Fatty Acids and Sterols in *Avena Sativa* L. in Yakutia Permafrost

V V Nokhsorov¹*, N K Chirikova¹ and D N Olenikov²

¹ North-Eastern Federal University, 58 Belinsky str., Yakutsk 677000 Russia
² The Institute of General and Experimental Biology, 6 Sakhyanovoy str., Ulan-Ude 670047 Russia

E-mail: vv.nokhsorov@s-vfu.ru

**Abstract.** The authors of this study examined the seasonal changes in qualitative and quantitative contents of fatty acids, total lipids, phospholipids, and sterols of the common oat (*Avena sativa* L.) that were sown at different times, grow in Yakutia permafrost and undergo inter-season cold hardening. During the first phase of cold hardening (fall period), the total lipid content in oat biomass grows considerably. Moreover, the content of phospholipids and polyunsaturated fatty acids increased, compared them to other summer fodder plants. In fall, the amount of phosphatidylcholine in oats increased 3.9 times compared to summer. Alpha-linolenic polyunsaturated acid was predominant in summer and fall (14.8 mg/g and 28.4 mg/g in dry mass, respectively). The inter-season (summer-fall) dominance of polyunsaturated fatty acids at low above-zero temperatures allows keeping the membrane fluidity at a necessary level. The total sterol content in *A. sativa* biomass was 0.8%. The authors concluded that fall vegetation is the primary source of pre-winter fattening of the Far North herbivorous animals.

**Keywords:** *Avena sativa* · Biomass · Fatty acids · Sterols · Gas-liquid chromatography · Permafrost · Cold hardening

1. Introduction

Fodder production in the Far North, especially on Yakutia’s vast territory (3.1 mln. sq. km), is challenging due to the extreme environment [7]. The main characteristics of northern ecosystems are subarctic climate, ubiquitous permafrost, and solar radiation budget dependence. These factors affect the dynamics of plant development and growth. During the short growth period, the plants are subjected to high solar radiation, moisture deficit, and cold snaps at the beginning of summer and fall. Native plants adapted to the environment by shortening all stages of ontogenesis [5]. Permafrost ecosystems host more than 2000 species of vascular plants [10]. Some of them are crucial to animal fodder, the meat and milk of which, in turn, form the local population’s staple diet.

The bulk of Yakutia’s permafrost grass grows in the first half of summer. However, the northern plant communities are often flooded, grazed on, or mowed for hay at this time. After being wounded, the plants go dormant, freezing in winter, and unable to produce and spread the seeds. The bulk of autumn grassy plants is formed by sedges, cotton grasses, horsetails, and grain plants. The latter keep up to 80% of their green mass under the snow, especially if grown on last-year decayed grass [6]. Another way to get naturally hardened green biomass is to plant annual grasses late. In ontogenesis and cold hardening, vascular plants store cold-shock proteins, soluble carbohydrates, unsaturated fatty acids (lipids), fat-soluble substances, carotenoids (lutein, zeaxanthin), vitamins, and other secondary
metabolites. Humans and herbivores use lipid-rich plants to provide their bodies with energy and essential fatty acids (FA) [8]. Steroid glycosides are also important due to a wide range of their biological effects.

This study aims to examine the seasonal dynamics of fatty acids, total lipids, sterols in *Avena sativa* L., which is a summer and autumn fodder plant, in Yakutia’s permafrost environment.

### 2. Materials and Methods

Common oats (*Avena sativa* L., “Nyubrinsky” variety) were sown two times: standard early sowing in 31.05.2014, and experimental late sowing in 15.07.2014. The experiments were conducted on field plots in Central Yakutia (near Yakutsk, 62°N, 130°E). Samples from the control group and experimental plants were taken 4–5 times during the growing season, depending on growth and cold hardening phases.

Total lipid analysis samples were taken from 07.07.14 to 25.07.14 for the first group, and from 25.07.14 to 30.09.14 for the second group. Fatty-acid and sterol analysis samples were taken at 07.07.14 for the first group, and at 25.09.14 for the second group.

The samples were immediately flash-frozen using liquid nitrogen.

Total lipids were extracted using chloroform-methanol 2:1 mixture. The total lipid amount was measured using gravimetric analysis [1]. To separate phospholipids, the authors employed two-dimensional thin-layer chromatography. Phospholipid amount was determined by inorganic phosphorus content using spectrophotometry [2]. Fatty acid methyl esters (FAME) were determined by gas-liquid chromatography using 5973/6890N MSD/DS gas chromatographer–mass spectrometer (Agilent Technologies, USA).

The tables and figures show the averages of three to six biological replications and their standard errors. Statistical data on the experiment was analyzed using Analysis ToolPak of Microsoft Office Excel 2010. The statistical significance of mean values was evaluated using t-test (at P<0.05). Shapiro–Wilk test was used to determine distribution normality.

### 3. Results

The absolute content of total lipids in oat leaves was determined by the gravimetric method in 1 gr of dry mass. The qualitative range of total lipids is one of the main characteristics of plant chemical composition. Total lipids in oat leaves contain other hydrophobic compounds, like hydrocarbons and sterols.

The total lipid amount in early and late oat leaves increased during their growth and development. When the daily average temperature decreased from 9 °C to 1 °C and -3 °C (first and second cold hardenings), the total lipid amount increased 1.2 times compared to the control group of the same sowing time.

After the first hardening in the pruned autumn grasses, total lipids were 1.3 times higher than in the comparable control group.

In common oat (*Avena sativa*), we discovered the following phospholipids (PL):

- phosphatidylcholine (PC);
- phosphatidylinositol (PI);
- phosphatidylethanolamine (PE);
- phosphatidylglycerol (PG);
- phosphatidic acid (PA);
- diphosphatidylglycerol (DPG).

Among these, PC and PG were dominant (see Figure 1).

In fall, during the first hardening (average temperature of -3°C), PC contents increased 3.9 times if compared to summer-sowed oats.
Table 1. Seasonal dynamics of total lipid content in *Avena sativa* leaves of different sowing periods (2014).

| Sample date | t, °С* | Development (hardening) stage | Contents, mg/g dry mass |
|-------------|--------|-------------------------------|------------------------|
|             | minimal | average                       |                        |
| Control group - first sowing period (31.05) |
| 07.07       | 14      | 18                            | leaf-tube formation    | 98.9 ± 6.9               |
| 11.07       | 13      | 21                            | leaf-tube formation    | 113.5 ± 7.2              |
| 14.07       | 17      | 21                            | ear emergence          | 126.7 ± 9.3              |
| 25.07       | 16      | 21                            | ripening               | 129.3 ± 8.9              |
|             |         |                               |                        |                        |
| Experimental group - second sowing period (15.07) |
| 25.07       | 16      | 21                            | sprouting              | 72.5 ± 5.3               |
| 11.09       | 1       | 9                             | leaf-tube formation, ear emergence | 128.2 ± 8.3 |
| 25.09       | -4      | 1                             | first hardening        | 153.9 ± 10.3             |
| 30.09       | -7      | -3                            | second hardening       | 155.1 ± 9.9              |

* – air temperature.

Source: Compiled by the authors.

Compared to the control groups, the content of total lipids and membrane phospholipids, especially PC, increased considerably in hardened plants.

Figure 1. Absolute content of phospholipids in summer (25.07.14) and fall (03.10.14) *Avena sativa* plants (mg/g dry mass). Source: Compiled by the authors.

The fatty acid profile contained 13 fatty acids (see table 2). The absolute content of FAME in fall *A. sativa* was 2.1 times higher (49.6 mg/g dry mass) than in the summer one. Alpha-linolenic acid C18:3(n-3) was predominant among the polyunsaturated fatty acids. Among the saturated acids, palmitic acid C16:0 predominated.

Comparing the main fatty acid classes in the summer-fall period has shown that the unsaturated FA content increased in fall, at -0.6°C average temperature. The complete range of Alpha-linolenic acid C18:3(n-3) was 14.8 mg/g in summer, and 28.4 mg/g in fall. Polyunsaturated fatty acids, like linoleic C18:2(n-6) and alpha-linolenic C18:3(n-3), help maintain cell membrane fluidity during hypothermic periods (Los, 2014). Among the diene-containing fatty acids, linoleic C18:2(n-6) was dominant. In fall oats, its contents were 2.2 times higher than in the control group. During fall hardening, the absolute content of unsaturated FA was three times higher than that of the saturated ones.
The sterols in the above-ground part of A. sativa mainly include avenacosides A and B, and their deglucosylated derivatives [3]. They were present during the whole growth period.

Table 2. Seasonal dynamics of fatty acid contents in Avena sativa leaves of different sowing periods (2014).

| Fatty acids | Summer period (07.07.14) | Fall period (25.09.14) |
|-------------|--------------------------|-------------------------|
|             | t, °C* = 10.9 °C         | t, °C* = -0.6 °C        |
| C12:0       | 0.0 ± 0.0                | 0.0 ± 0.0               |
| C14:0       | 0.1 ± 0.0                | 0.1 ± 0.0               |
| C15:0       | 0.0 ± 0.0                | 0.0 ± 0.0               |
| C16:0       | 3.4 ± 0.6                | 15.3 ± 0.9              |
| C16:1(n-9)  | 0.1 ± 0.0                | 0.8 ± 0.1               |
| C16:1(n-7)  | 0.6 ± 0.1                | 2.6 ± 0.1               |
| C16:1(n-5)  | -                        | -                       |
| C17:0       | 0.0 ± 0.0                | 0.0 ± 0.0               |
| C18:0       | 0.1 ± 0.0                | 0.1 ± 0.0               |
| C18:1(n-9)  | 0.4 ± 0.0                | 1.0 ± 0.3               |
| C18:1(n-7)  | 0.1 ± 0.0                | 0.2 ± 0.0               |
| C18:2(n-6)  | 2.3 ± 0.3                | 5.1 ± 0.3               |
| C18:3(n-3)  | 14.8 ± 2.2               | 28.4 ± 1.8              |
| C20:0       | 0.0 ± 0.0                | 0.1 ± 0.0               |
| C20:1(n-11)| -                        | -                       |
| C22:0       | 0.1 ± 0.0                | 0.2 ± 0.0               |
| ∑           | 22.3 ± 3.2               | 100.0                   |
|  ∑ of saturated | 4.0 ± 0.5          | 11.1 ± 1.8              |
|  ∑ of unsaturated | 18.3 ± 2.7        | 35.8 ± 2.4              |
| IN          | -                        | 2.2                     |
| SDR         | -                        | 0.5                     |
| ODR         | -                        | 0.9                     |
| LDR         | -                        | 0.8                     |

* – air temperature.

Source: Compiled by the authors.

The total sterol content in A. sativa biomass was 0.8%. To determine their qualitative composition, the authors used the densitometric method of High-Efficiency Thin Layer Chromatography (HETLC). As a result, the existence of four compounds A-D was established. To identify the compounds, the authors used chromatographic mobility data and mass-spectra (ES(+)-MC) with authentic terpene samples (see Figure 2). The compounds were identified as avenacoside A (B), avenacoside B (A), 26-desglucoavenacoside A (D), and 26-desglucoavenacoside B (C). The ratio of these compounds was 22:34:10:29 (B:A:D:C).
**Figure 2.** HETLC-densitometrogram of A. sativa above-ground part (SiO$_2$; CHCl$_3$-MeOH-H$_2$O in 14:7:1.1 ratio; detected using anise aldehyde/H$_2$SO$_4$, ES(+)-MS spectrum of zone A (avenacoside B) and structure formulae of tri-terpene glycosides. A – avenacoside B, B – Avenacoside A, C – 26-desglucoavenacoside B, D – 26-desglucoavenacoside A. Source: Compiled by the authors.

4. Discussion

Evolutionary-formed adaptation complexes are transforming due to global climate changes. In recent years, the molecular and genetic studies have revealed that all herbaceous plants respond to cold shock by activating signal systems and target-genes. Environmental temperature reduction negatively affects the cell membrane fluidity in plants, which leads to the up-regulation of FA-desaturation genes [4]. Increasing the unsaturated FA ratio helps stabilize the membrane fluidity in plants. Our data on the increase of unsaturated FAs in oat leaves sown in autumn is consistent with data on other plants [9]. The increase in the content of total lipids, phospholipids, and whole FA suggests that these substances, along with sugars, proteins, antioxidants, and carotenoids (β-carotene, pigments of the violaxanthin cycle), are involved in the cold adaptation of Yakutia’s permafrost fall plants [9]. Same as A. sativa, other herbaceous plants (Bromopsis inermis, Elytrigia repens, Equisetum variegatum, and Equisetum scirpoides) stored more FAs in vegetative organs in cold adaptation periods [5].

5. Conclusion

In the Far North environment, fall fodder plants, rich in nutrition and bioactive substances, play a significant role in the thermogenesis of cold-adapted mammals. Thus, the high amount of total lipids, phospholipids (PC and PG), and polyunsaturated FAs (C18:2 (n-6) and C18:3(n-3)) in fall-sown common oats and naturally-frozen plants are crucial to the pre-winter fattening of aboriginal animals.

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