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Energy Utilization of By-Products from the Soybean Oil Industry by Broiler Chickens: Acidulated Soapstock, Lecithin, Glycerol and Their Mixture

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ABSTRACT

Acidulated soy soapstock (ASS) and lecithin (LEC) are by-products from processing soy oil (SO) for human consumption, whereas glycerol (GLY) can be obtained through the transesterification of vegetable oils during the production of biodiesel. These are valuable by-products for poultry feeds that have been underutilized in many parts of the world. One study was conducted to estimate the AME_n of ASS, LEC, GLY as well as of their mixture (MIX: 85% ASS, 5% LEC and 10% GLY). Two hundred and sixty Cobb 500 female broilers of 20 days of age were housed in steel wire battery cages in a controlled temperature room for broilers. A completely *randomized* (energy sources x fat inclusion level) factorial design was applied, with 4 replicates of three birds per treatment. Birds were fed a corn-soybean meal control diet without supplemental fat or with the addition of 2, 4, or 6 % of the four energy sources. Total excreta collection was performed from 26 to 28 days. The AME_n values of by-products were calculated using regression analysis as well as by the difference method. The average AME_n values calculated by regression analysis were: 9,232, 7,502, 5,447 and 8,404, whereas results with the difference method were: 7,951, 6,579, 3,979 and 8,101 kcal/kg for, in both cases for ASS, LEC, GLY and MIX, respectively. It is concluded that these energy sources can be for broilers and that there are significant differences between the methods used to estimate AME_n of fats.

INTRODUCTION

Several by-products are obtained during the processing of soy oil (SO) for human consumption, as well as through its transesterification required for biodiesel production. By-products from these processes are generally cheap and are sometimes used in poultry feeds and include lecithin (LEC), acidulated soy soapstock (ASS), and glycerol (GLY).

Lecithin is removed by centrifuging crude soy oil, representing around 1.5 to 3.1 % of the original source (Overland *et al.*, 1994). As for its use by the animal, LEC has important roles in the phosphate and energy metabolism, in addition of being an important source of choline (Menten *et al.*, 1997; Woerfel *et al.*, 1981).

In most markets, SO needs to be previously neutralized before being sold for human consumption. This neutralization entails a chemical reaction with alkali, and as a result, around 6% of the original fat is a mixture of free fatty acids and mono and diglycerides (Vieira *et al.*, 2002). Values of AME_n of ASS for poultry vary with their oil of origin (Pardio *et al.*, 2001; Vieira *et al.*, 2002; Machado *et al.*, 2003). Important amounts of tocopherols can also be found in ASS (Pardio *et al.*, 2001).

Glycerol is a by-product of biodiesel production, and each litter of biodiesel yields obtained as around 79 g of GLY (Min *et al.*, 2010). As a precursor of glyceraldehyde 3-phosphate, GLY produces energy through the glycolytic and tricarboxylic-acid pathways in animals and, therefore, it can also be used as an energy source in poultry diets. Studies have been reported varying AME_n values for poultry (Nelson & Cox, 2002).

Despite the increasing availability of the by-products of SO processing and biodiesel production, their use in poultry nutrition is still limited. There are few literature reports on their AME_n values, especially when more than one of these by-product are added to poultry diets. Synergistic effects in energy utilization by broilers have been shown when GLY and ASS simultaneosuly are added into the same feed (Sklan, 1979). However, it is less common to add the three mentioned by-products simultaneously in a same poultry feed.

The objective of this study was to determine the apparent metabolizable energy (AME_n) values of LEC, ASS and GLY, as well as their mixture in a proportion that resembles their individual proportion in the original crude soy oil (Nelson & Cox, 2002; Overland *et al.*, 1994; Vieira *et al.*, 2002).

MATERIALS AND METHODS

All procedures of this study were approved by the Ethics and Research Committee of Universidade Federal do Rio Grande do Sul, Brazil.

Bird husbandry

A total of two hundred and sixty day-old Cobb X Cobb 500 slow-feathering female broiler chicks, vaccinated for Marek's disease, were obtained from a local hatchery (Frangosul S.A., Montenegro, Brazil).

Chicks were housed on day 1 on floor pens, where they remained until 20 days old. On day 21, birds were randomly allocated into 52 steel battery cages (0.40 m x 0.90 m, five birds per cage) equipped with one feeder and one drinker each. Room temperature was controlled to maintain bird comfort throughout the study. Lighting was continuous and feed and water were supplied *ad libitum*.

Experimental diets

A basal corn-soybean meal diet without fat supplementation was formulated (Table 1) as well as twelve other treatments diets consisting of the inclusion of LEC, ASS and GLY individually or as their respective combination (MIX: 5%, 85% and 10%, respectively LEC, ASS and LEC) in the basal diet. Fat sources were added to the feeds at the ratios of 2, 4 and 6% at the expense of the entire basal feed.

Birds were distributed in a completely randomized factorial arrangement of four energy sources and three levels of inclusion, totaling four replicates of five birds per treatment.

Table 1 – Basal diet composition.

Item	Basal diet
Ingredients (%)	
Corn	61.15
Soybean meal	35.09
Limestone	0.92
Dicalcium phosphate	1.79
Salt	0.48
L-Lysine HCL	0.11
DL-Methionine	0.25
Choline chloride 60%	0.05
Vitamin and Mineral premix[1]	0.16
Calculated nutrient composition (% or as noted)	
AME_n[3], (kcal/kg)	2876.00
Crude protein	21.00
Calcium	0.90
Available phosphorus	0.45
Sodium	0.21
DEB (mEq/kg)[3]	210.00
Choline	1650.00
Digestible Lysine	1.10
Digestible total sulfur amino acids	0.82
Digestible threonine	0.69
Digestible valine	0.88
Digestible isoleucine	0.81
Digestible arginine	1.31
Digestible tryptophan	0.22

[1]Supplied per kg of feed: vitamin A: 8000 IU; vitamin D_3: 2000 IU; vitamin E: 30 IU; vitamin K_3: 2 mg; thiamine: 2mg; riboflavin: 6mg; pyridoxine: 2.5 mg; vitamin B_{12}: 0.012 mg; pantothenic acid: 15 mg; niacin: 35 mg; folacin: 1mg; biotin: 0.08 mg; Fe: 40 mg; Zn: 80 mg; Mn: 80 mg; Cu: 10 mg; I:0.7 mg; Se: 0.3 mg; monensin sodium (CobanTM 40%, Elanco Animal Health): 275 mg.
[2]AME_n: apparent metabolizable energy corrected for retained nitrogen
[3] DEB: Dietary electrolyte balance

The ASS and LEC used in this study were obtained from the Energy Solutions for Animal Nutrition - ESAN (Londrina, PR, Brazil), whereas GLY (99%) was obtained from INAQUIM Indústria e Comercio Ltda. (Canoas, RS, Brazil). All fat sources were analyzed prior to the beginning of the study according to the methods of the AOAC (1995, 2005, 2000) and to ASTM (2006) and are detailed in Table 2.

Table 2 – Chemical characterization of the energy sources utilized to determine apparent metabolizable energy corrected for retained nitrogen (AMEn) values, % or as noted.

Analysis	ASS[1]	LEC[2]	GLY[3]	Analytical method
Moisture Karl Fischer	0.53	0.23	0.17	AOAC 984.20
Moisture and volatiles	1.06	5.28	0.90	AOAC 926.12
Crude Protein	0.12	3.42	0.00	AOAC 990.03
Ether Extract	99.90	99.94	0.41	AOAC 920.39
Peroxide value, mEq/kg fat)	0.00	0.00	0.00	AOAC 965.33
Acidity in oleic acid	77.30	10.50	9.30	AOAC Cd 3d-63
Iodine value, g/100g	11.47	94.82	-	AOAC 993.20
pH aqueous solution	2.90	3.70	6.90	AOCS G 7-56
Ash	1.27	8.09	0.03	AOAC 942.05
Phosphorus	0.03	1.93	-	AOAC 956.01
Sodium	0.003	0.021	0.280	AOAC 956.01
Glycerol	-	-	99.20	ASTM 2006
Methanol, mg/L	-	-	9.50	AOAC 958.04
Gross energy, (kcal/g)	9.14	7.78	4.30	Calorimeter

[1]Acidulated soapstock
[2]Lecithin soybean
[3]Glycerol 99%

Excreta Collection

Birds had five days of adaptation to the experimental diets. Starting on day 25, total excreta were collected for 72 h, and then homogenized, weighed, and frozen (-18 °C). Feed intake was calculated as the difference between the amounts of feed offered and left in the feeders at the end of the study. Calorimetry was performed using representative samples of the feeds and the excreta that were previously dried at 60 °C for 72 h using standard methods (AOAC, 1990).

The energy of the by-products was estimated by two methods: by regression analysis, where AME_n intake was regressed against feed intake with the slope representing the AME_n content of the by-products, adapting the procedure of Dozier *et al.* (2008), and by the difference method according to the equation of Campbell *et al.* (1983):

AME_n I= GEI – (EO_T – (1-X)EO_R)/ X

Where: (GEI = Gross energy of the test ingredient, EO_T = Energy output test diet, EO_R = Energy output reference diet, X= % Inclusion).

STATISTICAL ANALYSIS

Data were analyzed using the ANOVA procedure of SAS (2001). The Tukey's HSD test (Tukey, 1991) was applied to determine differences among means, considering a significance level at 5%. The PROC REG procedure of SAS (Statistical Analysis System, version 9.2) was used for regression analysis, which was conducted for each energy source, considering the basal diet without supplemental fat and the three levels of each added fat source.

RESULTS AND DISCUSSION

Average AME_n levels of the diets and by-products are presented in Table 3, and average values for each fat source are shown in Table 4. No interaction between fat source and level (p>0.05) was observed. However, fat level had a significant effect on AME_n content of diets and fat sources (p<0.01), whereas AME_n content of supplemental fat was significantly affected only by fat sources (p<0.01).

Average AME_n values of the fat sources calculated using regression analysis were 9232, 7502, 5447, and 8404, whereas the values obtained using the difference method were 7951, 6579, 3979, and 8101 kcal/kg for ASS, LEC, GLY and MIX, respectively (Table 3).

The addition of the soybean fat by-products in the present study led to a concurrent increase in AME_n of diets. This increase was associated with the level of inclusion and with the energy content of each fat source. However, the AME_n values estimated using regression analysis were higher than those calculated using the difference method. The difference method (Campbel *et al.*, 1983) was originally proposed to generate AME_n values using a single level of fat supplementation. According to Wiseman and Salvador and Dozier *et al.* (1991, 2008), one of the advantages of using multiple fat inclusion levels when determining AME content of fats is that the method does not depend on the individual rates of inclusion of the evaluated fats, thereby reducing the uncertainty of the dietary energy values. Furthermore, the use of multiple inclusion levels allows assessing the effects of inclusion rates. Lower standard errors energy values of the fats investigated are expected when regression equations are applied. In contrast, when low levels of fat are included, such as when the difference method is used, the likelihood of underestimating the AME_n values of the ingredient being studied increases. Depending on the energy source and of its dietary inclusion levels, the response in terms of energy contribution to the animal may be linear, curvilinear, or exceed its gross energy content (Sibbald and Kramer, 1978). Therefore, the methodology proposed by Campbell *et al.* (1983) may be more suitable for the calculation of AME_n contents when fat sources are added at low levels to a reference diet, allowing reducing the negative effects of possible procedure errors made during laboratory analyses.

The two methods applied in the present study, yielded different AME_n estimates of the evaluated fat sources. The obtained values also show some discrepancies when compared with published data. For instance, Gaiotto *et al.* (2000) reported 6715 kcal/kg for ASS fed to 5- to 6-d-old broilers, whereas Vieira *et al.* (2002) and Raber *et al.* (2008) found ASS energy values of 8115 and 8477 kcal/kg for 28 and 31-d-old broilers, respectively. These differences may be attributed to the age of birds because the physiological capacity of fat utilization is still poorly developed in young birds; an increase in the fat utilization as birds age are, however, expected (Wiseman & Salvador, 1989; Blanch *et al.*, 1995; Sakomura *et al.*, 2004). Moreover, determination methods may also affect AME_n estimates. For instance, most of the recent AME_n determinations for ASS were performed using a single inclusion level of this fat source (Gaiotto et. al., 2000; Vieira *et al.*, 2002; Raber *et al.*, 2008).

The NRC (1994) and Rostagno *et al.* (2011) reported AME_n of 6440 and 6036 kcal/kg for LEC, respectively. The commercial availability of LEC is sometimes limited, but this may be a valuable feed ingredient depending on its market price. In addition to its utilization as an energy source in poultry diets, supplementing LEC in poultry feeds also supplies important amounts of choline (14800 ppm as determined by the authors) and phosphorus, as shown in Table 2.

In the present study, the AME_n content of GLY was estimated as 5447 and 3979 kcal/kg by the difference method and by linear regression, respectively. Similar values were obtained by Cerrate *et al.* (2006) using a single inclusion level, and by Dozier *et al.* (2008) using linear regression, who determined AME_n values of 3596 kcal/kg for 17- to 24-d-old broilers and 3434 kcal/kg for 38- to 45-d-old broilers, respectively. Lammers *et al.* (2008) reported an AME_n value of 3805 kcal/kg for laying hens when replacing GLY for glucose.

The estimated AME_n value of the MIX was higher compared with sum of the values of each individual by-product. Values of 8101 and 8404 kcal/kg were determined for the MIX using the difference method and linear regression, respectively. In comparison, the sum of AME_n individual values estimated for each fat source was 7485 and 8767 using both methods. Data reported by Sklan (1979) suggest that the use of combinations of fat by-products can be translated

Table 3 – Apparent metabolizable energy corrected for retained nitrogen (AME_n) of fat sources and diets with inclusion of by-products of soybean oil processing, kcal/kg.

		AME_n, kcal/kg	
Fat Source		Diet[5]	Fat source[6]
ASS [1]		3277[a]	7951[ab]
LEC [2]		3175[b]	6579[b]
GLY[3]		3074[c]	3979[c]
MIX[4]		3232[a]	8101[a]
Level			
2%		3121[c]	6344
4%		3176[b]	6631
6%		3276[a]	6923
Fat source*Level			
ASS[1]	2	3195	7849
ASS[1]	4	3250	7904
ASS[1]	6	3386	8075
LEC[2]	2	3102	6332
LEC[2]	4	3171	6566
LEC[2]	6	3252	6835
GLY[3]	2	3010	3507
GLY[3]	4	3098	3845
GLY[3]	6	3116	4465
MIX[4]	2	3176	7692
MIX[4]	4	3185	8193
MIX[4]	6	3335	8315
Mean		3194	6652
Probability (ANOVA)			
Fat source		<0.0001	<0.0001
Level		<0.0001	0.4396
Fat source*Level		0.1447	0.9991
SEM		15.32	297.76

[-c]Means within the same column without common letters differ significantly ($p<0.05$).
[1] Acidulated soybean soapstock
[2] Lecithin
[3] Glycerol
[4]Mixture containing 85% ASS, 10% GLY and 5% LEC.
[5]AMEn = [GEI – GEE] – [8.22 × (NI –NE)] ÷ FI GEI = Gross energy intake, GEE = Gross energy output in the excreta, NI = nitrogen intake from the diet, NE = nitrogen output in the excreta, FI = feed intake, and 8.73 = nitrogen correction factor
[6]AME_n I= GEI – (EO_T – (1-X)EO_R)/ X, where: GEI = Gross energy of the fat source, EO_T = Energy output of test diet, EO_R = Energy output of the reference diet, X= % inclusion of the fat source.

into higher AME_n values because of a synergism when the different sources are consumed together by birds. In addition, fats containing different proportions of unsaturated and saturated fatty acids can lead to higher AME_n values (Renner & Hill, 1960; Wiseman and Lessire, 1987). The capacity of polar solutes to increase the micellar solubility of non-polar solutes, such as long-chain fatty acids, has been proposed as the mechanism responsible for this synergism (Freeman, 1984). The results of the present study demonstrate that ASS, LEC, and GLY at the respective proportions of 85%, 5%, and 10%, increased the AME_n value finally obtained for broilers using the difference method. As with the other fat sources evaluated, values were numerically higher when linear regression was compared with the difference method.

The increasing market value of energy has affected the cost of energy sources for poultry feeds, especially fats. Using methods of AME_n determination that adequately address the factors that impact fat utilization by the birds, such as inclusion rate, allow for more precise feed formulation. The two methods used in the present study present different degrees of complexity for their implementation. The liner regression method captured the impact of increasing fat inclusion rates in the feeds; however, this method clearly overestimated AME_n of GLY, because the determined AME_n value was higher than its gross energy value (Table 2).

The higher AME_n value of the MIX when compared with that obtained by the sum of the individual fat sources as determined by the difference method represents a competitive economic alternative to supply energy for broilers because of the large volumes of GLY currently offered in the market. The failure of the two methods to provide numerically closer AME_n values was an outcome limitation from the present study and remains to be clarified.

Table 4 – Average apparent metabolizable energy corrected for retained nitrogen (AME_n) of the soybean oil by-products estimated by two methods, kcal/kg.

Ingredient	AME_n, kcal/kg	
	Regression Analysis[5]	Difference Method[6]
ASS[1]	9232	7951
LEC[2]	7502	6579
GLY[3]	5447	3979
MIX[4]	8404	8101

[1]Acidulated soybean soapstock
[2]Lecithin
[3]Glycerol
[4]Mixture containing 85% ASS, 10% GLY and 5% LEC.
[5]$AME_n = y = a + bx$, GLY Y= 24,69x + 2978 ($p<0.0001$; r^2= 0,77), ASS Y= 62,21x + 3013 ($p<0.0001$; r^2= 0,88), LEC Y= 45,09x + 2993 ($p<0.0001$; r^2= 0.86), MIX Y= 53,99x + 3007 ($p<0.0001$; r^2= 0,78).
[6]AME_n I= GEI – (EO_T – (1-X)EO_R)/ X, where: GEI = Gross energy of the fat source, EO_T = Energy output of test diet, EO_R = Energy output of the reference diet, X= % Inclusion of the fat source.

CONCLUSIONS

The AME_n values of the soybean by-products estimated by regression analysis are 9232, 7502, 5447, and 8404 kcal/kg, whereas values estimated by the difference method are 7951, 6579, 3979, and 8101 kcal/kg for acidulated soybean soapstock, lecithin, glycerol, and a mixture of these three at the proportions of 85%, 5% and 10%, respectively.

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