FURTHER ASPECTS ON THE EFFECT OF DIFFERENT n-3 AND n-6 FATTY ACID SOURCES IN BROILER DIETS

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Summary

The motivation for this research work was to explore further effects of some different n-3 and n-6 fatty acid rich sources on broiler performance and some other related meat quality parameters, other than the frequently studied fatty acid profile of carcass, when the polyunsaturated fatty acids (PUFA's) rich vegetable oils; linseed oil (n-3 series) and sunflower oil and cottonseed oil (n-6 series), independently and simultaneously, substitute for the ideal long chain n-3 fatty acid-fish oil. Differences in oil inclusion levels were in an attempt to obtain iso-caloric-iso-nitrogenous diets, and calculations were based on that sunflower oil added at the rate of 3%, had the highest ME values. Fish oil was incorporated at the rate of 3.48% in the basal diet, and served as a control for the following seven experimental diets containing linseed oil (LO) at 3.45%, sunflower seed oil (SO) at 3.00%, cottonseed oil (CO) at 3.33%, LO+SO at 1.72+1.50%, resp., LO+CO at 1.72+ 1.66%, SO+CO at 1.5+1.66%, resp., and LO+SO+CO at 1.15+1.00+1.10%, resp%. (diets 2 up to 8). Parameters to be considered in this study were growth performance using 240 Ross broiler chicks, allocated among 8 groups, of 30 birds, each, carcass and meat measurements, besides some meat acceptability constituents were included in the study.

Results obtained indicate that broilers fed diets enriched with in n-6 PUFA's series, independently or simultaneously in doubles, were better than other tested forms, including the control group and the effects upon growth traits were significant (p<0.01) for the entire study period. Abdominal fat % varied significantly (p<0.05) between the studied

groups with no clear trend towards specific PUFA's category. Sunflower oil and fish oil, equally, had the lowest meat triglycerides values ($p<0.01$). But finally, while, sunflower oil alone, indicate the best meat sensory traits in terms of smile and flavour, fish oil inclusion worsened these traits ($p<0.01$). Consequently, when different PUFA's to be compared, the long chain n-3 PUFA's as fish oil, is recommended for better meat quality (low meat triglycerides and cholesterol) for human consumption, and when the choice is to obtain higher weight gains and better sensory meat quality, the n-6 PUFA's as sunflower oil is prerequisite.

Introduction

The increased concern on the production of high quality poultry meat, as functional food, is a growing approach. The health benefits associated with the nutrition of polyunsaturated fatty acids (PUFA's) of *n*-3 and *n*-6 series, and especially the role they have in protecting the human body against cardiovascular diseases (Grashorn, 2007), besides the amenability of the fatty acid composition of broiler meat to change, following a given modification in fatty acid composition of the diet (Hargis and Van Elswyk, 1993), have been the motivation and focus point of meritorious research work devoted to study the relationship between dietary fatty acid profile, and the resultant modification in the fatty acid profile of the carcass and adipose tissues of broiler chicks (Valavan *et al.*, 2006, Hung *et al.*, 2007, and Kralik *et al.*, 2008). In these and in too more earlier studies, sunflower oil, rich in linoleic acid ($C_{18:2:n\text{-}6}$), on one hand, and linseed oil, rich in linolenic acid ($C_{18:3:n\text{-}3}$), and fish oil, rich in the long chain *n*-3 fatty acids (LC *n*-3 FA's), in particular, eicosapentaenoic acid ($C_{20:5:n\text{-}3}$; EPA) and docosahexaenoic acid ($C_{22:6:n\text{-}3}$; DHA), on the other hand, these vegetable and marine oils, are the most *n*-6 and *n*-3 PUFAs sources studied, respectively. When these sources are compared, fish oil due to its high content of EPA and DHA posses extra appreciable roles in lowering the incidence of atherosclerosis, coronary heart diseases and plasma triglycerides (Leskanich and Noble, 1997). In this concern, some poultry studies, have suggested that α-linolenic acid (ω-3 fatty acid series) from linseed oil is further converted to EPA and DHA by desaturation and elongation with Δ^6 and Δ^5 enzymes and, hence, can be substituted for fish oil (Simopoulos, 1999). Although, some other reports (López-Ferrer *et al.,* 1999), proposed that poultry have a limited capacity to desaturate and elongate α-linolenic acid (López-Ferrer *et al.,* 2001).

Inclusion of fish oil in broiler diets has been reported to improve (Alparslan and Özdogan, 2006), no effect (Abas *et al.,* 2004), or, negatively affect (Hulan *et al.*, 1988) the growth performance of broilers that changed from a standard diet to one enriched with fish oil. When different PUFA's sources were compared; fish oil *vs.* linseed oil (Choct *et al.*, 2000), or fish oil *vs.* sunflower oil (Newman, 2000), no significant differences for the contrasts studied in growth performance, carcass traits, could be emerged. More interestingly, the inclusion of these high quality PUFA's sources in broiler diets are substantially linked with two meat processing dilemma; the off-odours and off-flavours associated with inclusion of high levels fish oil in the diet (Leskanich and Noble, 1997).

Little research work has been done to further describe the quality traits that are not less important the fatty acid composition of the produced meat, and need to be clarified when PUFA's are introduced in broiler diets. In Komprda *et al.,* (2003) study, working on turkeys, they reported no significant differences in total lipids content of meat from diets supplemented with linseed, sunflower seed and fish oils, however, meat cholesterol was noticeably decreased in linseed oil group. Recently, Roy *et al.*, (2008) reported that for growing chicks, inclusion of long chain PUFA's fish oil increased significantly the HDL-cholesterol of the meat.

Consequently, the study was planned to investigate the roles that dietary vegetable PUFA's from *n*-3 and *n*-6 series would have when substituted the fish marine oil of long chain *n*-3 rich source on some growth performance, meat quality traits and meat sensory traits of broilers chicks.

Materials and Methods

Before starting the biological experiment, the fatty acid profile of tested oil was carried out (AOAC, 2000). Data are provided in Table (1).

Two hundred forty-1 day-old unsexed Ross broiler chicks, divided equally into eight groups of three replicates each, were reared on wire battery cages and had the same managerial procedures throughout the growth trial term. Birds were given *ad libitum* access to water and the diets described in Table (2). Three-phased diets were formulated to meet the Ross 308 breeder requirements. Three Phases diets was applied. All diets were formulated to cover the strain requirements.. To obtain iso-caloric and nearly iso-nitrogenous diets, oils studied, other than sunflower oil, were added in proportions equivalent to the amount of energy supplied by 3% sunflower oil. Based on that sunflower oil has the highest ME value of these oils (9660 kcal/kg). Consequently, 3.48% fish oil (FO) in the corn-soybean meal based diet served as the control, was replaced with the following vegetable oils; linseed oil (LO), sunflower seed oil (SO), cottonseed oil (CO), LO+SO, LO+CO, SO+CO, and LO+SO+CO, resp.. To avoid the fatty acids oxidation of the used oils, α-tocopheryl acetate was added at the rate of 400 mg/kg diet. Growth performance was recorded for each growth phase.

At the termination of the growth trial period, six representing birds of each experimental group were overnight fasted, slaughtered and eviscerated. Weights of hot carcasses, liver, heart and abdominal fat were proportioned to the live body weight upon slaughtering.

Three right thigh muscles without skin of each experimental group of just slaughtered birds were kept refrigerated for 24 hours at 4ºC. Next day, samples used to prepare meat extract solution using phosphate buffer solution (pH 7.4). Clear homogenate solution was stored at -20ºC, for further determination of total lipids, triglycerides and cholesterol, following the method ascribed by Zollner and Kirsch (1962).

On slaughter, three whole carcasses of each treatment were stored refrigerated at -2ºC to the next day, where breast meat samples (without skin) of each treatment were prepared according to López-Ferrer *et al.*, (1999). Samples were wrapped in two aluminum foil layers, and then stored refrigerated at -20ºC untill the sensory test was carried out, after which the samples were thawed, than grilled on a double plated grill at 200ºC. Ten assessors were asked to rank the overall acceptability (smile and flavor) using a 9-point scale (where 1= very bad and 9= very good).

Data were subjected to a one-way analysis using SAS procedures (1990). Variables having significant differences were compared using Duncan's Multiple Range Test (Steel and Torrie, 1960).

Results and discussion

Fatty acid profile of tested oils

The fatty acid profile for the dietary oil supplements is given in Table (1). As would be expected, fish oil contained a high LC *n*-3 PUFA's compared to the vegetable oils. The principal *n*-3 FA's for fish oil were EPA ($C_{20:5\,n\text{-}3}$) and DHA, ($C_{22:6\,n\text{-}3}$). The two FA's were not present in LO and CO, and 0.2% EPA in SO. In contrast, the predominant fatty acid in LO was linolenic acid ($C_{18:3\ n\text{-}3}$), and in SO, it was linoleic acid ($C_{18:2\ n\text{-}6}$), followed by the, MUFA, oleic acid ($C_{18:1\ n\text{-}9}$). Whereas, cottonseed oil, mainly, consisted of linoleic and oleic acids, in proportions, relatively lower than those reported for SO.

Growth performance

Data provided in Tables (3) indicate that where diets were rich in *n*-6 PUFA's series (sunflower and cottonseed oils), independently or simultaneously in doubles, were better than other tested forms, and generally the effects upon growth traits (live body weights gains and feed conversion ratio) were significant ($p<0.05$) during most studied intervals. Also, these variables in most cases were negatively affected by *n*-3 oil forms (fish and linseed oils). Our results corroborate with Wongsuthavas (2007) who reported that high-α linolenic acid (linseed oil) diet lowered average daily gain and total feed intake compared to high linoleic acid (soybean oil) diet. Also, agree with the contentions of López-Ferrer *et al.*, (1999) who fed broiler chicks on diets supplemented with 8.2% fish oil or linseed oil, Choct *et al.*, (2000) reared broiler chicks on diets supplemented with 2% or 4% fish oil or linseed oil and, Valavan *et al.*, (2006) fortified the chicks on diets with 3% fish oil or linseed oil, all these studies, reported no significant difference effects on live weight gain between the two *n*-3 PUFA's oils. While, the improvements in growth traits recorded with sunflower oil supplementation are not in conformity with results reported by Newman (2000) who detected no significant differences in growth traits between broiler groups fed on diets with fish oil or sunflower oil. Also, our results counteract those of Soliman *et al.*, (2003) who found that growth of broilers was significantly better on a double or triple mixture of an equal parts of linseed, corn and sunflower oils to form 3% of the total broiler diet, compared to corn and sunflower seed oils, each alone. The obvious decrease in feed of fish oil (control) group, following linseed oil fed group compared with their counterparts on the individual PUFA's *n*-6 oils runs parallel with previous findings, where Fritsch *et al.*, (1991) reported a decrease in feed intake in linseed oil fed broiler chickens that they were attributed to the presence of some no*n*-saponifiables expeller fraction of the linseed oil used in their study. Alike, Abas *et al.*, (2004), and Alparslan and Özdogan (2006) attributed the decrease in feed intake as incorporating upper levels of fish oil to the sensitivity of chicks to fishy smell that the unwillingness in feed consumption of chickens could be attributed to, especially with the increase in the inclusion level.

Carcass traits

Results in Table (4) indicate that both abdominal fat % ($p<0.05$) and heart % ($p<0.01$), while, neither carcass yield % nor liver % ($p=0.07$) were significantly affected by oils evaluated. Fish oil and cottonseed oil, which had the highest saturated FA's % showed the highest third and first abdominal fat content in the present study, with no clear reason for the extraordinary fat content observed in the triple oil mixture. However, linseed oil + sunflower seed oil, together, showed the lowest favoured abdominal fat %. Choct *et al.*, (2000) reported that linseed oil fed broilers had heavier abdominal fat weights compared to others fed fish oil-diet fed group. Wongsuthavas (2007) reported that the amount of abdominal fat tended to decrease, whereas the liver weights were heavier in the birds fed high linolenic acid compared to high linoleic acid diet.

Generally, the experimental data indicate a lower abdominal fat deposition than reported for other studies tried conventional or supplemented with saturated or mono-unsaturated fatty acid diets (Sanz *et al.*, 2000 and Crespo and Esteve-Garcia, 2002). In this concern, Newman *et al.*, (2000), and Sanz *et al.*, (2000), attributed the decrease in abdominal fat percentage when broiler chicks fed enriched mono or polyunsaturated fatty acids to the increase in lipid oxidation rate and the reduction in endogenous fatty acid synthesis. Those authors reported a high activity in some enzymes of the lipid catabolism and a lower activity in the liver fatty acid synthetase in chickens fed on unsaturated fatty acid containing diets. So, the fluctuation in abdominal fat % among studied PUFA's sources could be attributed to the discrepancies in oxidation rate for these oils. However, it could not be ignored that no clear interpretation has to be suggested for the significant differences in abdominal fat percentages among treatments, since the energetic density, or in other words, the calorie/protein ratio of the experimental diets is comparable, as pointed by Lessire *et al.*, (1996). It worthy noting that any reduction in the amount of abdominal fat is considered to be positive by the producers and consumers, because it is lost during carcass cut-up, it contributes little to meat quality, and there is an increasing consumer resistance to eating fatty foods (Sanz *et al.*, 2000).

Meat measurements

Presented in Table (5) are meat quality traits (total lipids, triglycerides, cholesterol, and HDL-cholesterol) as affected by the oils studied. It is quite clear that sunflower oil, alone stand at the same quality values recorded for fish oil, also close results are seen for cottonseed oil, alone and less interestingly that associate between linseed oil and sunflower oil. Linseed oil and the triple mixture shared the worst findings. The results imply that *n*-6 PUFA can play comparable role to that attributed to the long chain *n*-3 fatty acids (EPA and DHA) from fish oil in preventing the cardiovascular diseases and participate to improve the health conditions of the human consumed the meat they contain. As we have too little work on the effect of PUFA's on meat quality traits, the study carried out by Komprda *et al.,* (2003) working on turkeys, reported no significant differences in total lipids content of meat from birds fed diets supplemented with 5% linseed, sunflower seed or fish oils. However, linseed oil decreased the cholesterol content of the meat *vs.* sunflower seed oil or fish oil. Also, Roy *et al.*, (2008) reported that for growing chicks, inclusion of long chain PUFA's high fish oil significantly, increased, the HDL-cholesterol of the meat.

Sensory meat traits

Data provided in Table (6) shows the objective meat quality parameters of flavour and smile as they were affected by oils under investigation. As it was accepted and reviewed, fish oil and linseed oil were ranked first and second to worsen the acceptability of the broiler meat. While, sunflower oil, alone surpassed other oils to enhance these traits. EPA and DHA from fish oil are prone to oxidation, and, consequently, their use in meat enrichment may produce off-tastes and off-odours, thereby reducing consumer acceptability (Bou *et al.*, 2005). On the other, whereas the inclusion of linseed oil as alternative source to *n*-3 fatty acids involves a lesser degree of off-flavours and odours, it also results in diminished deposits of EPA and DHA in the animal's tissues (Ajuyah *et al.*, 1993). Whereas linoleic acid is associated with milder flavours (Wilson, 2006). López-Ferrer *et al.*, (1999) reported that sensory quality of meat was improved when linseed oil or rapeseed oil substituted fish oil in broiler chicken meat (8.2%).

Conclusions

Consequently, the results in the current study involve that different PUFA's sources have distinguished effect on broiler studied criteria, and what is best for broiler chicks, does not imply the convenience for human consumption. Also, further studies are needed to explore the associative effect between the long chain *n*-3 PUFA's from fish oil and the *n*-6 PUFA's from its oil sources (sunflower seed, soybean, cottonseed) to promote meat products of added values, while keeping the performance of broilers.

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Table (1): Fatty acid profile (% of total) of experimental oils.

Fatty acid	Fish oil	Linseed oil	Sunflower seed oil	Cottonseed oil
$C_{14:0}$	9.72	-	-	-
$C_{16:0}$	20.64	5.80	6.00	12.9
$C_{16:1\ n\text{-}7}$	14.30	-	-	-
$C_{18:0}$	4.07	4.30	3.20	3.90
$C_{18:1\ n\text{-}9}$	10.86	17.70	28.70	21.20
$C_{18:2\ n\text{-}6}$	1.36	13.40	59.30	53.80
$C_{18:3\ n\text{-}3}$	0.70	57.70	0.10	5.80
$C_{20:4\ n\text{-}6}$	1.85	-	-	0.30
$C_{20:.5\ n\text{-}3,}$ EPA	14.15	-	0.20	-
$C_{21:5\ n\text{-}6}$	1.57	-	0.10	-
$C_{22:4\ n\text{-}6}$	0.59	-	-	-
$C_{22:5\ n\text{-}6}$	1.22	-	-	-
$C_{22:6\ n\text{-}3}$, DHA	9.10	-	-	-
Others	7.47	5.10	2.40	2.10
Saturated	34.43	10.10	9.40	17.10
∑ MUF's	25.16	18.50	29.08	22.80
∑ PUF's	32.94	71.10	60.80	59.60
∑ *n*-6	5.02	13.40	60.7	53.80
∑ *n*-3	23.95	57.70	0.30	5.80
ME (kcal kg^{-1}oil)	8340	8400	9660	8700

Table (2): composition and calculated analyses of the experimental diets.

Items	Starter (1-10 day)	Grower (11-28 day)	Finisher (29-40 day)
Yellow corn	52.70	61.00	66.15
Soybean meal 44%	34.12	23.00	23.00
Corn glutin	6.00	9.00	4.00
Sunflower oil*	3.0	3.0	3.0
Di-Ca-P	1.70	1.69	1.65
Vit.& Min. premix**	0.25	0.25	0.25
Lysine-HCl	0.23	0.37	0.20
DL-methionine	0.27	0.19	0.20
$CaCO_3$	1.30	1.10	1.10
NaCl	0.43	0.40	0.45
Total	100.0	100.0	100.0
	Calculated nutrient analyses		
Crude protein %	23.2	20.9	18.3
ME ($kcal/kg^{-1}$diet)	3056	3199	3188
Ether extract %	5.7	5.9	5.9
Ca %	1.0	0.90	0.90
Available P %	0.46	0.44	0.43
Lysine %	1.37	1.20	1.17
Methionine %	0.64	0.61	0.55
Methionine+Cystine %	1.07	0.96	0.85

*Sunflower oil was substituted as follows; Fish oil (FO) at 3.48%, linseed oil (LO) at 3.45%, sunflower seed oil (SO) at 3.00%, cottonseed oil (CO) at 3.33%, LO+SO at 1.72+1.50%, resp., LO+CO at 1.72+ 1.66%, SO+CO at 1.5+1.66%, resp., and LO+SO+CO at 1.15+1.00+1.10%, resp. %.

**Each 2.5 kg contains; 12000000 IU vit.A; 2000000 IU vit. D_3; 10000 mg vit. E; 1000 mg vit. K_3; 1000 mg vit. B_1; 5000 mg vit. B_2; 1500 mg vit. B_6; 10 mg vit. B_{12}; 30000 mg vit. PP; 10000 mg vit. B_5; 50 mg B_8; 1000 mg B_9; 250000 mg choline chloride; 60000 mg Mn; 30000 mg Fe; 50000 mg Zn; 10000 mg Cu; 1000 mg I; 100 mg Se and 100 mg Co.

Table (3): Effect of different PUFA's profile on live body weight gain and feed conversion ratio of broiler chicks.

Items	live body weight gain (g)				Feed conversion ratio(g feed/ g gain)			
	Starter 0-10 d	Grower 11-28d	Finisher 29-40d	Total 0-40d	Starter 0-10 d	Grower 11-28d	Finisher 29-40d	Total 0-40d
1- FO	144^{abc}	987	559^{b}	1690^{bc}	1.29^{a}	1.58	2.17^{c}	1.75^{b}
2- LO	123^{cd}	1011	499^{b}	1633^{c}	1.70^{bc}	1.60	2.56^{e}	1.89^{c}
3- SO	153^{ab}	1090	567^{ab}	1810^{a}	1.51^{ab}	1.50	2.30^{bc}	1.75^{ab}
4- CO	159^{a}	984	665^{a}	1808^{a}	1.46^{ab}	1.67	2.00^{bc}	1.77^{b}
5- LO+SO	140^{abcd}	943	722^{a}	1805^{a}	1.56^{abc}	1.64	1.74^{a}	1.67^{a}
6- LO+CO	120^{cd}	949	706^{a}	1775^{ab}	1.84^{c}	1.67	1.73^{a}	1.70^{ab}
7- SO+CO	132^{bcd}	987	667^{a}	1786^{ab}	1.64^{bc}	1.60	1.89^{ab}	1.71^{ab}
8- LO+SO+CO	119^{d}	961	543^{b}	1623^{c}	1.56^{abc}	1.60	2.63^{e}	1.94^{c}
Pooled SE	3.74	7.66	15.33	18.49	0.04	0.01	0.06	0.02
Significance	**	ns	**	**	*	ns	**	**

Means in the same column within each factor differently superscripted are significantly different.
ns: not significant, *: (p<0.05), and **:(p<0.01),

Table (4): Effect of different PUFA's profile on some carcass relative weights of broiler chicks.

Items	Carcass yield %	Abdominal fat %	Liver %	Heart %
1- FO (Control)	68.9	2.69ab	3.57	0.59d
2- LO	69.4	2.49abc	4.16	0.59d
3- SO	69.3	2.32abc	3.58	0.61d
4- CO	69.3	2.92a	3.50	0.64cd
5- LO+SO	69.8	1.65c	3.15	0.68bcd
6- LO+CO	70.4	2.21abc	3.39	0.73abc
7- SO+CO	71.3	1.80bc	3.47	0.83a
8- LO+SO+CO	71.4	2.81a	3.12	0.78ab
Pooled SE	0.29	0.12	0.09	0.02
Significance	ns	*	ns	**

Means in the same column within each factor differently superscripted are significantly different.
ns: not significant, *: (p<0.05), and **:(p<0.01),

Table (5): Effect of different PUFA's profile on some meat measurements of broiler chicks (mg/dl).

Items	Total lipids	Triglycerides	Cholesterol	HDL
1- FO (Control)	370	321[c]	9.1[d]	2.9[d]
2- LO	409	361[ab]	19.1[a]	6.2[a]
3- SO	381	318[c]	11.1[cd]	3.6[cd]
4- CO	386	335[bc]	13.5[bc]	4.4[bc]
5- LO+SO	358	346[abc]	14.0[bc]	4.5[bc]
6- LO+CO	391	367[ab]	15.9[ab]	5.4[ab]
7- SO+CO	398	382[a]	16.6[ab]	5.5[ab]
8- LO+SO+CO	395	372[ab]	19.1[a]	6.2[a]
Pooled SE	5.42	5.78	0.79	0.26
Significance	ns	**	**	**

Means in the same column within each factor differently superscripted are significantly different.
ns: not significant, and **:(p<0.01).

Table (6): Effect of different PUFA's profile on falvour and smile properties of broiler meat.

Items	FO	LO	SO	CO	LO+SO	LO+CO	SO+CO	LO+SO+CO	Pooled SE	Sig.
Flavour	6.44[e]	7.30[d]	8.55[a]	8.00[abc]	7.54[cd]	8.00[abc]	7.88[bcd]	8.20[ab]	0.14	**
Smile	6.07[c]	7.20[bc]	8.55[a]	7.88[ab]	7.73[ab]	8.33[a]	7.44[b]	8.10[a]	0.13	**

**Means in the same row within each factor differently superscripted are significantly different (p<0.01).