



## ABSTRACT

Production of chicken meat is an important branch of food industry. Generally, poultry meat farmers aim to produce birds with superior body weight and feed conversion ratio. However, in line with current developments, there are other parameters that must to be taken into account such as lower body fat deposition and improvement in the nutrient composition of products corresponding to consumer requirements. Nowadays, extra focus has been given to designing and enrichment foods as functional foods, with components that have beneficial effects on human wellbeing. In definition, functional foods contain particular nutrients and / or non-nutrients that have an effect on human health, beyond what is usually known as nutritional effects. There are several compounds in foods that improve consumer's health status. Among them the polyunsaturated fatty acids (PUFAs, n-6 and n-3 series) and conjugated linoleic acid (CLA) are the most functional and bioactives components of lipids. Chicken meat has been a common objective of nutritional modification because the absorbed dietary fatty acids from the small intestine directly enter into the chicken tissue lipids. This paper tries to review the recent findings in the area of chicken meat enrichment using dietary fats.

KEY WORDS chicken meat, CLA, enrichment, n-3 PUFAs.

### INTRODUCTION

It seems that in human rations and most animal feeds there is an unbalanced fatty acid composition, so that the content of n-3 has decreased whereas the content of n-6 fatty acids has increased. One approach to restore this balance is by supplementing food with fish originated fats, which are rich in long chain n-3 PUFA (Bezard *et al.* 1994; Tuncer *et al.* 1987; Manilla *et al.* 1999 and Lopez Ferrer *et al.* 2001). Fatty acids originated from diet can be directly integrated into body tissues intact (Klasing, 1998), a fact sometimes known as verify feeding grounds using fatty acid signatures (Williams and Buck, 2010). Moreover, dietary fats are recognized to determine the composition of various bird tissues, ranging from muscle membranes to eicosanoids (McCue *et al.* 2009). The importance of human diet in the maintenance of the healthy status is an ancient subject. In 500 before christ (BC), Hippocrates was quoted to say that "Food is medicine, and medicine is food". Human foods as a lifestyle factor, is involved in the incidence of many types of diseases such as cardiovascular diseases and cancer. Actually, atherogenesis and occurrence of different types of cancers can be influenced by human foods and nutrition pattern (Russo, 2009). Recent evidence suggests that not only the quantity but also the composition of dietary fat is important factor to preventsuch diseases (Sealls *et al.* 2008). Tables 1 and 2 show the recommended intake levels of some important fatty acids in human ratiosas well as the

majour fatty acids groups' concentrations in some common foods, respectivelly.

### Fats and fatty acids

Fats are the main storage source of energy in animal body. These components are important dietary ingredients with important roles in cell membranes structure and synthesis of important regulatory molecules (Goldman, 1994). The lipid is a general term that describes a group of fatty substances insoluble in water (or poorly soluble) and soluble in non-polar organic solvents (Gunstone et al. 1994). Triglycerides, as the common fats, are consisted of three fatty acids estrified to a 3-carbon molecule glycerol. The fatty acids unit in the structure of triglycerides determines fats texture, taste, and physical properties. There is a variety of naturally occurring fatty acids, which basic structure consists of a carboxyl group and a methyl group at the two ends of a long hydrocarbon chain. The most common natural fatty acids have straight chains of an even number of carbon atoms.

Fatty acids based on the number of double bonds in the molecule are also classified as saturated or unsaturated fatty acids. Saturated fatty acids (SFA) are free of double bond and unsaturated fatty acids (USFA) acids contain at least one double bond. Monounsaturated fatty acids (MUFA) containing only one double bond and fatty acids with more than one double bond are named polyunsaturated fatty acids (PUFA). The position and the number of double bonds define the chemical properties of fatty acids (Gunstone *et al.* 1994). Delta-9 desaturase enzyme converts SFA such as stearic acid (18:0) to MUFA. The synthesis of MUFA in animals and plants takes place in the same manner, but further desaturation to PUFA uses different mechanisms (Neuringer and Connor, 1986).

#### **Essential fatty acids**

Linoleic acid (LA; 18:2 n-6), linolenic acid (LNA; 18:3 n-3) and arachidonic acids (AA; 20:4 n-6) cannot be synthesized by either mammalian or avian cells and therefore must be supplied in diet (Stevens, 1996). These fatty acids are called "essential fatty acids" (EFA). Basically, animals lack the delta-12 and delta-15 desaturase enzymatic activity required to insert double bonds at n-6 or n-3 positions of fatty acids to *de novo* synthesis of EFAs (Sargent *et al.* 2002). Therefore, palmitic acids (16:0) or stearic acid (18:0) cannot be transformed to n-6 or n-3 series fatty acids by the *de novo* synthesis pathway (Field, 2003; Trugo and Torres, 2003).

Biochemical pathways for EFAs synthesis exist only in the chloroplasts of plant cells; thus plants are the source of EFAs in foods. Animals are only able to elongate and desaturate the parental EFAs to synthetise long-chain polyunsaturated fatty acids (LCPUFAs). In the endoplasmic reticulum, the delta-6 desaturases activity is responsible for the conversion of PUFA to LCPUFA (Barceló-Coblijn and Murphy, 2009). The *de novo* synthesis of PUFA is dependent on some hormones, on the relative concentration of precursor fatty acids, and their intermediated products. Arachidonic acid, the 20 carbons PUFA, is particularly important because of its function in membrane synthesis and embryonic development (Holman, 1986; Ching, 2000).

Parental essential fatty acids are substrate for other useful derivatives too. The 20 °C derivative, arachidonic acid (AA) is produced from linoleic acid.  $\alpha$ -linolenic acid (ALA) undergoes elongation and desaturation to form n-3 PUFAs, the eicosapentaenoic acid (EPA 20:3 n-3) and docosahexaenoic acid (DHA 22:5 n-3). These conversions are take place mainly in liver, lung, heart, kidney and brain (Ricardo and Valenzuela, 2000). The special biological effects of marine foods and human milk are based on the direct supply of EPA and DHA, by passing the controlling step of the delta-6 desaturase (Ricardo and Valenzuela, 2000).

Animals lack the enzymes required to convert n-3 and n-6 fatty acids into each other; consequently, the dietary ratio of 18:2 n-6 to 18:3 n-3 fatty acids is important (Barceló-Coblijn and Murphy, 2009).

In humans, the recommend ratio of n-6:n-3 EFAs ranges from 4:1- 10:1 (Sanchez and Malo, 1995). There is an interaction between different fatty acids so that the n-3 and n-6 fatty acid families suppress the metabolism of each other and both families suppress the metabolism of the n-9 fatty acids (Darshan, 2001).

Liquid vegetable oils originated form soybean, corn, sunflower, safflower, cottonseed and nuts and grains are dietary sources of n-6 PUFA, while, linseed and canola oils are rich in n-3 PUFAs. Marine animal's oils are the richest sources of n-3 series of LCPUFAs, EPA and DHA (Russo, 2009). Poultry, like most other animals, have an absolute dietary requirement for linoleic and  $\alpha$ -linolenic acids. Dietary deficiency in EFAs can cause lower growth and harmful effects on membrane biology, nervous system, bone formation, visual function and reproduction (Watkins, 1995; Calder, 1997). The NRC (1994) recommends a minimum of 1% of linoleic acid in chicken diets, but there is no recommendation for lenolenic acid requirements of chicken.

# Effects of dietary fats on the fatty acid composition of poultry tissues

The fatty acid profile of chicken meat can simply be modified by feed and chicken products could then offer a more economical source of health effective fatty acids (Phetteplace and Watkins, 1989).

	Omega-3 fatty acids			Omega-6 fatty acids
Most common forms	Eicosapentaenoic (EPA), Docosahexaenoic (DHA) and α-linolenic acids (ALA)		Linoleic acid (LA) accounts for 85 percent to 90 percent of dietary omega-6 fatty acids	
Common food sources	EPPA and DHA-fatty fish such as salmon, white tuna, mackeret, rainbow trout, herring, and sardines ALA-canola or soybean oil, walnuts, and ground flaxseed or flaxseed oil		Vegetable oils (e.g. corn, sunflower, safflower and soy), salad dressin, nuts, whole wheat bread and chicken	
2005 dietary reference intake (DRI) Adequate identified as (AI)	ALA recommendations 1.6 grams per day for men 19 years or older 1.1 grams per day for women 19 years or older		Linoleic acid (LA) recommendations	
			17 grams per day for men between 19 and 50 years old	
			14 grams per day if over 50 for men	
			12 grams per day for women between 19 and 50 years old	
			11 gra	ms per day if over 50 for women
Research suggests potential health promoting benefits	Reduce inflammation in heart disease, inflammatory bowel disease, and rheumatoid arthritis		Neutral or lo	wer levels of inflammatory markers
			Replacing saturated and trans- fat with omega06 fatty	
	Help prevent blood from clotting and sticking to artery walls		acids accociate	ed with decreasing risk of heart disease
	Prevent hardening of the arteries		Improve Insulir	resistance and reduce the incidence of
	Decrease risk of sudden death and abnormal heart rates			diabetes
	Decrease triglyceride levels		Lower blood pressure	
	Lower blood pressure		Lower cholesterol levels	
Source: USDA (1988); available at: http://	fnic.nal.usda.gov/food-composition/macronutrie	nts/fats-and-cholester	<u>ol</u> .	
Table 2 Percentage of different fatty	acids in some common fats			
Common fats / oils	Saturated fatty acids (%)	Monounsaturated fa	tty acids (%)	Polyunsaturated fatty acids (%)
Corn	13	25		55

 Table 1
 Common forms, recommended intakes and research findings related to Omega-3 and Omega-6 fatty acids

Common fats / oils	Saturated fatty acids (%)	Monounsaturated fatty acids (%)	Polyunsaturated fatty acids (%)
Corn	13	25	55
Olive	17	71	10
Palm	52	38	10
Rapeseed (canola oil)	7	53	22
Soybean	15	23	51
Butter	65	26	1-3
Lard	42	46	6-8
Tallow	53	42	2

Source: USDA (1988); available at: http://fnic.nal.usda.gov/food-composition/macronutrients/fats-and-cholesterol

This meat is considered to be functional i.e. to exert, at least potentially, beneficial effects on human health and resistance to disease (Pisulewski *et al.* 2002; Pisulewski and Kostogrys 2003; Pisulewski, 2005). Regardless of the differences in fat content and fatty acid composition, thigh and breast meat of poultry have more than 35% saturated and about 25% mono-unsaturated fatty acids (Cantor *et al.* 2000).

Generally, chicken meat contains lower levels of SFA and MUFA, but higher concentrations of PUFA compared to pork or beef (USDA, 1988). Indeed, SFA and MUFA are mainly synthesized endogenously, and their concentration in the carcass is fairly influenced by dietary fats (Barton *et al.* 2007).

In contrast, there is no indigenous synthesis of PUFAs in birds, thus its concentrations in chicken meat respond rapidly to the dietary manipulation. Therefore, the fatty acid profile of thigh and breast meat typically reflects the composition of dietary fatty acids (Pisulewski, 2005).

As noted earlier, these compounds could also affect chicken production efficiency. High fat deposition in chicken body reduces the effective energy use and can lead to lower feed efficiency (Grashorn, 2007). There are reports that PUFAs and CLA could reduce fat deposition, but saturated fatty acids from animal origin tend to deposit more fat in chicken body (Crespo and Esteve-Garcia, 2002; Zanini *et al.* 2006). These reports obviously showed that dietary fats are important factors that influence both the functional properties of chicken meat and the efficiency of chicken production. It has been hypothesized that the dietary n-3 fatty acids and CLA would induce changes in different aspect of lipid metabolism in broiler chickens. This would alter both the fatty acid profile of the produced meat, and whole body fat deposition.

# n-3 polyunsaturated fatty acids for chicken meat enrichment

Today, there is more attention on the effect of dietary choices on long-term human health. Consuming higher levels of n-3 PUFAs, may reduce the risk of cardiovascular diseases (Gebauer *et al.* 2006; Harris *et al.* 2007; Von Schacky and Harris, 2007). The most important n-3 fatty acids in human nutrition are eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), which are called long chain n-3 polyunsaturated fatty acids (LC-n3 PUFA).  $\alpha$ -linolenic (LNA; 18:3 n-3) serves as a

precursor for the synthesis of EPA and DHA (Burdge, 2004; Arterburn *et al.* 2006; Gebauer *et al.* 2006).

Chicken meats normally have higher n-3 PUFAs content than other meats (Ackman *et al.* 1988). The effects of dietary n-3 PUFAs on manipulation of the fatty acids profile of chicken meat have beenexamined in several studies (Hulan *et al.* 1988; Phetteplace and Watkins, 1989; Ajuyah *et al.* 1993), most of them developed with the aim of improving the dietary intake of n-3 PUFAs in human consumers. The n-3 PUFAs level in chicken meat can be increased to levels comparable to the flesh of a lean fish such as cod (Ackman *et al.* 1988; Hulan *et al.* 1988; Ratnayake *et al.* 1989).

Royan et al. (2013) showed a remarkable increase of long chain n-3 fatty acids (EPA and DHA) levels in the meat of birds fed with diets containing fish oil. Apparently, an interaction between CLA and long chain n-3 fatty acids resulted in an increased deposition of EPA and DHA in breast tissue of chickens fed the diet containing the mixture of CLA + fish oil, without a similar effect on thigh. More lipids in breast tissue (white meat) are as phospholipids, but in thigh tissue (dark meat), the main lipids are triglycerides (Gonzalez-Esquerra and Leeson, 2001). Then, because of its smaller potential for fat deposition, the enrichment of breast meat with n-3 PUFAs may be more difficult than thigh meat enrichement. According to CFIA (2003) to label a product as n-3 PUFA-enriched, concentration of total n-3 PUFA must be at least 300 mg per 100 g of meat. The considerable reduced n-6 / n-3 fatty acids ratios in the meat of birds fed fish oil containing diets is reported (Miller and Robisch, 1969; Royan et al. 2013) and it was more obvious in breast tissue than in thigh. This difference was mainly because of the higher DHA concentration in the extracted fat of breast tissue than of thigh tissue. It is well known that an increase in n-6 / n-3 ratio in dietary fat results in pathological changes in humans. For this reason, nutritionists recommend that not more than 30% of human dietary energy should supply from fat; and the most favorite dietary fat has a 1:1:1 ratio between saturated, monounsaturated and polyunsaturated fatty acids, and an n-6 / n-3 ratio lower than 5:1 (Grashorn, 2007).

Linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) act as precursor molecules which are transformed to the other n-6 or n-3 fatty acids through a series of elongation and saturation reactions. An enzymatic system including fatty acyl-CoA synthetases, D-6 and D-5 desaturases and respective elongases is involved in this conversion (Wang *et al.* 2005). It's known that these two fatty acid families share and even compete for the same enzymes (Mohrhauer *et al.* 1967). Arachidonic acid is the most important metabolite of linoleic acid. Therefore, arachidonic acid content in phospholipids membranes normally decreases when dietary ratio of long chain n-3 PUFAs increases (Komprda *et* 

*al.* 2005). This opposite alteration in dietary n-3 PUFAs supply and arachidonic acid concentrations in chicken meat was observed in the study of Royan *et al.* (2013), so that arachidonic acid decreased in breast and thigh meat fat in chicks fed dietary fish oil. It seems that in broiler chickens fed with dietary fish oil the conversion of 18:2n6 to 20:4n6 is decreased while in birds fed the diet contained high levels of 18:2n6 (soybean oil containing diets) formation of 20:4n6 is increased.

Holmer and Beare-Rogers (1985) and Phetteplace and Watkins (1989) found that fish oil effectively reduced the 20:4n6 content in rat and chicken meat. In the body, EPA (C20:5 n- 3; precursor of series 3 eicosanoids) is produced from linolenic acid and it has been observed that an opposite change take place in EPA / linolenic acid and arachidonic acid / linoleic acid ratios (Benatti, 2004). In a report on the effect of n-3 fatty acids on changing the  $\Delta$ 5-desaturase activity, Betti *et al.* (2009) showed a reduced  $\Delta$ 5-desaturase activity with duration of flaxseed feeding as n-3 fatty acid source which indicates that linolenic acid suppressed bioconversion of arachidonic acid from linoleic acid (Garge*et al.* 1988).

#### Conjugated linoleic acid for chicken meat enrichment

Supplementation of chicken diets with CLA has been an attractive approach to improve the meat quality and simultaneously increase the production of CLA enrichedmeat and meat products (Suksombat et al. 2007). It's known that the CLA metabolism in biological systems is similar to that of other fatty acids. CLA incorporate into both triacylglyceride and phospholipid fractions (Park et al. 1999; Sisk et al. 2001). There are different recommendations for human CLA daily requirement. Ip et al. (1994) reported that the daily CLA intake of the occidental population is about 1 g/day. According to Grashorn (2007), the recommended daily intake of CLA in humans is 0.1% of daily food, which in Europe is estimated as 2.4 kg in males and 2.0 kg in females, resulting in a daily CLA intake of 2.4 and 2 g, respectively. Whilst some authors have suggested a range of 0.5-1.5 g/day for CLA intake (Fritsche et al. 1998), some other reports indicated considerably lower intakes, including a Swedish report suggesting CLA intakes in the range of 0.16 g/day (Jiang et al. 1999). There are reports of higher CLA levels in the meat of chickens fed CLA containing diets (Du et al. 2001; Sirri, et al. 2003; Royan et al. 2013).

Banni *et al.* (1995) reported that CLA isomers of diet are absorbed and entered in both the adipose and membrane phospholipids and also that the deposition of CLA in animal tissues is a function of the dietary CLA level and length of consumption. It was also reported that the meat fat possess greatly higher levels of CLA than lean meat (Fogerty *et al.* 1988).

In the study of Royan et al. (2013), the fat content of breast and thigh tissues in birds fed the diet containing 2.1% CLA + 3.5% soybean oil was higher than in the diets containing 4.2% CLA and they suggested that the increase in CLA deposits in the tissue is not simply a consequence of increased fat deposition. Similar interactions between dietary CLA and soybean oil were also reported by Zanini et al. (2008), who concluded that the intake of soybean oil, associated with increasing levels of CLA, resulted in arise in lipid deposition in edible portions, as observed by an increase in the overall content of fatty acids, including CLA. Apparently, they did not observe differences in CLA content of meat as a percent of total fatty acids of extracted oil. Royan et al. (2013), found that the diet containing 4.2% CLA and especially the combination of 2.1% CLA + 3.5%soybean oil in the diet of broiler chickens enhanced the CLA content of breast and thigh tissues up to 161-176 and 207-235 mg/100 g meat respectively, which is considerably higher than the common values found in chicken meat and is comparable to the values reported for veal, but still lower than the common values of beef and lamb (Chin et al. 1992). They found a possible interaction between dietary fats so that CLA in combination with other fats (2.1% CLA+3.5% fish oil and 2.1% CLA+3.5% soybean oil diets) was more effective on reducing the oleic acid and increasing the stearic acid contents of the both breast and thigh tissues; while the 4.2% CLA diet increased more effectively the C16:1 deposition in breast compared with the diets containing CLA in combination with fish oil or soybean oil.

The CLA and CLA + fish oil diets have more efficiently increase SFA content in tissues than the CLA + soybean oil diet. Apparently, n-6 fatty acids reduce the CLA effect on SFA deposition as a result of more deposited CLA and PUFA in the meat of birds fed CLA + soybean oil diet. This finding was in agreement with previous reports on the effect of CLA on increasing the SFA content of meat (Choi et al. 2000; Du et al. 2001; Eder et al. 2002; Suksombat et al. 2007). Park et al. (2000) suggested that it is possible tha part of the effects of CLA act through a decrease in the synthesis of MUFAs which are necessary components for phospholipid and triglyceride synthesis.  $\Delta 9$ -cis desaturation of several fatty acids is carry out by the stearoyl-CoA desaturase enzyme (Cook, 1991), which typical substrates are palmitic (C16:0) and stearic (C18:0) acids that are transformed to palmitoleic (C16:1) and oleic acids (C18:1), respectively. Desaturation of these saturated fatty acids is required to produce mono-unsaturated fatty acids important for incorporation into the sn-2 position of triglyceride (Choi et al. 2000). ACLA down-regulation effect on the  $\Delta 9$ desaturase activity is observed in chicken (Du et al. 1999; Li and Watkins, 1998) and rat Lee et al. (1998). It seems that CLA inhibits either the stearoyl-CoA desaturase ( $\Delta$ 9desaturase) gene expression or its activity in adipocytes (Choi et al. 2000). The ratios of palmitoleic acid to palmitic acid (16:1/16:0) and oleic acid to stearic acid (18:1/18:0) are suggested as the potential indices for stearoyl-CoA desaturase activity (Ntambi, 1999). The decrease in the ratio of palmitoleate: palmitate and oleate: stearate could be the main reasons for decreased stearoyl-CoA desaturase activity. There are reports that the CLA isomers are somewhat different on their effects. Bretillon et al. (1999) found that the  $\Delta 6$ -desaturation of linoleic acid is reduced when the ratio of c9, t11 CLA isomer to linoleic acid increased, while the effect of t10, c12 CLA isomer on desaturation was slight. Enrichment of poultry meat with CLA may also affect meat quality. It has confirmed that the higher SFA deposition in tissues enriched with CLA resulted in more penetration resistance values of white meat (Du and Ahn, 2002). Peroxidizability index is a measure of the relative susceptibility of different fatty acids to autoxidation (Arakawa and Sagai, 1986) and is calculated using the equation PI= (% monoenoic×0.025) + (% dienoic×1) + (% trienoic×2) + (% tetraenoic×4) + (% pentaenoic×6) + (% hexaenoic×8), where the concentration of fatty acids in a food or oil is expressed as percentage. This equation shows that the more unsaturated a fatty acid, the higher its susceptibility to oxidation and thereby, the more probable to occur an "off" flavor in a food. Royan et al. (2013) compared CLA and n-3 fatty acid concentrations in meat of experimental birds and suggested that tissue enrichment with CLA in is not as effective as the enrichment with n-3 PU-FAs. These authors also suggested that the enrichment of chicken meat with n-3 PUFA or CLA is not always in a dose dependent manner and can affect by the dietary fat composition.

# CONCLUSION

This review suggest that the dietary fats, especially n-3 PUFAa and CLA could be a useful tool to produce enriched chicken meat as a functional food for human consumption, however there are inconsistencies in the results of the different authors that need to be clarified in future research.

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