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ORIGINAL ARTICLE

Significance of Coprophagy for the Fatty Acid Profile in Body Tissues of Rabbits Fed Different Diets

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Abstract Four groups of eight New Zealand hybrid rabbits were fattened with ad libitum access to the following pelleted experimental diets: ryegrass meal or alfalfa meal fed either alone or with oats meal in a ratio of 1:1. After 25 weeks they were slaughtered and dissected. Fatty acid (FA) profiles of caecotrophs (re-ingested fermentation products of the caecum), perirenal adipose tissue and intramuscular fat in the *Musculus quadriceps* were determined. With high proportions of branched-chain FA (BFA) and *trans* FA, and increased proportions of saturated FA relative to the diets, the caecotroph FA profile showed a clear fingerprint of anaerobe microbial lipid metabolism including biohydrogenation. By contrast, the FA profiles of adipose and lean tissue comprised high proportions of polyunsaturated FA (PUFA), whilst BFA and *trans* FA occurred in much lower proportions compared to the caecotrophs. Thus, coprophagy did not substantially modify the FA composition of the tissues investigated. Use of forage-only diets, compared to the oats supplemented diets, led to extraordinary high proportions of n-3 PUFA (including 18:3 and long-chain n-3) in the fat of adipose (21.3 vs. 6.7%) and lean tissue (15.4 vs. 5.7%). The forage type diet (grass vs. alfalfa) had smaller effects on the FA profiles. Indications of diet effects on endogenous desaturation, chain elongation and differential distribution of functional FA between the two tissues investigated were found.

Keywords Rabbit · Coprophagy · Caecum · Meat · Microbial lipid metabolism · Branched chain fatty acids · *trans*-Fatty acids · N-3 fatty acids · Forage · Oats

Abbreviations

ALA	α-linolenic acid
BFA	Branched chain fatty acids
CLA	Conjugated linoleic acids
FA	Fatty acids
FAME	Fatty acid methyl ester
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
TVA	*trans*-Vaccenic acid 11*trans*-18:1

Introduction

The significance of microbial lipid digestion in rabbits and other small herbivores with significant microbial fermentation in the hindgut (caecum) [1], is not yet well known. Information on the effect of the microbial influence on lipids in the digestive tract mostly comes from ruminant animals with their large forestomach [2, 3]. The microbial fermentation of the ingested feed is associated with biohydrogenation and isomerisation of unsaturated FA. This results in the production of considerable amounts of several *trans*-FA, conjugated linolenic acids (CLA) and stearic acid [2, 4]. Another particularity of ruminal digestion is the de novo synthesis of branched-chain FA [3]. Therefore, microbial digestion results in characteristic FA profiles in

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the tissues of ruminants, which were subject to extensive research work aimed to improve the nutritional quality of animal products [2, 4–8]. In rabbits, digestive fermentation takes place in the hindgut, namely the caecum. Ingesta undigested so far—predominantly plant cell walls—are fermented and relevant nutrients including B vitamins and protein are anaerobically synthesised by the hindgut microbes [1, 9]. Because nutrient absorption from the hindgut is limited, these animals produce a particular kind of soft feces in the caecum, the caecotrophs, which are re-ingested [9, 10]. In wild living rabbits, the main part of the unabsorbed ingesta is recycled by this way [9].

Lipids produced by bacteria in the caecum should share the typical microbial FA profile as described for the foregut of ruminants. Potentially, such lipids could reach the duodenum and the blood via coprophagial re-ingestion. High amounts of *trans*-FA and branched-chain FA (BFA), as found in the tissues of beavers [11, 12], could be explained by this behavior. However, in-depth research on the FA profile of caecotrophs and an evaluation of its influence on the endogenous FA metabolism of such animals has not yet been performed. This could be useful in comparative physiological research on wild animals in order to develop a marker for coprophagy [10]. It could also elucidate the significance of coprophagy for the endogenous metabolism of the animal and, consequently, for the use of rodents and rabbits, as animal models in lipid metabolism research. These species could, by coprophagy alone, ingest an uncontrolled amount of these FA in experimental studies, which may cause particular metabolic responses [13], thus partially questioning their use to simulate potential effects of *trans* FA and BFA in humans. Finally, coprophagy could influence the nutritional value of rabbit meat if the ingested hindgut microbe-derived FA result in a substantial modification of the tissue lipids. This context has not yet been investigated.

Another important question is how the diets fed to rabbits interact with the microbial metabolism in the caecum resulting in a specific pattern of FA supply. In comparison, n-3 PUFA and 9*c*,11*t*-18:2 CLA in meat and milk from ruminants increase when animals are fed diets containing high proportions of forage and only low proportions of cereal or legume grain crops [6–8], i.e. diets which are relatively close to the natural feeding behavior of these species. A similar situation might be found in rabbits which are grazing animals as well [14]. Accordingly, in the tissues of wild rabbits, concentrations of ALA and long-chain n-3 PUFA were found [15] to be clearly higher than those reported from rabbits fattened on farm [16]. Rabbits in agricultural production systems are commonly fed diets which largely differ from that of wild rabbits because they contain high cereal proportions [17–19]. The question remains whether the nutritional quality of the rabbit meat lipids could be increased with high-forage diets which are closer to the natural diets, and to which extent an interaction with coprophagy is relevant. Although a number of publications on the fat quality of rabbit meat exist [15–17, 19–23], no reports on controlled studies of the effects of high-forage diets are available. Diet type, especially the proportion of concentrate, was shown to influence the amount of caecotroph formation [18, 24] and, thus, indirectly the ingestion of microbially modified FA. Finally, the forage type might be important in this respect. From ruminant research, there are indications that legumes such as alfalfa, although having lower ALA proportions in the lipids than ryegrass [25], could inhibit microbial biohydrogenation [2, 5] and thus could increase supply with ALA. In case caecotrophy substantially influences FA intake, such an effect could be important in rabbits, too.

The hypotheses to be tested by an experiment with growing rabbits included: (1) that the profile of long-chain FA in rabbit's caecotrophs closely resembles the FA profile of ruminant digesta, (2) that traces or larger amounts of those FA which are typical for microbial fermentation can be recovered in the body tissue of rabbits, (3) that high-forage diets have a direct (more ALA) or caecotrophy-mediated (more 9*c*,11*t*-18:2 CLA and TVA) influence on tissue FA composition, and (4) that a leguminous forage type has a significant influence in this respect.

Experimental Procedure

Four groups of eight New Zealand hybrid type rabbits were fattened on different diets for 25 weeks. The four diets were pelleted, consisting either of pure ryegrass meal (G), pure alfalfa meal (A), grass meal and oats meal 1:1 (GO) or alfalfa meal and oats meal 1:1 (AO). The composition of the main dietary nutrients as analyzed according to Naumann and Bassler [26] is given in Table 1, and their FA profile in Table 2. The rabbits originated from one breeding facility for meat rabbits. At the beginning of treatment feeding, they were between 5 and 6 weeks old with a body mass between 1,124 and 1,930 g. Groups proportionately consisted of female and castrated male animals, and allocation to treatment was balanced with respect to the average of and variation in initial body mass. Animals of each group were kept together on woodchips in enclosures of 5.4 m^2, fitted with a sleeping box of 0.59 m^2. Feed and water was provided with ad libitum access. Daily intake was recorded as the average per group. Feed consumption of the groups was monitored daily by weighing the feed refusals before filling the troughs for the next day. No measures were taken to prevent coprophagy in the animals. The animals were also weighed before slaughter.

Table 1 Nutrient composition of the experimental diets as well as food intake and body masses development in the experimental groups (means)

Diets[a]	G	A	GO	AO
Dry matter (DM; g/100 g as fed)	89.5	90.4	90.8	89.4
Nutrients (g/100 g DM)				
Organic matter	85.3	85.5	90.8	91.8
Crude protein	18.1	16.4	13.5	14.6
Ether extract	4.0	1.9	5.1	3.5
Neutral detergent fibre	45.8	46.3	33.3	38.2
Food intake (g as fed/animal/day)[b]	274 ± 60[c]	283 ± 58	210 ± 54	211 ± 46
Initial body mass (g)	1,493 ± 209[d]	1,525 ± 209	1,474 ± 233	1,482 ± 199
Body mass at slaughter (g)	4,776 ± 364[d]	4,637 ± 315	5,286 ± 425	5,206 ± 375
Dressed carcass mass (g)[e]	2,320 ± 258[d]	2,259 ± 165	2,517 ± 182	2,495 ± 131

[a] *G* grass only; *A* alfalfa only; *GO* grass and oats 1:1; *AO* alfalfa and oats 1:1

[b] Average over the complete feeding period; data obtained as total intake per group

[c] SD based on between day variation

[d] SD based on between animal variation

[e] Body mass minus feet, head, skin, internal organs, and discrete adipose tissue

Immediately after slaughter, the animals were dissected, and samples of abdominal (perirenal) fat and of the central quadriceps muscle of the left hind leg were taken. Additionally, the gastrointestinal tract was dissected, opened, and controlled for the presence of caecotrophs. In the colon, caecotrophs can be differentiated from hard faeces based on their smaller size, and softer and moister consistency [1]. Due to a tough mucoprotein membrane, which resists digestion in the stomach for several hours [9], re-ingested caecotrophs can be identified from shape, form and consistency. Therefore, we assumed that caecotrophs in the stomach that still were not disintegrated were equal in composition to caecotrophs from the caecum and colon. No caecotrophs were collected from the small intestine. Samples of adipose and muscle tissue and caecotrophs were immediately sealed in plastic bags and stored at −20°C until analysis.

Lipid Extraction from the Samples

Lipids from feeds and caecotrophs were extracted by accelerated solvent extraction (ASE 200; Dionex Corp., Sunnyvale CA) using hexane/isopropanol (2:1 vol/vol) and transformed into FA methyl esters (FAME) according to Wettstein et al. [27]. The FAME were dissolved in hexane and purified on a silica gel column.

Lipid extraction from lean and adipose tissue was performed after homogenisation of the samples. Lipids from *M. quadriceps* were extracted by dissolving 1 g of homogenisate in 5 ml hexane-isopropanol (3:2). The lipids from adipose tissue were extracted from 0.2 g homogenisate with 12 ml hexane. Both solvents contained triundecanin and phosphatidylcholin (Fluka Chemie, Buchs, Switzerland) as internal standards. Subsequently, the fatty acids in the extracts were converted to methyl esters according to the official IUPAC method [28] with slight modifications (2 ml 0.5 M methanolic NaOH boiled for 3 min in a reaction tube, 3 ml 1.3 M boron trifluoride boiled for 4 min). A cleaning step was carried out to prevent artifacts caused by cholesterol when analyzing the *trans*-FA isomers. The FAME were sprayed onto a thin layer chromatography glass plate coated with silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany). Subsequently, the plates were run in a chamber saturated with a solution of hexane, diethylether and acetic acid in a ratio of 85:15:0.2 for 30 min until the solvent had reached the upper border of the plate. The FAME were identified and marked in UV light (440 nm) after having sprayed the plate with a fluorescent dye (2′,7′-Dichlorofluorescein, Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). The identified zone was scratched off, dissolved with a 1:1 mixture of chloroform and hexane and filtered through a silica gel column.

Fatty Acid Analysis

All samples were analyzed for individual FAME on a gas chromatograph (HP 6890, Agilent Technologies Inc., Wilmington, DE) equipped with an FID detector and a Supelcowax™ 10 capillary column (30 m × 0.32 mm, 0.25 µm film thickness; Supelco, Bellefonte, PA). An amount of 1 µl was injected by split injection (1:30, split temperature 260°C). Hydrogen was used as a carrier gas at a flow rate of 2.2 ml/min and a pressure of 55.8 kPa. The initial oven temperature was 160°C for 0.5 min. The temperature program was as follows; increase of 20°C/min up

Table 2 Total amount and individual proportions of FAME (g/100 g FAME) in the experimental diets (means of duplicate analyses; deviation always below 0.05)

Diets[a]	G	A	GO	AO
FAME, g/100 g diet	3.34	1.55	4.35	3.20
Total SFA	19.3	27.0	21.4	24.8
12:0	0.18	0.27	0.07	0.11
14:0	0.45	0.82	0.44	0.71
15:0	0.11	0.20	0.07	0.18
16:0	13.7	18.2	16.7	18.7
17:0	0.14	0.34	0.13	0.19
18:0	2.78	3.58	2.87	3.67
20:0	0.49	0.86	0.37	0.39
22:0	0.73	1.06	0.33	0.36
24:0	0.63	1.41	0.38	0.47
Total MUFA	19.8	8.3	34.2	29.2
16:1n-7	0.44	0.65	0.33	0.61
17:1	0.13	0.19	0.09	0.10
9*c*-18:1	17.4	6.5	32.2	26.7
*t*18:1[b]	1.41	0.72	0.96	1.12
20:1n-9	0.39	0.23	0.63	0.65
Total PUFA	58.9	61.9	43.0	44.6
18:2n-6	25.5	21.8	29.4	31.9
18:3n-3	32.9	39.2	13.3	12.0
20:2n-6	0.11	0.33	0.07	0.14
20:3n-3	0.05	0.10	0.03	0.03
20:5n-3	0.16	0.26	0.13	0.31
22:6n-3	0.22	0.16	0.09	0.18
Total n-6 FA	25.6	22.1	29.4	32.0
Total n-3 FA	33.3	39.7	13.6	12.6
n-6/n-3 ratio	0.77	0.56	2.17	2.55

FAME fatty acid methyl esters; *SFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids

[a] *G* grass only, *A* alfalfa only; *GO* grass and oats; *AO* alfalfa and oats

[b] Not fully separated peaks of *t*10- and *t*11- 18:1

to 190°C; increase of 7°C/min up to 230°C; isotherm at 230°C for 5.3 min; increase of 20°C/min up to 250°C; isotherm for 6 min. The detector temperature was 270°C. For a clearer separation of *trans* and *cis*18:1 isomers, extracts from caecotrophs, adipose and lean tissue were further analyzed on a 200 m × 0.25 mm size CP7421 capillary column (Varian Inc., CA, USA). An amount of 1 µl was split injected (1:50; split temperature 270°C). Again hydrogen was used as a carrier gas, and gas flow was 1.7 ml/min for 59 min and, subsequently, 1.3 ml/min for 42 min. The initial temperature was 181°C for 60 min. Subsequently the temperature was increased by 5°C/min up to 230°C; isotherm for 32 min, then increase by 5°C/min up to 250°C, then kept isothermic for 12 min. The detector temperature was 300°C.

Statistical Evaluation

Linear multivariate ANOVA was carried out with the SPSS© 14.0 software. Fixed factors were oats supplementation (− vs. + oats) and forage type (ryegrass vs. alfalfa), and the model included the interaction of both factors.

Results

Alfalfa contained less lipids than the ryegrass. The SFA were higher and MUFA were lower in alfalfa compared to ryegrass. The PUFA proportions were similar in both forages. (Tables 1, 2). By contrast, the addition of oats increased the ether extract content of the diets and massively shifted the FA profile from ALA to monounsaturated FA. BFA occurred for all diets only at the detection threshold in feed and are therefore not given in Table 2. *trans*18:1 FA occurred at a low level. Due to the higher energy density (less fibre) in the oats supplemented diets, voluntary food intake was lower while gains and dressing percentage (48.2% on average) remained similar. Differences found in body mass at slaughter between the groups were mostly due to variations of discrete adipose tissue. Caecotrophs were found with similar frequency in all groups (in 4, 4, 4 and 3 animals of groups G, A, GO and AO, respectively).

Fatty Acid Profile of the Caecotrophs and Effects of Diet

In the caecotrophs, the saturated FA (SFA) represented the most abundant group of FA, followed by monounsaturated FA (MUFA) and PUFA (Table 3). The proportion of the branched-chain saturated FA (BFA) was also rather high, ranging from 7 to 13 g/100 g FAME on average. The FA 15:0a (anteiso) and 16:0i (iso) contributed clearly more than half of all BFA. Oats supplementation had significant effects on proportions of various FA in the caecotrophs. With diets GO and AO, total SFA were significantly higher compared to diets G and A, due to higher proportions of 18:0, while BFA, *trans*18:1 FA and n-3 PUFA were lower. Overall total MUFA, PUFA and n-6 PUFA were not affected ($P > 0.05$) by dietary oats. Among the two most abundant 18:1 FA, 9*c*-18:1 was higher, while *trans*-vaccenic acid (11*t*-18:1; TVA) was lower ($P < 0.05$) when feeding oats. With diets GO and AO, the proportion of 9*c*,11*t*-CLA in the caecotroph FAME was reduced to below 0.1 g/100 g. The largest proportionate reduction with oats was found in 18:3n-3 leading to a significant increase of the n-6/n-3 ratio from about one to 2–4. The use of alfalfa instead of grass meal led to significantly lower proportions of 18:0 both in diets with and without oats. Most of the

Table 3 Concentration of total FAME (g/100 g wet weight) and fatty acid profile (g/100 g total FAME) in the caecotrophs of rabbits fed different diets ($n = 4$ for groups G, A, GO; $n = 3$ for group AO)

	Diets[a]					*P* levels[b]		
	G	A	GO	AO	SEM[c]	O	F	O × F
Total FAME	1.03	0.39	1.05	0.83	0.172	ns	<0.05	ns
Total SFA	49.8	54.8	63.9	53.1	2.01	<0.05	ns	<0.01
12:0	0.523	0.892	0.573	0.717	0.0549	ns	<0.01	ns
13:0	0.180	0.406	0.215	0.235	0.0433	ns	<0.05	<0.05
14:0	2.21	3.81	1.92	2.87	0.224	<0.05	<0.001	ns
15:0	4.35	5.37	2.84	3.08	0.377	<0.001	ns	ns
16:0	18.5	22.2	21.2	22.7	1.554	ns	ns	ns
17:0	1.010	2.308	0.962	1.073	0.1345	<0.01	<0.001	<0.01
18:0	19.0	12.1	33.1	18.8	2.059	<0.001	<0.001	ns
19:0	0.177	0.343	0.084	0.117	0.0431	<0.01	<0.05	ns
20:0	0.998	2.188	0.994	1.108	0.1160	<0.01	<0.001	<0.01
22:0	1.53	2.66	1.05	1.18	0.131	<0.001	<0.01	<0.01
23:0	0.333	0.986	0.277	0.344	0.0624	<0.001	<0.001	<0.01
24:0	1.55	2.89	1.20	1.45	0.135	<0.001	<0.001	<0.01
Total BFA	9.18	13.06	6.64	9.48	0.757	<0.01	<0.01	ns
14:0i	1.31	1.85	1.05	1.28	0.158	<0.05	<0.05	ns
15:0i	1.137	1.824	0.863	1.302	0.1370	<0.05	<0.01	ns
15:0a	2.61	4.21	1.87	3.15	0.380	<0.05	<0.01	ns
16:0i	3.06	3.65	2.20	2.89	0.285	<0.05	ns	ns
17:0i	0.650	0.813	0.269	0.360	0.0466	<0.001	<0.05	ns
17:0a	0.417	0.710	0.385	0.485	0.0682	ns	<0.05	ns
Total MUFA	21.0	16.8	18.6	22.3	1.11	ns	ns	<0.01
15:1	0.896	1.871	0.496	0.948	0.1686	<0.01	<0.01	ns
16:1n-7	0.512	0.678	0.253	0.353	0.0902	<0.05	ns	ns
17:1	0.430	0.632	0.367	0.407	0.0840	ns	ns	ns
5*t* -18:1	0.057	0.138	0.026	0.049	0.0063	<0.001	<0.001	<0.01
6*t*/8*t*-18:1[d]	0.191	0.130	0.135	0.151	0.0208	ns	ns	ns
9*t*-18:1	0.197	0.270	0.164	0.187	0.0123	<0.01	<0.01	ns
10*t*-18:1	0.157	0.137	0.129	0.121	0.0132	ns	ns	ns
11*t*-18:1	7.39	5.48	3.61	4.19	0.775	<0.05	ns	ns
12*t*-18:1	0.312	0.283	0.260	0.279	0.0200	ns	ns	ns
13*t*/14*t*-18:1[e]	0.483	0.388	0.419	0.429	0.0233	ns	ns	ns
16*t*-18:1	0.234	0.249	0.136	0.167	0.0191	<0.01	ns	ns
9*c*/15*t*-18:1[e]	7.15	4.63	10.06	11.77	0.940	<0.01	ns	ns
11*c*-18:1	1.544	0.842	0.812	0.985	0.1299	ns	ns	<0.05
12*c*-18:1	0.639	0.350	0.886	1.362	0.0778	<0.001	ns	<0.01
13*c*-18:1	0.073	0.172	0.067	0.073	0.0248	ns	ns	ns
14*c*-18:1	0.264	0.245	0.307	0.227	0.0313	ns	ns	ns
15*c*-18:1	0.113	0.156	0.032	0.064	0.0140	<0.001	<0.05	ns
20:1n-9	0.246	0.155	0.321	0.482	0.0586	<0.01	ns	ns
22:1	0.063	0.004	0.067	0.029	0.0221	ns	ns	ns
24:1	0.030	0.000	0.043	0.011	0.0192	ns	ns	ns
Total PUFA	15.6	10.2	8.8	12.2	1.38	ns	ns	<0.05
18:2n-6	7.24	5.75	6.09	9.53	0.840	ns	ns	<0.05
9*c*11*t*-18:2	0.115	0.183	0.059	0.088	0.0302	<0.05	ns	ns
18:3n-6	0.050	0.051	0.170	0.067	0.0445	ns	ns	ns

Table 3 continued

	Diets[a]					*P* levels[b]		
	G	A	GO	AO	SEM[c]	O	F	O × F
18:3n-3	7.33	4.78	2.15	2.12	0.715	<0.001	<0.05	<0.05
20:3n-6	0.000	0.000	0.012	0.000	0.0061	ns	ns	ns
20:3n-3	0.036	0.014	0.012	0.010	0.0091	ns	ns	ns
20:4n-3	0.078	0.090	0.183	0.319	0.1836	ns	ns	ns
20:5n-3	0.040	0.021	0.022	0.015	0.0142	ns	ns	ns
22:6n-3	0.076	0.000	0.092	0.021	0.0460	ns	ns	ns
Total n-3	7.67	4.89	2.46	2.49	0.715	<0.001	ns	ns
Total n-6	7.29	5.80	6.27	9.60	0.680	ns	ns	<0.05
n-6/n-3	0.95	1.18	2.55	3.86	0.203	<0.001	<0.001	0.0300

FAME fatty acid methyl esters; *SEM* standard error of means; *SFA* saturated fatty acids; *BFA* branched-chain fatty acids; *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids

[a] *G* grass only; *A* Alfalfa only; *GO* grass and oats 1:1; *AO* alfalfa and oats 1.1

[b] *O* oats effect (with vs. without); *F* forage type effect (grass vs. alfalfa)

[c] Average SEM within groups

[d] These fatty acids are co-eluting

[e] Sum of two peaks containing unidentified 18:2 isomers

BFA and some minor SFA were significantly higher with the pure alfalfa diet, but this was reversed for the SFA in the presence of the oats (O × F interaction, $P < 0.01$). Interactions among the two dietary factors were also found for total MUFA and PUFA with the result that no clear overall forage type effect remained. Alfalfa resulted in a lower 18:3n-3 proportion than the grass ($P < 0.05$), but only in the non supplemented diets.

Fatty Acid Profile of the Body Tissues and Effects of Diet

In the perirenal adipose tissue, total SFA, MUFA and PUFA occurred at relatively similar levels. BFA, TVA and CLA, indicative for microbial lipid sources, occurred at very low proportions (Table 4). Most of the FA were significantly influenced by oats supplementation. For the SFA, this effect was marginal, while MUFA were clearly elevated by oats. This resulted mainly from higher values of the 9*c*-18:1/15*t*-18:1 peak ($P < 0.001$); but also 16:1n-7 was considerably higher ($P < 0.01$) with the oats diets. Total PUFA declined with diets GO and AO compared to G and A, exclusively resulting from a highly significant drop in n-3 PUFA. Thus, the average n-6/n-3 ratio increased from 0.7 with G and O to 2.7 with GO and AO ($P < 0.001$). The proportions of total and individual BFA were lower by about half with oats diets ($P < 0.001$). Total SFA significantly increased and total MUFA and PUFA significantly declined with the alfalfa compared to the grass diet, but this effect occurred mainly in the diets without oats. Total and some individual BFA were significantly higher in proportion with alfalfa instead of the grass, while 18:3n-3 was lower ($P < 0.01$). Alfalfa significantly increased long chain n-3 PUFA; this, however, at a low level.

The FA profile of the intramuscular fat and its variation with diet were relatively similar in nature to those found in the adipose tissue (Table 5). However, particularly for individual PUFA, the effects of oats addition were not as distinct, and significant effects of alfalfa were absent. Intramuscular lipids proportion of 18:3n-3 was considerably lower, and the long-chain PUFA (>3 double bonds) occurred with five- to ten-fold higher proportions than in the FAME of the adipose tissue. Particularly arachidonic acid (20:4n-6) and docosapentaenoic acid (22:5n-3) made up a considerable proportion of total PUFA in intramuscular fat, which was not the case in perirenal adipose tissue.

Differences in Fatty Acid Profiles of Food, Caecotrophs and Body Tissues

In contrast to the body tissue lipids, caecotroph lipids contained comparably high proportions of BFA and *trans*-18:1 FA. These FA occurred only in traces or low amounts in feeds and body tissues. Although the diet effects on BFA were similar in direction in the caecotrophs and in the body tissues, the relative proportions between the individual BFA in caecotrophs differed. The relative proportions of 17:0i and 17:0a within BFA were clearly higher in the body tissues than in the caecotrophs. A second property,

Table 4 Concentration of FAME (g/100 g wet weight) and fatty acid profile (g/100 g total FAME) in the perirenal adipose tissue of rabbits fed different diets ($n = 8$ per group)

	Diets[a]					*P* levels[b]		
	G	A	GO	AO	SEM[c]	O	F	O × F
Total SFA	34.0	41.6	36.2	37.1	0.59	ns	<0.001	<0.001
10:0	0.019	0.023	0.015	0.016	0.0020	<0.01	ns	ns
12:0	0.066	0.075	0.053	0.057	0.0032	<0.001	ns	ns
14:0	2.11	2.79	2.42	2.72	0.086	ns	<0.001	<0.05
15:0	0.555	0.990	0.397	0.502	0.0248	<0.001	<0.001	<0.001
16:0	23.5	28.5	25.8	26.6	0.49	ns	<0.001	<0.001
17:0	0.670	1.097	0.493	0.591	0.0154	<0.001	<0.001	<0.001
18:0	6.60	7.62	6.73	6.33	0.288	ns	ns	<0.05
19:0	0.205	0.247	0.140	0.150	0.0055	<0.001	<0.001	<0.01
20:0	0.183	0.185	0.134	0.111	0.0035	<0.001	<0.01	<0.01
22:0	0.022	0.028	0.008	0.008	0.0012	<0.001	<0.01	<0.01
24:0	0.048	0.049	0.027	0.022	0.0023	<0.001	ns	<0.05
Total BFA	0.713	0.957	0.499	0.524	0.0219	<0.001	<0.001	<0.001
14:0i	0.051	0.065	0.036	0.032	0.0028	<0.001	ns	<0.01
15:0i	0.097	0.136	0.072	0.062	0.0130	<0.01	ns	ns
15:0a	0.117	0.168	0.065	0.080	0.0048	<0.001	<0.001	<0.01
16:0i	0.207	0.303	0.150	0.157	0.0103	<0.001	<0.001	<0.001
17:0i	0.064	0.067	0.043	0.043	0.0017	<0.001	ns	ns
17:0a	0.170	0.208	0.127	0.143	0.0044	<0.001	<0.001	<0.05
Total MUFA	26.21	23.52	37.91	37.27	0.66	<0.001	<0.05	ns
15:1	0.136	0.419	0.066	0.119	0.0090	<0.001	<0.001	<0.001
16:1n-7	4.02	4.55	5.29	5.73	0.418	<0.01	ns	ns
17:1	0.416	0.576	0.310	0.386	0.0080	<0.001	<0.001	<0.001
5*t* -18:1	0.000	0.009	0.000	0.000	0.0033	ns	ns	ns
6*t*/8*t*-18:1[d]	0.033	0.037	0.023	0.020	0.0033	<0.001	ns	ns
9*t*-18:1	0.062	0.047	0.059	0.059	0.0044	ns	ns	ns
10*t*-18:1	0.046	0.023	0.017	0.018	0.0046	<0.01	<0.05	<0.05
11*t*-18:1	0.183	0.134	0.131	0.109	0.0092	<0.001	<0.001	ns
12*t*-18:1	0.025	0.021	0.021	0.024	0.0042	ns	ns	ns
13*t*/14*t*-18:1[e]	0.068	0.065	0.035	0.053	0.0041	<0.001	ns	<0.05
16*t*-18:1	0.028	0.032	0.022	0.022	0.0014	<0.001	ns	ns
9*c*/15*t*-18:1[e]	19.1	15.9	29.5	28.4	0.38	<0.001	<0.001	<0.05
11*c*-18:1	0.99	0.72	1.05	1.22	0.038	<0.001	ns	<0.001
12*c*-18:1	0.095	0.093	0.089	0.084	0.0052	ns	ns	ns
13*c*-18:1	0.074	0.068	0.067	0.086	0.0051	ns	ns	<0.05
14*c*-18:1	0.015	0.019	0.014	0.016	0.0009	ns	<0.01	ns
15*c*-18:1	0.008	0.008	0.004	0.004	0.0008	<0.001	ns	ns
19:1	nd	nd	nd	nd				
20:1n-9	0.212	0.145	0.360	0.351	0.0053	<0.001	<0.001	<0.001
22:1	0.012	0.008	0.022	0.017	0.0011	<0.001	<0.001	ns
Total PUFA	38.48	33.51	24.89	24.47	0.77	<0.001	<0.01	<0.01
18:2n-6	15.8	12.6	17.5	17.8	0.42	<0.001	<0.01	<0.001
9*c*11*t*-18:2	0.072	0.060	0.056	0.066	0.0036	ns	ns	<0.01
18:2[c]	0.080	0.071	0.045	0.048	0.0021	<0.001	ns	<0.01
18:3n-3	21.64	19.50	6.94	5.87	0.500	<0.001	<0.01	ns
20:3n-6	0.033	0.035	0.040	0.041	0.0015	<0.001	ns	ns

Table 4 continued

	Diets[a]					*P* levels[b]		
	G	A	GO	AO	SEM[c]	O	F	O × F
20:3n-3	0.230	0.245	0.088	0.088	0.0090	<0.001	ns	ns
20:4n-6	0.107	0.104	0.113	0.120	0.0035	<0.01	ns	ns
20:4n-3	0.043	0.037	0.022	0.020	0.0014	<0.001	<0.01	ns
20:5n-3	0.086	0.106	0.037	0.055	0.0035	<0.001	<0.001	ns
22:4n-6	0.022	0.027	0.016	0.011	0.0008	<0.001	ns	<0.001
22:5n-3	0.202	0.238	0.094	0.112	0.0114	<0.001	<0.05	ns
22:6n-3	0.070	0.072	0.017	0.051	0.0042	<0.001	<0.001	<0.001
Total n-3	22.13	20.44	7.19	6.20	0.457	<0.001	<0.01	ns
Total n-6	16.15	12.92	17.57	18.14	0.403	<0.001	<0.01	<0.001
n-6/n-3	0.73	0.63	2.45	2.93	0.036	<0.001	<0.001	<0.001

For footnotes see Table 3

characterising the caecotrophs vs. the tissue lipids, was the high proportion of SFA (including most individual SFA). The proportion of SFA in total FAME was clearly above 50 g/100 g for caecotrophs and below 40 g/100 g for the perirenal adipose and intramuscular fat. Complementary, PUFA were distinctly higher in the investigated tissues than in the caecotrophs.

Discussion

Characteristics of the Caecotroph Fatty Acid Profile, and Effects of Diet Type

In general, comparing the FA profiles of the caecotroph lipids with those of the corresponding feeds indicated an active anaerobe microbial lipid metabolism. A first crude indicator was the ratio of SFA, in particular 18:0, to PUFA, being higher in caecotrophs than in the diets. As described for the forestomach of ruminant animals [2], 18:0 is the terminal product of microbial biohydrogenation of dietary 18:3 and 18:2. A second indicator was the high concentration of *trans* FA, mainly 11*trans*18:1, found in the caecotrophs. These FA are also the result of microbial lipid metabolism in the digestive tract [2, 29]. Finally, the substantial proportions of BFA and odd-chain FA found in the caecotroph lipids support the same conclusion [3].

High proportions of forages in the diet are known to increase BFA formation in ruminant digestion [3]. In the caecotrophs, the same effect was indicated by the elevated proportions of BFA and odd-chain FA found in the rabbits receiving the forage-only diets (G and A). Alfalfa diets obviously did not have the inhibitory effect on ALA biohydrogenation anticipated from replacing grass by legumes [2, 5]. By contrast, when feeding the pure alfalfa diet the reduction of the ALA proportion was even more pronounced than with the ryegrass diet, suggesting increased biohydrogenation and isomerisation. Higher BFA proportions with the alfalfa diets suggest increased microbial activity, too, contrasting to what is known from ruminants [5, 30].

However, an increased biohydrogenation should not only reduce ALA or linoleic acid proportions, but also increase CLA, octadecanoic MUFA and 18:0 [2]. In this respect, the results on the caecotroph FA profiles were apparently inconsistent because neither 18:1 nor 18:0 were higher with diet A compared to G. A confounding between discriminating absorption in the duodenum and biohydrogenation in the caecum cannot be excluded. The interactions of the two dietary factors give no clear picture for SFA, MUFA and PUFA in the caecotrophs, either. Significant interactions of the sums of FA groups have no corresponding counterpart in interactions in all single FA. They appear rather to be artifacts. Thus, the clearest information about diet effects on microbial activity in the caecum apparently may be obtained from BFA which are synthesised de novo. This is reflected by the fact that the diet effects on all FA of this group were almost parallel.

Signature of the Caecotroph FA Profile as Found in the Body Tissues

FA profiles typical for microbial fermentation are found in both ruminating and non-ruminating foregut-fermenting herbivores, e.g. kangaroos [2, 31–33]. By contrast, monogastric herbivores have no forestomach with microbial fermentation; instead, microbial digestion occurs in the caecum and the colon of such species. In most aspects, the bacterial fermentation in the hindgut is comparable to that in the rumen [34] and similar effects of microbial lipid metabolism were demonstrated in the hindgut and the faeces, as e.g. by Hartman et al. [31] for horse faeces.

Table 5 Concentration of FAME (g/100 g wet weight) and fatty acid profile (g/100 g total FAME) in the intramuscular fat of the *M. quadriceps* of rabbits fed different diets ($n = 8$ per group)

	Diets[a]					*P* levels[b]		
	G	A	GO	AO	SEM[c]	O	F	O × F
Total FAME	2.15	2.93	3.15	2.56	0.302	ns	ns	<0.05
Total SFA	35.2	39.2	35.7	36.2	0.52	<0.05	<0.001	<0.01
10:0	0.032	0.036	0.029	0.028	0.0038	ns	ns	ns
12:0	0.059	0.067	0.060	0.058	0.0037	ns	ns	ns
14:0	2.02	2.47	2.51	2.52	0.111	<0.05	<0.05	ns
15:0	0.427	0.700	0.317	0.371	0.0217	<0.001	<0.001	<0.001
16:0	23.3	25.9	24.9	25.0	0.453	ns	<0.01	<0.01
17:0	0.598	0.898	0.389	0.462	0.0217	<0.001	<0.001	<0.001
18:0	8.36	8.52	7.04	7.26	0.295	<0.001	ns	ns
19:0	0.282	0.307	0.233	0.256	0.0120	<0.001	ns	ns
20:0	0.116	0.132	0.089	0.083	0.0064	<0.001	ns	ns
22:0	0.051	0.104	0.064	0.083	0.0135	ns	<0.05	ns
24:0	0.023	0.027	0.021	0.015	0.0040	ns	ns	ns
Total BFA	0.477	0.622	0.351	0.362	0.0217	<0.001	<0.01	<0.01
14:0i	0.033	0.042	0.027	0.022	0.0028	<0.001	ns	<0.05
15:0i	0.056	0.069	0.040	0.041	0.0026	<0.001	<0.05	<0.05
15:0a	0.072	0.102	0.045	0.051	0.0040	<0.001	<0.001	<0.01
16:0i	0.147	0.205	0.107	0.109	0.0089	<0.001	<0.01	<0.01
17:0i	0.048	0.053	0.037	0.040	0.0021	<0.001	ns	ns
17:0a	0.122	0.152	0.095	0.099	0.0051	<0.001	<0.01	<0.05
Total MUFA	28.5	26.8	37.0	35.8	1.23	<0.001	ns	ns
15:1	0.088	0.259	0.050	0.079	0.0082	<0.001	<0.001	<0.001
16:1n-7	5.28	5.58	7.49	7.42	0.568	<0.01	ns	ns
17:1	0.405	0.557	0.330	0.374	0.0161	<0.001	<0.001	<0.01
5*t* -18:1	0.036	0.042	0.053	0.051	0.0100	ns	ns	ns
6*t*/8*t*-18:1[d]	0.006	0.018	0.005	0.011	0.0037	ns	<0.05	ns
9*t*-18:1	0.063	0.039	0.039	0.037	0.0106	ns	ns	ns
10*t*-18:1	0.056	0.057	0.044	0.064	0.0137	ns	ns	ns
11*t*-18:1	0.121	0.107	0.090	0.062	0.0134	<0.05	ns	ns
12*t*-18:1	0.047	0.022	0.017	0.027	0.0068	ns	ns	<0.05
13*t*/14*t*-18:1[e]	0.036	0.061	0.034	0.030	0.0040	<0.001	<0.05	<0.01
16*t*-18:1	0.110	0.165	0.191	0.181	0.0226	<0.05	ns	ns
9*c*/15*t*-18:1[e]	20.3	18.3	26.4	24.6	0.741	<0.001	<0.05	ns
11*c*-18:1	1.23	0.95	1.39	1.58	0.058	<0.001	ns	<0.001
12*c*-18:1	0.066	0.062	0.062	0.054	0.0038	ns	ns	ns
13*c*-18:1	0.046	0.068	0.082	0.089	0.0099	<0.01	ns	ns
14*c*-18:1	0.042	0.018	0.011	0.009	0.0060	<0.01	<0.05	ns
15*c*-18:1	0.008	0.009	0.003	0.008	0.0018	ns	ns	ns
19:1	0.112	0.091	0.068	0.063	0.0033	<0.001	<0.001	<0.05
20:1n-9	0.141	0.099	0.242	0.221	0.0112	<0.001	<0.05	ns
22:1	0.017	0.020	0.022	0.018	0.0040	ns	ns	ns
Total PUFA	34.8	32.4	25.8	26.4	1.08	<0.001	ns	ns
18:2n-6	14.9	12.8	15.2	15.7	0.46	<0.01	ns	<0.05
9*c*11*t*-18:2	0.078	0.065	0.071	0.072	0.0038	ns	ns	ns
18:2[c]	0.071	0.067	0.047	0.063	0.0111	ns	ns	ns
18:3n-3	11.48	12.37	4.41	3.40	0.456	<0.001	ns	<0.05

Table 5 continued

	Diets[a]					*P* levels[b]		
	G	A	GO	AO	SEM[c]	O	F	O × F
20:3n-6	0.368	0.321	0.293	0.361	0.0351	ns	ns	ns
20:3n-3	0.185	0.216	0.079	0.076	0.0066	<0.001	ns	<0.05
20:4n-6	3.60	2.78	3.14	4.10	0.437	ns	ns	ns
20:4n-3	0.056	0.061	0.032	0.030	0.0047	<0.001	ns	ns
20:5n-3	0.596	0.604	0.156	0.205	0.0382	<0.001	ns	ns
22:4n-6	0.186	0.167	0.381	0.532	0.0365	<0.001	ns	<0.05
22:5n-3	2.10	1.83	1.16	1.30	0.170	<0.001	ns	ns
22:6n-3	0.694	0.539	0.242	0.464	0.0493	<0.001	ns	<0.001
Total n-3	15.11	15.62	6.07	5.39	0.379	<0.001	ns	ns
Total n-6	19.2	16.3	19.2	20.5	0.87	<0.05	ns	<0.05
n-6/n-3	1.28	1.04	3.16	3.79	0.098	<0.001	ns	<0.001

For footnotes see Table 3

However, as these processes in monogastric animals occur after the digesta has passed the duodenum, the major site of absorption for lipids, they do not have a major influence on the lipid composition of body tissues. This is reflected by higher proportions of PUFA in the tissues of horses, pigs and other hindgut fermenters as compared to ruminants and non-ruminant foregut fermenters [35–38].

Due to this dichotomy—absorption of 'ruminant-specific' FA in foregut fermenters, no absorption of such FA in hindgut fermenters—the endogenous presence of 'ruminant specific' FA has been considered as an indicator of foregut fermentation [39]. Thus, Käkela et al. [11] interpreted the endogenous presence of BFA and *trans* FA in tissue lipids of beavers (*Castor canadensis*) as an indication of a 'ruminant-like' bacterial fermentation in the gastrointestinal tract. However, the same research group [12] described PUFA levels in beaver tissues exceeding those found in ruminants by far. The beaver, therefore, shares similarities with ruminants with respect to BFA and *trans* FA and with hindgut-only fermenters concerning PUFA. This phenomenon could be explained by the digestive strategy of coprophagy of rodents and other small herbivores [9]. Coprophagy has been studied particularly well in rabbits [18, 24, 40, 41]. Hirakawa [9] describes that wild rabbits recycle almost the entire undigested matter in the form of caecotrophs. Based on the values published by Belenguer et al. [24], coprophagy could have made up approximately 10% of nutrient intake in our study. The proportion of total lipid absorption covered by coprophagy is not clear from the literature.

One central question of the present study was to which extent coprophagy can alter rabbit tissue FA composition. If the microbial lipids contain functional FA like CLA, even a proportion of 10% of the absorbed FA would be valuable in terms of the nutritional quality of the meat. If, on the other hand, with a more natural feeding strategy, coprophagy would be intensified [9, 24], the related increase of SFA might counterbalance such positive effects. The results show that both were only marginally the case. With its extraordinary high proportions of PUFA, the rabbits' tissues had nothing in common with those of ruminants, which consistently comprise much lower proportions of PUFA [4, 6–8, 29, 30]. It seems likely that a high proportion of dietary FA was absorbed in the small intestine before any biohydrogenation processes could take place in caecum and colon. This is consistent with the reduction found in total FAME from diets to caecotrophs, which was in the magnitude of 2/3. In this respect, the rabbits of this experiment were not only able to corroborate some previous findings on tissue PUFA content in rabbits [15, 19], but also the observations of the high PUFA content in beaver tissues [12].

However, certain amounts of BFA, odd-chain FA and *trans*18:1 FA were still found in the rabbits' tissues. Although endogenous BFA synthesis cannot be excluded, this is expected to take place to a low extent [3]. It seems reasonable to assume that the majority of BFA found in the tissues are of caecotroph origin. Compared to that reported for sheep [42], the BFA content of intramuscular fat was only approximately three times lower. Considering the high impact of ruminal processes on the endogenous FA metabolism in sheep [2], the amounts of BFA in the rabbits' tissues indicate that the contribution of microbially modified lipids to the total dietary lipids was not negligible. The considerably higher BFA proportions in tissues of the groups fed without oats are obviously a reflection of the diet effects on caecotrophs. With increasing dietary fibre proportions, the caecal microbial fermentation in rabbits

and their intake of microbially fermented matter increases [18, 24]. Based on what is known from ruminant digestion [3], it seems likely that the increased BFA proportions in the tissues are an indicator of increased coprophagial activity by the rabbits fed forage-only diets. In this context, the increase in SFA in the tissues of the forage-only groups compared to the oats-groups could be explained by an enhanced intake of caecotrophs as these contained proportionately more SFA than the diets. By contrast, the low and insignificant amounts of *trans*-FA found in the tissues do not allow the same conclusion.

We conclude that, although on a low level, FA originating from the caecotrophs are traceable in the rabbits' metabolism, but to a different degree for individual FA. Provided these results are confirmed with other diets and under different conditions, markers for the occurrence of coprophagy in wild animals could be developed on the basis of certain BFA and *trans* FA. Under free-ranging conditions, on a less consistent supply of high-quality forage, coprophagy might contribute even more to the animal's nutrient intake. This would lead to higher tissue proportions of the discussed FA. For example, the higher proportions of *trans* FA in free-ranging beavers [12], as compared to the rabbits of the present study, could indicate a higher proportionate intake of caecotrophs.

Influence of Excluding Concentrate on the FA Profile of the Body Tissues

Feeding the forage-oats diets resulted in a recovery of the characteristically high MUFA content of oats. It also led to high concentrations of n-3 PUFA, particularly of 18:3n-3, in the lipids of perirenal adipose and muscle tissue. These concentrations were in the upper range of data reported in the majority of other studies about rabbit meat quality [16, 17, 21–23]. By feeding a high n-3 oil supplement, Dal Bosco et al. [43] achieved n-3 PUFA concentrations in the range found in the present study with the oats diets. Cobos et al. [15] found such values in wild rabbits and hares from central Spain. For long chain n-3 PUFA in intramuscular lipids, comparable or higher values were described by Forrester-Anderson et al. [19] for grazing rabbits consuming an unknown amount of additional concentrate. Comparably high n-3 FA proportions in the rabbit meat had never been achieved in the other studies reviewed. Those studies always used dietary forage proportions of less than 50%, the lowest value applied in the present investigation.

When increasing the forage proportion from 50 to 100% in the present study, the 18:3n-3 proportions were significantly higher and amounted to 15.4 and 21.3% of the intramuscular and the adipose fat of the rabbits, respectively. This reflects the favorably high 18:3n-3/18:2n-6 ratio in diets G and A, and illustrates the limited role coprophagy played for the FA composition of the rabbits' tissues. Positive effects of the forage-only diets were also noted on the proportions of the long-chain n-3 PUFA, particularly in the intramuscular lipids. This indicates considerable chain elongation processes, converting ingested 18:3n-3 into long-chain n-3 PUFA. Elongation of 18:2n-6 and 18:3n-3 competes for the same enzymes and depends on the respective concentration of both substrates [44]. Thus, the effects of diets G and A on n-3 PUFA confirmed our anticipation. The expected decrease of 20:4n-6, however, was lacking. The efficiency of endogenous elongation is crucial for the estimation of health effects of dietary 18:3n-3 in human diets [45], and the findings are controversial in this respect [46, 47]. The present data show that the endogenous concentrations of long-chain n-3 PUFA may depend considerably on the intake of 18:3n-3, at least in rabbits. An open question is whether the source of dietary 18:3n-3, other dietary factors or both have a confounding influence on the elongation processes.

In a broad range of animal species, body tissues were found to have higher proportions of n-3 PUFA under free-ranging conditions as compared to animals kept in captivity [48]. Our data, as well as data for cattle [8, 29], suggest that this 'wilderness effect' can be mimicked in domesticated herbivore species by high-forage diets. Overall, the present data show that a forage-only diet increases the sum of n-3 PUFA in rabbit meat to a level similarly high as that found in free-ranging conspecifics [15]. Compared to domesticated animals, including ruminants [36], free-range grazing pigs [49] and the horse [36], the enhancement of total of n-3 PUFA by a forage-only diet was particularly high in the rabbits of the present study. Even under extensive organic feeding conditions neither pork [49, 50] nor beef [8] reached these concentrations. Provided these results can be generalised for various rabbit rearing conditions, meat from forage-only fed rabbits could represent a particularly healthy food. Although feed conversion efficiency is lower with forage-only diets due to the high contents of indigestible fibre [24], the economic success of forage-only feeding systems could be substantial, if the nutritional value of such a high n-3 meat is paid for.

Another significant effect of the forage-only compared to the oats-supplemented diets was the lower proportion of 9*c*-16:1. Since this MUFA only occurred in traces in feeds and caecotrophs, it is likely that the high tissue level was caused by endogenous δ-9-desaturation [51]. This would imply that forage-only diets might reduce tissue desaturase activity. One explanation for this phenomenon could be the higher exogenous intake of dietary PUFA making endogenous desaturation less important.

Influence of the Forage Type on the FA Profile of the Body Tissues

The comparison of alfalfa versus grass as dietary forages did not reveal substantial effects on tissue FA profiles. Besides the influence on BFA, which was discussed above, the most obvious effect of dietary alfalfa compared to ryegrass was a small but significant reduction in 18:3n-3 and an increase in long-chain n-3 PUFA. This suggests a positive effect of alfalfa on the endogenous chain elongation. This effect only occurred in adipose tissue. The distribution of n-3 PUFA across the entire organism depends on several factors as described for other small herbivores [46], pigs [47], and humans [45]. Thus, any explanation for the alfalfa effect on adipose n-3 PUFA remains speculative. It is, however, interesting to note that total n-3 PUFA did not differ much between grass- and alfalfa-fed rabbits although total lipid and, accordingly, total n-3 content of the alfalfa-containing diets was lower by a factor of two than with the grass.

Conclusion

1. The FA profiles of caecotroph lipids of rabbits showed typical properties of an anaerobic microbial lipid metabolism comparable to that occurring during ruminal digestion.
2. Small amounts of BFA and *trans*-FA from caecotrophs are obviously ingested and metabolised. This could have significance for the evaluation of FA digestion and metabolism with small herbivore animal models. It seems possible to develop markers for the occurrence of significant levels of coprophagy in non-ruminant herbivores particularly on the basis of BFA and *trans*18:1 FA.
3. Considering the nutritive value of rabbit meat, the contribution of coprophagy to the tissue FA profiles appears to be marginal. This holds true for SFA concentrations, which are more similar to horse meat than to beef, as well as for CLA, which were nearly absent in the tissues of the rabbits in the present study.
4. The endogenous FA metabolism, with respect to chain elongation, desaturation and distribution between different tissues, to some extent depends on dietary factors such as proportions and type of forage.
5. The effect of high dietary forage proportions on the n-3 contents of rabbit meat is so substantial that it seems worthwhile confirming these data with various forages and developing forage-based feeding strategies for rabbit meat production.

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