.37[b]
P value	0.002	0.412	0.132	0.000	0.014
SEM	24.68	149.51	76.36	10.66	51.17
DM Digestibility (%)	86.29 ± 2.04	87.34 ± 0.51	89.13 ± 5.66	90.07 ± 2.15	91.12 ± 3.79

[a,b] Means within the same column with different superscripts differ significantly ($p < 0.05$); [ns] Means non-significance

Table 3. GE, AMEn, and TMEn value of crude glycerines (kcal kg^{-1}).

Energy	CG1		CG2		*P* value	SEM
	10%	20%	10%	20%		
GE[1]	4065.18 ± 169.93[b]		5928.09 ± 5.41[a]		0.004	60.11
AMEn	2926.59 ± 48.42[b]	3046.61 ± 158.54[b]	3893.90 ± 111.95[a]	4010.11 ± 73.65[a]	0.000	30.77
TMEn	3068.73 ± 96.24[b]	3194.06 ± 278.37[b]	3970.15 ± 127.56[a]	4054.52 ± 90.63[a]	0.000	48.14
% of GE	75.42	78.57	66.97	68.40		

[1] Gross energy of each crude glycerine were analysed using adiabatic bomb calorimeter method. [a,b] Means within the row with different superscripts differ significantly ($p < 0.05$).

High inclusion of crude glycerine (10 and 20%) was suggested in the Silveira et al. (2015) work, also based on Matterson et al. (1965), It suggested to use high level of crude glycerine inclusion to get a betterestimationand low variation of the energy value, due to the difference of 100 kcal in the experimental feed will make a high variation in the estimation of the value when using Matterson et al. (1965) equation.

Table 3 presented GE content as a result from using adiabatic bomb calorimeter method and the estimated AMEn and TMEn of crude glycerine based on Matterson et al. (1965) equation. Gross energy between both crude glycerines was significantly differrent due to the high amount of crude fat in the CG2. Kerr et al. (2011) reported that the amount of fatty acid was the main factor that influence the gross energy value in the crude glycerine, the energy value will be increased as the fatty acid content increased. Even though, this study did not determine the fatty acid content, it can be replaced by crude fat, as it is also known as the ether extract or the free lipid content, the lipid materials may include triglycerides, diglycerides, monoglycerides, phospholipids, steroids, and free fatty acids (Association of American Feed Control Officials [AAFCO], 2014). The same results were found at Dozier et al. (2011) which determined the AME of crude glycerine on broiler from yellow grease with 34.84% and poultry fat with 24.28% fatty acidscontents. The crude glycerines had 68.7% and 62.3 % of AME as percentage of GE, respectively similar to the result of CG2.

CG2 has lower ME as a percentage of GE compared to CG1, it means the CG2 is poorer to digest than CG1 even though it has high GE content. The reduce ability to utilize crude glycerine in the high crude fat content is explained by its fatty acids. Triglycerides together with free fatty acids can be absorbed better than only free fatty acids. This is due to the support of a a monoglyceride backbone.The amount of fatty acids entering micellular solution may decrease because of the relatively low concentration of monoglycerides in the duodenum. In addition, 2-monoglyceride support water solubility, which result in a mixed bile salt-monoglyceride fatty acid micelle that can help the lipid absorption (McMurry & Begley, 2005; Senior, 1964).

CG1 has similar properties with crude glycerin from Dozier et al. (2011) with high glycerol, moisture, and low fat content, but the crude glycerine from Dozier et al. (2011) has 92.8% of GE meanwhile the CG1 is 75.42 to 78.57%. This difference may be cause by the processing standard. The difference between both crude glycerine was CG1 using RBD palm oil, meanwhile crude glycerine from Dozier et al. (2011) was using soybean oil. Dozier et al. (2011)also estimated the AME content in poultry using the value of GE by multiplying to 65.6% if the total fatty acid range from 25 to 35% which close to the result of CG2.

Conclusion

From this study it could be concluded that crude glycerine inclusion in the diet increased the dry matter digestibility. High level crude glycerine inclusion (more than 10%) tend to decrease the metabolizable energy. The GE, AMEn, and TMEn content of CG1 were 4065.18, 2926.59, and 3068.73 kcal kg^{-1} and for CG2 were 5928.09, 4010.11, and 4054.52 kcal kg^{-1}, respectively.

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