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Development of a Technology for the Directed Modification of Fatty Acid Composition of Beef in Grass-Fed Feeding

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Abstract**: *The article presents the results of the influence of studying the formation of a fatty acid profile in cattle meat using forage herbs rich in secondary metabolites (polyphenol oxidase, saponins, tannins and catecholamines) on lipolysis and biohydrogenation in animal rumen. The experiment planning matrix was performed using the Latin square method. The main factor studied was the effect of polyphenol oxidase on biohydrogenation processes in animals. Based on the studies, recommendations are made on the optimal composition of grassland in the pastures of the South of Russia in order to improve the fatty acid composition of beef.***

Keywords**: *technology, unsaturated fatty acids, biohydrogenation, forage grasses.***

I. INTRODUCTION

Grass-fed beef production strategy is the most effective and affordable approach to increase n-3 PUFA content in it. The transformation of PUFAs from feed to meat depends on two important processes:

1) an increase in the level of PUFA in the feed (and, therefore, in the fat of the animal);

2) reduction of the processes of their biohydrogenation (cleavage) in the rumen (Scollan N.D., Hocquette J.F., Nuernberg K., Dannenberger D., Richardson I. and Moloney A. (2006) Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Science 74: 17-33.).

The experience of researchers at the University of Aberystwyth shows that the diversity of forage grasses, the stage of growth and the method of conservation (silage and hay, degree of drying, etc.) affect the concentration of 18: 3n-3 (Dewhurst et al., 2006). The study of the degree of genetic variation, on the total content of lipids, or the composition of fatty acids of these lipids will allow us to select types of herbs with a high content of MUFA and PUFA. If this variability is possible, this can provide an opportunity to choose herbs with a high lipid content and a higher content of 18: 3n-3, which would increase the involvement of these fatty acids in the food chain.

Our previous studies have established that seasonal and environmental factors play a significant role in the phenotypic variation in the content of fatty acids, which in turn will require adjustment of the fatty acid composition of the feed during the growing process.

An in vitro experiment to study the rate of absorption, digestion and release of chloroplasts by rumen microflora of the rumen was not possible, because of the problems of viability and concentration of protozoa, it was concluded that control over the intake / digestion / excretion. An in vivo experiment similar in purpose showed that the absorption of plant chloroplasts by the protozoa is fast 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.

Assessment of the degree of preservation of PUFA before it enters the duodenum showed that this process is associated with a whole complex of factors and interactions that occur between the components of forage grasses and the microflora of animal rumen.

The obtained experimental data made it possible to establish the effect of feeding on the content in protozoa C18: 3n-3, respectively, the next step is to find ways to increase the protozoan flow into the small intestine, while maintaining a steady concentration of protozoa in the rumen. It is possible to achieve these results when studying the "alpine factor", because animals in alpine meadows have reduced biohydrogenation processes, which may be associated with secondary metabolites of plants, including polyphenol oxidase (PPO), saponins, tannins and catecholamines. These compounds can potentially affect lipolysis or biohydrogenation processes.

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II. MATERIALS AND METHODS

To assess the effect of PPO on the preservation of C18: 3n-3 and C18: 4n-4 in the rumen, the experiment was conducted on six healthy animals of the Kalmyk and Kazakh white-headed breeds of the Volga type with fistulas of the rumen and duodenum. The animals were fed silage from ryegrass (PRG, low PPO), clover (RC, medium PPO), and hedgehogs (CF, high PPO), each diet was supplemented with Echium spp (Psyllium bruise) seed oil, which is rich in PUFA (table 1).

Table - I: The fatty acid composition of the oil from the seeds of Echium spp

Fatty acid	The content in oil,%
Palmitic acid (16: 0)	6-8
Stearic acid (18: 0)	3-5
Oleic acid (18: 1)	15-19
Linoleic acid (18: 2n-6)	14-18
Gamma linoleic acid (18: 3n-6)	9-12
Alpha linolenic acid (18: 3n-3)	28-33
Stearidonic Acid (18: 4n-3)	10-14

The experiment was carried out by the method of Latin squares 3 × 3 (table 2).

Table – II: The matrix of the planning of the experiment by the method of Latin square 3 × 3

A	B	C
B	C	A
C	A	B

The degree of biohydrogenation and conservation of 18: 4n-3 with each diet were determined by the duodenal flow of fatty acids.

The effect of secondary plant compounds (PPO, saponins, tannins and catecholamines) on the biohydrogenation of n-3 PUFAs and the microbial ecosystem of the rumen was evaluated by in vitro method. Key protozoan species involved in biohydrogenation were evaluated using both denaturing gradient gel electrophoresis (DGGE) and qPCR. The relationships between fatty acid metabolism and protozoan species were established using canonical correlation analysis.

Silage was fed on the basis of a fixed rate of 15 g per 1 kg of live weight. The construction of the experiment was carried out on two 3 × 3 Latin squares, each period was 21 days, consisting of 14 days of adaptation to the diet, 4 days for collecting feces, 2 days for selecting biota of the duodenum and 1 day for selecting biota of the scar. All silos were well preserved with an average dry matter (DM) content of 34.4, 55.3 and 45.4% for clover, ryegrass and hedgehogs, respectively. PPO activity in silos was underestimated due to deactivation, but nevertheless differed between types of silage: it was higher in clover than ryegrass or hedgehog, 0.15, 0.05 and 0.08 μkat / g CB, respectively ($P < 0,05$). To assess the degree of oxidation, the value of the protein bound to phenol (mg / g CB) was used; therefore, the PPO activity was as expected: the highest in the hedgehog (15.9), the lowest in ryegrass (10.1) with an intermediate result in clover (12.2).

III. RESULTS AND DISCUSSION

Data on the level of consumption of dry matter, fatty acids, fatty acid flow and the degree of biohydrogenation are presented in table 3.

Table – III: Effect of grass silage with contrasting levels of PPO compared with clover silo on intake and metabolism of fatty acids in the rumen

	CF	PRG	RC	Error, P
Dry matter consumed (kg / day)	5.87	6.58	7.55	<0.001
Fatty Acids Taken (g / day)				
C18: 0 stearic acid	1.52	2.62	4.04	<0.001
C18: 2n-6 linoleic acid	16.6	21.0	32.5	<0.001
C18: 3n-3 alpha-linolenic acid	43.5	55.1	59.6	<0.001
Total Fatty Acids	93.4	119	144	<0.001
Duodenal flow (g / day)				
C18: 0 stearic acid	71.1	75.7	77.1	No data
C18: 1 trans	8.97a	12.6	13.5	<0.001
C18: 2 CLA	0.22a	0.26	0.79	<0.001
C18: 2n-6 linoleic acid	1.55	2.54	7.22	<0.001
C18: 3n-3 alpha-linolenic acid	2.43	4.44	13.5	<0.001
Total Fatty Acids	148	160	187	<0.01
Biohydrogenation (%)				
C18: 2n	90.6	87.9	77.9	<0.001
C18: 3n	94.4	92.1	77.6	<0.001

When assessing the migration of fatty acids into the duodenum, the degree of animal feed intake was taken into account. Migration C18: 0 was comparable with the highest flows of C18: 1 trans, CLA and C18 PUFAs when feeding hedgehog silos with an intermediate value for ryegrass and the lowest value for feeding clover. The biohydrogenation of C18 PUFA was significantly lower on hedgehog fattening compared to other silos, and in animals consuming clover higher than in animals consuming ryegrass.

Feeding the hedgehog led to lower biohydrogenation of C18 PUFA. Feeding with clover containing a lower level of PPO did not increase the level of duodenal flow of C18 PUFA in relation to the control consuming ryegrass with the lowest PPO level.

Thus, these results indicate that PPO grass has a limited potential for improving the fatty acid profiles of lipids in ruminant meat.

The effect of plant secondary compounds on lipolysis, biohydrogenation, and the rumen microbial ecosystem was evaluated in an in vitro culture group. The study consisted of two experiments:

Experiment A - the effect of catecholamines and saponins;

Experiment B - the effect of polyphenol oxidase and tannins.

Experiment A included 36 incubations, each experiment contained 1 g of SV lyophilized material (ryegrass) as the main nutrient with or without the addition of catecholamine (dopamine 100 µg / g) or saponin (1% deodorase).

Experiment B included 48 incubations and four plant species were used: Trifolium pratenses (meadow clover) versus Trifolium pratenses (meadow clover with the gene modification of the PPO gene) and Lotus Japonicus (low tannin Japanese lamb) against Lotus pedunculatus (high tannin lamb duck) Each type was incubated (in triplicate) in a rumen inoculum at 39 ° C with analysis at 4 time points (0, 2, 6, and 24 hours). Lots of feed crops were prepared by thorough homogenization. Samples were analyzed for total lipid and lipid fractions to determine the level of C18 PUFA lipolysis and biohydrogenation. Samples were also taken to profile microflora, using RNA as a marker. Evaluation of samples of 2 species of red clover, differing in the content of PPO, showed the absence of the effect of catecholamines. Saponins, bound phenols and tannins led to a significant decrease in lipolysis in the batch of cultures and had a direct effect on the reduction of biohydrogenation 18: 3n-3 (table 4).

Table – IV: C18: 3n-3 biohydrogenation in the presence and without saponins or catecholamines, (%)

Time	Control	Catecholamine	Saponin
2	43,9	40,2	24,9
6	45,7	52,2	32,2
24	56,9	55,5	50,8

Saponins and bound phenols appeared to have a greater effect during early fermentation (2-6 hours), while the effect of tannin was maintained throughout all incubations (table 5).

Table – V: C18: 3n-3 biohydrogenation with high (+) and low (-) tannin content (%)

Time	Tannin +	Tannin -
2	0,00	17,2
6	8,00	23,2
24	16,6	26,5

These results may be associated with the depletion of secondary plant metabolites or adaptation of the microbial population. Evaluation of the diversity of microorganisms revealed changes in the bacterial community after the addition of saponin, which are consistent with the data on the content of fatty acids in that they showed clustering of 2 and 6 hour samples separately for the control, but after 24 hours there was a similarity between the saponin and the control samples . Reverse transcription of RNA to cDNA for experiments with concentrated tannin showed limited bacterial activity, and therefore the extraction of cDNA with a sufficiently high concentration for the analysis of bacterial diversity was impossible. It is likely that the concentrated tannins present in L. Pedunculatus (lamb chafer) were very toxic to the microflora of the rumen.

Thus, based on the data obtained, it can be concluded that the addition of deodorase (yucca extract) when feeding forage grasses is most promising for a favorable change in the lipid metabolism of the scar.

IV. CONCLUSION

The study of the possibility of reducing the biohydrogenation processes due to the so-called "alpine factor" was examined from the perspective of the influence of secondary plant metabolites, including polyphenol oxidase (PPO), saponins, tannins and catecholamines. It has been established that feeding herbs with different levels of polyphenol oxidase content has limited potential for improving the fatty acid profile of lipids in ruminant meat due to the lack of a significant level of reduction in biohydrogenation processes. The best results were shown by feeding animals silage grass hedgehogs with a high level of polyphenol oxidase.

A significantly greater inhibition of biohydrogenation is ensured by the inclusion in the diet of forage grasses containing saponins (1% deodorase) and tannins (lamb lamb). However, the effectiveness of the presence of tannins is probably due to the fact that the concentrated tannins present in L. Pedunculatus (lamb's lamb) were very toxic to the microflora of the rumen.

Thus, taking into account the requirements for the selection of grasses on the rangelands of southern Russia, the following grasses can be recommended: ryegrass + hedgehog + clover + perennial sorghum. Feeding these types of herbs in a fresh and canned form, with the addition of deodorase as a source of saponins, is most promising for a favorable change in the lipid metabolism of the scar.

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