



Mixtures of mono-, di- and tri-glycerides as energy supplements to broilers' diets

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ABSTRACT

Mixtures of mono-, di- and tri-glycerides from olive oil (MDT) were added to: palm oil (PO), olive oil (OO), soybean oil (SO), free fatty acids from palm oil (PFA), free fatty acids from olive oil (OFA). The compound mixtures were used as energy supplements in the diets of broiler chickens in comparison with plain SO and plain animal fat (AF). Two hundred and ten birds were randomly allotted to 7 dietary treatments with the diverse oil sources: 6 birds per cage, 5 cages per treatment. The effects of the treatments on growth rates, feed/gain ratios and acidic composition of abdominal fat of hybrid Ross 308 female chickens were studied. The animals were slaughtered at the end of the trial, at day 35. The breast meat quality was then evaluated by a panel of 15 trained members and analysed for shelf life duration. The AF treatment gave the highest weight gain, but only in the first week. MDT + OO (50/50) resulted the best combination, with slight, non significant, better performances and a decidedly better quality in terms of acidic composition of abdominal fat, taste and juiciness of breast meat and shelf life.

Key Words: Fat supplements, Olive oil, Glycerides, Broiler feeding.

RIASSUNTO

MISCELE DI MONO-, DI- E TRI-GLICERIDI DELL'OLIO DI OLIVA
COME INTEGRATORI ENERGETICI NELLA DIETA DEI BROILER

Delle miscele di mono-, di- e tri-gliceridi dell'olio di oliva (MDT) sono state aggiunte a: olio di palma (PO), olio di oliva (OO), olio di soia (SO), acidi grassi liberi dell'olio di palma (PFA), acidi grassi liberi dell'olio di oliva (OFA). Le miscele composte sono state usate come integratori energetici nella dieta di broiler e poste a confronto con l' SO puro e con il grasso animale (AF) puro. Duecentodieci pulcini sono stati casualmente assegnati a 7 trattamenti dietetici con le diverse fonti lipidiche: 6 pulcini per gabbia, 5 gabbie per trattamento. Si sono studiati gli effetti dei trattamenti sugli accrescimenti, sugli indici di conversione alimentare e sulla composizione in acidi grassi del grasso addominale di femmine broiler ibride Ross 308. Gli animali sono stati macellati alla fine della prova, a 35 giorni. La qualità della carne del petto è stata poi valutata da un panel di 15 membri professionisti e analizzata per la durata della shelf life. Il trattamento AF ha dato il più elevato incremento di peso, ma solo nella prima settimana. La miscela MDT + OO (50/50) è risultata la migliore combinazione con lievi, anche se non significative migliori performance e decisamente migliore in termini di composizione in acidi grassi del grasso addominale, gusto e sugosità della carne del petto e durata della shelf life.

Parole chiave: Integratori lipidici, Olio di oliva, Gliceridi, Alimentazione dei broiler.

Introduction

Fats of animal origin, once broadly used in the broiler industry in Europe as energy supplements, are no longer well accepted because of the psychological consequences of BSE. Plant oils have replaced animal fats, but still some of them raise problems: the highly unsaturated ones (e.g. soybean oil) result in "oily" meat, with a relatively short shelf life, thus commercially not appealing, whereas more saturated oils (e.g. palm oil) are not so readily absorbable, at least during the first week of life of chicks. Garrett and Young (1975) demonstrated that monoglycerides are more readily absorbed than triglycerides by the chickens' gut. In particular, the monoesters of lauric acid (C_{12:0}) and myristic acid (C_{14:0}) are characterised by the maximum absorption rate. In general, the longer the chain, the lower the absorption. Besides, the authors confirmed that free fatty acids need monoglycerides to be absorbed as micelles with the bile salts and that the monoglyceride of oleic acid (C_{18:1 cis 9}) is the most efficient glyceride in carrying through the gut wall the poorly absorbable non polar molecules, such as palmitic acid (C_{16:0}). Because in Europe antimicrobial drugs are about to be banned in the near term, another important aspect deserving attention is that the mixtures of glycerides and free fatty acids are reported in the literature as potent selective antimicrobial factors (Cañas-Rodríguez and Smith, 1966; Isaacs *et al.*, 1995; Bergsson *et al.*, 2002; Dierick *et al.*, 2002; Sun *et al.*, 2002, 2003).

The scope of the present work was to study the effect of different mixtures of mono- di- and triglycerides and free fatty acids in the diet of broilers on growth performance traits, on the quality of abdominal fat and breast meat and on the shelf life.

Material and methods

Animals, growth trials and treatments

The animals were Ross 308 hybrid female chickens. They were kept for 5 weeks in cages, in order to be able to control feed intake and weight gain. Each cage contained 6 birds and each experimental group was made up with 5 cages (5 replicates), so that 30 birds were allotted to each treatment. The cages were 100 cm large, 30 cm high

and 55 cm deep. The group of birds fed the diet supplemented with soybean oil (SO) was considered the control group, to be compared against the others. One broad feeding trial was carried out, aimed at testing 6 lipid mixtures against SO. The theses were: control with SO; treatment 1 with animal fat (AF) discarded from poultry carcasses; treatment 2 with a mixture of mono-, di- and triglycerides of olive oil (25%, 50%, 25%, MDT) and palm oil (PO); treatment 3 with MDT and olive oil (OO); treatment 4 with MDT and SO; treatment 5 with MDT, PO and palm oil free fatty acids (PFA) and treatment 6 with MDT, OO and olive oil free fatty acids (OFA) (see footnote of Table 1).

The shelf life and the taste of the breast meat, judged by a panel test of trained members, were measured and evaluated relatively to 5 treatments only out of 7 (Table 4).

The diets were changed three times, accordingly to 3 periods: 0-7 d; 8-21 d and 22-35 d. The ingredient composition and chemical traits of the diets were those indicated in Table 1. The chemical composition and metabolizable energy content of the diets were calculated according to Sauvant *et al.* (2002). The acidic composition of the tested lipid supplements is presented in Table 2. The mixture of mono-, di- and triglycerides of olive oil is referred to as MDT.

Measurements and samplings

Weight gain and feed intake were recorded each week and referred to the single periods, even though these data are not present in Table 3 for the sake of brevity. At the end of the experiment, all the birds were slaughtered at the slaughterhouse and samples of separable abdominal fat were collected for the analysis of acidic composition and iodine absorption number (AOAC, 1990).

The carcasses of 6 birds per treatment, fed: a) the control diet; b) the diet with AF (treatment 1); c) the diet with MDT and PO (treatment 2); d) the diet with MDT + OO (treatment 3) and e) the diet with MDT + PO + PFA (treatment 5), were packed in transparent film and exposed to direct artificial light reproducing the light conditions of a market shelf, for 6 days and then analysed for the colour and oxidative rancidity.

Instrumental colour analysis was based on

Table 1. Ingredient composition and major traits of diets.

	Diets			
	0-7 d	8-21 d	22-35 d	
Ingredients (%):				
Maize meal	52.2	57.0	58.2	
Soybean meal 48	35.5	33.2	31.0	
Maize gluten feed	3.0	-	-	
CaHPO ₄ ·2H ₂ O	1.9	1.9	1.9	
CaCO ₃	1.5	1.2	1.2	
NaHCO ₃	0.25	0.25	0.25	
NaCl	0.25	0.25	0.25	
DL Methionine	0.25	0.25	0.25	
Lysine HCl	0.15	0.15	0.15	
Lipid supplement ¹	4.5	5.3	6.3	
Mineral vitamin supplement ²	0.5	0.5	0.5	
Estimated composition ³ :				
Crude Protein	%	21.2	19.9	19.0
Metabolizable Energy	kcal/kg ⁴	2900	3010	3100
Calcium	%	1.2	1.1	1.0
Phosphorus	"	0.7	0.7	0.7
Phosphorus, available	"	0.3	0.3	0.3
Lysine	"	1.3	1.2	1.1
Methionine + Cysteine	"	0.9	0.8	0.8

¹ Lipid supplements:

- control birds, soybean oil (SO);
- treatment 1, animal fat (AF);
- treatment 2, glycerides of olive oil (MDT) + palm oil (PO), 50:50;
- treatment 3, MDT + olive oil (OO), 50:50;
- treatment 4, MDT + SO, 50:50;
- treatment 5, MDT + PO + palm oil free fatty acids (PFA), 50:15:35;
- treatment 6, MDT + OO + olive oil free fatty acids (OFA), 50:15:35.

² Mineral vitamin supplement (per kg): vitamin A, 10,000 U; D₃, 1,000 U; E, 50 mg; K, 3 mg; riboflavin, 4 mg; Ca pantothenate, 10 mg; nicotinic acid, 40 mg; choline HCl, 150 mg; B₁₂, 6 mg; B₆, 4 mg; biotin, 0.10 mg; thiamine, 1 mg; Mn, 50 mg; Zn, 45 mg; Fe, 30 mg; Cu, 4 mg; I, 2 mg; Se, 1 mg.

³ Sauvant et al., 2002.

⁴ Average value, depending on the composition of the lipid supplement.

the measurements of light reflected from the breast sample and converted into values with the L*, a*, b* system (ASPA, 1996). An automated Minolta Chroma meter Cr 200 (Minolta Camera Co. Ltd, 2-Chrome, Osaka, Japan) with diffuse illumination with 8 mm diameter measuring area was used to measure L* (lightness), a* (redness), b* (yellowness). The colour of the 6 samples

from 6 different birds per treatment was measured in 3 different sites on the sixth day from slaughtering. Metric hue angle (T) and Chroma (C) were then calculated.

The extent of oxidative rancidity was evaluated by the thiobarbituric acid (TBA) test. The thiobarbituric acid reactive substances (TBARS) were measured according to Sørensen and

Table 2. Acidic composition, peroxide and iodine numbers of basal lipid supplements.

	SO	AF	OO	MDT	PO
Acid (g/100 g fatty acids):					
Palmitic (16:0)	11.18	23.88	12.90	13.17	46.20
Stearic (18:0)	3.82	11.45	3.20	2.82	4.50
Oleic (18:1)	24.32	45.98	70.40	70.72	39.28
Linoleic (18:2)	53.10	8.37	10.80	11.17	7.80
Linolenic (18:3)	6.13	-	0.60	0.31	0.20
Peroxide number	5.50	4.72	8.00	2.80	18.00
Iodine number	132.0	60.9	85.0	86.5	53.3

Table 3. Live performance traits, iodine number and acidic composition of abdominal fat (g/100 g fat).

	Treatments							SE
	control	1	2	3	4	5	6	
First week (0-7 d):								
weight gain g	134 ^{CDE}	173 ^{AB}	150 ^{ACD}	142	165 ^{AC}	114 ^E	121 ^{DE}	8.04
feed intake/gain	1.15 ^{AB}	1.10 ^{AB}	1.11 ^{AB}	1.16 ^A	1.14 ^{AB}	1.07 ^{BC}	1.02 ^C	0.02
Whole growth period (0-35 d):								
weight gain g	1,782	1,734	1,822 ^A	1,825 ^A	1,783	1,698 ^B	1,707 ^B	25.60
feed intake/gain	1.75 ^{AB}	1.74 ^{AB}	1.59 ^{DE}	1.65 ^{CD}	1.68 ^{BC}	1.53 ^E	1.54 ^E	0.02
Acidic composition of abdominal fat:								
palmitic (16:0)	19.60 ^{DE}	25.36 ^A	24.27 ^{BC}	19.32 ^E	20.31 ^D	24.81 ^{AB}	18.90 ^E	0.20
palmitoleic (16:1)	5.56 ^D	7.15 ^A	6.13 ^{BC}	6.08 ^C	2.50 ^G	4.35 ^E	3.14 ^F	0.12
stearic (18:0)	3.65 ^{BC}	7.06 ^A	3.10 ^{CD}	2.46 ^D	6.61 ^A	3.37 ^C	4.34 ^B	0.18
oleic (18:1)	35.34 ^F	42.36 ^D	48.49 ^B	53.98 ^A	38.43 ^E	46.33 ^C	53.36 ^A	0.26
linoleic (18:2)	33.79 ^A	14.14 ^F	16.38 ^E	17.22 ^D	29.26 ^B	19.01 ^C	17.43 ^D	0.18
linolenic (18:3)	1.50 ^B	0.60 ^D	0.41 ^{EF}	0.38 ^F	2.15 ^A	0.61 ^{CD}	0.68 ^C	0.02
Iodine number	102.2 ^A	72.50 ^F	80.60 ^E	86.9 ^C	95.8 ^B	81.6 ^E	84.5 ^D	0.48

Different superscripts within the same row indicate statistically significant differences ($p < 0.01$).

Treatments:

- control birds, soybean oil (SO);
- treatment 1, animal fat (AF);
- treatment 2, glycerides of olive oil (MDT) + palm oil (PO), 50:50;
- treatment 3, MDT + olive oil (OO), 50:50;
- treatment 4, MDT + SO, 50:50;
- treatment 5, MDT + PO + palm oil free fatty acids (PFA), 50:15:35;
- treatment 6, MDT + OO + olive oil free fatty acids (OFA), 50:15:35.

Table 4. Colour and shelf life traits on broiled breast meat.

	Treatments					SE
	control	1	2	3	5	
Instrumental colour analysis of the skin:						
Lightness (L)	67.30	67.43	65.54	65.05	66.10	2.57
Redness (a)	- 0.49	- 1.86	- 1.58	- 1.73	- 1.76	1.28
Yellowness (b)	19.50 ^{Aa}	17.15 ^{Ab}	14.42 ^b	12.17 ^B	10.27 ^B	3.41
Chroma (C)	19.57 ^{Aa}	17.28 ^{Ab}	14.52 ^b	12.34 ^B	10.47 ^B	3.41
Metric hue angle (T)	- 0.35	- 1.08	- 1.06	- 1.01	- 1.01	0.85
Instrumental colour analysis of the breast meat:						
Lightness (L)	53.54	57.96	54.14	52.08	53.61	3.60
Redness (a)	1.85	0.53	0.51	0.85	1.13	1.12
Yellowness (b)	10.73 ^{Aa}	10.42 ^A	9.42 ^{ab}	7.18 ^{bc}	5.89 ^{Bc}	2.23
Chroma (C)	10.98 ^{Aa}	10.49 ^A	9.44 ^{ab}	7.29 ^{bc}	6.21 ^{Bc}	2.24
Metric hue angle (T)	0.88	0.05	0.99	1.45	2.24	2.00
TBA analysis, six days past slaughter:						
µg malonaldehyde/g meat	0.180 ^A	0.125 ^B	0.051 ^{Ca}	0.032 ^C	0.029 ^{Cb}	0.01
Panel test means of mark scores ¹ :						
Stringiness	3.27	3.33	3.33	3.93	3.33	0.22
Juiciness	3.20	3.07 ^b	3.27	3.73 ^a	3.80 ^a	0.22
Flavour	3.20 ^b	2.93 ^b	3.33 ^b	4.00 ^a	3.73 ^a	0.21
General opinion	3.27 ^b	3.00 ^b	3.33 ^b	4.07 ^a	3.80 ^a	0.21

Different superscripts within the same row indicate statistically significant differences (capital letters: $p < 0.01$; small letters: $p < 0.05$).

Treatments:

- control birds, soybean oil (SO);
- treatment 1, animal fat (AF);
- treatment 2, glycerides of olive oil (MDT) + palm oil (PO), 50:50;
- treatment 3, MDT + olive oil (OO), 50:50;
- treatment 5, MDT + PO + palm oil free fatty acids (PFA), 50:15:35.

¹1 = very poor; 2 = poor; 3 = sufficient; 4 = good; 5 = very good.

Jørgensen (1996). Absorbance was measured at 525 nm (Perkin Elmer spectrometer Lambda EZ 150) against a blank made up of distilled water (5 ml) and TBA reagent (5 ml). TBARS, expressed as malonaldehyde (mg/g meat), were evaluated by a standard curve for 1,1,3,3-tetraethoxypropane (TEP).

Analysis of fatty acids

Samples of abdominal separable fat (25 g) were extracted according to Folch *et al.* (1957), evaporated under nitrogen and kept anhydrous on

silica gel under vacuum (Roach *et al.*, 2002). The methyl esters of fatty acids were prepared by means of trans-esterification of glycerides with sodium methylate in methanol (MeO-Na/MeOH, 0.5 M) according to Christie (1982) and Roach *et al.* (2002). The fatty acids of the lipid supplements were esterified again according to Christie (1982) with C19:0 (nonadecanoic acid) as the internal standard.

Methyl esters of medium and long chain fatty acids were analysed by means of a gas-chromatograph apparatus (Dani, Milano, Italy) equipped

with a FID detector and a high polar fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, the Netherlands; 50m x 0.25 mm internal diameter; film thickness 0.20 µm). The column temperature programming was 120°C for 1 min, then up to 180°C by 5°C/min, held for 18 min, then up to 200°C by 2°C/min, held for 1 min, finally up to 230°C, again by 2°C/min. The injector and detector temperatures were 270°C and 300°C, respectively. Helium was the carrier gas with a flux of 1 ml/min and a split ratio of 1/100 (Kramer *et al.*, 1999; Sehat *et al.*, 1999). Nonadecanoic methyl ester was used as the internal standard.

Panel test

Sensory evaluation was performed by a professional panel of 15 trained members. One bit of broiled breast meat from each of 6 birds per treatment (5 treatments: control diet, diet 1, diet 2, diet 3 and diet 5) was examined for stringiness, juiciness, taste and overall opinion. A discrete scale between 1 and 5 (1 = very poor, 2 = poor, 3 = sufficient, 4 = good, 5 = very good) was used for the evaluation of each trait. The test was carried out on the sixth day from slaughtering on the carcasses kept under transparent film on a refrigerated shelf (current shop conditions).

Statistical analyses

All data referable to the different treatments were compared with one another by the Statistical Analysis System (SAS, 1990) by means of one-way ANOVA, keeping the factor "diet" as the fixed one.

Results and discussion

Weight gain and feed efficiency ratios (Table 3) refer to the first week and to the whole growth period separately, because the first week is the critical one in terms of development of the gut morphology and physiology. The differences are considered statistically different at the $p < 0.01$ level only, in order to over complicate the discussion.

At the end of the first week, the largest gains were those of the birds fed diets 1 (AF) and 4 (MDT + SO), different from the gains of birds fed diet 3 (MDT + OO), at the $p < 0.05$ level only, not indicated in the table. The lowest gains were obtained with diets 5 and 6, containing free fatty

acids, with the lowest feed/gain ratios. Both gain and feed efficiency may be explained with the low intake (bad taste?). In the long period of 35 days, AF resulted comparable with SO of the control group. Diets 2 and 3, supplemented with MDT, resulted in gains above 1.8 kg, statistically not different from the control diet and diet 1, but significantly better than diets with PFA and OFA. The lowest feed/gain ratios were, again those of the diets with PFA and OFA, because of the low intake. As a general comment to the results of live performances, it may be said that the mixture of MDT appeared an efficient vehicle, enhancing the digestibility and absorption of a difficult oil such as PO. Actually, the high weight gain of birds fed diet 2 was associated with the statistically lowest feed/gain ratio. The results of the above cited Garrett and Young (1975), who found that monoolein is very highly absorbed (98%) in the gut of chickens, easily carrying PA from palm oil, were confirmed. The presence of free acids appeared not useful in this respect.

The nature of supplemented fats was quite closely reflected in the acidic composition of the analysed abdominal fat, depicted in the second part of Table 3, thus confirming the findings of Fisher (1984), Pinchasov and Nir (1992), Scaife *et al.* (1994). In fact, the acidic composition of the body fat of chickens is described as partly due to the hepatic endogenous lipogenesis of palmitic acid (PA) and oleic acid (OA) and partly attributable to the exogenous dietary inlet of linoleic acid (LA) and linolenic acid (LNA). If this is the correct interpretation of what happened in our birds, it means that when huge amounts of LA and LNA have been introduced with the diet, the relative amounts of PA and OA have been "diluted". On the contrary, the high concentrations of PA and OA were the consequence of the relatively low amounts of LA and LNA in the diet. In any case, it was confirmed that the acidic composition of the abdominal fat reflects the composition of the supplemented dietary fat or oil. What we were able to observe is that:

- PA was highest in the abdominal fat of the birds fed PO (treatments 2 and 5), comparable to that of the birds fed AF (treatment 1);

- the levels of stearic acid (SA) were highest with AF and MDT + SO (treatments 1 and 4);

- the presence of OO in the diet, even under the form of free acids (OFA), was reflected in the highest percentages of OA found in the fat samples: more than 50% with treatments 3 and 6 and close to 50% with treatments 2 and 5;

- the influence of SO was clear with treatment 4, due to the relatively poor concentration of OA and to the high concentrations of linoleic acid (LA) and linolenic acid (LNA) in SO (see table 2).

If we consider that saturated acids must be kept low in the diets of consumers, and among the unsaturated acids, the monounsaturated OA is less easily oxidised than the polyunsaturated LA and LNA, we may conclude that the more desirable results were obtained with MDT + OO (treatment 3): high weight gain, low feed/gain ratio, healthy acidic composition of carcass fat.

The colour of both skin and breast meat of the birds fed MDT (treatments 2, 3 and 5 in Table 4), was less brilliant in its yellow component than the colour of skin and meat of birds fed SO and AF, but still fully acceptable by the consumer. Anyway, the measured values for the yellow component of colour were comparable with the results reported by Bilgili *et al.* (1998).

The TBA analysis, performed after six days of exposure to the light, temperature and air conditions of a market shelf, clearly indicated the better preservation toward oxidation ($p < 0.01$) of the MDT treated meat as compared with SO and AF. The relative enrichment of the monounsaturated component of tissues fat at the expenses of polyunsaturated fatty acids is reported in the literature (Diplock *et al.*, 1988; Hsieh *et al.*, 2002) as the prime factor responsible for the improved tissue oxidative stability.

Finally, the members of the panel test judged the cooked breast meat of the birds fed MDT + OO (treatment 3) more juicy, more tasty and generally better ($p < 0.05$) than the meat of the birds fed both SO and AF.

Conclusions

Aim of the whole work was testing different lipids as energy supplements to the diets of broilers in order to find out the best alternative to: i) soybean oil, a good supplement but yielding oily, slippery meat and suspected of not being GMO free; ii) animal fat, again a good one but no longer popular because of the BSE problem; and iii) palm oil, yielding acceptable carcasses but poorly digestible in the chickens' gut.

The experimental results clearly demonstrated that:

- the mixture MDT olive is a good carrier to enhance palm oil utilisation;

- the mixture MDT olive associated with OO is a good fat supplement as well, in terms of performance traits;

- it is confirmed that the quality of supplemented fat in terms of acidic composition closely reflects the composition of the abdominal depot fat in the birds. It is therefore possible to manipulate it in order to improve its potential safety for the consumers' health, by lowering palmitic acid level and enhancing oleic acid;

- the use of the mixture MDT olive + OO was acknowledged by 15 trained panel members as the best supplement in terms of taste and juiciness of meat;

- again, the same mixture resulted in a meat with a low level of oxidation products (TBARS) after a shelf exposure of 6 days.

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