

## The effect of short term feeding with organic and conventional diets on selected immune parameters in rat

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**Key words:** Organic food, Conventional food, Immunity, Splenocyte proliferation.

### Abstract

*There is currently no evidence for beneficial health impacts being associated with the consumption of organic rather than conventional foods. This preliminary study was therefore aimed at using haematological parameters, white blood cell (WBC) number and splenocyte proliferation as sensitive assays to evaluate influence of the organic, low input and conventional components in the diet on rats' immune system function. The results of a short term feeding trial with two rat generations indicates a potential effect on immune system function, which has to be confirmed by longer-term exposure studies.*

### Introduction

Organic farming is an integrated system of agriculture based on ecological principles, promotion of biodiversity, biological cycles and organic matter recycling to maintain soil fertility. The regulations for organic crop cultivation prohibit the use of chemo-synthetic pesticides, mineral fertilizers, growth promoters and genetic engineering or Genetically Modified Organisms (Rosati and Aumaitre 2004). Despite the increasing interest in organic food production, the number of articles describing potential positive and negative effects of consumption of organic and conventional foodstuffs on human and animal health is still very small (Lund and Algers, 2003). The presence of chemical contaminants (e.g. pesticides) in conventional food is likely to have an influence on their concentration in the bodies of consumers, and some *in vitro* experiments indicate that they may cause immunosuppression (Finamore et al. 2004). On the other hand, it has been suggested that immune responses in farm animals fed either conventional or organic diets are similar (Millet et al. 2005). There is therefore currently no scientifically sound evidence that demonstrates health benefits associated with the consumption of organic rather than conventional foods. However, whether or not the consumption of organic foods has significant health impacts, deserves to be tested in well controlled experimental research. The aim of this study was, therefore, to assess the effect of diets, based on organically, low input and conventionally grown crops on selected immune parameters of rats in a short-term experimental feeding trial.

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## Materials and methods

Adult male and female Wistar rats were kept under conditions of controlled light (12-h light/12-h dark cycle) and temperature (22–23 °C) with free access to water and experimental feeds. The animals were randomly assigned to one of five experimental dietary groups, according to the protocol shown in Table 1. After three weeks of feeding animals were paired and bred. Paternal males were sacrificed one week later, while females were fed the respective diets during the pregnancy and suckling period (total 10 weeks) and sacrificed thereafter along with a part of the off-spring of both sexes. Six young males from each dietary group were left alive to be fed subsequently for 9 weeks and this part of the experiment is still in progress. Animals were anesthetized with Tiopental, blood was collected from heart and spleens isolated aseptically and used immediately for *in vitro* studies. All animal procedures were in accordance with the Guiding Principles for the Care and Use of Research Animals and had been approved by the First Warsaw Ethics Committee for Experiments on Animals. Experimental diets were prepared using components produced under different agronomy regimes (Leifert et al. 2007), and characterized in a paper submitted for presentation at this congress (Rembalkowska et al. 2007). The experimental diets used for the different groups are described in Table 1. Control rats were fed a standard feed for rodents (Labofeed, Andrzej Morawski Feed Production Plant, Kcynia near Bydgoszcz, Poland).

**Table 1: Experimental protocol (see Leifert et al. 2007 for details)**

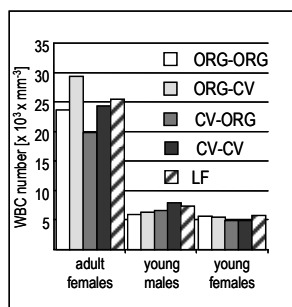
Exp. groups	Type of diet
ORG-ORG	no synthetic pesticides and no mineral fertilizers (organic farming)
ORG-CV	no synthetic pesticides and with mineral fertilizers (low input 1)
CV-ORG	with synthetic pesticides and no mineral fertilizers (low input 2)
CV-CV	with synthetic pesticides and with mineral fertilizers (conventional farming)
LF (control)	standard rodent's food – Labofeed

Hematological parameters (hematocrit value, RBC number and hemoglobin content) and WBC were assayed using standard laboratory methods. Splenocyte cultures were prepared according to a method used previously for rat lymphocytes (Bik et al. 2006). The following mitogens were then applied: Phytohemagglutinin A (PHA), Concanavalin A (ConA) or Lipopolysaccharide (LPS). Control cultures consisted of cells incubated with culture medium alone (spontaneous proliferation). Splenocyte proliferation *in vitro* was assessed by incorporation of <sup>3</sup>H-thymidine and expressed in counts per minute (cpm) as mean ( $\pm$  SD) and as stimulation index (SI). For statistical evaluation of differences between groups, ANOVA parametric followed by the Student-Neuman-Keuls test was used. Results were considered statistically significant when  $p < 0.05$ .

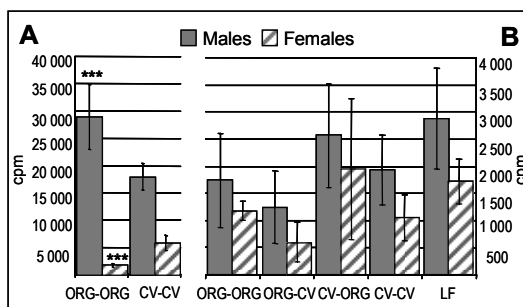
## Results

There were no significant differences in hematological parameters in rats of both sexes and generations and those fed different diets (data not shown). In adult female rats, the total WBC number were higher than in their 3-week-old off-spring of both sexes. In adult female rats WBC numbers were highest in the ORG-CV and lowest in the CV-ORG group (Fig. 1). In the parental generation only spontaneous proliferation

of splenocytes from ORG-ORG and CV-CV diet groups have been examined. Proliferation of splenocytes in rats on the ORG-ORG diet was higher for males but lower for females in comparison with rats in the CV-CV diet group (Fig. 2A). In 3-week-old rats no significant differences could be found between sexes of dietary groups; however, a trend towards lower levels in one of the low input (ORG-CV) and conventional (CV-CV) dietary groups was detected (Fig.2B).



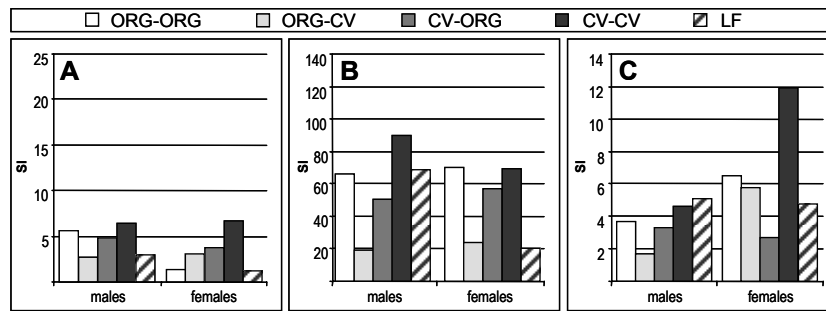
**Figure 1: The effect of diet on WBC number**



**Figure 2: Spontaneous proliferation of splenocytes from different diet groups of adult (A) and young (B) rats, expressed in cpm as mean ±SD for 4 replications of each culture variant. ORG-ORG vs CV-CV \*\*\*p<0.001.**

Mitogen-stimulated proliferation of splenocytes from young rats was examined over wide concentration range of both T-cell (PHA and ConA) and B-cell (LPS) specific mitogens. The response was dose-dependent and the effect of only one concentration of particular mitogen is shown on Fig. 3. The ability of splenocytes to be stimulated by T-cell specific mitogens was diet-dependent and seemed to be highest in splenocytes obtained from CV-CV and ORG-ORG rats. Moreover, a significantly reduced splenocyte proliferation in ORG-CV dietary groups was observed (Fig. 3A and 3B). Mitogenic response to LPS was much lower, especially in young males, which showed significantly reduced proliferation especially in the ORG-CV group. On the other hand, SI was particularly high in females on the CV-CV diet (Fig. 3C).

As feeding experiments of rats are still ongoing, no statistical test could yet be applied. To evaluate the effect of (and interactions between) (a) fertility management and (b) crop protection practices on the composition of crops and subsequent immune parameters in rats fed diets based on crops from different systems, 2-way ANOVA tests are planned to be carried out as soon as experiments have been completed.



**Figure 3: The influence of diet on mitogen-stimulated (PHA 2 µg/well – A; ConA 0.125 µg/well – B; LPS 4 µg/well – C) splenocyte proliferation in young rats of both sexes. Results are expressed as the mean of SI calculated for 4 replications of each culture variant.**

### Discussion

In this study we used hematological parameters, WBC number and splenocyte proliferation as sensitive measures to detect potential effects of diets based on crops produced by organic, low input and conventional methods, in the immune system of rats. Changes in the parameters examined are difficult to detect after only short term exposure to different diets. We found, however, highly elevated number of WBC and spontaneous splenocyte proliferation in parental females vs young rats, which seems to be related with the immaturity of the off-spring immune system (Spencer et al. 2006). Moreover, these results seem to be related to the immunomodulatory effect of prolactin, which was found to be elevated in lactating females and identified as a lymphocyte proliferation regulator (Clevenger et al. 1998). These observations suggest a necessity to repeat the experiment over a longer period and with the use of mature young animals. Proliferation of splenocytes from young rats appeared to be suppressed when diets based on crops grown with mineral fertiliser inputs (ORG-CV); also while spontaneous proliferation did not decreased significantly, the response to mitogenic stimulation was significantly less efficient.

### Conclusions

The results of the short term feeding of two generations of rats using the diets containing organically, low input and conventionally grown components indicate some changes in their immune system function in comparison to rats fed standard diet. To support the observed tendency of immunomodulatory activity of these diets, a long term feeding and associated *in vitro* studies are necessary.

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## Disclaimer

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