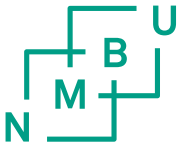
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Acknowledgement

This master thesis was written at Department of Animal and Aquacultural Sciences (IHA) at the University of Life Science (NMBU), spring 2015. This thesis is a part of a master degree in animal science with specialization in nutrition.

My interest for companion animals and nutrition made the choice of master degree simple. This thesis was chosen because I wanted to use up to date and relevant data from the feed industry to get a different perspective and immerse myself in the study of pet nutrition.

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"We keep moving forward, opening new doors and doing new things because we're curious. And curiosity keeps leading us down new paths." - Walt Disney

Department of Animal and Aquacultural Sciences
NMBU
Ås, 15.05.2015

……
Juni Solstad Karlsen

Summary

This thesis is divided into two sections: section 1 explaining the theory about fat and fatty acids functions, health effects and sources, section 2 includes a study of commercial extruded and raw dog foods.

Fat is the most energy dens nutrient and functions as energy, structural components in cell membranes, source of essential fatty acids (EFA), precursor to biological active substrates and carrier of fat-soluble vitamins. EFA cannot be synthesized by the animal, and needs to be added in the feed. EFA includes n6 fatty acids: linolenic acid (LA) and arachidonic acid (AA), and n3 fatty acids: α-linolenic acid (ALA), EPA and DHA. LA and ALA being precursors for AA, and EPA and DHA, respectively. The n6 and n3 families compete for the same enzymes for elongation and desaturation in the body. The n6 fatty acids, especially AA and its eicosanoids, have an inflammatory effect and induces responses to infections in the body. Whereas long chain n3 fatty acids and its eicosanoids are less biologically active and have anti-inflammatory responses. Therefore, the dietary concentrations of n6 and n3 fatty and dietary n6:n3 ratios can give different biologically responses through its eicosanoids. Vegetable oils are good sources for LA and ALA, marine sources are the only source for EPA and DHA, and AA is only found in animal sources. Recommended supply for LA to adult dogs are established, while supplementation for ALA, EPA and DHA is recommended by National Research Council (NRC), but not by the Association of American Feed Control Officials (AFFCO) or European Pet Food Industry Federation (FEDIAF). The two latter organisation have recommendations, besides LA, for puppies only. The scientific knowledge on EFA supply in dog foods is therefore not complete.

The present study was conducted to obtain information about the content of fat and fatty acid composition in 18 commercial dog foods (11 extruded and 7 raw foods) in the Norwegian market, by comparing low price (LP, n=4) and high price (HP, n=7) dry foods, and extruded and RWs (n=7). The extruded foods had substantially higher average concentrations of carbohydrates (49.6-56.0 %) on DM basis than the RW diets (9.7 %) and conversely, fat levels (42.4 %) in the RW group on DM basis, were significantly higher than the dry foods (11.9-15.8 %). The content of saturated fatty acid (SFA) was significantly higher in the RW group ($p<0.0001$), due to high amounts of beef tallow compared to the dry foods. All diets were above or met the NRC's recommendation of LA (0.67 g/MJ), except for two diets in the

RW group (0.35 and 0.33 g/MJ). All but one diet (0.01 g/MJ) met the recommendation level for ALA of 0.03 g/MJ. Several diets had levels high above recommendation, especially diets in the RW group (average of 0.2 g/MJ). The AA was present in all diets and varied from 0.02 in the LP group, 0.03 in the HP group to 0.06 g/MJ in the RW group. However, there were only significant difference ($p<0.005$) between the dry foods and the RWs. EPA and DHA concentrations varied greatly between diets (0.0-0.56 g/MJ), but was not significantly different between groups. One diet differed substantially from the others with the highest concentration of EPA and DHA of 0.56 g/MJ, this was approximately 18 times higher than the recommendation (0.03 g/MJ). Another diet deviated by not containing EPA or DHA, however, this diet contained the highest amounts of ALA (2.03 g/MJ) among the dry foods. Dietary n6:n3 ratio was significantly higher ($p<0.02$) for the LP group (8.3:1), compared to the HP (4.7:1) and RW (4.0:1) group. The ratio was especially low in one of the dry foods (1.2:1).

To conclude, the EFA and dietary n6:n3 ratios in individual diets varied substantially, irrespective of diet type, extruded or raw. The EFA content differed between the low price and high price group, but not significantly. Indicating that great individual differences between diets gave high variations within each group. Raw diets contained a higher content of fat (% DM) than the extruded diets, but had similar levels of EFA. AA was the only single fatty acid significantly higher in the raw foods, compared to the extruded diets.

Sammendrag

Denne masteroppgaven er delt inn i to deler: del 1 forklarer teorien om fett og fettsyrers funksjoner, helseeffekter og kilder, seksjon 2 omfatter en studie av kommersielle ekstruderte- og rå hundefôr.

Fett er det mest energirike næringsstoffet og fungerer som energi, strukturelle komponenter i cellemembraner, kilde til essensielle fettsyrer (EFA), forløperen til biologisk aktive substrater og bærer av fettløselige vitaminer. EFA kan ikke syntetiseres av dyr, og må tilsettes i fôret. EFA inkluderer n6 fettsyrer: linolensyre (LA) og arakidonsyre (AA), og n3 fettsyrer: α-linolensyre (ALA), EPA og DHA. LA og ALA er forløpere for henholdsvis AA og EPA og DHA. De to fettsyre familiene, n6 og n3, konkurrerer om de samme enzymene for forlengelse og desaturase i kroppen. Fettsyrer fra n6 familien, spesielt AA og dens eikosanoider, har en inflammatorisk virkning og induserer responser ved infeksjoner i kroppen. Derimot er langkjedede n3 fettsyrer og dens eikosanoider mindre biologisk aktive og har anti-inflammatoriske responser. Derfor kan konsentrasjonen av n6 og n3 fettsyrer og n6: n3 forholdet gi ulike biologiske responser gjennom sine eikosanoider. Vegetabilske oljer er gode kilder for LA og ALA, marine kilder er den eneste kilden for EPA og DHA, og AA finnes kun i animalske kilder. Anbefalt dosering for LA til voksne hunder er bestemt, mens tilskudd av ALA, EPA og DHA er anbefalt av National Research Council (NRC), men ikke av Association of American Feed Control Officials (AFFCO) eller European Pet Food Industry Federation (FEDIAF). De to sistnevnte organisasjon har anbefalinger utover LA kun for valper. Den vitenskapelige kunnskap om EFA doseringen i hundefôr er derfor ikke komplett.

Denne studien ble gjennomført for å få informasjon om innholdet av fett og fettsyresammensetning i 18 kommersielle hundefôr (11 ekstruderte- og 7 råfôr) i det norske markedet, ved å sammenligne lav pris (LP, n = 4) og høy pris (HP, n = 7) ekstruderte fôr, og ekstruderte fôr og RW (n = 7). De ekstruderte fôrene hadde betydelig høyere gjennomsnittskonsentrasjoner av karbohydrater (49,6 til 56,0%) på DM basis enn RW (9,7%) og omvendt, fettnivåene (42,4%) i RW gruppen på DM basis, var betydelig høyere enn de ekstruderte fôrene (11,9 til 15,8%). Innholdet av mettede fettsyre (SFA) var signifikant høyere i RW gruppen ($p < 0,0001$), på grunn av høye mengder av oksetalg i forhold til de ekstruderte fôrene. Alle fôrene var over eller tilfredsstilte NRC sine anbefaling for LA (0,67 g / MJ), med unntak av to fôr i RW-gruppen (0,35 og 0,33 g / MJ). Alle unntatt et fôr (0,01 g / MJ) møtte

anbefaling nivå på 0,03 g / MJ ALA. Flere fôr hadde nivåer høyt over anbefaling, spesielt fôr i RW gruppen (gjennomsnitt på 0,2 g/MJ). AA var til stede i alle fôr og varierte fra 0,02 i LP-gruppen, 0,03 i HP-gruppen til 0,06 g/MJ i RW gruppen. Likevel var det bare signifikant forskjell ($p < 0,005$) mellom de ekstruderte fôrene og RW. EPA og DHA konsentrasjoner varierte mye mellom fôrene (0,0 til 0,56 g/MJ), men var ikke signifikant forskjellig mellom gruppene. Et fôr skilte seg vesentlig fra de andre med den høyeste konsentrasjon av EPA og DHA på 0,56 g/MJ, og var omtrent 18 ganger høyere enn anbefaling (0,03 g/MJ). Et annet fôr avviker ved å ikke inneholde EPA eller DHA, men dette fôret inneholdt den høyeste mengden av ALA (2,03 g/MJ) blant de ekstruderte fôrene. Forholdet mellom n6: n3 var signifikant høyere ($p < 0,02$) for LP-gruppen (8,3:1), sammenlignet med HP (4,7:1) og RW (4,0:1) gruppe. Forholdet var spesielt lavt i en av ekstruderte fôrene (1,2: 1).

For å konkludere, EFA og forholdet mellom n6: n3 variert betydelig mellom individuelle fôr, uavhengig av type, ekstrudert eller rå. EFA innhold avvek mellom lav og høy pris gruppen, men ikke signifikant. Hvilket indikerer at store individuelle forskjeller mellom fôr ga høye variasjoner innenfor hver gruppe. Rått fôr inneholdt et høyere innhold av fett (% DM) enn de ekstruderte fôrene, men hadde tilsvarende nivåer av EFA. AA var den eneste fettsyren som var signifikant høyere i rå fôrene, i forhold til de ekstruderte fôrene.

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Abbreviations

EFA – Essential fatty acids

SFA – Saturated fatty acids

MUFA – Monounsaturated fatty acids

PUFA – Polyunsaturated fatty acids

n6 – omega 6 fatty acids

n3 – omega 3 fatty acids

FFA – Free fatty acids

LA – linoleic acid

ALA – α-linolenic acid

AA – arachidonic acid

GLA – γ-linolenic acid

EPA – Eicosapentaeonic acid

DHA – Docosahexanenoic acid

PG – Prostaglandins

LT – Leukotrienes

ATTD – Apparent total tract digestibility

ME – Metabolizable energy

CP- Crude protein

CF – Crude fat

CHO – Carbohydrate

LP – Low price food

HP – High price food

RW – Raw food

Introduction

In today's society, humans are concerned with general health and living a healthy lifestyle. This focus has been transferred to our companion animals, especially to the dog. Nutrition is a major aspect of this drive and proper nutrition is crucial for normal growth and biological functions in both humans and dogs. Although deficiency symptoms are rare as pets generally are fed well balanced diets, there are numerous differences between commercial foods, which could lead to deficiency.

Since the 1920's fatty acids have been considered vital for normal body function (Bauer et al. 1998). Fats associated with disorders and diseases have received more attention in recent years, especially since fat in pet foods has an impact on the dog's energy, fitness and development. Scientists have raised awareness about the importance of the essential fatty acids (EFA), whether it should be a requirement in dog foods, and whether linoleic acid (LA) and α-linolenic acids (ALA) alone are adequate. In commercial dog foods health claims based on content of nutrients e.g. EFA are often used for marketing.

The largest and most acknowledged dog food producers claims that their food supplies EFA according to current recommendations. Nutritional recommendations for dogs given by different institutions are normally similar, however, guidelines given by National Research Council (NRC) (2006), The Association of American Feed Control Officials (AAFCO) (2014) and The European Pet Food Federation (FEDIAF) (2014) are not consistent in their recommendations for n3 long-chain fatty acids. NRC (2006) recommends supplement for puppies and adult dogs, while AAFCO (2014) and FEDIAF (2014) only recommend supplement for puppies. These differences indicate that requirement for n3 EFA in dogs are yet to be established, as research is scarce and not explicit

This thesis is divided into two sections: section 1 explains the theory about fat and fatty acids functions, health effects and sources: section 2 includes a study of commercial extruded foods and raw foods (RW), comparing low and high price diets, and dry foods and RWs.

Section 1

Fat and fatty acids

Fat or lipids is one of the major biological substrates in the body. Fat functions as energy, structural components in cell membranes, source of essential fatty acids (EFA), precursor to biological active substrates and carrier of fat-soluble vitamins (Alexander 1998; Wiseman & Kendall 1984). Lipids account for 5-25 % or more of the body tissue (Mathews et al. 2000), and the fatty acid composition in adipose tissue and cell membranes are highly influenced by the diet (Sargent et al. 2002). Most fats are highly digestible, and the body's ability to store fat is almost unlimited compared to carbohydrate storage. On a weight basis, fat is a dense nutrient and provides more than twice the amount of energy compared to protein and carbohydrates. Fat is therefore important for adjusting the dietary energy content. Furthermore fat also gives texture and palatability to dog food (Wiseman & Kendall 1984).

The biologically important and most abundant lipids are triglycerides (TG), phospholipids (PL) and sterols (of which cholesterol (CE) is the major form). TG are stored in adipose tissue as an energy reserve and thermal insulation, PL are a major constituent of cell membranes (Mathews et al. 2000). Unlike most lipids, glycerol and fatty acids are absent in CE (Sargent et al. 2002). CE is mainly found in the plasma membrane in all mammalian cells. The physiological effect of CE reduces the permeability of small water-soluble molecules and prolong the membranes viscosity; in addition CE is a part of bile salt production (Vance & Vance 1985). Many complex lipids in the body, like CE and TG, cannot circulate in free form, as they are not water-soluble. Consequently, lipids are attached to proteins, making water-soluble lipoproteins. Lipids have lower density than proteins, so the balance between them determines the density of lipoprotein. Excess of lipids will give very low-density lipoproteins known as VLDL or low-density lipoprotein (LDL), as will an excess of protein give very high-density lipoprotein (VHDL) or high-density lipoprotein (HDL). LDL plays a role in transporting CE to adipose tissue, as HDL returns the excess of CE from adipose tissue back to the liver, for either metabolism or excretion (Mathews et al. 2000). Accumulation of CE over time may develop fatty sediments on the inside of coronary arteries, also called atherosclerotic plaques (Mathews et al. 2000). Elevated levels of CE in the blood increase the risk of heart disease in humans (Lamarche et al. 1997). About 2/3 of plasma CE in humans are LDL form (Mathews et al. 2000) and in swine, which are similar to humans, the majority of

CE is in the LDL form. Dogs however have HDL as the dominant portion of CE (Julien et al. 1981). The fact that dogs are adapted to high fat levels from a natural diet in the wild, presumably explains why CE in dogs are transported as HDL (Watson 1996). These differences describe why dogs can handle more fat in the diet than humans can, without increasing risk for atherosclerosis.

TG and PL are made up of fatty acids attached to glycerol, which are divided into classes according to their saturation; saturated (SFA) with no double bonds, monounsaturated (MUFA) with one double bond and polyunsaturated (PUFA) with two or more double bonds (Lenox & Bauer 2013). Fatty acids are also classified according to chain length, and position of the first double bond from the methyl end of the carbon chain, resulting in fatty acid families such as n3, n6 and n9 (Sargent et al. 2002). The n3 and n6 family is PUFAs, while most of the n9 is MUFAs (Alexander 1998).

Liver and adipose tissue are the two main tissues that produce fatty acids or de novo lipogenesis (Nguyen et al. 2008; Vance & Vance 1985). In adult dogs, the adipose tissue is considered to be the main organ of fatty acid synthesis (Stangassinger et al. 1986). Fatty acids with double bonds closer than carbon nine from the methyl end cannot be synthesized by the animal, and need to be added in the feed (McDonald et al. 2011). The ability to synthesize fatty acids into acetyl CoA in dogs and other animals stops at C16:0 (palmitic acid) and C18:0 (stearic acid) and the desaturation capacity is limited to synthesizing C16:1 n-7 (palmitoleic acid) and C18:9 n9 (oleic acid) by delta -9 desaturase (Sargent et al. 2002). Although mammals cannot synthesize LA (C18:2 n6) and ALA (C18:3 n3) they can further synthesize them by elongation into arachidonic acid (AA, C20:4 n6), eicosapentaenoic acid (EPA, C20:5 n3) and docosahexaenoic acid (DHA, C22:6 n3) respectively. Both n6 and n3 families compete for the same enzymes, however, ALA is a more preferred substrate for the enzyme Δ6-desaturase (Calder 2005).

By adding double bonds to the chain (desaturation) or by elongation of the acyl chain, enzymes remodels LA and ALA into long-chained PUFAs (Figure 1) (Calder 2006; Holman 1998). The rate of converting LA to γ-linoleic acid (GLA, 18:3 n-6) and ALA to stearidonic acid (SA, 18:4 n3) is depended on the availability of Δ6-desaturase (Maniongui et al. 1993). The conversion of ALA by Δ6-desaturase is far more efficient, than the conversion of LA. However, a study performed by Bauer et al. (1998) found that dogs given a diet with high

amounts of ALA, accumulated more LA. A possible theory was that ALA has a sparing effect on LA (Bauer 2007). Studies have shown that the conversion of ALA to EPA is more efficient than ALA to DHA (Bauer et al. 1998). It is debated whether ALA as a precursor is adequate, or if direct supplementation of EPA and DHA is needed. A study performed by Heinemann et al. (2005) indicated that ALA alone was not sufficient. Puppies suckling from a mother fed an EFA deficient diet was able to synthesize EPA and DHA from ALA when the requirement was high. After weaning, the conversion of ALA to DHA and EPA became less efficient. Bauer et al. (1998) found similar results in adult dogs. Dogs fed a diet with a moderate increase in ALA had a rapid increase of EPA and other n3 fatty acids in the plasma lipids, however, the amount DHA was unchanged. This indicates that the conversion of ALA to DHA is limited in the liver in adult animals (Bauer et al. 1998). In addition, a study conducted on cats, found that the brain tissue played an important part in the conversion of DPA (C22:5 n-3) to DHA (Pawlosky et al. 1994).

Both n6 and n3 families are precursors for eicosanoids from AA, EPA and DHA (Calder 2006; Vance & Vance 1985). Eicosanoids produced from the two families have similar structure, but different biological characteristics. Eicosanoids includes any C_{20} fatty acids and can be divided into two groups: one contains the prostaglandins (PG) and thromboxanes (TX), the second contains hydroxy- and hydroperoxy fatty acids and leukotrienes (LT) (Wander et al. 1997). In general, AA is the major component in eicosanoid synthesis. Fatty acids are released from the cell membrane phospholipids by phospholipase (A_2) and are desaturated to eicosanoids. AA are metabolised by cyclo-oxygenase, giving TX and PG of the 2-series and metabolised by lipoxygynase to LT 4-series (Figure 1).

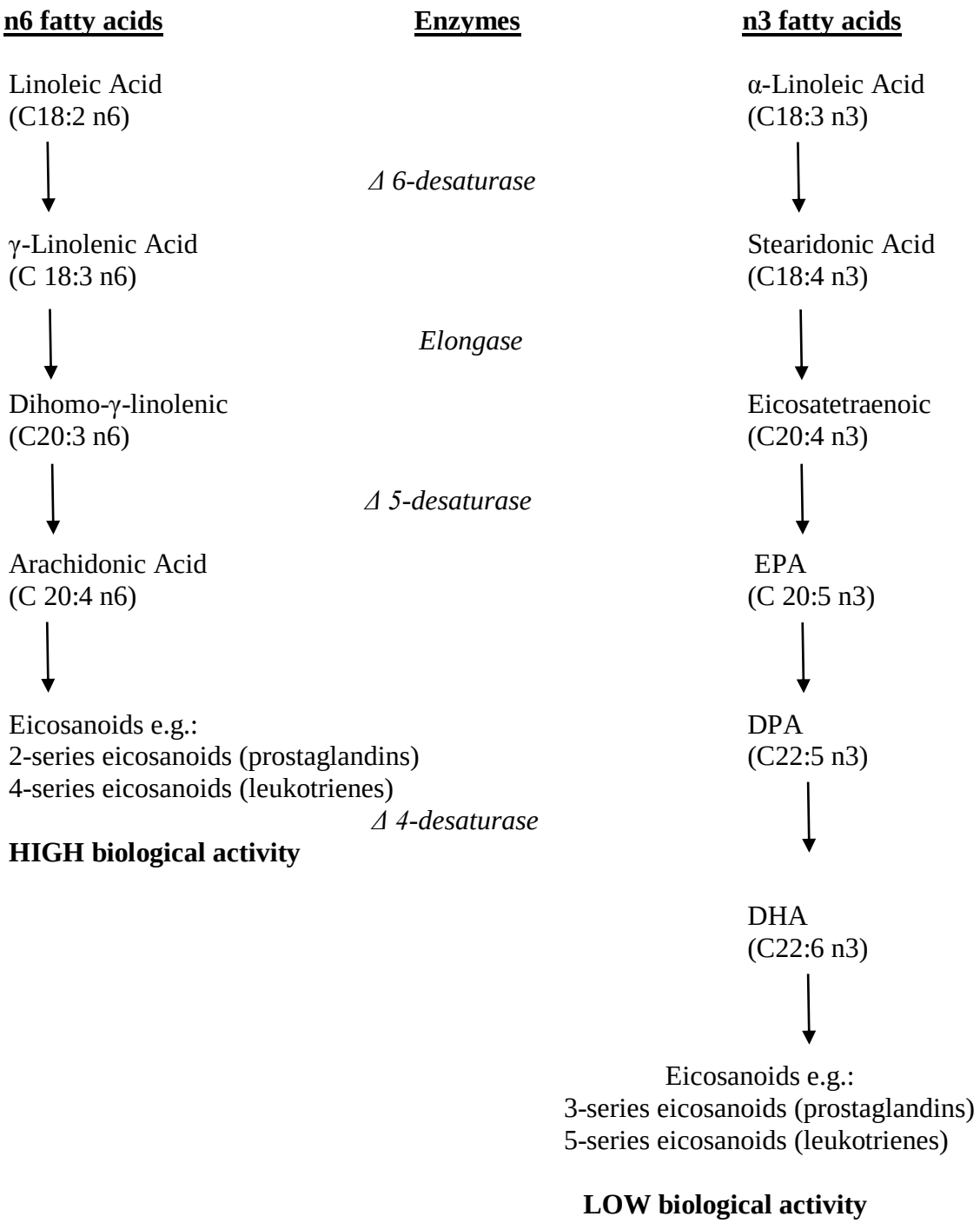

Figure 1: Schematically overview of the metabolism of n6 and n3 fatty acids.

When energy is needed, as in periods of starvation, adipose tissue is broken down to free fatty acids (FFA) and transported bound to albumin in plasma to muscle tissue for oxidation (Shug & Keene 1991). FFA are mainly utilized by β-oxidation in the mitochondria to acetyl-CoA and can then enter the citric acid cycle. The citric acid cycle is the endpoint for fatty acids catabolism, producing ATP. Citrate is the end product and is transported from the mitochondria to cytosol, as a substrate in fatty acid synthesis (Mathews et al. 2000). Dogs

have a great ability to utilize FFA as an energy source during rest and training, in addition, they can adapt to increased FFA utilization for muscle work with high fat diets in aerobic conditions (Grandjean 1994).

Essential fatty acids functions

It has become evident that n3 fatty acids is crucial in retinal-, brain- and neural-tissue development. A high amount of DHA in brain and retina tissue indicates that DHA has an especially important role in these tissues (Anderson et al. 1990; Litman et al. 2001). The n6 fatty acids, like LA, is essential to maintain the epidermal skin's ability to resist water and preserving a good skin and coat. GLA and AA produced from LA and its eicosanoids are generally found in phospholipids in the skin (Kirby et al. 2007).

Eicosanoids derived from n6 fatty acid (e.g. PG_2 and LT_4) are more reactive in biological responses than eicosanoids formed from n3 fatty acids (e.g. PG_3 and LT_5) (Alexander 1998). The eicosanoids have physiological effects such as immune response, inflammatory response, cardiovascular tone, renal function, blood clotting, neural function and reproduction. Eicosanoids are hormone-like compounds with a short half-life. Unlike hormones, eicosanoids are not stored, instead they are produced in specific cells (Sargent et al. 2002). PG_2 have several pro-inflammatory effects, including inducing fever and enhancing pain and oedema caused by other substrates. LT_4 increase vascular permeability and enhance production of cytokines among others. PG_3 and LT_5 derived from n3 fatty acids have less inflammatory effect or anti-inflammatory effect than the n6 (Calder 2006). By competing for the same conversion enzymes, n3 suppresses dome of the inflammatory response from n6 (Calder 2006).

Increased intake of n3 PUFAs gives elevated levels of EPA and DHA in inflammatory cell phospholipids at the expense of AA. Several cytokine productions are regulated by eicosanoids. A change in eicosanoid production as a result of increased n3 PUFAs would probably influence the cytokine production and its biological effect (Meydani et al. 1993). As cytokines main source and target are the immune system cells, an alteration in cytokine production could have an impact on the immune reaction (Wander et al. 1997), e.g. leading to reduced wound healing (Lenox & Bauer 2013). A balance between n6 and n3 is therefore vital because of their difference in biological strength.

Essential fatty acids and health effects

Deficiency in EFA is uncommon as most dogs are fed complete and balanced pet foods, however deficiency occurs occasionally, e.g. by reduced food intake, poor digestion, poorly formulated diets or long storage time of the food (Watson 1998). There are individual differences in biological responses to foods, which in some cases result in deficiency symptoms often reflected in the skin and coat (Wiese et al. 1966). As for most disorders and diseases, the level of EFA required in the body is dependent on interactions between genes and environment. Environment in this case being the diet (Sargent et al. 2002). Positive effects like improved skin and coat have been associated with n3 PUFAs, but adverse responses have also been reported. Effects and functions of n3 PUFAs are listed in Table 1.

*Table 1: Effect of increased content of long-chain n3 fatty acids in dogs and other species**

Organ/ Disorder	Effect	Reference
Skin	Improved skin and coat	Watson (1998), Logas and Kunkle (1995)
Hearth	Antiarrhythmic effect	Smith et al. (2007)
Retina	Normal development and function	Anderson et al. (1990)*, Heinemann et al. (2005)
Immune system	Supressed cell mediated immune response	Wander et al. (1997)
Neural system	- Normal development and function	Anderson et al. (1990), Pawlosky et al. (1994)*
	- Improved trainability in puppies	Hoffman et al. (2004)
Osteoarthritis	Improved movability	Roush et al. (2010)
Wound healing	Prolonged wound healing	McDaniel et al. (2008)*
Renal failure	- Reducing development, increasing longevity	Brown et al. (1998)
	- Worsening the condition	Logan et al. (1992)*

A study conducted on rats discovered that feeding an EFA deficient diet resulted in different fatty acids composition among the organs. The heart and muscles retained EPA and AA, while red bloods cells, liver and kidney showed reduced levels of AA and EPA (Moussa et al.

1996), implying that EFA play different roles for organ function and that the body can economize and distribute EFA to the where it is most needed when scarce.

Skin disorders or diseases are a one of the common problems detected by dog owners. The skin is the body's largest metabolically active organ. Dietary intake of nutrients are therefore highly important for maintenance of a healthy coat and skin (Watson 1998). Therapeutic effects are found by supplementation of PUFA, especially for pruritic skin diseases associated with hypersensitivity reactions, hypersensitivity to certain foods, atopic dermatitis and idiopathic pruritus (Watson 1998). Skin disorders related to deficiency can be improved by changing to a diet with higher fat content or by adding food oils or fatty acid supplementation (often vegetable or marine oils). Generally, supplements with high levels of LA may be useful for dry and dull skin and coat, without inflammation (Watson 1998). Supplementation with sources rich in EPA and DHA, replaces AA in the cell membrane, resulting in reduced production of n6 pro-inflammatory eicosanoids in favour of less inflammatory eicosanoids from n3 fatty acids (Logas & Kunkle 1995). Improved skin and coat may be due to changes in fatty acids alone but could also could also be affected by changes in protein, vitamin and zinc level (Watson 1998).

Special foods such as joint, renal and dermatological diets have an elevated amount of n3 PUFA compared to maintenance diets, yet, the amount may not be sufficient to prevent further development of several disorders (Lenox & Bauer 2013). It could be speculated if the various positive health effects is partly the reason why nutritional guidelines like NRC (2006) have specific recommendations for n3 fatty acids, and that producers have started supplementing and promote EFA in dog foods.

Essential fatty acid sources

Commercial dog foods often contain more than one fat source and are therefore a mixture of SFA, MUFA and PUFA, including EFA. Animal fats are generally more saturated than vegetable- and marine-oils, in addition, animal fat is the main source of AA. Fatty acids with a chain length of C_{16} to C_{18} is normally found in animal and plant tissue, while longer chains like C_{20} to C_{22} are common in marine oils (Austreng et al. 1979; Bauer et al. 1998; Rouvinen 1990). As synthesis of fatty acids in the body stops at one single double bond, the amount of MUFA is naturally high in animal sources. LA, AA and ALA content is generally low in beef

tallow, compared to chicken fat or lard. Most marine oils contain little LA, AA and ALA, but have high levels of EPA and DHA with some variations depending on fish species. Vegetable oils are rich in LA or ALA, or both, depending on the species. Proximate fatty acid composition and EFA content in different fat sources is summarized in Table 2.

Table 2: Fatty acid composition (% of total fatty acids) in common fat sources. Explanations of abbreviations see abbreviations page 3. (Source: Hand et al. (2000) and NRC (2006))

	SFA	MUFA	PUFA	LA	AA	ALA	EPA	DHA
Fats								
Beef tallow	47.4	40.2	4.0	2.0	0.8	0.6	nd	nd
Chicken fat	28.6	43.0	22.1	19.0	0.75	1.3	nd	nd
Lard	38.9	43.9	12.2	10.0	1.7	1.0	nd	nd
Marine oils								
Menhaden	30.5	24.8	26.6	25.0	nd	nd	15	9
Capelin	20.0	61.7	12.2	1.7	0.1	0.4	4.6	3.0
Salmon (sea caught)	18.6	41.2	33.5	1.2	0.9	0.6	12.0	13.8
Vegetable oils								
Rapeseed oil	5.8	56.3	33.2	14-22	nd	7-10	nd	nd
Flaxseed oil	9.4	20.2	66.0	16.0	nd	53.0	nd	nd
Safflower oil	8.6	12.1	74.5	76.0	nd	0.5	nd	nd
Soybean oil	14.2	23.0	57.8	54.0	nd	7.0	nd	nd
Sunflower	8.9	45.5	40.0	39.8	nd	0.2	nd	nd

nd = not detected in analysis

Nutritional recommendations

To date, LA, is the only fatty acid considered essential to dogs in all nutritional guidelines. Requirement for n3 fatty acids is not verified experimentally, although dogs may have a need for dietary n3 supplementation during different life stages (Ahlstrom et al. 2004; Bauer et al. 1998).

Recommendation are set in accordance with metabolizable energy (ME). The three corporations have the same recommended amount of total fat of 13.8 g/ 1000 kcal ME corresponding to 3.29 g/ MJ. NRC (2006) recommends 2.8 g/ 1000kcal LA, equal to 0.67 g/ MJ. AAFCO (2014) has the same recommendation for LA, whereas FEDIAF (2014) recommends 0.79 g/MJ. Only NRC (2006) lists ALA, EPA and DHA as essential and provide recommended levels. Recommended amount of ALA is 0.11 g ALA/ 1000 kcal ME, equivalent to 0.03 g/ MJ. Likewise, the recommended amount of EPA and DHA is 0.03 g/

MJ. EPA and DHA recommendations are combined by NRC (2006), estimating a mixture of 50-60 % EPA and 40-50% DHA. AAFCO (2014) states there is not sufficient scientific experiments supporting a specific required amount for adult animals. Instead of suggesting an amount, AAFCO sets an upper limit (LA+AA):(ALA+EPA+DHA) ratio of 30:1. The recommendations are summarized in Table 3.

Table 3: Recommendations (g/MJ) for NRC, AAFCO and FEDIAF for adult dogs.

	NRC	AAFCO	FEDIAF
Total fat	3.29	3.29	3.29
LA	0.67	0.67	0.79
ALA	0.03	-	-
EPA/DHA	0.03	-	-
n6:n3 (upper limit)	-	30:1	-

The optimal dietary n6:n3 ratio is difficult to interpret, as the scientific basis of the ratio is may be different. The n6:n3 ratio may only include LA and ALA, or the total content of n6 and n3 fatty acids (including AA, EPA, DHA and others). Two diets with the same n6:n3 ratio therefore may have different fatty acid composition and concentration levels (Hall et al. 2006; NRC 2006). The correct ratio or concentration of n6 or n3 fatty acids is therefore not yet determined for dogs (Wander et al. 1997).

Section 2

Analysis of commercial dog foods

Nutritional composition of foods is of vital importance to the health and well-being of our pets. The dog food producers also apply health claims similar to those in human nutrition in product marketing. Thus, dog owners have become more conscious of what they feed to their companion animals, causing the market to evolve into a broad variety of diets and food qualities. Dry foods have different standards and are often regarded as economy and premium diets. Economy diets are found in grocery stores and rely on easy access and low prices, targeting dog owners who want something simple or are conscious about price. Premium foods are sold in pet shops and at veterinarian clinics. Premium diets have a higher price range and focuses far more on health aspects and offering a broad variety of specialized foods. The target group is concerned owners, willing to pay more to keep their pets healthier

and to promote longevity. It is reasonable to think that the premium diets are more committed to nutritional performance of the feed, not just the basic requirement of the dog.

The demand for a more natural diet has led several dog owners to feed raw foods. Sled dogs and dogs with high energy requirements have used raw foods for many years, however ordinary pet owners started requesting the same for their companion dog in recent years. Raw diet's increased market share has resulted from dog owners demand for a less processed diet without heat treatment and preservatives, and with fewer, but more natural ingredients for their dog. Dry foods mainly contain processed ingredients that are reheated during extrusion. Dry foods also have a long shelf-life and, hence, added preservatives to secure satisfactory quality after several months of storage. Some dog owners also regard the high carbohydrate or grain content in dry foods to be negative, as dogs do not require carbohydrates in their diet. Similar to the focus in human nutrition, they believe that dietary carbohydrates could be harmful to health and cause diabetes and obesity in dogs. Few of the raw foods contain carbohydrates, but all normally have high fat content.

This section of the thesis examines the fat content, fatty acid composition and EFA content of commercial dog foods in the Norwegian market. The diets were grouped as followed: low price (LP), high price (HP) and raw foods (RW). In addition, fat digestibility in minks was determined for the extruded dry foods.

Aim of study: to compare differences in fatty acid composition between diets and evaluate whether fatty acid composition differ significantly between price groups (LP diets and HP diets) and between extruded and RW diets.

Predictions to be tested in the survey:

- Fatty acid composition would be substantially different among the dry foods.
- HP would contain higher levels of EFA compared to LP.
- In addition, the RWs would have higher fat content than the extruded dry foods, thereby show difference in fatty acid content and composition.

Material and methods

Diets

A total of 18 commercial dog foods: 11 extruded dry foods and 7 RW in the Norwegian market were used in the study (Table 4). The extruded dry foods were categorized into two groups; economic dry foods found in grocery shops (Low Price, LP), ranging from 13.9 kr/ kg to 25.2 kr/ kg and premium dry foods found in pet shops (High Price, HP) ranging from 38.8 kr/ kg to 65.2 kr/ kg. All extruded diets were intended for adult medium breeds, while the RW diets were for adult dogs irrespective of size.

Table 4: Diets divided by groups; low price dry foods (LP), high price dry foods (HP) and raw foods (RW), and producers.

Group	Diet	Produced by
LP	Doggy	Läntmannen Doggy AB, Vårgårda, Sweden
	Labb	Felleskjøpet Agri SA, Lillestrøm, Norway
	Pedigree	Mars Norge AS, Skøyen, Norway
	Snögg	Purina, Nestlé A/S, Oslo, Norway
HP	Appetitt	Felleskjøpet Agri SA, Lillestrøm, Norway
	Dr. Clauder	Dr.Clauder GmbH & Co. KG, Hamminkeln, Germany
	Eukanuba	Iams Europe BV., Coevórden, Nerherlands
	Fish4Dogs	Agri Marine Nutrition, Stavanger, Norway
	Hill's	Hill's Pet Nutrition, Lyngsby, Denmark
	Orijen	Champion Petfoods, Morinville, Alberta, Canada
	Royal Canin	Royal Canin S.A., Almarques, France

RW	MUSH Vaisto, pork, beef and salmon	MUSH, Finland
	Natures Menu, chicken, vegetables and rice	Natures Menu, Norfolk, England
	Natures Menu, tripe and chicken	Natures Menu, Norfolk, England
	Provit, tripe and beef	Norsk Dyremat AS, Rudshøgda, Norway
	Provit, tripe and lamb	Norsk Dyremat AS, Rudshøgda, Norway
	V&H, salmon	Vom&Hundemat, Trøgstad, Norway
	V&H, chicken and tripe	Vom&Hundemat, Trøgstad, Norway

From here on, the dog foods will be anonymous and referred to as diet 1-18.

Chemical analysis

The chemical analyses were carried out at two different laboratories. The laboratory at the department of Animal and Aquacultural Sciences, Ås, Norway analysed diets and faeces from the digestibility study for dry matter (DM) at 103°C and ash at 550°C until constant weight. Crude protein (CP) was determined by Kjeldahl-N *6.25 using Kjeltec applying AOAC method 2001.11. Starch was analysed by the same method as Mccleary et al. (1994). Eurofins laboratory in Moss, Norway, determined crude fat (CF) by hydrolysis with HCl-ether extraction in the diets and faeces and fatty acid composition by gas chromatography with flame ionization detector in the diets. Total carbohydrate (CHO) content was not analysed but calculated by subtracting CP, CF and ash from the DM content.

Digestibility study in mink

Fat digestibility values for the dry foods were determined by using mink. The study was conducted in 2014 at a laboratory at the research farm at Norwegian University of Life Sciences, Ås, Norway. The research laboratory is under supervision by the Norwegian Animal Research Authority, and the study was performed in accordance with institutional and national guidelines for the care and use of animals (the Norwegian Animal Welfare Act, and the Norwegian Regulation on Animal Experimentation).

The digestibility experiment was carried out in mink (*Neovison vison*) by quantitative measurement of dietary intake and faecal production in four animals per diet. Mink has shown to be a good model for main nutrient digestibility in dogs (Tjernsbekk et al. 2014; Vhile et al. 2005). Freeze-dried faeces from the dry foods were milled and sieved for hairs before being analysed. Further information about the protocol for the study can be obtained from Ahlstrom et al. (2004) and Tjernsbekk et al. (2014).

Apparent total tract digestibility (%) was calculated by:

[nutrient intake (g) – nutrient faecal output (g) /nutrient intake (g)] x 100.

Metabolizable energy determination

The chemical analysis of the diets, digestibility values of protein, fat and carbohydrate (by difference) was applied to determine metabolizable energy (ME) content of the dry diets.

Metabolizable energy (g/kcal) = [nutrient (g) x kcal[1]] x digestibility (%).

The factors applied were 4.45 kcal·g^{-1} for protein (5.7 kcal·g^{-1} corrected with 1.25 kcal·g^{-1} for nitrogen loss in urine), 9.4 kcal·g^{-1} for fat and 4.1 kcal·g^{-1} for carbohydrates (NRC 2006). For the RW, standard digestibility values of 91% for protein and 96 % for fat were used. These values are more similar to raw RW digestibility than standard values for dry food. Due to the low content of carbohydrate and absence of heat treatment in the RWs, the digestibility value was set to standard for dry foods, 85% (NRC 2006).

Statistical analyses

Statistical analysis were performed using SAS (2013), version 9.4 for Windows software. The general linear model procedure (GLM) for the analysis of variance was used. The model tested the fixed effect of groups (LP, HP and RW) on fat content and concentration of single EFA:

$$Y_{ijk} = \mu + \tau_i + \epsilon_{ijk}$$

where μ is the general mean, τ_i is the fixed effect of group and ε_{ijk} is the random error.

Results were stated as least-square means (LSMEANS), with the variance shown as pooled standard error of the means (SEM). Significance level was $p<0.05$.

Results

The results are presented in three sections: chemical composition, fatty acid composition and group comparison. The 18 diets were grouped into LP (n=4), HP (n=7) and RW (n=7). Dry foods refers to diet 1-11 in the results, which include LP diet 1-4 and HP 5-11. The RW follows with diet 12-18. Ingredient declared for the diets will be commented on, but is not presented in tables.

Chemical composition

Chemical composition on as fed basis

The chemical analyses confirmed that the chemical compositions were in accordance with the declaration for all diets (see attachments). There were minor differences in DM content among the dry foods (90.1-92.7 %), but substantial differences for the RWs (25.9-40.3 %) (Table 5). Differences between dry foods and RWs are due to great differences in DM content. Ash content was lower in the RWs compared to the dry foods, 1.0-4.1 to 4.8-7.7 % respectively. The CP values were similar amongst the dry foods (20.5-27.6% except for one diet of 35.2 %), and the RW (10.7-16.2 %). The lowest value of CF (8.2 %) was less than half that of the highest value (17.8 %) within the dry foods. The content of crude fat varied within the RWs by 9.4-20.4 %. The recommended allowance for dietary for adult is 3.29 g/MJ ME (NRC 2006). All the 18 diets contained fat above this level, 6.4 g/MJ for the lowest (diet 2) and 19.6 g/MJ (diet 16) for the highest (not shown). The content of CHO and starch was high in the dry foods, 31.9-56.3 % and 16- 40%, respectively, while only a few of the RW foods contained carbohydrate (0.5-10.2 %), starch was not determined in RWs. Starch generally constitutes to the largest fragment of carbohydrates in the dry foods.

Table 5: Content of dry matter (DM), ash, crude protein (CP), crude fat (CF), carbohydrate (CHO) and starch (%/ kg food) analysed for diet 1-18, divided into low price (LP), high price (HP) and raw foods (RW).

	Food No.	DM	Ash	CP	CF	CHO	Starch
LP	**1**	92.6	6.1	27.6	12.9	46.1	34.4
	2	92.0	6.9	20.6	8.2	56.3	39.7
	3	91.3	6.6	20.5	12.3	51.9	37.9
	4	90.1	7.7	21.6	10.1	50.6	36.8
HP	**5**	91.9	4.8	24.3	14.4	48.4	38.1
	6	92.3	6.8	22.6	13.6	49.3	32.0
	7	92.7	7.2	24.9	14.4	46.3	33.1
	8	92.0	5.7	24.8	14.0	47.5	37.6
	9	92.3	5.8	24.9	14.7	46.9	35.1
	10	90.9	6.0	35.2	17.8	31.9	15.9
	11	92.7	5.9	24.2	13.1	49.5	37.4
RW	**12**	36.0	2.4	11.5	18.4	3.7	nd
	13	25.9	1.0	14.5	10.5	0.0	nd
	14	36.7	3.9	16.2	17.1	0.0	nd
	15	36.0	4.1	15.2	17.0	0.0	nd
	16	40.3	3.9	15.5	20.4	0.5	nd
	17	33.8	3.4	10.8	9.4	10.2	nd
	18	32.6	2.5	10.7	10.8	8.6	nd

nd = not determined

Main nutrient content on dry matter basis

Carbohydrates accounted for 61.2 % (diet 2) of the DM, at the highest level and 35.1 % (diet 10) at the lowest among the dry foods (Figure 2). RWs differed from the dry food diets in that they contained less carbohydrate or no carbohydrate (diet 13, 14 and 15). Carbohydrate content varied between 1.2- 30.2 % for a few RW diets, so the highest level of carbohydrate content (30.2 %, diet 17) was similar to the lowest from the dry food groups. Because of the aforementioned low carbohydrate content for diet 10, both protein and fat levels were much higher, 38.8 and 19.6 %, resembling a BARF diet (bone and raw meat diet), similar to RWs. As expected, the highest carbohydrate content revealed the lowest protein content (22.4 %, diet 2 and 3), and fat content, (8.9 %, diet 2) for dry foods. The content of protein in the RW group were generally high and varied from 31.9-56.4 %. Fat levels in the RW group accounts for 51.1 % (diet 12) at the highest level and 27.8 % (diet 17) at the lowest, and is still higher than for the dry foods (8.9-19.6%). The distribution between protein, fat and carbohydrate was approximately equal for diet 17 and 18, which differed from the other diets in the present study.

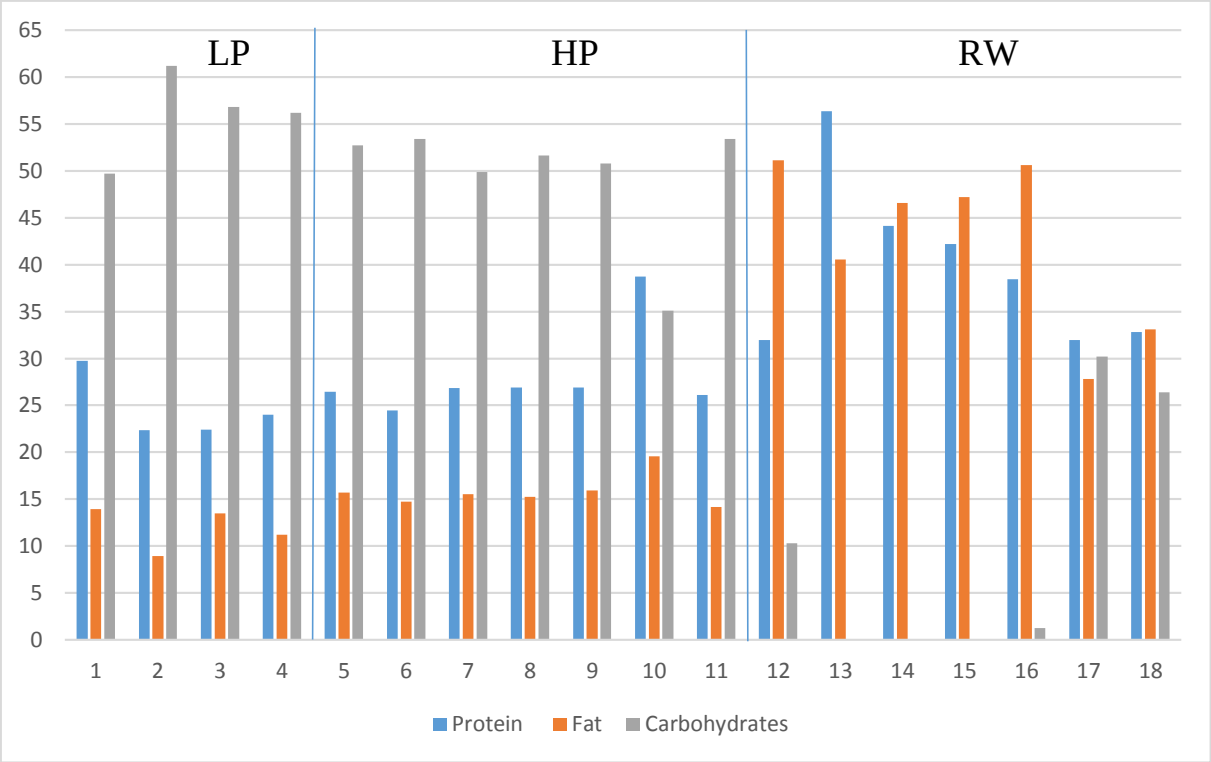

Figure 2: Dry matter (DM) content of protein, fat and carbohydrates (% of DM). Low price (LP): 1-4, high price (HP): 5-11, raw foods (RW): 12-18.

Contribution of metabolizable energy from main nutrients

Total ME content per kg food ranged from 13.0 to 15.6 MJ/kg for the dry foods and from 6.4 to 10.4 MJ/kg for the diets in the RW group (Figure 3). The lower ME content in the RWs than in the dry diets was due to the lower DM content in the RW diets (Table 5). Carbohydrates made up the largest concentration of ME in the dry foods (39.0-48.1 %), diet 10 and 2 deviated with the lowest level 21.5 % and the highest levels of 54.1 %. In addition, LP diets showed slightly higher levels of energy in form of carbohydrate, than the HP group. Conversely, diet 10 had most of the ME from fat (44.5 %), similar to the RWs (51.7-74.1 %). The highest level of ME coming from fat in the RW group (74.1 %, diet 16) was 1.5 times the amount from the highest of dry foods (diet 10). All the RWs had fat as the main source of energy. Content of energy from protein was similar between the dry foods ranging from 19.9-33.9 % and 20.7- 38.6 % for the RWs.

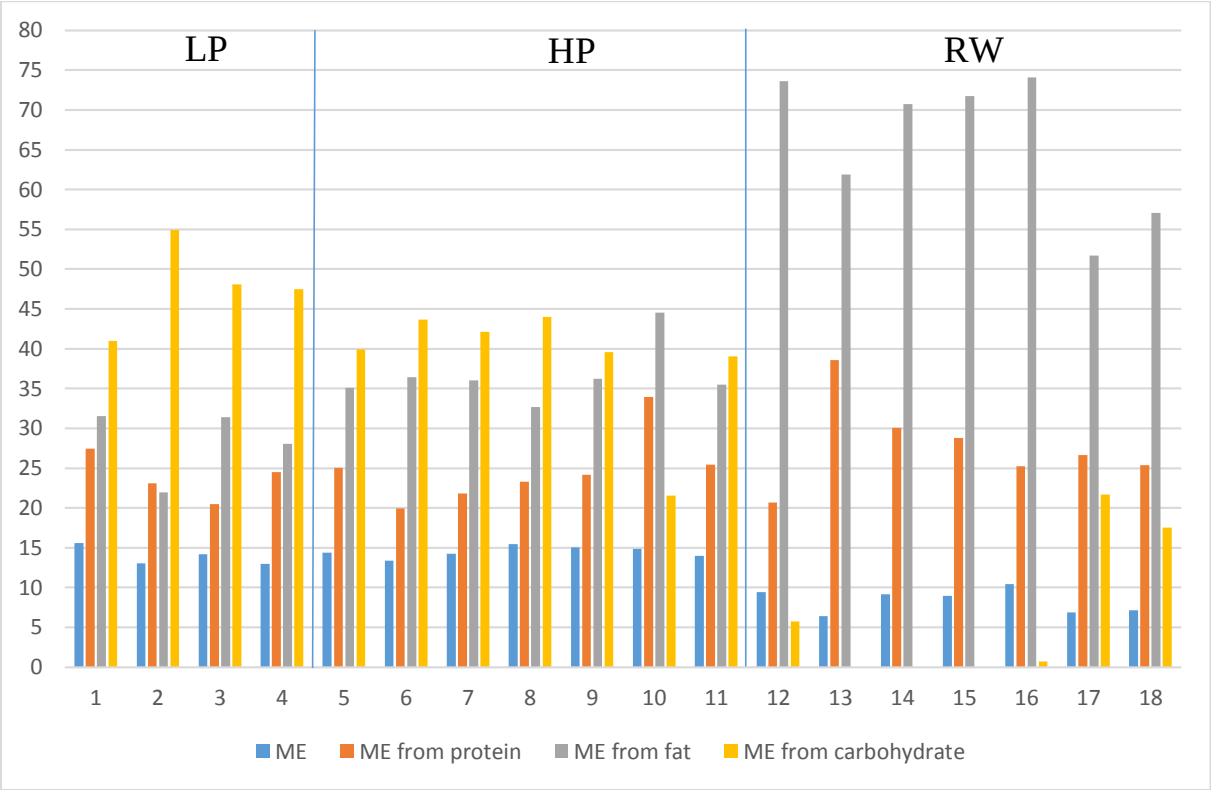

Figure 3: Metabolizable energy (MJ ME/ kg food), and ME from protein, fat and carbohydrate (% ME). Low price (LP): 1-4, high price (HP): 5-11, raw foods (RW): 12-18.

Fat digestibility

Individual fat digestibility values for the dry foods are presented in Figure 4, while digestibility of other nutrients will be presented in a separate article. Fat digestibility for RW was set to 96 %, (not shown in Figure 2), while fat digestibility determined by mink digestibility was applied to the dry foods. Apparent total tract digestibility (ATTD) values showed differences close to 10 % between the minimum (88.8 %, diet 2) and maximum (96.8 %, diet 1) digestibility. In addition, diet 2 had lower fat digestibility than the three other diets in the LP group (91.5-96.8 %). In the HP group, diet 5 revealed lower digestibility (89.1 %) compared to the rest of the group (90.6-96.4 %).

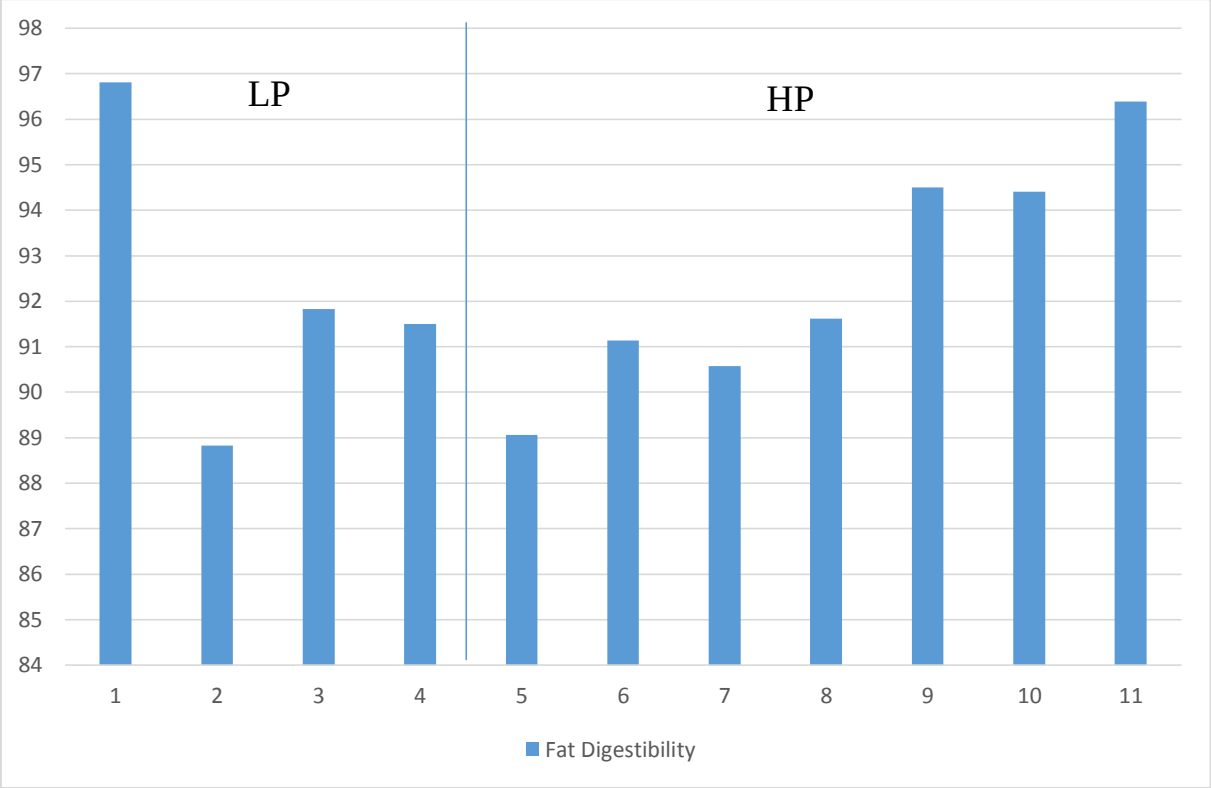

Figure 4: Fat digestibility of dry foods (%). Low price (LP): 1-4, high price (HP): 5-11.

Fatty acid composition

Fatty acid families

The pattern of the SFA, MUFA and PUFA content were similar for a majority of the dry foods showing the highest content of MUFA (1.6-4.5 g/MJ), lower for SFA (1.1- 2.9 g/MJ) and lowest for PUFA (0.8- 2.6 g/MJ) (Figure 5). However, some of the dry food diets had a similar or slightly higher SFA level (diet 2, 7 and 8). SFA was generally higher in the RWs (4.1- 9.5 g/MJ) compared to dry foods, although SFA levels within the RW group also differed markedly. Characteristics of diets containing high levels of SFA are the high content of palmitic acid (C16:0) and stearic acid (C18:0) (not shown). Amount of PUFA varied considerably, ranging from 0.8 to 2.6 g/MJ for the dry foods and 0.6 to 3.5 g/MJ for the RWs. Level of PUFA were noteworthy lower for the RW diet 12 and 13 (0.6 g/MJ) and for diet 2 among the dry foods (0.8 g/MJ).

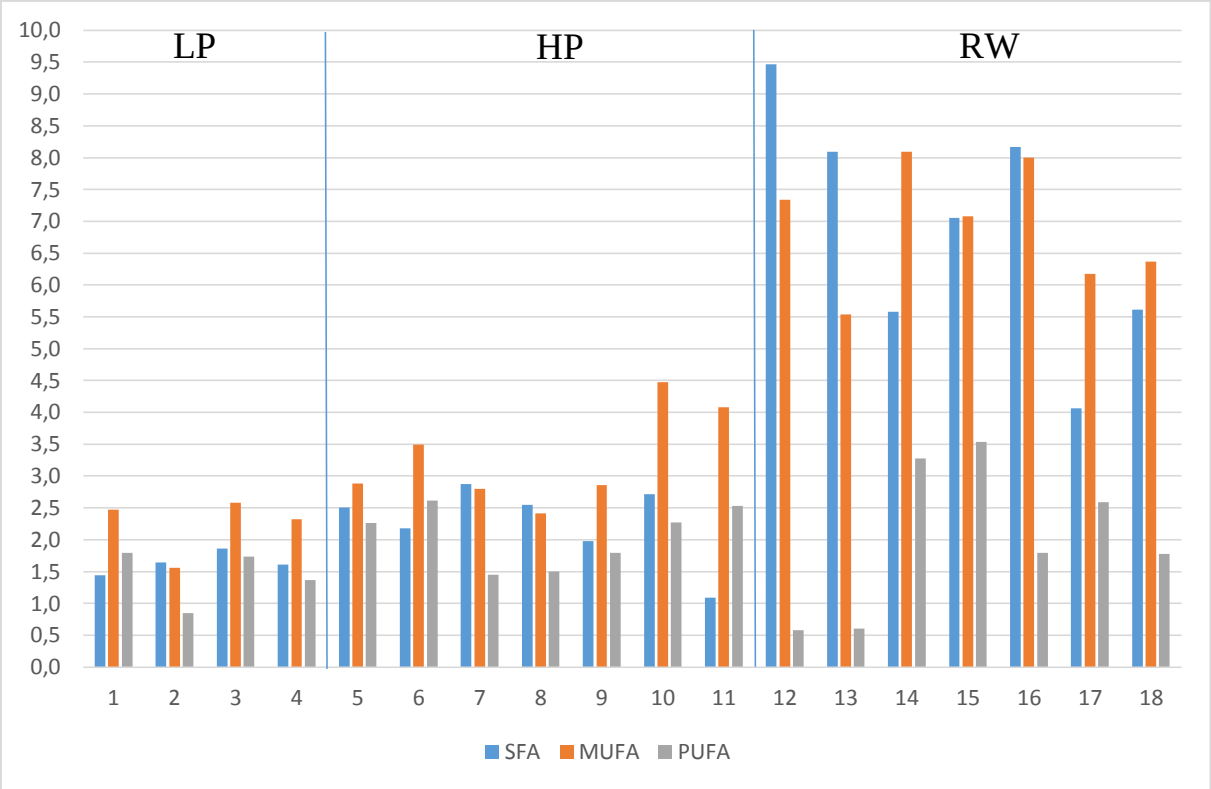

Figure 5: Content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (g/MJ ME). Low price (LP): 1-4, high price (HP): 5-11, raw foods (RW): 12-18.

Linolenic acid and α-linolenic acid content

The LA differences were distinct among the diets, ranging from 0.33 g/ MJ (diet 13) to 3.0 g/MJ (diet 15), both in the RW group (Figure 6). Levels of LA around 2.33, 2.05, 2.03 and 2.02 g/MJ (diet 14, 17, 5, and 6) were also notably high. NRC recommendation for LA (0.67 g/MJ) is indicated in Figure 6. All diets contain sufficient amounts to meet the recommended level for LA, except for diet 12 and 13, which only covered 0.35 and 0.33 g/MJ, respectively. In addition, diet 2 barely met the recommendation with 0.74 g/MJ. GLA levels were especially low or absent in in the diets. GLA was most frequently determined in diets from the HP group, 6 out of 7 contained a small amount (0.01-0.02 g/MJ).

The greatest amount of ALA, 0.45 g/MJ (diet 6), was much higher than the lowest, 0.01 g/MJ (diet 11). The content of ALA varied within and between the groups, with 0.01- 0.5 g/MJ for the dry foods and 0.1- 0.3 g/MJ for the RWs. Out of 18 diets, only one diet (diet 11) did not contain adequate content to meet the NRC's recommendation for ALA (0.03 g/MJ). Whereas

diet 6, 14 and 15 were noteworthy higher than the rest of the diets (0.5, 0.3 and 0.3 g/ MJ, respectively).

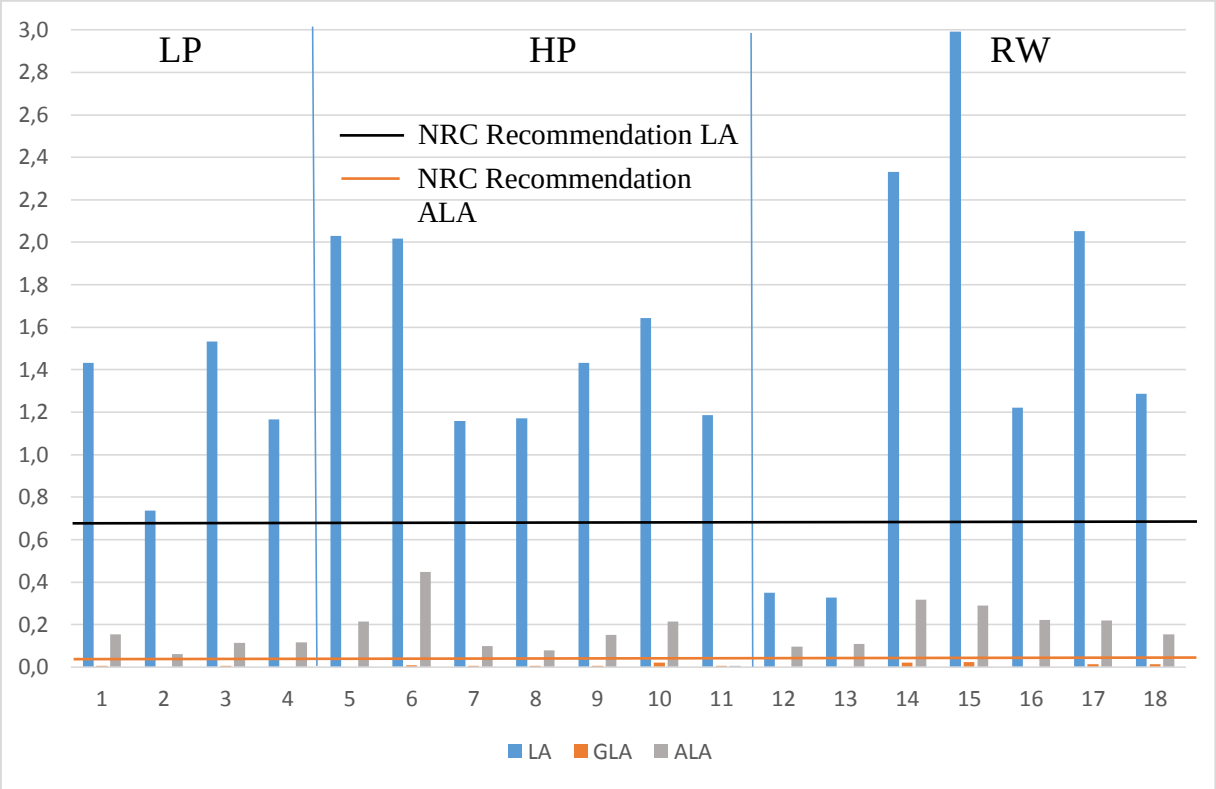

Figure 6: Content of linoleic acid (LA), gamma-linoleic acid (GLA), α-linolenic acid (ALA) (g/MJ). NRC recommendation for LA (0.67 g/MJ) and ALA (0.03 g/MJ presented as horizontal lines. Low price (LP): 1-4, high price (HP): 5-11, raw foods (RW): 12-18.

Arachidonic acid, EPA and DHA content

AA was present in all diets and ranged from 0.02 g/MJ (diet 2) to 0.08 g/MJ (diet 14 and 15). Figure 7 revealed a pattern, where LP had the lowest levels of AA, HP moderate and RW high levels. NRC have no dietary recommendation for AA in adult dogs.

The highest concentration of EPA and DHA, 0.56 g/MJ (diet 11), was approximately twice the amount of the second highest content 0.27 g/MJ (diet 14). The lowest level was close to detection level, 0.01 g/MJ (diet 2). Three of the diets (diet 2, 5 and 12) did not meet NRC's recommendation for EPA & DHA combined of 0.03 g/ MJ, containing 0.01, 0.00 and 0.02 g/MJ respectively. It is noteworthy that the majority of diets contained a mix of EPA/DHA, while diet 5 contained neither of them, EPA was absent in diet 2 and DHA was absent in diet

12 (not shown). The majority of the diets (12 of 18) contained concentrations above the recommendation for EPA and DHA.

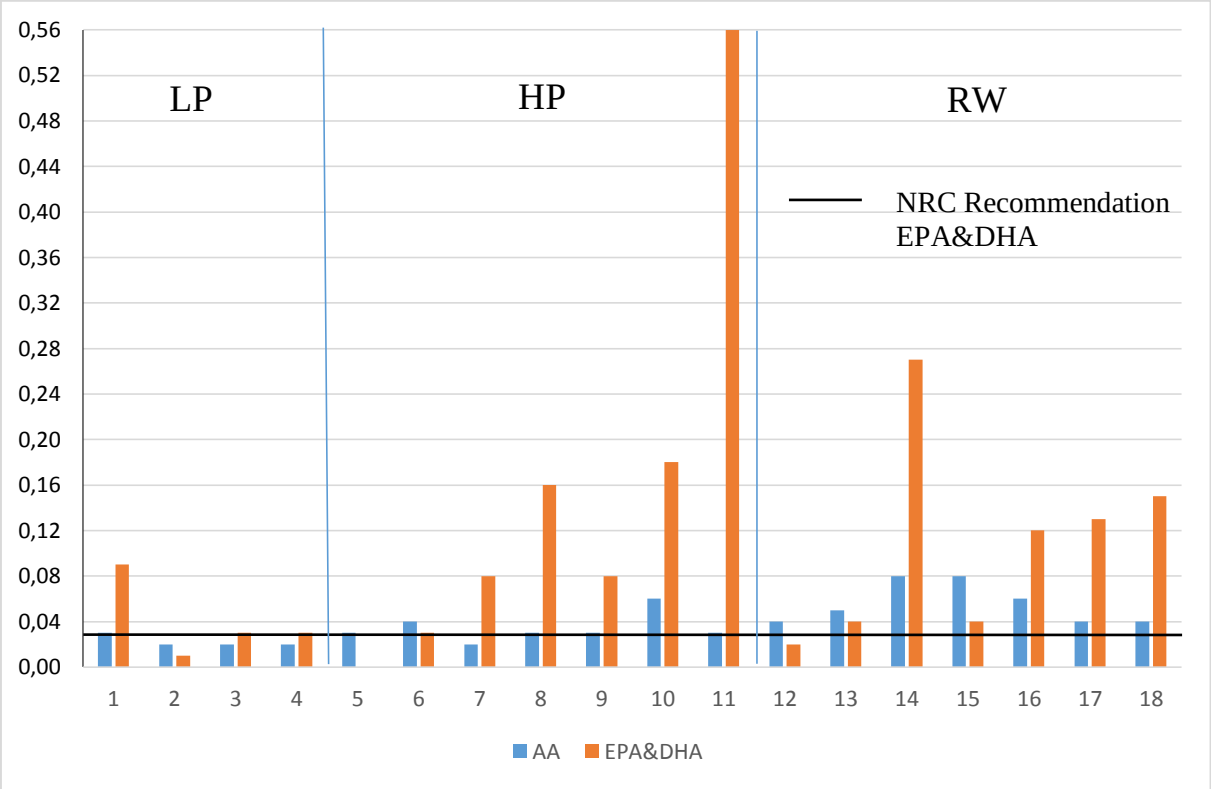

Figure 7: Arachidonic acid (AA), EPA and DHA (g/MJ) for diet1-18. NRC recommendation for EPA & DHA combined (0.03g/ MJ) are presented as horizontal lines. Low price (LP): 1-4, high price (HP): 5-11, raw foods (RW): 12-18.

Dietary n6:n3 ratio

Dietary n6:n3 ratios presented in Figure 8 revealed large variations with ratios from 1.2 to 10.1:1. The n6:n3 ratio was extremely low for diet 11 (1.2:1), due to the high content of total n3 from EPA and DHA (1.18 g/MJ). The highest n6:n3 ratio presented was 10.1:1 (diet 2 and 3), following the lowest content of n3, 0.08 g/MJ (diet 2). Highest content of n3 among the RWs were 0.8 g/MJ, and lowest was 0.2 g/MJ. Several of the diets (diet 1, 6, 7, 8, 9 and 17) had a medium level ratio (4.0-5.7:1), most of them in the HP group. The total n6 content varied from 0.4 g/MJ (diet 13) to 3.2 g/MJ (diet 15), both in the RW group. However, all the diets had lower ratios than the upper limit of 30:1 proposed by AAFCO.

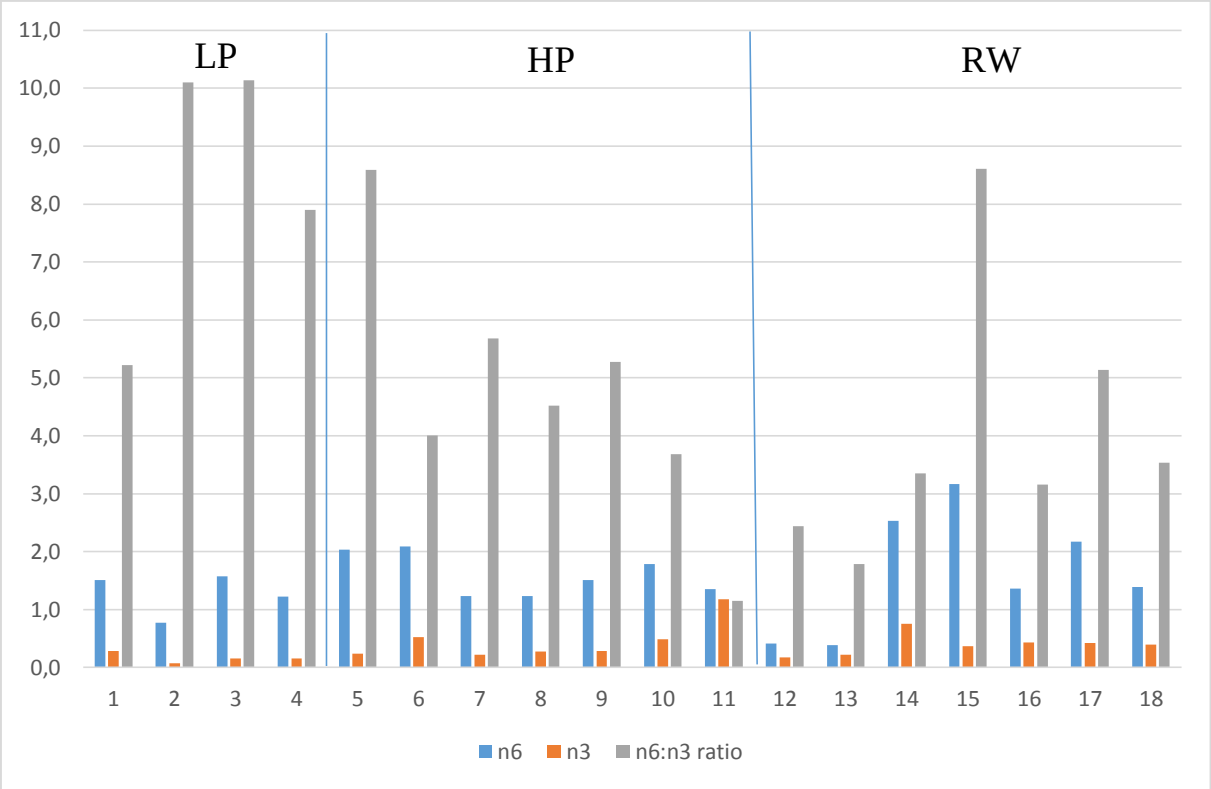

Figure 8: Total amount of n6 fatty acids, total amount of n3 fatty acids (g/ MJ) and n6:n3 ratio for diet 1-18. Low price (LP): 1-4, high price (HP): 5-11, raw foods (RW): 12-18.

Group comparisons

Main nutrient content on dry matter basis in groups

Means values for the nutrient content on DM basis and differences between groups are presented in Table 6. RW had a significantly higher protein ($p<0.0033$) and fat content ($p<0.0001$) than the dry foods on DM basis. Likewise the dry foods had a significantly higher carbohydrate content ($p<0.0001$) than the RWs.

Table 6: Crude protein (CP), crude fat (CF) and carbohydrate (CHO) (% of DM) and pooled SEM and p-value in the low price (LP), high price (HP) and raw food (RW) group.

	LP	HP	RW	Pooled SEM	P-value
CP	24.6^{b}	28.1^{b}	39.7^{a}	2.8	0.0033
CF	11.9^{b}	15.8^{b}	42.4^{a}	2.5	<.0001
CHO	56.0^{a}	49.6^{a}	9.7^{b}	4.0	<.0001

Values are least square means, total n=18, LP n=4, HP n=7, RW n=7.
^{a,b,c} Means not sharing the same superscript are significantly different at $p<0.05$.

Metabolizable energy content in groups

The ME per kg food was significantly different ($p < 0.0001$) between the dry foods and the RW group (Table 7), a result of lower DM content in the RWs (Table 5). On DM basis there was still a significant difference ($p < 0.0001$) between the dry foods and the RWs, but the ME content was higher for RWs than for the dry foods. Fat percentage of ME was significantly different for RW and the dry foods ($p < 0.0001$), in addition LP and HP tended to differ ($p=0.06$), (not shown in the table). The content of ME from carbohydrates was also differed significantly between the dry foods and RW ($p<0.0001$).

Table 7: Metabolizable energy per kg food (MJ ME/ kg food), metabolizable energy per kg dry matter (MJ ME/ kg DM) and energy from crude protein (CP), crude fat (CF), carbohydrate (CHO) (% of ME), pooled SEM and p-value in the low price (LP), high price (HP) and raw food (RW) group.

	LP	HP	RW	Pooled SEM	P-value
ME	13.9^{a}	14.5^{a}	8.3^{b}	0.5	<.0001
ME DM	15.2^{b}	15.7^{b}	24.1^{a}	0.6	<.0001
Protein	23.9	24.8	27.8	2.0	0.33
Fat	28.2^{b}	36.6^{b}	65.7^{a}	2.7	<.0001
Carbohydrate	47.9^{a}	38.6^{a}	6.5^{b}	3.4	<.0001

Values are least square means, total n=18, LP n=4, HP n=7, RW n=7.
^{a,b,c} Means not sharing the same superscript are significantly different at $p<0.05$.

Fat digestibility and fatty acid composition

Fat digestibility revealed no significant difference between the LP and the HP group (Table 8). RW was not considered, as the value was set to a high standard digestibility for fat. SFA content differed significantly between the RWs and the dry foods, while MUFA was significantly different for all the groups, revealing a pattern of LP with the lowest content, HP with intermediate and RW with the highest MUFA content. For the PUFA there was no significant differences.

Among the single EFA only AA was significantly different ($p<0.005$), as the RW group had a higher content than the LP and HP group. Generally, the LP diets had the lowest mean levels of EFAs. The n6:n3 ratio was significantly lower for the HP and RW diets compared to the LP diets ($p<0.02$).

Table 8: Fat digestibility, fatty acids composition, sum of n6 and n3 fatty acids (g/MJ), n6:n3 ratio, pooled SEM and p-value in low price (LP), high price (HP) and raw food (RW) group.

	LP	HP	RW	Pooled SEM	P-value
Fat digestibility	92.2	92.5	96.0*	0.9	0.02
SFA	1.6[b]	2.3[b]	6.9[a]	0.5	<.0001
MUFA	2.2[c]	3.3[b]	6.9[a]	0.3	<.0001
PUFA	1.4	2.1	2.0	0.3	0.45
LA	1.2	1.5	1.5	0.3	0.76
GLA	0.005	0.01	0.009	0.003	0.55
AA	0.02[b]	0.03[b]	0.06[a]	0.01	0.005
ALA	0.1	0.2	0.2	0.04	0.41
EPA	0.02	0.07	0.05	0.02	0.29
DHA	0.03	0.09	0.06	0.04	0.52
Sum n6	1.3	1.6	1.6	0.3	0.70
Sum n3	0.2	0.5	0.4	0.1	0.21
n6:n3 ratio	8.3[a]	4.7[b]	4.0[b]	1.0	0.02

Values are least square means, total n=18, LP n=4, HP n=7, RW n=7.
[a,b,c] Means not sharing the same superscript are significantly different at $p<0.05$.
* Estimated value

Discussion

Fatty acid composition in dog foods provides an indication of the fat's origin. Animal fat supplies higher amounts of SFA and MUFA, while vegetable and marine oil contain more PUFAs. Since the phospholipid portion of cell membranes and triglycerides throughout the body consist of fatty acids, the membranes' composition is effected by dietary intake (Kirby et al. 2007; Wiese et al. 1966). The composition of EFA in dog foods is therefore of great importance.

The diets selected for the study are a representative assortment from the Norwegian market; both economic and premium dry foods, in addition to raw foods were compared. Chemical compositions of the diets were in accordance with the declared content for all diets. The chemical composition was consistent with results by Krogdahl et al. (2004).

Main nutrient composition and ME content in the diets

The dry foods contained markedly higher concentrations of carbohydrates (49.6- 56.0 %) on DM basis than the RW group (9.7 %). Among the dry foods, there was a tendency of lower carbohydrate content in the HP group compared to the LP group, the difference was not significant ($p=0.06$). Several RW diets were carbohydrate free or contained low levels, causing the content of carbohydrate to be significantly different between the dry foods and the RWs ($p<0.0001$). Conversely, as expected, the RW group had significantly higher levels of fat (42.4 %) compared to the dry foods (11.9-15.8 %). One of the dry foods had elevated levels of protein (38.8 %) compared with the rest of the dry foods (22.4- 29.8 %) on DM basis. This resembles a dry BARF (bone and raw meat) diet, with more protein and fat, similar to all the RWs (31.9-56.4 % protein). BARF is a reference used about non processed raw diets, often homemade (Freeman & Michel 2001), however it appears that the philosophy has been attempted to be transferred to dry foods.

The concentration of ME per kg dog food ranged from 13.0 to 15.6 MJ ME/kg for the dry foods; this was similar to the values determined in 12 commercial dry foods (13.7-16.0 MJ ME/kg) by Ahlstrom et al. (2004). The ME per kg food was significantly higher for the dry foods than the RWs, this was due to the difference in DM. However, the MJ ME on DM basis showed higher amount of energy in the RWs than in the dry foods. In ME per kg, the two dry food groups had significantly higher lsmeans values for ME from carbohydrates (38.6-47.9

%), compared to the RWs (6.5 %). The HP group had a slightly lower level of energy from carbohydrate than LP, however not significantly different, but there was a tendency (p= 0.06). The tendency indicated higher concentrations of energy coming from fat or protein in the HP group. Content of energy supplied by carbohydrate in the individual dry foods ranged from 39.0 to 54.1 % in the present study, and agreed with the calculated carbohydrate content (44.5 and 49.7 %) in German et al. (2011).

Fat was the main energy source in the RW diets, and the fat level was significantly higher than for the dry foods. The fat content in the RW diets ranged from 51.7 to 74.1 % of ME. These values were similar to another study conducted with two commercial raw foods, which contained 65 and 74 % of ME from fat. In addition, the protein level in the current study (20.7- 38.6 %) was similar to the protein values presented (23-28 %) (Freeman & Michel 2001). The two commercial diets in Freeman and Michel (2001) contained only one or two ingredients; the ingredients thereby determined protein and fat content. The RWs in the present study also contained few ingredients, which is typical for these kind of dog foods. The fatty acid composition of the food will therefore be highly dependent on the fatty acid composition of very few ingredients, which may pose a risk of low EFA supply.

Two of the RW diets had close to equal distribution of energy from protein, fat and carbohydrate, which is uncommon in commercial diets. It could be questioned if the energy from carbohydrate was fully available for the dog as the foods were not heat treated, however the carbohydrates added could have been precooked. It is reasonable to assume that the two diets were focusing on marketing RW to normal companion dogs. Declaration revealed both vegetables and rice, which is often added in RWs to attract the owner, resembling the stomach content of a prey and giving the impression of a healthy and varied diet (Freeman & Michel 2001).

Fat digestibility

The fat digestibility in the present study was generally high and ranged from 88.8 to 96. 8 % for the LP group and 89.6 to 96.4 % for the HP. These values were similar to Krogdahl et al. (2004), at 83.9 to 91.7 % for low price foods and 76.4 to 95.8 % for high price foods, although the selection of diets in the two studies were slightly different. The digestibility values deviated little from NRC (2006) standard digestibility of 90 % for fat. Generally,

ATTD is high in mink (70-98 %) (Rouvinen 1990) and dogs (96.6 %) (Tjernsbekk et al. 2014). The small differences between diets in the present study could be dependent on differences in the level of saturation, chain length and melting point (Austreng et al. 1979; Rouvinen 1990). Austreng et al. (1979) found a clear relation between increased melting point of fat and fatty acids, and lower digestibility, in mink and rainbow trout. For chains up to C_{18} the digestibility decreases for every unit of increase in length. However, a chain length up to C_{22} will increase the digestibility (Austreng et al. 1979). Both PUFA and MUFA fatty acids have a lower melting point than their equivalent SFA, and therefore a higher digestibility. The digestibility of stearic acid (C18:0) is generally poor compared to other fatty acids, both for dogs and other species (Kritchevsky 1994; Rouvinen 1990). This is reflected in the poor digestibility of diet 2 which had a high amount of SFA, mainly palmitic acid (C16:0) and stearic acid (C18:0) (not shown in table). This could be due to selection of raw materials, as more animal by-products, especially beef tallow, contain high amounts of SFA (Rouvinen 1990). The fat digestibility value of 96 % applied for the RWs in the present study may therefore be overestimated, as the SFA content was highest in this group suggesting that beef tallow was a main fat source.

Fatty acid composition

The fatty acid pattern differed more among the RW diets, than the dry foods. Among others, SFA and MUFA was higher in the RWs than the dry foods, which is due to the use of the diets and ingredient list. Dry foods are mainly designed for normal companion dogs, while several of the RWs were in the first place intended for dogs with high energy requirement, such as sled dogs (Reynolds et al. 1994). Amount of SFA was significantly higher in RWs than the extruded diets ($p<0.0001$). The origin of the SFA in RWs was beef tallow or tripe, according to the declarations. Tripe is a good consistency and palatability enhancer in raw diets. However, tripe is less suitable in dry extruded diets because of its high melting point. Low amounts of SFA in dry foods could be a result of processing complications with saturated fat sources. Lin et al. (1997) showed that increasing the fat content decreased starch gelatinization. If more beef tallow or other sources high in SFA were added, less starch was gelatinized compared to adding more unsaturated fat, like poultry fat. Gelatinization is essential to make kibbles durable. As kibble texture is not a concern in the RW diets, the SFA level could be higher. In reality, it could be an advantage with high SFA in RW diets to obtain a more solid consistency. From the declarations, one could see that some of the RW diets

were often made of a mix of by-products from poultry, beef and salmon, while others were composed from only one of these ingredients. The selection of ingredients was clearly reflected in the saturation level of the diets and could explain the difference in the SFA, MUFA and PUFA patterns. Of all the18 diets, 11 had a significant higher level of MUFA than both SFA and PUFA, suggesting poultry and vegetables were the main source of fat (see Table 2), which is consistent with the declarations. The highest amount of MUFA compared to SFA and PUFA, has also been seen in the baseline diets in Hall et al. (2006) and in Ahlstrom et al. (2004). High levels of PUFA could lead to lipid oxidation causing rancidity during storage (Guy 2001). Thus, addition of antioxidants are important to avoid lipid peroxidation, but may increase the cost (Wander et al. 1997). Problems related to lipid peroxidation could explain the low levels of PUFA in some of the LP diets, however the group differences in PUFA levels were not significant.

Linolenic acid and α-linolenic acid

In most of the diets, content of LA was high and met the NRC (2006) recommendation; however not all had adequate concentrations. This was surprising because most animal fat and especially vegetable fat sources typically contain plenty of LA. Great variation within diets were also seen in Ahlstrom et al. (2004), but all diets had sufficient levels to meet the recommendations. The two diets inadequate in LA in the current study were in the RW group. According to the declaration of these diets, the fat source was beef, tripe and lamb, as mentioned, sources high in SFA with moderate levels of LA (see Table 2). Little or no addition of vegetable sources could lead to a diet being insufficient in several EFAs. It appears that producers of these diets had not considered EFA content when composing the diet. It is not possible to conclude if dogs fed these diets would develop deficiencies, as there was some LA present in both of them. However, there is a risk of deficiency if fed as the only diet for a long period.

A study done by Wiese et al. (1966) determined that symptoms of a diet deficient in LA became clear after 6-8 months and resulted in hair loss, scaling of epidermis, scruffy coat and shivering. Therefore, skin defects are typically seen as a symptom of LA or EFA deficiency. Skin defects can be alleviated by changing the diet or by feeding supplements, like fish oil or vegetable oil. In general increasing the total amount of fat may improve the coat and skin, but there are even more benefits if there is an increase in unsaturated fat (Bauer 2007; Watson

1998; Wiese et al. 1966). Hence, the two above-mentioned diets could have been completed to the EFA requirement with a small addition of vegetable oil.

ALA was present in all diets but in small amounts. Several declarations listed vegetable oils and were high above recommendations (0.03 g/MJ). The diet containing the highest amount of ALA (2.03 g/MJ) among the dry foods, are further discussed in the next section.

Arachidonic acid, EPA and DHA

The content of AA was distinctly different between the diets, ranging from 0.02 to 0.08 g/MJ. This range was similar to the results found in Ahlstrom et al. (2004). No recommendation for AA for adult dogs is given in any of the guidelines, presumably because LA supply is considered to be adequate to synthesize AA. Some of the RW diets in the present study had high levels of AA compared to the dry foods, which is due to the dominance of animal by-products being used as ingredients in the RW diets. Animal fat is the only source of AA, which explain why AA was the only single fatty acid that were significantly different between the RWs and the dry foods. Therefore, one could speculate whether the high AA content of the RW diets may partly compensate for the low LA content as LA function as a precursor for AA. If this applies, the EFA levels for the two RW diets concerning (mentioned in the section above), may satisfy the requirement for an adult dog.

Concentration of EPA and DHA ranged from zero to very high levels (0.56 g/MJ). Several diets revealed content well above the recommendation for sum EPA and DHA (0.03 g/ MJ), indicating that fat sources were of marine origin, as they were the only sources rich in EPA and DHA (Table 2). The diet with the highest level declared fish based ingredients with protein and fat from marine sources only, explaining the high levels of EPA and DHA. Generally, the levels of EPA and DHA were higher in the HP and RW group (0.05-0.07 and 0.06-0.09 g/MJ), but they were not significantly different from the LP group (0.02 and 0.03 g/MJ). NRC's recommendation for the combination of EPA & DHA were not met for two of the diets. These diets either lacked EPA and DHA completely, or contained only EPA or DHA (not shown). The diet not containing EPA or DHA had moderate to high amounts of ALA, but besides that, the fatty acid composition was quite similar to the other dry foods. Several of the diets in Ahlstrom et al. (2004) originated from the same producers used in the present study one of diets in both studies revealed the same EFA pattern, high ALA, but no

EPA and DHA. This suggests that food producers rely on ALA as the n3 fatty acid source for EPA and DHA synthesis using flaxseed oil or other vegetable oils as a source of ALA. However, AAFCO (2014) or FEDIAF (2014) do not have recommendations for ALA for adult dogs. One may also speculate if the very low level of EPA and DHA in the diet induces more of the enzymes responsible for the elongation from ALA to EPA, than if EPA/DHA had been present at higher levels (Gibson et al. 2013). Especially since one of the producers have continued using the same strategy supplying only ALA and no EPA or DHA. Studies supports the theory that only ALA is needed, however the amount is not determined. Bauer et al. (1998) reported increased levels of EPA and other n3 fatty acids in the plasma when adding a modest amount ALA, in the form of flaxseed oil. Indicating that ALA could be synthesized to chain EPA and other n3 fatty acids. However, as mentioned in the theory, studies indicate that DHA needs to be added directly, as ALA is not metabolically equal to EPA or DHA, and the conversion is inadequate (Bauer et al. 1998; Bauer 2007; de Deckere et al. 1998).

AA, EPA and DHA levels differed most between the HP and RW group and the LP group (Table 8). This could be explained by fish oil being expensive compared to vegetable oils, however, all the LP diets declared fish by-products or fish oil on the ingredient list. All but one diet declared fish as either oil or by-product, but the concentration or the amount added in the LP diets was obviously less than for the HP and RW groups.

Dietary n6:n3 ratio

The n6:n3 ratio for one diet was extremely low (1.2:1), similar to the low ratio, 1.4:1, used in the study by Wander et al. (1997). Results from Wander et al. (1997) indicated that diets with such low ratio could lead to reduced production of PG, lower levels of antioxidants (α-tocopherol) in the plasma and increased risk of lipid peroxidation. Positive effects could be reduced HDL in plasma and reduced cell-mediated immune response, especially in the skin. Various diets in the present study had content ranging from 4.0-5.6:1, similar to the medium ratio (5.4:1) in Wander et al. (1997), however, few significant differences were revealed between the groups given a medium ratio and a high ratio (Wander et al. 1997). The highest n6:n3 ratio (10.1:1) in the present study was much lower than AAFCO's (2014) recommended upper limit of 30:1. The ratio was also much lower than the high ratios used in other studies (30-40:1) (Hall et al. 2006; Wander et al. 1997). Dietary n6:n3 ratio differed significantly between the LP group and the HP and RW groups; the two latter groups having a

lower ratio than LP. The main reason for this was the lower n3 concentration in the LP diets. However, ratio is difficult to interpret and the total concentration of n3 and n6 is as important, if not more important, than the n6:n3 ratio (Hall et al. 2006).

Hall (2006) found that a concentration of 6.3 g total n3 fatty acids/kg food was the optimal content to reach maximum plasma levels of DHA, however several studies is needed to confirm this. If this is the optimal level, all but two diets had sufficient amounts of n3 fatty acids in the present study (data not shown). The optimal ratio or concentration to adult dogs is, as of today, not yet established due to conflicting results (Wander et al. 1997).

Conclusion

- The EFA and dietary n6:n3 ratios in individual diets varied substantially, irrespective of diet type, extruded or raw.
- The EFA content differed between the low price and high price group, but not significantly. High individual differences between diets gave high variations within each group.
- Raw diets contained a higher content of fat (% DM) than extruded diets, but had similar levels of EFA. AA was the only single fatty acid significantly higher in the raw foods, compared to the extruded diets. Feeding a raw diet do not necessarily cover the EFA requirement, because ingredient selection affects the fatty acid composition of the diet.
- Few of the diets had levels of n6 and n3 EFAs below NRC recommendations. The concentration n6 and n3 fatty acids or the n6:n3 ratio needed to optimize the benefits and to avoid negative effects is not explicit and require additional studies.

Attachments

Diet declarations

Table 9: Declaration for dry matter (DM), ash, crude protein (CP), crude fat (CF), n3 fatty acids (n3 FA), n6:n3 ratio, EPA/DHA, carbohydrate (CHO) and fiber (%/ kg) for diet 1-18, divided into low price (LP), high price (HP) and raw food (RW).

	Food No.	DM	Ash	CP	CF	n3 FA	n6:n3 ratio	DHA/EPA	CHO	Fiber
LP	**1**	-	6.8	25.0	15.0	-	-	-	-	1.3
	2	-	7.5	20.0	8.0	-	-	-	-	3.0
	3	-	7.0	21.0	13.0	-	2.6/0.3	-	-	2.5
	4	90.0	6.0	21.0	10.0	-	-	-	50,5	2.5
HP	**5**	-	4.6	22.0	15.0	0.43	-	-	-	1.7
	6	-	6.0	25.0	15.0	-	-	-	-	3.0
	7	92.0	7.1	26.0	15.0	-	2.4/0.37	-	-	2.5
	8	-	6.0	25.0	14.0	0.5	-	0.31	-	1.2
	9	-	6.4	25.0	16.0	-	-	-	-	1.4
	10	-	8.0	38.0	18.0	-	3.0/1.1	0.6/0.3	-	5.0
	11	-	7.4	26.0	12.0	1.0	-	-	-	2.5
RW	**12**	-	-	16.0	14.0	-	-	-	-	-
	13	-	-	16.0	14.0	-	-	-	-	-
	14	35.0-39.0	5.0	15.0	20.0	-	-	-	-	-
	15	35.0-39.0	5,0	15.0	19.0	-	-	-	-	-
	16	38.0	4.1	15.0	18.0	-	-	-	-	0.9
	17	30.0	2.0	12.0	6.0	-	-	-	-	1.0
	18	30.0	1.5	9.0	6.0	-	-	-	-	1.0

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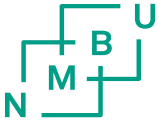

Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no