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Studying the mechanism of formation the beef' fatty acids composition by grass feeding.

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ABSTRACT

This article presents the results of the influence of secondary metabolites of forage grasses (polyphenol oxidase, saponins, tannins and catecholamines) on the processes of lipolysis and biohydrogenation in the rumen of animals. Based on the research, recommendations are given on the optimal composition of the herbage of the pastures of the South of Russia, to improve the fatty acid composition of beef.

Keywords: beef, unsaturated fatty acids, biohydrogenation, grass feeding.

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INTRODUCTION

The strategy for the production of beef with the use of herb fattening is the most effective and affordable approach for increasing the content of n-3 PUFAs in it. The transformation of PUFA from feed to meat depends on two important processes:

1) increase in the level of PUFA in feed (and, consequently, in animal fat);
2) decrease in the processes of their bio-hydrogenation (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 Meat Science 74: 17-33.) [1].

The experience of researchers at the University of Aberystwyth shows that the diversity of forage grasses, the growth stage and the method of conservation (silage and hay, the degree of withering, etc.) affect the concentration of 18: 3n-3 (Dewhurst et al., 2006). Investigation of the degree of genetic variability, the total lipid content, or the composition of fatty acids of these lipids will allow selection of herbs with elevated levels of MUFA and PUFA. If this variability is possible, then it can provide a choice of 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 [6-12].

Previous studies have allowed us to establish that seasonal and environmental factors play a significant role in the phenotypic variation in fatty acid content, which in turn will require adjusting the fatty acid composition of the feed in the growing process [2-5].

An experiment to study the rate of uptake, digestion and isolation of chloroplasts by rumen microflora was in vitro impossible, because of the problems of viability and concentration of protozoa, it was concluded that control of intake / digestion / secretion. A similar in purpose experiment in vivo showed that the absorption of the simplest scar 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.

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

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

MATERIAL AND METHODS

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

Table 1: Fatty acid composition of seed oil Echium spp

Fatty acid	Oil content, %
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 realized using the method of Latin squares 3 × 3 (Table 2).

Table 2: Matrix of experiment planning using the Latin square method 3×3

A	B	C
B	C	A
C	A	B

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

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 in vitro. The key types of protozoa involved in biohydrogenation were evaluated using both denaturing gradient gel electrophoresis (DGGE) and qPCR. Interrelations between the metabolism of fatty acids and species of protozoa 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 experiment was built on two Latin squares of 3 × 3, each period was 21 days, consisting of 14 days of adaptation to the diet, 4 days for collection of feces, 2 days for selection of biotics of the duodenum and 1 day for selection of biotics of the rumen. All the silos were well preserved with an average dry matter content of 34.4, 55.3 and 45.4% for clover, ryegrass and Dactylis, respectively. The activity of PPO in silos was understated due to deactivation, but nonetheless varied between silage species: higher in clover than ryegrass or Dactylis, 0.15, 0.05 and 0.08 µcat / g of CB, respectively (P <0 , 05). To estimate the degree of oxidation, the value of protein associated with phenol (mg / g CB) was used, hence the activity of PPO, as expected: the highest in Dactylis (15.9), the lowest in ryegrass (10.1) with an intermediate result in clover (12.2).

RESULTS AND DISCUSSION

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

Table 3: Effect of grass silage with contrasting levels of PPO in comparison with clover silo on reception and metabolism of fatty acids in rumen

	CF	PRG	RC	Error, P
Consumed dry matter (kg / day)	5.87	6.58	7.55	<0.001
Received fatty acids (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 acid content	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 acid content	148	160	187	<0.01
Biohydrogenation (%)				
C18: 2n-6 linoleic acid	90.6	87.9	77.9	<0.001
C18: 3n-3 alpha-linolenic acid	94.4	92.1	77.6	<0.001

When assessing the migration of fatty acids to the duodenum, the degree of feed intake in animals was taken into account. The C18: 0 migration was comparable to the largest C18: 1 trans, CLA and C18 PUFAs when fed with Dactylis silo with an intermediate value in ryegrass and the lowest value for clover feeding. The biohydrogenation of C18 PUFAs was significantly lower in the fattening of Dactylis than in other silos, and in animals consuming clover it was higher than in animals consuming ryegrass.

Feeding Dactylis resulted in a lower biohydrogenation of C18 PUFA. Feeding clover containing a less high level of PPO did not increase the level of duodenal flow of C18 PUFA relative to control consuming ryegrass with the lowest level of PPO.

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

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

Experiment A - effect of catecholamines and saponins;
Experiment B - effect of polyphenol oxidase and tannins.

Experiment A consisted of 36 incubations, each experiment containing 1 g of CB 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 versus Trifolium pratenses (Lotus clover with gene modification of the PPO gene) and Lotus Japonicus (Japanese tadpole with low tannin content) versus Lotus pedunculatus (a tall plumage with high tannin content). Each type was incubated (in triplicates) in a rumen inoculum at 39 ° C with analysis at 4 time points (0, 2, 6 and 24 h). Fodder crops were prepared by careful homogenization. Samples were analyzed for total lipid and lipid fractions to determine the level of lipolysis and biohydrogenation of C18 PUFAs. Samples were also selected for profiling the microflora using RNA as a marker. Evaluation of samples of 2 species of red clover, differing in content of PPO, showed no effect from catecholamines. Saponins, bound phenols and tannins led to a significant reduction in lipolysis in the batch of cultures and had a direct effect on a decrease in the 18: 3n-3 biohydrogenation (Table 4).

Table 4: Biohydrogenation of C18: 3n-3 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), whereas the effect of tannin was maintained throughout all incubations (Table 5).

Table 5: Biohydrogenation of C18: 3n-3 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 the adaptation of a microbial population. Assessment 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 the clustering of 2 and 6 hour samples separately to the control, but after 24 h there was a similarity between saponin and control samples . Reverse transcription of RNA to cDNA for experiments with concentrated tannin showed limited bacterial activity, and therefore extraction of cDNA with a sufficiently high concentration for bacterial diversity analysis was impossible. It is likely that the concentrated tannins present in L. Pedunculatus (lapwing drowned), were very toxic to the microflora of the rumen.

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

CONCLUSION

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

Significantly large inhibition of biohydrogenation provides for the inclusion in the diet of forage grasses containing saponins (1% deodorases) and tannins (lapwing drowned). However, the efficacy of the presence of tannins is probably due to the fact that the concentrated tannins present in L. Pedunculatus (lapwen drowned) were very toxic to the microflora of the rumen.

Thus, taking into account the requirements for selection of grasses in pasture lands of the South of Russia, the following grass can be recommended: ryegrass + Dactylis + clover + perennial sorghum. Feeding these herbs in 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 rumen.

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