Aus dem Department für Tierernährung

der Veterinärmedizinischen Universität Wien

Institut für Tierernährung und Funktionelle Pflanzenstoffe

(Leiter: Univ.-Prof. Dr.sc.agr. Qendrim Zebeli)

Efficacy of dietary polyunsaturated fatty acids in modulating adipose tissue lipids in young calves

Diplomarbeit

Veterinärmedizinische Universität Wien

vorgelegt von

Maria Janzen

Wien, im Juni 2023

Betreuerin: Dr.Sc. Ratchaneewan Khiaosa-Ard
Tierernährung und Funktionelle Pflanzenstoffe
Department für Nutztiere und öffentliches Gesundheitswesen in der Veterinärmedizin
Veterinärmedizinische Universität Wien

Gutachter: Univ.-Prof. Dr.med.vet. Johannes Khol, Dipl.ECBHM
Universitätsklinik für Wiederkäuer
Department für Nutztiere und öffentliches Gesundheitswesen in der Veterinärmedizin
Veterinärmedizinische Universität Wien

Contents

Acknowledgement

At this point I would like to thank all the people who supported me in the preparation of this thesis.

Special thanks go to my supervisor Dr.Sc. Ratchaneewan Khiaosa-Ard, who always showed understanding for my questions and schedule and provided me with her assistance and support at all times.

An important contribution was also made by Suchitra Sharma, BSc. who, as a technical assistant, carried out almost all the laboratory work and gave me a little insight into her work.

Finally, I would also like to thank my boyfriend Arman, who has always encouraged me not to give up even in difficult phases and to consistently pursue my goal. He always took care of the indispensable little things of everyday life and made sure that I physically - and more importantly mentally - survived this intensive time well.

1 Introduction

Ruminants naturally live and eat on pastures. Forages are the closest feed to the natural diets of ruminants. But nowadays, cows are fed rations with high proportions of concentrated feed, consisting of grains and oilseed meals, in order to achieve higher growth rates and milk production, which could compromise animal health. Concentrates differ from forages in carbohydrates, lipids and their fatty acid composition. Usually concentrates are high in omega-6 (n6) polyunsaturated fatty acids (PUFA) while forages are high in omega-3 (n3) PUFA. In humans, lab animals and swine, the ratios of the n6 and n3 PUFA have already been shown to influence cell metabolism and immune responses (Leskanich and Noble 1999, Simopoulos 2002, Innes and Calder 2018). Because n6 and n3 PUFA are involved, among other functions, in regulating inflammatory responses, modifying tissue fatty acids can be instrumental to animal health. With this aspect, adipose tissue is of interest since it is a metabolically and endocrinologically important organ, therefore it has a major impact on overall body homeostasis (Louveau et al. 2016). Modification of dietary n6 to n3 PUFA have been shown to alter the fatty acid profile of various tissues including adipose tissue in farm animals (Fincham et al. 2009, Liméa et al. 2012). However, in ruminants, the metabolism of dietary lipids depends greatly on microbiological processes taking place in the rumen (Martins et al. 2011) prior to the absorption by the host animal. Thus, the impact of dietary fatty acids on tissue fatty acids is less predictable compared to monogastric animals. In general, the knowledge of fatty acid shifts in ruminant adipose tissue due to dietary influence is still limited, with even less data on calves than on adult cows (Masmeijer et al. 2020). It is known that the rumen of young ruminants undergoes maturity (Khan et al. 2016) and so ruminal microbial processes might not be equal to those of adult ruminants. Because of this gap of research, the present thesis is dedicated to study the influence of dietary fatty acids on the fatty acid composition of adipose tissue in calves.

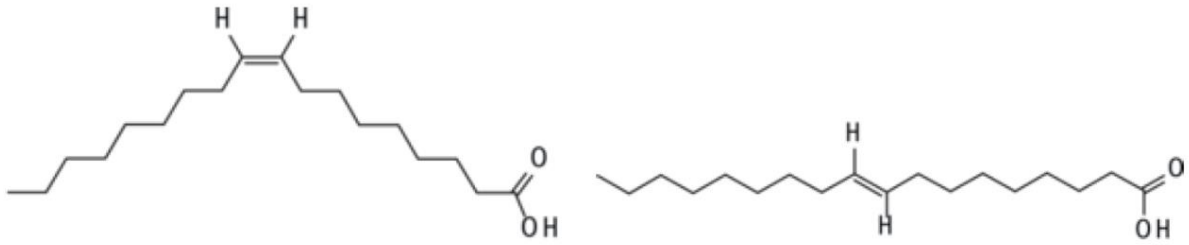
2 Literature review

2.1 Biochemistry of fatty acids

Fatty acids are the building blocks of lipids. All fatty acids consist of hydrocarbon chains with different length. On one end of the chain there is a methyl group (CH-3) and on the other end of the chain there is a carboxylic group (-COOH) (Koolman et al. 1998) (Table 1). Fatty acids can be classified according to various criteria based on the synthesis ability of the organism or based on the chemical structure including, length of the hydrocarbon chain, degree of saturation and configuration at the double bond. Based on chemical structure, the length of hydrocarbon chains can be categorized in four groups. The short chain fatty acids include fatty acids with 2-4 carbon atoms in the chain, medium chain fatty acids include fatty acids with 6-10 carbon atoms, long chain fatty acids include fatty acids with 12-18 carbon atoms and very long chain fatty acids include fatty acids with 20-30 carbon atoms in the chain (Tvrzicka et al. 2011). It is well known that a distinction is made between saturated and unsaturated fatty acids. Saturated fatty acids (SFA) have only single bonds between the carbon atoms whereas unsaturated fatty acids have one or more double bonds. The unsaturated fatty acids can further be differentiated in monounsaturated fatty acids (MUFA) with only one double bond and polyunsaturated fatty acids (PUFA) with two or more double bonds. The position of the first double bond from the methyl end gives the fatty acids part of their name. For example, if the third carbon bond is a double bond it is called an n3 PUFA, if the sixth carbon bond is a double bond it is called an n6 PUFA (Tvrzicka et al. 2011). Besides the position of the first double bond also the number of carbon atoms and the number of double bonds are indicated for naming fatty acids (Tvrzicka et al. 2011).

Fatty acids also differ in the configuration of the double bonds. If the hydrogen molecules of two C-atoms that lie next to each other and are connected by double bonds point to the same side this fatty acid is called a cis-fatty acid. This configuration leads to a new structure of the molecule because the hydrocarbon chain bends at the point of the double bond. This makes cis-fatty acids more voluminous. If the hydrogen molecules point to opposite sides this fatty acid is a trans-fatty acid (trans-FA). Trans-FA are straight just like SFA (Tvrzicka et al. 2011). Figure 1 shows an example for visual orientation. Trans-FA are not common in a “natural environment” or in unprocessed products except for the rumen in ruminants from where these fatty acids enter the gastrointestinal tract and metabolic processes (Ferlay et al. 2017). So, trans-FA can be found in dairy products but also in industrial fats that have been catalytically

hardened (Cholewski et al. 2018), although the profile of trans-FA of dairy products differs from that of industrially hydrogenated fats (Stender et al. 2008).



Oleic acid (C18:1 cis 9) Elaidic acid (C18:1 trans 9)

Figure 1: Chemical structure of oleic acid and elaidic acid (Deutsche Gesellschaft für Ernährung e.V. 2016)

Based on the synthesis ability of the organism, fatty acids that can be synthesized endogenously are called non-essential fatty acids and those that cannot be synthesized by the organism itself are essential fatty acids and they must be supplied through nutrition. For mammals these are linoleic acid (18:2 n6) and α-linolenic acid (18:3 n3) (Koolman et al. 1998). A particularly important function of these two essential fatty acids is the formation of arachidonic acid and eicosapentaenoic acid, which serve to synthesise eicosanoids – the oxidized derivatives of 20-carbon PUFA. These are local mediator molecules responsible for processes such as pain sensitivity, inflammatory reactions and allergic processes (Koolman et al. 1998). It is known that eicosanoid derivatives of n3 PUFA have an anti-inflammatory effect and while many of eicosanoid derivatives of n6 PUFA exhibit a pro-inflammatory effect on organisms (Simopoulos 2002, Gurr et al. 2016). Fatty acids especially PUFA are needed for basic development of growth, skin, nerves, hormones and more (Klein 2002).

Table 1: List of some important fatty acids with their different nomenclature systems (Tvrzicka et al. 2011)

Formular	Systematic name	Trivial name	Structure
16:0	Hexadecanoic acid	Palmitic acid	CH3-C-C-C-C-C-C-C-C-C-C-C-C-C-C-COOH
18:0	Octadecenoic acid	Stearic acid	CH3-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-COOH
18:1 n9	cis-9-Octadecenoic acid	Oleic acid	CH3-C-C-C-C-C-C-C-C=C-C-C-C-C-C-C-COOH

18:2 n6	cis-9,12-Octadecadienoic acid	Linoleic acid (LA)	CH3-C-C-C-C-C=C-C-C=C-C-C-C-C-C-C-C-COOH
18:2 n7	cis-9,trans-11-Octadecenoic acid	Rumenic acid	CH3-C-C-C-C-C-C=C-C=C-C-C-C-C-C-C-C-COOH
18:3 n3	cis-9,12,15-Octadecenoic acid	α-Linolenic acid (ALA)	CH3-C-C=C-C-C=C-C-C=C-C-C-C-C-C-C-C-COOH
20:4 n6	cis-5,8,11,14-Eicosatetraenoic acid methyl ester	Arachidonic acid	CH3-C-C-C-C-C=C-C-C=C-C-C=C-C-C=C-C-C-C-COOH
20:5 n3	cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	Eicosapentaenoic acid	CH3-C-C=C-C-C=C-C-C=C-C-C=C-C-C=C-C-C-C-COOH

The properties and function of lipids depend on the fatty acids in the structure. The fatty acid chains are hydrophobic and the longer the hydrocarbon chain is, the more predominant are the hydrophobic properties (Tvrzicka et al. 2011). The melting point and thus the consistency of fatty acids depends on a combination of various factors including the degree of saturation, the chain length and the cis-trans configuration of the double bonds. The more double bonds a fatty acid contains, the lower is its melting point. The longer the chain of a fatty acids is, the higher is its melting point. Cis-configured fatty acids occupy more space and lipids that contain more of those are usually soft or even fluid. Whereas trans-FA are linear, more immobile, and therefore in more solid lipids (Tvrzicka et al. 2011).

2.2 Metabolism of lipids in the rumen

In ruminants, the majority of ingested lipids is subjected to microbial action in the rumen prior to the host enzymatic action and absorption in the lower digestive tract. Ingested lipids are first split off through lipolysis in the rumen. Various lipases are active in the rumen that break down the lipids contained in the feed into glycerol and free fatty acids having a free carboxyl group which is necessary for the following hydrogenation step. These lipases are largely released by rumen bacteria but are also present in fresh plant materials (Bionaz et al. 2020). Rumen protozoa and possibly fungi may also play a role in lipolysis. However, this role has not yet been sufficiently researched and is not very significant according to current knowledge (Bionaz et al. 2020).

Subsequently, due to their toxicity to many rumen bacteria, the released PUFA are hydrogenated to SFA (Lock et al. 2006) – the process which is called biohydrogenation, before passing through to the lower digestive tract to finally be absorbed in the small intestine (Ferlay et al. 2017). Biohydrogenation consists of a cascade of transconfiguration and hydrogenation, which requires the activity of several bacteria in the rumen for the biohydrogenation process to take place completely and properly. These biohydrogenating bacteria can be divided into two groups, A and B (Kemp and Lander 1984). Bacteria of group A hydrogenate 18:2 n6 and 18:3 n3 via several intermediate steps to 18:1 isomers, while bacteria of group B hydrogenate 18:1 fatty acids to 18:0. Figure 2 shows the main pathway of the conversion from 18:2 n6 and 18:3 n3 to the final product 18:0 (Ferlay et al. 2017).

Linoleic acid (cis9, cis12-18:2)	α-Linolenic acid (cis9, cis12, cis15-18:3)
↓ Group A	↓ Group A
Rumenic acid/CLA (cis9, trans11-18:2)	CLnA (cis9, trans11, cis15-18:3)
↓ Group A	↓ Group A
Vaccenic acid (trans11-18:1)	trans11, cis15-18:2
↓ Group B	↓ Group A
Stearic acid (18:0)	Vaccenic acid (trans11-18:1) / cis15-18:1 / trans15-18:1
	↓ Group B
	Stearic acid (18:0)

CLA= conjugated linoleic acid, CLnA= conjugated linolenic acid

Figure 2: Conversion from linoleic and α-linolenic acid to stearic acid

Since not many bacteria are capable of the last step of biohydrogenation, this step proceeds slowly (Lock et al. 2006). For this reason, the intermediate products accumulate in the rumen and can escape the rumen before they are subjected to the completion of the biohydrogenation process (Beam et al. 2000). This results in a mixture of different C18 fatty acids leaving the rumen, passing through the abomasum, entering the duodenum for absorption and subsequently being transported to body tissues of animals (Beam et al. 2000). Oil supplementation, depending on the concentration, can regulate the rate of biohydrogenation and lipolysis in the rumen and thus also the amount of unsaturated fatty acids leaving the

rumen (Beam et al. 2000). Usually, large proportions (> 90%) of C18 PUFA are lost to the biohydrogenation process at the expense of the increase of 18:0 and numerous intermediates of biohydrogenation (various isomers of 18:1, 18:2 and 18:3). However, research has shown that 18:3 n3 is biohydrogenated to a greater extent than 18:2 n6 (Beam et al. 2000, Lock et al. 2006). In the intestine, these free fatty acids leaving the rumen bind to tiny feed particles or form bi-layer disks called micelles with bile salts and lysolecithin. Micelle formation attaches to enterocytes and allows fatty acids to be absorbed by diffusion or active transport (Bionaz et al. 2020). The free fatty acids are reattached to glycerol to form triglycerides once again in enterocytes (Lock et al. 2006). To be transported through the blood to their target cells in the organism the triglycerides must cross a bio membrane again. For this purpose, the triglycerides are encased in protein with other lipid-like substances and converted into chylomicrons that are also called very low density lipoproteins. However, the lipoproteins are too large to be absorbed directly into the blood vessels, so they are transported via the lymph to the large veins of the heart. Here they can be absorbed into the bloodstream and get distributed to their destinations in the organism. In the capillaries of the target organ like adipose tissue the lipoproteins are converted to free fatty acids by enzymes and can be used for their actual purpose like supplying energy or getting build in membranes (Wu et al. 2021). Owing to the ruminal biohydrogenation, the lipids of ruminants contain relatively high proportions of MUFA and SFA but very low in PUFA, in opposite to pork and chicken meat (Muzolf-Panek and Kaczmarek 2021) (Table 2). Some of the PUFA found in animal tissues are conjugated linoleic acid (CLA). Unlike other animal products, ruminant products are the natural food source of CLA, which are considered particularly healthy for human nutrition (Hartigh 2019, Şanlier et al. 2019). When comparing beef, pork and chicken, beef contains about 3 mg CLA per g fat and pork and chicken only contain 0.6 and 0.9 mg/g (Guo 2009).

Table 2: Fatty acid composition (%) of raw meat (Muzolf-Panek and Kaczmarek 2021)

	SFA	MUFA	PUFA
Beef	47.38	48.27	3.73
Pork	41.15	48.99	9.14
Chicken	30.40	46.15	23.29

SFA = saturated fatty acids

MUFA = monounsaturated fatty acids

PUFA = polyunsaturated fatty acids

2.3 Metabolism of fatty acids in the body

One primary biological function of fatty acids is that they can supply energy in a process called ß-oxidation. In mitochondria fatty acids are degraded to Acetyl-CoA which is a mandatory step of the citrate cycle – one of the most important components of the metabolism in a living being (Koolman et al. 1998). But in times when an organism is sufficiently supplied with energy, surplus fatty acids can be stored in the body for later energy production. This process is called lipogenesis. For this process, activated glycerol gets esterified with three fatty acids to form triacylglycerol, also called triglyceride. Triacylglycerols can be then stored water-free and thus space-saving in the form of lipid droplets in adipose tissue. The property of lipid droplets is not only to store energy but also to regulate "membrane trafficking, recycling of phospholipids, intracellular protein metabolism, cell signalling and response to starvation" (de Carvalho and Caramujo 2018). Adipose tissue is formed from preadipocytes, which develop from mesenchymal stem cells and undergo proliferation. This step is known as hyperplasia. It is the stage in which the cells proliferate but remain the same size. After differentiation into adipocytes, the fat cells only increase in size, which is called hypertrophy (Lei et al. 2021). The whole process of producing adipocytes is the adipogenesis. However, there is no rigid programme for this, according to which it always runs exactly, but adipogenesis, like so many other processes of life, can be influenced by the environment. If something changes, such as temperature or nutrients, this information is passed on via intracellular signals and causes short- or long-term adjustments in the adipocytes (Garton and Duncan 1969). Ruminant adipocytes grow at different rates (Cianzio et al. 1985). The fastest growing cells are those of the adipose tissue of the kidneys, followed by those of the mesentery, those of the subcutaneous tissue, those of the intermuscular adipose tissue, those of the intramuscular adipose tissue and the slowest growing adipocytes, those of the brisket fat (Cianzio et al. 1985). The different adipose tissue depots vary also in their fatty acid composition. For example, kidney fat has more SFA than subcutaneous fat (Hilditch and Williams 1964). In humans, fatty acid uptake from food has been studied intensively and is known to occur more efficiently in the upper body fat than lower body fat and more efficiently in the visceral fat than subcutaneous fat (Tchkonia et al. 2013). Not only there is depot variation but also regional differences within the same depot. For instance, in mice, a high-fat diet has been shown to affect the regulation of fatty acid metabolism but not uniformly between the adipose regions of the same depot. Accordingly, the expression of de-novo-lipogenesis-genes was 70% lower in the proximal part of the epididymal adipose tissue than in the distal part. In addition, the

proportion of essential fatty acids increased in the epididymal adipose tissue compared to the mesenteric adipose tissue (Caesar et al. 2010).

Fatty acids are also important components of membranes of all cells in the body. Glycerol is esterified with only two fatty acids and phosphoric acid to form phospholipids which are the main components of biomembranes. Besides phospholipids, there are also glycolipids as part of membranes, and they can especially function as receptors. Glycolipids are composed of sphingosine, oligosaccharide and one fatty acid (Koolman et al. 1998). Membrane fatty acids can vary and thus influence the properties of membranes including membrane fluidity, permeability and flexibility (Weijers 2012, de Carvalho and Caramujo 2018). Furthermore, membrane fatty acids also serve as a storehouse of signalling molecules between cells like anti-inflammatory signalling upon activation (de Carvalho and Caramujo 2018). Important derivatives are eicosanoids that are synthesized by modification of the 20-carbon basic structure of arachidonic acid (Calder 2006). Eicosanoids are local hormones that regulate, apart from inflammatory responses, also reproduction processes, blood pressure, digestion processes and further processes. Besides the eicosanoid derivatives, research has revealed a wide range of 18-carbon derivatives that also function as lipid mediators (Gabbs et al. 2015). In small quantities, however, fatty acids are also found unesterified e.g., in the blood. They are then referred to as free fatty acids (Surette 2008). Because fatty acids can be so diverse in composition, they are well suited as biomarkers and are sometimes used in this field (Surette 2008, de Carvalho and Caramujo 2018).

2.4 From grass to grain: feeding of dairy cattle and the shifts in dietary fatty acids

The natural feeding behaviour of cattle would be to eat mainly forages considering that wild ruminants live on pastures. Cattle feed can be divided into two categories: forage and concentrated feed. Due to the aims in modern dairy farming when the profitability is clearly in the foreground, dairy cattle are fed rations containing a large proportion of concentrates for a higher growth and milk yield and better cost efficiency (Chen et al. 2021). Concentrated feed contains many easily digestible carbohydrates that are quickly fermented in the rumen by microorganisms. This leads to increased feed intake, more production of propionate and butyrate (Chen et al. 2021), more milk production, and higher protein content of the milk (Phipps et al. 1995). However, the shift from grazing or forage-only to feeding high concentrate diets also has disadvantages. Many metabolic and inflammation-related problems such as subacute rumen acidosis, laminitis or fatty liver in dairy cows have been associated with

feeding high concentrate diets (Chen et al. 2021). Ruminal degradation of starchy concentrate, more of volatile free fatty acids are produced in a faster rate than when fibrous feed is degrade, which then lowers rumen pH (Khafipour et al. 2016). Subsequently, this sets off a cascade that leads to a higher proinflammatory cytokine release (Khafipour et al. 2016).

Physiological effects of calf feed have not yet been as well researched as that of adult cows. After initial feeding with milk or milk replacers, calves are gradually switched to solid feed with so-called starter diets. These feed mixtures are usually high in concentrate feed with a low fat content and low in forages (Hill et al. 2009). This kind of starter diets are common because concentrates have been shown to trigger an increased production of butyrate in the rumen and this in turn is known to promote the development of the rumen epithelium (Khan et al. 2016). Only when the rumen is fully developed calves can be nourished by solid feed alone. Farmers want the calf to be weaned as quickly as possible, thus saving costs for milk or milk replacer, so they use these starter diets. In addition to the complications of adult cows feeding mentioned above, clumping of papillae, acidosis, rumenitis and parakeratosis of rumen epithelium (Khan et al. 2016) are also described for calves.

The proportions of concentrate and forage vary greatly depending on whether the feed mixture is made for fresh lactating cows, late lactating cows, dry cows or for calves. Forage and concentrate feed differ in the physical property as well as the nutritional value. Concentrate feed is mainly rich in starch (Martins et al. 2011) and usually also contains more fat and protein (Chishti et al. 2022) than forages and has therefore a higher energy density. Forages, on the other hand, contain much less energy per kg feed dry matter (Rabelo et al. 2001), but is particularly rich in fibre. In ruminant nutrition, dietary fibre is measured in neutral detergent fibre (NDF), which is crucial for healthy and stable rumen function (Zebeli et al. 2012). When starting to eat solid feed, young calves consume very little fibre-rich ingredients (Omidi-Mirzaei et al. 2018). Both types of feed do not contain very much fat, but the small amount and especially the fatty acids contained are very important for a balanced diet. Cereal grains and forages differ in lipid type and more importantly the fatty acid composition. Concentrates contain more triglycerides and forages contain more glycolipids and phospholipids (Drackley 2007, Harfoot 1981). As far as fatty acids are concerned, SFA are more abundant in concentrates and PUFA in forages (Martins et al. 2011). Tables 3 and 4 list the most abundant fatty acids of some common feed ingredients in dairy rations, including the forage sources such as grass in various forms (fresh, ensiled, dried), corn silage, alfalfa and wheat straw (Arfuso et al. 2017, Bodkowski et al. 2016, Havlin et al. 2016) and concentrate feed sources

such as corn, soybean meal, rapeseed meal, barley and wheat bran (Braun et al. 2018, Dehghan et al. 2013, Hill et al. 2009, Mesilati et al. 2012).

Table 3: Fatty acid composition of common forages in dairy cattle diets

Fatty acids in%	Fresh grass (Huuskonen et al. 2010)	Grass silage (Huuskonen et al. 2010)	Hay (Valvo et al. 2006)	Corn silage (Ranathunga et al. 2010)	Alfalfa (Ranathunga et al. 2010)	Wheat straw (Mavrommatis et al. 2021)
16:0	14.3	16.6	18.33	19.3	22.8	29.88
16:1 cis-9	2.2	2.1	0.45	0.12	0.15	
18:0	2.1	1.5	4.31	2.3	3.0	4.86
18:1 cis-9	2.0	4.4	8.29	11.3	4.1	34.77
18:2 n6	15.1	21.8	16.98	26.8	17.4	21.95
18:3 n3	59.4	49.3	48.77	20.1	25.5	1.86

Table 4: Fatty acid composition of common concentrate feed ingredients in dairy cattle diets

Fatty acids in%	Corn (Lee et al. 2013)	Soybean meal (Lee et al. 2013)	Rapeseed meal (Di Lena et al. 2021)	Barley (Huuskonen et al. 2010)	Wheat bran (Maiorino et al. 1986)
16:0	11.25	13.06	9.66	18.4	18.68
16:1 cis-9	0.14	0.13	0.81	0.1	0.42
18:0	2.07	4.08	1.11	1.0	1.83
18:1 cis-9	23.74	16.19	53.86	17.4	22.09
18:2 n6	59.41	55.20	29.17	51.3	50.58
18:3 n3	1.62	10.09	4.53	9.1	4.5

In Table 3 nd 4 it can be seen that most of the forage components are rich in n3 PUFA whereas concentrate components are rich in n6 PUFA. This means that modern dairy cattle fed with high proportions of concentrate feed have significantly higher intake of n6 to n3 PUFA ratios in comparison to when feeding their natural diets. For instance, the n6 to n3 PUFA ratio of a

grass-only diet was 0.25 (Huuskonen et al. 2010) or pasture only diet had a ratio of 0.27 (Fredriksson Eriksson and Pickova 2007), whereas in a total mixed ration with 60% concentrate for dairy cattle the n6 to n3 PUFA ratio raised to 3.9-5.9 (Greco et al. 2015). Depending on the source and proportion of cereal grains as well as oil addition, calve starter diets can have an n6 to n3 PUFA ratio up to 15 (Jolazadeh et al. 2019).

2.5 Effects of dietary n6 and n3 PUFA on the fatty acid composition of tissues in ruminants

Despite the biohydrogenation process of PUFA, 18:2 n6 and 18:3 n3 can still be enriched in ruminant tissues through diet. The majority of 18:2 n6 and 18:3 n3 are transformed during the process of biohydrogenation, however, some of them escape the process, are absorbed and subsequently enter the tissues such as milk, muscle or adipose tissue. Modification of dietary PUFA via feeding strategy can therefore modify the enrichment of these PUFA in different tissues (Lock et al. 2006).

A meta-analysis of 82 feeding trials comparing grazing, forage-based indoor feeding and concentrate-based indoor feeding showed that 18:2 n6, 18:3 n3 and total C18 fatty acid contents always increased in milk fat when it was elevated in the diet (Khiaosa-Ard et al. 2015). Accordingly, grazing and forage-based indoor feeding achieved a higher enrichment of 18:3 n3 in milk than concentrate-based indoor feeding. And concentrate-based indoor feeding achieved a higher enrichment of 18:2 n6 in milk.

Feeding also has a profound effect on the fatty acid composition of muscle and adipose tissues. For instance, in muscle tissue of grass-fed cattle, 18:2 n6 accounted for 3.41% of total fatty acids and in grain-fed cattle without access to pasture it was 6.19%. On the contrary, 18:3 n3 accounted for 1.3% of total fatty acids in grass-fed cattle and only 0.28% in grain-fed cattle (Garcia et al. 2008). If the high n3 PUFA contents of grass and the high n6 PUFA contents of grain are taken into account, this clearly indicates a correlation in Garcia's study. Liméa et al. (2012) performed a feeding trial in goats testing four diets differing in the amount of concentrate from 0 to 340 g per day. They showed that the more concentrate fed, the higher the n6 PUFA content in muscle and adipose tissue, but also the lower the n3 PUFA content in these tissues. A lower n3 PUFA content could be detected in the adipose tissue of cattle that were fed a feedlot diet of maize silage and cereals in contrast to cattle that were exclusively pastured (Fincham et al. 2009). In the tissue, not only does the content of fatty acids such as 18:2 n6 and 18:3 n3, which are increased or decreased in the diet, change, but the content of other fatty acids such as CLA or trans 18:1 is also altered when PUFA intake changes. This is

attributed to both the alteration in biohydrogenation and the enzyme activity in converting trans 18:1 to CLA in the tissue (Garcia et al. 2008). The muscle tissue of grass-fed cattle contained 0.72% CLA, while the muscle tissue of grain-fed cattle contained only 0.31%. Trans 18:1, on the other hand, was lower in grass-fed cattle (3.22%) than in grain-fed cattle (4.35%).

Not only the amount of forage but also the quality of forage influences the PUFA content of the forage. To obtain a high-quality hay it needs a soil that provides sufficient nutrients. The hay must be harvested at a time when the nutrient content of the grass is at its highest and it must be carefully dried indoor to prevent losses of the nutrients, like n3 PUFA, during drying (Oksanen and Thafvelin 1965, Wyss 2012). This achieves hay with higher contents of lipids, crude protein and water-soluble carbohydrates, which defines high-quality hay (Kleefisch et al. 2016). Studies in early lactating cows showed that feeding high quality hay instead of regular hay supported the intake, milk production (Kleefisch et al. 2018) and enriched n3 PUFA in the milk fat (Khiaosa-ard et al. 2020).

The effect of dietary fatty acids on the fatty acid composition of tissues in calves is generally less studied than in adult cows. A rapid growth and development of adipose tissue is part of animal growth of young calves. Adipocyte hyperplasia occurs mainly in the last weeks before birth and up to 250 days after birth, which can be affected by the mother's diet. For instance, maternal transcription factors that regulate the differentiation of fat cells in the adipose tissue of the foetus were influenced by the maternal diet (Ladeira et al. 2018). Furthermore, the fatty acid composition of the mother's milk, thus the intake of PUFA of the calf, is influenced by the diet of the dam and consequently this affects the fatty acid composition of tissue lipids. The supplementation of 18:2 n6 and 18:3 n3 and CLA in the dam, nine weeks before and one week after birth, was reflected in the adipose tissue of the calves (Dahl et al. 2020). PUFA in solid feed becomes increasingly important as calves approach weaning and thereafter, as they account for the majority of nutrient intake. But this topic is not much researched so far. It must be noted that studies usually perform an extraction of total lipids and do not distinguish between triglycerides (the storage lipids) and phospholipids (the membrane lipids). Thus, the effect of feeding on the enrichment of PUFA adipose and muscle tissues predominately reflect the effect on triglycerides since proportionally more storage lipids than membrane lipids are present in tissues, especially for adipose tissue. Indeed, the fatty acid composition of membrane phospholipids also respond to dietary PUFA intake (Clandinin et al. 1985, Jenkins 1994), although it is more resistant to change compared to the triglyceride as shown in rats (Abbott et al. 2012). In general, membrane lipids are still poorly researched. But because shifts in membrane PUFA have health relevance, for adipose tissue research differentiating between

storage and membrane lipids are crucial to understand the health aspect of feeding. Accordingly, this study differentiates between membrane and storage lipids in investigating the effect of dietary PUFA on modulating tissue fatty acid composition in young dairy calves.

3 Aims and Hypothesis

This study expected to find more n3 PUFA and less n6 PUFA in the adipose tissue of young dairy calves fed higher levels of n3 PUFA and lower levels of n6 PUFA, whereby the shift of these fatty acids was expected to be reflected more in storage lipids than in membrane lipids. The aim of this thesis was therefore to evaluate the effects of four diets in young dairy calves differing in hay proportion and quality on PUFA intake and corresponding changes in fatty acid composition of the adipose tissue, which was differentiated between storage and membrane lipids.

4 Materials and methods

4.1 Diet, feeding, and housing of calves

Data used in this thesis were derived from 20 Holstein calves (17 males, 3 females) which were part of the "Healthy Calf" project. Details about that experiment and several research findings are described in earlier papers (Hartinger et al. 2022, Poier et al. 2022, Terler et al. 2022, Terler et al. 2023). The calves were housed in individual compartments with straw and were kept strictly according to the animal treatment and handling protocols approved by the national authority (GZ:BMBWF-66.019/0016-V/3b/2019). After birth and colostrum feeding, calves were randomly assigned to one of the four experimental diets including 100% regular hay (NH), 100% high-quality hay (also known as “Zuckerheu”, ZH), 30% regular hay + 70% calf concentrate (NH+Conc) and 30% high-quality hay + 70% calf concentrate (ZH+Conc). Ingredients of the concentrate were 35% barley, 36% wheat, 17% soybean meal, 10% linseed meal and 3% mineral supplement. NH was grass harvested as the second cut of a permanent grassland containing a mixture of approximately 75% grasses, 15% clover and 10% herbs and dried in a hay drying facility. The ZH was primarily perennial ryegrass harvested as first and second cut at the beginning of ear emergence and dried in an indoor drying facility as described by Terler et al. (2022). The nutrient contents on a dry matter basis of the grasses and concentrate mix are listed in Table 5.

Table 5: Nutrient contents on a dry matter basis of high (ZH) and medium quality hay (NH), concentrate (Conc) and milk used in the feeding experiment

	% crude protein	% water-soluble carbohy-drates	% non-fibre carbohy-drates	% lactose	% neutral detergent fibre	% fat	MJ metabolizable energy/kg dry matter
ZH	21	20.5			45.5	2.4	11.2
NH	14.9	12.4			52.2	1.8	9.4
Conc	19.3		54.7		20.4	1.8	13.5
Milk	26			36		32.2	19.2

On the first day of life, each calf received at least 2.5 litres of its mother's colostrum. After that, the calves received acidified milk *ad libitum* for the next four weeks. In the next eight weeks milk allowance was reduced step by step for all calves until weaning at the end of week 12. Already in the first week, calves were offered their experimental solid diet. The milk, feed and water leftovers were accurately weighed daily and compared with the milk, feed and water

offered to determine the daily consumption of each individual calf. As already described by Terler et al. (2022), in the first seven weeks of life calves in all feeding groups drank less than 2.5 liter of water per day. From then until the eleventh week ZH calves drank the most water. From the eleventh week on NH drank the fewest water. In the last week NH, ZH, NH+Conc and ZH+Conc had a daily water intake of 10.9, 13.9, 12.7, and 13.5 liter (Terler et al. 2022).

4.2 Sampling and performance data record

Monthly interval feed samples were taken for the analysis of the fatty acid composition and content, which were subsequently used for the estimation of the daily n6 to n3 PUFA intake. The calves were weighed after birth and before slaughter and from this the daily gain was calculated. Fat and protein deposition (kg/d) was calculated with the growth rate using a formula taken from the study by Robelin and Chilliard (1989). After about 15 weeks (94-111 days of life), the calves were sacrificed. They were numbed with a bolt gun and killed by opening the jugular vein. After death, the skin and internal organs were removed. The weight of the kidney and kidney fat were documented. Maximum 30 minutes after numbing, adipose samples were taken from two regions of the perirenal adipose tissue. This adipose tissue depot was selected because it is the fastest growing adipose tissue in cattle (Cianzio et al. 1985). Adipose tissue sample of the inner region was taken from the area near the helium, sample of the outer region was taken from a large fat pad distal to the kidney. After sampling, the tissue was cut into small pieces, then shock-frozen in liquid nitrogen and after that preserved under -80 °C until analysis.

4.3 Fatty acid composition analysis of feed samples and adipose tissue

The fatty acid analysis of feed samples was done using gas chromatography (GC), but before fatty acid salts and acyl components of the lipids had to be converted to fatty acid methyl esters (FAME). For this a one-step extraction-methylation was performed according to the method of Palmquist and Jenkins (2003). At first the reagents and internal standard mixture had to be prepared. For that, a 10% methanolic HCl 20 ml of acetyl chloride (Sigma Aldrich, Merck KGaA, Darmstadt, Deutschland) was added to 100 ml of methanol on ice dropwise due to the exothermic reaction, while stirring on a magnetic stirring plate. When finished the solution was stored at room temperature away from light. 60 g potassium carbonate was dissolved in distilled water and produced to 1000 ml of 6% potassium carbonate-solution, which was stored at room temperature. For the internal standard, 40 mg of heptadecanoic acid (17:0; Sigma

Aldrich, Merck KGaA, Darmstadt, Deutschland; #H3500) was prepared in 50 ml heptane. The internal standard was stored at 4°C.

Before the feed samples were subjected to lipid extraction, they were air dried and pulverised. Then about 500 mg of each feed sample were weighed into a 35 ml glass tube with screw cap and the precise weight was documented. Subsequently, 2 ml internal standard was added followed by 3 ml of 10% methanolic HCl which acted as the catalyst. The tubes were sealed leakage-free, vortexed and heated for two hours in a 90 °C water bath. In between, they were repeatedly checked for leaks. The tubes were then left to cool at room temperature for 10-15 minutes. Subsequently 1 ml of heptane was added and mixed gently. The next step was to add 10 ml of 6% K_2CO_3. Careful execution was necessary to prevent any loss of samples. After that the sample was vortexed again and for ten minutes centrifuged at room temperature at approximately 1600 × *g* (Eppendorf Centrifuge 5810R = 4000 rpm) to separate liquid layers. Then the top layer was transferred to a 2-ml Eppendorf tube and 50-100 mg of sodium sulfate (or same amount of charcoal if the sample was highly colored) was added. After that the sample was vortexed again and for ten minutes centrifuged at room temperature at approximately 21380 × *g* (Hermle Z 326 K; 21380 x *g* = 15000 rpm). Finally, the solvent layer was transferred into a glass vial (with/without insert) and the cap was closed. The vials were stored at +4 °C until GC analysis to the maximum four weeks for quality reasons.

GC analysis was used to determine the composition and amount of fatty acids contained in the sample. A device with a fused silica column from Phenomenex was used. The injector had a temperature of 250 °C and the detector was 260 °C. The carrier gas to bring the FAMEs through the column was helium. A 37-component FAME mixture was used as an external standard to evaluate the FAME peaks. With the help of this external and the internal standard, the amount of fatty acids was calculated. The official AOAC method was used for the calculation (AOAC 2012).

Before the fatty acids of the adipose tissue could be examined, they first had to go through several steps of preparation. To do this, frozen tissue was finely ground while being kept frozen with liquid nitrogen into a homogeneous powder with a mortar and pestle. Next, the lipids were then extracted from the ground tissue samples using a chloroform-methanol mixture following the protocol of Folch et al. (1957) with minor modifications. The extraction was carried out at room temperature for 2.5 hours. Occasionally, it was gently shaken by hand. In a new glass tube it was mixed with 1.8 ml of a 0.88% KCl solution until a homogeneous liquid was obtained. After this was centrifuged at 3000 rpm (1811 × *g*) for 10 minutes at room temperature, and the upper aqueous phase was decanted. The lower layer contained the extracted lipids and was

dried under N_2. Finally, a 2% suspension was prepared from the lipids and hexane. The extracted lipids were separated into neutral lipids, phospholipids and free fatty acids using a solid phase extraction. Each fraction was recovered and then prepared for GC analysis. Accordingly, the individual fractions were first mixed with 0.5 ml hexane, then heptadecanoic acid (17:0) was added as an internal standard. The recovered mixture was then subjected to an acid-catalysed methylation to form FAMEs. After that, it also got analysed and quantified by GC like described above for the feed samples.

4.4 Statistical analysis

All data analyses were performed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Thus values presented are least squares means and the standard error of the mean (SEM) are reported along. Daily feed intake and fatty acid intake were transformed to a weekly average and were subsequently used for data analysis. For this, we tested fixed effects of feed, week and their interaction. One way ANOVA to test the diet effect on different performance variables including the average daily fatty acids intake from week 12 till end of life was examined. For adipose tissue fatty acids, we tested the fixed effects of feed, adipose region and their interaction. Because there were differences in gender distribution to treatments and time of sampling, these factors were also considered as the random factor in the analysis. Differences among the least squares means were compared according to Tukey's test. Effects and group differences were called when a P level was below 0.05.

5 Results

5.1 Solid feed intake

The daily feed intake, on a weekly average basis, from first week of life until slaughter are shown in Figure 3. The concentrate and hay intake showed a significant effect of diet group ($P < 0.001$), week of life ($P < 0.001$) and the interaction between feed and week of life ($P < 0.001$). The concentrate intake in NH+Conc and ZH+Conc increased after it was offered from the fifth week onwards, slowly at first, but then from the tenth week it increased by about 50 g per week up to 3.15 kg/d in NH+Conc and 2.66 kg in ZH+Conc. The hay intake increased almost equally in all groups from the fifth week onwards. It was not until the ninth week that the calves on both hay-only diets consumed significantly more hay with each week of life than calves that were offered the high concentrate diets. Since then, hay intake increased rapidly in both hay-only diets from about 0.4 to 3.34 kg/d for ZH and 2.5 kg/d for NH. In the Conc groups, hay intake also increased steadily from the ninth week onwards, although not nearly as much as in the hay-only groups. ZH+Conc calves consumed up to 1.19 kg/d hay, while NH+Conc calves only ate up to 0.75 kg/d. Differences in milk intake were detected transiently only in the week 3 and 4 of life when the NH calves drank the least milk intake of about 8.5 kg/d while the other groups drank about 10.7-11.6 kg/calf/d. After that, when the milk allowance was restricted, there was no differences in milk intake. Accordingly, the milk intake in each group decreased simultaneously according to the amount of milk offered, which sank evenly from 8 kg to 0 kg in seven weeks.

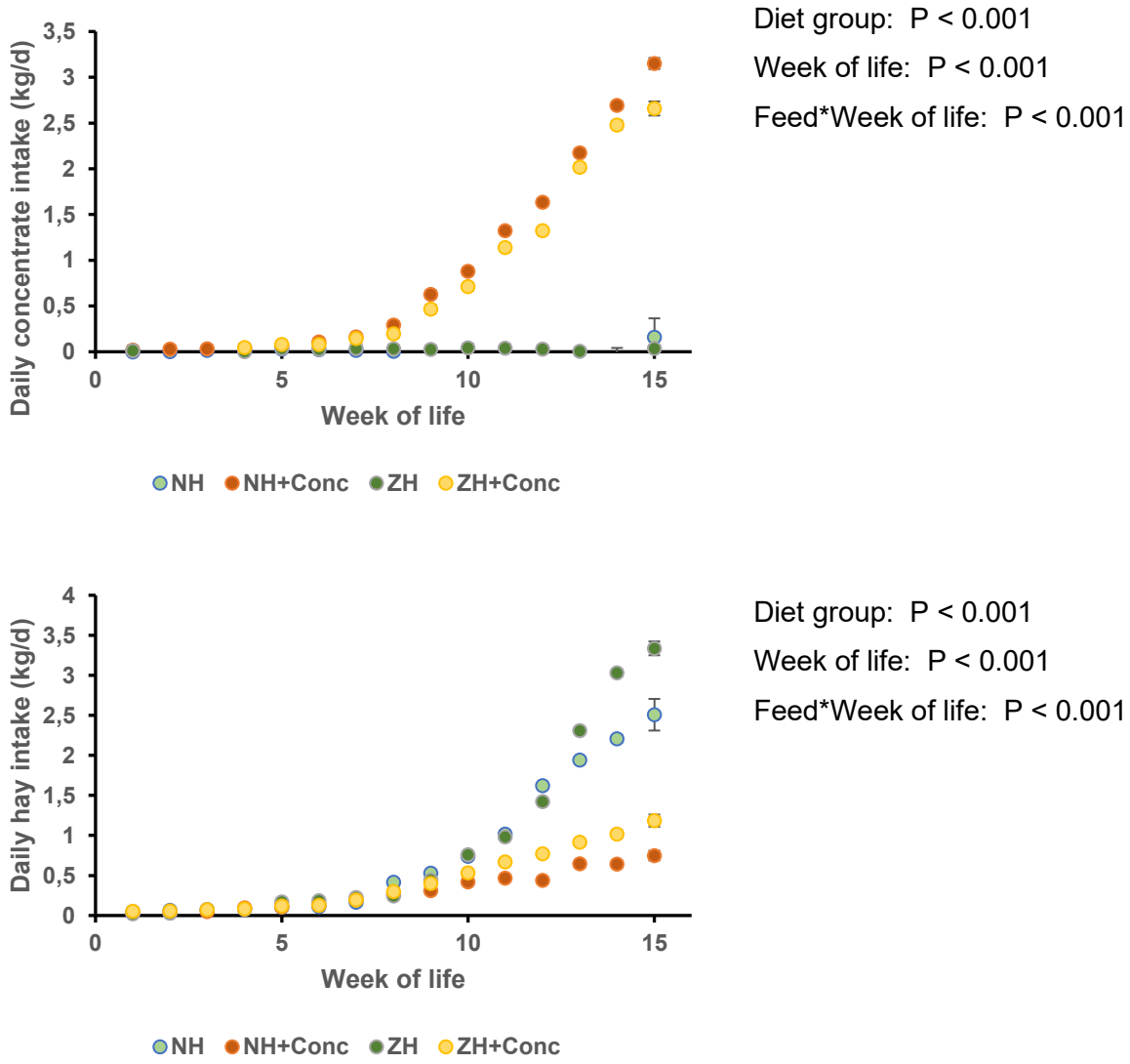


Figure 3: Daily concentrate and hay intake, on a weekly average, in young dairy calves fed diets differing in the proportion and quality of hay (NH = 100% regular hay, ZH = 100% high-quality hay, NH+Conc = 30% regular hay + 70% calf concentrate, ZH+Conc = 30% high-quality hay + 70% calf concentrate)

5.2 Dietary fatty acid composition, fatty acid intake and performance

Table 6 shows the results of the feed analysis. The acidified milk contained proportionally low n6 and n3 PUFA overall (< 2% of total fatty acids). Concentrate was rich in n6 PUFA, whereas hay was rich in n3 PUFA. It is striking that ZH contained almost 10% more n3 PUFA than NH. Combining these results with the data of daily concentrate and hay intake, we obtained the daily consumed n6 to n3 PUFA ratio. Figure 4 shows the n6 to n3 PUFA intake in all four diet groups on a weekly average. There was an effect of diet which changed over time as

underlined by the significant interaction of diet and time ($P < 0.001$). In the first four weeks of life, when calves consume mainly milk and little solid feed (Figure 3), the n6 to n3 PUFA intake in all four diet groups was approximately the same, at about 2:1 (Figure 4). From the fifth week onwards, the n6 to n3 PUFA ratio varied more and more among the groups. The NH+Conc group exhibited the highest n6 to n3 PUFA intake, reaching up to 5.7. In the ZH+Conc group, the n6 to n3 PUFA intake only went up to 3.2 and even dropped to 2.2 in the last week. In the groups fed hay only, the n6 to n3 PUFA intake was always the lowest and decreased continuously with the decrease of milk intake and the increase of solid feed intake. There was only a slight difference in the n6 to n3 PUFA intake between the two types of hay. In the group with NH the intake dropped to 0.38 and in the ZH group it even dropped to 0.28.

Table 6: Fatty acid content (g/100 g, on a DM basis, for solid feed components) and composition (% of total fatty acids) of milk, concentrate, regular hay and high-quality hay (mean ± SD)

	Milk	Concentrate	NH	ZH
Total fatty acid content g/100 g	3.15 ± 0.24	2.27 ± 0.17	1.37 ± 0.23	2.03 ± 0.23
16:0	30.53 ± 1.27	20.41 ± 1.78	18.92 ±6.45	19.77 ± 2.9
18:0	9.86 ± 0.67	1.87 ± 0.16	2.21 ± 0.35	1.62 ± 0.2
18:1 n9	21.15 ± 1.99	15.01 ± 0.62	4.76 ± 1.13	2.55 ± 0.23
18:2 (all isomers)	1.42 ± 0.12	55.35 ± 1.11	17.13 ± 1.23	12.76 ± 0.51
18:3 n3	0.68 ± 0.09	4.47 ± 0.22	40.74 ± 5.95	50.43 ± 3.01

NH = 100% regular hay

ZH = 100% high-quality hay

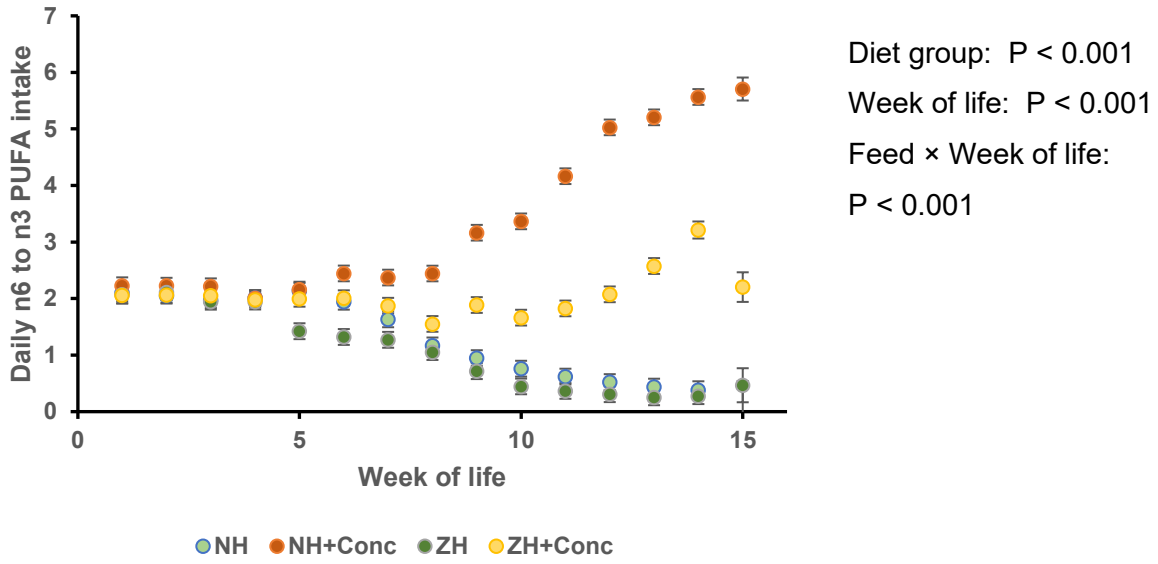


Figure 4: Daily n6 to n3 PUFA intake, on a weekly average, in young dairy calves fed diets differing in the proportion and quality of hay (NH = 100% regular hay, ZH = 100% high-quality hay, NH+Conc = 30% regular hay + 70% calf concentrate, ZH+Conc = 30% high-quality hay + 70% calf concentrate)

Table 7 shows the recorded performance data of the calves in the different groups. The diet showed a significant effect on all variables ($P < 0.05$) except the birth weight ($P = 0.37$) and dry matter intake of the last three to four weeks of life ($P = 0.063$). Calves fed the two concentrate diets were on average about 30 kg heavier than the NH-fed calves ($P < 0.05$), while calves in ZH showed the intermediate value. Concentrate-fed calves gained about 0.3 kg/d more than NH-fed calves ($P < 0.05$). Again, ZH-fed calved showed the intermediate value. Estimated lipid deposition was the highest in the concentrate groups. In calves fed NH the lipid deposition was 100 g/d lower ($P < 0.05$) and in ZH calves the value was intermediate. Estimated protein deposition was the highest in the concentrate groups too. In calves fed NH the protein deposition was about 40 g/d lower than in the Conc groups ($P < 0.05$) and ZH calves had an intermediate value again. The kidney weight was almost the same in all groups except for NH calves, whose kidney weighed about 0.1 kg less ($P < 0.05$). The kidney fat weight was intermediate in the ZH group, about 0.2 kg heavier in the two concentrate groups and 0.3 kg lighter in the NH group. During the three to four last weeks of life when the solid feed was the calves' primary diet, the intake of total n3 PUFA was lowest in NH+Conc, NH and ZH+Conc did not have significantly higher values and calves of the ZH group had the highest total n3 PUFA intake. Total n6 PUFA intake was around 20 g/d lower in the two hay-only diets ($P < 0.05$) than in both Conc diets during this period. Consequently, the n6 to n3 PUFA intake was highest in NH+Conc and only half as high in ZH+Conc ($P < 0.05$). The two hay diets had

the lowest n6 to n3 PUFA intake ($P < 0.05$) but showed no significant differences between each other.

Table 7: Intake of dry matter and fatty acids (FA), growth performance and the fatty acid composition (% of total fatty acids) separated by the lipid class extracted from adipose tissue samples.

(NH = 100% regular hay, ZH = 100% high-quality hay, NH+Conc = 30% regular hay + 70% calf concentrate, ZH+Conc = 30% high-quality hay + 70% calf concentrate)

Item	NH	NH+Conc	ZH	ZH+Conc	Standard error	P value
Birth weight, kg	40	46	39	42	4.1	0.370
Slaughter weight, kg	110^{b}	138^{a}	127^{ab}	139^{a}	6.2	0.014
Daily gain, kg/d	0.68^{b}	0.91^{a}	0.85^{ab}	0.97^{a}	0.06	0.012
Lipid deposition, g/d	69^{b}	162^{a}	122^{ab}	189^{a}	28	0.017
Protein deposition, g/d	131^{b}	165^{a}	154^{ab}	175^{a}	8.4	0.005
Kidney weight, kg	0.36^{b}	0.58^{a}	0.48^{a}	0.55^{a}	0.03	<0.001
Kidney fat weight, kg	0.49^{b}	0.96^{a}	0.79^{ab}	1.05^{a}	0.117	0.021
Solid feed intake (week 12-15)						
dry matter intake, kg/d	1.95	2.77	2.25	2.8	0.24	0.063
Total n3 PUFA, g/d	11.06^{b}	4.95^{b}	25.19^{a}	10.82^{b}	2.82	<0.001
Total n6 PUFA, g/d	4.81^{b}	27.64^{a}	6.54^{b}	25.64^{a}	1.99	<0.001
n6 to n3 PUFA intake	0.59^{c}	5.47^{a}	0.45^{c}	2.64^{b}	0.6	<0.001

Groups sharing no common superscripts differ significantly ($P < 0.05$)

5.3 Adipose tissue fatty acid composition

Table 8 and Figure 5 summarize the fatty acid composition of two different regions of the kidney adipose tissue. Overall, there was a strong effect of diet and adipose region on the fatty acid composition but there was no significant interaction between diet and tissue region on the proportion of key fatty acids and n6 to n3 PUFA ratio (Table 8). With respect to the regional difference, the neutral lipids of the inner adipose tissue region contained more of 16:0 and 18:1 and less of 18:0 than of the outer region. The phospholipids of the inner adipose tissue region contained more of 16:0 but less of 18:1, 18:3 n3, 20:4 n6 and n6 PUFA and had a lower n6 to n3 PUFA ratio than phospholipids of the outer region. The other measured fatty acids showed no significant differences between the region in the adipose tissue.

Figure 5 depicts the proportion of key PUFA in the adipose tissue as affected by the diet. Accordingly, the diet showed a significant effect on the proportions of 18:2 n6, 18:3 n3, n3 PUFA, n6 PUFA and n6 to n3 PUFA ratio in total fatty acids of neutral lipids ($P < 0.05$) and on 18:2 n6, 18:3 n3, 20:5 n3, n3 PUFA and n6 to n3 PUFA ratio of phospholipids ($P < 0.05$). The proportion of 18:2 n6 as well as the proportion of total n6 PUFA in the neutral lipids of the calves in the concentrate-fed groups was almost two times higher than in the hay-fed groups

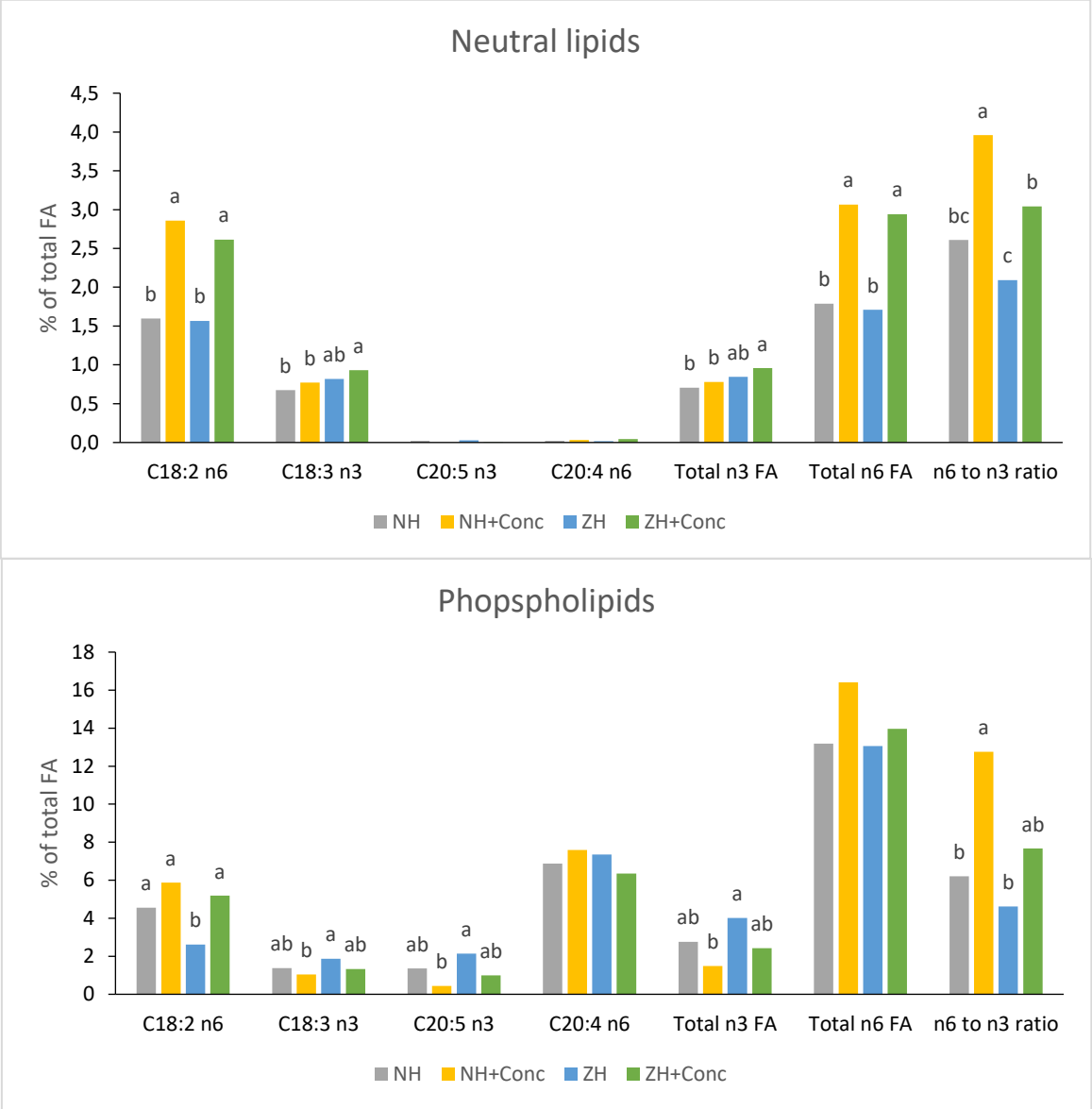
($P < 0.05$). In contrast, differences among diets in the proportions of total n3 PUFA in the neutral lipids were not as drastic in magnitude though statistically significant. ZH+Conc led to the higher proportion of 18:3 n3 and the total n3 PUFA than NH and NH+Conc ($P < 0.05$) while ZH showed the intermediate proportion. 20:5 n3 and 20:4 n6 in neutral lipids were in all groups very low and showed no significant differences ($P > 0.05$). The ratio of n6 to n3 PUFA was highest with 3.96 in NH+Conc and followed in descending order ZH+Conc, NH and ZH.

Phospholipids contained generally higher proportions of PUFA, especially n6 PUFA, than neutral lipids. In the phospholipids total n6 PUFA was lowest in ZH and NH and highest in NH+Conc ($P = 0.097$). 18:2 n6 proportion in the phospholipids was lowest in ZH at 2.62% being significantly lower than all other diet groups ($P < 0.05$). In contrast, 18:3 n3 was highest in the phospholipids of the ZH calves being significantly higher than NH+Conc ($P < 0.05$), while NH and ZH+Conc showed intermediate values. This distribution was also true for 20:5 n3 and total n3 PUFA. 20:4 n6 was in all four groups at about 7% ($P = 0.44$). With a similar pattern found in the neutral lipids, the ratio of n6 to n3 PUFA was highest in NH+Conc, lower in ZH+Conc and NH and lowest in ZH.

Table 8: Fatty acid composition (% of total fatty acids (FA)) of two different regions of the kidney adipose tissue in young dairy calves fed diets differing in the proportion and quality of hay

	Tissue region			P-value		
Fatty acid	Inner	Outer	SEM	Feed[1]	Tissue	Feed×Tissue
Neutral lipids						
16:0	29.45	27.86	0.64	0.782	0.001	0.446
18:0	16.21	20.53	0.85	0.265	<0.001	0.132
18:1	36.80	35.24	0.73	0.180	0.005	0.630
18:2 n6	2.009	2.308	0.219	<0.001	0.063	0.456
18:3 n3	0.783	0.815	0.066	0.001	0.440	0.671
20:5 n3	0.001	0.025	0.017	0.319	0.061	0.251
20:4 n6	0.023	0.034	0.011	0.075	0.171	0.997
Total n3 FA	0.799	0.847	0.064	0.001	0.254	0.754
Total n6 FA	2.181	2.569	0.271	<0.001	0.053	0.601
n6 to n3 ratio	2.776	3.077	0.226	<0.001	0.070	0.458
Phospholipids						
16:0	29.02	24.28	1.71	0.064	0.001	0.787
18:0	24.17	25.48	1.39	0.164	0.135	0.190
18:1	16.21	19.08	1.17	0.205	0.002	0.877
18:2 n6	4.479	4.639	0.855	<0.001	0.660	0.506
18:3 n3	1.199	1.611	0.211	0.005	0.010	0.795
20:5 n3	1.540	0.925	0.497	0.013	0.091	0.496
20:4 n6	4.997	9.093	0.840	0.440	<0.001	0.695
Total n3 FA	2.799	2.537	0.635	0.004	0.563	0.626
Total n6 FA	11.310	17.000	1.730	0.097	<0.001	0.376
n6 to n3 ratio	5.798	9.826	1.919	0.001	0.006	0.455

[1]See Figure 5 for the means values of dietary treatments



Groups sharing no common superscripts differ significantly ($P < 0.05$)

Figure 5: Fatty acid composition of neutral lipids and phospholipids in kidney adipose tissue in young dairy calves fed diets differing in the proportion and quality of hay. P values and standard error of the mean are shown in Table 8.

(NH = 100% regular hay, ZH = 100% high-quality hay, NH+Conc = 30% regular hay + 70% calf concentrate, ZH+Conc = 30% high-quality hay + 70% calf concentrate)

6 Discussion

6.1 Regional differences in adipose tissue fatty acid composition

Until now, ruminant research has revealed differences in fatty acid composition among different tissues, like muscle and adipose tissue (Wang et al. 2019, Gravador et al. 2020) and for adipose tissue also among adipose depots (Berthelot et al. 2001). The current results of the kidney adipose tissue of young dairy calves indicate that different regions of the same adipose tissue depot also differ in the fatty acid compositions . This is probably partly due to the regulation of expression of de novo lipogenesis genes as shown in mice (Caesar et al. 2010) but remains to be proven in cattle. The regional difference of neutral lipids was mainly for 16:0, 18:0 and 18:1. Only phospholipids showed differences of n6 PUFA, especially 20:4 n6, between the two regions. Since n6 PUFA express physiological effects (Calder 2006), changes occurring to the membrane phospholipids of particular location could be crucial to tissue inflammation when the location is exposed to a stressor. For instance, adipose tissue in close proximity to the GIT is more susceptible to inflammation due to increased exposure to microbial toxins that infiltrate through a compromised gut barrier, initiating localized inflammation (Khiaosa-ard and Zebeli 2018). It will be necessary for future research projects to consider not only the depot differences but also where tissue samples within the depot are taken. Although fatty acid composition differed significantly between tissue regions and between different diet groups, there was no interaction between diet and adipose tissue region shown here in young dairy calves. This means that diet indeed has a significant effect on the fatty acid composition in the adipose tissue, but not on how the fatty acids are distributed within the tissue. It must depend on factors other than diet how much of a particular fatty acid accumulates in a particular region of adipose tissue.

6.2 Dietary effects on performance and adipose tissue fatty acid composition

Intake of solid feed is clearly dependent on the duration and quantity of milk offered (Silva et al. 2019, Terler et al. 2022). Correspondingly, in the current experiment milk intake transiently differed only in weeks three and four and did not differ once milk supply was limited. That means that detected differences in nutrient intake, performance or adipose tissue fatty acids in this study were likely driven by the solid feed intake.

Overall, calves fed ZH ate more hay than those fed NH. When comparing between the concentrate-fed groups, the calves fed ZH also ate more hay and consumed slightly less concentrate instead. Therefore, it can be postulated that the decisive factor for hay consumption is the palatability of the hay and that is the reason why ZH was presumably

preferred to NH. Furthermore, ZH was also more energy-dense with 1.8 MJ more metabolizable energy per kg feed compared to NH. The higher energy content was a result of the higher contents of crude protein, carbohydrates and fat. Forage NDF content affects rumen filling, and thus lowering NDF content increases the DM intake (Fustini et al. 2017). Likewise, ZH contained less NDF than NH and this may also explain more solid feed intake of ZH-fed calves. Usually, ruminants fed concentrates show higher performance than animals fed forages only (Bu et al. 2021, Moran et al. 2017). In line with that, the positive effect of concentrate feeding on promoting growth was also observed in the present work. Notably, while NH led to the lowest performance of the calves ZH was not significantly lower compared to NH+Conc. Still, ZH+Conc provided the best performance results. Studies on dry and lactating cows (Kleefisch et al. 2016, Kleefisch et al. 2018) showed that feeding high-quality hay with reduced concentrate is optimal for dairy cows. Similarly, and in line with the results of this study, it also seems to be an optimal diet for dairy calves.

The feeding of hay and concentrates substantially determines the composition of PUFA intake (Drackley 2007, Harfoot 1981, Martins et al. 2011). Between the hay-only diets, the n6 to n3 ratio was only slightly lower in ZH than in NH. Nevertheless, the lipid content in ZH and thus the total PUFA intake was higher when the animals were fed ZH than when they were fed NH. Proportions of forages in diets determine the profile of PUFA intake (Garcia et al. 2008, Khiaosa-Ard et al. 2015, Liméa et al. 2012). This was clearly shown in the present work as well. At the end of week twelve ZH+Conc had a n6 to n3 PUFA intake five times higher than in the hay groups and NH+Conc had a n6 to n3 PUFA intake ten times higher than in the hay groups. The gap was higher in NH+Conc because the calves also ate relatively more concentrate in their offered diet, which raised the n6 PUFA intake and thus the n6 to n3 ratio. This was because the majority of ingredients in the concentrate were n6 PUFA rich. Concentrate included barley, wheat, and soybean meal, all together accounting for 88% of the concentrate. Barley, wheat, and soybean meal each contained more than 50% n6 PUFA and about 10% n3 PUFA (Huuskonen et al. 2010, Lee et al. 2013, Maiorino et al. 1986).

The effect of diet on group differences in the n6 to n3 PUFA intake was also reflected in the neutral lipids and phospholipids of the adipose tissue of young calves. In both neutral lipids and phospholipids, hay-only calves showed lower n6 to n3 ratios than those of concentrate-fed calves. And as well as in the dietary intake the n6 to n3 PUFA ratio was lower in ZH+Conc than in NH+Conc.

Previous studies also have established the advantages of forage feeding for increasing n3 PUFA content in ruminant lipids, which is considered beneficial for consumer health. For

example, Garcia et al. (2008) studied the muscle tissue of grass-fed and grain-fed cattle. In the grass-fed cattle, there was an almost 10-fold decrease in the n6 to n3 ratio in the muscle tissue, while also the grass had a significantly lower n6 to n3 ratio than the grain. Liméa et al. (2012) showed that the lower the percentage of concentrates in goats' diets, the lower the n6 to n3 ratio in muscle and adipose tissue. Fincham et al. (2009) also found a lower n6 to n3 ratio in the adipose tissue of cattle grazed only compared to the adipose tissue of cattle fed concentrates.

Compared to the neutral lipids, phospholipids contain much more n6 and n3 PUFA (Beak et al. 2019, Wood et al. 2008). The present results underline that dietary PUFA intake also influences the PUFA profile of phospholipids. The incorporation of dietary n3 PUFA into cell membranes competes with n6 PUFA (Surette 2008). On closer examination, it can be seen that the gap in n6 to n3 PUFA ratio of adipose tissue lipids among diets was mainly influenced by the changes in n6 PUFA proportion, while changes in n3 PUFA proportions were less drastic. This pattern was, however, stronger in neutral lipid fraction than phospholipids. This is partly due to biohydrogenation, as studies have shown that 18:3 n3 is more biohydrogenated than 18:2 n6 (Beam et al. 2000, Lock et al. 2006). Moreover, the current data hint that adipose tissues have a higher capacity to store n6 PUFA while the capacity for n3 PUFA in the adipose tissue can be saturated at some point. Likewise, some studies in rats have shown that tissues more preferably store n6 PUFA than n3 PUFA (Abbott et al. 2012). In milk of dairy cows (Khiaosa-Ard et al. 2015), in muscle of cattle (Garcia et al. 2008) and in adipose tissue of lambs (Willems et al. 2014) n6 PUFA are enhanced to a greater extent than n3 PUFA by the effect of dietary PUFA. This suggests that in order to efficiently reduce the n6 to n3 ratio of tissues, it might be more important to reduce the n6 intake instead of increasing the n3 intake in diets which already provide plenty n6 PUFA intake. A reduction is being strived for, because low n6 to n3 ratios correlate with a more effective immune reaction (Leskanish and Noble 1999), lower inflammation rate (Masmeijer et al. 2020), higher bacterial diversity in the rumen microbiome (Cristobal-Carballo et al. 2021) and positive effects on the reproduction tract (Tran et al. 2016). Still, the available scientific evidence on this issue is limited and requires further research. In the author’s opinion a combination of high-quality hay and concentrate is recommended for a good balance of performance and health. However, high concentrate diets were used in the current experiment. It should be subject to further research whether the concentrate proportion can be further reduced and raise the hay proportion to a level where a stronger effect on tissue fatty acid composition can be obtained without adverse effects on the performance due to the loss of energy.

In this study more n3 PUFA and less n6 PUFA were found in the adipose tissue of young dairy calves fed higher levels of n3 PUFA and lower levels of n6 PUFA, whereby the shift of these fatty acids were reflected more in storage lipids than in membrane lipids.

7 Summary

Calves are usually fed starter diets rich in concentrates to support rapid growth and rumen development. Their natural feed would be pastures which is similar to forages and, depending on their age, milk. Forages and concentrates differ in their fatty acid composition. Forages are rich in omega-3 polyunsaturated fatty acids whereas most concentrates are rich in omega-6 polyunsaturated fatty acids. While omega-6 polyunsaturated fatty acids are known to be pro-inflammatory, omega-3 polyunsaturated fatty acids are reported to be anti-inflammatory. Shifting polyunsaturated fatty acids profile could be highly relevant for adipose tissue which is a metabolically and endocrinologically active organ. Understanding effects of feeding on modification of adipose tissue polyunsaturated fatty acids is an important topic in nutrition but has been understudied in ruminants. This study investigated the influence of dietary polyunsaturated fatty acids on neutral lipids and phospholipids of the adipose tissue of young calves. From birth to approximately 15 weeks of life, 20 Holstein calves were assigned to one of the four different diets (100% regular hay (NH), 100% high-quality hay (ZH), 30% regular hay + 70% calf concentrate (NH+Conc) and 30% high-quality hay + 70% calf concentrate (ZH+Conc)). Calves fed ZH ate more hay than calves fed NH. Even when the calves were fed concentrates, those in the ZH+Conc group ate more hay than those in the NH+Conc group. Conversely, the ZH+Conc calves ate less concentrate than the NH+Conc calves. Fatty acid composition of the different feed types and two different regions of the kidney adipose tissue were analysed. ZH contained more total lipids and 10% more omega-3 polyunsaturated fatty acids than NH. Birth weight, slaughter weight, daily body weight gain, rate of lipid deposition, rate of protein deposition, kidney weight and kidney fat weight were recorded. These performance data were higher in the calves of both Concentrate groups, but also ZH calves had hardly lower values than NH+Conc calves. The omega-6 to omega-3 polyunsaturated fatty acids intake was highest in NH+Conc, was in the middle in ZH+Conc and lowest in both hay-only groups. The omega-6 to omega-3 polyunsaturated fatty acids ratio in the adipose tissue corresponded to the intake. This was true for both neutral lipids (storage lipids) and phospholipids (membrane lipids). Moreover, adipose tissue showed a preferable storage of dietary omega-6 polyunsaturated fatty acids over the omega-3 polyunsaturated fatty acids. In conclusion, changes in the polyunsaturated fatty acids composition of the diet of young dairy calves can affect not only the profile of storage lipids but also the membrane lipids of the adipose tissue of these calves. A reduction in the proportion of concentrate as well as the use of high-quality hay are instrumental in bringing a lower intake of omega-6 polyunsaturated fatty

acids and a higher intake of omega-3 polyunsaturated fatty acids, which may provide health benefits particularly related to inflammation.

8 Zusammenfassung

Kälber werden in der Regel mit einem kraftfutterreichen Starterfutter gefüttert, um ein schnelles Wachstum und die Pansenentwicklung zu fördern. Ihr natürliches Futter wären eigentlich Weiden, die dem Grünfutter ähnlich sind, und abhängig vom Alter, Milch. Grünfutter und Kraftfutter unterscheiden sich in ihrer Fettsäurezusammensetzung. Grünfutter ist reich an mehrfach ungesättigten Omega-3-Fettsäuren, während die meisten Kraftfutter reich an mehrfach ungesättigten Omega-6-Fettsäuren sind. Während mehrfach ungesättigte Omega-6-Fettsäuren als entzündungsfördernd bekannt sind, sollen mehrfach ungesättigte Omega-3-Fettsäuren entzündungshemmend wirken. Eine Verschiebung des Profils der mehrfach ungesättigten Fettsäuren könnte für das Fettgewebe, das ein metabolisch und endokrinologisch aktives Organ ist, daher von großer Bedeutung sein. Das Verständnis der Auswirkungen der Fütterung auf die Veränderung der mehrfach ungesättigten Fettsäuren im Fettgewebe ist ein wichtiges Thema in der Ernährung, wurde aber bei Wiederkäuern bisher nur unzureichend untersucht. In dieser Studie wurde der Einfluss von mehrfach ungesättigten Fettsäuren in der Nahrung auf die neutralen Lipide und Phospholipide des Fettgewebes junger Kälber untersucht. Von der Geburt bis zum Alter von ca. 15 Wochen wurden 20 Holstein-Kälber einem von vier verschiedenen Futtergruppen zugeteilt (100% normales Heu (NH), 100% hochwertiges Heu (ZH), 30% normales Heu + 70% Kälberkraftfutter (NH+Conc) und 30% hochwertiges Heu + 70% Kälberkraftfutter (ZH+Conc)). Kälber, die mit ZH gefüttert wurden, fraßen mehr Heu als Kälber, die mit NH gefüttert wurden. Selbst wenn die Kälber mit Kraftfutter gefüttert wurden, fraßen die Kälber in der ZH+Conc-Gruppe mehr Heu als die Kälber in der NH+Conc-Gruppe. Umgekehrt fraßen die ZH+Conc-Kälber weniger Kraftfutter als die NH+Conc-Kälber. Im Rahmen der Studie wurden die Fettsäurenzusammensetzung der verschiedenen Futtertypen und zweier verschiedener Regionen des Nierenfettgewebes analysiert. ZH enthielt mehr Gesamtfette und 10% mehr mehrfach ungesättigte Omega-3-Fettsäuren als NH. Es wurden das Geburtsgewicht, das Schlachtgewicht, die tägliche Gewichtszunahme, die Rate der Fettablagerung, die Rate der Proteinablagerung, das Nierengewicht und das Nierenfettgewicht erfasst. Diese Leistungsdaten waren bei den Kälbern beider Kraftfuttergruppen höher, aber auch die ZH-Kälber hatten kaum niedrigere Werte als die NH+Conc-Kälber. Die Aufnahme von mehrfach ungesättigten Omega-6- zu Omega-3-Fettsäuren war bei NH+Conc am höchsten, lag bei ZH+Conc im Mittelfeld und war bei beiden Heu-Gruppen am niedrigsten. Das Verhältnis von Omega-6- zu Omega-3-mehrfach ungesättigten Fettsäuren im Fettgewebe entsprach dem Verhältnis in der Aufnahme. Dies galt sowohl für neutrale Lipide (Speicherlipide) als auch für Phospholipide (Membranlipide).

Darüber hinaus zeigte das Fettgewebe eine bevorzugte Speicherung von mehrfach ungesättigten Omega-6-Fettsäuren gegenüber den mehrfach ungesättigten Omega-3-Fettsäuren aus der Nahrung. Zusammenfassend lässt sich feststellen, dass Veränderungen in der Zusammensetzung der mehrfach ungesättigten Fettsäuren in der Ernährung von jungen Milchkälbern nicht nur das Profil der Speicherlipide, sondern auch die Membranlipide des Fettgewebes dieser Kälber beeinflussen können. Eine Verringerung des Kraftfutteranteils sowie die Verwendung von hochwertigem Heu tragen zu einer geringeren Aufnahme von mehrfach ungesättigten Omega-6-Fettsäuren und einer höheren Aufnahme von mehrfach ungesättigten Omega-3-Fettsäuren bei, was insbesondere im Zusammenhang mit Entzündungen gesundheitliche Vorteile mit sich bringen kann.

9 Abbreviations

n6 = omega-6

n3 = omega-3

PUFA = polyunsaturated fatty acids

MUFA = monounsaturated fatty acids

trans-FA = trans-fatty acid

FA = fatty acids

SFA = saturated fatty acids

NDF = neutral detergent fibre

18:2 n6 = linoleic acid

18:3 n3 = α-linolenic acid

CLA = conjugated linoleic acid

NH = 100% regular hay

ZH = 100% high-quality hay

NH+Conc = 30% regular hay + 70% calf concentrate

ZH+Conc = 30% high-quality hay + 70% calf concentrate

GC = gas chromatography

FAME = fatty acid methyl esters

SEM = standard error of the mean

10 References

Abbott SK, Else PL, Atkins TA, Hulbert AJ. Fatty acid composition of membrane bilayers: importance of diet polyunsaturated fat balance. Biochim Biophys Acta. 2012;1818(5):1309–17. doi:10.1016/j.bbamem.2012.01.011.

AOAC. Official Methods of Analysis. AOAC International. 2012(19th ed. Gaithersburg).

Arfuso F, Fazio F, Panzera M, Giannetto C, Di Pietro S, Giudice E, Piccione G. Lipid and lipoprotein profile changes in newborn calves in response to the perinatal period. Acta Veterinaria. 2017;67(1):25–32. doi:10.1515/acve-2017-0003.

Beak S-H, Lee Y, Lee EB, Kim KH, Kim JG, Bok JD, Kang S-K. Study on the fatty acid profile of phospholipid and neutral lipid in Hanwoo beef and their relationship to genetic variation. J Anim Sci Technol. 2019;61(2):69–76. doi:10.5187/jast.2019.61.2.69.

Beam TM, Jenkins TC, Moate PJ, Kohn RA, Palmquist DL. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. J Dairy Sci. 2000;83(11):2564–73. doi:10.3168/jds.S0022-0302(00)75149-6.

Berthelot V, Bas P, Schmidely P, Duvaux-Ponter C. Effect of dietary propionate on intake patterns and fatty acid composition of adipose tissues in lambs. Small Ruminant Research. 2001;40(1):29–39. doi:10.1016/S0921-4488(00)00217-0.

Bionaz M, Vargas-Bello-Pérez E, Busato S. Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. J Anim Sci Biotechnol. 2020;11(1):110. doi:10.1186/s40104-020-00512-8.

Bodkowski R, Czyż K, Kupczyński R, Patkowska-Sokoła B, Nowakowski P, Wiliczkiewicz A. Lipid complex effect on fatty acid profile and chemical composition of cow milk and cheese. J Dairy Sci. 2016;99(1):57–67. doi:10.3168/jds.2015-9321.

Braun H-S, Schrapers KT, Mahlkow-Nerge K, Stumpff F, Rosendahl J. Dietary supplementation of essential oils in dairy cows: evidence for stimulatory effects on nutrient absorption. Animal. 2018;13(3):518–23. doi:10.1017/S1751731118001696.

Bu Z, Ge G, Jia Y, Du S. Effect of hay with or without concentrate or pellets on growth performance and meat quality of Ujimqin lambs on the Inner Mongolian Plateau. Anim Sci J. 2021;92(1):e13553. doi:10.1111/asj.13553.

Caesar R, Manieri M, Kelder T, Boekschoten M, Evelo C, Müller M, et al. A combined transcriptomics and lipidomics analysis of subcutaneous, epididymal and mesenteric adipose tissue reveals marked functional differences. PLoS One. 2010;5(7):e11525. doi:10.1371/journal.pone.0011525.

Calder PC. Polyunsaturated fatty acids and inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2006;75(3):197–202. doi:10.1016/j.plefa.2006.05.012.

Carvalho CCCR de, Caramujo MJ. The Various Roles of Fatty Acids. Molecules 2018. doi:10.3390/molecules23102583.

Chen H, Wang C, Huasai S, Chen A. Effects of dietary forage to concentrate ratio on nutrient digestibility, ruminal fermentation and rumen bacterial composition in Angus cows. Sci Rep. 2021;11(1):17023. doi:10.1038/s41598-021-96580-5.

Chishti MFA, Rahman MAU, Jatta K, Khan S, Riaz M, Bilal Q, et al. Effect of forage to concentrate ratio on growth performance and feeding behavior of Thalli lambs. Trop Anim Health Prod. 2022;54(4):236. doi:10.1007/s11250-022-03226-3.

Cholewski M, Tomczykowa M, Tomczyk M. A Comprehensive Review of Chemistry, Sources and Bioavailability of Omega-3 Fatty Acids. Nutrients 2018. doi:10.3390/nu10111662.

Cianzio DS, Topel DG, Whitehurst GB, Beitz DC, Self HL. Adipose tissue growth and cellularity: changes in bovine adipocyte size and number. J Anim Sci. 1985;60(4):970–6. doi:10.2527/jas1985.604970x.

Clandinin MT, Field CJ, Hargreaves K, Morson L, Zsigmond E. Role of diet fat in subcellular structure and function. Canadian Journal of Physiology and Pharmacology. 1985;63(5):546–56. doi:10.1139/y85-094.

Cristobal-Carballo O, McCoard SA, Cookson AL, Laven RA, Ganesh S, Lewis SJ, Muetzel S. Effect of Divergent Feeding Regimes During Early Life on the Rumen Microbiota in Calves. Front Microbiol. 2021;12:711040. doi:10.3389/fmicb.2021.711040.

Dahl N, Albrecht E, Dannenberger D, Uken KL, Hammon HM, Maak S. Consequences of Maternal Essential Fatty Acid and Conjugated Linoleic Acid Supplementation on the Development of Calf Muscle and Adipose Tissue. Animals (Basel) 2020. doi:10.3390/ani10091598.

Dehghan-Banadaky M, Ebrahimi M, Motameny R, Heidari SR. Effects of live yeast supplementation on mid-lactation dairy cows performances, milk composition, rumen digestion and plasma metabolites during hot season. Journal of Applied Animal Research. 2013;41(2):137–42. doi:10.1080/09712119.2012.739085.

Deutsche Gesellschaft für Ernährung e.V. trans-Fettsäuren und die Gesundheit. 2016. https://www.dge.de/wissenschaft/fachinformationen/trans-fettsaeuren-und-die-gesundheit/. Accessed 22 May 2023.

Di Lena G, Sanchez Del Pulgar J, Lucarini M, Durazzo A, Ondrejíčková P, Oancea F, et al. Valorization Potentials of Rapeseed Meal in a Biorefinery Perspective: Focus on Nutritional and Bioactive Components. Molecules 2021. doi:10.3390/molecules26226787.

Drackley JK. Overview of fat digestion and metabolism in dairy cows; 2007:1-9.

Ferlay A, Bernard L, Meynadier A, Malpuech-Brugère C. Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: A review. Biochimie. 2017;141:107–20. doi:10.1016/j.biochi.2017.08.006.

Fincham JR, Fontenot JP, Swecker WS, Herbein JH, Neel JPS, Scaglia G, et al. Fatty acid metabolism and deposition in subcutaneous adipose tissue of pasture- and feedlot-finished cattle. J Anim Sci. 2009;87(10):3259–77. doi:10.2527/jas.2008-1277.

Folch J, Lees M, Stanley GS. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226(1):497–509. doi:10.1016/S0021-9258(18)64849-5.

Fredriksson Eriksson S, Pickova J. Fatty acids and tocopherol levels in M. Longissimus dorsi of beef cattle in Sweden - A comparison between seasonal diets. Meat Science. 2007;76(4):746–54. doi:10.1016/j.meatsci.2007.02.021.

Fustini M, Palmonari A, Canestrari G, Bonfante E, Mammi L, Pacchioli MT, et al. Effect of undigested neutral detergent fiber content of alfalfa hay on lactating dairy cows: Feeding behavior, fiber digestibility, and lactation performance. J Dairy Sci. 2017;100(6):4475–83. doi:10.3168/jds.2016-12266.

Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM. Advances in Our Understanding of Oxylipins Derived from Dietary PUFAs. Adv Nutr. 2015;6(5):513–40. doi:10.3945/an.114.007732.

Garcia PT, Pensel NA, Sancho AM, Latimori NJ, Kloster AM, Amigone MA, Casal JJ. Beef lipids in relation to animal breed and nutrition in Argentina. Meat Science. 2008;79(3):500–8. doi:10.1016/j.meatsci.2007.10.019.

Garton GA, Duncan WR. Effect of diet and rumen development on the composition of adipose tissue triglycerides of the calf. Br J Nutr. 1969;23(2):421–7. doi:10.1079/bjn19690049.

Gravador RS, Brunton NP, Fahey AG, Gkarane V, Claffey NA, Moloney AP, et al. Effects of dietary fat sources on the intramuscular and subcutaneous adipose tissue fatty acid composition, and consumer acceptability of lamb. J Sci Food Agric. 2020;100(5):2176–84. doi:10.1002/jsfa.10242.

Greco LF, Neves Neto JT, Pedrico A, Ferrazza RA, Lima FS, Bisinotto RS, et al. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on performance and inflammatory responses to a lipopolysaccharide challenge in lactating Holstein cows. J Dairy Sci. 2015;98(1):602–17. doi:10.3168/jds.2014-8805.

Guo M. Lipids and lipid related functional foods. In: Functional Foods. Elsevier; 2009. p. 161–196. doi:10.1533/9781845696078.161.

Gurr MI, Harwood JL, Frayn KN, Murphy DJ, Michell R. H., editors. Lipids: Biochemistry, Biotechnology and Health. 6th ed. Oxford, UK: Wiley Blackwell; 2016.

Harfoot CG. Lipid metabolism in the rumen. In: Pergamon Press, ed. Lipid Metabolism in Ruminant Animals. Elsevier; 1981. p. 21–55. doi:10.1016/B978-0-08-023789-3.50006-4.
Hartigh LJ den. Conjugated Linoleic Acid Effects on Cancer, Obesity, and Atherosclerosis: A Review of Pre-Clinical and Human Trials with Current Perspectives. Nutrients 2019. doi:10.3390/nu11020370.
Hartinger T, Pacífico C, Poier G, Terler G, Klevenhusen F, Zebeli Q. Shift of dietary carbohydrate source from milk to various solid feeds reshapes the rumen and fecal microbiome in calves. Sci Rep. 2022;12(1):12383. doi:10.1038/s41598-022-16052-2.
Havlin JM, Robinson PH, Garrett JE. Niacin feeding to fresh dairy cows: immediate effects on health and milk production. Anim. Prod. Sci. 2017;57(6):1069. doi:10.1071/AN15419.
Hilditch TP, Williams PN. The chemical constitution of natural fats: Fourth Edition. New York: John Wiley & Sons; 1964.
Hill TM, Bateman HG, Aldrich JM, Schlotterbeck RL. Effects of changing the essential and functional fatty acid intake of dairy calves. J Dairy Sci. 2009;92(2):670–6. doi:10.3168/jds.2008-1368.
Huuskonen A, Jansson S, Honkavaara M, Tuomisto L, Kauppinen R, Joki-Tokola E. Meat colour, fatty acid profile and carcass characteristics of Hereford bulls finished on grazed pasture or grass silage-based diets with similar concentrate allowance. Livestock Science. 2010;131(1):125–9. doi:10.1016/j.livsci.2010.02.019.
Innes JK, Calder PC. Omega-6 fatty acids and inflammation. Prostaglandins Leukot Essent Fatty Acids. 2018;132:41–8. doi:10.1016/j.plefa.2018.03.004.
Jenkins TC. Regulation of lipid metabolism in the rumen. J Nutr. 1994;124(8 Suppl):1372S-1376G. doi:10.1093/jn/124.suppl_8.1372S.
Jolazadeh AR, Mohammadabadi T, Dehghan-Banadaky M, Chaji M, Garcia M. Effect of supplementation fat during the last 3 weeks of uterine life and the preweaning period on performance, ruminal fermentation, blood metabolites, passive immunity and health of the newborn calf. Br J Nutr. 2019;122(12):1346–58. doi:10.1017/S0007114519002174.
Kemp P, Lander DJ. Hydrogenation in vitro of -Linolenic Acid to Stearic Acid by Mixed Cultures of Pure Strains of Rumen Bacteria. Microbiology. 1984;130(3):527–33. doi:10.1099/00221287-130-3-527.
Khafipour E, Li S, Tun HM, Derakhshani H, Moossavi S, Plaizier JC. Effects of grain feeding on microbiota in the digestive tract of cattle. Anim Fron. 2016;6(2):13–9. doi:10.2527/af.2016-0018.
Khan MA, Bach A, Weary DM, Keyserlingk MAG von. Invited review: Transitioning from milk to solid feed in dairy heifers. J Dairy Sci. 2016;99(2):885–902. doi:10.3168/jds.2015-9975.
Khiaosa-ard R, Kleefisch M-T, Zebeli Q, Klevenhusen F. Milk fatty acid composition reflects metabolic adaptation of early lactation cows fed hay rich in water-soluble carbohydrates with or without concentrates. Animal Feed Science and Technology. 2020;264:114470. doi:10.1016/j.anifeedsci.2020.114470.
Khiaosa-ard R, Kreuzer M, Leiber F. Apparent recovery of C18 polyunsaturated fatty acids from feed in cow milk: A meta-analysis of the importance of dietary fatty acids and feeding regimens in diets without fat supplementation. J Dairy Sci. 2015;98(9):6399–414. doi:10.3168/jds.2015-9459.
Khiaosa-Ard R, Zebeli Q. Diet-induced inflammation: From gut to metabolic organs and the consequences for the health and longevity of ruminants. Res Vet Sci. 2018;120:17–27. doi:10.1016/j.rvsc.2018.08.005.
Kleefisch M-T, Zebeli Q, Humer E, Gruber L, Klevenhusen F. Effects of feeding high-quality hay with graded amounts of concentrate on feed intake, performance and blood metabolites of cows in early lactation. Arch Anim Nutr. 2018;72(4):290–307. doi:10.1080/1745039X.2018.1474004.
Kleefisch M-T, Zebeli Q, Humer E, Kröger I, Ertl P, Klevenhusen F. Effects of the replacement of concentrate and fibre-rich hay by high-quality hay on chewing, rumination and nutrient digestibility in non-lactating Holstein cows. Arch Anim Nutr. 2016;71(1):21–36. doi:10.1080/1745039X.2016.1253227.

Klein CJ. Nutrient requirements for preterm infant formulas. J Nutr. 2002;132(6 Suppl 1):1395S-577S. doi:10.1093/jn/132.6.1395S.
Koolman J, Röhm K-H, Wirth J. Taschenatlas der Biochemie. 2nd ed. Stuttgart: Thieme; 1998.
Ladeira MM, Schoonmaker JP, Swanson KC, Duckett SK, Gionbelli MP, Rodrigues LM, Teixeira PD. Review: Nutrigenomics of marbling and fatty acid profile in ruminant meat. Animal. 2018;12(s2):s282-s294. doi:10.1017/S1751731118001933.
Lee JW, Kil DY, Keever BD, Killefer J, McKeith FK, Sulabo RC, Stein HH. Carcass fat quality of pigs is not improved by adding corn germ, beef tallow, palm kernel oil, or glycerol to finishing diets containing distillers dried grains with solubles. J Anim Sci. 2013;91(5):2426–37. doi:10.2527/jas.2012-5328.
Lei Z, Wu H, Xiong Y, Wei D, Wang X, Luoreng Z, et al. ncRNAs regulate bovine adipose tissue deposition. Mol Cell Biochem. 2021;476(7):2837–45. doi:10.1007/s11010-021-04132-2.
Leskanich CO, Noble RC. The comparative roles of polyunsaturated fatty acids in pig neonatal development. Br J Nutr. 1999;81(2):87–106. doi:10.1017/s0007114599000215.
Liméa L, Alexandre G, Berthelot V. Fatty acid composition of muscle and adipose tissues of indigenous Caribbean goats under varying nutritional densities. J Anim Sci. 2012;90(2):605–15. doi:10.2527/jas.2010-3624.
Lock AL, Harvatine KJ, Drackley JK, Bauman DE. Concepts in fat and fatty acid digestion in ruminants. Proceedings Intermountain Nutrition Conference. 2006:85–100.
Louveau I, Perruchot M-H, Bonnet M, Gondret F. Invited review: Pre- and postnatal adipose tissue development in farm animals: from stem cells to adipocyte physiology. Animal. 2016;10(11):1839–47. doi:10.1017/S1751731116000872.
Maiorino PM, Al-Hozab AA, Mitchell R, Reid BL. Animal fat effects on nutrient utilization. Poult Sci. 1986;65(12):2304–13. doi:10.3382/ps.0652304.
Martins AP, Lopes PA, Costa ASH, Martins SV, Santos NC, Prates JAM, et al. Differential mesenteric fat deposition in bovines fed on silage or concentrate is independent of glycerol membrane permeability. Animal. 2011;5(12):1949–56. doi:10.1017/S1751731111001091.
Masmeijer C, van Leenen K, Cremer L de, Deprez P, Cox E, Devriendt B, Pardon B. Effects of omega-3 fatty acids on immune, health and growth variables in veal calves. Prev Vet Med. 2020;179:104979. doi:10.1016/j.prevetmed.2020.104979.
Mavrommatis A, Skliros D, Sotirakoglou K, Flemetakis E, Tsiplakou E. The Effect of Forage-to-Concentrate Ratio on Schizochytrium spp.-Supplemented Goats: Modifying Rumen Microbiota. Animals (Basel) 2021. doi:10.3390/ani11092746.
Mesilati-Stahy R, Malka H, Argov-Argaman N. Association of plasma insulin concentration to fatty acid distribution between milk fat and membrane synthesis. J Dairy Sci. 2012;95(4):1767–75. doi:10.3168/jds.2011-4583.
Moran L, O'Sullivan MG, Kerry JP, Picard B, McGee M, O'Riordan EG, Moloney AP. Effect of a grazing period prior to finishing on a high concentrate diet on meat quality from bulls and steers. Meat Science. 2017;125:76–83. doi:10.1016/j.meatsci.2016.11.021.
Muzolf-Panek M, Kaczmarek A. Chemometric Analysis of Fatty Acid Composition of Raw Chicken, Beef, and Pork Meat with Plant Extract Addition during Refrigerated Storage. Molecules 2021. doi:10.3390/molecules26164952.
Oksanen HE, Thafvelin B. Fatty acid composition of hay. J Dairy Sci. 1965;48(10):1305–9. doi:10.3168/jds.S0022-0302(65)88453-3.
Omidi-Mirzaei H, Azarfar A, Kiani A, Mirzaei M, Ghaffari MH. Interaction between the physical forms of starter and forage source on growth performance and blood metabolites of Holstein dairy calves. J Dairy Sci. 2018;101(7):6074–84. doi:10.3168/jds.2017-13892.
Palmquist DL, Jenkins TC. Challenges with fats and fatty acid methods. J Anim Sci. 2003;81(12):3250–4. doi:10.2527/2003.81123250x.
Phipps RH, Sutton JD, Jones BA. Forage mixtures for dairy cows: the effect on dry-matter intake and milk production of incorporating either fermented or urea-treated whole-crop wheat, brewers'

grains, fodder beet or maize silage into diets based on grass silage. Animal Science. 1995 Dec;61(3):491-6.
Poier G, Terler G, Klevenhusen F, Sharma S, Zebeli Q. Replacing concentrates with a high-quality hay in the starter feed of dairy calves: II. Effects on the development of chewing and gut fermentation, and selected systemic health variables. J Dairy Sci. 2022;105(4):3113–28. doi:10.3168/jds.2021-21346.
Rabelo E, Bertics SJ, Mackovic J, Grummer RR. Strategies for increasing energy density of dry cow diets. J Dairy Sci. 2001;84(10):2240–9. doi:10.3168/jds.S0022-0302(01)74671-1.
Ranathunga SD, Kalscheur KF, Hippen AR, Schingoethe DJ. Replacement of starch from corn with nonforage fiber from distillers grains and soyhulls in diets of lactating dairy cows. J Dairy Sci. 2010;93(3):1086–97. doi:10.3168/jds.2009-2332.
Robelin J, Chilliard Y. Short-term and long-term effects of early nutritional deprivation on adipose tissue growth and metabolism in calves. J Dairy Sci. 1989;72(2):505–13. doi:10.3168/jds.S0022-0302(89)79133-5.
Şanlier N, Gökcen BB, Sezgin AC. Health benefits of fermented foods. Crit Rev Food Sci Nutr. 2019;59(3):506–27. doi:10.1080/10408398.2017.1383355.
Silva AL, DeVries TJ, Tedeschi LO, Marcondes MI. Development of equations, based on milk intake, to predict starter feed intake of preweaned dairy calves. Animal. 2019;13(1):83–9. doi:10.1017/S1751731118000666.
Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002;56(8):365–79. doi:10.1016/s0753-3322(02)00253-6.
Stender S, Astrup A, Dyerberg J. Ruminant and industrially produced trans fatty acids: health aspects. Food Nutr Res 2008. doi:10.3402/fnr.v52i0.1651.
Surette ME. The science behind dietary omega-3 fatty acids. CMAJ. 2008;178(2):177–80. doi:10.1503/cmaj.071356.
Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, Kirkland JL. Mechanisms and metabolic implications of regional differences among fat depots. Cell Metab. 2013;17(5):644–56. doi:10.1016/j.cmet.2013.03.008.
Terler G, Poier G, Klevenhusen F, Zebeli Q. Replacing concentrates with a high-quality hay in the starter feed in dairy calves: I. Effects on nutrient intake, growth performance, and blood metabolic profile. J Dairy Sci. 2022;105(3):2326–42. doi:10.3168/jds.2021-21078.
Terler G, Velik M, Poier G, Sener-Aydemir A, Klevenhusen F, Zebeli Q. Feeding concentrate with hay of different qualities modulates rumen histology, development of digestive tract as well as slaughter performance and meat quality of young dairy calves. Arch Anim Nutr. 2023:1–16. doi:10.1080/1745039X.2023.2197633.
Tran LV, Malla BA, Sharma AN, Kumar S, Tyagi N, Tyagi AK. Effect of omega-3 and omega-6 polyunsaturated fatty acid enriched diet on plasma IGF-1 and testosterone concentration, puberty and semen quality in male buffalo. Anim Reprod Sci. 2016;173:63–72. doi:10.1016/j.anireprosci.2016.08.012.
Tvrzicka E, Kremmyda L-S, Stankova B, Zak Atvr functions. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2011;155(2):117–30. doi:10.5507/bp.2011.038.
Valvo MA, Bella M, Scerra M, Biondi L. Effects of ewe feeding system (grass vs concentrate) on milk fatty acid composition: AU - PY - 2006/01/01 SP - T1 - VL - 74 JO - ER -. Options Mediterraneennes, series A. 2006(74).
Wang X, Yan S, Shi B, Zhang Y, Guo X. Effects of concentrate supplementation on fatty acid composition and expression of lipogenic genes of meat and adipose tissues in grazing lambs. Italian Journal of Animal Science. 2019;18(1):554–63. doi:10.1080/1828051X.2018.1547666.
Weijers RNM. Lipid composition of cell membranes and its relevance in type 2 diabetes mellitus. Curr Diabetes Rev. 2012;8(5):390–400. doi:10.2174/157339912802083531.
Willems H, Kreuzer M, Leiber F. Alpha-linolenic and linoleic acid in meat and adipose tissue of grazing lambs differ among alpine pasture types with contrasting plant species and phenolic compound

composition. Small Ruminant Research. 2014;116(2-3):153–64. doi:10.1016/j.smallrumres.2013.11.002.

Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, et al. Fat deposition, fatty acid composition and meat quality: A review. Meat Science. 2008;78(4):343–58. doi:10.1016/j.meatsci.2007.07.019.

Wu SA, Kersten S, Qi L. Lipoprotein Lipase and Its Regulators: An Unfolding Story. Trends Endocrinol Metab. 2020;32(1):48–61. doi:10.1016/j.tem.2020.11.005.

Wyss U. Influence of conservation method on fatty acid composition of herbages of a permanent grassland meadow. Grassland Science Europe. 2012(17):323–5.

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN, Drochner W. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J Dairy Sci. 2012;95(3):1041–56. doi:10.3168/jds.2011-4421.