The influence of colored wheat Konini feeding on antioxidant activity parameters in rats

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Abstract: The influence of feeding of purple wheat KONINI (14.01 mg/g of anthocyanins) on antioxidant activity measured in the liver tissue of rats was determined. 64 male rats of Wistar Albino strain were used in the experiment. Experimental group was fed by ration which content 100% of purple wheat KONINI. Control group was fed by ration with 100% of common wheat. Antioxidant activity measured by different methods DPPH, FR, FRAP and ABTS were higher in experimental group (10.06 ± 0.26; 543.88 ± 23.61; 39.56 ± 1.01; 458.76 ± 3.58, respectively) than in control group (9.20 ± 0.31; 482.46 ± 15.56; 36.73 ± 0.72; 445.38 ± 3.13, respectively. Differences were significant ($P<0.05$).

*Key-Words:*purple wheat Konini, rats, antioxidant activity

Introduction

Wheat is one of the most widely grown grain crops in the world, and durum and bread wheat represent staple foods for human nutrition, especially in the Mediterranean area [1]. The anthocyanins also have therapeutic roles for humans, against tissue inflammation, capillary fragility, cardiovascular disease, cancer, hyperglycaemia, and oxidative liver damage [2]. Unlike the carotenoids, for which plant breeding is mainly in response to the needs of the pasta producers, the anthocyanins represent a new target for genetic improvement due to consumer demand for foods with greater health benefits [1]. Several investigations of the anthocyanin content in coloured grain of spring wheat genotypes under spring-sown conditions were performed [3, 4, 5, 6, 7]. The purple colour is caused by anthocyanins accumulated in the pericarp [8]. It is well known that herbal anthocyanins are functioning as antioxidants and, in addition, they have anti-bacterial and anti-carcinogenic effects as well [9].
It is necessary to monitor the influence of anthocyanins in the food to human and animal organism.

Material and Methods

64 selected male laboratory rats of Wistar Albino strain at the age of 6 weeks were divided into 2 groups. Rats were marked by shaving of specific areas and keep in plastic bags with 8 rats per one. Average live weight of rats was 243 g. Room temperature (20 - 23°C) and humidity (50 - 60%) were controlled. Lighting system was 16 hours light and 8 hours dark. The experimental group (N = 32) was fed with dried granules of 100% wheat meal from purple wheat KONINI with content of Crude protein (CP) 16.8%. The control group (N = 32) was fed with dried granules of 100% wheat meal from common wheat in which content of CP was increased using wheat gluten to the identical in KONINI. Body weight gain was followed in two-day intervals and feed consumption was followed daily. At the age of 69 days they were killed using diethylether and liver tissue was taken for determination of antioxidant activity measured by different methods: DPPH (free radical 2,2-diphenyl-1-picrylhydrazyl), FR (Free Radicals*)*, FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

Determination of antioxidant capacity using the DPPH• test

The DPPH• test is based on the ability of the stable 2,2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors. The DPPH• radical displays an intense UV-VIS absorption spectrum. In this test, a solution of radical is decolourized after reduction with an antioxidant (AH) or a radical (R•) in accordance with the following scheme: DPPH• + AH → DPPH•-H + A•, DPPH• + R• → DPPH•-R [10].

Determination of antioxidant activity by Free Radicals method

This method is based on ability of chlorophyllin (the sodium-copper salt of chlorophyll) to accept and donate electrons with a stable change of maximum absorption. This effect is conditioned by an alkaline environment and the addition of catalyst [11].

Determination of antioxidant activity by FRAP method

By the [12]: "The FRAP method (Ferric Reducing Antioxidant Power) is based on the reduction of complexes of 2,4,6-tripyridyl-s-triazine (TPTZ) with ferric chloride hexahydrate (FeCl3·6H2O), which are almost colourless, and eventually slightly brownish. This chemical forms blue ferrous complexes after its reduction. The method has its limitations, especially in measurements under non-physiological pH values (3.6). In addition, this method is not able to detect slowly reactive polyphenolic compounds and thiols."

Determination of antioxidant activity by ABTS test

The ABTS radical method is one of the most used assays for the determination of the concentration of free radicals. It is based on the neutralization of a radical-cation arising from the one-electron oxidation of the synthetic chromophore 2.2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•): ABTS• – e- ABTS•+. This reaction is monitored spectrophotometrically by the change of the absorption spectrum. Results obtained using this method are usually recalculated to Trolox® concentration and are described as "Trolox® Equivalent Antioxidant Capacity" (TEAC). For chemically pure compounds, TEAC is defined as the micromolar concentration of Trolox® equivalents demonstrating the same antioxidant activity as a tested compound (at 1 mmol·L-1concentration) [13].

The data were processed through the use of MICROSOFT EXCEL (USA) and STATISTICA.CZ Version 10.0 (Czech Republic).

Results and Discussion

The results of antioxidant activity measured by DPPH, FR, FRAP and ABTS methods are in Table 1. Significant differences *(P<0.05)* between experimental group Konini and Control group was found using all method. DPPH Inhibition of 10.06 ± 0.26% on experimental group and inhibition of 9.20 ± 0.31% was measured on control group. In experiment [14] aimed at effect on inclusion of yellow Citrus wheat on antioxidant activity higher values were measured however these differences were insignificant.

Table 1 Antioxidant activity measured by different methods in the liver tissue of experimental rats

Methods	*KONINI*	*CONTROL*
DPPH	10.06 ± 0.26 [a]	9.20 ± 0.31 [b]
FR	543.88 ± 23.61 [a]	482.46 ± 15.56 [b]
FRAP	39.56 ± 1.01 [a]	36.73 ± 0.72 [b]
ABTS	458.76 ± 3.58 [a]	445.38 ± 3.13 [b]

DPPH is expressed in % of inhibition; FR, FRAP and ABTS values are expressed in gallic acid equivalent (GAE mg/l).

When using method Free Radicals (FR) values were higher in experimental rats fed Konini wheat (543.88 ± 23.61 GAE mg/l) than that Control wheat (482.46 ± 15.56 GAE mg/l). When using method FRAP, between experimental Konini group (39.56 ± 1.01 GAE mg/l) and control group (36.73 ± 0.72 GAE mg/l) significant differences were measured, which means that experimental Konini wheat has provable higher effect on antioxidant activity compared to control wheat. In experiment with yellow Citrus wheat [14] slightly higher values were measured in experimental group than in control.

Statistically significant higher value measured by method ABTS in experimental group Konini (458.76 ± 3.58 GAE mg/l) than that in control group (which achieved 445.38 ± 3.13 GAE mg/l) was discovered. In the experiment of Bendová [14] lower values were discovered in favour of experimental group but these values were not statistically provable.

Conclusion

In this experiment antioxidant activity on wheat Konini with higher anthocyanin representation was observed. Experiment took place on model animals of Wistar albino laboratory rat. When consuming this variety, effect of this wheat was discovered on

antioxidant activity when using methods DPPH, FR, FRAP and ABTS. Thus, it is possible to use this variety for feeding of farm animals as rats are model animals for pigs. It is possible to use the new variety Konini wheat in food processing industry also since consumer demand for functional food is getting higher. Because Konini wheat contains higher volume of anthocyanin which has positive impact on consumers' health condition, thus it is usable for production of functional foods.

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