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Ciclo XXIV

Fatty acid composition of different tissues of newborn and suckling piglets

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Chapter 1

PREFACE AND GENERAL INTRODUCTION

Preface

In pigs, the fetal growth is mostly affected by the very composite interaction involving sow, placenta and fetus, just after birth. Newborn piglet has to make several physiological adjustments, very quickly, to adapt its metabolism to the extra-uterine life.

In swine production, the mortality of newborn piglets is a highly critical occurrence that affects the effectiveness of the farm and the improvement of the survival of piglets have a great relevance in commercial swine production. This objective is commonly pursued taking care the intricate interactions between the genetic and environmental factors affecting the performance of sow and the piglets.

The availability of body energy reserves are determinant to ensure newborn piglets survival during the first few days of life. Essentially, the mobilization of body's reserves is vital for piglets that have to maintain homeostasis of glucose until when their energy requirement can be satisfied by the ingestion of colostrum and milk. This explains, even if not completely, why the mortality rates are greatest in small-size newborn piglets.

Moreover, the wide-ranging selection for more prolific sows and lean pigs has resulted in an increase of the number of newborn piglets that are less mature at birth. Actually, the physiological immaturity of newborns is the main factor that affect the mortality of piglets during the weaning period.

During the last decades some studies reported that, also in pigs, during the intra uterine life the growth of several fetal tissues changed. However, the fatty acid composition of lipids in the fetal tissues during gestation, and in the first phase of extra uterine life have not been well-known.

In modern swine farms, cereals are the most commonly used raw material used as components of sow diets. In those feeds, the mainly constituents of the lipid fraction are

the monounsaturated FA (MUFA) and n-6 polyunsaturated FA (PUFA). Usually, diets for gestating sows were formulate in order to meet the protein, vitamins, minerals and energy requirements of animals. Even if the relevance of essential fatty acids in animal diets seems adequately well-known in animal nutrition, fat requirements and fatty acids composition are not commonly considered in the formulation of diets.

The availability of data about the fatty acids composition in different tissues of fetus and newborn piglets seems essential for the evaluation of nutrient requirements of gestating/lactating sows.

The main focus of my thesis is the study of the fatty acid composition of various tissues of newborn piglets and during the subsequent suckling time.

In the introduction session is a overview of the main relevant background of scientific literature regarding the overall and specific objectives of the doctoral thesis.

The experimental session contains the descriptions of three separate experiments that have specific objectives:

1 to investigate the fatty acid composition of lipids of different tissues of newborn pre-suckling piglets;

2 to study the changes of the fatty composition in lipids of several internal organs and tissues of piglet during the suckling time;

3 to investigate the relationship between maternal and offspring fatty acid profile in blood.

In the last session are described, and detailed, the analytical methods used for the chemical analysis and the calculations performed to make the experimental datasets.

General introduction

Growth of the swine fetus

The gestation in sows lasts about 114±2 days. During this period fetus development is not constant. The gestation could be divided in two main phases; during the first phase (until the sixtieth day) the weight gain of fetus is very low conversely to the second phase when the weight gain of fetus is more rapid. Those observations are well represented by the results reported by Gortner (1945) and summarized in figure 1. In this case the averages of swine fetuses weight during the gestation time are well fitted by a positive exponential function.

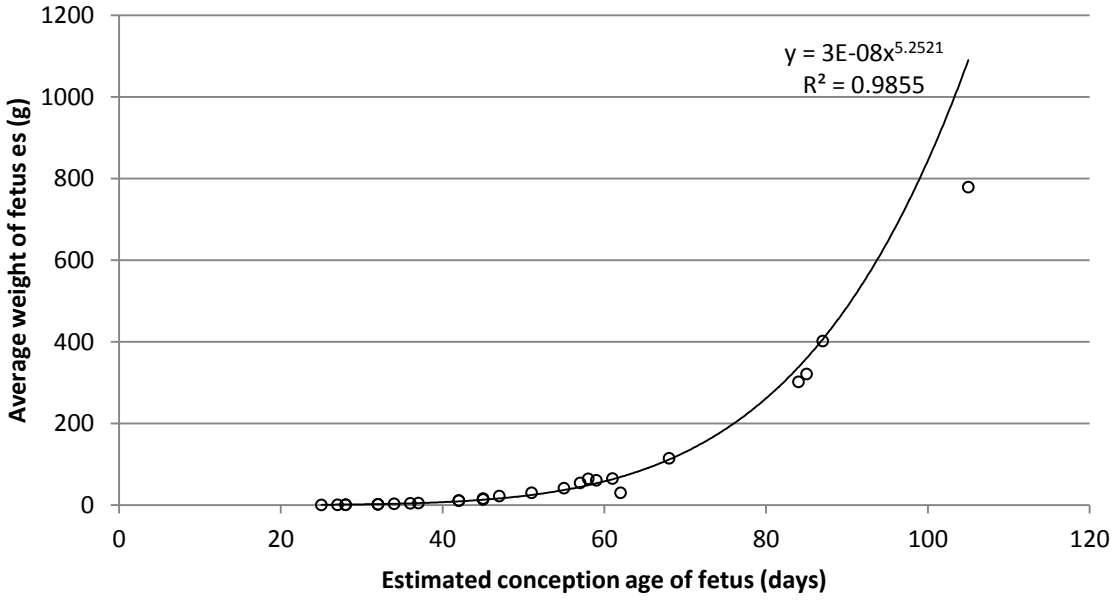


Figure 1. Evolution of average weight of fetus during gestation (data from Gortner, 1945).

During the gestation time also for internal organs of fetus occur significant change. In table 2 are reported the means of weight of different organs and carcass in fetus of piglets obtained by McPherson et al. (2004). Those results show that in the gestation

time after the 60th day the weight of all organs grow statistically. While, no difference was shown in weight of organ at 45th and 60th days.

Table 1. Weight of different organs of piglets at different days during the gestation (data from McPherson et al., 2004).

Organ Weight (g)	Days of gestation						SEM
	45	**60**	**75**	**90**	**102**	**110**	
carcass	17.27[a]	114.49[ab]	288.35[b]	631.15[c]	864.92[d]	1258.8[e]	84.81
gast. tract	0.52[a]	4.08[ab]	13.19[b]	38.19[c]	55.39[d]	90.63[e]	6.01
liver	2.19[a]	8.93[ab]	14.47[b]	27.84[c]	29.51[c]	44.06[d]	2.95
heart	0.28[a]	1.06[ab]	2.31[b]	6.46[c]	8.88[d]	12.08[e]	0.84
lung	0.51[a]	5.37[ab]	11.25[b]	25.23[c]	33.24[d]	49.68[e]	3.35
brain	-	3.13[a]	3.57[a]	9.88[ab]	17.45[b]	25.46[c]	2.15

Means within a row with different superscripts differ significantly ($P < 0.05$).

However, even if all organs grow in the second half of gestation, the contribution of each organ to define the total weight of the fetus is very different. In fact, looking the percentage repartition of fetal weights among its principal organs, and carcass, during pregnancy (figure 2) is possible take some interesting indications:

- the relevance of carcass on fetus weight increases up to about 70th day, after this time, the carcass accounts for about 85-86% of the fetal weight until the end of gestation.
- the relative weight of gastrointestinal tract increases during the whole gestation period reaching the 6% at the 110th day.
- the liver is the organ with the greatest impact on the weight of fetus during the first half of gestation, but its percentage contribution decreases dramatically in the definition of the weight of fetus in the second one.

the relevance of the weight of the lungs and the brain is greatest in fetus at 60th day, then their values remain practically constant under 6 and 2% respectively.

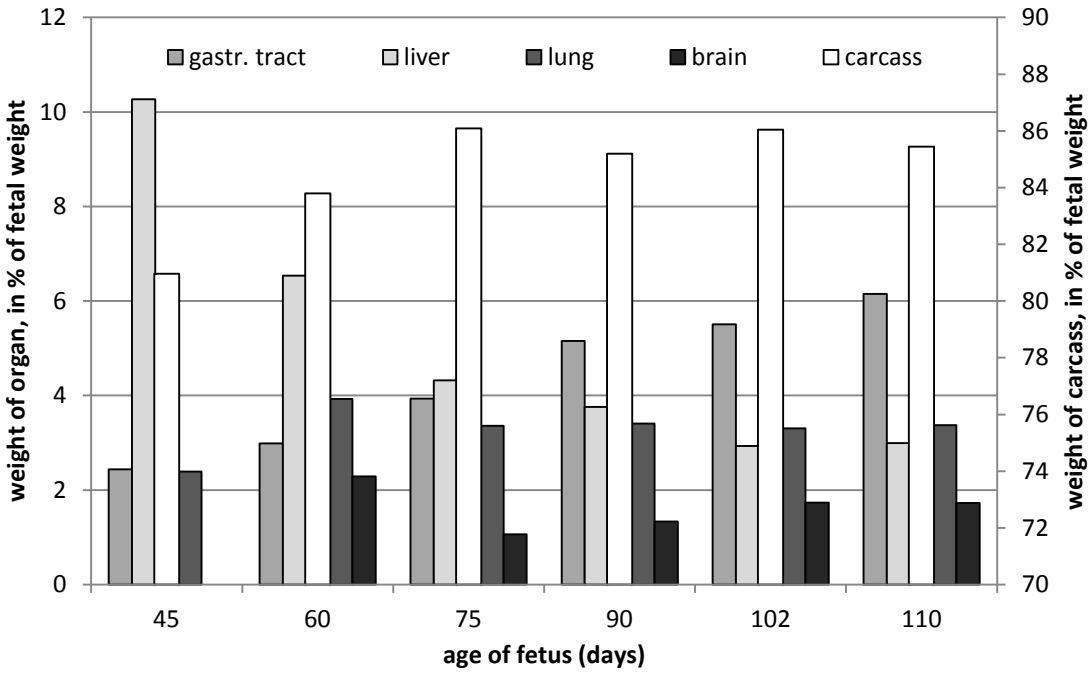


Figure 2. Weight of different organs, and carcass, of fetus at different time of gestation (data from McPherson et al., 2004).

The experimental results of McPherson et al. (2004) allow to evaluate the trend of protein and fat weight deposited in piglets during the intrauterine life. The amount of protein (in grams) deposited in fetus piglets for each day after the 45th day of gestation until the birth are plotted in Figure 3. Data showed that in swine the body protein and fat increase occurs essentially during the last trimester of intrauterine life.

Maximum protein and fat deposition in fetus occurs during the second half of gestation (Figure 3). These data reflect the increasing feed requirements of sows in late of gestation time. McPherson et al. (2004) reported that protein and fat daily gain in fetus is about 0.25 and 0.06 g/d during the first part of gestation (before 69th day), whereas in the second one (after 69th day) those daily gain are about 4.63 and 1.09 g/d, respectively. The best fitting equations calculated with the means reported by McPherson et al. (2004) show that the daily deposition of fat and protein in body fetus

have a trend that is very similar, as indicated by the values of exponent in the two equations. However, protein deposition during fetal life is 4 time than that fat, and their ratio remains constant along the fetal development.

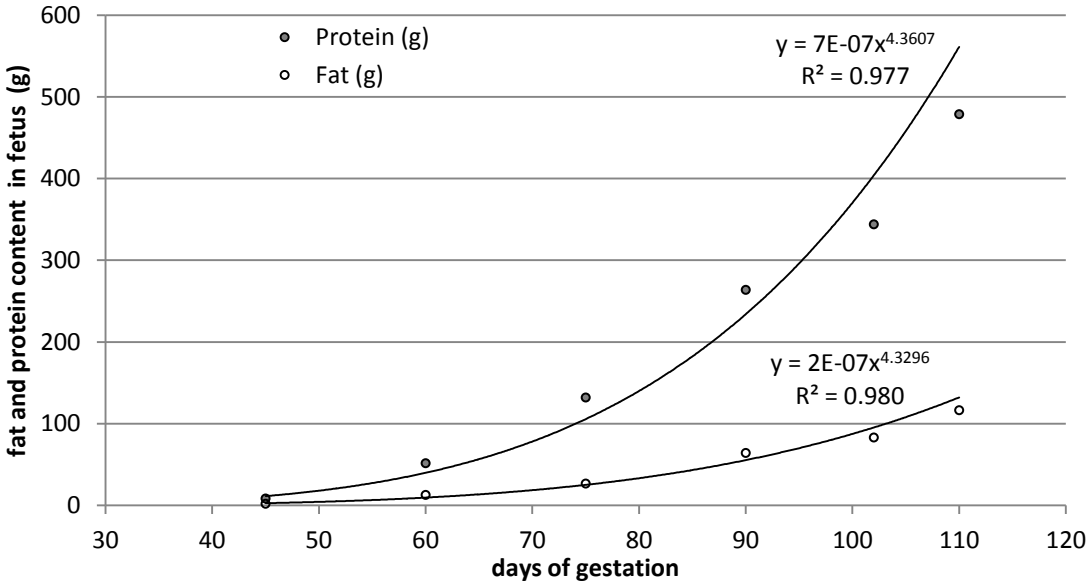


Figure 3. Evolution of protein and fat content in fetus during gestation (Data from McPhearson et al., 2004).

Fat deposition in fetal piglets is positively related in a linear way with fetal carcass weight evolution how highlighted by reworking of data from Mc Phearson (McPherson et al., 2004) (Figure 4). The parameters of the equation are useful to estimate, with good accuracy, the fat content considering the weight of fetus. in particular, we can estimate that about 9% of weight gain in fetus of piglets is due to fat deposition.

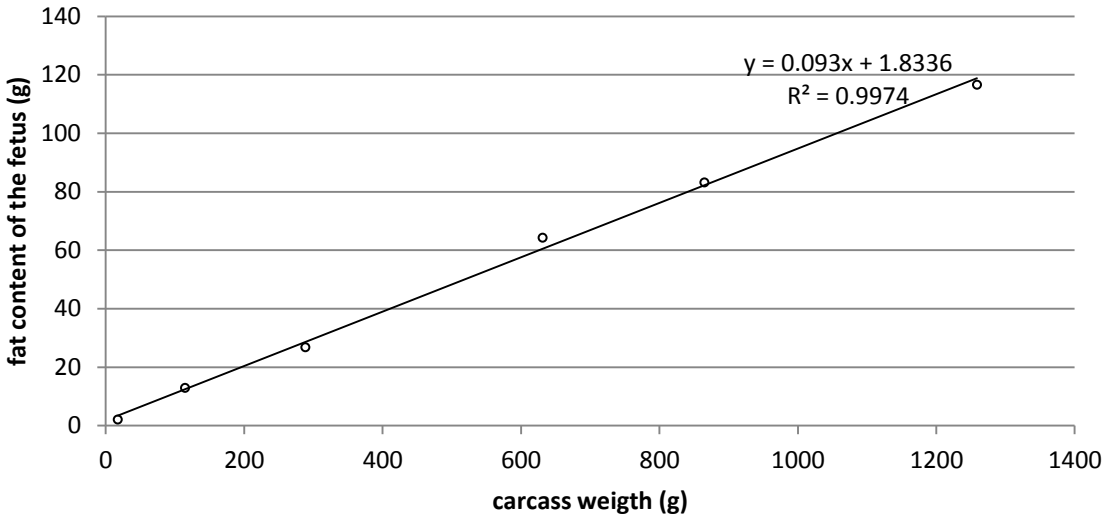


Figure 4. Relationship between fat amount and carcass weight of fetus during gestation. (Data from McPhearson et al., 2004).

The lipid fractions in the growing swine fetus

The composition of lipids in fetus is not constant during gestation (Figure 5). Free fatty acid percentage reach the maximum in early gestation when fetus development begins, phospholipids show little change during the time. Unsaponifiable lipids decrease from the beginning to the end of fetus growth, more markedly in the first phase; cholesterol, being a fraction of unsaponifiable lipids, follows the same trend whereas the cholesterol percentage does not vary (Gortner, 1945). Neutral lipid fraction (glycerides) is constant and low until middle gestation and, from this point it increases until the parturition (McPherson et al., 2004). Moreover, Farnworth and Kramer (1989) showed that the lipid fraction composition markedly differed among the tissues in piglet fetus. In figure 6, are reported the percentages of the different lipid fractions in fetus of piglets at 110th days of gestation. In all the tissues considered the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions were the largest lipid classes represented. The lipids extracted from the liver is characterized for the significant higher value of fraction

obtained combining the triglycerides (TG) with free fatty acids (FFA), where FFA values levels were lower than l%.

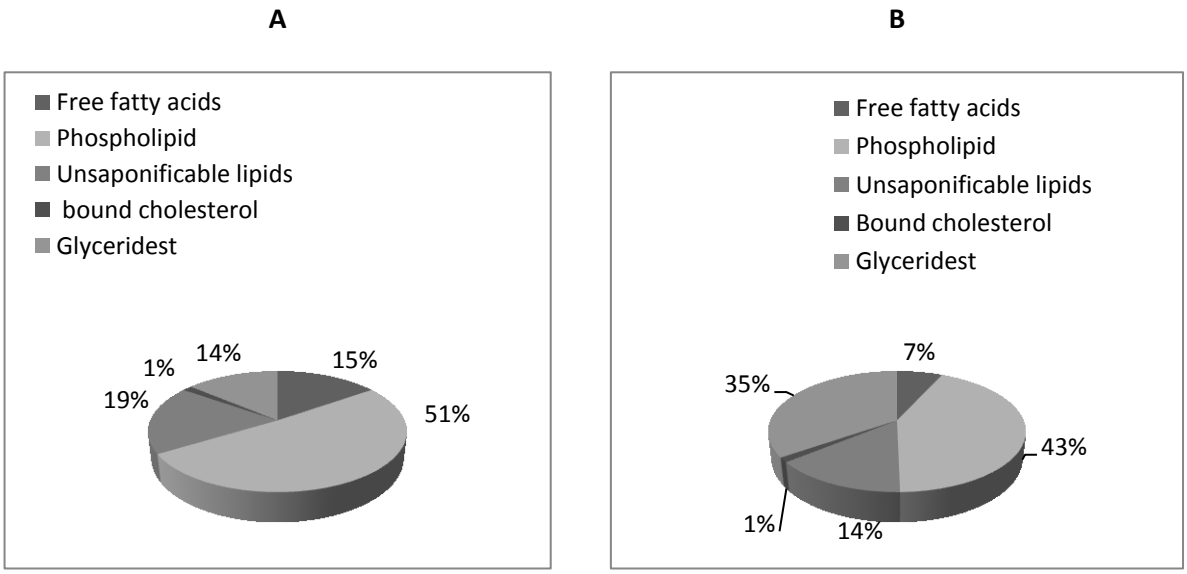


Figure 5. Proportions of lipid fraction in fetus at day 25th (A) and at day 105th (B) (Data from Gortner, 1945).

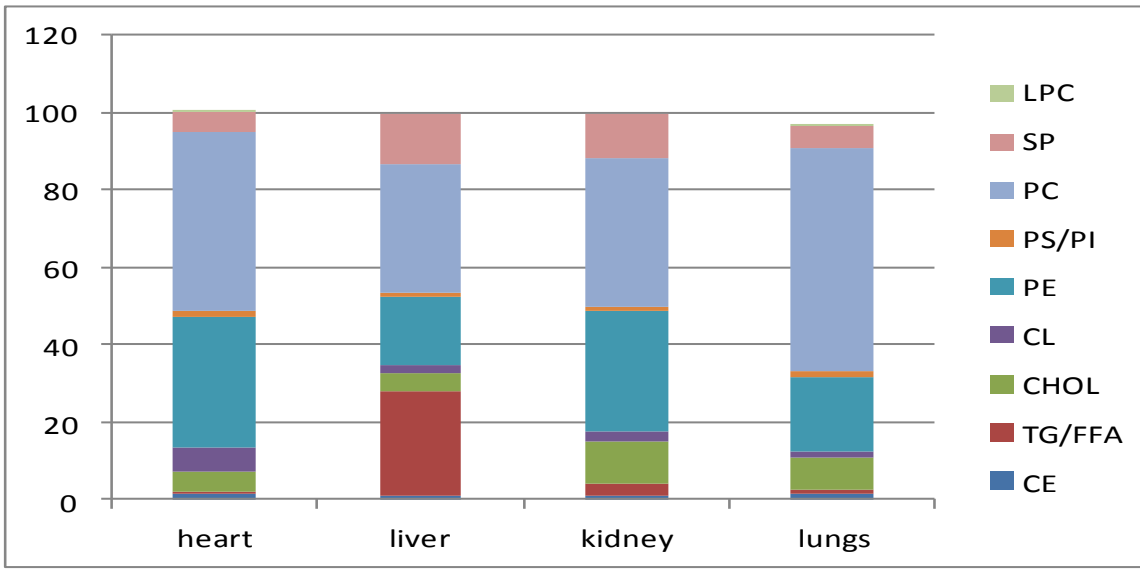


Figure 6. Proportions of lipid fraction in pig fetus at 110 days of gestation (Data from Farnworth and Kramer, 1989).

Synthesis of lipid in swine fetus

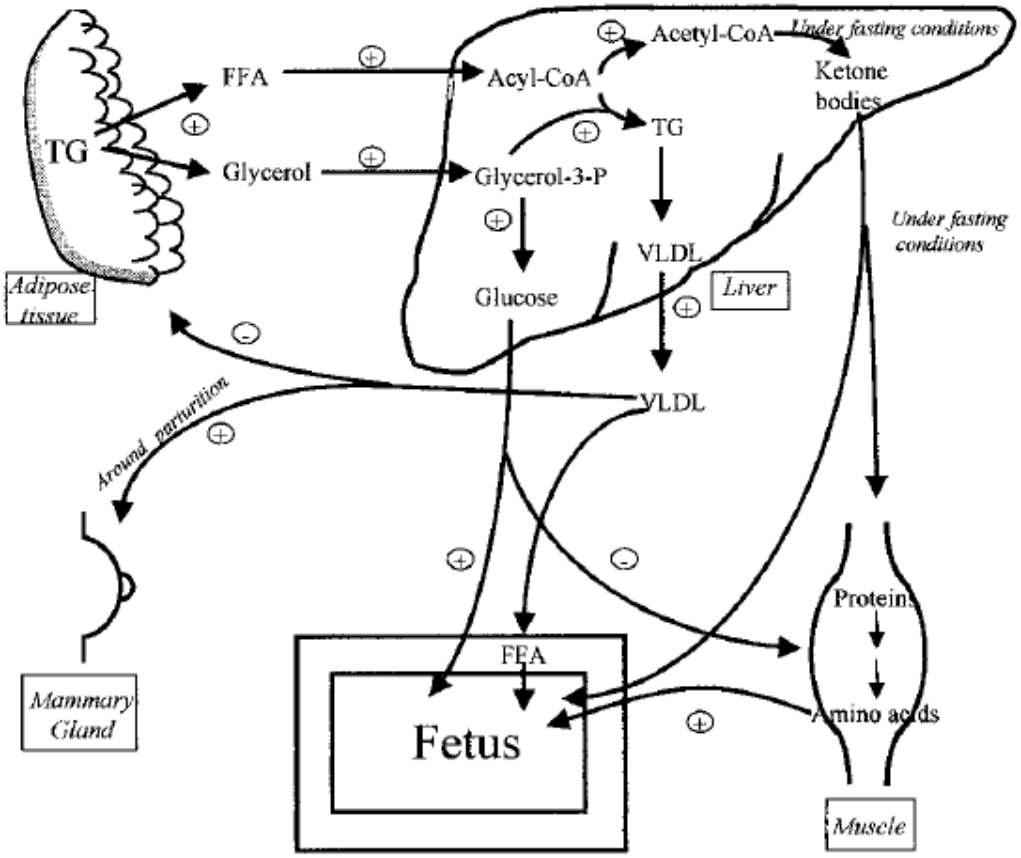


Figure 7. Representation of interaction of maternal lipid metabolism during the last trimester of gestation (Herrera and Amusquivar, 2000).

During gestation period fetal development depends to nutrients that arrive from maternal circulation across the placenta. This fact leads to changes in maternal metabolism in order to compensate the continuous loss of substrates. In women (Villar et al., 1992) and rats (Lopezluna et al., 1986) from early to two-thirds of gestation changes in endocrine system and hyperphagia increased in net body weight. In subsequent periods of gestation high consumption of fat depotis due to the enhanced lipolytic activity of hormone sensitive lipase (Martinhidalgo et al., 1994). Glucose, and amino acids, FFA and glycerol across the placenta more than fatty acids (Hay, 1994).

Differences among species can be highlighted, in human for example, there are high levels of fat depot at birth, maybe due to high permeability of placenta during fetal period. Fat depots in human derived from maternal fat and de-novo synthesis is relegated in the end of gestation. In the rat liver it has been shown that there is an increase in synthesis or oxidation of glycerides followed by synthesis of ketone bodies from plasma FFAs in fasted mother during late gestation (Scow et al., 1964). The same levels of ketone bodies were found in maternal and fetal plasma, maybe bevause ketone bodies can across very easily the placenta and are used as energetic substrates and for lipid synthesis.(Shambaugh, 1985) (Edmond, 1974). As mentioned above, the main function of glycerol is as substrates for glucose synthesis. In the end of gestation hypertriglyceridemia develops in sow, due to increase production of VLDL-triglycerides from liver (Wasfi et al., 1980) and the transfer of triglycerides among fraction of lipoprotein increases. Also absorption of dietary lipids increases (Argiles and Herrera, 1989), the extraephatich lipoprotein lipase and circulating lipoprotein rich in triglycerides decrease (Martinhidalgo et al., 1994).Maternal tryglicerides can not across the placenta but is source of essential fatty acids for fetus. Liver in adults rats and women has not lipoprotein lipase (LPL) expression, but this capacity was seen in pregnant rats and women during fasting (Testar et al., 1985) (Vilaro et al., 1990). Liver in pregnant rats and women has different conduct respect in non pregnant, in first case liver is storage organ for triglycerides contributing to increase maternal ketonemia, in second case is exporter of triglycerides. This situation leads to availability to ketone bodies for fetus and restrict consumption of other maternal substrates like amino acids and glucose. Insulin resistance is present in the last third of gestation and increases lipolysis from adipose tissue (Ramos and Herrera, 1995). Insulin resistance is negatively correlated to lipoprotein lipase activity (enzyme that hydrolyze triglycerides to FA and

glycerol) and consequently with fat accumulation (because FA can not across cell (Ramos and Herrera, 1996). In summary, in the end of gestation there are increase in levels of estrogen; decrease in hepatic lipase activity; increase of VLDL from liver and HDL increase in triglycerides.

Availability of FA from maternal plasma for fetus depends to the presence in placenta of lipoprotein receptors (Albrecht et al., 1995) and activity of lipase.(Elphick and Hull, 1977) This process allows the hydrolysis of triglycerides and redistribution of FA for fetus liver. The cellular absorption of FFA is mediated to facilitated membrane translocation processes with particular protein that binding membrane of fatty acid in plasma (Abumrad et al., 1984).

The human placenta has particular affinity to LCPUFA I fact same of last these are in higher concentration in fetus circulation than in maternal circulation (Crawford et al., 1976), this mechanism allow the preferential passage of AA and DHA in fetus tissues in last trimester of gestation, when the request for build neural and vascular system are major (Innis, 1991).

Fatty acids (FA) are fundamental components of lipids, they are a long carbon atom chain (aliphatic chain) with just one carboxylic group at the end of the chain (figure 10). Usually the chain is linear and its length is extremely important because it affects physical and chemical features of FA. FA can be classified, according to the number of carbon atoms that constitute the carbon chain, in short chain fatty acids (SCFA, less than 6 carbon atoms), medium chain fatty acids (MCFA, from 6 to 12 carbon atoms), long chain fatty acids (LCFA, from 12 to 22 carbon atoms) and very long chain fatty acids (VLCFA, more than 22 carbon atoms). FA may have one or more double bound in aliphatic chain, the presence of these bounds influence the fusion temperature of FA. From this point of view FA can be classified in saturated fatty acids (SFA) if there are

no double bounds, mono unsaturated fatty acids (MUFA) if there is one double bound in aliphatic chain and polyunsaturated fatty acids (PUFA) if there is more then one double bound in carbon chain.

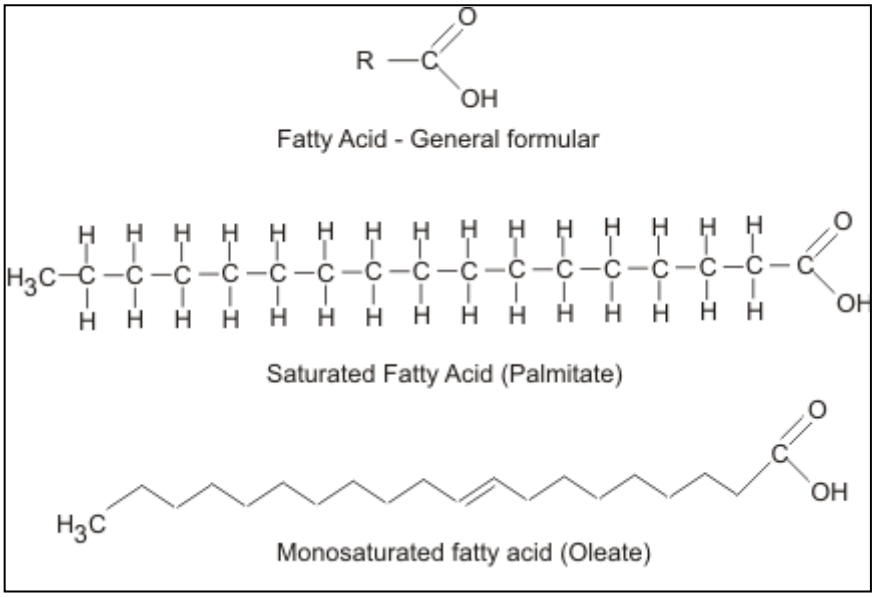


Figure 8. Examples of saturated and unsaturated fatty acids.

Essential fatty acids (EFA) linoleic acid C18:2 n-6 (LNA) and alpha linolenic acid C18:3 n-3 (ALA), are not synthesized by the mammalian body and should be taken with diet. EFA are classified, according to the position of last double bound, in n-3 (table 2), n-6 (table 3) and n-9 (table 4) FA if last double bound is situated in the third, the sixth and the ninth carbon atom respectively, starting to count atoms from the end of the carbon chain.

Table 2. n-3 fatty acids.

Common name	**Lipid**	**Iupac name**
Hexadecatrienoic acid (HTA)	16:3 (n-3)	all-cis 7,10,13-hexadecatrienoic acid
Alpha-linolenic acid (ALA)	18:3 (n-3)	all-cis-9,12,15-octadecatrienoic acid
Stearidonic acid (SDA)	18:4 (n-3)	all-cis-6,9,12,15,-octadecatetraenoic acid
Eicosatrienoic acid (ETE)	20:3 (n-3)	all-cis-11,14,17-eicosatrienoic acid

Eicosatetraenoic acid (ETA)	20:4 (n-3)	all-cis-8,11,14,17-eicosatetraenoic acid
Eicosapentaenoic acid (EPA, Timnodonic	20:5 (n-3)	all-cis-5,8,11,14,17-eicosapentaenoic
Heneicosapentaenoic acid (HPA)	21:5 (n-3)	all-cis-6,9,12,15,18-heneicosapentaenoic
Docosapentaenoic acid (DPA,	22:5 (n-3)	all-cis-7,10,13,16,19-docosapentaenoic
Docosahexaenoic acid (DHA, Cervonic	22:6 (n-3)	all-cis-4,7,10,13,16,19-docosahexaenoic
Tetracosapentaenoic acid	24:5 (n-3)	all-cis-9,12,15,18,21-tetracosapentaenoic
Tetracosahexaenoic acid (Nisinic acid)	24:6 (n-3)	all-cis-6,9,12,15,18,21-

Table 3. n-6 fatty acids.

Common name	**Lipid numbers**	**Iupac name**
Linoleic acid	18:2 (n-6)	all-cis-9,12-octadecadienoic acid
Gamma-linolenic acid (GLA)	18:3 (n-6)	all-cis-6,9,12-octadecatrienoic acid
Eicosadienoic acid	20:2 (n-6)	all-cis-11,14-eicosadienoic acid
Dihomo-gamma-linolenic acid (DGLA)	20:3 (n-6)	all-cis-8,11,14-eicosatrienoic acid
Arachidonic acid (AA)	20:4 (n-6)	all-cis-5,8,11,14-eicosatetraenoic acid
Docosadienoic acid	22:2 (n-6)	all-cis-13,16-docosadienoic acid
Adrenic acid	22:4 (n-6)	all-cis-7,10,13,16-docosatetraenoic acid
Docosapentaenoic acid (Osbond acid)	22:5 (n-6)	all-cis-4,7,10,13,16-docosapentaenoic acid
Tetracosatetraenoic acid	24:4 (n-6)	all-cis-9,12,15,18-tetracosatetraenoic acid
Tetracosapentaenoic acid	24:5 (n-6)	all-cis-6,9,12,15,18-tetracosapentaenoic acid

Table 4. n-9 fatty acids.

Common name	**Lipid numbers**	**Iupac name**
Oleic acid	18:1 (n-9)	cis-9-octadecenoic acid
Eicosenoic acid	20:1 (n-9)	cis-11-eicosenoic acid
Mead acid	20:3 (n-9)	all-cis-5,8,11-eicosatrienoic acid
Erucic acid	22:1 (n-9)	cis-13-docosenoic acid
Nervonic acid	24:1 (n-9)	cis-15-tetracosenoic acid

When the FA are introduced into mammalian cells they undergo a series of elongation and desaturation reactions which lead to the formation of new long chain PUFA (LC-PUFA) like docosapentaenoic acid (C22:5 n-6) from linoleic acid (LNA) , and docosahexaenoic acid (C22:6 n-3) from alpha linolenic acid (ALA) (Schmitz and Ecker, 2008). The same pool of enzymes makes a reaction that produces eicosanoids as intermediate (Fig.11). n-3 and n-6 fatty acids compete for these enzymes (Cho et al.,

1999a) (Cho et al., 1999b), and for this reason it is important maintain a right balance between n-3 and n-6 substrates.

Figure 9. n-3 and n-6 essential fatty acids metabolism (data from Schmitz and Ecker, 2008)

It is important to highlight that the synthesis ex- novo of FA like docosahexaenoic acid (DHA) is a process that happens thanks to different type of cellular organelles, the microsomes and the peroxisomes.

The synthesis of new FA, is possible thanks to the Δ-5 and Δ-6 desaturase with elongate enzymes. that convert linoleic acid (C18:2 n-6) to γ-linoleic acid (C18:3 n-6) and in dihomo-linolenic acid (C20:3 n-6). Subsequently arachidonic acid (ARA, C20:4 n-6) is formed. ARA is eicosanoid precursor (Figure 8) but could be converted in

docosapentanoic acid (DPA , C22:5 n-6) through elongase, Δ-6 desaturase and β-oxidation action. The intermediates of these reaction are docosatetraenoic acid (C22:4 n-6), tetracosatetraenoic acid (C24:4 n-6), tetracosapentaenoic acid (C24:5 n-6). Parallel alpha- linoleic acid (ALA, C18:3 n-3) is converted with the same enzyme to stearidonic acid (C18:4 n-3) and eicosatetraenoic acid (C20:4 n-3) and subsequently converted to eicosapentanoic acid (EPA, C20:5 n-3), precursor of eicosanoid, or converted to docosahexaenoic acid (DPA, C22:6 n-3). The intermediates are docosapentaenoic acid (C22:5 n-3), tetracosapentaenoic acid (C24:5 n-3), tetracosahexaenoic acid (C24:6 n-3) (Figure 11).

The synthesis activity from these organelles is possible only after the born of the animal (Li et al., 2000). The reactions of elongation and desaturation it's possible only in the microsome and the beta-oxidation only in peroxisome that is essential for the DHA synthesis. In a study peroxisomes and microsomes prepared whit labeled FA in different boxes, did not show presence of labeled DHA, if the two mixture of peroxisome and microsome were mixed, DHA has been shown (Li et al., 2000). This fact should mean that the DHA found in the fetal piglet comes from maternal placenta, and it is just accumulated in the tissue. In fact the PUFA found in the fetus is stored from the placental transfer, then it is correct to say that PUFA found in the fetus body it is originated from maternal synthesis. Results are according to the study of Sprecher which suggests that there is a place in the cell for elongation and desaturation, and another one where the carbon chain undergoes beta-oxidation (Sprecher et al., 1995) (Voss et al., 1991). Another example could be syndromes of Zelleweger (Martinez, 1989) and Adrenoleukodystrophy, where there are total absence of peroxisome in the cells, with consequent incapacity to synthesize DHA. This fact suggests that peroxisomes are necessary to complete DHA synthesis. When ALA arrives in a

particular tissue, it could undergo two chemical reactions: the beta-oxidation with recycling carbon chain (Cunnane and Anderson, 1997), or the elongation and desaturation making the new PUFA n-3. In fact there are two types of FA that can be found in the different tissues of piglets. FA of depot from the diet or FA ex-novo formed from the fatty acid content in the diet. Their content is tissue specific, for example in the brain there is a high percentage (60-85%) of ALA.

Placenta as regulator organ

Recent studies have demonstrated that there is a correlation between selection traits of sow (like weight of fetuses at birth, number of fetuses for litter, postnatal survivor) and placental structure (Akdag et al., 2009, Beaulieu et al., 2010).

The sow's uterus has two horns, each horn is served by an artery that supply blood with nutrients to each fetus, proceeding from the end of the horn to the end of cervix (Merck 2000). The endometrium is the intern lining of uterus and it is very rich in glandular, secretion of which provides embryo development and placental formation (Reece., 1997). In swine, the placental development occurs between the 20^{th} and 30^{th} days of pregnancy (Knight et al., 1977). The placenta have the function to transport all nutrients required by the fetus and respiratory gases and to move away all waste's components from fetus metabolism (Reynolds and Redmer, 1995). Furthermore it produces essential hormones for development and their receptors (Bauer et al., 1998). The position in uterus horn where the fetus grows, affects fetal growth rate, birth weight and survivor rate after birth (Wise et al., 1997). If the fetus has developed in the end of cervical area its dimension would be smaller than that one of a fetus developed in uterine crowd. Moreover the sow's features like age and body mass may influence conception and gestation time (Giesemann et al., 1998). For example, the average intake of feed in sows

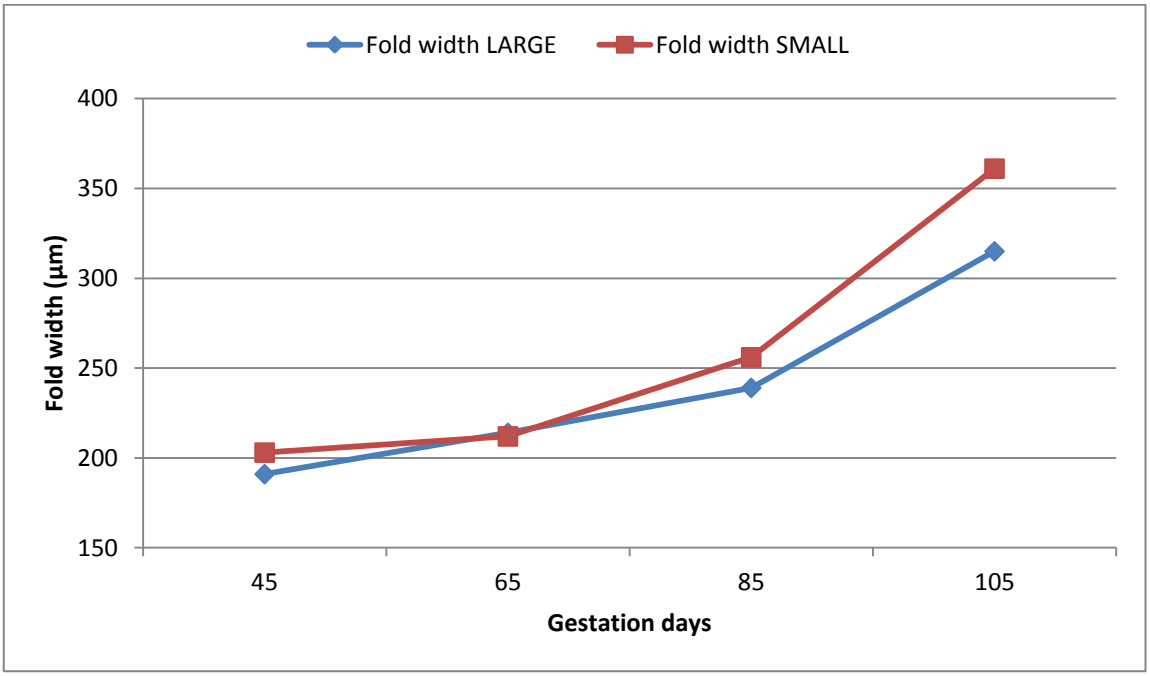
is higher than in gilts, and this aspect is important for litter development of fetus in uterus and for the subsequent productions of colostrum and milk. At the begin of lactation the body conditions of sows have to allow to contrast the excessive lose of protein, during the lactation period, to obtain the best possible performace of litter at weaning and support the increasing requirements of ovarian activity (Clowes et al., 2003).

The passage of fatty acids in placenta as nonesterified free fatty acids (if the FA are esterified into triacylglycerols these before are hydrolyzed to free FA), and their circulation in fetus, is possible because their are carried in plasma bound to proteins, mainly serum albumin (Stephenson et al., 1993) and alpha-fetoprotein (Naval et al., 1992) carrying-out to a major concentration of DHA and AA (arachidonic acid) in fetal tissues than in maternal tissues (Crawford et al., 1976) (Neuringer et al., 1984) (Stammers et al., 1991). The placenta can affect this process because it has the enzymatic pool which lipolytic and esterification activity that allows the passage of FA from maternal PFs and TGs to fetal circulation (Thomas and Lowy, 1982) (Ramsay et al., 1991) (Stammers et al., 1995). For example in brain the accumulation of FA is mediated by their arrive as free FA bounded with particular proteins of plasma (Sastry, 1985) or into phospholipids or triacilglicerides (previous esterification) in a lot of lipoprotein (Scott and Bazan, 1989). In fetus' brain has been shown the mechanism for accumulation of DHA by docosahexaenoyl-CoA synthetase, that block the egress of DHA from brain cell (Moore, 1994) and in postnatal brain of different species the capacity of this organ to make ex novo synthesis of DHA from process of elongation and desaturation to essential fatty acids has been demonstrated.(Cunnane et al., 1994), (Green and Yavin, 1993). Brain can metabolize LNA to DHA in astrocities and celebrovascular endotelium cell (Moore, 1994), the DHA passes from these cells to

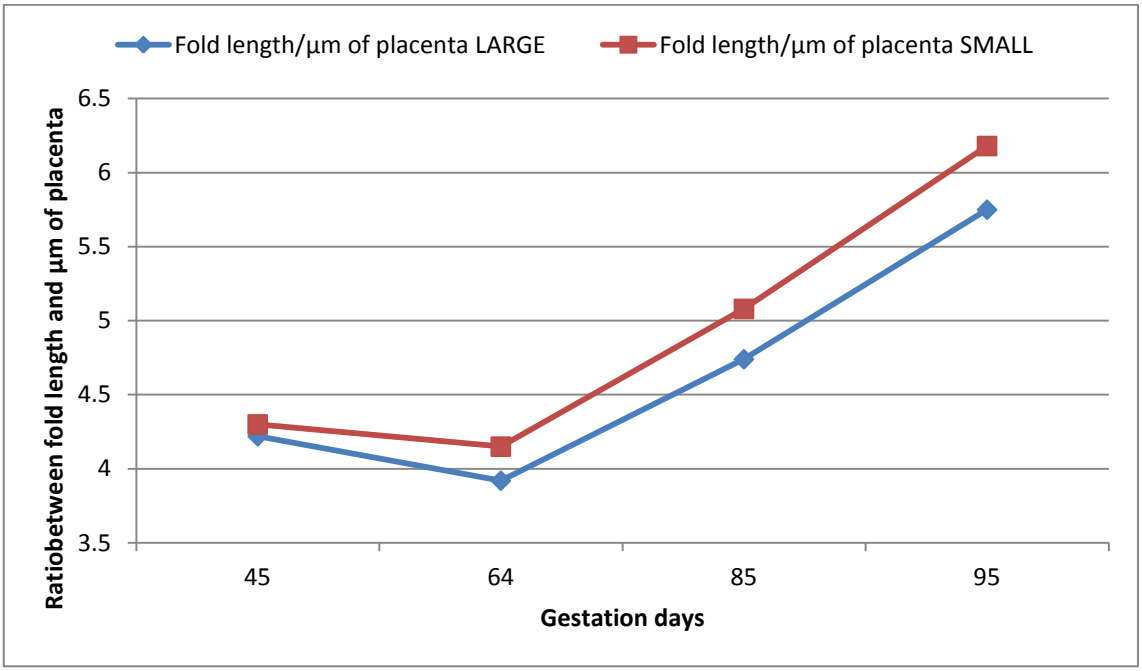
neurons that can not obtain DHA from LNA (Moore et al., 1991). Brain can accumulate DHA from this process in pre (Green and Yavin, 1993) and postnatal (Cunnane and Chen, 1992) (Anderson et al., 1994) life. Another important factor that limits the transport of fatty acids from placenta to the fetus can be low rate of esterification by lipoprotein lipase (Ramsay et al., 1991). Sow can synthesize n-3 LC-PUFA from essential diet's n-3 PUFA and increase them during gestation (Brazle et al., 2009). The same happened in women, where there are an increase of C22:6 n-3 in plasma maintaining fixed level of essential FA in dietary intake (Otto et al., 2001). It has been observed that an increase of PUFA in sow's diet results in an increase of C20:5 n-3 and C20:6 n-3 in cord plasma of piglets at birth (Rooke et al., 2000) (Rooke et al., 2001b). This process is similar to human species, where the n-3 LC PUFA are transported from maternal placenta to fetus through specific transporter protein. The amount of C22:6 n-6 in piglets ,which mother has been fed with a diet enriched in C18:3 n-3, was higher than in control piglets of 14 days of age (in liver and brain was 54% and 24% higher respectively). Even in piglets of 1 day of age it has been shown how maternal diet rich in C18:3 n-3 affects the content increase of C22:6 n-6 (1,2 time more) (Farmer and Petit, 2009), and the presence of linseed oil for mother alimentation leads to an increase in C20:5 n-3 and C22:6 n-3 in piglet's plasma at birth (Rooke et al., 2000).

The low activity of enzyme pool for elongation and desaturation in liver fetuses respect to activity in suckling piglets confirms the theory that LC-PUFA in piglets at birth from maternal source (Clandinin et al., 1981) (Clandinin et al., 1985). Even in this case the human species does not differ, in fact administration of C18:3 n-3 in pregnant women leads to the twice amount of C20:5 n-3 in newborn piglets (de Groot et al., 2004). The first effect of supplementation in placenta and fetuses is observable in the first forty days of gestation (Brazle et al., 2009).

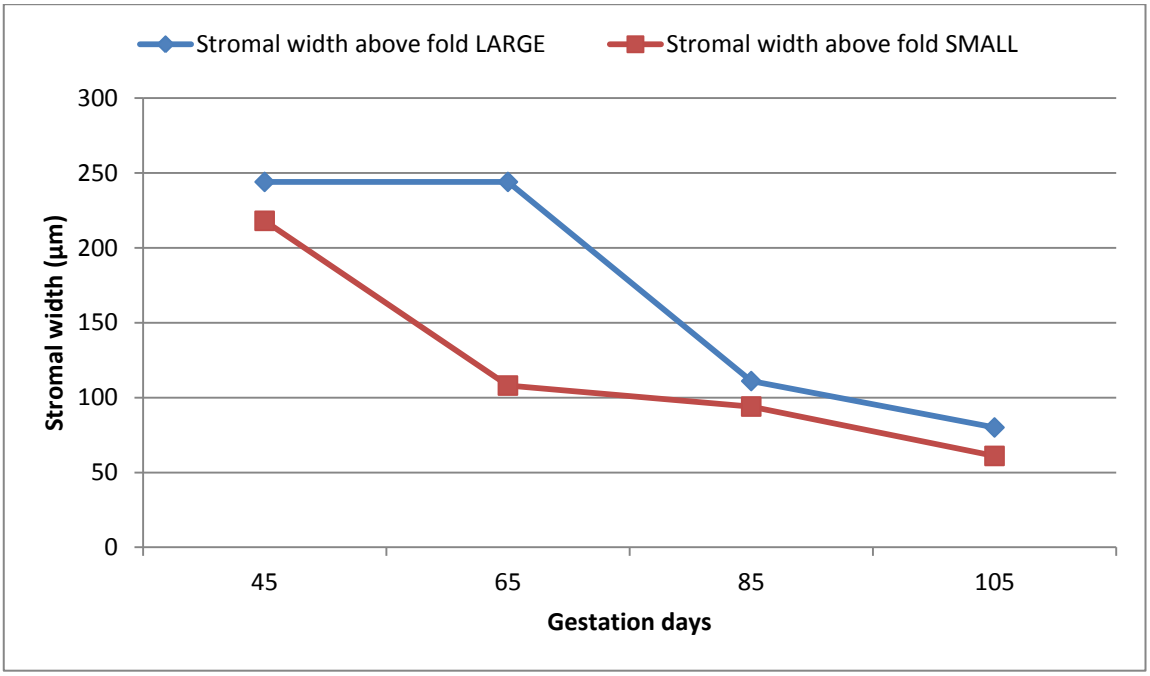
Intrauterine crowding affects the dimension of microscopic placental folds (the place where maternal and fetal blood meet) and the number of fetus in uterus and their dimension. In particular, if microscopic placental folds is excessive fetal losses may occur (Vallet and Freking, 2007). The increased width of microscopic fold (Figure 12), the ratio between length of placental epithelial bilayer and unit length of the placenta (Figure 13) and the reduction of stroma width above folder (Figure 14) could be able to compensate uterine crowding.



Significance between days (p < 0.01); significance between fetal size (p < 0,05).
Figure 12. Evolution of microscopic fold width during gestation between litters with large and small fetuses (data from Vallet and Freking, 2007).



Significance between days (p < 0.01); significance between fetal size (p < 0,01).
Figure 13. Evolution of ratio between fold length and µm of placenta during gestation between litters with large and small fetuses (data from Vallet and Freking, 2007).



Significance days x fetal size (p < 0.01).
Figure 14. Evolution of stromal width above fold during gestation between litters with large and small fetuses (data from Vallet and Freking, 2007).

Actually, also the diet of sows during the pre-mating time can affect the fetus development. In fact, during the period between the weaning and the following ovulation the sow's diet would influence the insulin level in blood's circulation whit stimulation of LH from brain and successive stimulation of growth on follicle, oocite (Koketsu et al., 1996) (van den Brand et al., 2001) and ovum (Poretsky and Kalin, 1987) (Quesnel et al., 2007). Right insulin level in pre mating period allows the level of progesterone in the first 10 days of pregnancy to stay high (Wientjes et al., 2011).

Effect of gestation sow feeding on piglet acidic profile

Increasing of FA in maternal diet leads to an increased FA amount in fetus blood. In fact abnormalities in physiological and metabolic status in sow leads to changes in adipose tissues formation in fetus (Hausman et al., 1982) (Ezekwe et al., 1984) (table 5).

Table 5. effect of diabetes and fasting on sow in different adipose tissues characteristic in piglets.(Hausman et al., 1982)

	FCS	FFS	FDS	p-value
Body Wt (g)	1.077±69	1.085±41	1.013±54	
Adipose thickness (mm)	3.7±0.2^{b}	5.0±0.2^{a}	5.1±0.5^{a}	<0,05
Inner fat cell layer (μm)	18.8±1^{b}	18.7±0.4^{b}	28.7±2^{a}	<0.05
Other fat cell layer (μm)	18.5±0.7^{b}	8.3±0.9^{b}	29.2±2.9^{a}	<0.05

FCS(fetus control sow); FFS(fetus fasted sow); FDS (fetus diabetic sow).

There is a strong relationship between The maternal diet strongly affects fetus and litters (Barker, 1995). Fetus and piglets acidic profile in tissues is strongly related whit n-3 fatty acids in maternal diet (Rooke et al., 2001b), (Spencer et al., 2004), (figures 15; 16).

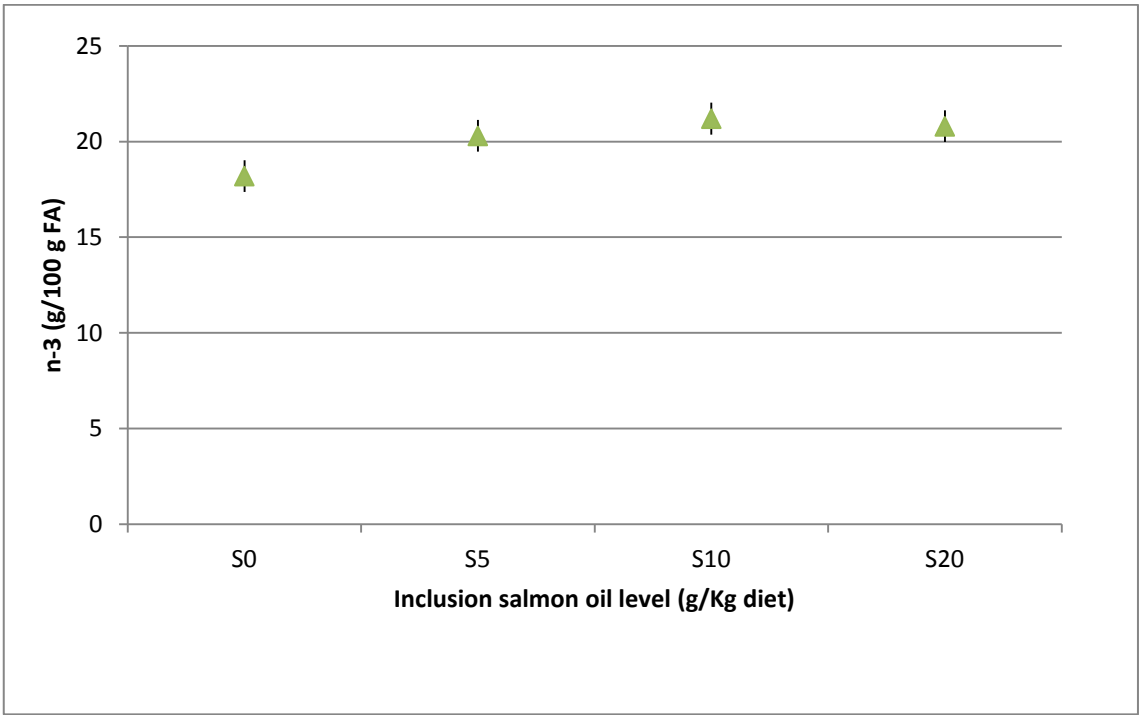


Figure 15. Effect of different level of salmon oil in dam's diet on total n-3 FA in piglet's brain (data from Rooke et al., 2001).

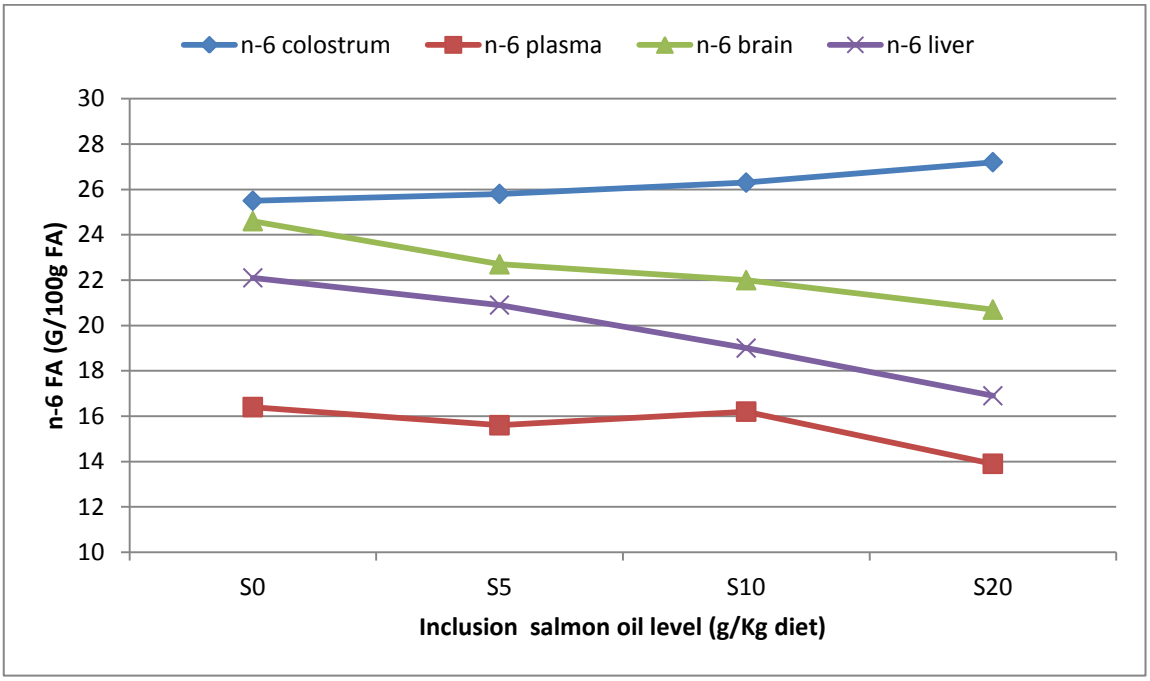


Figure 16. Effect of different level of salmon oil in dam's diet on total n-6 FA in different piglet's tissues (data from Rooke et al., 2001).

As mentioned the presence of n-3 FA in the sow diet in late gestation affects only slightly changing in piglet's PUFA profile, but if the supplementing diet start from early

gestation there will be a larger difference in fetus and piglets body composition (Rigau et al., 1995), (Rooke et al., 1999) (Rooke et al., 2001b). For this reason these types of studies give different results. If the infusion is confined in late gestation the enzymatic system has not time to explain its action and the amount in n-3 FA in fetus tissues is dependent to n-3 presence in diet of sow (Brazle et al., 2009) (tables 6; 7).

Table 6. effect of different diets with different amount of n-3 fatty acids in endometrium and fetus tissues in 40-43 days of gestation (mg/g of dry tissue) (data from Brazle et al., 2009).

	CONTROL	PFA	FLAX	p-value
Endometrium				
C20:5 n-3	0.10[a]	0.14[a]	0.24[b]	0.001
C22:5 n-3	0.85[a]	0.87[a]	1.21[b]	0.029
C22:6 n-3	0.35[a]	0.69[b]	0.46[a]	0.001
Chorioallantois				
C20:5 n-3	0.061	0.064	0.087	0.606
C22:5 n-3	0.33	0.35	0.37	0.916
C22:6 n-3	0.60[a]	0.82[b]	0.85[b]	0.040
Fetus				
CLA 9-cis,11 trans	0.004[a]	0.015[b]	0.002[a]	0.026
C20:5 n-3	0.14[a]	0.21[a]	0.25[b]	0.055
C22:5 n-3	0.59	0.75	0.78	0.089
C22:6 n-3	4.04[a]	5.23[b]	4.18[a]	0.046

CONTROL (corn, soybean meal diet); PFA (control+protected fish oil rich in n-3); flax (control+ground flax).

Table 7. effect of different diets with different amount of n-3 fatty acids in endometrium, embryo tissues and maternal plasma in 11-19 days of gestation (mg/g of dry tissue) (data from Brazle et al., 2009).

	CONTROL	PFA	p-value
Maternal plasma			
C20:5 n-3	4.61	6.73	0.006
C22:5 n-3	22.08	24.04	0.192
C22:6 n-3	9.61	12.65	0.001
Endometrium			
C20:5 n-3	0.032	0.082	<0.001
C22:5 n-3	0.57	0.81	<0.001
C22:6 n-3	0.24	0.56	<0.001
Embryo			
C20:5 n-3	0.90	0.46	0.415
C22:5 n-3	0.13	0.11	0.662
C22:6 n-3	0.20	0.30	0.016

CONTROL (corn, soybean meal diet); PFA (control+protected fish oil rich in n-3).

It is interesting to see how different sources of n-3 FA act in different way on different tissue, for example flax , very rich in alpha-linolenic acid, leads to an increase EPA but not DHA in fetus and endometrium, but flax increase DHA amounts in chorioallanthois it seems that the enzymatic system has different functioning among different tissues. Results from experiment of Brazle indicated that the changing in diet sow is affective from nineteenth day of gestation and the accumulation in the fetus is evident from day fortieth of gestation, data suggest that the first accumulation of DHA is assigned to chorioallantoich placenta formed from nineteenth day. In human transfer and accumulation of PUFA in fetal tissue are very similar to swine model. The levelsof DHA and LA (linoleic acid C18:2 n-6) in maternal plasma reflects the level of the same FA in fetal plasma. It would be a selective transfer of DHA from mother to fetus (Elias and Innis, 2001) and in human like in animal the diet of mother influences the PUFA contained in fetus tissue (Elias and Innis, 2001).

Effect of lactation sow diet in blood and milk

One of the best ways that allows to influence acidic profile in piglet tissues is to modify the maternal diet. In fact changes in sow diet are strongly related to milk quality and quantity and, in particular to fetus development.

The mother's diet can influence important features of piglets as birth weight, weaning weight. Furthermore survivor at weaning can influence parameters in subsequent growth phases about carcass quality as growth rate and organoleptic characteristics of meat.

Fish oil is one of the most common supplement used to increase PUFA level in the diet. Results obtained from the use of fish oil can be positive or negative, in the latter case it has been observed that diet affects osmotic fragility and oxidative stability (Stagsted and Young, 2002) of eritrocitie' s membrane in sows and pig even if administrated for a little time (Cools et al., 2011).

Being the life-time of eritrocyties of 72 days (Withrow and Bell, 1969), the encapsulation of PUFA should occur after cell's formation. For this reason the antioxidant needs of pigs increases because membrane cells are more susceptible to peroxidation of lipid and to attacks by free radicals (Sarkadi-Nagy et al., 2003).

In a recent study (Cools et al., 2011) the amount of PUFA in the diet affects the oxidative and not the osmotic stability and the amount of oxidation substrate is directly correlated to the amount of antioxidant capacity in plasma due to regulation of anthyoxidant-pathway.

Also the ratio between n-3 or n-6 FA is an important parameter to consider because, as mentioned above, their effect on cells is very different (inflammatory vs. anti-inflammatory). High or low level of n-6/n-3 ratio in diet leads to different results

(Papadopoulos et al., 2009)as different feed intake: lower in the group fed whit high n-6/n-3 ratio than in group whit low n-6/n-3 ratio (maybe due to the high leptin level in first group (Barb et al., 1998) and to the increase of insulin resistance (Behme, 1996)). Maybe it is related to lower catabolic status in lactating sows and consequent low milk production there is a strong relationship between piglet's performance and insulin and leptin level in pre-farrowing sow's period, like shown in a study about rats where leptin presence was correlated whit reduced litter weight (Woodside et al., 2000).

Relationship between n-3 fatty acid level and protein content in sows diets has been investigated, making difference between first and second parity sows (Mateo et al., 2009). Results showed that there are not difference in sow's performance for high or low level of protein, conversely to results from other studies (Spencer et al., 2004) where positively results about increase in litter size have been found. In this case n-3 fatty acids have been administated 30d before breeding. Mateo's study highlighted higher grown performances in piglets from first parity which mothers diets were supplemented just with n-3 than those which mothers diets were supplemented with n-3 and high level of protein, (like in second parity situation).

Birth weight is a very important parameter because it affects the rate of mortality before birth and it is related to the content of fat and glycogen, essential component for cold's resistance, at birth.(Varley, 1995) (Herpin et al., 2002). Maternal dietary supplementation can improve piglet's performance at birth as body weight and content of subcutaneous fat independent to the type of fatty acid administered and period of gestation (early or late gestation). MUFA in maternal diet can reduce low body weight at birth differently from PUFA (Laws et al., 2009).

The same component can make different effect when is used in different form. Flax (one of most important plant foods as source of n-3 fatty acid) can be used in diets of

sow in different forms: oil, seed and meal and affects fatty acid profiles of sow's blood and milk and of piglets' carcass and brain tissues. The results found highlighted the importance to administer flax like oil or seed because meal did not produced responses, the effect seems to be strongly related to oil presence in the food (Farmer and Petit, 2009). Different authors reported difference in response between liver and brain related to the different time to exposure at experimental diet, brain needed more time than liver to show significant difference in amount of n-3 FA.

Improving sow diet with fat from 35th days of gestation (to the end of lactation) can reduce mortality rate at birth because the increased energy reserve and the improved quality of colostrums and milk affects the amount of fat deposition in subcutaneous piglet's fat. In this work differences in piglet's performance like feed efficiency and features of carcasses between groups fed whit fat or starch in sow's diet have not been found, but the group fed with fat seemed to have a better improvement of fat cells in muscle tissues (Quiniou et al., 2008).

It is very important to understand when, during gestation time, the effect of diet is most affective and dividing gestation in different fraction time could help to do it. How is know the activity of low lipoprotein lipase (LPL) varies during gestation, in fact it is higher in the first half of gestation with high fat accumulation in adipose tissues of dam. In the last third of gestation LPL activity decreases leading to a consequent collapse in fat accumulation and contemporary increase of lipolitic activity. During this period the LPL activity is opposite in mammary gland where this protein allows fat accumulation for milk synthesis (Ramirez et al., 1983). The supplementation of fatty acid on diet in first half of gestation can influence the composition of colostrums and milk. For example ALA (alpha linolenic acid) is converted in DHA and accumulated in adipose tissues and subsequently made available during lactation. In the last third of gestation

sow is in a catabolic condition and in diets administrated in this period AA contents is very low and its synthesis is contrasting to elevate amount of n-3 fatty acids that share the same enzymatic pool. The difference in n-3 PUFA plasma of suckling piglets in first days of lactation is higher if the sows have received fish oil in diet than olive oil. This difference is not definite in the end of lactation for both group (Tables 8; 9), early gestation (G1) and last gestation (G2) (Amusquivar et al., 2010).

Table 8. n-3 PUFA from different source at 3th day of lactation (data from Amusquivar et al., 2010).

	n-3 PUFA from Olive oil	n-3 PUFA from Fish oil
G1	1.8 ± 0.1	2.5 ± 0.2**
G2	2.1 ± 0.3	4.7 ± 0.4***

*G1 n-3 administered in early gestation; G2 n-3 administered in last gestation; * p<0.05; ** p<0.01; *** p<0.001*

Table 9. n-3 PUFA from different source at 21th day of lactation (data from Amusquivar et al., 2010).

	n-3 PUFA from Olive oil	n-3 PUFA from Fish oil
G1	3.3 ± 0.3	3.7 ± 0.1
G2	2.6 ± 0.43	3.5 ± 0.3

*G1 n-3 administered in early gestation; G2 n-3 administered in last gestation; * p<0.05; ** p<0.01; *** p<0.001*

Other studies aim to find a maternal diet able to decrease stillborn piglets and the death rate from birth to weaning. Azain (Azain, 1993), fed sows with one control diet, and two diets enriched in fat, one with long chain triglycerides (LCT) from soybean oil and one whit medium chain triglycerides (MCT). These treatments do not were significant on weight from birth to weaning, not significant on stillborn rate (figure 17). Survivor rate to weaning was improved with diet containing MCT The higher effect of MCT has been seen on piglets under 900 g how showed in figure 18.

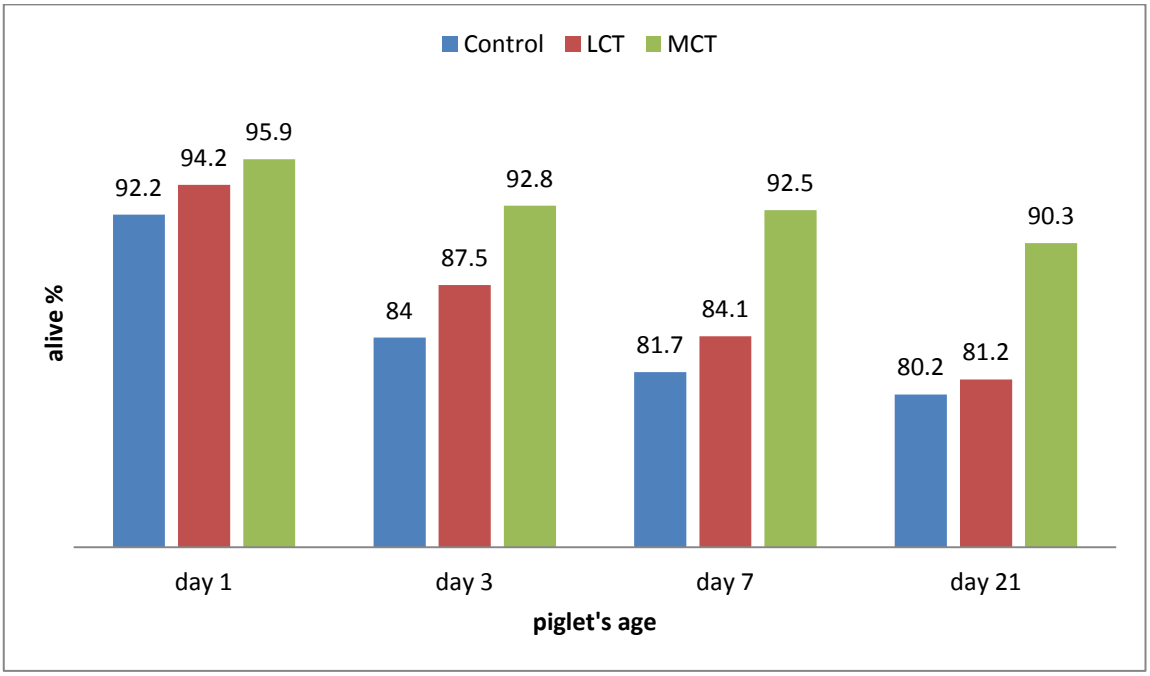


Figure 17. Survivor from birth to weaning with different type of diets In theend of lactation the statistical significance between MCT and control was p < 0.01, and between MCT and LCT was p < 0,05. (data from Azain, 1993).

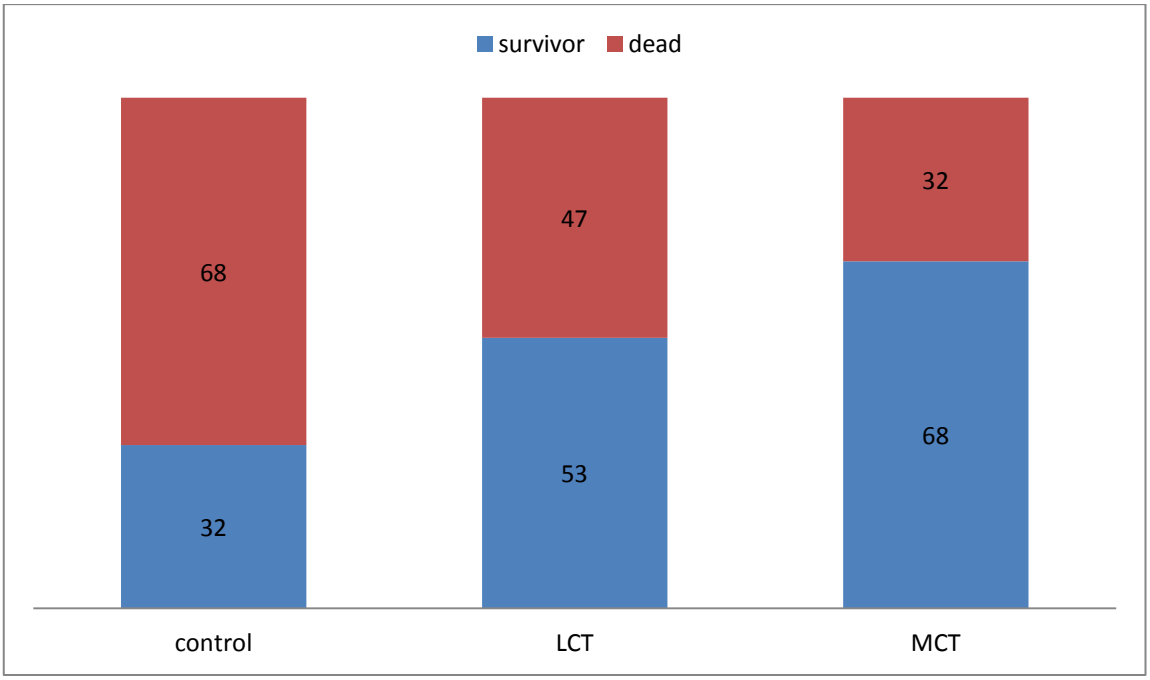


Figure 18. Effect of late gestation diet in class piglet weigh under 900 g (data from Azain, 1993).

Effect of maternal milk in suckling piglet acidic profile

In a study by de Quelen (de Quelen et al., 2010) the low level of C22:6 n-3 derived from C22:5 n-3 caused by the excess in C18:3 n-3 which processes are mediated by same enzyme (Δ6 desaturase), does not follow the same behavior for al tissues. For example brain is the preferential site of accumulation of C22:6 n-3 and for this reason the level of this FA is influenced in a different way in its tissue compared to the liver. Moreover the high capacity of suckling piglets to convert C18:3 n-3 in n-3 LC-PUFA is very important for brain, in fact constant level of C18:3 n-3 and C20:5 n-3 in the first week corresponded to high level of C22:6 n-3. Researcher supposed capacity of brain to accumulate this FA from other tissues through blood circulation.

The inclusion of lineseed oil in sow's diet mainly affects the increase of placental content of LC-PUFA, and allows to product piglets whit major proportion of C22:6 n-3 in brain and carcasses and this effect is reflected during lactation. In fact high content of C18:3 n-3 in sow's milk allows to maintain high levels of LC-PUFA in these tissues in suckling piglets (de Quelen et al., 2010).

The presence of fish oil in maternal diet carried out to an increase in C22:3 n-3 in piglet's tissues and a reduction of C20:4 n-6. For this reason it is very important to find the quantitative of fish oil able to increase C22:3 n-3 and in the meantime to not reduce C20:4 n-6. Among the various levels of fish oil to administer with the diet 10g of fish (salmon) oil for Kg of diet gave the best results(that is ingestion of 2.4 g of C20:5 n-3 and 3.6 g of C22:6 n-3 for day). (Rooke et al., 2001b)

A study by Akdag (Akdag et al., 2009) showed the effect of birth weight and the parity in weaning weight and survivor to weaning. 851 piglets have been divided into 2 groups: low weight (Lw) when the piglet was 100g under medium weight of litter and high weight (Hw)from the medium weight to over. Results showed several obvious

correlation among different parameters: the positive correlation of the birth weigh with weaning weigh and with weaning of survivor; the negative correlation of the litter size with birth weigh, weaning weigh and weaning of survivor piglets

Most of studies about PUFA profiles aims to find the way to change and manipulate them. The swine is the best model for human studies, in fact a lot of experiment aim to improve knowledge about physiology and metabolic processes and subsequently improve human health. For example the DHA content in retina of suckling piglets fed with different types of artificially milk compared with maternal milk could be important also for human studies (Alessandri et al., 1998).

How showed in results there a clear increase in DHA level, when diet with fish oil or jolk were used ($p < 0.0001$ sow milk Vs diet whit salmon oil presents the most significative difference), conversely AA seems not be affected by type of diet (just in phosphatidylcholine). In phosphatidylethanolamine AA has been affected by fish oil with EPA C20:5 n-3 (it probably decreases because these two fatty acids share the same enzymatic pool). Another very important factor that allows to increase DHA content in piglet tissues is the maintenance of LA/ALA ratio (figure 19). The administration in piglets three days older, of foods with different ratio of these essential fatty acids for three weeks, brings different accumulation in neural tissues how showed in the figure below.

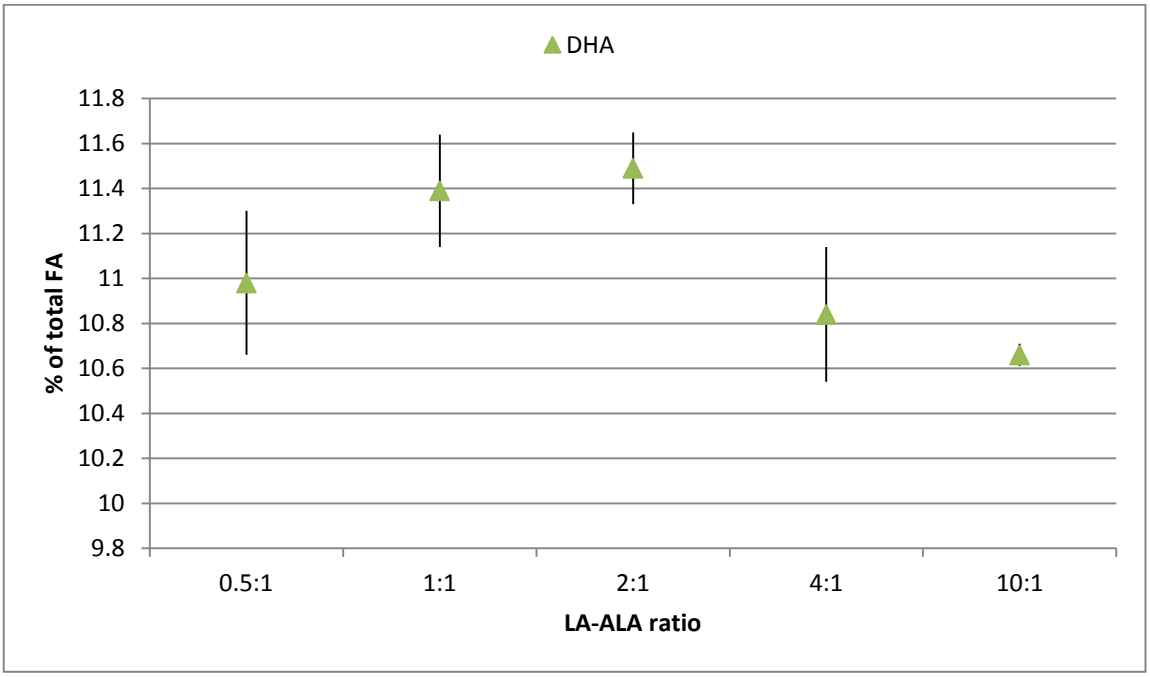


Figure 19. Variation of DHA content in brain from piglet fed diets with different LA-ALA ratio (data from Blank et al., 2002).

Relationship between LA-ALA ratio is not linear and the maximum amount of DHA in brain tissues is obtained for medium values among those texted. Brain tissue had the minimum response in DHA increase compared with liver, plasma and eritrocites how showed in figure 20.

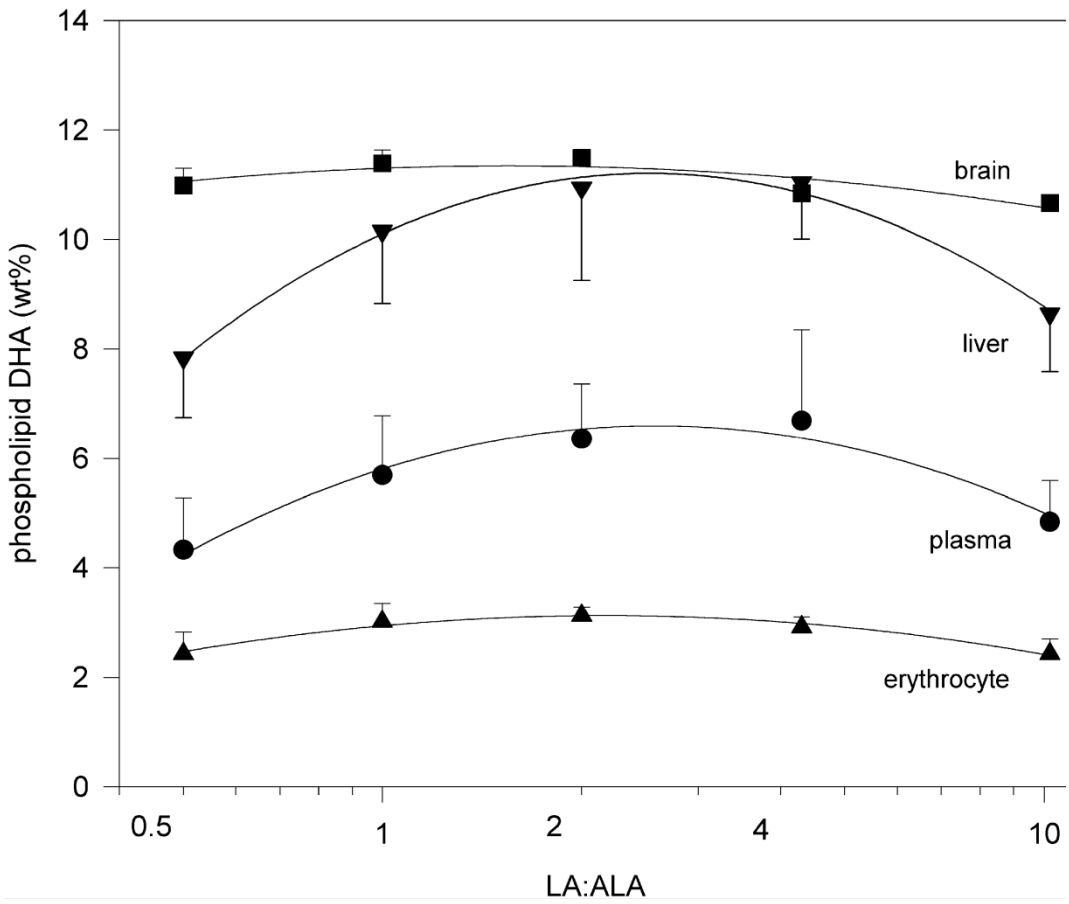


Figure 20. Variation of DHA content in different tissues of piglets fed with different LA-ALA ratio on diet (data from Blank et al., 2002).

In this study amount of LA was 13% of total fats in all diets and LNA varied from 1.3% to 26.8%, and showed clearly that there are not benefits to drop under 4:1 ratios.

The first source of energy and fatty acid for piglets is the colostrums and subsequently milk. They are very important for the development of immune system and of some tissues as the nervous one and the retina that is very rich in DHA and AA (Lauritzen et al., 2001) . It has been demonstrated that it is possible to influence the composition of piglet tissues through sow's diet (Amusquivar et al., 2010) (Lauridsen and Jensen, 2007) (Missotten et al., 2009). So far in a composition of a sow diet, it is important to consider foods for the content in energy, protein, vitamin and mineral but not for type and amount of fatty acidic profile. The base for feed's pork are cereal (corn, usually)

that have high amount in FA and in PUFA n-6 but have not right proportion between n-6 and n-3 fatty acids, in fact this ratio results very high until 10:1 while the rate recommended for human is 1-4 (Simopoulos, 2002).Several studies showed the importance of n-3 fatty acids in infant diet, early at fetus level, and later in colostrums and milk for lactating piglet (Innis, 1991) (Innis et al., 1999), this is very important because the higher development of brain occurs between the last period of pregnancy and first period of postnatal life (Passingham, 1985).

The digestibility of unsaturated fatty acids is higher than saturated fatty acids (Powles et al., 1994). This is very important in little animal that have very few body reserve of fat and which only source of fat is maternal milk. The transfer of FA from mother to piglet depends on the type of FA, for example piglets receive ALA mostly from milk whereas DHA is transported whit bloodstream to the fetus (Sampels et al., 2011).

After weaning

The effect of features as birth weight, birth order, litter size on following performance for growth, composition muscle and meat quality has been investigated in many studies. An interesting study by Beaulieu (Beaulieu et al., 2010) (table 10) analyzes the importance of body weight at birth because a lot of studies (Quiniou et al., 2002) (Herpin et al., 2002) have found negative correlation between this trait and the subsequently phases of piglets growth. In this work a negative relationship between litter size and body weigh has been found.

Table 10. effect of litter size in body weigh at birth, days to market and carcass composition of marketed pigs. (data from Beaulieu et al., 2010).

	Pigs for litter			P value
	3 to 10	11 to 13	14 to 19	
Av. body weigh	1.57[a]	1.37[b]	1.27[b]	< 0.001
Days to market	154.3	155.3	153.5	0.52
Dressed wt, kg	94.5	94.3	94.6	0.82
Yield, %	60.3	60.5	60.2	0.64
Loin, mm	66.4	66.9	66.6	0.81
Fat, mm	19.8	19.7	20.2	0.09

Furthermore results showed that litter size (across body weigh at birth) was correlated with the time needed to prepare the animal for sale, to calculate the effective commercial gain. This study highlights that there is not negative correlation among litter size (body weigh at birth) and meat's features, but the pig from high litters size must remains in farm for a greater number of days.

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Chapter 2

EXPERIMENT 1

FATTY ACID PROFILE IN DIFFERENT TISSUES OF PRE-SUCKLING PIGLETS

Introduction

In contrast with others mammalian species the newborn of pigs has a very low amount lipid as body component (Widdowson, 1950) The availability of body fat reserve is one of the most important factors affecting the chances of survival of newborn piglet when feed supply is not adequate to the metabolic requirement.

In swine fetus, the fat body deposition is very low and occurs mainly during the second half of gestation time. This lipogenic activity is supported by the strong placental transfer of glucose and fatty acids. However, the placental transfer of triglycerides in swine is about null, and the essential fatty acids (EFAs) for the fetus are derived from sow diet, and become available as a result of joint actions of lipoprotein receptors and lipase activities in the placenta (Amusquivar et al., 2010). A lipogenic activity has been demonstrated to occur de novo in piglet fetal tissues in particular for the long-chain PUFA (LC-PUFA). Nevertheless, the de novo lipogenesis in the fetal pig by fatty acid uptaked via the placenta have not been exhaustively assessed (Leskanich and Noble, 1999). Recently, research focused on determining the beneficial roles of LC-PUFA that are important for visual, neural, and brain development of infant (Uauy and Dangour, 2006). The EFAs as linoleic acid (LN, C18:2 n-6) and alpha linolenic acid (ALA, C18:3 n-3) are the main precursors for synthesis of LC-PUFA. This explain the relevance of those EFAs in diet of mammalian species. The enzymatic pools of mammalian cells can synthesize the LC-PUFA like EPA and DHA from ALA; whereas ARA and DPA synthesis is from LN (Sprecher, 2000). Not just increase in n-3 fatty acid or decrease in n-6 FA are enough for right health status, but ratio between last these should be considerate (Palmquist).

Study of lipid metabolism in pigs requires knowledge of fatty acid (FA) composition of various tissues of piglets at birth. In suckling piglets, FA profile of tissues is highly dependent

on FA composition of dam diet (Farmer and Petit, 2009). Some mammalian tissues, specially liver and brain can synthesize LC-PUFA from EFA by reactions of desaturation and elongation. Study of FA composition of different organs or tissues in newborn piglets may be a valuable model to increase knowledge of the function of some essential FA in human metabolism, in fact swine species have strong similarity to respond human medicine during childbirth (Mota-Rojas et al., 2011).

Aims: objective of the present study was to investigate fatty acid composition in different tissues of newborn pre-suckling piglets.

Materials And Methods

Animals: the experiment was carried out, in a commercial farm located in north-west Sardinia, according to the European Union regulations of the Animals (Scientific Procedures) Act, 1986.

In the farm, during gestation sows were individually housed in crates and daily fed 2.5 kg of a standard commercial diet for gestating/lactating sows, divided into two meals. The diet was formulated according to meet the requirements of sow and fetal development. A sample of diet was collected for the chemical analysis.

At 110 days of gestation sows are housed in individual farrowing crates.

Six multiparous (second or third parity) sows of a commercial genotype (Landrace × Large White) artificially inseminated with the same pooled Large White semen were used for the experiment.

Immediately after delivery one piglet per litter was weighed, stunned and exsanguinated before any suckling. The choice of animal was dictated by their weights, representative of the average weight of litter. Immediately after the piglet was dissected and internal organs like brain, hearth, kidney, liver and thigh muscle were collected and stored after washing with deionized water to remove blood. Samples were weighed before being stored at -80°C before their processing for chemical analysis.

Chemical analysis of sow diet: Chemical analysis of sow diet are described in Appendix section.

Fatty acid composition of tissues: after the lyophilisation process, samples of tissues were weighed to calculate the dry matter, and finally processed with grinder machine. Fatty acid (FA) profile of brain, hearth, kidney, liver and thigh muscle of piglets and concentrate were analyzed by GC as reported by Nudda et al. (2008). Content of each FA in lipids is expressed as a percentage of total FAME. Those analytical procedures are detailed in Appendix section.

Statistical analysis

Calculations: the fatty acids concentrations were grouped according to the length of carbon chain in three different classes: short chain fatty acids (SC-FA) under 14 carbon atoms. Medium chain fatty acids (MC-FA), from 14 to 17 carbon atoms and long chain fatty acids (LC-FA) from 18 carbon.

Moreover, the fatty acids concentrations of each sample were grouped in the following classes: Saturated Fatty Acids (SFA); MonoUnsaturated Fatty Acids (MUFA) and PolyUnsaturated Fatty Acids (PUFA); PUFA n-3 and PUFA n-6 as reported in tables 5.1; 5.2; 5.3 and 5.4 in Appendix chemical analysis section.

The One-way ANOVA was carried out for the fatty acids profile of tissues using tissue as the main effect. Differences were considered significant at P≤0.05. Statistical analysis were performed using MINITAB® software (Version 16, Minitab, State College, PA, USA).

Results And Discussion

The mean value (n 6) for piglet weight was 1.31 kg (st. dev. 0.06). The chemical composition and the energy content of the gestating sows is reported in table 1.1.

Table1.1. Chemical composition of sow diet.

Dry Matter (%)	87.6
Crude protein (g/kg of DM)	149
Ether extract (g /kg of DM)	48
Digestible energy (MJ/kg DM)	14.8

The diet composition was very similar to the most common diet used for gestating sows in Italy (Colin et al., 2005). Fatty acid composition of sow diet is reported in table 1.2. The LN (C18:2 n-6) represents more than half of the total FA in the feed used in this experiment, the other most relevant FA are palmitic (C16:0) and oleic acid (C18:1 c9). This FA profile of diet suggests that this has been achieved by vegetal oil and meal, and can be obtained by a mix of the most commonly ingredients (like corn, soy meal and barley) used for swine diets in West Europe.

Table 1.2 Fatty acids composition of sow diet.

Fatty acid	**(g/100g of FAME)**
< C16:0	0.15
C16:0	15.66
C18:0	1.57
C18:1 cis 9	22.26
C18:2 ω6	54.64
C 18:3 ω3	3.98

As expected, the ether extract content differed significantly between the tissues. Samples of brain and kidney tissues have higher lipid content than those of heart and muscle, and this concentration has intermediate values in the liver tissue (Table 1.3). In different tissues the means of each FA family vary in a significant manner. High levels of SFA were found in brain and liver, low level is recorded in heart. MUFA is abundant in liver and skeletal muscle and poor in brain. PUFA acids is higher in heart and low in liver and skeletal muscle. The consequence is that the value of ratio SFA/UFA is lover than 1:1 in all tissues. Its highest value is obtained in lipids of muscle, brain and liver were the amount of SFA and UFA are comparables (0.9, 0.89 and 0.8, respectively), while the lowest value (0.58) for this ratio is in lipid fraction of hearth tissue. Levels of SFA, MUFA and PUFA in brain are very similar with the results reported by de Quelen et al. (2010) in brain of 1-d-old piglets from sows fed diets with added lard or linseed, whereas, Farmer and Petit (2009) in brain of 1-d-old piglets from sows fed a standard diet or diets supplemented with different forms of flax reported values of PUFA more high (> 30%) and, consequently, lower values of SFA and MUFA (about 43 and 22 %, respectively).

Table 1.3. Ether extract and families of fatty acid concentrations in different tissues of newborn piglets.

	Tissue					
	Brain	**Hearth**	**Kidney**	**Liver**	**Muscle**	**P**
EE (% of DM)	7.80^{a}	5.30^{b}	8.02^{a}	7.04^{ab}	4.96^{b}	**
Fatty acid (g/100 g of FAME)						
SFA	47.18^{a}	36.86^{c}	41.92^{b}	44.62^{ab}	47.54^{a}	**
UFA	52.82^{c}	63.14^{a}	58.08^{b}	55.38^{bc}	52.46^{c}	**
MUFA	26.80^{c}	31.90^{bc}	32.92^{b}	38.79^{a}	36.77^{ab}	**
PUFA	26.02^{b}	31.24^{a}	25.16^{b}	16.59^{c}	15.69^{c}	**
MUFA/SFA	0.57^{b}	0.77^{a}	0.86^{a}	0.87^{a}	0.79^{a}	**
PUFA/SFA	0.55^{b}	0.34^{c}	0.86^{a}	0.38^{c}	0.61^{b}	**
SFA/UFA	0.90^{a}	0.91^{a}	0.58^{c}	0.81^{a}	0.73^{b}	**

Means followed by different letters within each row are significantly different.
***:P≤0.01.*

FA with carbon chain shorter than 14 C have not been detected in any samples of tissue. The means values of all the fatty acid detected with the GC analysis were significantly affected by the kind of tissue (Table 1.4). The myristic acid (C14:0), even if present in all tissues, is significantly more abundant in liver and muscle than in other tissues. The highest value of palmitic acid (C16:0), that is one of the most relevant FA of all tissues, is in lipids extracted from the muscle and is at its lowest in brain and hearth. More than 20% of the FAMEs extracted by brain tissues is represented by stearic acid (C18:0), while the concentration this FA is only more than 10% in muscle. Oleic acid (C18:1 c9) is most abundant in liver and muscle and least in brain (25.16, 23.35 and 17.60 g/100 g of FAME, respectively). The highest level of LA (C18:2 n-6) was found in hearth, its level in kidney and muscle is similar and lowest amount was detected in brain. ALA (C18:3 n-3) was found in trace in brain and kidney, its level is higher in muscle, hearth and liver. Arachidonic acid (AA, C20:4 n-6) showed highest levels in brain, heart and kidney than in liver and muscle. Interesting is high level of DHA (C22:6 n-3) in brain respect others tissues, in fact in brain there are about 70%

of total. Moreover, the high concentration of AA and DHA in brain confirms that those FA are incorporated into the nervous tissue of the brain during its growth, which mainly occurs during the second half of gestation as results of ex novo synthesis by the activity of elongase and desaturase enzymes (Palmquist, 2009).

Table 1.4. Fatty acid composition of lipid extract of different tissues of newborn piglets.

Fatty acid	***Tissue***					
(% of Fame)	**Brain**	**Muscle**	**Hearth**	**Liver**	**Kidney**	***P***
C14:0	0.39^{c}	2.82^{a}	0.35^{c}	2.51^{a}	1.07^{b}	**
C14:1c9	0.00^{b}	0.05^{a}	0.00^{b}	0.03^{ab}	0.00^{b}	**
C15:0	0.01^{d}	1.26a	0.25^{c}	0.38^{b}	0.57^{b}	**
C16:0	18.94^{c}	28.61^{a}	16.13^{c}	24.14^{b}	23.33^{b}	**
C17:0	0.18^{c}	1.08^{a}	0.65^{b}	0.85^{ab}	0.82^{b}	**
C17:1 c10	0.06^{c}	0.83^{a}	0.46^{b}	0.71^{a}	0.00^{c}	**
C18:0	22.53^{a}	11.75^{c}	16.65^{b}	15.04^{b}	16.12^{b}	**
C18:1 c9	17.60^{c}	23.35^{a}	21.06^{ab}	25.16^{a}	19.52^{bc}	**
C18:2 n-6	0.44^{d}	6.63^{b}	13.13^{a}	4.04^{c}	5.57^{b}	**
C18:3 n-6	0.01^{c}	0.41^{a}	0.34^{a}	0.19^{b}	0.01^{c}	**
C18:3 n-3	0.01^{b}	0.06^{a}	0.04^{a}	0.04^{a}	0.01^{b}	*
CLA (tot.)	0.78^{a}	0.62^{a}	0.22^{b}	0.65^{a}	0.03^{c}	**
C20:4 n-6	14.63^{a}	7.22^{c}	15.24^{a}	9.61^{b}	17.20°	**
C20:5 n-3	0.06^{bc}	0.14^{ab}	0.25^{a}	0.03^{bc}	0.00^{c}	**
C24:0	5.13^{a}	1.79^{c}	2.73^{b}	1.59^{c}	0.00^{d}	**
C22:5 n-3	0.10^{c}	0.42^{b}	0.67^{a}	0.27^{bc}	0.39^{b}	**
C22:6 n3	10.20^{a}	0.61^{c}	1.12^{bc}	1.87^{b}	1.18^{bc}	**
PUFA n-3	10.35^{a}	1.23^{b}	2.08^{b}	2.21^{b}	1.57^{b}	<0.001
PUFA n-6	15.66^{c}	14.87^{c}	29.50^{a}	14.56^{c}	23.59^{b}	<0.001
n-6/n-3	1.54^{c}	12.39^{a}	14.61^{a}	7.31^{b}	15.38°	<0.001

Means followed by different letters within each row are significantly different (P< 0.05). ** P≤0.01; *P ≤ 0,05.

The FA profile in brain and liver are very similar with the data reported by Rooke et al. (2000) (Rooke et al., 2000) from piglets borne to sows fed diets containing maize throughout pregnancy, but very different from the values obtained from piglets borne to sows fed diets

containing fish or linseed oils. This confirm that the fatty acid profile in lipids of the different tissues reported in this experiment represent the common range in piglets borne to commercial farms where sows are fed diets no-enriched with oils high in PUFA. Moreover, our data agree with (Rooke et al., 2001b) when consider the FA composition of brain tissues of newborn piglets from sows fed the basal diet to which no oil was added. In particular our accordance with Rooke et al. (2001b) is evident for LA (0.44 *vs.* 0.4, respectively) and for the content of AA (14.6 *vs.* 15.1, respectively). Whereas, in our experiment the values PUFA n-3 in brain tissue are lower than reported by Rooke et al. (2001b). This discrepancy is probably due to the different value of ratio n-6/n-3 in diet used during gestation for sows in our experiment (13.7 *vs* 10, respectively). The ALA in the brain tissue was found even if in trace, whereas Rooke et al. (2001b) did not show the data for this FA. The FA profile of lipids extracted by liver differed from results of Rooke et al. (2001b) for this tissue in piglets from sows fed the basal diet. The most relevant discrepancies are for n-6/n-3 that is higher in our results (7.21 *vs.* 2.8), and for ALA and AA concentrations that are lower our experiment (0.04 *vs.* 0.2 and 9.6 *vs.* 13.5, respectively). All these differences affect the PUFA n-3 value that is lower in our liver samples than in Rooke et al. (2001b). The DHA is about 98 and 85 % of the total PUFA n-3 in lipids of brain and liver. Those results are in accordance with the data reported by Rooke et al (2001b) and similar with the other data of the same research group in another experiment (Rooke et al. 2001a) (Rooke et al., 2001a). Moreover, Rooke et al. (2001a) (Rooke et al., 2001a) reported for, lipids in liver, a n-6/n-3 value lower than that obtained in our experiment (2.1 *vs.* 7.21, respectively). The aim to increase the content of PUFA n-3 in tissues of newborn piglets, through the modulation of the ratio n-6/n-3 in the diet of gestating sows, has been pursued in several experiments. The concentrations of C22:5 n-3 and C22:6 n3 in brain of piglets from sows fed linseed based-diet, where PUFAn-6/PUFAn-3 was about 2:1, are significant higher than in piglets sows fed linseed based-diet, where PUFAn-6/PUFAn-3 was

about 8.5:1 (de Quelen et al., 2010) or about 11:1 (Sampels et al., 2011). Similar results are obtained by Farmer and Petit (2009) comparing 1-d-old piglets from sows fed a standard diet, where PUFAn-6/PUFAn-3 was about 7:1, with others supplemented with different forms of flax where the PUFAn-6/PUFAn-3 was about 1.1:1. However, these authors did not observe significant differences when the standard diet was compared with one where PUFAn-6/PUFAn-3 was about 4.7:1.

The percentage distribution of FA classes, according to their length, is significantly affected by the tissue (Table 1.5). The highest concentration for the class C <16 was obtained in lipids from muscle (4.2 %) and the lowest in brain and hearth (0.40 and 0.59, respectively). Whereas, the highest value of C16-18 class is in liver and muscle, and the lowest in brain. The class of longer chain (C >18) FA has a stronger presence in lipids form brain, where are more than 30%, and the lower value in muscle and liver.

Table 1.5. Classes of fatty acids of different tissues of newborn piglets.

	Tissue					
FA class	**Brain**	**Muscle**	**Hearth**	**Liver**	**Kidney**	*P*
C <16	0.40^{d}	4.20^{a}	0.59^{d}	2.94^{b}	1.65^{c}	<0.001
C 16-18	68.23^{c}	84.45^{a}	78.36^{b}	82.32^{ab}	78.71^{b}	<0.001
C >18	31.37^{a}	11.35^{d}	21.05^{b}	14.74^{cd}	19.65^{bc}	<0.001

The percentage distribution of FA classes in lipids of brain is in accordance with the composition of FA profile reported by Farmer and Petit (2009) of 1-d-old piglets from sows fed, during late gestation, a standard diet or diets supplemented with different forms of flax. Although those authors reported that the diet affect in a significant manner the FA profile in lipid of brain.

Conclusion

The results of this experiment show that the fatty acid composition differed between tissues, especially for FA synthesized ex-novo. The high concentration of DHA in brain confirms the ex novo synthesis of LC-PUFA n-3 in this tissue by the activity of elongase and desaturase enzymes.

These results represent a contribution to basic knowledge on FA composition of different tissues of newborn piglets from sows fed the most commonly diets used in pig farms in Italy.

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Chapter 3

EXPERIMENT 2

FATTY ACID COMPOSITION OF SEVERAL TISSUES OF SUCKLING PIGLETS OF DIFFERENT AGE

Introduction

The positive effects of supplementation of fat in diets of high productive sows has been documented (Quiniou et al., 2008). However, the optimal dietary level of fat has not been determined. The most commonly diets used in European swine farms are based on cereals and others raw materials which contain a lipid fraction mostly represented by monounsaturated FA (MUFA) and n-6 polyunsaturated FA (PUFA). In suckling piglets the dietary essential fatty acids (EFA) as linoleic acid (LA,C18:2 n-6) and a-linolenic acid (ALA, C18:3 n-3) source is represented by the sow's milk. In lactating sows the FA profile of milk is strongly affected by FA compositions of fat in their diet (Bazinet et al., 2003; Lauridsen and Jensen, 2007). The optimal dietary recommendations for EFA in lactating sows has not been determined. In fact: the NRC (1998) suggest, for all swine classes, that the dietary requirement is 0.1% of diet; Whittemore (1998) suggests 0.5 – 5%0 of diet. Whereas, no specific recommendations are reported for ALA. The EFA are the necessary precursors of a series of longer chain PUFA (LC-PUFA) such as C20: n-6 (AA), C22:5 n-3 (DPA) and C22:6 n-3 (DHA) which play important roles in neural, and brain growth of fetus and newborn (Lauritzen and Carlson, 2011). Actually, some mammalian tissues, specially liver and brain can synthesize LC-PUFA from EFA by reactions of desaturation and elongation. Although, some information are available about the relationship between the FA diet of sows and the FA profile in brain of suckling piglets, there is little information on the evolution of the FA profile in different tissues of piglets during suckling time. Study of FA composition of different organs or tissues in newborn pre and post suckling piglets may be a precious implement to improve the knowledge of the function of EFA in piglet metabolism, and moreover, can represents a valuable basic knowledge for studies on infants metabolism.

Aims: The aim of this experiment is to study the changes of the fatty composition in lipids of several internal organs and tissues of piglet during the suckling time

Material And Methods

Animals: the experiment was carried out, in a commercial farm located in north-west Sardinia, according to according to the European Union regulations of the Animals (Scientific Procedures) Act, 1986.

In the farm, during gestation sows were housed in individual crates and daily fed 2.5 kg of a standard commercial diet for gestating/lactating sows, divided into two meals. The diet was formulated according to meet the requirements of sow and fetal development. At 110 days of gestation, all pregnant sows were transferred to individual farrowing crates. Three sows, at third gestation, of the same commercial genotype (Landrace × Large White) artificially inseminated with the same pooled Large White semen were chosen for the experiment.

All farrowings were attended and immediately after delivery piglets were assisted for colostrums assumption from respective dam. The temperature of farrowing crates was maintained at about 25°C. In each crate, a portable heat lamp supplemented heat for piglets. Two days after delivery, four piglets per litter were chosen (for a total of 12 piglets) and tagged. The choice of animal was dictated by their weights, representative of the average weight of litter. Piglets were weighed daily until the 28st day. During the entire 28 days of lactation, no creep feed or milk replacer were offered to piglets.

For all the lactation period the same standard commercial feed used during the gestation was available *ad libitum* for sows. Feed used for lactating sows was from the same batch that was used for during the gestation and already sampled and analyzed for the previous experiment.

At 7, 14, 21 and 28 days of age one piglet per litter was stunned and exsanguinated. Immediately after, each piglet was dissected and internal organs like brain, hearth, kidney, liver and longissimus muscle were collected and stored after washing with deionized water to

remove blood. Samples were weighed before being stored at -80°C before their processing for chemical analysis.

Chemical analysis of sow diet: Chemical analysis of sow diet is described in the Appendix section.

Fatty acid composition of tissues: after the lyophilisation process, samples of tissues were weighed to calculate the dry matter, and finally processed with grinder machine. Fatty acid (FA) profile of brain, hearth, kidney, liver and thigh muscle of piglets and concentrate were analyzed by GC as reported by Nudda (Nudda et al., 2008). Content of each FA in lipids is expressed as a percentage of total FAMEs.

Calculations: the FA concentrations were grouped according to the length of carbon chain in three different classes: short chain fatty acids (SC-FA) under 14 carbon atoms. Medium chain fatty acids (MC-FA), from 14 to 17 carbon atoms and long chain fatty acids (LC-FA) from 18 carbon.

Moreover, the FA concentrations of each sample were grouped in the following classes: Saturated Fatty Acids (SFA); MonoUnsaturated Fatty Acids (MUFA) and PolyUnsaturated Fatty Acids (PUFA); PUFA n-3 and PUFA n-6 as reported in tables 5.1; 5.2; 5.3 and 5.4 in chemical analysis section.

The ANOVA was carried out for the fatty acids profile of each tissues using age of piglet as the main effect. Differences were considered significant at $P \leq 0.05$. Statistical analysis were performed using MINITAB® software (Version 16, Minitab, State College, PA, USA).

Results

Chemical composition, energy content and fatty acids composition of the lactation sow feed is reported in table 1.1 and 1.2 of previous experiment.

The evolution of the weight of piglets used in the experiment (Figure 2.1) the equation estimating the relationship between the age (in days) and the weight of the piglets indicate that the average daily weight gain (ADG) is about 0.264 kg. These ADG values indicate that the milk yield of sows was sufficient to support very well the weight gain of piglets along the suckling time.

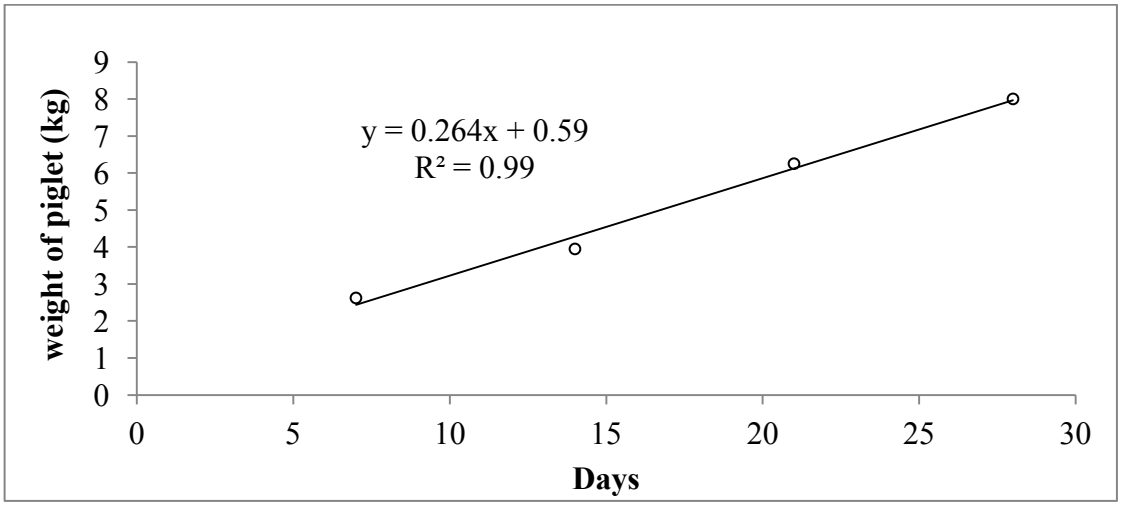

Figure 2.1 Average weights of suckling piglets at different age

The weight averages of for each internal organ dissected by piglets at the different age are reported in Figure 2.2. Obviously, the weight of each organ increase with the age of the animals. However, the magnitude of weight gain is not the same for all organs. In fact, the weight of the liver at 28 days is about seven times that at 7 days, while, during the same range time, kidney and heart doubled their weight.

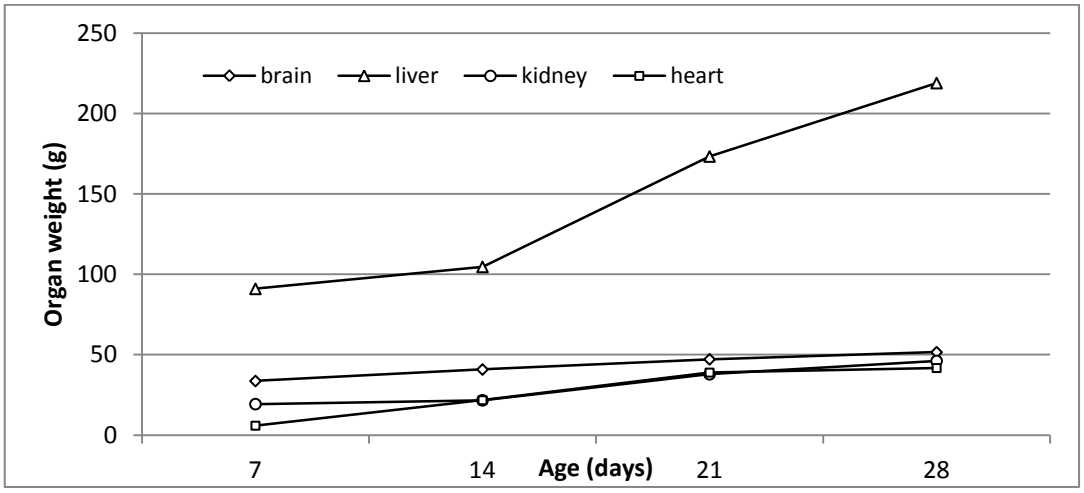

Figure 2.2. Average weights of internal organs of suckling piglets at different age

The concentration of ether extract values along the experimental time (figure 2.3) show a high variability of this chemical component between tissues (P = < 0.001) and the age (P = 0.017) of animals, whereas the interaction tissue *x* age is not significant (P = 0.12).

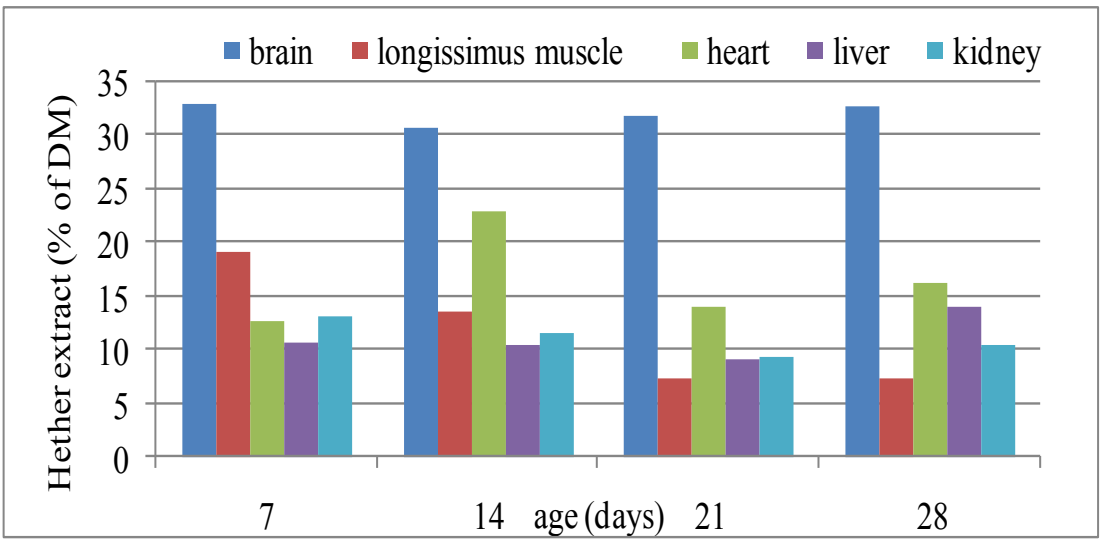

Figure 2.3. Mean concentrations of ether extract (% of DM) in different tissues of suckling piglets at different age (days).

As expected the tissue with the highest fat concentration is the brain. In particular, in all samples of brain tissue analysed more than 30% of the dry matter is represented by the ether extract, and this value remain constant along the observation period.

The differences in the concentration of the mains fatty acid families between the tissues and the age of piglets were highly significant how show in Tables 2.1 and 2.2. However, the interaction of the two factors was always significant indicating that the effect tissue was not the same at the different age of the piglets.

In figure 2.1 are reported the FA profiles of lipids extracted by brain of piglets at different time. The age of piglets affect significantly only few FA: the concentrations of C14:0, C16:1 c7, decrease as age of piglets rose; the concentrations of C18:1 c11, and CLA increase with the age; whereas, the C18:3 n-9 and C22:0 are represented in traces with a not definite trend as the concentration of C20:3 n-6. The stearic acid (C18:0) is the more represented in brain lipids (about 20%) along the time. Whereas, the oleic acid (C18:1 c9) concentrations tend to increase linearly (P = 0.07) as age, passing from about 16% of 7^{th} day to about 19 % of 28^{th} day. The presence of AA during the experiment remains at about 10%. Very similar values of AA are reported by Bazinet et al. (2003) in brain of piglets 14 days old suckling from sows fed two different diets where the concentration of C18:2 n-6 was 48.6 and 33.5% of the total FA. De Quelen et al. (2010), in brain of suckling piglets at different ages (0, 3, 7, 21 and 32 days) shows mean values of AA, which are slightly higher than ours, that are not affected by the sow diets (oil-based *vs* lard-based diets) but decreasing with age. In brain lipids is relevant the concentration of C22:4 n-6 (more than 5%), which is classically accepted as a product-step of the pathway for the biosynthesis of LCPUFA n-6 series originating LA (Li et al., 2000). This result is similar with the values reported by Farmer and Petit (2009) for piglet brain from 1-d-old piglets from sows fed diet where the LN concentration was comparable to that of our experiment.

Table 2.1 Means of fatty acids concentrations in lipids of brain of suckling piglets at different age.

Fatty acid	*Age (days)*					
(% of FAMEs)	**7**	**14**	**21**	**28**	SEM	P
C14:0	0.29^{a}	0.37^{a}	0.29^{a}	0.17^{b}	0.04	< 0.01
C15:0	0.05	0.01	0.00	0.00	0.03	0.14
C16:0	17.38	15.98	14.63	16.05	2.34	0.58
C16:1 t8	0.08	0.11	0.09	0.07	0.02	0.11
C16:1 c7	1.09^{a}	1.07^{a}	0.95^{ab}	0.82^{b}	0.09	0.02
C16:1 c9	1.11	1.19	1.14	1.01	0.07	0.07
C17:0	0.20	0.16	0.14	0.15	0.02	0.09
C17:1 c9	0.00	0.04	0.00	0.00	0.03	0.47
C18:0	22.01	21.94	21.73	21.82	0.58	0.94
C18:1 t11	0.16	0.17	0.06	0.28	0.15	0.39
C18:1 c9	17.51	18.58	19.69	20.37	1.08	0.05
C18:1 c11	5.12^{b}	5.35^{ab}	5.55^{ab}	5.69^{a}	0.20	0.04
C18:2 n-6	1.60	1.76	1.57	1.58	0.23	0.72
C18:3 n-6	0.04	0.00	0.00	0.02	0.02	0.12
C18:3 n-9	0.00^{b}	0.07^{a}	0.05^{ab}	0.00^{b}	0.02	0.01
C18:3 n-3	0.00	0.00	0.00	0.01	0.01	0.44
CLA (tot.)	1.42^{b}	1.08^{c}	1.52^{b}	2.07^{a}	0.13	< 0.01
C20:2 n-6	0.20	0.20	0.28	0.25	0.05	0.05
C20:3 n-9	0.39	0.44	0.29	0.45	0.14	0.14
C20:3 n-6	0.63^{b}	0.72^{ab}	0.79^{a}	0.74^{a}	0.04	0.04
C20:4 n-6	11.67	12.50	12.41	11.37	0.76	0.76
C22:0	0.00^{b}	0.08^{a}	0.07^{a}	0.00^{b}	0.02	0.02
C22:1 n-11	0.04	0.09	0.11	0.02	0.04	0.04
C22:1 n-9	0.04	0.00	0.10	0.20	0.13	0.13
C20:5 n-3	0.13	0.23	0.26	0.14	0.11	0.11
C22:2 n-6	0.00	0.03	0.05	0.05	0.04	0.04
C22:4 n-6	5.92	5.79	6.02	5.53	0.41	0.41
C24:0	3.44	3.65	3.15	3.02	0.37	0.37
C22:5 n-3	0.35	0.39	0.44	0.41	0.06	0.06
C22:6 n-3	8.83	7.95	8.49	7.39	0.64	0.64

Means followed by different letters within each row are significantly different (P<0.05).

We have not analyzed the milk, however, others authors showed a clear influence of sows diet FA composition on milk FA along lactation, in particular regarding the concentrations of LA, ALA, EPA, DPA and DHA (Lauridsen and Jensen, 2007). Therefore we can consider that FA

profile of diets fed by our sows similarly reflected in the FA composition of lipids in sows milk in this study.

In brain the concentration of the LC-PUFA originating from ALA, such as EPA (C20:5 n-3), DPA (C22:5 n-3) and DHA (C22:6 n-3), did not vary with the age of piglets. In fact, the mean range of their concentrations are: 0.12-0.24% for EPA, 0.33-0.41% for DPA and 7.0-8.28% for DHA. De Quelen et al. (2010) show mean values of EPA, DPA and DHA, in brain of suckling piglets, that are slightly higher than ours when lactating sows fed a lard-based diet. However, those authors observed a significant reduction of DHA concentration as age increases. Moreover, the concentrations of DPA and EPA are significantly higher in brain of piglets from sows fed linseed-based diet when compared with piglets from sow fed lard-based diet.

In table 2.2 are summarized the mean concentrations of the FA families in brain of piglets with different age. The age of piglets did not significantly affect any family of AF. This because the individual FA that were affected by age were those present in very low concentrations in lipids of brain. However, the means of MUFA show a tendency to increase as the age of piglets (P = 0.08). This is due to the significant increase of C18:1,c11 and the tendency to increase of C18:1,t9 which is the main monounsaturated FA in lipid of brain. De Quelen et al. (2010) in brain of suckling piglets obtained a significant effect of the age (0, 3, 7, 21 and 32 days) for the decrease of SFA and PUFA, and, consequently for the increase of MUFA. In our data the PUFA n-6 has been more than double PUFA n-3 for brain of all ages of the piglets. This result is not agree with those reported by De Quelen (2010) who obtained nearly equivalent concentrations for the two families of PUFA. This discrepancy maybe due to the different value of C18:2 n-6/C18:3 n-3 of sow diets.

Table 2.2 Means of fatty acids families in lipids of brain of suckling piglets at different age.

	Age (days)					
	7	**14**	**21**	**28**	SEM	P
PUFA n-3 *(%)*	9.30	8.57	9.19	7.96	0.69	0.14
PUFA n-6 *(%)*	20.07	21.00	21.13	19.54	1.08	0.29
PUFA tot *(%)*	29.37	29.57	30.32	27.50	1.55	0.23
n-6/n-3	2.17	2.45	2.30	2.46	0.16	0.16
SFA *(%)*	43.42	42.24	40.12	41.25	2.12	0.34
UFA *(%)*	56.58	57.76	59.88	58.75	2.12	0.34
MUFA *(%)*	27.21	28.19	29.56	31.25	1.50	0.05
MUFA/SFA	0.63	0.67	0.74	0.76	0.07	0.11
PUFA/SFA	0.68	0.70	0.76	0.67	0.06	0.42
SFA/UFA	0.77	0.73	0.67	0.71	0.06	0.36

The age of piglets has significantly influenced many of the FA in lipids of *Longissimus dorsi* muscle (Table 2.3). In fact, the C16:0 increases significantly from about 25% in 7-day old piglets to more than 30% in 28-day old, while at the same time the value of C18:3 decreases from about 27% to about 19%. In lipids extracted from *Longisimus dorsi* muscle the means of C18:2 n-6 differed significantly within the ages of piglets ($P < 0.01$). However, its trend is not definite along the ages, in fact the lowest value (about 15%) is observed in 14 –day old piglets and the value numerically highest (about 18%) is in muscle of the oldest piglets. A very similar trend was observed for the concentrations of C20:4 n-6 and C22:4 n-6 that are the most relevant LCPUFAs originating for elongation and desaturation of C18:2 n-6. This confirm the very strong relationship between the concentration of this EFA precursor and its derivatives LCPUFA n-6.

Table 2.3 Means of fatty acids concentrations in lipids of muscle (*longissimus d.*) of suckling piglets at different age.

Fatty acid	*Age (days)*					
(% of FAMEs)	**7**	**14**	**21**	**28**	SEM	P
C14:0	1.47	2.16	1.89	1.74	0.25	0.05
C14:1c9	0.01	0.07	0.04	0.06	0.03	0.10
C15:0	0.13	0.11	0.10	0.09	0.02	0.10
C16:0	25.78^{b}	30.95^{ab}	32.59^{a}	31.44^{ab}	2.17	0.02
C16:1 c7	1.12	0.26	0.00	0.44	0.24	< 0.01
C16:1 c9	4.68	5.52	5.06	5.40	0.96	0.72
C17:0	0.22	0.19	0.19	0.18	0.03	0.62
C17:1 c9	0.00^{a}	0.14^{b}	0.18^{ab}	0.00^{b}	0.04	< 0.01
C18:0	8.85	8.39	10.31	9.52	1.04	0.20
C18:1 c9	27.73	25.51	22.24	19.93	2.90	0.05
C18:1 c11	4.80^{a}	4.10^{b}	4.52^{ab}	4.18^{b}	0.22	0.01
C18:2 n-6	17.62^{ab}	15.82^{b}	16.58^{ab}	18.75^{a}	0.93	0.02
C18:3 n-6	0.18	0.12	0.09	0.11	0.04	0.06
C18:3 n-9	0.00	0.07	0.04	0.00	0.02	0.03
C18:3 n-3	0.43	0.61	0.51	0.59	0.20	0.70
CLA (tot.)	0.68^{ab}	0.95^{a}	0.64^{ab}	0.38^{b}	0.18	0.03
C20:2 n-6	0.73^{a}	0.06^{b}	0.05^{b}	0.57^{a}	0.08	< 0.01
C20:3 n-9	0.13^{a}	0.00^{b}	0.00^{b}	0.07^{ab}	0.05	0.03
C20:3 n-6	0.46	0.34	0.36	0.47	0.05	0.04
C20:4 n-6	2.97^{ab}	1.80^{b}	1.74^{b}	3.80^{a}	0.76	0.03
C20:3 n-3	0.00	0.11	0.10	0.00	0.02	< 0.01
C22:0	0.00	0.04	0.06	0.00	0.02	0.02
C22:1 n-9	0.00^{b}	0.11^{a}	0.07^{a}	0.00^{b}	0.02	< 0.01
C20:5 n-3	0.14^{a}	0.00^{b}	0.00^{b}	0.14^{a}	0.03	< 0.01
C22:2 n-6	0.01	0.00	0.00	0.00	0.01	0.44
C22:4 n-6	0.64	0.48	0.50	0.73	0.16	0.26
C24:0	0.23	0.17	0.14	0.18	0.12	0.84
C24:1 c15	0.00^{b}	0.09^{a}	0.06^{ab}	0.00^{b}	0.03	0.01
C22:5 n-3	0.47	0.40	0.39	0.63	0.12	0.11
C22:6 n-3	0.23	0.14	0.11	0.30	0.08	0.07

Means followed by different letters within each row are significantly different (P<0.05).

The C18:3 n-3 is detected in all ages, it isn't affected by the age and its concentration is always less than the 1%. A similar trend is reported for the others components of the PUFA n-

3 family, like C20:5 n-3, C22:5 n-3 and C22:6 n-3. These results confirm, also for the PUFA n-3 family, the strong relationship between the concentration of the precursor and those of its derivatives.

When consider the distribution of FA in classes (table 2.4) we note that the age of piglets significantly affect the concentrations of SFA and PUFA and UFA, as consequence. The lowest SFA value is reported in the youngest piglets, while its concentration isn't significant different in piglets 14, 21 and 28 days old. An opposite trend is obtained UFA concentration. The anomalous changes of C18:2 n-6 along the suckling time, previously described, affect the consequent anomalous trend of the PUFA n-6 family. These trends, which are not always clearly defined, are likely due to the continuous modification of the composition of the muscle during the first part of extrauterine life of piglets.

Table 2.4 Means of fatty acids families in lipids of muscle (*longissimus d.*) of suckling piglets at different age.

Fatty acid	*Age (days)*					
(% of FAMEs)	**7**	**14**	**21**	**28**	SEM	P
PUFA n-3 *(%)*	1.27	1.26	1.11	1.65	0.23	0.09
PUFA n-6 *(%)*	22.62^{ab}	18.62^{c}	19.32^{bc}	24.43^{a}	1.49	< 0.01
PUFA tot *(%)*	23.88^{ab}	19.88^{b}	20.43^{b}	26.08^{a}	1.66	0.01
n-6/n-3	18.42	14.88	17.76	14.90	2.52	0.26
SFA *(%)*	36.68^{b}	42.72^{ab}	46.03^{a}	43.18^{ab}	2.79	0.02
UFA *(%)*	63.32	57.28	53.97	56.82	2.79	0.02
MUFA *(%)*	39.43	37.40	33.55	30.73	3.59	0.07
MUFA/SFA	1.08	0.88	0.74	0.72	0.13	0.04
PUFA/SFA	0.65^{a}	0.47^{b}	0.45^{b}	0.60^{a}	0.05	< 0.01
SFA/UFA	0.58^{b}	0.75^{ab}	0.86^{a}	0.76^{ab}	0.09	0.03

Means followed by different letters within each row are significantly different (P<0.05).

The means of FA extracted from lipids of hearth are reported in Table 2.5.

Table 2.5 Means of fatty acids concentrations in lipids of heart of suckling piglets at different age.

Fatty acid	*Age (days)*					
(% of FAMEs)	**7**	**14**	**21**	**28**	SEM	P
C14:0	1.05	1.49	1.37	1.62	0.15	0.07
C14:1c9	0.02	0.03	0.04	0.04	0.03	0.94
C15:0	0.06	0.04	0.07	0.07	0.03	0.58
C16:0	20.77^{b}	23.95^{ab}	23.99^{ab}	25.99^{a}	1.16	0.04
C16:1 c7	0.48	0.44	0.41	0.35	0.05	0.17
C16:1 c9	3.91^{b}	4.70^{ab}	4.52^{b}	5.41^{a}	0.30	0.02
C17:0	0.18	0.15	0.18	0.19	0.05	0.78
C17:1 c9	0.00	0.13	0.17	0.10	0.08	0.38
C18:0	12.66	11.00	11.16	10.65	0.59	0.12
C18:1 t11	26.66	27.58	25.77	25.24	1.45	0.32
C18:1 c11	4.33	3.85	3.69	3.60	0.44	0.55
C18:2 n-6	17.03	16.33	16.44	16.38	0.92	0.92
C18:3 n-6	0.00	0.03	0.03	0.05	0.05	0.83
C18:3 n-9	0.06	0.04	0.05	0.04	0.03	0.84
C18:3 n3	0.52	0.56	0.56	0.66	0.08	0.34
CLA (tot.)	0.67	0.55	0.59	0.53	0.16	0.88
C20:2 n-6	0.15	0.04	0.05	0.22	0.16	0.54
C20:3 n-9	0.00	0.00	0.00	0.01	0.01	0.59
C20:3 n-6	0.51	0.41	0.44	0.40	0.04	0.20
C20:4 n-6	7.22	5.78	7.19	5.98	1.13	0.42
C20:3 n-3	0.14	0.13	0.12	0.11	0.02	0.62
C22:0	0.04	0.02	0.02	0.01	0.01	0.17
C22:1 n-9	0.17	0.08	0.14	0.14	0.04	0.22
C20:5 n-3	0.00	0.00	0.00	0.01	0.01	0.59
C22:2 n-6	0.00	0.01	0.00	0.00	0.01	0.59
C22:4 n-6	0.84	0.54	0.61	0.34	0.19	0.19
C24:0	0.50^{a}	0.25^{b}	0.22^{b}	0.18^{b}	0.06	0.02
C22:5 n-3	0.84	0.64	0.89	0.77	0.13	0.25
C22:6 n-3	0.42	0.34	0.45	0.38	0.09	0.45

Means followed by different letters within each row are significantly different (P<0.05).

The FA profile of lipids extracted from hearth seems enough stable along the ages of suckling piglets (Table 2.5). However, the mean of C16:o (palmitic acid) increases significantly with

the age and the C16:1 c9 too. This is counterbalanced by a tendencies to decrease of the others saturated FA like C18:0 and C20:0. The changes of C18: 2n-6 did not reach significant value, as for its main derivatives of elongation and desaturation (C20:4 n-6; and C22:4 n-6). The C18:3 n-3 and its long chain derivatives as C22:5 n-3 and C22:6 n-3 are detected in all ages, and their concentration doesn't vary with the age of the suckling piglets. The C20:5 n-3 is present in very low concentrations which are near the detection limit of the analytical method. The changes of singles saturated FA, previously described, represent the cause of tendency to increase of the SFA family (table 2.6). Whereas the age of piglets did not affect the values of the other FA families.

These results suggest that the lipid composition of the heart in piglets is not affected by relevant changes during the suckling time considered in this experiment.

Table 2.6 Means of fatty acids families in lipids heart of suckling piglets at different age.

	Age (days)					
	7	**14**	**21**	**28**	SEM	P
PUFA n-3 *(%)*	1.92	1.75	2.03	1.94	0.23	0.55
PUFA n-6 *(%)*	25.74	23.14	24.76	23.37	1.77	0.51
PUFA tot *(%)*	27.66	24.89	26.78	25.31	1.94	0.52
n-6/n-3	13.42	13.26	12.33	12.08	0.99	0.45
SFA *(%)*	36.03	37.66	37.69	39.18	1.00	0.13
UFA *(%)*	63.97	62.34	62.31	60.82	1.00	0.13
MUFA *(%)*	36.30	37.44	35.53	35.52	1.98	0.63
MUFA/SFA	1.01	1.00	0.94	0.91	0.06	0.38
PUFA/SFA	0.77	0.66	0.71	0.65	0.06	0.30
SFA/UFA	0.56	0.60	0.61	0.64	0.03	0.13

Means followed by different letters within each row are significantly different (P<0.05).

Also the FA composition of lipids extracted from the liver seems to be quite stable during the suckling time (Table 2.7).

Table 2.7 Means of fatty acids concentrations in lipids of liver of suckling piglets at different age.

Fatty acid	*Age (days)*					
(% of FAMEs)	**7**	**14**	**21**	**28**	SEM	P
C14:0	0.15	0.44	0.37	0.32	0.21	0.41
C15:0	0.00	0.03	0.03	0.01	0.02	0.19
C16:0	16.44	19.01	20.80	18.34	3.11	0.44
C16:1 c7	0.26	0.19	0.20	0.07	0.11	0.31
C16:1 c9	1.56	1.94	2.02	2.37	0.53	0.38
C17:0	0.22	0.22	0.20	0.19	0.03	0.61
C17:1 c9	0.00^{b}	0.08^{a}	0.03^{ab}	0.00^{b}	0.02	0.01
C18:0	24.70	25.86	28.13	25.20	2.77	0.48
C18:1 c9	12.10	12.29	9.92	9.69	1.84	0.25
C18:1 c11	2.71	2.47	2.12	2.27	0.22	0.06
C18:2 n-6	13.64^{b}	14.29^{ab}	13.77^{b}	15.95^{a}	0.67	0.01
C18:3 n-6	0.18	0.14	0.09	0.07	0.05	0.08
C18:3 n-9	0.01	0.03	0.00	0.01	0.02	0.35
C18:3 n-3	0.11	0.16	0.12	0.17	0.04	0.18
CLA (tot.)	0.10	0.27	0.18	0.10	0.08	0.09
C20:2 n-6	0.46	0.20	0.16	0.28	0.13	0.08
C20:3 n-9	0.21^{a}	0.00^{b}	0.00^{b}	0.11^{ab}	0.05	< 0.01
C20:3 n-6	0.63^{b}	0.74^{ab}	0.76^{ab}	1.17^{a}	0.19	0.04
C20:4 n-6	18.70	14.59	13.99	16.31	4.60	0.62
C20:3 n-3	0.06	0.06	0.07	0.04	0.04	0.90
C22:0	0.00	0.03	0.01	0.00	0.02	0.17
C22:1 n-9	0.08	0.00	0.00	0.05	0.08	0.58
C20:5 n-3	0.06	0.14	0.09	0.06	0.09	0.64
C22:4 n-6	1.09	0.95	1.00	1.27	0.33	0.66
C24:0	0.84	0.75	0.84	0.91	0.41	0.97
C24:1 c15	0.00	0.02	0.00	0.00	0.01	0.08
C22: n-3	1.93	1.43	1.68	2.06	0.62	0.63
C22:6 n-3	3.75	3.05	3.01	2.91	1.26	0.84

Means followed by different letters within each row are significantly different (P<0.05).

In fact, the changes that have achieved significance in the statistical analysis are for FA that haven't substantial interest or the means of others FA with a no-definite trend along the age of the animals such as C18:1 c11. The only one FA affect by age in clear manner is C20:3 n-6

that increases linearly. These FA profiles are very similar with the data reported by Lauridsen and Jensen (2007) in liver of piglets from lactating sows that fed the control diet. This accordance maybe due to the high similarity of FA profile in diets of sows. However, it is important to note that those authors (Lauridsen and Jensen, 2007) observed significant changes in the concentrations of some FA when they compared the data of 4-day-old piglets with those of 18 and 24 days. The discrepancy is, probably, explained considering that we studied the FA profile of nursing piglets from their 7th day of age, while those authors began with youngest animals (4 days old).

In table 2.8 are reported the concentrations of the FA families in lipids of liver. The amounts of UFA are higher than SFA for all ages. This agree with the data obtained in liver of piglets from lactating sows that fed the control in the experiment of Lauridsen and Jensen (2007).

Table 2.8 Means of fatty acids families in lipids liver of suckling piglets at different age.

	Age (days)					
	7	**14**	**21**	**28**	SEM	P
PUFA n-3 *(%)*	5.91	4.84	4.97	5.24	1.93	0.91
PUFA n-6 *(%)*	34.70	30.91	29.76	35.05	5.43	0.57
PUFA tot *(%)*	40.61	35.74	34.73	40.29	7.34	0.68
n-6/n-3	5.89	6.47	11.37	6.73	5.47	0.61
SFA *(%)*	42.34	46.79	50.79	44.98	5.31	0.33
UFA *(%)*	57.66	53.21	49.21	55.02	5.31	0.33
MUFA *(%)*	17.05	17.46	14.49	14.73	2.60	0.42
MUFA/SFA	0.40^{a}	0.37^{ab}	0.28^{b}	0.33^{ab}	0.04	0.04
PUFA/SFA	0.96	0.77	0.73	0.90	0.20	0.51
SFA/UFA	0.73	0.88	1.10	0.82	0.25	0.39

The FA profile of lipids extracted by kidney are reported in table 2.9. the age of piglets affects significantly some FA, as previous show in others organs or tissues.

Table 2.9 Means of fatty acids concentrations in lipids of kidney of suckling piglets at different age.

Fatty acid	*Age (days)*					
(% of FAMEs)	**7**	**14**	**21**	**28**	SEM	P
C14:0	0.85^{a}	0.74^{ab}	0.40^{b}	0.54^{ab}	0.14	0.02
C15:0	0.05	0.07	0.02	0.05	0.04	0.62
C16:0	21.23	22.11	20.06	21.32	1.98	0.71
C16:1 t8	0.00	0.03	0.00	0.09	0.09	0.55
C16:1 c7	0.53^{a}	0.36^{b}	0.27^{b}	0.32^{b}	0.05	< 0.01
C16:1 c9	2.57	2.32	1.43	1.77	0.45	0.06
C17:0	0.13	0.15	0.15	0.17	0.06	0.87
C17:1 c9	0.00	0.00	0.05	0.04	0.06	0.67
C18:0	0.00	0.00	0.05	0.04	0.06	0.67
C18:1 c9	23.27^{a}	18.68^{ab}	14.20^{b}	13.14^{b}	1.94	< 0.01
C18:1 c11	4.44	3.95	4.38	4.41	0.31	0.39
C18:2 n-6	16.23	13.58	14.84	16.15	1.61	0.31
C18:3 n-6	0.13	0.09	0.05	0.05	0.03	0.07
C18:3 n-9	0.02	0.05	0.02	0.02	0.03	0.65
C18:3 n-3	0.47^{a}	0.24^{ab}	0.18^{b}	0.29^{ab}	0.11	0.05
CLA (tot.)	0.65	0.56	0.45	0.37	0.11	0.08
C20:2 n-6	0.10	0.17	0.11	0.09	0.09	0.77
C20:3 n-9	0.04	0.00	0.00	0.02	0.05	0.65
C20:3 n-6	0.98	1.21	1.40	1.48	0.22	0.11
C20:4 n-6	10.86^{b}	16.20^{ab}	19.74^{a}	18.68^{a}	2.21	0.01
C20:3 n-3	0.13^{b}	0.17^{ab}	0.20^{a}	0.19^{ab}	0.02	0.04
C22:0	0.09	0.15	0.18	0.14	0.04	0.10
C22:1 n-11	0.00	0.01	0.02	0.02	0.02	0.78
C22:1 n-9	0.25^{b}	0.40^{ab}	0.56^{a}	0.62^{a}	0.08	< 0.01
C22:2 n-6	0.03	0.00	0.00	0.00	0.02	0.51
C22:4 n-6	1.53	1.88	2.55	2.13	0.38	0.07
C24:0	0.43	0.38	0.41	0.37	0.06	0.61
C24:1 c15	0.02	0.02	0.00	0.00	0.03	0.54
C22: n-3	0.84	0.81	1.16	1.02	0.15	0.09
C22:6 n-3	0.75^{b}	1.17^{ab}	1.57^{a}	1.46^{a}	0.23	0.01

Means followed by different letters within each row are significantly different (P<0.05).

The concentration of C18:1 c9, decreases dramatically with the age in lipids of kidney, while an opposite trend is obtained for its saturated FA (C18:0). A very consistent chance is

documented for the concentration of C20:4 n-6 which has the lowest value in at 7 days, while the next are much higher and not change. A similar trend was also recorded for concentrations of C20:3 n-6 and C22:4 n-6. The value of C18: 3 n-3 was high in the first age and much lower in subsequent, while the trend DHA is completely opposite even if this FA represents one of its most important derivatives of elongation.

The changes observed for individual FA affect the means of the respective families (table 2.10). In fact, concentration of PUFA, both n-3 and n-6, increases in lipids of the kidney with the age, and this change is offset by the significant decrease of MUFA family.

Table 2.10 Means of fatty acids families in kidney of suckling piglets at different age.

	Age (days)					
	7	**14**	**21**	**28**	SEM	P
PUFA n-3 *(%)*	2.20	2.38	3.12	2.95	0.36	0.05
PUFA n-6 *(%)*	29.86^{b}	33.13^{ab}	38.67^{a}	38.58^{a}	2.07	< 0.01
PUFA tot *(%)*	32.06^{b}	35.50^{ab}	41.79^{a}	41.53^{a}	2.19	< 0.01
n-6/n-3	13.65	13.96	12.61	13.25	1.62	0.80
SFA *(%)*	36.13	38.09	36.79	37.62	1.55	0.52
UFA *(%)*	63.87	61.91	63.21	62.38	1.55	0.52
MUFA *(%)*	31.82^{a}	26.41^{ab}	21.42^{b}	20.85^{b}	2.06	< 0.01
MUFA/SFA	0.89^{a}	0.69^{ab}	0.58^{b}	0.55^{b}	0.08	< 0.01
PUFA/SFA	0.89^{b}	0.93^{ab}	1.14^{a}	1.11^{ab}	0.09	0.03
SFA/UFA	0.57	0.62	0.58	0.60	0.04	0.52

Means followed by different letters within each row are significantly different (P<0.05).

Conclusion

During the time considered in this experiment, piglets fed FA only through the milk suckled by their mothers, and the diet of sows does not change. Therefore, changes in FA composition of lipids of different organs or tissues that we observed are considered as mainly due to their development during the suckling time of piglets. These changes in profile of FA were not the same in all tissues. This suggests that, during the suckling time, the lipid metabolism must meet the different needs of growing organs or tissues. for FA involved in the pathway of PUFA n-3 and n-6, in many cases, it was noted the strong relationship between the concentration of precursors, such C18:2 n-6 and C18:3 n-3, and their LCPUFA.

The data of this experiment can be useful to know the effect of age on lipid metabolism of suckling piglets. This may assist to think, more carefully, about the real requirements of FA, in particular of EFAs, in diets of lactating sows.

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Chapter 4

EXPERIMENT 3

COMPARISON OF FATTY ACID COMPOSITION OF LIPIDS IN SERUM OF LACTATING SOWS AND THEIR SUCKLING PIGLETS

Introduction

Study of lipid metabolism in piglets requires knowledge of the dietary essential fatty acids (EFAs), linoleic acid (LA, 18:2n-6) and a–linolenic acid (ALA, 18:3n-3) and their long chain derivatives (long chain polyunsaturated fatty acids , LCPUFAs) that play important roles in neural, and brain growth of fetus and newborn (Lauritzen et al., 2001). The main factors that affect the maternal transfer of precursor EFA and LCPUFA to the fetus and suckling newborns include placental function, maternal LCPUFA status such as intake and circulating levels of fatty acids in gestating and lactating sows. However, some mammalian tissues, specially liver and brain can synthesize LC PUFA from essential FA by desaturation and elongation pathway. Study of FA composition of different organs or tissues in newborn pre and post suckling piglets may be a valuable model to increase knowledge of the function of EFAs in human metabolism.

Aims: objective of the present study was to investigate the relationship between the fatty acid composition in lipids of lactating sows and of those of suckling piglets.

Material And Methods

Animals: the experiment was carried out, in a commercial farm located in north-west Sardinia, according to according to the European Union regulations of the Animals (Scientific Procedures) Act, 1986.

During gestation sows were housed in individual crates and daily fed a standard commercial diet for gestating/lactating sows. The diet was formulated according to meet the requirements of sow and fetal development.

Five days before their third-parity, three sows, of the same commercial genotype (Landrace × Large White) inseminated with the same pooled Large White semen were chosen for the experiment and transferred to individual farrowing crates.

All farrowings were attended and immediately after delivery piglets were assisted for colostrums assumption from respective dam. Two days after delivery three piglets per litter were chosen (for a total of 9 piglets) and tagged. The choice of animal was dictated by their weights, representative of the average weight of litter. Piglets were weighed daily until the 21st day. During the entire 21 days of lactation, no creep feed or milk replacer were offered to piglets.

For all the lactation period the same standard commercial feed used during the gestation was available *ad libitum* for sows. Feed used for lactating sows was from the same batch that was used for during the gestation and already sampled and analyzed for the previous experiments.

At 7, 14, and 21 days of age one piglet per litter was stunned, exanguinated via jugular vein and blood was collected into 10-ml vacutainer tubes (Becton Dickinson, Le Pont Claix, France) containing lithium heparin. From each sow blood sample was collected at 7, 14, and 21 days postpartum by jugular on ear venipuncture. After separation by centrifugation (2000 rpm for 10 min at 4 °C), plasma was collected and frozen at -20°C until being analyzed.

Chemical analysis of sow diet: Chemical analysis of sow diet are detailed in section 5.

Fatty acid composition of plasma: Fat extraction and esterification procedure for fatty acid analysis were performed as reported in chapter 6. The chromatographic conditions and fatty acid identification were carried out as reported in chapter 6 The relative

amount of each fatty acid (% of total FAME) is reported as a percentage of total peak area for all fatty acids.

Calculations: the fatty acids concentrations were grouped according to the length of carbon chain in the following different classes: short chain fatty acids (SC-FA) under 14 carbon atoms; medium chain fatty acids (MC-FA), from 14 to 17 carbon atoms and long chain fatty acids (LC-FA) from 18 carbon. In addition, the sum of saturated fatty acids (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), polyunsaturated n-3 (PUFA n-3), polyunsaturated and n-6 fatty acids (PUFA n-6) were calculated as reported in tables 5.1; 5.2; 5.3 and 5.4 in chemical analysis section (chapter 6)

Statistical analysis

The ANOVA was carried out for the fatty acids profile of plasma using the animal (piglet or sow), time of sampling (7, 14 or 21 days) and their interactions as the main effects. The Statistical analysis were performed using MINITAB® software (Version 16, Minitab, State College, PA, USA).

Differences were considered significant at $P \leq 0.05$. The Pearson correlation coefficients between the fatty acids concentration in plasma, separately for sow and piglets, were calculated.

Results

The fatty acid (FA) profile of sow diet is reported in Table 3.1. The concentrations of the most relevant FA indicate that the diet was obtained by a mixture of the most commons ingredients used for swine diets in Europe, such cereals. The low content of linolenic acid (C 18:3 n-3), if compared with the C18:2 n-6, confirms that the diet has not been added fats, or oils, which can increase its concentration.

Table 3.1 Fatty acids composition of diet administered to lactating sows.

Fatty acid	**(g/100g of FAME)**
< C16	0.15
C16:0	15.66
C18:0	1.57
C18:1 cis 9	22.26
C18:2 n-6	54.64
C 18:3 n-3	3.98

The means of FA concentrations in lipids obtained from plasma of lactating sows and piglets were reported in table 3.2.

In plasma of lactating sow the C18:2 n-6 is the most abundant FA and represent about 50% of the total FAMEs detected. The others FA well represented in sow's plasma were C16:0 (about 25% of total FA) and C18:1 c9 (17.4%). The plasma concentration of LA is markedly higher than values observed in previous paper (Farmer and Petit, 2009). The only long-chain PUFA (LCPUFA) identified in sow plasma is the C20:4 n-6 which comes from the elongation and desaturation of its precursor C18:2 n-6. The high concentration of LA, followed by palmitic and oleic acids in the diet of the sows explain the plasma concentration of those fatty acid in their plasma. The results confirm the close relation of FA in plasma of swine with the composition of lipids in diet as

observed in other experiments (Rooke et al., 1999, Farmer and Petit, 2009, Amusquivar et al., 2010). Infact, the absence of LCPUFA n-3 in plasma of sows is explained by the absence of those FA in their diets, as supported by the positive effects of omega3 supplementation on omega3 concentration in the plasma (Rooke et al., 1999).

The FA profile of lipids from plasma of sows is very different from their suckling piglets. Actually, some FA that have been detected in piglets were not quantified in the plasma of their mothers like C14:0, C18:1 c11, C18:3 n-3, C24:0, C22:5 n-3, and C22:6 n-3.

The FA composition of piglet's plasma differs from the mothers for the higher concentration of C16:0, C16:1 c7, C18:0 and C18:3 n-6, and for the lower concentration of C18:2 n-6. Moreover, in the plasma of piglets were also quantified the presence of n-3 LCPUFA derived from the elongation of C18: 3 n-3 as the C22:5 n-3 and C22:6 n-3. These results suggest that in tissues of piglets occurs the synthesis ex novo LCPUFA n-3 through a series of elongation and desaturation steps.

The sampling time affect significantly affects only the concentration of C18:2 n-6, which increased with the suckling time. This trend is common for plasma of sow and piglets, as explained by not significant interaction animal x time (P = 0.17).

Table 3.2. Means of fatty acids concentrations in plasma of lactating sows and suckling piglets at different time

Fatty acid	***Animal***		***Time***			***P***		
(% of FAMEs	*Sow*	*Piglet*	*7*	*14*	*21*	*A*	*T*	*AxT*
C14:0	n.d.	1.17	0.64	0.49	0.63	-	0.81	0.81
C16:0	24.66	29.43	24.53	28.35	28.26	0.02	0.17	0.45
C16:1 c7	0.67	5.05	3.00	2.65	2.93	0.01	0.85	0.67
C18:0	3.03	8.10	6.73	5.02	4.94	0.01	0.39	0.76
C18:1 c9	17.43	19.93	22.23	19.23	14.57	0.34	0.09	0.38
C18:1 c11	n.d.	2.06	1.33	0.82	0.94	-	0.44	0.44
C18:2 n-6	48.40	26.49	34.14^{b}	37.43^{ab}	40.78^{a}	0.01	0.02	0.17
C18:3 n-6	n.d.	0.36	0.26	0.12	0.21	-	0.42	0.70
C18:3 n-3	n.d.	0.52	0.30	0.18	0.30	-	0.29	0.29
C20:4 n-6	5.77	5.70	6.01	5.50	5.69	0.92	0.79	0.99
C24:0	n.d.	0.22	0.14	0.05	0.14	-	0.29	0.29
C22:5 n-3	n.d.	0.34	0.25	0.07	0.19	-	0.10	0.10
C22:6 n-3	n.d.	0.44	0.28	0.10	0.27	-	0.16	0.16

Means followed by different letters within each row are significantly different (P<0.05).

In plasma of piglets a strong inverse correlations between the C16:0 and C18:0 were found. This could be explained by the extension pathway of C16:0 to C18:0 that may have cause a reduction of palmitic acid and the correspondent increase of stearic acid.

The C18:3 n3 was positively correlated with EPA and DHA. Both EPA and DHA could be either provided in diet or synthesized in the body from the essential fatty acid α-linolenic acid (18:3n–3). On the other hand, no correlation was found among C18:2 n-6 and arachidonic acid (C20:4 n6) (P = 0.145). Because the production of ARA, EPA, and DHA require the same enzymes of elongations and desaturations (delta-6-desaturase and delta-5-desaturase) a competition could be expected. However, large concentrations of linoleic acid do not inhibit the ability of EPA and DHA to be produced from low concentrations of C18:3 n3 (Burke et al., 2001). This could explain the positive

correlation founded between ARA and DHA. Lignoceric acid (C24:0), that is present in plant and therefore originate from the diet, was strongly correlated with EPA and DHA.

Table 3.2. Correlation coefficients of fatty acid concentrations in plasma of piglets during the suckling time

	C14:0	C16:0	C16:1 n7	C18:0	C18:1 c9	C18:1 c11	C18:2 n6	C18:3 n6	C18:3 n3	C20-4 n6	C24:0	DPA
C16:0	-0.338											
	0.212											
C16:1 n7	0.426	0.380										
	0.168	*0.223*										
C18:0	0.462	-0.920	-0.378									
	0.131	*0.000*	*0.225*									
C18:1 c9	0.484	-0.681	0.369	0.589								
	0.111	*0.015*	*0.237*	*0.044*								
C18:1 c11	0.649	-0.709	0.362	0.728	0.871							
	0.022	*0.010*	*0.247*	*0.007*	*0.000*							
C18:2 n6	-0.684	0.481	-0.161	-0.552	-0.841	-0.901						
	0.014	*0.113*	*0.618*	*0.063*	*0.001*	*0.000*						
C18:3 n6	0.152	-0.640	-0.270	0.490	0.355	0.457	-0.231					
	0.637	*0.025*	*0.369*	*0.106*	*0.258*	*0.135*	*0.470*					
C18:3 n3	0.587	-0.931	0.207	0.852	0.647	0.764	-0.543	0.636				
	0.045	*0.000*	*0.519*	*0.000*	*0.023*	*0.004*	*0.068*	*0.026*				
C20-4 n6	-0.582	-0.400	-0.692	0.287	0.085	0.017	0.118	0.447	0.227			
	0.047	*0.197*	*0.013*	*0.365*	*0.792*	*0.958*	*0.716*	*0.145*	*0.477*			
C24:0	0.222	-0.549	-0.336	0.587	-0.365	0.593	-0.547	0.451	0.607	0.534		
	0.487	*0.064*	*0.243*	*0.045*	*0.243*	*0.042*	*0.065*	*0.142*	*0.036*	*0.074*		
DPA	0.256	-0.653	-0.464	0.710	0.591	0.702	-0.698	0.395	0.589	0.461	0.841	
	0.422	*0.021*	*0.128*	*0.010*	*0.043*	*0.011*	*0.012*	*0.204*	*0.044*	*0.131*	*0.001*	

DHA	0.124	-0.613	-0.493	0.587	0.465	0.620	-0.542	0.658	0.599	0.640	0.922	0.887
	0.701	*0.034*	*0.104*	*0.045*	*0.128*	*0.031*	*0.069*	*0.020*	*0.039*	*0.025*	*0.000*	*0.000*

Table 3.3. Correlation coefficients of fatty acid concentrations in plasma of sows during the lactation time

	C16:0	**C16:1 c7**	**C18:0**	**C18:1 c9**	**C18:2 n-6**	**C18:3 n-6**
C16:1	-0.057					
	0.892					
C18:0	0.115	-0.637				
	0.786	*0.089*				
C18:1 c9	-0.914	0.323	-0.157			
	0.002	*0.436*	*0.71*			
C18:2 n-6	0.54	-0.559	-0.001	-0.8		
	0.167	*0.149*	*0.998*	*0.017*		
C18:3 n-6	-0.15	0.794	-0.478	0.326	-0.601	
	0.724	*0.019*	*0.231*	*0.43*	*0.115*	
C20:4 n-6	0.259	0.312	-0.15	-0.222	-0.159	0.669
	0.535	*0.452*	*0.724*	*0.598*	*0.707*	*0.07*

Conclusions

The results of the present trial suggest that following conclusions:

the concentration of FA in plasma of swine was markedly influenced by the composition of lipids in diet as observed in other experiments. The FA profile of lipids from plasma of sows is very different from their suckling piglets, in particular some FA that have been detected in piglets were not quantified in the plasma of their mothers. The Pearson correlation analysis evidenced interesting relationship among FA that need further investigation.

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Chapter 5

GENERAL CONCLUSION

Results of the experimental activities reported in this thesis highlight three cardinal points regarding the lipids composition in tissues of newborn and suckling piglets. First study shows fatty acids profile in newborn un-suckling piglets. The main results from the data analyzed show strong heterogeneity among different tissues, especially for FA synthesized *ex novo* like long chain polyunsaturated fatty acids (PUFA). The lipids of Brain are the most rich of this FA, this indicates elongase and desaturase enzyme activity during foetal development.

In the second study we observe the evolution of fatty acids profile in lipids extracted from different internal organs of tissues of piglets along the suckling time. Most relevant results show that the fatty acid profile in changes with the age of suckling animals. These changes are different between tissues, indicating that the growth of each organ or tissues in body of newborn piglets follows a different route and therefore requires distinct combination of fatty acids. In many tissues there are a strong relationship between the concentration of LCPUFA and their precursors that are the essential fatty acids C18:2 n-6 and C18:3 n-3. This indicate that, during the suckling time, piglet metabolism plays a key role in the utilization of milk substrates, also in terms of ex novo synthesis of fatty acids.

Last experiment shows the comparison of fatty acids profile of plasma from sows and their suckling piglets. The two fatty acids profile strongly differed. The concentration of FA in swine plasma was markedly influenced by lipid composition of the diet. In plasma of piglets there are some FA, like LCPUFA which are not detected in plasma of their mothers. Finally, Pearson correlation analysis of the fatty acids concentrations evidenced interesting relationship between them.

In all experiment were analyzed tissues from muscle and mayn internal organs,

To provide a complete vision about fat deposition during study period

Further studies are needs for better understand relationship between sow diet and piglet lipid metabolism in foetal and pre weaning period development. Moreover from another important point of view swine represent interestig model for comprehend and delve knowledge in human lipid metabolism.

Chapter 6

LIPID ANALYSIS.

Tissues

Fat extraction

Organs and muscles tissues just collected were stored at – 80°C for 2 days. After 2 days samples were placed in lyophilizer LyoLab 3000 (Heto) for 3 days. Lyophilizer samples were ground by domestic grinder machine and stored at -20 °C until analysis.

Lipids were extracted from 1 g of samples with chloroform-methanol (2:1 v/v) according to the method of Folch et al. (1957) modified. 1 g of tissue sample was placed into 50 ml tube and 30 ml of chloroform-methanol (2:1 v/v) were added. The tube was capped, shaken by hand for 30 seconds approximately. The tubes were placed for 5 min in ultrasonic bath (BRANSON 2510, BRANSONIC®) and afterwards centrifuged at 1500 rpm × g for 10min at room temperature for separate 2 phases. The upper phase was removed using a water aspirator and discarded while the chloroform extract layer was evaporated under vacuum in a rotary evaporator. Extracted fat samples were collected with different hexane amounts for obtain samples with fat concentration around 25 mg/ml.

Esterification procedures.

The fatty acids methyl esters (FAME) were prepared with a base-catalyzed trans esterification according the FIL-IDF standard procedure (1999). Hexane evaporate from 1 ml of samples under hydrogen current, for obtained approximately 25 mg of lipid extract. These 25 mg of lipid were mixed with 500 µl of of sodium methoxide in methanol, and vortexed for 2 min. 1 ml of hexane containing internal standard (0.5 mg/ml) was added and vortexed for 2 minutes. After the separation in two phases the upper phase was collected for run in gas chromatograph.

Blood

Fat extraction

Blood samples were collected in vacutainer tubes coated with lithium heparin. Samples were immediately centrifuged at 2000 rpm for 10 min at 4 °C to recover plasma which was immediately stored at -20°C until required for analysis. Lipids were extracted from 2 ml of plasma how described from Corl (Corl et al., 2001), with 3 ml of exan-isopropanol (3:2 v/v) and 2 ml of sodium –solfate solution (67 g/l). After recovered supernatant sodium-solfate was put into this and dried in nitrogen current.

Esterification procedures.

Lipid extract was mixed with 500 µl of hexane, 40 µl of methyl acetate and 40 µl of methylation reagent (1,75 ml of methanol : 0.4 ml of sodium methoxide 5.4 M) and vortexed for 1 minute. After 24 hours mixed with 60 µl of termination reagent (1g of oxalic acid : 30 ml of diethilic ether). Mixed with few grain of calcium chloride for remove methanol and extract the samples for GC.

Feed

The dry matter content of feed was determined by oven drying at 105 °C for 24 hours. Ash was determined leaving feed samples in muffle at 550 °C for 24 hours (AOAC 920.153. 1999). Crude protein (CP) and fat (EE) content was determined with Kjeldahl and Folch method (Folch et al., 1957) respectively.

Two step methylation procedure.

Lipids were extracted from 1 g of sow concentrate feed, added 1 mL exhane and 2 mL of sodium methoxide (0.5 M in methanol) solution. Samples were vortexed and incubated in water bath at temperature of 50°C. When the samples were removed from bath and cooling for 5 minutes. 3 ml of methanolic HCl were added, vortexed and incubated in water bath for 30 minutes at 60°C. After samples were cooled for 7 minutes, were added 3 mL of exhane and 7.5 mL of K2CO3 (6%), vortexed and centrifuged for make the separation in two phases.

Gas-chromatograph condition

Fatty acids methyl esters were separated in a capillary column (CP-select CB for Fame; 100 m×0.32 mm i.d., 0.25-µm film thickness, Varian Inc., Palo Alto, CA) and quantified using nonadecanoic acid (C19:0) methyl ester (Sigma Chemical Co., St. Louis, MO). The injector and FID temperatures were 255 °C. For all samples the temperature program was as follows: 75 °C for 1 min, increased at 8 °C/min to 165 °C, held for 35 min, increased at 5.5 °C/min to 210 °C, held for 1 min, and finally increased at 15 °C/min to 240 °C held for 15 min. The split ratio was 1:40 and He was the carrier gas with a pressure of 37 psi.

FA have been identified in "chromatographic run" thanks FA standard used for recognize its conduct. Internal standards used for quantify relative amount of other FA were C5:0 and C19:0.from integrate of different peach we can know amount of each FA in acidic profile of sample (Figure 5.1).

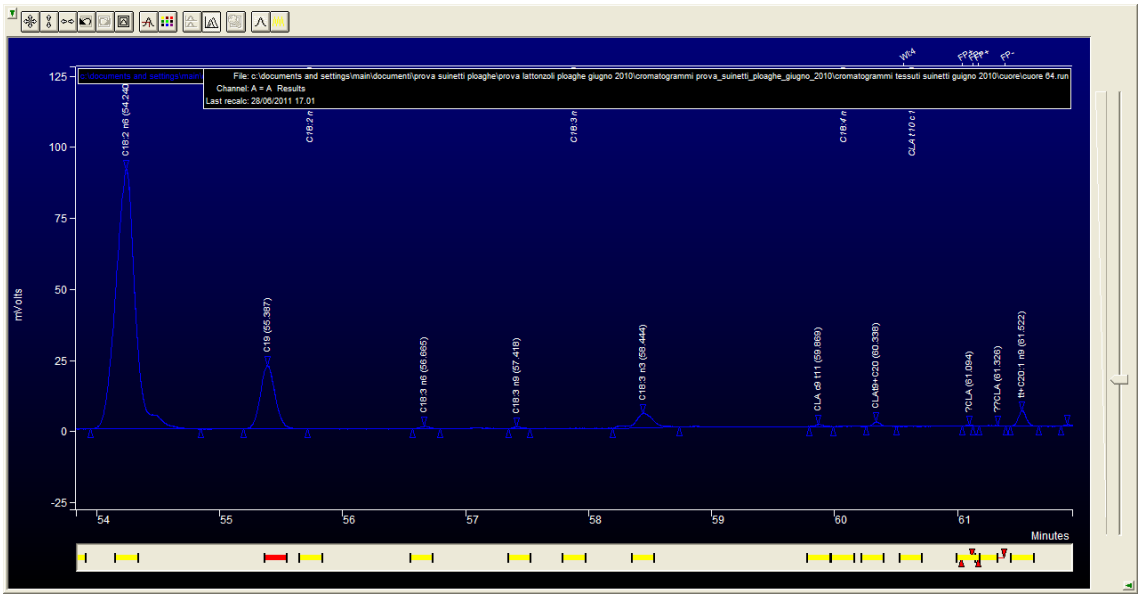

Figure 5.1. a view of chromatographic window work.

Table 5.1. Saturated fatty acids

Lipid Numbers	Common Name	IUPAC name	Structural Formula
C5:0	Valeric acid	Pentanoic acid	$CH_3(CH_2)_3COOH$
C6:0	Caproic acid	Hexanoic acid	$CH_3(CH_2)_4COOH$
C8:0	Caprylic acid	Octanoic acid	$CH_3(CH_2)_6COOH$
C10:0	Capric acid	Decanoic acid	$CH_3(CH_2)_8COOH$
C11:0	Undecylic acid	Undecanoic acid	$CH_3(CH_2)_9COOH$
C12:0	Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$
C13:0	Tridecylic acid	Tridecanoic acid	$CH_3(CH_2)_{11}COOH$
C14:0	Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$
C15:0	Pentadecylic acid	Pentadecanoic acid	$CH_3(CH_2)_{13}COOH$
C16:0	Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$
C17:0	Margaric acid	Heptadecanoic acid	$CH_3(CH_2)_{15}COOH$
C18:0	Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$
C21:0	Heneicosylic acid	Heneicosanoic acid	$CH_3(CH_2)_{19}COOH$
C22:0	Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$
C24:0	Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$

Table 5.2. Monounsaturated fatty acids.

Lipid	Double bound	Common	Iupac name	Structural formula
C16:1	7	Palmitoleic	hexadec-9-enoic	$CH_3(CH_2)_5CH=CH(CH_2)$
C18:1	c9	Oleic acid	(9Z)-Octadec-9-	$CH_3(CH_2)_7CH=CH(CH_2)$
C18:1	t9	Elaidic acid	(E)-octadec-9-	$CH_3(CH_2)_7CH=CH(CH_2)$
C18:1	11	Vaccenic	(E)-Octadec-11-	$CH_3(CH_2)_5CH=CH(CH_2)$
C20:1	11	Eicosenoic	(*Z*)-Eicos-11-	$CH_3(CH_2)_7CH=CH(CH_2)$
C22:1	11	cetoleic	(Z)-docos-11-	$CH_3(CH_2)_9CH=CH(CH2$
C22:1	13	euricic acid	(Z)-docos-13-	$CH_3(CH_2)_7CH=CH(CH_2)$
C24:1	15	nervonic	(Z)-tetracos-15-	$CH_3(CH_2)_7CH=CH(CH_2)$

Table 5.3. Poliunsaturated fatty acids.

Lipid number	Double bound	Common name	Iupac name	Structural formula
C18:2	9-12	linoleic acid	(9Z,12Z)-octadeca-9,12-dienoic acid	$C_{18}H_{32}O_2$
C18:3	9- 12- 15	alpha-linolenic	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	$C_{18}H_{30}O_2$
C20:3	8- 11- 14	eicosatrienoic acid	(8Z,11Z,14Z)-icosa-8,11,14-trienoic acid	$C_{20}H_{34}O_2$
C20:4	5- 8- 11- 14	arachidonic acid	(6Z,9Z,12Z,15Z)-octadeca-6,9,12,15-tetraenoic acid	$C_{20}H_{32}O_2$
C20:5	4- 8- 12- 15- 18	eicosapentanoic acid	(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid	$C_{20}H_{30}O_2$
C22:5	4- 8- 12- 15- 19	docosapentaenoic acid	(7Z,10Z,13Z,16Z,19Z)-docosa-7,10,13,16,19-pentaenoic acid	$C_{22}H_{34}O_2$
C22:6	4- 7- 10- 13- 16- 19	docosahexaenoic acid	(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid	$C_{22}H_{32}O_2$

Table 5.4. Fatty acids n-3.

Lipid number	Double bound	Common name	Iupac name	Structural formula
C18:3	9- 12- 15	alpha-linolenic	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	$C_{18}H_{30}O_2$
C20:5	4- 8- 12- 15- 18	eicosapentaenoic acid	(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid	$C_{20}H_{30}O_2$
C22:5	4- 8- 12- 15- 19	docosapentaenoic acid	(7Z,10Z,13Z,16Z,19Z)-docosa-7,10,13,16,19-pentaenoic acid	$C_{22}H_{34}O_2$
C22:6	4- 7- 10- 13- 16- 19	docosahexaenoic acid	(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid	$C_{22}H_{32}O_2$

Table 5.5. Fatty acids n-6.

Lipid numbers	Double bound position	Common name	Iupac name	Structural formula
C18:2	9-12	linoleic acid	(9Z,12Z)-octadeca-9,12-dienoic acid	$C_{18}H_{32}O_2$
C20:3	8- 11- 14	eicosatrienoic acid	(8Z,11Z,14Z)-icosa-8,11,14-trienoic acid	$C_{20}H_{34}O_2$
C20:4	5- 8- 11- 14	arachidonic acid	(6Z,9Z,12Z,15Z)-octadeca-6,9,12,15-tetraenoic acid	$C_{20}H_{32}O_2$

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