

## The content of bioactive compounds in rat experimental diets based on organic, low-input and conventional plant materials

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**Key words:** rat feed, organic, conventional, low input, polyphenols

### Abstract

*Rat feed based on raw plant materials was produced according to the nutritional recommendations for rat feeding trials. Rat feeds produced from crops grown under 4 different production systems were used: (a) organic, (b) conventional, (c) low input 1 (organic plant protection was used in combination with mineral fertilizers) and (d) low input 2 (conventional pest management and only organic fertilizers were used). The results showed that rat feeds prepared from the organically produced plants contained more total polyphenols and the nutritionally desirable, bioactive compound lutein. The main objective of analyzing bioactive compounds in the rat feed is to determine whether the differences in composition of feed materials from different production systems could explain any measured differences in impact on the health status of rats.*

### Introduction

Organic foods are perceived by many consumers as being safer and healthier than conventional ones. Organic farming principles and standards aim to enhance the long-term natural fertility of soils, to minimize water and soil pollution, to avoid the use of mineral fertilizers and chemical pesticides, and this has been hypothesized to lead to positive health effects in livestock and humans consuming organic foods/feeds.

Fruits and vegetables have positive health benefits and contain significant amounts of biologically active components that may be responsible for these effects. Since organic production systems do not use synthetic pesticides, crop plants have to rely upon their own inherent resistance mechanisms/strategies to defend against the diseases and pests. A common strategy is to produce more phytochemicals (plant secondary metabolites) in response to lower levels of soluble plant nutrients. It is therefore thought, that crops grown under organic management may have an increased phytochemical content due to the more constant release of soluble nutrients throughout the growing season (Brandt and Mølgaard 2006).

For example, oxidized juice from organic spinach was found to have 120% higher antioxidant activity than similarly prepared material from conventionally grown spinach

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(Ren et al. 2001). Juices from organic Welsh onion and Chinese cabbage had 20 – 50% higher antioxidant activity than from corresponding conventional leafy vegetables. Green pepper was the only vegetable, for which significant difference in antioxidants content between juices from organic and conventionally grown crops could not be detected (Ren et al. 2001).

For carotenoids there is currently insufficient research data to draw general conclusions. Also, results may be variable. For example, Rembialkowska (2003) showed that organic carrots contained less carotenoids in some and higher concentration in other field experiments (Rembialkowska 2003). An experiment performed by Warman and Havard (1997) showed that conventional carrots contained significantly more beta-carotene: (102 mg kg<sup>-1</sup>) compared to conventional carrots (94.6 mg kg<sup>-1</sup>).

Overall, there is still insufficient data comparing the levels of potentially health promoting compounds (vitamins, minerals, phytochemicals etc) in plant foods produced by organic, low input and conventional methods and there are virtually no studies evaluating the effect of consumption of organic and low input foods.

### Materials and methods

The study was carried out in 2006 in the Division of Organic Foodstuff at Warsaw Agricultural University. Wheat, potatoes, carrots and onions produced under organic, conventional and low input production protocols in the Nafferton Factorial Systems Comparison Experiments (see Leifert et al. 2007) were dried to a dry matter content of 89%, irrespective of the initial water content, with a moderate temperature in order to keep their nutritive value. Rat feed based on these materials was produced according to the nutritional recommendations for rat feeding trials (Table 1).

**Tab. 1: Composition of rat experimental diet**

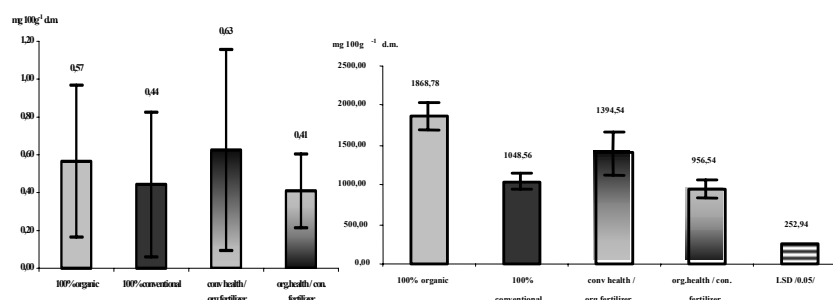
Component of diet	%	Proteins [g/kg]	Fibre [g/kg]	Lis [g/kg]	Met+Cys [g/kg]	Tre [g/kg]	Try [g/kg]	Ca [g/kg]	P [g/kg]
Lacto-albumin	6,80	3,430		0,487	0,256	0,358	0,103		
Casein	11,03	6,800		0,739	0,272	0,353	0,118		
Wheat	54,50	2,336	0,530	0,061	0,080	0,061	0,022	0,012	0,065
Potato	10,20	0,245	0,200	0,015	0,005	0,003	0,004	0,000	0,008
Carrot	3,92	0,038	0,138	0,002	0,002	0,000	0,000	0,001	0,001
Onion	0,95	0,014	0,014	0,001	0,000	0,000	0,000	0,000	0,000
Rape oil	5,79								
Min+Vit	6,81								
<b>Total</b>	<b>100,00</b>	<b>12,863</b>	<b>0,882</b>	<b>1,305</b>	<b>0,615</b>	<b>0,775</b>	<b>0,248</b>		

Four types of rat feeds based on crops grown under 4 different production systems: (a) organic, (b) conventional, (c) low input 1 (organic plant protection in combination with mineral fertilizers based fertilisation) and (d) low input 2 (conventional pesticide based crop protection with organic fertilization protocols) were used (see Leifert et al. 2007 for details of the agronomic methods used). The following composition analyses of the rat feeds were carried out: dry matter using the scale method (PN-91/R-87019), total flavonoids according to the Christ-Müller methods described by Strzelecka et. al

(1978), total polyphenols by the Folin – Ciocalteu colorimetric methods described by Singleton and Rossi (1965), beta-carotene and lutein by liquid column chromatography as described by Saniawski and Czapski (1983), and total antioxidant activity using the colorimetric method described by Ren et al. (1999). The results of these qualitative characteristics of each of the different rat feeds were statistically evaluated using Statgraphics 5.1 program specifically Tukey's test at  $\alpha = 0.05$ .

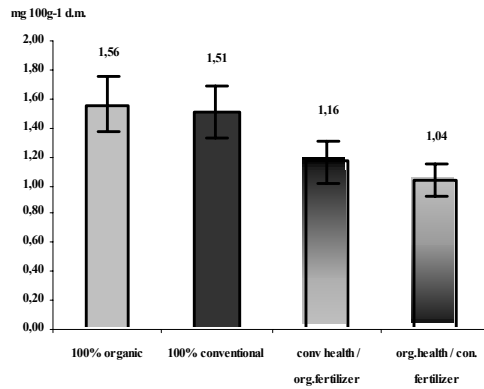
## Results and discussion

Since the feed was dried before being mixed, all examined rat feeds contained similar contents of dry matter. In previous studies organic vegetables were often found to contain higher levels of dry matter than conventional ones (Rembalkowska 2003, Rembalkowska 2004), but this was not tested in the study reported here. For the total flavonoid content the differences between feeds were not statistically significant (Fig.1).



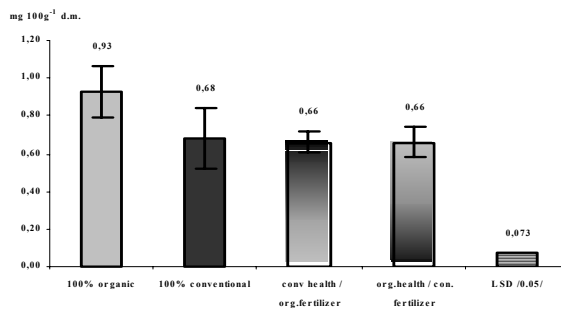
**Figure 1: Total flavonoid (left) and total polyphenol content (right) in rat feed with ingredients from organic, conventional and low input production**

There were significant differences in content of total polyphenols between organic, conventional and low input rat feeds ( $P=?$ ) (Fig. 1). The highest levels were detected in organic rat feed, and the lowest level of total polyphenols was found in feed produced from crops grown in low input system 2 (oh/cf) (Fig. 1). The content in the materials depends on the cultivar, processing methods, fertilization method, light conditions and plant protection protocols used. It seems that the fertilization methods had a greater effect on polyphenol contents than the plant protection protocols. These results are in line with many other studies comparing different nutrient supply strategies (Brandt and Mølgaard 2006). In contrast, studies comparing different plant protection methods while keeping the nutrient supply similar, such as Young et al. (2005) tend to hypothesize, that different levels of pest attack are responsible for the higher content of polyphenols in organically produced leafy vegetables. This can however be dismissed based on the general observation that crops from successful organic farms are not more affected by pests and diseases than corresponding conventional ones (van Bruggen 1995).



**Figure 2: Total beta-carotene-content in rat feed with ingredients from organic, conventional and low input production**

The organic and conventional rat feeds contained the highest levels of beta-carotene while levels in feeds based on crops produced under two low-input system conditions were lower, but this difference was only marginally significant ( $P = ?$ ). Beta-carotene was found in rat feeds due to the inclusion of carrots as a vegetable component. Previous studies into the  $\beta$ -carotene content of carrots reported contradictory results. For example Rembialkowska (2003) found lower levels of beta-carotene in organic carrots, Evers (1989) reported that the level of nitrogen fertilization had no significant impact on the content of beta-carotene in carrots, and (Leclerc et al. (1991) found higher levels of beta-carotene in the organic carrots.



**Figure 3: Lutein content in rat feed with ingredients from organic, conventional and low-input production**

The highest level of lutein was found in organic rat feed, with all other rat feeds having lower levels (Fig. 3). There are virtually no studies comparing the lutein content in vegetables from organic and conventional production systems. The high lutein content in the organic rat feed may have a potentially health-promoting effect, because lutein, which accumulates in the retina, is regarded as an important natural protector against

light-induced damage to the eyes (Alves-Rodrigues and Shao 2004). However, this effect has only been shown in humans and other primates, it is not yet known if it is relevant for nocturnal animals such as rats.

There were no significant differences in the total antioxidant activity of rat feeds made from crops produced in different production systems. In the current study the higher amount of polyphenols in organic feeds was therefore not correlated with a higher antioxidant status. There is not always a positive correlation between polyphenol content and antioxidant activity. For example, Cai et al. (2004) showed that some medicinal plants contained high levels of polyphenols but relatively low total antioxidant activity when compared to other plants with lower polyphenol levels. The results obtained showed that the level of some bioactive compounds (total polyphenol compounds, lutein, beta-carotene) was significantly higher in rat feeds prepared from the organic raw materials, but that at the same time the level of total anti-oxidant activity was lower in organic feeds than in low-input feeds. There are two possible explanations of these results:

The anti-oxidant activity of the rat feeds has been only measured for water-soluble bioactive compounds; it wasn't measured for the fat-soluble compounds, such as polyphenols, lutein and beta-carotene. Therefore the measured anti-oxidant activity could have been underestimated.

The chemical structure of polyphenols and the substitution patterns and groups have a big effect on the subsequent antioxidant and free radical scavenging activities of these plant compounds (Plumb et al. 1998). The majority of flavonoids, have moderate to high direct antioxidant activity as aglycones. However, aglycones are only formed when plant material is subjected to harsh processing conditions, so in the present study, the natural substitutions to the phenolic hydroxyl groups are probably still intact, resulting in a lower activity (Cano et al. 2002, Plumb et al. 1998, Williamson et al. 1999). Therefore in the future it is planned to **(a)** perform HPLC analyses to profile polyphenols and then determine if the concentrations of specific compounds can be related to the antioxidant activity and **(b)** measure anti-oxidant activity of both water- and fat-soluble compounds.

### **Conclusions**

Rat feeds prepared from the organically fertilized plants contained higher levels of certain bioactive compounds, especially total polyphenols and lutein.

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