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Using Grass Feeding to Enhance Level of Omega-3 Fatty Acids in Beef.

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ABSTRACT

The paper presents the results of studies on the enrichment of polyunsaturated fatty acids of beef. The fat-and-fat composition of herbs has been studied. The significant role of seasonal and ecological factors in the phenotypic variation of the fatty acid content is established, which in turn will require correction of the fatty acid composition of the feed in the growing process. In vivo and in vitro studies on simple scars and their effect on plant chloroplasts have been carried out. It was found that, due to viability problems and protozoa, the in vivo intake / digestion / isolation control. Influence of feeding on the content in protozoa C18: 3n-3 was established, respectively, the next step is to increase the protozoal flow into the small intestine, while maintaining a stable density of scars.

Keywords:beef, polyunsaturated fatty acids, forage grasses, fattening

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INTRODUCTION

Products enriched with omega fatty acids, are increasingly appearing on the shelves of stores: bread, milk mixtures for children, nursing and pregnant women and much more.

The human body is able to synthesize only omega-9 fatty acids; Omega-3 and Omega-6 fatty acids are not able to be produced in the body and must come with food, as they are also necessary for life.

The relatively small differences in the structure of the molecules cause Omega-6 and Omega-3 to act on the human body in completely different ways. The "minus" of Omega-6 acid is the products of its metabolism.

The human body needs both kinds of fatty acids, although it has already been established that an excess of omega-6 can lead to unfortunate consequences. Consumption of omega-6 and omega-3 should occur in a proportion of 1: 1 to 4: 1 [1, 2].

The quality of food is becoming increasingly important for consumers. For meat, the definition of quality is becoming more complex, as it encompasses not only the physical aspects of meat, such as tenderness, juiciness, taste, but also includes more recent issues such as safety, traceability, preventive properties and the production environment. Consumers are gradually becoming aware of the relationship between nutrition and health, especially with regard to cancer and atherosclerosis. Knowledge of these relationships has strengthened consumers' interest in the nutritional quality of foods, so this becomes a more important aspect of product quality [3].

Beef is considered to be very nutritious and valuable food. The importance of meat as a source of protein with a high biological value (including, for example, vitamins A, B6, B12, D, E, iron, zinc, selenium) is well known. However, over the past 10-15 years, these positive qualities have often been overshadowed by several negative attributes. The latter include the perception that beef contains a large amount of fat, rich in saturated fatty acids, associations between red meat and cancer, and non-nutritional issues, such as harmful effects on animal health (BSE syndrome) [2].

Beef is a natural carrier of useful n-3 PUFAs (eicosapentaenoic acid (EPA, C20: 5n-3) and dosagehexaenoic acid (DHA, C22: 6n-3), as well as CLA and an important source of beneficial fats for humans. acids in beef will improve the nutritional value of this important meat for the consumer.

Intramuscular fat is the most important fatty depot of fatty acids for humans. The ratio of NLC, HOAFA and PUFA on average is from 0.45 to 0.48, from 0.35 to 0.45 and up to 0.05 of the total amount of fatty acids. The ratio of polyunsaturated fatty acids to saturated for beef is usually small and is about 0.1. The n-6: n-3 ratio for beef is advantageously low, usually less than 3 to 1, which reflects significant amounts of useful n-3 PUFAs, especially C18: 3n-3, as well as EPA and DHA. Beef also contains CLA and, in particular, cis-9, trans-11 and trans-10, cis-12 CLA. The anticarcinogenic and antiatherogenic effects of cis-9, trans-11, as well as anticonvulsant effects of trans-10, cis-12 are reflected in the work of BeluryMA [1, 2, 3].

MATERIALS AND METHODS

Plant-based feeding strategies are the most appropriate and sustainable approach to increasing the content of n-3 PUFAs in beef. The transformation of C18: 3n-3 from feed to meat depends on two important processes:

- increase in the level of C18: 3n-3 in the feed (and, consequently, in the animal)
- reduction of biohydrogenation processes in the rumen.

The experience of researchers at the University of Aberystwyth shows that the variety of grass, the stage of growth and the way of conservation (silage and hay, the degree of wilt, etc.) affect the concentration of C18: 3n-3 [4, 5, 6].

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Taking into account the requirements for the creation of pastures with high yields, productive longevity, rapid achievement of pasture ripeness, resistance to grazing and trampling, and high content of unsaturated fatty acids, 3 types of grasses have been selected: ryegrass + perennial sorghum + sand oats. The plants were grown on the territory of a training pilot farm with a cut every 6 weeks and an annual fertilizer at a rate of 250 kg N per 1 hectare for 3 years. Collection of herbal material for the analysis of fatty acids was carried out in June and September 2014, 2015 and 2016 [7, 8, 9].

The fatty acid content was determined in 1 g of lyophilized material using methyl ester of gneisnosanoic acid (C21: 0) as the internal standard (Sigma-Aldrich Co, St Louis, MO, USA) and one-stage extraction by the esterification method. Methyl esters of fatty acids (FAME) were separated and quantified using gas chromatography. A linear mixed model was used to estimate the dispersion components. The analysis was carried out using the REML (limited maximum likelihood) analysis in Genstat (14th edition, VSN International Ltd, Hemel Hempstead, UK).

RESULTS AND DISCUSSION

In Table 1, for each year out of three, the mean and standard deviations for the five major fatty acids (C16: 0, C18: 0, C18: 1n-9, C18: 2n-6 and C18: 3n-3) are shown. The number of C18: 2n-6 and C18: 3n-3 was the largest percentage of the total number, and their number was the highest in the 2nd year, while the three minor components tend to increase in each slant.

Table 1: Fatty acid content in the test material, (mg / g^{-1} LM)

Year	Name	C16:0	C18:0	C18:1n-9	C18:1n-9	C18:2n-6	C18:3n-3
During all this time	Ryegrass	4.26±0.620	0.39±0.078	0.38±0.139	2.73±0.633	15.02±4.015	25.03±5.253
During all this time	Sorghum	3.92±0.693	0.38±0.103	0.46±0.270	2.65±0.846	12.09±3.708	22.95±8.706
During all this time	Sandy oats	4.01±0.579	0.39±0.073	0.46±0.133	2.94±0.705	11.61±2.889	22.42±6.123
1	Ryegrass	3.77±0.513	0.36±0.073	0.35±0.116	2.71±0.447	14.66±2.440	23.41±3.141
1	Sorghum	2.89±0.140	0.32±0.036	0.42±0.082	2.52±0.267	9.12±1.305	16.58±1.059
1	Sandy oats	3.26±0.544	0.34±0.041	0.33±0.044	2.69±0.420	10.17±2.385	18.29±3.141
2	Ryegrass	4.20±0.389	0.39±0.060	0.37±0.123	3.14±0.372	18.03±3.193	28.96±4.581
2	Sorghum	3.88±0.428	0.42±0.135	0.58±0.501	3.30±1.047	14.53±4.336	29.59±12.764
2	Sandy oats	3.91±0.220	0.42±0.075	0.57±0.119	3.56±0.558	13.44±2.874	27.10±8.462
3	Ryegrass	4.75±0.513	0.42±0.088	0.40±0.167	2.35±0.732	12.35±3.861	22.55±5.128
3	Sorghum	4.45±0.247	0.39±0.102	0.40±0.059	2.28±0.722	11.96±3.311	21.71±4.718
3	Sandy oats	4.44±0.301	0.40±0.079	0.46±0.117	2.64±0.698	11.11±2.929	21.37±3.741

Table 1 also shows that five fatty acids are present in all samples. Analysis of the variance components using the REML model in all six quarries has demonstrated a significant effect of this factor.

On the basis of the results obtained, it can be concluded that seasonal and environmental factors play a significant role in the phenotypic variation in the content of fatty acids, which in turn will require an adjustment of the fatty acid composition of the feed in the growing process.

Reducing the degree of biogenesis in the rumen can increase the processes of transformation of PUFA into muscle and adipose tissue. PUFAs are rapidly hydrogenated with the microbiotic of the rumen, which leads to the formation of NLC (mainly 18: 0), but also to the formation of intermediate CLA and trans monoenes (mainly trans-vaccinic acid TVA). This is one of the main reasons why ruminant fats are mainly saturated fatty acids. Lipolysis in the rumen is a prerequisite for microbial hydrogenation (biohydrogenation) of unsaturated fatty acids. The degree to which biohydrogenation is "complete" affects the amount of EFA produced in the rumen, but also by the number of CLA and TVA. The determination of interactions between plant components and the biology of the rumen (lipolysis and biohydrogenation) is necessary for the directed improvement of the fatty acid composition of beef and other products of ruminant animals.

PUFA is contained in plant phospholipids. Phospholipids are present in large amounts in chloroplast membranes, and understanding the processes affecting the content of chloroplasts in the rumen can open additional opportunities for improving the composition of beef fatty acids.

For this purpose, 2 experiments A and B were performed: the rate of absorption, digestion and release of chloroplasts by the method of invitro was evaluated by invitro, and by invivo an evaluation of the preservation of PUFA on the way to the duodenum.

Experiment A. In vitro studies were conducted to better understand the fate of chloroplasts rich in PUFA in the rumen and, in particular, the processes taking place between the chloroplasts and the biotics of the rumen. Intact chloroplasts were obtained from spinach leaves (Spinaciaoleracea) using standard methods. The resulting chloroplasts were processed by the simplest Epidiniaspp. and Entodiniaspp. Intracellular and extracellular chlorophyll as well as fatty acid content in protozoa were monitored at time intervals of 0, 0.5, 1, 2, 4 and 8 hours. The intracellular location of autofluorescing chloroplast protozoa was assessed using fluorescence confocal microscopy.

A number of unforeseen technical problems hampered the success of these approaches in vitro. At the initial stage of the research, an attempt was made to purify the protozoa as possible from a larger number of intracellular chloroplasts, so that at the time 0 h they contained only a very low amount of intracellular chloroplast. First of all, fractionation of protozoa, and then anaerobic incubation, was used to control the time necessary for them to isolate intracellular chloroplasts. Control of density (density) of protozoa, vitality and the presence of intracellular chloroplast was carried out for 24 hours. The viability of protozoa was steadily reduced to several live cells after 6 hours, and microscopy showed that protozoa have many intracellular chloroplasts even after 24 hours. Following this, an attempt was made to replace intracellular chloroplasts rich in C18: 3n-3 with chloroplasts rich in C16: 3n-3, feeding corn after fractionation. This experiment again showed that the protozoa are not very active after 6 hours, so the removal of chloroplasts rich in C18: 3n-3 and the experiment itself must be done during this time, which is impossible. As a result, a decision was made to obtain protozoa from cows on straw fattening (low C18: 3n-3 and chlorophyll content) 1 week before the experiment, and use them directly after fractionation in experiments. The experiments were established in invitro with a ratio of 1: 100 (1 × 104 protozoa / ml: 1 × 106 chloroplasts / ml). According to the developed experiment, the amount of protozoa that could be extracted from the rumen fluid was limited to a density (density) of 1 × 104 protozoa / ml. Unfortunately, the use of this density, did not allow the detection of fatty acids and chlorophyll. Thus, due to problems of viability and density, it is concluded that control of ingestion / digestion / isolation by in vitro method is impossible.

Subsequently, it was decided to conduct the experiment by invivo method, 6 healthy animals of Kalmyk and Kazakh white-headed Zavolzhsky type (average live weight 250 kg) with fistulae of the rumen and duodenum were put on the fattening of straw and mixed fodder for 14 days before transferring to fresh grass , only for one day (morning feed only). Sampling of the feed was conducted daily and weekly combined to freeze, while grass samples were taken in the morning exclusively for the experiment (approximately 1 kg) and frozen. Selection of protozoa, plankton and attached bacteria was carried out 1 hour before the change in the diet and 2 and 6 hours after the change. The content of chlorophyll and fatty acids in each microbial fraction was analyzed and the fixed samples were monitored for the content of intracellular chloroplasts using confocal microscopy and transmission electron microscopy. The ration on the basis of straw and mixed fodder had more ADF, NDF, C18: 1n-9 and fewer WSC, fatty acids, C16: 0, C18: 2n-6 and C18: 3n-6 compared to the diet on fresh grass, which was predictable.

The obtained data on fatty acids showed that the protozoa were significantly enriched in C16: 0, C18: 0, C18: 2n-6, C18: 3n-6, C18: 1 trans-11, 2 hours after feeding with fresh grass, and the level each of these fatty acids began to decrease in part within 6 hours, although the C16: 0, C18: 0 and C18: 3n-3 intraprotosic concentrations remained significantly higher than the values obtained 1 hour prior to fresh grass feeding (Table 2).

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Table 2: Fatty acid content (mg / g^{-1}LM) at various time intervals before and after feeding with fresh grass

Fatty acid	Time			SED	P
	1	2	6		
18:3n-3	0.052a	0.604b	0.511b	0.062	<0.001
18:2n-6	0.955a	2.805b	1.586a	0.298	<0.001
18:2 cis-9, trans-11	0.316a	0.298a	0.334a	0.070	0.879
18:1 trans-11	1.166a	2.792c	1.681b	0.206	<0.001
18:0	5.728b	7.479c	4.516a	0.400	<0.001
16:0	5.281a	11.625c	8.201b	0.585	<0.001

The microscopic data showed that, 2 hours after feeding with fresh grass, the protozoa had significantly more intracellular chloroplasts, which remained high and 6 hours after feeding, so the fatty acid data correlated with the intraprotozoic chloroplast content. These data illustrate that the absorption of protozoa mainly of plant chloroplasts occurs rapidly and the intracellular level of chloroplasts is maintained for at least 6 hours. Thus, protozoa quickly become the main reservoir of chloroplasts, and then useful sources of PUFA.

Experiment B. Six healthy animals of Kalmyk and Kazakh white-headed Zawolzian type with fistula of the cicatrix and duodenum were selected for the in vivo experiment.

Animals were kept on a limited diet of fresh grass (a diet high in chloroplasts) and hay (a diet low in chloroplasts). The duodenal flow of C18: 2n-6 and C18: 3n-3 was determined by both diets. The biota of the rumen was collected and purified to quantify both the intracellular chlorophyll content and the standard for quantitative PCR (qPCR). The protozoal flow to the duodenum in both diets was quantified using qPCR. Knowing the chlorophyll content in protozoa and their course in the duodenum, the flow of intracellular chloroplast into the duodenum was assessed. The relationship between PUFA and protozoa on the way to the duodenum provides a proof of the connection with the intracellular chloroplast, and was carried out using the canonical correlation analysis.

Table 3: Daily intake and duodenal fluxes of dry matter (DM), organic matter (OM) and nitrogen (N) (g / d)

	Diet		SED	*P*
	S : C	PRG		
Dry matter (kg / day)	8.80	9.42	0.54	NS
Total nitrogen	191	207	11.8	NS
Water-soluble carbohydrates (WSC)	607	1488	52.2	<0.001
Neutral food fibers (NDF)	4136	4481	255	NS
Acidic dietary fiber (ADF)	2439	2571	149	NS
Accepted fatty acids	0.28	0.26	0.02	NS
C12:0	-	-	-	-
C14:0	0.75	1.10	0.10	0.007
C16:0	27.0	36.0	1.80	0.008
C16:1n-7	1.4	0.4	0.07	<0.001
C18:0	3.26	2.73	0.20	0.05
C18:1n-9	54.0	4.2	2.59	<0.001
C18:2n-6	50.7	35.0	3.21	0.008
C18:3n-3	15.1	153	3.71	<0.001
The duodenal flow	-	-	-	-
Dry matter (kg / day)	12.2	12.1	0.74	NS
OM (kg / day)	11.8	11.5	0.63	NS
Total nitrogen	125	169	31.5	NS
Protozoa	34.4	0.70	9.84	0.027

Six healthy animals of the Kalmyk and Kazakh white-headed Zawolgian type with fistulae of the rumen and duodenum were grown according to a two-stage variable fattening plan: straw: mixed feed (60:40, dry weight, S: C, low chloroplast content) or fresh grass (PRG, high the content of chloroplasts). After the 12th adaptation to the diet, samples of biotics of the rumen and duodenum were selected. The content of nitrogen

and fatty acids of biotics of the rumen and duodenum was evaluated and PCR analysis of 18 S rDNA protozoa was performed, which allowed calculating the protozoal nitrogen flux (Table 3).

Data on the content of fatty acids in protozoa and microscopic observations showed that the protozoa were enriched with C18: 3n-3 after feeding with fresh grass compared to the S: C diet due to an increase in the intracellular chloroplast content. However, the duodenal concentration of protozoa 18S rDNA after fattening by grass was low, indicating retention of the protozoa by the scar. The consequence of such cicatricial retention after fattening of fresh grass was that a small amount of C18: 3n-3 or any other fatty acid entered the duodenum compared to the values obtained after fattening straw: feed (table 4).

Table 4: Total fatty acids and protozoan-bound fatty acids of the duodenal flow in animals for fattening straw: feed (S: C) or fresh grass (PRG)

	The duodenal flow (g / day)				Protozoal flow (g / day)				Contribution*	
	S:C	PRG	SED	*P*	S:C	PRG	SED	*P*	S:C	PRG
C14:0	2.29	1.72	0.21	0.024	0.26	0.00	0.07	<0.001	11.4	0.33
C15:0	1.44	1.27	0.15	<0.001	0.32	0.00	0.19	<0.001	52.8	3.15
C16:0	24.9	25.5	1.30	0.008	5.36	0.16	3.91	0.114	21.5	0.63
C17:0	1.32	1.56	0.15	<0.001	0.13	0.00	0.09	<0.001	9.85	0.23
C18:0	88.6	102	9.77	0.006	8.76	0.26	7.66	0.193	9.89	0.25
C18:1 *trans-11*	5.20	24.0	2.34	<0.001	1.36	0.07	0.90	<0.001	26.2	0.29
C18:2n-6	10.2	2.21	0.33	<0.001	1.32	0.02	0.88	<0.001	12.9	0.09
C18:3n-3	1.66	3.06	0.23	<0.001	0.14	0.01	0.11	<0.001	8.43	0.33
cis-9, *trans-11* CLA	0.10	0.08	0.02	<0.001	0.08	0.00	0.134	<0.001	80.0	2.00
trans-10, *cis-12* CLA	0.00	0.06	0.01	<0.001	0.00	0.00	0.00	<0.001	50.0	0.00
Total amount of fatty acids	173	196	15.7	0.002	23.8	0.46	16.4	0.096	13.8	0.23

CONCLUSIONS

Thus, the study of the effect of feeding on the content in protozoa C18: 3n-3 showed that the next step is to increase the protozoal flow into the small intestine, while maintaining a stable density of scars. Achieve these results is possible when studying the "alpine factor", because animals on alpine meadows have reduced biohydrogenation processes, this may be associated with secondary plant metabolites, including PPO, saponins, tannins and catecholamines. These compounds can potentially influence the processes of lipolysis or biohydrogenation and improve the bovine fatty acid composition.

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