

THE USE OF PREDICTED APPARENT METABOLIZABLE ENERGY VALUES TO UNDERSTAND THE OIL AND FAT VARIABILITY IN BROILERS

Agnes THNG✉, Jun Xiang TING, Hui Ru TAY, Chin Yi SOH, Hwee Ching ONG, David TEY

Kemin Industries (Asia) Pte Limited, Animal Health and Nutrition, 12 Senoko Drive, Singapore 758200, Singapore

*Email: agnes.thng@kemin.com

ABSTRACT: The objective of this study was to analyze the predicted apparent metabolizable energy (AME) of different oil samples across Asia Pacific region and investigate the AME values in broilers of different ages (< 21 or > 21 days old). A total of 635 oil and fat samples consisting of 93 fish oils, 36 coconut oils, 70 crude palm oils, 42 refined palm oils, 43 soybean oils, 147 rice bran oils, 163 tallows and 41 lards were collected and analyzed over a span of eight years (2011 to 2018). The free fatty acid (FFA) content of oil and fat samples were analyzed through acid-base titration and the degree of saturation (ratio of unsaturation to saturated fatty acids; U:S) were determined with Gas Chromatography with Flame Ionization Detector (GC-FID). The FFA and U:S of the samples were then incorporated into the Wiseman equation to correlate the oil and fat qualities with the AME. Our survey revealed AME variations were prevalent in most of the oil types studied, with fish oils and tallows showing the largest energy gap within oil samples. The results showed that the predicted AME values for oil and fat samples differ across countries, even within batches from the same supplier. Taken together, our investigation suggests that there is a considerable variation in the AME values of oils and fats, which may affect the feed formulation precision.

Keywords: Dietary energy, Fatty acid composition, Lipids, Oil quality, Poultry

INTRODUCTION

Vegetable oils and animal fats are usually added to animal diets to increase dietary energy concentration (Ravindran et al., 2016). Since oils and fats confer at least twice as much energy as other food nutrients such as carbohydrates and proteins (Ahiwe et al., 2018; Blair, 2018), there is a greater demand in optimizing the use of these products to meet the energy requirements of poultry (Ravindran et al., 2016). Furthermore, high fat feeding in poultry has been proven to improve the digestibility and absorption of non-lipid constituents (Blair, 2018). However, the quality of oils and fats are highly variable, and their digestibility are dependent on their chemical structures (Codony et al., 2017). Poor processing and storage conditions can also cause structural changes in oils and fats, leading to high fluctuations in the nutritional values (FAO/WHO, 2001; Gibson and Newsham, 2018).

Fat digestion consists of the emulsification of dietary fat with bile salt, followed by the enzymatic hydrolysis of triglycerides. The 2-monoglycerides, formed from partial hydrolysis of triglycerides, improve the solubility and absorption of free fatty acids through the formation of micelles (Pond et al., 2004; Scanes et al., 2019). As such, low levels of 2-monoglycerides will result in incomplete micellar solubilization of free fatty acids. It was previously reported that the total micellar fatty acids were lowest in the duodenum of free fatty acid (FFA) - fed chicks where monoglycerides were present at trace level (Hofmann and Borgstrom, 1962; Sklan, 1979). In addition, fat digestion is also highly dependent on the degree of fatty acid saturation where Tancharoenrat et al. (2014) reported a higher digestibility with unsaturated fatty acids such as oleic acid and linoleic acid in comparison to saturated fatty acids such as palmitic and stearic acids. Additionally, the natural emulsifying properties of unsaturated fatty acids could also aid in mixed micelle formation and absorption, resulting in better utilization of saturated fatty acids (Rodriguez-Sanchez et al., 2019). Given the importance of FFA and the degree of saturation of oil (ratio of unsaturated to saturated fatty acids; U:S) in oil digestion and absorption, the Wiseman equation incorporates both of these parameters into one general equation to predict the energy values of different sources of oils and fats (Wiseman and Blanch, 1994).

As these macromolecules are important energy sources for animals, it is imperative for us to understand the variation of oil quality based on apparent metabolizable energy (AME) across countries and oil types, and its impacts on broilers. Previous reports showed that the fat utilization in broilers was age dependent where fat utilization improved with age (Rodriguez-Sanchez et al., 2019). Animal nutritionists often struggle to formulate feed with adequate energy intake due to the variation of the nutritional values in oil and fat samples that can lead to reduction in the performances of the

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animals and substantial economic losses (Niu et al., 2009; Ahiwe et al., 2018). A better understanding of the AME of different oil and fat samples can be gained by incorporating the FFA and U:S data into the Wiseman equation to generate information on the quality of the oil and fat samples; this will allow nutritionists to make informed decisions on their use for feed formulation to achieve consistent animal performance. In this study, the AME for oil and fat samples were determined based on the Wiseman equation to highlight the importance of accurate information on dietary energy value of feed.

MATERIALS AND METHODS

Instrumentation

DL 50 GRAPHIX auto-titrator (Mettler-Toledo, Ohio, United States) and DG113-SC glass electrode (Mettler-Toledo) were used to determine free fatty acid content. 7890B GC-FID (Agilent Technologies, California, United States) with Supelco SPTM-2560 (L × I.D. 100 m × 0.25 mm, df 0.20 µm thickness) (Sigma-Aldrich, Missouri, United States) was used for chromatographic separation of fatty acid methyl esters (FAME).

Sample collection and preparation

A total of 635 oil and fat samples with plant and animal origins were collected across the Asia Pacific region and analyzed over a span of eight years from year 2011 to 2018. These samples included tallow, rice bran oil, fish oil, palm oil (crude and refined palm oil), soybean oil, lard and coconut oil. All samples were stored in plastic containers upon receipt and kept in the chiller at 2 °C to 6 °C. Before analysis, the samples were either thawed at room temperature or melted in the oven at 60 °C. All samples were analyzed within one week from the collection date.

Free Fatty Acid (FFA) content

The FFA content of oil and fat samples were determined with an in-house method, modified from the Association of Official Analytical Chemists (AOAC) method (AOAC, 2012). Fifty (50) mL of 95% ethanol (Aik Moh Paints and Chemical Pte Ltd, Singapore) was added to 1.0 g of oil or fat sample in a titration cup. The sample was stirred for 60 s under stirring speed of 50% with an auto-titrator. After stirring, titration was done with 0.1 N sodium hydroxide (Merck KGaA, Darmstadt, Germany) as the titrant using a pH sensor with measurement mode set as equilibrium controlled. The result was calculated from the volume consumption of the sodium hydroxide titrant and its concentration. Based on the oil type, the FFA content is expressed either as % oleic acid, % palmitic acid or % lauric acid.

Fatty Acid Methyl Esters (FAME) composition analyses

FAME composition of oil and fat samples were determined using an in-house method, with modification from Association of Official Analytical Chemists (AOAC) method (AOAC, 2012). Four mL of 2% (w/v) methanolic sodium hydroxide (Merck) was added to 40 mg of fat or oil sample and refluxed until there were no visible fat globules. 5 mL of 14% boron trifluoride in methanol (Sigma-Aldrich) was added and refluxed for another 2 mins. Finally, 10 mL of heptane (Sigma-Aldrich) was added and refluxed for another 1 min. Subsequently, the content was cooled to room temperature. Next, 15 mL of 26% (w/v) sodium chloride (Merck) was added and swirled vigorously. The top organic layer (heptane) was filtered through sodium sulphate (Merck) and injected into the GC-FID for chromatographic separation. Extracted samples were analyzed with helium at a flow rate of 0.85 mL/min as carrier gas and a split ratio of 40:1. Injection volume was set at 0.4 µL with injection port temperature set at 260 °C. The GC oven temperature was programmed at 140 °C for the first 5 mins and raised to 235 °C at 5 °C/min for 15 mins, followed by 15 °C/min to 250 °C for 5 mins. The total run time was 45 mins. Percentage composition of each FAMES in oil and fat samples were calculated with Supelco 37 component Fatty Acid Methyl Esters (FAME) Mix certified reference material (CRM) (Sigma-Aldrich) as reference standard.

Data analysis

Prediction of Apparent Metabolizable Energy (AME) using Wiseman equation

AME of samples were predicted using a general equation (Equation 1) with A, B, C and D based on the values shown in Table 1 (Wiseman and Blanch, 1994; Wiseman et al., 1998).

$$\text{AME (MJ/kg fat)} = A + B \cdot \text{FFA} + C \cdot e^{D(U/S)} \quad (1)$$

Apparent Metabolizable Energy (AME) variation

AME range was calculated as the difference between the highest and lowest predicted AME values whereas relative variations were calculated as the ratio of calculated range against lowest predicted AME or literature AME.

Statistical analyses

Single measurement data were calculated for the AME of each oil type. Descriptive statistics were calculated using Microsoft Excel 365 and presented in Table 3.

Table 1 – Empirical values of constants A – D used in Wiseman equation to predict the apparent metabolizable energy (AME) values of poultry at different ages

Constant (unit)	Young broilers (< 21 days) ^a	Old broilers (> 21 days) ^a
A (MJ/kg)	38.112 ± 1.418	39.025 ± 0.557
B (MJ/kg)	-0.009 ± 0.002	-0.006 ± 0.001
C (MJ/kg)	-15.337 ± 2.636	-8.505 ± 0.746
D	-0.506 ± 1.186	-0.403 ± 0.088

^a Empirical values of constants A – D were categorized into two groups, young broilers (aged < 21 days) and old broilers (aged > 21 days). All young broilers (aged < 21 days) followed the same empirical values for constants A – D, likewise for old broilers (aged > 21 days).

RESULTS

Predicted Apparent Metabolizable Energy (AME) values for all samples

Using GC-FID and acid-base titration, all samples were analyzed for their lipid composition and FFA content (Table 2). Descriptive analysis of eight different oil types were presented in Table 3. AME of young broilers (aged < 21 days) and old broilers (aged > 21 days) were studied in this paper. Based on the GC-FID analyses, it was determined that the U:S for crude palm oil was lowest amongst all samples analyzed while the U:S for soybean oil was the highest, with relatively low FFA content of 1.01% oleic acid recorded (Table 3). When the data was further extrapolated using Equation 1, it was found that the highest predicted mean AME values were from soybean oil, at 8362 kcal/kg (young broilers) and 8672 kcal/kg (old broilers). On the other hand, the lowest predicted mean AME values were from crude palm oil with the predicted AME values at 6617 kcal/kg (young broilers) and 7669 kcal/kg (old broilers) (Table 3).

It was apparent that the predicted AME values were inconsistent across all oil samples. In particular, a large spread of AME for fish oil samples for different age groups of broilers was observed. The energy gaps for young (< 21 days old) and old broilers (> 21 days old) were 2295 kcal/kg and 1417 kcal/kg, with a relative variation of 36% and 19% respectively (Table 3). The AME gap for crude palm oil for young (< 21 days old) and old broilers (> 21 days old) were found to be 1057 kcal/kg and 540 kcal/kg with relative variations of 17% and 7% (Table 3). Comparatively, refined palm oil also showed a smaller AME spread relative to crude palm oil, with 506 kcal/kg for young broilers (8% variation) and 250 kcal/kg for old broilers (3% variation) (Table 3). As the three major oil groups (e.g. tallow, rice bran oil, and fish oil) accounted for 63% of the total oil and fat samples collected and represented the majority of the oil and fat products (Table 2), the data for these groups were further analyzed (Table 4).

Tallow

Large AME discrepancy of 2670 kcal/kg for young broilers with relative variation of 49% and 1565 kcal/kg for old broilers with a relative variation of 23% were observed (Table 3). Out of 163 samples, 85% of the samples were received from five different sources originating from South Korea (Table 4). Majority of the samples were from the same source, supplier 1, where it accounted for approximately 78% of the tallow samples received from South Korea. Large spread of AME was observed for supplier 1, at 1248 kcal/kg with a relative variation of 20% for young broilers and 626 kcal/kg with a relative variation of 8% for old broilers (Figure 1). As such, supplier 1 from South Korea was singled out with samples collected in eight batches over a span of five years, from year 2012 to 2016. The AME values observed were inconsistent even within batches where the energy spread was in the range of 230 kcal/kg to 1063 kcal/kg with relative variation of 3% to 17% (Table 5). Likewise, AME values for tallow samples from supplier 3 were inconsistent as well, with energy spread at 1362 kcal/kg for young broilers (aged < 21 days) and 740 kcal/kg for old broilers (aged > 21 days) (Figure 1). This translated to relative variations of 20% for young broilers (aged < 21 days) and 10% for old broilers (aged > 21 days).

Rice bran oil

All rice bran oil samples received were from Thailand since 2012. From 2012 to 2014, the predicted ME values were highly variable as shown in Figure 2. However, from 2015 onwards, the predicted AME values were calculated to be more consistent where the energy values ranged from 7500 kcal/kg to 8000 kcal/kg (relative variation of 7%) with only seven outlier samples. High FFA content of 12.50% oleic acid was observed (Table 3).

Fish oil

Majority of the fish-based oil samples were from Indonesia and Thailand (63% of fish oil samples). Figure 3 showed that fish oils from Thailand consisted of large energy gaps of 2295 kcal/kg (young broilers) and 1417 kcal/kg (old broilers). Similarly, when the AME for different batches of fish oils from the same supplier (supplier A) in Thailand were determined, it was found that the AME ranged from 6442 kcal/kg to 8738 kcal/kg with relative variation of 36% in young broilers (Table 4). Likewise, a difference of 1926 kcal/kg in terms of AME variation (30%) was observed between fish oil samples from Indonesia (Table 4).

DISCUSSION

The quality and efficiency of feed formulations are highly dependent on two main factors, the extent and accuracy of animal nutritionists' knowledge on raw materials' qualities and compositions, as well as the nutrient requirements of targeted species (Lall and Dumas, 2015). Animal nutritionists struggle to formulate feed with adequate energy when lipid energy values stated in traditional feed tables often deviate from the actual energy value due to various reasons such as poor storage and processing conditions. It is also likely that these values did not account for the species and age dependent metabolism. While Baião et al. (2005) reported that the AME for tallow was in the range of 7000 kcal/kg, Figures 1(A) and 1(B) indicated that regardless of animal age groups, inconsistency in AME values of tallow were apparent where energy variations occurred even within the same supplier with relative variation as high as 17% for the same batch of tallow samples. Without proper lipid quality evaluations, this will eventually lead to poorer animals' performances and economic losses.

The predicted mean AME of soybean oil is the highest as compared to other oil types. One of the main contributing factors is the presence of high unsaturated fatty acids where the recorded U:S ratio was 4.71 (Table 3). Consistent to a previous study conducted by Rodriguez-Sanchez et al. (2019) where it was reported that hydrolysis in unsaturated diets were relatively more efficient than saturated diets which results in higher digestibility and absorption. The AME of soybean oil ranged from 6665 kcal/kg to 8796 kcal/kg for young broilers (aged < 21 days) and 7716 kcal/kg to 8997 kcal/kg for old broilers (aged > 21 days). In comparison, a study showed that the AME of soybean oil is at 8790 kcal/kg (Baião et al., 2005), demonstrating 12% relative variation from the literature value. Low fatty acid content (1.01% oleic acid) was also observed for soybean oil. The presence of high FFA decreases bile secretion which in turns reduces micellar formation, leading to poor absorption of digested materials (Ravindran et al., 2016; Rodriguez-Sanchez et al., 2019). A study conducted by Wiseman and Salvador (1991) showed that AME is inversely proportional to the FFA content, with the effect being more pronounced in younger broilers. In agreement with the study conducted by Wiseman and Salvador, our survey also showed that the AME values of young broilers were more divergent as compared to older broilers, demonstrating their sensitivity to oil quality variations possibly due to less developed physiological capacity in fat utilization (Rodriguez-Sanchez et al., 2019).

Our results showed that from year 2015 onwards, the AME values for rice bran oil samples collected from Thailand were more consistent (Figure 2). One plausible reason could be due to technological improvements made to the manufacturing or transporting processes in Thailand. While more consistent AME values were observed over the years, the FFA content of rice bran oil remains the highest among the eight oil types analyzed (Table 3). As rice bran contains endogenous lipase capable of digesting and hydrolyzing the triglycerides present to form FFA (Goffman and Bergman, 2003, Vallabha et al., 2015), it is possible that the samples collected were likely to be extracted from poor quality rice bran where the triglycerides had been hydrolyzed (Rajan and Krishna, 2009). Interestingly, while high FFA content was observed in rice bran oil (Table 3), its AME remains one of the highest among the other oil types. One of the reasons could be due to the relatively higher U:S where the presence of unsaturated fats aid in the solubilization and absorption of FFA (Hofmann and Borgstrom, 1962).

Large energy gaps were observed in fish oil with 36% variation in young broilers (< 21 days) and 19% variation in old broilers (> 21 days). This is likely due to the presence of different fish oils with different oil quality grades such as salmon fish oil and crude tuna fish oil. There are different standards for different fatty acid compositions of different fish origins. For instance, while the standard for C22:6 (n-3) docosahexaenoic acid of tuna oil ranges from 21.0 – 42.5% of total fatty acids, similar standard for wild salmon oil ranges from 6.0 – 14.0% (FAO/WHO, 2017). Fish oils are also susceptible to lipid oxidation due to the high degree of unsaturation (European Food Safety Authority (EFSA, 2010) where unsaturated fatty acids are prone to oxidation (Dominguez et al., 2019). Comparatively, refined palm oil has lower AME spread as compared to crude palm oil. This is likely due to the refining processes that may have possibly removed the impurities, and therefore confers a more consistent oil quality in refined palm oil.

Given these analyses, it is evident that having a proper lipid analysis in place is fundamental for accurate estimation of dietary energy in feed formulations.

Table 2 – Number of oils and fats collected across Asia Pacific region, per oil type

Oils and fats	Count
Tallow	163
Rice bran oil	147
Fish oil	93
Crude palm oil	70
Soybean oil	43
Refined palm oil	42
Lard	41
Coconut oil	36

Table 3 – Descriptive analysis data of the eight different oil types for broilers

Apparent Metabolizable Energy (AME) of poultry (broiler)																
Item	Tallow		Rice bran oil		Fish oil		Crude palm oil		Soybean oil		Refined palm oil		Lard		Coconut oil	
	<21	>21	<21	>21	<21	>21	<21	>21	<21	>21	<21	>21	<21	>21	<21	>21
Statistics (kcal/kg)																
Minimum	5448	6930	6709	7566	6442	7530	6359	7524	6665	7716	6533	7652	6002	7399	6685	7705
Maximum	8118	8495	8138	8503	8738	8947	7416	8064	8796	8997	7038	7902	7825	8320	7914	8375
Range	2670	1565	1429	937	2295	1417	1057	540	2131	1282	506	250	1822	922	1229	670
1 st quartile	6742	7744	7608	8332	6912	7826	6509	7616	8321	8624	6858	7810	6996	7878	7159	7926
Median	6949	7853	7710	8488	7183	7965	6601	7666	8579	8807	6908	7835	7115	7934	7493	8121
3 rd quartile	7087	7913	7787	8567	7916	8373	6674	7704	8589	8814	6964	7862	7347	8060	7664	8229
Mean	6886	7820	7635	8194	7370	8076	6617	7669	8362	8672	6886	7824	7084	7925	7411	8075
SE of mean	29	15	38	12	60	34	21	11	49	43	18	9	62	31	54	32
Standard deviation, σ	374	191	265	147	581	329	172	88	463	279	114	56	398	200	323	192
Nutritional parameters																
FFA (%) ^a	2.66		12.50		4.62		7.07		1.01		0.34		1.35		8.45	
U:S ratio	1.28		2.70		2.14		1.10		4.71		1.20		1.48		2.10	

< 21 = Young broilers of age less than 21 days; > 21 = Old broilers of age more than 21 days; SE = Standard error; FFA = Free fatty acid content; U:S = unsaturated: saturated fatty acid. ^a Free fatty acid content is expressed as % palmitic acid for crude and refined palm oil, % lauric acid for coconut oil and % oleic acid for the rest of the oil types.

Table 4 – Details of samples collected from the different countries for the three major oil types (Tallow, rice bran oil and fish oil), with minimum, maximum, range and mean apparent metabolizable energy (AME) of young broilers (< 21 days)

Country	Tallow							Rice bran oil						Fish oil					
	AME (< 21 days) (kcal/kg)							AME (< 21 days) (kcal/kg)						AME (< 21 days) (kcal/kg)					
	Min	Max	R	Mean	n	n _s	Min	Max	R	Mean	n	n _s	Min	Max	R	Mean	n	n _s	
Thailand	-	-	-	-	0	-	6709	8138	1429	7635	147	7	6442	8738	2295	7367	25	5	
Indonesia	-	-	-	-	0	-	-	-	-	-	0	-	6499	8425	1926	7416	34	4	
Vietnam	6299	6333	35	6316	2	-	-	-	-	0	-	6575	8321	1746	7103	24	9		
Philippines	5448	6561	1112	5989	6	1	-	-	-	0	-	7919	8233	313	8028	3	1		
Singapore	5826	6372	546	6138	3	1	-	-	-	0	-	7199	7199	0	7199	1	1		
Taiwan	6002	6640	638	6345	10	2	-	-	-	0	-	7440	7826	386	7633	2	1		
South Korea	6240	8118	1878	7003	138	5	-	-	-	0	-	7948	7948	0	7948	1	1		
New Zealand	6073	6520	448	6370	3	2	-	-	-	0	-	7861	8309	448	8031	3	1		
India	6503	6503	0	6503	1	1	-	-	-	0	-	-	-	-	-	0	-		
Total	5448	8118	2670	6886	163	12	6709	8138	1429	7635	147	7	6442	8738	2295	7370	93	23	

AME (< 21 days) = Apparent metabolizable energy for young broilers of age less than 21 days; Min = Minimum; Max = Maximum; R = Range; n = number of observations; n_s = number of suppliers.

Table 5 – Details of tallow samples collected from South Korea, Supplier 1, with minimum, maximum, range calculated for apparent metabolizable energy (AME) of young broilers (< 21 days)

Batch	AME (< 21 days) (kcal/kg)	Count	Min	Max	R	Percentage variation (%)
Batch 1		26	6240	7303	1063	17
Batch 2		7	6546	7124	578	9
Batch 3		24	6612	7428	815	12
Batch 4		22	6710	7488	778	12
Batch 5		18	6630	7413	783	12
Batch 6		1	7258	7258	0	NA
Batch 7		1	7173	7173	0	NA
Batch 8		9	6908	7137	230	3

AME (< 21 days) = Apparent metabolizable energy for young broilers of age less than 21 days; Min = Minimum; Max = Maximum; R = Range; NA = Not Applicable

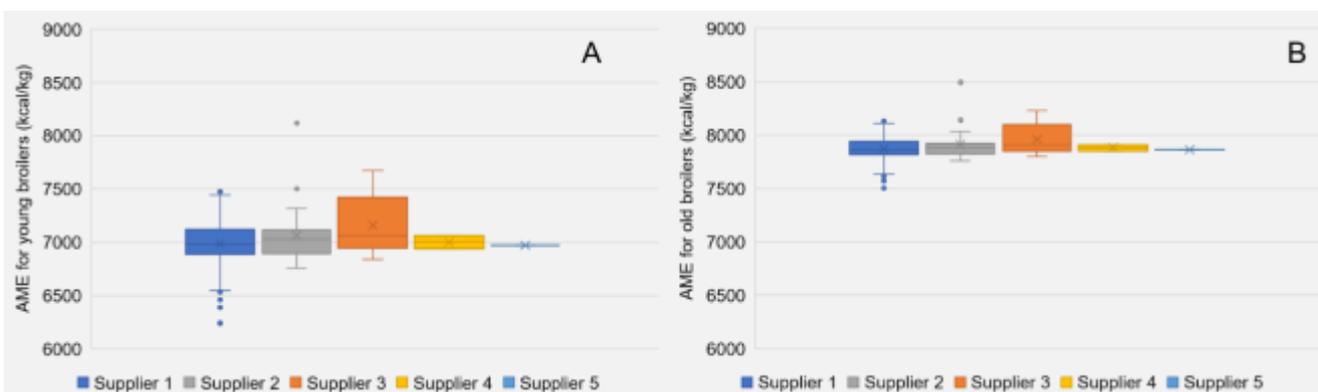


Figure 1 - Variations in minimum, first quartile, median, third quartile and maximum in predicted apparent metabolizable energy (AME) for both (A) young broilers (aged < 21 days) and (B) old broilers (aged > 21 days) differentiated by the different tallow suppliers in South Korea.

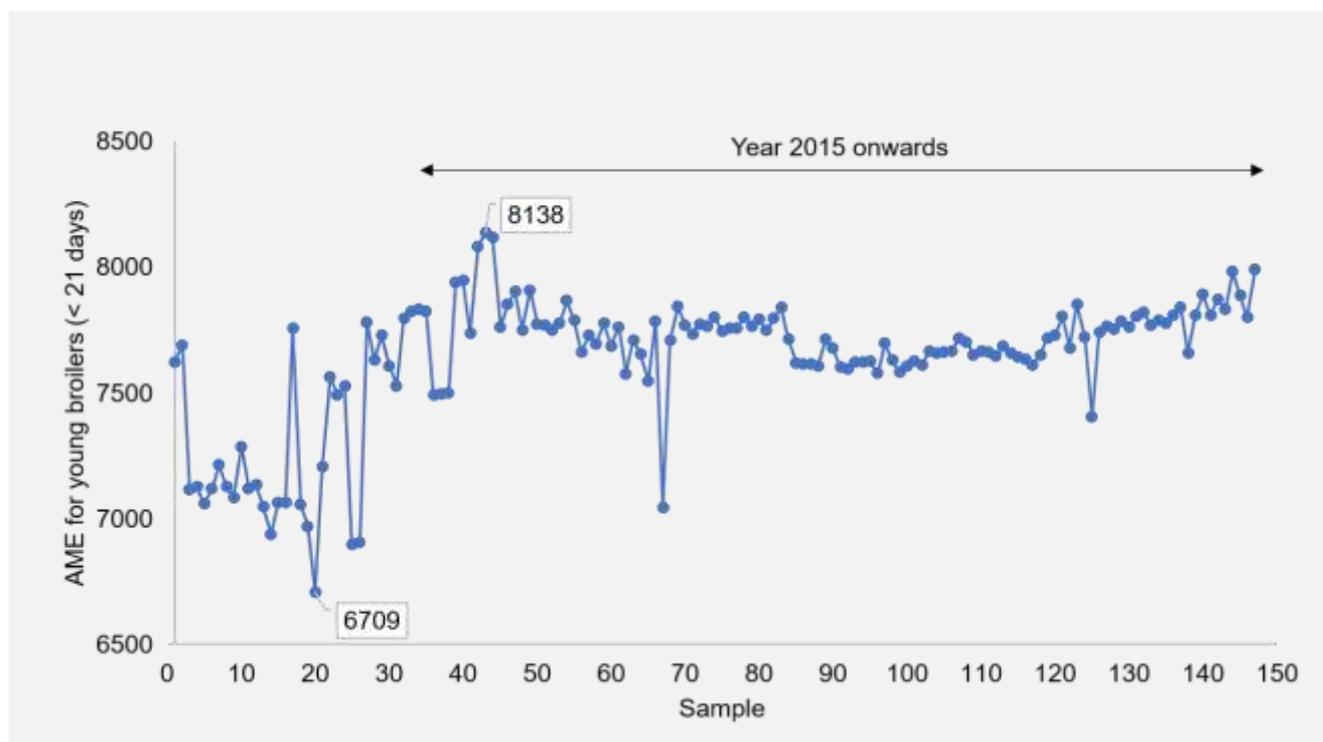


Figure 2 - Predicted apparent metabolizable energy (AME) trend graph for young broilers (aged < 21 days) with emphasis on year 2015 onwards, for rice bran oils.

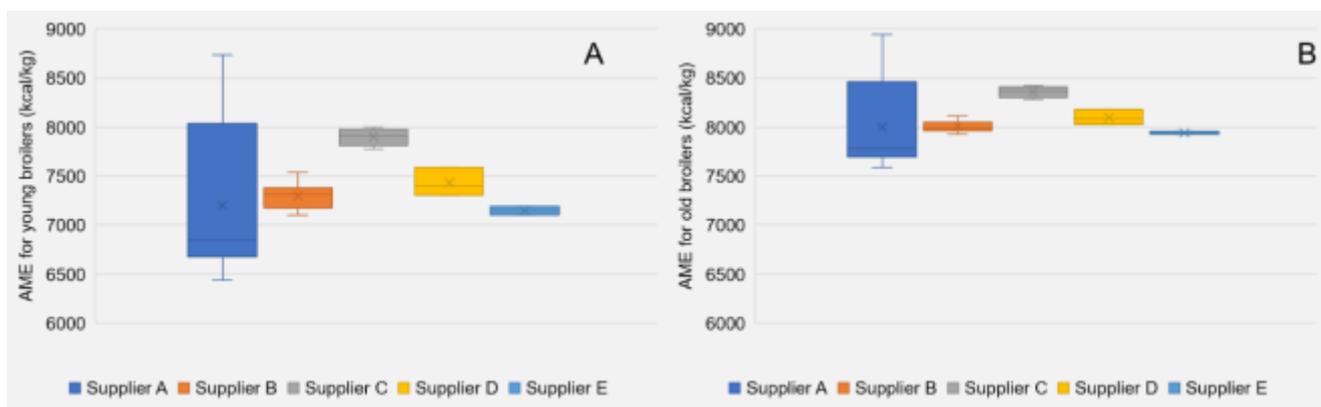


Figure 3 - Variations in minimum, first quartile, median, third quartile and maximum in predicted apparent metabolizable energy (AME) for both (A) young broilers (aged < 21 days) and (B) old broilers (aged > 21 days) differentiated by the different fish oil suppliers in Thailand.

CONCLUSION

In conclusion, our data suggested that there is a considerable variation of the AME values in oils and fats. The AME variation that existed across oil samples from different regions and even within batches from similar suppliers may affect the feed formulation precision if the variation remains unaccounted for. Generic lipid energy values extracted from the traditional feed table were typically inaccurate as the animal species and age dependent metabolism were likely not considered in these tables. Furthermore, poor storage and processing conditions may deteriorate the oil quality as well. Inevitably, inconsistent AME values will not only contribute to huge economic losses but may also impact the animal performances adversely due to inaccurate feed formulations that fail to meet the caloric requirements of the animals. In view of these concerns, it is important to have a proper lipid evaluation tests in place for a more accurate lipid profile (e.g. AME value) estimation. Additionally, oil quality parameters such as peroxide and *p*-anisidine values should also be considered for oxidative stability evaluation as oil and fat quality may deteriorate over time. To improve oil and fat qualities, bio-emulsifiers and antioxidants can be used concurrently to improve oil and fat qualities in the context of oxidative stability and feed fat variability control.

DECLARATIONS

Corresponding Authors

Agnes Thng, e-mail: agnes.thng@kemin.com. Jun Xiang Ting, email: junxiang.ting@kemin.com. Hui Ru Tay, email: huiru.tay@kemin.com. Chin Yi Soh, email: chinyi.soh@kemin.com. Hwee Ching Ong, email: hweeching.ong@kemin.com. David Tey, email: david.tey@kemin.com.

Authors' Contribution

A. Thng proposed the design of study, prepared the manuscript and performed the laboratory analysis. J.X. Ting, H.R. Tay, C.Y. Soh and H.C. Ong assisted with the laboratory analyses. D. Tey reviewed and edited the manuscript.

Conflict of Interests

The authors declared that there is no conflict in this study.

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