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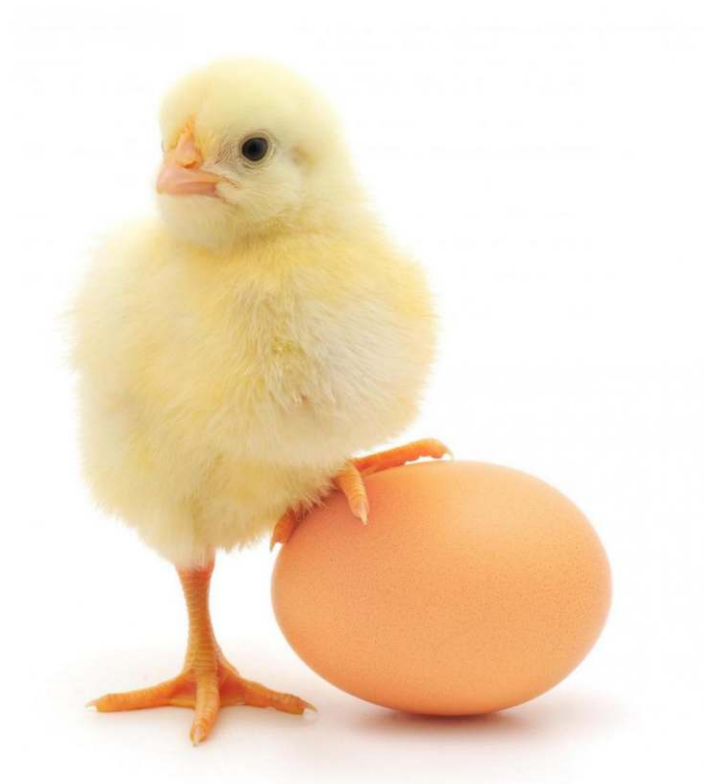

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Determination of Metabolizable Energy of Rich Unsaturated Fatty Acids Dry-Fat in Chicken Diets Using Chemical, Biological and Mathematical Methods

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Abstract: Two biological experiments were conducted to determine apparent Metabolizable Energy (AME) of rich unsaturated fatty acids dry-fat (Polyfat® as an example, PF) using corn or corn-soybean meal diets with different inclusion levels of PF in adult cockerel diets. The biological experiments designed to follow procedures of excreta total collection method (TCM). The chemical evaluation of PF included determination of peroxide No., acid No., fatty acid profile and gross energy. Then different mathematical equations were applied to calculate AME of PF based on the chemical evaluation. The determined values of AME of PF using chemical methods were 7160 and 7188 kcal/kg for PF. Among biological experiments, using restricted quantity of corn diet with high levels of PF (25 or 50% of diet) resulted in lower AME values (4724 and 3992kcal/kg PF). While applying *ad libitium* consumption of corn-soybean practical diet containing 3% soybean oil (as reference oil), 3.8% of PF or 50:50 mixture of both (1.5% SO+1.9% PF), gave more realistic value of 6973 kcal/kg for PF. Mixing PF with soybean oil showed clear synergism effect and added caloric value of 9.6% (737 kcal) to the mixture above the expected value. Applying simple regression procedures on results of chemical and biological evaluations showed highly significant ($p<0.001$) relationships between AME of PF and either digestible fat, dietary saturated fatty acids (%) or supplemental stearic acid (%) in diet. These relationships have been presented as high confidant prediction equations with r^2 values ranged between 0.9355 and 0.9997.

Key words: Metabolizable energy, dry fat, polyfat®

INTRODUCTION

Although, fats and oils are commonly added to poultry diets to reach the high energy requirements of modern strains especially broiler chicks, limited reports about using vegetable oils in the form of Ca-salts of fatty acids (dry-fat) are available. Furthermore, chemical analysis (especially fatty acid profile) and Metabolizable Energy (ME) of dry-fat products depend on its components from vegetable oils which resulted in unevenly ME values and biological response to dry-fat products (Blanch *et al.*, 1995; Mendlik *et al.*, 1999; Dewi *et al.*, 2011). Therefore reports about using dry-fat products should specify the product and its chemical and biological characteristics. Polyfat® is an example of modern modified dry-fat product which consists of calcium salts of 70% palm oil fatty acids, 25% sunflower+corn oils and 5% soybean oil and provided by Norel-Misr, Egypt (Boulos *et al.*, 2011). Although Polyfat® contains more unsaturated fatty acids than traditional dry-fat products. Yet, there was no available scientific reports about its ME value and fatty acid utilization. Labib *et al.* (2005) reviewed factors affect on the determination of ME such as the determination method, composition of basal diet and inclusion level of fat source (Dale and Fuller, 1982; Ketels *et al.*, 1986; Wiseman *et al.*, 1986, 1991). The objective of the present study was to determine the chemical composition of Polyfat® and its ME value by different methods to obtain more reliable value for Polyfat®. Also the study aimed to examine the probable synergism between Polyfat® and Soybean oil.

MATERIALS AND METHODS

Determination of Metabolizable energy was carried out by two different feeding procedures (restricted quantity or *ad libitum*) and diets (corn or practical). The Total Collection Method (TCM) reported by Lessire *et al.* (1985) used to measure AME of Polyfat® (PF) using adult cockerels. The data was subjected to different mathematical equations to identify the most appropriate one to determine the ME.

Chemical evaluation of polyfat® (PF): Chemical evaluation of PF included; Gross energy [GE, using Isoperibol automatic bomb calorimeter (Parr1261) 1997], fatty acids profile (using the AOAC official methods of analysis No. 969.33), peroxide No. and acidic No. Values of PF ME were calculated using the model of Wiseman *et al.* (1991) based on fatty acid analysis of PF and the ratio between unsaturated and saturated fatty acids (UFA and SFA) as:

$$\text{AME (MJ/Kg)} = 37.046 - 11.994 \times e^{[-0.675X\,(U:S)]}$$

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Table 1: Chemical analysis and calculated ME values of PF

Item	Value
Gross Energy (KCal/Kg)	7688±92
Peroxide No. (mmol O_2/Kg)	3.00
Acid No. (%)	0.35
Fatty acid Profile (% of total fatty acids)	
Capric (C 10:0)	0.2
Lauric (C 12:0)	3.3
Myristic (C 14:0)	2.2
Palmitic (C 16:0)	46.4
Palmitioleic (C 16:1 w7)	0.2
Stearic (C 18:0)	4.1
Vaccinic (C 18:1 w7)	0.7
Oleic (C 18:1n9)	33.4
Linoleic (C 18:2)	9.0
Linolenic (C 18:3)	0.2
Octadecatetraenoic (C 18:4 w3)	0.3
SFA	56.2
UFA	43.8
UFA:SFA	0.78
Oleic: Plamitic (O:P)	0.72
Linoleic: Plamitic (L:P)	0.19
(L+O)/P	0.91
ME of PF (kcal/kg; Based on UFA:SFA)[1]	7160
ME of PF (kcal/kg; Based on% C 18:2)[2]	7188

1: Model reported by Wiseman *et al.* (1991)
2: Model reported by Halloran and Sibbald (1979)

where, U was (%) of unsaturated fatty acids in PF; S was (%) of saturated fatty acids in PF.
Also the suggested mathematical model by Halloran and Sibbald (1979) based on the (%) of linoleic acid (C 18:2) applied to get AME value of PF as:

$$\text{AME in Kcal/Kg} = 6540+72\ (\%\ \text{linoleic})$$

The analysis of PF and calculated values of ME based on fatty acids contents are summarized in Table 1.

Biological study

Experiment 1: Twelve one year old Golden Montazah cockerels (developed Egyptian local strain) with an average live body weight of 1743 g were used throughout this experiment. Birds were distributed equally into three treatments (T) of 4 replicates each. Metabolizable energy was determined using the method of Lessire *et al.* (1985) using corn diet and modification of Sibbald (1976) about restricted quantity of feed (80 g) and excreta collection period (48 h). Birds of T1 fed on yellow corn grain 100% while those of T2 and T3 fed on mixture of 75% corn: 25% PF and 50% corn: 50% PF, respectively. The TCM was applied where, birds fed on the experimental diets for 3 days *ad libitium* for acclimation with new modified diets, then fasted for 24 h to insure that the digestive tract was empty from residues of feed used before carrying out the trial. Only 80 g of the experimental diets were offered for each bird and excreta were collected for 48 h including the feeding and fasting period (Table 2). Birds were reared in individual wire battery cages and received the same managerial procedures throughout the experimental period. Birds had *ad libitium* access to water. Feed intake and excreta weight of each bird were recorded. Excreta samples were dried on 60°C/48 h then GE was determined. Values of AME of the experimental diets were calculated using the model reported by Labib *et al.* (2005) Eq. (7)

where:

AME of diet = (EI - EE) / FI (g)
EI = (GE/g of feed) x FI (g)
EE = (GE/g excreta x weight of excreta (g)

Where:

EI = Energy intake
EE = Energy excreted
FI = Feed intake

To calculate the values of AME of tested material (PF) the equation reported by Sibbald *et al.* (1960) was applied as:

AME/g tested material (Kcal) = [AME / g test diet - (AME/g basal diet) x (% basal in test diet) / 100] x 100 / % test material in test diet

where, basal diet was corn and tested material was PF. Calculation of TME was done by applying the mathematical equation of Sibbald *et al.* (1976) as:

TME = [Energy intake - (Energy excreted of fed birds-Endogenous excreted energy)] / g of feed intake

where, endogenous energy factor (8 kcal/bird/24 h) reported previously by Lessire *et al.* (1985) was used.

Experiment 2: Eighteen 10 month old Golden Montazah (developed Egyptian local strain) cockerels with an average live body weight of 2217 g were used throughout the experiment. Birds were distributed equally into three treatments (T) of six birds each and individually reared in wire battery cages. Metabolizable energy was determined using the method of Lessire *et al.* (1985) in which using practical corn-Soybean meal diet instead of corn diet in EXP. 1 to be as similar as the practical conditions of diet and supplemental levels of fat/oil. The examined levels of SO or PF were used as described previously by Boulos *et al.* (2011) for broiler chicks during growing period. Birds of T1 fed on the experimental diet contains 3% SO, while those of T2 and T3 fed on mixture of 1.5% SO+1.9%PF and 3.8% PF, respectively. Composition and calculated values of experimental diets are presented in Table 3 and 4. The TCM was applied where, birds fed on the experimental diets for 3 days (acclimation period) *ad libitium* followed by 24 h of fasting, then starting fed on the experimental diets (feeding period) *ad libitium* for 48 h followed by 24h of fasting. The excreta collection period was 72h

Table 2: Experimental design, diet composition, calculated and determined values of Exp 1 diets

Diet composition and experimental design	T1	T2	T3
Corn (Basal)%	100	75	50
PolyFat (Tested)%	-	25	50
Feed allowance (g/bird during the exp.)	80	80	80
Excreta collection period (hrs)	48	48	48
Gross energy (kcal/kg diet)	3807	4777	5747

Calculated values of fatty acids (%)

	T1	T2			T3		
Fatty acids	Total (Corn)	Basal (Corn)	Tested (PF)	Total	Basal (Corn)	Tested (PF)	Total
C 16:0	0.62	0.47	11.60	12.07	0.31	23.20	23.51
C 18:0	0.1	0.08	1.02	1.10	0.05	2.05	2.10
C 18:1	1.17	0.88	8.35	9.23	0.59	16.70	17.29
C 18:2	1.82	1.37	2.25	3.62	0.91	4.50	5.41
C 18.3	0.09	0.07	0.05	0.12	0.05	0.10	0.15
SFA	0.72	0.55	12.63	13.17	0.36	25.25	25.61
UFA	3.08	2.32	10.65	12.97	1.55	21.30	22.86
O:P	1.89	1.87	0.72	0.76	1.90	0.72	0.74
L:P	2.94	2.94	0.19	0.30	2.94	0.19	0.23
UFA:SFA	4.28	4.28	0.84	0.98	4.31	0.84	0.89
Total FA	3.80	2.87	23.28	26.14	1.91	46.55	48.46
SFA tested:basal				22.96		70.14	
USFA tested:basal				4.59		13.74	
FA tested:basal				8.11		24.50	

starting with the feeding period (48 h) and ending at the end of the second fasting period (24 h). All birds were individually weighed at the beginning of feeding period and at the end of the experiment. Feed consumption and excreta were weighed at the end of experiment and then excreta were dried on 60°C/48 h. The proximate chemical analysis of both feed and excreta samples were done using procedures of AOAC (1990). The digestibility of nutrients and Total Digestible Nutrients (TDN) values were calculated using models of Scott *et al.* (1976, P: 537). The reported model by (Titus, 1971) to calculate AME of diet as:

$$AME\ (kcal/kg) = (TDN \times 4.185 \times 1000) / 100$$

where, then TME values of the experimental diets were obtained by using the factor of endogenous energy (8 kcal/bird/24 h) reported by Lessire *et al.* (1985). The AME of fat/oil source were calculated by applying two methods. The first one, by subtracting the AME of the rest of diet from the total value of diet AME, then divided by the proportion of fat/oil source in the diet. The second calculation method by using the equation reported by Wiseman and Salvador (1991) as:

$$ME\ of\ fat = GE\ of\ g\ of\ fat\ (amount\ of\ absorbed\ fat)$$

where, the utilization of the fat in basal diet was assumed to be constant as mentioned previously by Ketels and De Groote (1989).

Mathematical and statistical procedures: Data of each experiment were subjected to a one-way analysis of variance using SAS (1990). Variables having significant differences were compared using Duncan's Multiple Range Test (Steel and Torrie, 1960). According to Ketels and De Groote (1989), to compute the ideal mathematical relationships between AME of PF and either (Digestible fat) DFAT%, dietary SFA% or supplemental stearic acid (%), regression analysis was done. The best fit equations were selected out of linear, logarithmic, power or polynomial by p-value and the highest value of r^2.

RESULTS AND DISCUSSION

Chemical evaluation of PF: The data presented in Table 1 showed that PF used in the present study contained 7688 kcal of GE/kg and 0.35% of acid number. The FA profile showed that PF contained 9% of Linoleic acid, 43.82% of UFA and 56.2% of SFA from total FA contents. Based on this fatty acid profile, the calculated value of ME of PF based on UFA/SFA using the model reported by Wiseman *et al.* (1991) was 7160 while that value based on contents of Linoleic acid (Halloran and Sibbald, 1979) was 7188 Kcal/Kg of the product. The determined value of acid No. (an indicator of free fatty acids) in PF was (0.35%) acceptable value for fat/oil source. According to the reported results of AME prediction of fats in chicken diets by Wiseman and Salvador (1991) and Wiseman *et al.* (1991) clearly showed that increasing free FA in fat source (from 14.4 to 952.1 g free FA/Kg of fat) caused a progressive reduction in AME value of fats and the degree of reduction influenced by the level of saturation in the fat. The low percentage of free FA in PF had no negative influence on the ME value.

Table 3: Experimental design, composition and calculated analysis of diets in Exp. 2

	T1	T2	T3
Feed allowance	*ad libitium.*	*ad libitium*	*ad libitium*
Feeding period (h)	48	48	48
Excreta collection period (h)	72	72	72
Diet composition[1]:			
Corn	58.67	58.27	57.87
Soybean meal 44%	16.7	16.7	16.7
Corn glutein 60%	4.7	4.7	4.7
Wheat bran	6.7	6.7	6.7
Soybean oil	3	1.5	-
PolyFat	-	1.9	3.8
Di-Ca-P	1.39	1.39	1.39
Lime stone	8.16	8.16	8.16
DL-methionine	0.01	0.01	0.01
Vit. and min. premix[2]	0.3	0.3	0.3
Nacl	0.37	0.37	0.37
Total	100	100	100
Calculated values:			
Crude protein (%)	15.78	15.75	15.72
ME (kcal/kg diet)	2850	2836	2824
Ether extract (%)	5.85	5.93	6.01
Calcium (%)	3.42	3.58	3.74
Available phosphor (%)	0.38	0.38	0.38
Lysine (%)	0.72	0.72	0.72
Methionine (%)	0.32	0.32	0.31
Methionine+Cystine (%)	0.60	0.60	0.59
Sodium (%)	0.16	0.16	0.16

[1]Diets were formulated to cover the requirements of Egyptian developed local chicken strains from all nutrients

[2]Each 3 kg of Vit. and Min. Mixture contains:

Vit. A 12000,000 IU
Vit. D_3 2000,000 IU
Vit. E 10,000 mg
Vit. K_3 2000 mg
Vit. B1 1000 mg
Vit. B2 5000 mg
Vit. B6 1500 mg
Vit. B12 10 mg
Pantothenic acid 10,000 mg
Niacin 30,000 mg
Folic acid 1000 mg
Biotin 50 mg
Choline 300,000 mg
Manganese 60,000 mg
Zinc 50,000 mg
Copper 10,000 mg
Iron 30,000, Iodine 1000 mg
Selenium 100 mg
Cobalt 100 mg
$CaCO_3$ to 3,000 g

Biological study

Experiment 1: The results of Exp. 1 (Table 5) showed lower values for AME and TME of experimental diets even corn diet (T1). The determined values of AME and TME of PF were lower than those values which calculated based on the FA content of PF. Values of diets AME and TME (Fig. 1) significantly increased by increasing the proportion of PF in the diet from 0 (T1) to 25% (T2) and 50% (T3), while those of PF decreased

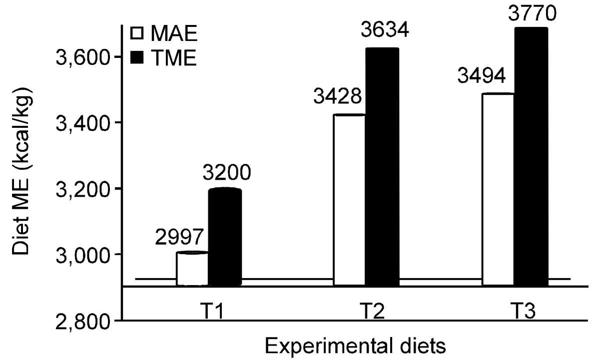

Fig. 1: Values of AME and TME of experimental diets (EXP 1). T1: 100% corn, T2: 75% corn+25%PF and T3: 50% corn+50%PF. Showed that increasing inclusion level of PF from 0 to 50% in experimental diets increasing the determined values of AME and TME of diets

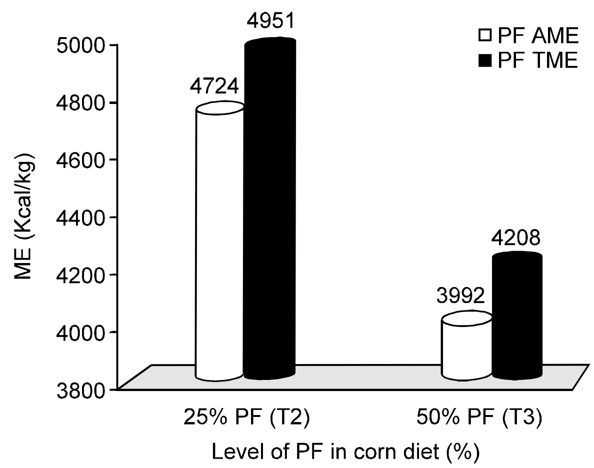

Fig. 2: Values of AME and TME of PF at levels 25 and 50% in corn diet (Exp1). T2: 75% corn+25%PF and T3: 50% corn+50%PF. Showed that increasing inclusion level of PF from 25 to 50% decreased the determined values of AME and TME of PF

(Fig. 2). Adding PF at 25 or 50% of the diet resulted in a significant decrease of feed consumption by 2.53% and 25.32% compared to the consumed feed of corn diet group (80 g). This reduction of feed consumption correlated with numerical decrease in body weight by 2.01 and 2.66% compared with 0.17% loss in body weight of birds in corn diet group. These reductions in feed consumption and body weight during the experimental period (48 h) and the widening gap between the determined values and the theoretical (based on FA content of PF) values of AME and TME indicated that the procedures of determination was incompatible to the purpose of the study. The high

Table 4: Calculated values of FA in experimental Exp2 diets

		Added FA			Total FA		
FA	Ingr. FA[1]	T 1	T 2	T 3	T 1	T 2	T 3
C 16:0	0.45	0.35	0.91	1.48	0.80	1.36	1.93
C 18:0	0.07	0.12	0.12	0.13	0.19	0.20	0.20
C 18:1	0.80	0.59	0.83	1.07	1.39	1.63	1.87
C 18:2	1.32	1.74	1.01	0.29	3.05	2.33	1.60
C 18.3	0.07	0.20	0.11	0.01	0.27	0.17	0.08
SFA	0.52	0.47	1.13	1.79	0.99	1.56	2.13
UFA	2.18	2.54	1.97	1.40	4.72	4.13	3.54
O:P	1.79	1.71	0.91	0.72	1.75	1.20	0.97
L:P	2.94	4.99	1.11	0.19	3.84	1.71	0.83
UFA:SFA	4.20	5.45	1.74	0.78	4.79	2.65	1.66
Total FA	2.70	3.00	2.99	2.97	5.70	5.69	5.68
Added FA: Ingr. FA					1.109	1.104	1.099
SFA (added:Ingr.)					0.89	2.17	3.45
UFA (added:Ingr.)					1.16	0.90	0.64

[1]Ingr. FA is fatty acids of diet ingredients except oil/dry fat

Table 5: Experimental results of Exp. 1

	T1 (0% PF)	T2 (25% PF)	T3 (50% PF)	p-value
Initial BW (g)	1804±88.5	1742±69.3	1692±71.2	0.6028
Final BW (g)	1801±94.3	1706±68.5	1647±74.6	0.4221
BW Loss (%)	0.17	2.01	2.66	
Feed Intake (g)	78.8±1.20[a]	77.0±1.44[a]	59.0±4.77[b]	0.0017
Chang in FI (Relative to T1,%)	-	-2.01	-25.32	
AME of diet (kcal/kg)[1]	2997±41.1[b]	3428±49.66[a]	3494±156.8[a]	0.0116
AME of PF (kcal/kg)[2]		4724	3992	
TME of diet (kcal/kg)[3]	3200±43.8[b]	3634.4±51.61[a]	3770±141.9[a]	0.0041
TME of PF (kcal/kg)[2]		4951	4208	

[1]Labib *et al*. (2005); AME= (Energy intake-Energy excreted)/feed intake. (EQ.7)

[2]Sibbald *et al*. (1960); AME/g tested material=(AME/g test diet-(AME/g basal diet*(%basal in test diet)/100)*100)/% test material in test diet

[3]Sibbald *et al*. (1976); TME=(GE of feed intake-(Excreta energy of fed birds-endogenous excreted energy))/g of feed intake; the factor reported by Lessire *et al*. (1985) for endogenous energy (8 Kcal/bird/24 h) is used

proportion of PF in diets (25 or 50%) seemed to be very high levels that inversely influenced on the birds palatability of diets.

The gradual reduction of AME and TME values of PF at levels 25% (4724 and 4951 kcal/kg) and 50% (3992 and 4208 kcal/kg) could be due to the increased SFA content of the experimental diet from 13.17 to 25.61% and decreasing UFA:SFA ratio from 0.98 to 0.89 for T2 and T3 beside the slight decrease in L: P ratio from 0.30 to 0.23. Labib *et al*. (2005) reviewed the effect of added fat level to basal diet on ME value and reported that increasing the proportion of some added fats reduces their ME values (Dale and Fuller, 1982; Wiseman *et al*., 1991).

Early studies proved that AME values of fats and oils are influenced by their chemical structures (Young, 1961; Renner and Hill, 1960). Therefore many researchers reported different prediction equations to estimate the ME values based on this scientific fact. The equations based on the proportion of SFA and UFA (Ketels and De Groote, 1989) Linoleic acid (Halloran and Sibbald, 1979) or UFA:SFA and FFA contents (Wiseman *et al*., 1991). Freeman (1984) reviewed that chemical structure of fats and oils have an influence on their ME values through its effect on digestion and absorption of FA.

The reported reduction of ME values of PF in this experiment, as a result of increasing SFA content of the diet, have previously reported by many workers (Garrett and Young, 1975; Leeson and Summers, 1976; Ketels and De Groote, 1988, 1989; Wiseman and Salvador, 1991) and was explained by the limited absorption of long chain SFA compared with long chain UFA and short chain FA (Hakansson, 1974). Consequently, gradual increase of amounts of fats or oils rich in SFA resulted in decreasing in ME values (Halloran and Sibbald, 1979; Ketels *et al*., 1986; Ketels and De Groote, 1989) and this confirmed our findings.

However, in the present experiment, the O:P ratio of T2 and T3 were approximately similar (0.76 and 0.74), so it could not be an effective factor for the depressing of PF ME, while the L:P decreased from 0.30 to 0.23 in T2 and T3 diets and based on the results of Young and Garrett (1963) that reduction of L:P ratio could have an adverse effect on absorbability of P, O and L. Also that report explained the acceptable difference between the determined ME values of corn and its standard values (3350 kcal/kg) while the gap was wider in case of PF by high O:P and L:P ratios (1.89 and 2.94) in corn diet which save maximum digestion and absorption of FA. The authors showed that when palmetic and stearic acids fed together tended to depress the absorption of each other. This previous study of fatty acid absorption confirmed the depressed ME values of PF in T2 and T3 whereas stearic acid levels were 1.1 and 2.1% while corn diet (T1) contained 0.1% of stearic acid.

Generally the recorded increasing of ME of PF than corn and the decreasing of ME of PF at levels 25 and 50% of diet compared to the obtained values by chemical evaluation, seemed to be affected by the amount of fatty acids more than the ratios between them.

Experiment 2: Results of Exp 2 (Table 6) showed that the experimental diets had not significant adverse effects neither on feed intake or body weight. There was no reduction in body weight due to PF inclusion in diets as obtained in Exp 1. Birds of T3 (PF 3.8%) recorded the lowest numerical value of feed consumption (220 g) while those of T2 (1.5 SO+1.9% PF) consumed the biggest quantity of feed (311 g). No significant differences detected among experimental treatments in all digestible nutrients (digestible organic matter, DOM; DFAT and TDN) except nitrogen retention values (NR%). The determined values of diets AME based on TDN values were in a narrow range (2752, 2776 and 2750 kcal/kg diet for T1, T2 and T3, respectively). The same trend recorded for diets TME values (2853, 2864 and 2848 kcal/kg diet for T1, T2 and T3, respectively) which showed that the differences between AME and TME values were (101, 88 and 98 Kcal for T1, T2 and T3, respectively) smaller than those determined in Exp 1(144, 166 and 180 Kcal for T1, T2 and T3). The correction of AME and TME to nitrogen excretion did not make a valuable change.

Using practical corn-Soybean meal diet containing applicable levels of SO or/and PF resulted in more realistic determined ME values of PF. The determined value of PF AME in T3 was 6973 kcal/kg PF which became closer to the calculated values based on PF chemical structure (7160 and 7188 Kcal/Kg PF, Table 1). The outcome of the second calculation method for PF ME using the DFAT% and GE of PF was 7151 Kcal/Kg PF. Mixing PF with SO resulted in large increase of AME value of the mixture (Table 7). The determined value of SO and PF mixture (8411 Kcal) was 9.6% more than the theoretical value (7674 Kcal). This increased value indicated a synergism effect between PF and SO in corn-soybean diet resulted in raising AME of PF from 6973 to 8473 Kcal/Kg.

According to the obtained results of Exps 1 and 2, the determined values of ME for PF changed by using different basal diets and inclusion rates of PF. This finding reported previously by many researchers who stated that there is an effect of experimental diet and the level of added fat on the determined ME (Leeson and Summers, 1976; Sibbald and Kreamer, 1980; Mateos and Sell, 1980,1981; Dale and Fuller, 1982; Ketels *et al.*, 1986; Wiseman *et al.*, 1986). Matios and Sell (1981) clearly stated that the level of examined fat and determination and calculation method had an effect on the determined values of ME when birds fed on practical diets containing 0, 5, 10, 15, 20, 25, or 30% yellow grease. Complementary, Dale and Fuller (1982) recorded an improvement in TME values of SO (22.4%) and tallow (34.9 to 43.0% for low and high stearic content) when used practical corn-soybean meal basal

Table 6: Experimental results of Exp. 2

	T1	T2	T3	p-value
Initial BW (g)	2219±59	2242±153	2190±111	0.9489
Final BW (g)	2217±58	2245±150	2184±111	0.9300
BW Loss (%)	0.09	+0.12	-0.025	
Feed Intake (g/bird)	257±16.7	311±24.8	220±36.1	0.0891
Chang in FI (Relative to T1,%)		+21.0	-14.4	
DOM%	69.4±2.5	67.7±1.6	69.6±1.9	0.7755
NR%	20.7±6.7[b]	40.6±3.2[a]	45.0±8.5[a]	0.0429
DFAT2%	93.5±1.5	90.9±1.6	94.1±1.0	0.2663
TDN[1]	64.6±2.1	63.9±1.3	64.2±1.6	0.9619
AME of diet (kcal/kg)[2]	2752±82.7	2776±52.8	2750±68.2	0.9618
TME of diet (kcal/kg)[3]	2853±90.2	2864±58.6	2848±64.7	0.9209
AMEn of diet (kcal/kg)[4]	2752±87.7	2778±52.8	2751±68.2	0.9633
TMEn of diet (kcal/kg)	2853±90.4	2864±58.7	2850±64.5	0.9225
AME of oil/fat source	8375	8411	6973	
AME of PF (kcal/kg, based on TDN)		8473	6973	
AME of PF (kcal/kg, based on DF)[5]			7151	

[1]Scott *et al.* (1976)

[2]The model reported by Titus (1971)

[3]Using the factor of endogenous energy (8 Kcal/bird/24 h) reported by Lessire *et al.* (1985)

[4]Nitrogen correction factor reported by (Titus, 1956)

[5]The model reported by Wiseman and Salvador (1991)

at low level (2.5%) than those values obtained by using purified diet with high level of fat inclusion up to 15%. They explained the improvement in the TME of the tallow by an interaction with fatty acids in the practical basal ingredients that may be obscured at higher levels of inclusion. This results in agreement with our results whereas AME of PF was improved by 47.6% when used at level 3.8% in corn-soy practical diet compared with the determined value at inclusion level 25% in corn diet (difference between 6973 and 4724 kcal/kg for Exp2 and Exp1). Values of DFAT% in this study ranged from 90.9 and 94.1% and were closer than those reported by Sibbald and Kreamer (1980) for corn oil and tallow in corn based diet (93 and 75.8%), while the utilization of individual fatty acids varied between fatty acids and within same acids in different dietary treatments. Unsaturated fatty acids were well utilized (85 to 96%) while saturated fatty acids were less utilized (59 to 90%), particularly at high levels of input (15%). This variation in availability of fatty acids within the same diet confirmed our finding.

Results of Exp2 showed that although, there were no significant difference detected in TDN or DFAT% between treatments, the determined value of AME PF in T2 and T3 were varied than the chemically determined values. The AME determined value of PF in T3 (6973 kcal/kg) group was lower than chemically determined values (7160 and 7188 kcal/kg, Table 1) and this could be due to the highest level of SFA (2.13%) and lowest levels of UFA and U:S ratio (3.54% and 1.66) than values of T1 and T2 (Table 4).

Early studies showed a reduction of fat digestibility when Calcium content in chicks' diets increased (Fedde *et al.*, 1960; Sibbald and Price, 1977). Although, some researchers proved significant decrease of SFA absorption in presence of high calcium concentration in aqueous media (Renner and Hill, 1960; Patton and Cary, 1979; Allen, 1982; Brink *et al.*, 1995), others reported that dietary calcium might alert FA absorption because it is an efficient process. The interference by calcium is probably due to the ionic properties of calcium and the release of FA from the 1, 3 position of the triacyl-glycerol moiety after lipase-mediated hydrolysis of a fat. Therefore small amount of Ca is needed to enhance FA digestion and absorption (Shiau, 1987). These reports explain our results whereas inclusion of PF at 25 or 50% of the diet in the first experiment correlated with lower DFAT% (61.4 and 51.9%, un shown data) and consequently lower ME values of PF compared to the determined values at low applied (1.9 and 3.8%) inclusion levels in the second experiment whereas DFAT% were higher (90.9 and 94.1%).

Table 7: Synergism effect between SO and PF in practical corn-soybean diets

Oil/Fat	AME (Kcal/Kg)
SO	8375
PF	6973
SO:PF	Theoretical 7674
(50:50)	Determined 8411
	Synergism effect
	737 Kcal = 9.6%

On the other hand, the determined AME value of PF in T2 (8473 kcal/kg, Exp 2, Table 6) was higher than chemically determined values (7160 and 7188 kcal/kg, Table 1) indicated a synergism effect between fatty acids of PF and SO (Lall and Slinger, 1973; Muztar *et al.*, 1981). Early studies of Lall and Slinger (1973), Sibbald (1978) and Ketels and De Groote (1989) showed that replacing 10 or 20% of Tallow by vegetable oils was sufficient to increase fat AMEn to levels greater than anticipated. Alzueta *et al.* (2007) confirmed the synergism between palm oil and high-oleic sunflower seeds in broiler chicks and suggested that improvement based on oleic acid mainly. Freeman (1984) showed that the mechanism responsible for synergism between fats is the capacity of polar solutes, whereas USFA increased the micelles solubility of non-polar solutes such as long chain SFA. The degree of synergism depends on the relative concentration of U:S to get the maximum synergism effect on fat digestibility and AME value 2:1, by Danicke *et al.* (2000) and Alzueta *et al.* (2007). The recorded synergism effect between SO and PF confirmed our previous results (Boulos *et al.*, 2011) about using PF in broiler diets at the same inclusion levels during growing period. Broilers fed on PF recorded feed conversion ratio significantly equal to that recorded for SO (1.74 and 1.77). While chicks fed diets contained SO:PF at ratio 50:50 recorded the best significant feed conversion ratio (1.68).

Determination of PF ME by mathematical equations: Results of regression analysis of the relationships between AME of PF and DFAT%, added SFA% or supplemental stearic acid (C18:0)% as independent variables (Fig. 3, 4 and 5). All examined relationships were highly significant and the best fit regression equations are listed in Table 8. The best fit relations between AME of PF and DFA% were linear (p = 0.0001 and r^2 = 0.9997), best fit relations between AME of PF

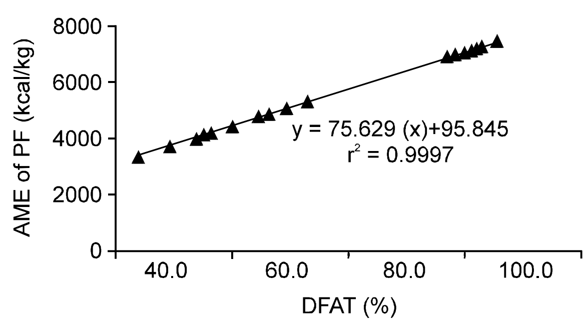

Fig. 3: Relationship between DFAT% and AME of PF

Table 8: Mathematical relationships between AME of PF and some biological and chemical independent variables

Dependent Variable (X)	Best fit relation	Equation	r^2	p-value
DFA (%)	Linear	y = 75.629(X)+95.845	0.9997	0.0001
Dietary SFA (%)	Logarithmic	y = -1289 ln (X)+8118.2	0.9355	0.0001
Supplemental Stearic (%)	Polynomial	y = 954.04 (X^2) -3784.9 (X) + 7732.7	0.9367	0.0001

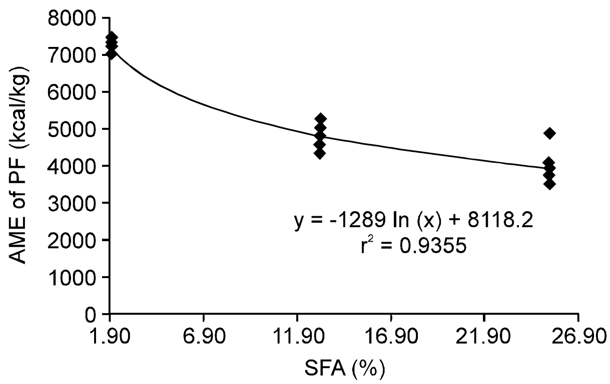

Fig. 4: Relationship between dietary SFA% and AME of PF

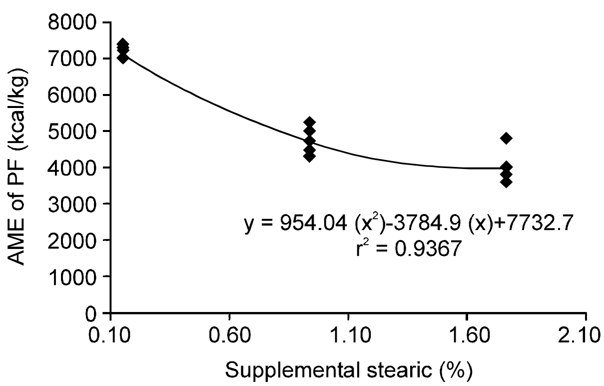

Fig. 5: Relationship between added Stearic% and AME of PF

Table 9: Determined values of PF ME from EXP 1 and 2

Method	AME (kcal/kg)
Corn diet (25% of diet)	4724
Practical corn-soybean diet	
-Single source: (based on TDN)	6973
: (based on DFAT)	7151
-Mix 50:50 with SO	8473
Based on FA profile	
-UFA:SFA	7160
-C 18:2	7188

and dietary SFA% were logarithmic (p = 0.0001 and r^2 = 0.9355), while best fit relations between AME of PF and supplemental stearic (C18:0)% were polynomial (p = 0.0001 and r^2 = 0.9367). The obtained high values of r^2 indicating that prediction equations can be a mathematical method for determination AME of dry fat by the knowing of any of independent variables and by saving the same experimental conditions. Using equations to predict the AME of poultry feeds and feed ingredients have been reported by many researchers (Palic *et al*., 2012).

Finally, the overall determined values of AME of PF from both Exps 1 and 2 (Table 9) ranged from 3992 and 8473 kcal/kg (depending on diet composition and inclusion level of PF and period of excreta collection during the experiment). Results of Exp 2 using practical corn soybean meal diet with applicable inclusion level of PF were more realistic.

Conclusion: The calculated AME value of Polyfat® based on fatty acids profile were 7160 or 7188 kcal/kg PF.

Using one ingredient diet with restricted amount of feed and high inclusion rate of PF (25 or 50%) to determine AME gained unreal and lower values than those determined chemically (3992 or 4724 kcal/kg PF), while using practical corn-soybean meal diet included applicable level of PF (3.8%) gave more reliable AME value (6973 kcal/kg PF).

Mixing PF with SO (50:50) improved the AME value (9.6%) of the mixture from 7674 to 8411 Kcal/Kg.

It could be predict the AME value of fat source (Y) using mathematical equations using some characters of fat such as:

y = 75.629(x)+95.845, where x = digestible fat%
y = -1289 ln (X)+8118.2, where x = dietary SFA% of dry fat
y = 954.04 (X^2)-3784.9 (X)+7732.7

where, x = supplemental Stearic% of dry fat.

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