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Effect of Dietary Calcium Level on True Metabolizable Energy Value of Various Fat Sources Determined by Precision Fed Rooster Assay

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Abstract: An experiment was conducted to determine the effect of dietary Ca levels on True Metabolizable Energy (TME) and TME with nitrogen correction (TME_n) values of 3 fat sources (cotton seed oil, animal fat and a 50:50 mixture). The fats were each added at the level of 0, 3, 6 and 9% to 2 basal low and high Ca content diets. The true metabolizable energy of experimental diets was determined using precision fed rooster assay method. The TME and TME_n of 3 fat sources were obtained through regression and extrapolation to 100% fat replacement in basal diets. The addition of Ca carbonate to the zero added fat diet significantly ($p<0.05$) reduced the TME_n of diet (3348 vs. 3294 kcal kg^{-1}). The true metabolizable energy of animal fat was significantly ($p<0.05$) lower than that of cotton seed oil (8619 vs. 9974 kcal kg^{-1}). However, the TME_n of fat and oil mixture was similar to that of oil value (9974 vs. 9915 kcal kg^{-1}). The TME_n of fat decreased as the level of fat increased ($p<0.01$) when it was calculated by the difference method. Addition of Ca carbonate to the diets contained any sources of fat significantly ($p<0.05$) reduced the TME_n value of fat. The metabolizable energy of fat linearly ($p<0.05$) reduced in response to increase in dietary fat levels as determined by difference method.

Key words: Fat, calcium, true metabolizable energy, rooster assay

INTRODUCTION

Many factors affect the utilization of dietary fat in chickens. Such factors include: age (Wiseman and Salvador, 1989), breed of bird (Katongole and March, 1980), fatty acid saturation, chain length and free fatty acids (Wiseman, 1990; Wiseman and Salvador, 1991), fatty acid position on the glycerol molecule, level of fat in diet (Leeson and Summers, 2005; Wiseman, 1990), inhibitors and anti-nutritional factors (Longstaff and McNab, 1991; Tani *et al*., 1994) and mineral level.

When fats are digested, free fatty acids may produce a complex with minerals and form soaps that may or may not be soluble. If insoluble soaps are formed, there is the possibility that both fatty acid and mineral will be unavailable to the bird (Leeson and Summers, 2005). There are numerous reports indicating that fat retention and diet AME decreased when poultry diets contained high level of fat and Ca (Sibbald and Price, 1977; Atteh and Leeson, 1984, 1985b). Atteh and Leeson (1985b) revealed that an increase in the diet Ca level in broiler chickens may increase the proportion of digesta fat that is appeared as soap. They also, reported that the proportion of digesta and excreta fat presented as soaps is depend on the type of fatty acid supplementation.

Although, the lower digestibility of fatty acids can be related to high level of dietary minerals, the fatty acids may also, reduce the bioavailability of minerals including calcium and magnesium. These minerals are important nutrients and their deficiencies may have a negative effect on performance of birds.

There is limited number of documents on the effect of dietary Ca on TME and TME_n value of fat sources. The objective of this study was to determine the effect of dietary Ca level on the TME and TME_n value of 3 different fat sources, widely differed in chemical composition and structure.

MATERIALS AND METHODS

One hundred sixty eight individually caged, 29 weeks old White Leghorn cockerels of Hy-line strain were used for this study. The fats sources were cottonseed oil, animal fat and a 50/50 (w w^{-1}) mixture of the 2 sources.

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Each of the fat sources was added at the level of 0, 3, 6 and 9% to the 2 basal diets of low and high Ca content. The low Ca basal diet that were designed to serve as fat carrier, consisted of corn 64.3%, soybean meal 32.2%, salt 0.3%, D-L methionine 0.15%, calcium carbonate 1%, dicalcium phosphate 1.75 and 0.5% vitamin-mineral premix. The high Ca basal diet contained 2% additional calcium carbonate that was replaced with 2% corn, so the level of calcium carbonate in this diet was 3% as compared to 1% in the low Ca diet. Each of the fat sources was incorporated at the expense of an equal weight of the basal diets.

Twenty four diets (3 fat sources × 4 fat level × 2 Ca level) were assayed for TME and TME_n using precision fed rooster assay method (Sibbald, 1986). Each diet was force fed at the level of 30 g $bird^{-1}$ to 8 adult S.C.W.L. cockerels and there were 8 starved control birds, which were used to determine the endogenous energy and nitrogen losses.

Nitrogen in feed and excreta samples was determined by Kjeldhale procedure. Gross energy was measured in an adiabatic oxygen bomb calorimeter and the fatty acid profile of fats was determined by gas chromatograph. peroxide value, acid value, saponification and unsaponifiable value and iodine index were also determined according to AOAC (1990). The TME and TME_n value of experimental diets were calculated according to the following equations:

$$TME/g \text{ of feed} = [(F_i \times GE_f) - (E \times GE_e) + (FE_m + UE_e)]/F_i$$

$$TME_n/g \text{ of feed} = [((F_i \times GE_f) - (E \times GE_e) - (NR \times K)) + ((FE_m + UE_e) + (NR_o \times K))]/F_i$$

where:

F_i = The feed intake (g)
GE_f = The gross energy g^{-1} of feed
E = The excreta output
GE_e = The gross energy g^{-1} of excreta
$F_mE + U_eE$ = The mean of the energy voided by the unfed birds
NR, NR_o = The estimates of nitrogen retention for fed and fasted birds, respectively

The TME and TME_n value of fats at each level of inclusion were calculated by the difference method according to the following equation:

$$ED = (P \times EF) + (1\text{-}P)\ EB$$

where:

ED = The ME of the experimental diet
P = Level of fat in the experimental diet
EF = ME of fat (1-P) is level of basal diet in experimental diet
EB = The ME of basal diet

The TME and TME_n of fat was also determined by regression and extrapolation to 100% fat in basal diet.

The TME and TME_n values obtained from factorial design experiment (3×4×2, fat sources x fat level x Ca-level) were statistically analyzed using ANOVA of SAS (SAS Institute, 2003). Significant differences among treatment means were determined using Duncan's multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

Animal fat had higher peroxide, acid and unsaponifiable value than of cotton seed oil, thus its quality was lower than of oil source (Table 1). The most dominant fatty acid in animal fat and cotton seed oil was oleic (55.1%) and linoleic acid (56.5%), respectively.

The TME and TME_n values of the experimental diets containing cotton seed oil and blended fat did not differ significantly ($p>0.05$), but basal diets contained animal fat had a significantly ($p<0.05$) lower TME and TME_n values than that in either of basal diets contained oil or mixture of oil and fat (Table 2). The metabolizable energy of diets containing either oil or fat was significantly ($p<0.05$) increased as the level of fat increased in the diet when measured by difference method. But the effect was lower for animal fat and this is why there is an interaction between fat level and fat sources (Table 2). The metabolizable energy of diets contained higher level of Ca-carbonate was significantly ($p<0.05$) lower than diet contained lower level of calcium. This was expected because of 2% of Ca-carbonate replacement for corn in the lower calcium diet. None of the interactions except for fat source x fat level were significant ($p>0.05$).

The TME and TME_n values of animal fat calculated by difference were significantly ($p<0.05$) lower than that of other fat sources (Table 2). Animal fat usually has more saturated fatty acids, so mixing cottonseed oil with animal fat did increase the digestibility of animal fat and

Table 1: Fatty acid composition and chemical characteristics of cotton seed oil and animal fat

Characteristics	Cotton seed oil	Animal fat
Dry matter	99.95	99.870
Unsaponifiable value	0.460	1.4500
Iodine index	107.6	35.150
Saponification index	190.0	211.00
Acid value	2.130	4.5000
Peroxide value	4.000	8.0000
Fatty acid composition		
C18:3	0.228	nd[1]
C18:2	56.50	1.6900
C18:1	15.75	55.100
C18:0	1.620	1.7600
C16:0	24.94	31.210
C12:0	0.990	4.4800

[1]Not detected, below the level of sensitivity of the method used

Table 2: Effect of fat source, fat level and Ca content of the basal diet on TME and TME_n values of the experimental diets and fat or oil and/or 50:50 mixture

	Experimental diets (Kcal kg^{-1})		Fat sources (Kcal kg^{-1})	
Characteristics	TME	TME_n	TME	TME_n
Fat source	**	**	*	*
Cotton seed oil	3545[a]	3354[a]	10130[a]	9974[a]
Animal fat	3449[b]	3258[b]	8810[b]	8619[b]
50:50 mixture	3541[a]	3343[a]	10245[a]	9915[a]
Fat level (%)	**	**	*	*
0	3242[d]	3051[d]	-	-
3	3472[c]	3282[c]	10917[a]	10765[c]
6	3596[b]	3400[b]	9146[b]	8924[b]
9	3777[a]	3580[a]	9188[b]	8865[a]
Calcium level	*	*	-	*
Low Ca	3545[a]	3348[a]	9879	9615[a]
High Ca	3484[b]	3294[b]	9690	9495[b]
Fat source x fat level	*	*	ns	ns
Fat source x Ca	ns	ns	ns	ns
Fat level x Ca	ns	ns	ns	ns
Fat source x fat level x ca	ns	ns	ns	ns
SE	22.8	22.2	260.3	234.5

[a-d]Values within a column with no common superscript differed significantly ($p<0.05$); **Level of significant ($p<0.01$); *Level of significant ($p<0.05$); ns: not significant

Table 3: Regression equations for the effect of fat level (X) on TME of different fat sources (Y) when diet supplemented with low or high calcium

Ca level	Fat source	Intercept (a)	b	r^2	Level of significance	Fat TME (X = 100)
Low	Cotton seed oil	3275±37.5[1]	65.95±6.7	0.87	**	9870
Low	Animal fat	3300±29.9	44.55±5.7	0.83	**	7755
Low	50:50 mixture	3302±40.9	60.34±7.3	0.83	**	9336
High	Cotton seed oil	3216±36.4	67.16±6.4	0.88	**	9932
High	Animal fat	3247±39.9	41.57±7.6	0.71	**	7404
High	50:50 mixture	3241±37.3	59.47±6.6	0.85	**	9188

consequently increased the ME of the blended fat. The ME of fat values calculated by difference method at 3% of dietary level was significantly ($p<0.01$) higher than that when using 6 or 9% of diet. This results are in agreement with other reports (Fuller and Dale, 1982; Wiseman, 1984, 1990; Wiseman and Lessire, 1987).

Higher Ca level in diet significantly ($p<0.05$) reduced the TME and TME_n values of fat as compared to low Ca-diet (9495 vs. 9615). Atteh and Leeson (1983-1985a) reported a substantial differences in broiler chick and laying hen digesta fatty acids, which were appeared as soap. This may partly explain the differences in fatty acid utilization by young and adult birds. The fatty acid utilization by broiler chicks depend on dietary Ca level, whereas fatty acid utilization by laying hen was independent of dietary Ca level and there are indications that long chain fatty acids (palmitic and stearic acids) form soaps more easily than of oleic acid during the digestion processes. This effect is probably due to the ability of the adult bird to produce more bile acids than young chicks.

There is an indication that while, soaps are formed in the upper digestive tract in older birds, they are subsequently solubilized in the lower tract due to changes in pH. Under these conditions both the fatty acid and mineral are available to the bird. Control over digesta pH may, therefore, be an important parameter for control over soap formation (Leeson and Summers, 2005). Usayran *et al.* (2001) showed supplementation of laying hen diet with 4% vegetable oil has no effect on egg shell thickness and tibia ash. It seems in laying hens, a large portion of unabsorbed fat is appeared as soap and this soap was formed mainly in post-absorptive area of the gut, in which case any detrimental effect of soap formation are neutralized (Horani and Sell, 1977). Thus, laying hen does not seem to have problems associated with fatty acid supplementation except, where palmitic acid is the only source of fat (Atteh and Leeson, 1985c).

According to Sibbald and Price (1977), an increase in the level of dietary Ca decreased the TME value of tallow in adult Leghorn cockerels, but had a much smaller effect on the TME value of soybean oil. The researchers believed that the effects were complex and varied with the level of fat inclusion in diet. Rising *et al.* (1990) revealed that the fatty acid availability of animal fat reduced by 14% and TME was reduced from 9.63-9.36 Kcal g^{-1} when diet with 3.8% Ca was fed to laying hens.

The use of graded level of fat in fat metabolizable energy determination is considered a more appropriate procedure because, in addition to allowing the estimation

Table 4: Regression equations for the effect of fat level (X) on TME_n of different fat sources (Y) when diet supplemented with low or high calcium

Ca level	Fat source	Intercept (a)	b	r^2	Level of significance	Fat TME_n (X = 100)
Low	Cotton seed oil	3090±34.3[1]	64.45±6.1	0.89	**	9535
Low	Animal fat	3107±28.2	44.51±5.3	0.85	**	7558
Low	50:50 mixture	3112±32.6	56.97±5.8	0.87	**	8809
High	Cotton seed oil	3026±36.7	67.31±6.5	0.88	**	9757
High	Animal fat	3058±38.3	41.85±7.2	0.73	**	7243
High	50:50 mixture	3050±37.0	59.17±6.2	0.85	**	8967

of any non-linear trends, they permit the derivation of any dietary energy value of fats through regression analysis (Wiseman, 1990). In this experiment, there was no significant departure from linearity in response of dietary ME to added fat (Table 3 and 4) and animal fat tended to give lower TME and TME_n when high Ca diet is compared to low Ca diet.

CONCLUSION

Dietary Ca level does lower the ME of fats in adult cockerels. Although, more saturated fat (animal fat) showed lower ME value and more pronounced effect is observed when high Ca diet is fed. Long-term supplementation of various dietary lipids may also, alter birds bone chemical and physical properties (Liu *et al*., 2003a, b, 2004).

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