Comparative study of photosynthetic pigments in the leaves of forage plants of the cryolithozone of Yakutia

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Abstract. Thin-layer chromatography and spectrophotometry were used to determine the content of photosynthetic pigments (chlorophylls a and b, carotenoids) in green leaves of forage plants. It was found that the content of carotenoids was high for forage grasses with a high content of green pigments. It was revealed that β-carotene plays the most important role since it is a precursor of vitamin A and lutein, which has a high antioxidant (protective) property. According to modern concepts, lutein and β-carotene actively function in various organs of herbivores. They play a special role in the functioning of the mammalian visual apparatus, protecting this organ from the light factor. In addition, lutein is one of the physiologically active substances that make up cow's milk.

1. Introduction

Green leaves of higher plants contain molecules of photosynthetic pigments: chlorophylls a, b and carotenoids, which are isoprenoid C₄₀-polyenes with conjugated double bonds that have typical absorption spectra in the ultraviolet region. Chlorophylls and carotenoids are some of the most important chemical compounds in plants because they affect the rate of photosynthesis and the production of plant biomass [1]. Chlorophyll plays a key role in the processes of biosynthesis since it is instrumental in the conversion of light energy into the energy of chemical bonds in the process of photosynthesis [2]. It was found that chlorophyll pigments determine the intensity of plant color. The pigment content also affects the chemical composition of plants and is used in various statistical correlations related to their physiology and phytochemistry [3]. The content of photosynthetic pigments in plants is also considered an indicator of their response to the environment, weather and anthropogenic conditions [4]. Besides chlorophylls, carotenoids are another most common group of plant pigments. Carotenoids are secondary plant metabolites, which are divided into two groups: hydrocarbon carotenes, such as β-carotene, and xanthophylls, i.e. oxygenated derivatives of carotenes. It is a group of lipophilic compounds that range in color from yellow to orange and red. Carotenoids are auxiliary pigments in photosynthesis, they transfer absorbed energy to chlorophyll with an efficiency of 15–90%, and protect chlorophyll from the excessive light intensity. β-Carotene (provitamin A) is a source of vitamin A for humans and animals [5].

Forage production in the North of Russia, including the vast territory of Yakutia (area of 3.1 mln. km²), faces extreme natural and climatic factors [6]. These include a short growing season, extremely low air temperatures in winter (up to -60 °C), high temperatures in summer (up to +40 °C), and the presence of permafrost. During a short growing season, plants are exposed to heavy solar radiation,
moisture deficit, and short-term frosts on the soil surface in early summer and autumn. Many forage plants growing in the cryolithic zone of Yakutia play an important role as a forage base for herbivores, whose meat and milk form the basis of the traditional diet of local residents.

In Yakutia, natural forage lands (meadows and pastures) form the basis of the forage base. The specificity of the seasonal growth and development of the bulk of herbaceous vegetation in the permafrost zone is that its intensive growth occurs in the first half of summer.

This work aims at a comparative study of the composition and content of photosynthetic pigments in the leaves of some forage plants of Yakutia.

2. Materials and Methods

The objects of the study were leaf samples of forage plants: meadow fescue (*Festuca ovina* L.), Siberian bluegrass (*Poa sibirica* Roshev.), Langsdorf reed grass (*Calamagrostis langsdorffii* (Link) Trin.), Alpine foxtail (*Alopecurus alpinus* Smith). The plants were collected over the terrace of the river valley Lena, Central Yakutia (62°15′ N, 129°37′ E). Experiments with cryo-feed (seed oats) were carried out in field plots in the conditions of Central Yakutia (environs of Yakutsk, 62° N, 130° E). The annual cereal oats (*Avena sativa* L., variety Nyurbinsky) were sown at two times: standard (early) on May 31 and experimental (late) on July 15.

Pigments were extracted from green leaves of the fresh plant material using 100% acetone at 8–10 °C in low light. The homogenate was centrifuged for 20 min at 8000 g at 4 °C. The content of chlorophylls (a+b) and carotenoids in the supernatant was determined spectrophotometrically using the Agilent 8453 E spectrophotometer (Agilent Technologies Deutschland GmbH, Germany) by recording the optical density at wavelengths of 662, 644, and 470 nm.

Separation of individual carotenoids was carried out using thin-layer chromatography (TLC) in accordance with the modified method [7] after extraction of lyophilisates by 100% acetone.

The pigments were determined in 3 biological and two analytical replicates. The content of pigments was determined on a dry weight and expressed in mg/g for chlorophylls and μg/g for carotenoids. The dry weight of the plant material was determined by drying the samples to constant weight in a drying cabinet at 100 °C.

3. Results and discussion

![Graph showing the content of chlorophylls a and b in the leaves of forage plants of the cryolithozone of Yakutia.](image)

**Fig. 1.** The content of chlorophylls a and b in the leaves of forage plants of the cryolithozone of Yakutia.
Fig. 2. The content of carotenoids in the leaves of some forage plants of the cryolithozone of Yakutia.

Analysis of the content of photosynthetic pigments using high-performance thin-layer chromatography in forage grasses of the Republic of Sakha (Yakutia), which territory lies entirely on permafrost, showed the presence of chlorophylls a and b, as well as individual yellow pigments (carotenoids) such as neoxanthin, violaxanthin, lutein, etc. β-carotene. Fig. 1 shows that the level of chlorophyll a content is 2.5 times higher than the level of chlorophyll b. The highest content of the fund of green pigments (chlorophylls a, b) was found in the leaves of *Alopecurus alpinus*. The data obtained showed that the content of green pigments in all studied forage grasses in terms of food value was close to the content of chlorophylls found in the leaves of *Alopecurus alpinus*. In terms of the content of photosynthetic pigments present in forage grasses, the level of green pigments in cryo-feed from *Avena sativa* differs significantly. The content of photosynthetic pigments in the green leaves of *Avena sativa*, frozen by the natural climatic cold of the cryolithozone of Yakutia during the autumn-winter period, is 1.5–2 times higher than in all other forage grasses. This cryo-feed from *Avena sativa* has a high nutritional value, as it is rich in the content of photosynthetic pigments, especially β-carotene (provitamin A) and lutein.

The samples of forage grasses of hay, in addition to green pigments, also contained the yellow pigments - carotenoids. It was found that the content of carotenoids was high in forage grasses with a high content of chlorophylls. Thus, it was shown that the leaves of the studied forage plant species of Central Yakutia maintained a high level of neoxanthin, violaxanthin, lutein, and β-carotene. It should be noted that of the above-mentioned carotenoids, the most important role have β-carotene, since it is a precursor of vitamin A, and lutein, which has a high antioxidant (protective) property [8]. These substances are not synthesized in the body of animals and humans [9]. According to modern concepts, lutein and β-carotene actively function in various organs of herbivores [10-2]. They play a special role in the functioning of the mammalian visual apparatus, protecting this organ from the light factor. In addition, lutein is one of the physiologically active substances in cow's milk [13]. For example, this pigment is found in large quantities in cow colostrum, which is why its color in the first 7-10 days has a yellow-orange color. During this period, the newborn calf feeds by colostrum rich in lutein and β-carotene, which helps it to adapt to an unfavorable external environment [14–15].

4. Conclusions
High-performance thin-layer chromatography was used to isolate chlorophylls a and b, carotenoids from the leaves of forage plants of the cryolithozone of Yakutia. The levels of photosynthetic pigments in green leaves of forage grasses were determined using spectrophotometry. It was found
that cryo-feed from *Avena sativa* has a higher content of photosynthetic pigments (by 1.5-2 times), which prolongs the functioning of the photosynthetic apparatus at low positive temperatures. It was revealed that the leaves of *Alopecurus alpinus* have a higher nutritional value in terms of the content of green pigments than other types of forage grasses growing in natural conditions in the cryolithozone of Yakutia. In general, based on the results obtained, the authors concluded that the level of photosynthetic pigments in the leaves of forage grasses plays one of the key roles in the regulation of the vital activity of herbivores in the Far North.

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