Methods of enrichment of the qualitative composition of a beef with polyunsaturated fatty acids in ecologically unstable agrarian territories

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Abstract. Beef is a natural carrier of beneficial n-3 PUFA (eicosapentaenoic acid (EPA, C20: 5n-3) and docosahexaenoic acid (DHA, C22: 6n-3), it is also an important source of healthy fats for human. The plant-based feeding strategies can be considered as the most appropriate and sustainable approach to increasing the content of n-3 PUFAs in beef. Taking into account the requirements for creating the pastures with high yields, productive longevity, a rapid achievement of pasture ripeness, resistance to grazing and trampling, as well as a high content of unsaturated fatty acids, a total of 3 types of herbs are selected: oat-grass + perennial sorghum + leymus. The plants were grown on the territory of the experimental pilot farm with a cut every 6 weeks and an annual fertilizer at the rate of 250 kg N per 1 hectare for 3 years. The collection of herbal material for the analysis of fatty acids was carried out in June and September 2014, 2015, and 2016. Our microscopic data showed that 2 hours after feeding on fresh grass, the protozoa had a significantly larger number of intracellular chloroplasts, which remained high and 6 hours after feeding. Therefore, data on fatty acids were correlated with the content of intraprotitzoal chloroplast. These data illustrate that the absorption by protozoa of mainly plant chloroplasts occurs rapidly. More than that, the intracellular level of chloroplasts is maintained for at least 6 hours. Thus, the protozoa quickly become the main reservoir of chloroplasts and useful sources of PUFAs.

1. Introduction
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. Relatively small differences in the structure of molecules make Omega-6 and Omega-3 acting in the human body in completely different ways. The “minus” of Omega-6 acid is a product of its metabolism. The Omega-6 metabolic by-products contribute to inflammation, blood clots, tumor growth.

The Omega-3 metabolism products have opposite effects. As soon as Omega-3 fats enter the body, they invade cells, affecting their structure and activity. Because of this, they have such a variety of useful properties. These acids improve the functioning of the heart, brain, eyes and joints; reduce the level of harmful cholesterol; are excellent antioxidants; prevent and improve eczema, allergies, asthma, Alzheimer’s disease, depression and nervous diseases, diabetes, hyperactivity of children, psoriasis,
Food quality is becoming increasingly important for consumers. Determining quality is becoming more difficult for meat. It covers 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 working environment. Gradually, consumers become aware of the relationship between nutrition and health, especially with regard to cancer and atherosclerosis. Knowledge of these relationships has increased consumer interest in the nutritional quality of food, so that this becomes a more important aspect of food quality.

Beef is considered 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 clouded due to 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 detrimental effects on animal health (BSE syndrome) [2]. Intramuscular fat is the most important fat storage depot for humans. The ratio of NLC, MFA, 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 fatty acids. As a rule, the ratio of polyunsaturated fatty acids to saturated for beef is small (about 0.1). The n-6: n-3 ratio for beef is 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. Also, beef contains CLA and, in particular, cis-9, trans-11 and trans-10, cis-12 CLA. The anticarcinogenic and anti-atherogenic effects of cis-9, trans-11, as well as the anticonvulsant effects of trans-10, cis-12 are reflected in the work of Belury M A [1, 2].

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: (a) an increased level of C18:3n-3 in the feed (and, therefore, in the animal); (b) reduction of the processes of biohydrogenation in the rumen.

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

2. Materials and Methods
Taking into account the requirements for creating the pastures with high yields, productive longevity, the rapid achievement of pasture ripeness, resistance to grazing and trampling, as well as a high content of unsaturated fatty acids, 3 types of herbs are selected: oat-grass + perennial sorghum + leymus. The plants were grown on the territory of the experimental pilot farm with a cut every 6 weeks and an annual fertilizer at the rate of 250 kg N per hectare for 3 years. The collection of herbal material for the analysis of fatty acids was carried out in June and September 2014, 2015, and 2016 [5], [6], [9]. The content of fatty acids was determined in 1 g of the lyophilized material using heneicosylic acid methyl ester (C21:0) as an internal standard (Sigma-Aldrich Co., St Louis, MO, USA) and one-step extraction by transesterification. Fatty acid methyl esters (FAME) were separated and quantified using gas chromatography. To evaluate the components of the variance, a linear mixed model was used. The analysis was performed using REML (Restricted maximum likelihood) analysis at Genstat (14th edition, VSN International Ltd, Hemel Hempstead, UK).

3. Results
In Table 1, for each of the three, mean and standard deviations are shown for the five essential fatty acids (C16:0, C18:0, C18:1n-9, C18:2n-6, and C18:3n-3). The number of C18:2n-6 and C18:3n-3 was the largest percentage of the total, and their number was the highest in the 2nd year, while three minor components tend to increase in each bevel, as a rule.
made that it is impossible to control the intake/digestion/treatment protozoa from straw and the experiment itself should be carried out during this time, which is impossible due to problems of viability and density, the conclusion is inevitable.

Thus, due to problems of viability and density, the conclusion is inevitable. However, the use of this density did not allow to detect fatty acids and chlorophyll. Thus, due to problems of viability and density, the conclusion is made that it is impossible to control the intake/digestion/excretion by the in vitro method.

Table 1. The content of fatty acids 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 |
|-------|--------|-------|-------|----------|----------|----------|----------|
|       | Oat-grass | 4.26±0.620 | 0.39±0.078 | 0.38±0.139 | 2.73±0.633 | 15.02±4.015 | 25.03±5.253 |
|       | Sorghum  | 3.92±0.693 | 0.38±0.103 | 0.46±0.270 | 2.65±0.846 | 12.90±3.708 | 22.95±8.706 |
|       | Leymus   | 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     | Oat-grass | 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     | Leymus   | 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     | Oat-grass | 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     | Leymus   | 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     | Oat-grass | 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     | Leymus   | 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 the five fatty acids are present in all samples. Analysis of the components of the dispersion using the REML model in all six cuttings demonstrated a significant influence of this factor.

Determining the interactions between plant components and rumen biotics (lipolysis and biohydrogenation) are necessary for the directional improvement of the fatty acid composition of beef and other products of ruminants. The PUFA content in plant phospholipids. Phospholipids are found in large quantities in chloroplast membranes. Understanding the processes that affect the chloroplast content in the rumen may open up additional possibilities for improving the composition of beef fatty acids.

For this purpose, the in vitro method was used to estimate the rate of absorption, digestion, and release of chloroplasts by the biotics of the rumen. In vitro studies were conducted to more clearly understand the fate of chloroplasts rich in PUFA in the rumen and, in particular, the processes between chloroplasts and rumen biotics. Intact chloroplasts were obtained from spinach leaves (Spinacia oleracea) using standard methods. The resulting chloroplasts were treated with protozoa Epidinia spp. and Entodinia spp. Intracellular and extracellular chlorophyll, as well as the content of fatty acids in protozoa were monitored with time intervals (0, 0.5, 1, 2, 4, and 8 h). The intracellular position of autofluorescent protozoa chloroplasts was evaluated using fluorescent confocal microscopy.

A number of unforeseen technical problems hindered the success of these in vitro approaches. At the initial stage of research, an attempt to clear the rumen’s protozoa as much as possible from a larger number of intracellular chloroplasts was made, so that at the time point 0h they only contained a very low amount of intracellular chloroplast. First of all, the protozoa fractionation was used, and then its anaerobic incubation was applied to control the time they needed to isolate intracellular chloroplasts. Protozoa density, viability and the presence of intracellular chloroplast were monitored for 24 hours. The protozoa viability steadily declined to several living cells after 6 hours, and microscopy showed that the protozoa had a lot of intracellular chloroplast 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 by feeding on corn after fractionation. This experiment again showed that the protozoa were not very active after 6 hours. Therefore, the removal of chloroplasts rich in C18:3n-3 and the experiment itself should be carried out during this time, which is impossible. As a result, the decision was made to receive protozoa from straw-fed cows (a low content of C18:3n-3 and chlorophyll) 1 week before the experiment and using them immediately after fractionation in experiments. The experiments were established in vitro 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 of 1×104 protozoa/ml. Unfortunately, the use of this density did not allow to detect fatty acids and chlorophyll. Thus, due to problems of viability and density, the conclusion is made that it is impossible to control the intake/digestion/excretion by the in vitro method.
Subsequently, the decision to conduct an in vivo experiment was made. A total of 6 healthy animals of the Kalmyk and Kazakh white-headed breed of the Trans-Volga type (with an average live weight of 250 kg) with fistulas of the rumen and duodenum were fed with straw for 14 days. The feed samples were taken daily and weekly pooled for freezing, while grass samples were taken in the morning exclusively for the experiment (approximately 1 kg) and frozen. The selection of protozoa, planktonic and attached bacteria was carried out 1 hour before the change in diet and 2 and 6 hours after the change. The content of chlorophyll and fatty acids in each microbial fraction was analyzed, and fixed samples were monitored for the content of intracellular chloroplasts using confocal microscopy and transmission electron microscopy. The diet based on straw and feed had more ADF, NDF, C18:1n-9 and less WSC, fatty acids, C16: 0, C18: 2n-6 and C18: 3n-6 compared to the ration on fresh grass, which was predictable.

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

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

4. Discussion

Based on the results obtained (Table 1), the conclusion can be drawn that seasonal and environmental factors have a significant impact on the phenotypic variation of the fatty acid content, which in turn would require an adjustment of the fatty acid composition of the feed during the growing process.

Reducing the degree of biogenization in the rumen can increase the processes of transformation of PUFA into muscle and adipose tissue. PUFAs are rapidly hydrogenated by rumen microbiotics, which leads to the formation of SFA (mainly 18:0), but also to the formation of intermediate compounds CLA and trans monoens (mainly trans-vaccenic acid TVA). This is one of the main reasons why the fats of ruminants are represented mainly by 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 SFA produced in the rumen, but also the amount of CLA and TVA. The microscopic data showed that 2 hours after feeding on fresh grass, the protozoa had a significantly larger number of intracellular chloroplasts, which remained high and 6 hours after feeding. Therefore, data on fatty acids were correlated with the content of intraprotozoal chloroplast.

These data illustrate that the absorption by protozoa of mainly 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 PUFAs.

5. Conclusion

The absorption of protozoa by mainly 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 PUFAs.
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