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Novel Approach for Food Safety Evaluation. Results of a Pilot Experiment To Evaluate Organic and Conventional Foods

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There is evidence that organic food often contains relatively high amounts of natural toxic compounds produced by fungi or plants, whereas corresponding conventional food tends to contain more synthetic toxins such as pesticide residues, but only a few studies have evaluated the impact of their consumption on health. This study proposes a novel approach to evaluate the potential health risk of organic compared to conventional food consumption, that is, the assay of sensitive markers of cell function in vulnerable conditions. The markers utilized were intestinal and splenic lymphocyte proliferative capacity and liver acute-phase reaction, both responding to the presence of toxins. The vulnerable conditions in which body defenses can be less efficient were weaning and protein-energy malnutrition. This study reports the results of a pilot experiment on one sample of eight varieties of organically and conventionally grown wheat. Weaned rats were assigned to two groups fed conventional (CV) or organic (ORG) wheat for 30 days. Each group was divided in two subgroups of well-nourished (WN) or protein-energy-malnourished (PEM) rats. For each rat, the lymphocyte proliferation was assayed by [^{3}H]thymidine incorporation after stimulation of cells with a mitogen, in a culture medium containing either commercial fetal calf serum (FCS) or the corresponding rat serum (RS) to mimic the in vivo proliferative response. The acute-phase proteins (albumin, transthyretin, transferrin, ceruloplasmin, retinol-binding protein) were measured in plasma by Western blotting and immunostaining with specific antibodies. The proliferative response of lymphocytes cultured with FCS and the amount of acute-phase proteins of rats fed the ORG wheat sample, either WN or PEM, did not differ from those of rats fed the CV wheat sample. However, the proliferative response of lymphocytes cultured with RS was inhibited in PEM-CV compared with PEM-ORG. The content of mycotoxins was highest in the organic sample, and therefore the immunotoxic effect was probably due to other contaminants in the CV wheat. In conclusion, these results indicate that the conventional wheat sample tested represented a higher risk for lymphocyte function than the wheat sample organically grown, at least in vulnerable conditions.

KEYWORDS: Organic wheat; conventional wheat; lymphocyte proliferation; acute-phase proteins; rats

INTRODUCTION

A large use of chemical substances in conventional agricultural production, such as insecticides, fungicides, and pesticides, can be seriously dangerous for human health and environment. Although the use of naturally occurring pesticides is allowed in organic agriculture, in general, organic foods coming from crops grown without synthetic pesticides are free of pesticide residues. Besides this aspect, there is some evidence that organic crops tend to have a higher nutrient content than conventional crops, at least in terms of vitamin C, nitrate, and antioxidant compounds content (*1*−*3*). Moreover, feeding animals with organic food has been reported to improve general health condition and disease resistance as compared to conventional feeding (*4*−*6*). Thus, many consumers are turning to organic foods, perceiving them as healthier than conventional foods (*7*, *8*). Indeed, the demand for organic food has increased markedly over the past 5−10 years (*9*, *10)*. However, scientific evidence supporting the issue that organically grown foodstuffs are safer than those conventionally grown is still lacking (*11*). In general, organic farming practices, which include the absence of synthetic fertilizers, use of organic amendments such as animal or green manure, and long crop rotation, are thought to reduce the risk of plant infection by pathogens (*12*). However, there is some evidence that the reduced use of fungicides and pesticides may lead to a greater contamination by toxins in organic than in conventional food (*13*). On the contrary, other studies have found a higher extent of contamination in conventional than in organic food (*14*−*16*). Moreover, the use of herbicides can lead to the development of plant root pathogens (*17*), whereas the

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long crop rotation practiced in organic cultivation can avoid the development of fungus and nematode-transmitted virus disease (*12*).

Grains can be contaminated by the fungus *Fusarium*, which produces a variety of mycotoxins; among them, deoxynivalenol (DON) is the most frequently detected in wheat (*18*). Ochratoxin A (OTA) is another contaminant of wheat and is produced by toxigenic strains of *Aspergillus* and *Penicillium* (*19*, *20*). Several factors may lead to either an increase or decrease of contamination in cereal-based food. The use of certain fungicides, such as tebuconazole and metconazole, controls *Fusarium* infection and DON production, whereas other fungicides such as azoxystrobin and related molecules are less effective or even result in an enhanced DON production (*18*). Climatic conditions may also influence the occurrence of fungi infection, as reported in a study showing a high incidence of *Fusarium* contamination in wheat caused by rainfall during the flowering season (*16*). Furthermore, appropriate environmental conditions may cause fungi survival; it has been shown in recent studies that OTA-producing *Penicillium verrucosum* was able to proliferate on soil with waste grain (*21*). In conventional agricultural techniques, the use of high levels of nitrogen increases the risk of mycotoxin formation (*22*). On the other hand, improper storage, including too high temperature, poor drying, and elevated moisture, which are often associated with organic agricultural enterprises, favors the development of fungi and their metabolites in cereal grains (*18*, *19*).

OTA and DON, other than being genotoxic, carcinogenic, and nephrotoxic, exert an immunotoxic activity (*23*−*27*). The majority of studies aimed at investigating the risk for health of organic foods have compared the amount of microorganisms, toxins, or pesticide residues present in organically and conventionally produced foods. Only a few studies have evaluated the effect of the consumption of organic versus conventional food on health, by analyzing mortality rate, body and organ weight, reproductive performance, and fertility of animals (*4*−*6*, *28*, *29*). The results of these studies have indicated a slight improvement of these parameters after the animals had been fed with organic compared to conventional food. No data are available on the effect of organic or conventional food contaminants on physiological parameters of cell function in relation to subject conditions and duration of food consumption.

In the present study, we propose a novel approach to evaluate the potential health risk induced by long-term consumption of organic versus conventional cereals, that is, the assay of sensitive markers of cell function in vulnerable conditions, in which cell defense can be less efficient. As sensitive markers of cell function, we have measured the intestinal and peripheral immune response and the liver acute-phase reaction. Indeed, the intestinal mucosa is continuously exposed to a myriad of food antigens and ingested toxic substances. The intestinal immune system (*30*), together with splenic lymphocytes, should provide a prompt defense against insults. However, the lymphocyte proliferative capacity can be seriously affected by several toxins, which make the cells unable to exert their protective function against insults (*14*, *26*). The liver, an organ with a central role in nutrition, responds to disturbances of cell homeostasis through the acute-phase reaction, by increasing the synthesis of some plasma proteins and decreasing the synthesis of others (*31*, *32*). As vulnerable conditions, we have utilized the weaning period and protein-energy malnutrition. Indeed, several studies have shown that protein-energy malnutrition induces severe alterations in different organs and tissues including the liver (*33*, *34*) and immune system (*35*, *36*), especially during development (*33*, *37*), causing a predisposition to damage by food contaminants. With the model proposed, it is possible to evaluate the combined effects of toxic compounds, their metabolites, or other unknown contaminants that cannot be analyzed by chemical analysis and the potential damages induced by them on some fundamental cell functions.

The present paper reports on some pilot experiments carried out to explore to what extent the model proposed is able to detect subpathological toxic effects of organic and conventional wheat samples through the use of cell function markers.

MATERIALS AND METHODS

Organic and conventional wheat samples were each a mixture of the same amount of eight different varieties grown in neighboring fields. They were cultured in northern Italy by G. Boggini (Istituto Sperimentale per la Cerealicoltura, S. Angelo Lodigiano, Milan, Italy), as previously reported (*38*). The organic agricultural techniques included no pesticides and fungicides, crop rotation (sowing cereal grains after previous cultivation of sunflower), and fertilization with mature manure (2.73×10^4 kg/ha) 1 month before sowing. The soil characteristics included 50 g/kg total $CaCO_3$, 15 g/kg active $CaCO_3$, 31.5 g/kg organic matter, C/N 10.2, and pH 8.1. For conventional agriculture, Inex herbicide (9% linuron and 16% pendimetalin; 5 L/ha; DuPont, Milan, Italy) was applied about 1 month after the sowing. Moreover, crop rotation with *Lolium multiflorum*, a mixture of nitrogen, phosphorus, and potassium as fertilizer at sowing (400 kg/ha in 1:2:4 ratio), followed by a dose of NH_4NO_3 (50 units/ha) after 3 and 4 months, were used. The soil characteristics included 350 g/kg total $CaCO_3$, 70 g/kg active $CaCO_3$, 30 g/kg organic matter, C/N 36, and pH 8.1. For both organic and conventional cultivation, sowing was made in late October, and water was provided by rainfall, which was on the average of seasonal rainfalls. After harvesting, all wheat samples were stored at 12−14 °C.

The analysis of fungal species in the wheat samples was made using a light microscope according to the method of Nelson et al. (*39*) and was provided by La Torre et al. (*40*). The percentage of both organic and conventional kernels infected was never more than 30%. In organic wheat, *Fusarium* was found in four of the eight samples, whereas *Penicillium* infected one sample. In conventional wheat, two of the eight samples were infected with *Fusarium*, whereas only one of eight samples was infected with *Penicillium*. No significant contamination by *Aspergillus* was detected in either organic and conventional wheat. Considering the type of contamination, the samples were assayed for DON content. The results of DON level in the wheat samples, analyzed by HPLC, were provided by Brera et al. (*41*). DON was detected in three organic samples (150, 290, and 543 μg/kg, respectively) and in one conventional sample (300 μg/kg). The contents of DON in the eight pooled wheat samples were 123 μg/kg for the organic samples and 37.5 μg/kg for the conventional samples.

Animals. Male Sprague−Dawley rats (Charles River, Como, Italy) at weaning were housed in stainless steel cages and maintained at 23 °C with a 12 h light−dark cycle. They were randomly assigned to two groups of rats fed conventional (CV) or organic (ORG) wheat. Each group was divided in two subgroups of well-nourished (WN) and protein-energy-malnourished (PEM) rats. The WN-CV and WN-ORG subgroups were fed a complete balanced diet containing 24% casein and 8% wheat protein. The PEM-CV and PEM-ORG subgroups were fed a casein-free diet in which 8% of wheat protein represented the only protein source. On day 30, rats were anesthetized by pentobarbital injection (10 mg/kg), and blood was collected for serum preparation. Mesenteric lymph nodes (MLN) and spleen were dissected in sterile conditions under a laminar flow hood (Celbio, Milan, Italy) and placed in RPMI-1640 medium (Euroclone, Milan, Italy).

As preliminary experiments to test if OTA and DON elicited an acute-phase reaction, young adult rats (200 ± 20 g) were fed ad libitum a complete semisynthetic diet (A rats). They were divided into three groups: one group (A + OTA) received daily 1 μg/day of OTA (Sigma, Milan, Italy) in 10% ethanol by gavage; another group (A + DON) received 10 μg/day of DON; the last group (A) received 10% ethanol.

The amount of OTA was chosen on the basis of the average content in wheat samples (0.8 μg/kg) reported in the literature (*20*, *44*). Animals were fed for 21 days, which was the time in which the acute-phase reaction was elicited. Rats were anesthetized, and blood was collected for serum preparation. Serum was stored in aliquots at −20 °C until use.

Weight of animals and food intake were controlled twice per week. Care and use of animals were approved by the Animal Care and Ethics Committee of INRAN (Rome, Italy).

Assessment of in Vitro Lymphocyte Proliferative Response. The collected MLN were scraped on a metallic grid (0.5−1 mm mesh) and the released lymphocytes filtered on a piece of nylon sheet. Spleens were smashed with a 1 mL plastic syringe piston to release lymphocytes into the medium. The MLN and splenic lymphocytes were centrifuged at 250*g* for 5 min, washed, and suspended in RPMI-1640 medium supplemented with penicillin (1×10^5 units/L), streptomycin (100 mg/L), 4 mM glutamine, 1% nonessential amino acids, and 50 mM 2-mercaptoethanol (Sigma). For each rat, cells were cultured at 3×10^5/well in 96-well flat-bottom plates (Costar, Rome, Italy) in RPMI-1640 medium containing 10% of either heat-inactivated fetal calf serum (FCS; Euroclone) or serum prepared from the corresponding rat (RS), at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was measured by trypan blue exclusion and was similar in all groups. Cells were stimulated with 2.5 mg/L of concanavalin A (ConA; Sigma) for 72 h and pulse-labeled with 5 mCi/L of [^{3}H]thymidine (6.7 Ci/mmol; NEN, Zaventem, Belgium) for the last 18 h of incubation. After harvesting, radioactivity was counted in a scintillator counter (Beckman, Milan, Italy).

Analysis of Acute-Phase Proteins. The acute-phase proteins were assayed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting followed by immunostaining. Serum proteins from all groups of rats were fractionated on a SDS-PAGE gel under denaturing conditions. Acrylamide concentrations varied from 8 to 15%, according to the molecular weight of the protein to be analyzed: from 8% acrylamide for ceruloplasmin, molecular mass 132 kDa, to 15% acrylamide for transthyretin (TTR), molecular mass 15 kD-. The amount of serum utilized varied from 0.02 to 1 μL, according to the concentration of the proteins. Serum proteins were measured according to the method of Bradford (*42*). Proteins fractionated by SDS-PAGE were transferred onto nitrocellulose filters according to the method of Bellovino et al. (*43*). The intensity of protein bands on films was quantified by densitometry. The polyclonal antibodies anti-rat TTR were raised in rabbit by injecting the protein purified as previously described (*43*). The antibodies anti-rat albumin and transferrin in rabbit were purchased by Cappel (ICN, Milan, Italy). Anti-human ceruloplasmin in goat and anti-human retinol-binding protein (RBP) in rabbit were from Dako (Glostrup, Denmark) and cross-reacted well with the rat proteins.

Statistical Analysis. The significance of the differences was evaluated by one-way ANOVA followed by Fisher's test. Differences with P values <0.05 were considered to be significant.

RESULTS

Body Weight, Food Intake, and Total Serum Proteins. Final body weight and food intake of WN-ORG rats did not differ from that of WN-CV rats. Final body weight of PEM rats was lower than that of WN rats, but PEM-CV and PEM-ORG rats did not differ in body weight and food intake (**Table 1**). According to the average level of DON of the wheat samples, the amounts of DON in the diets for the WN-ORG and WN-CV rats were 49.2 and 15.0 μg/kg of diet, respectively, and for the PEM-ORG and PEM-CV rats it was 108.7 and 33.11 μg/kg of diet, respectively.

Within A rats, the untreated and treated rats (A + OTA and A + DON) did not differ in body weight, food intake, and total serum proteins (**Table 2**).

Proliferative Capacity. To evaluate the potential health risk of organic versus conventional wheat, the proliferative capacity of lymphocytes was measured. The results are reported as cpm of [^{3}H]thymidine incorporation in unstimulated and ConA-stimulated lymphocytes (**Tables 3** and **4**): the proliferation of unstimulated lymphocytes reflects their in vivo status of proliferation, whereas that of ConA-stimulated cells indicates their capacity to respond to an external stimulus. For each rat, the proliferation was measured in lymphocytes cultured in

Table 1. Effects of Diets Containing Conventional (CV) or Organic (ORG) Wheat on Body Weight and Food Intake of Well-Nourished (WN) and Protein-Energy-Malnourished (PEM) Rats[a]

	body wt (g)		
	initial	final	food intake[b]
WN-CV	49.3 ± 4.6	248.0 ± 56.3	11.3 ± 1.2
WN-ORG	52.0 ± 3.5	249.3 ± 23.7	11.8 ± 0.5
PEM-CV	55.5 ± 7.5	94.0 ± 16.1	9.4 ± 1.3
PEM-ORG	56.5 ± 4.1	92.0 ± 11.9	8.4 ± 1.3

[a] Data are the means ± SD of at least 10 rats for each group. [b] Gtams per day.

Table 2. Effect of Ochratoxin A (OTA) and Deoxynivalenol (DON) on Body Weight and Serum Protein Content[a]

	body wt (g)		
rats	initial	final[b]	serum proteins[c] (g)
A	100.0 ± 5.0	200.0 ± 8.1	7.6 ± 0.3
A + OTA[d]	100.0 ± 5.2	197.0 ± 9.0	7.5 ± 0.5
A + DON[e]	105.0 ± 6.3	203.0 ± 8.5	7.5 ± 0.7

[a] Data are the means ± SD of at least 10 rats for each group. [b] After 21 days of OTA or DON treatment. [c] Grams per 100 mL. [d] One microgram per day. [e] Ten micrograms per day.

Table 3. Proliferative Response to Concanavalin A (ConA) of Lymphocytes of Spleen and Mesenteric Lymph Nodes (MLN) of Well-Nourished (WN) Rats Fed a Diet Containing Conventional (CV) or Organic (ORG) Wheat[a]

	MLN		spleen	
	cpm − ConA	cpm + ConA	cpm − ConA	cpm + ConA
FCS[b]				
WN-CV	2223 ± 563	169236 ± 15862	2384 ± 614	141883 ± 34408
WN-ORG	2065 ± 358	232639 ± 55652	1654 ± 575	136016 ± 15204
RS[c]				
WN-CV	467 ± 110	41116 ± 6888	775 ± 118	41012 ± 12405
WN-ORG	606 ± 152	38702 ± 12005	657 ± 266	32578 ± 12493

[a] Proliferative response was analyzed after 18 h of [^{3}H]thymidine incorporation. Data are the means ± SD of at least 10 rats for each group. [b] Fetal calf serum. [c] Rat serum.

Table 4. Proliferative Response to Concanavalin A (ConA) of Lymphocytes of Spleen and Mesenteric Lymph Nodes (MLN) of Protein-Energy-Malnourished (PEM) Rats Fed a Diet Containing Conventional (CV) or Organic (ORG) Wheat[a]

	MLN		spleen	
	cpm − ConA	cpm + ConA	cpm − ConA	cpm + ConA
FCS[b]				
PEM-CV	1427 ± 286	113454 ± 22690	2894 ± 578	160547 ± 18027
PEM-ORG	1513 ± 303	107916 ± 21584	1583 ± 316	144336 ± 22506
RS[c]				
PEM-CV	734 ± 146	17260 ± 3452	1243 ± 248	45744 ± 8005
PEM-ORG	456 ± 91	26370 ± 5274	1325 ± 264	76934 ± 12360

[a] Proliferative response was analyzed after 18 h of [^{3}H]thymidine incorporation. Data are the means ± SD of at least 10 rats for each group. [b] Fetal calf serum. [c] Rat serum.

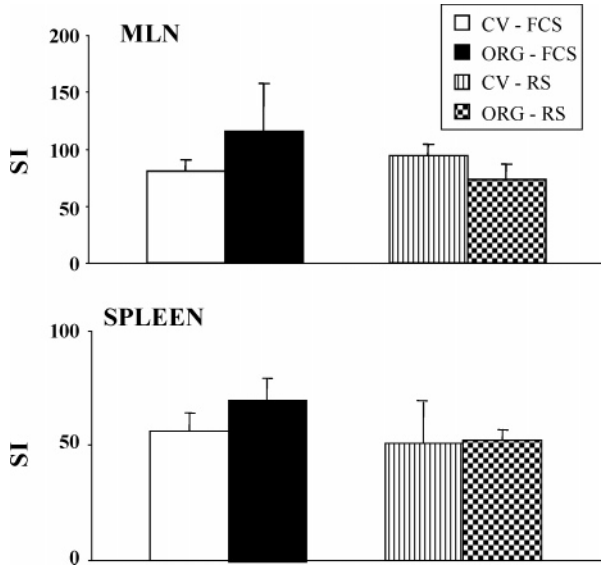

Figure 1. Stimulation index (SI) of lymphocytes of mesenteric lymph nodes (MLN) and spleen of well-nourished rats fed a diet containing conventional (CV) or organic (ORG) wheat. Cells were cultured in medium containing fetal calf serum (FCS) or rat serium (RS). SI indicates the ratio of cpm of ConA stimulated/cpm unstimulated cells. FCS, fetal calf serum; RS, rat serum. Data are the means ± SD of at least 10 rats for each group.

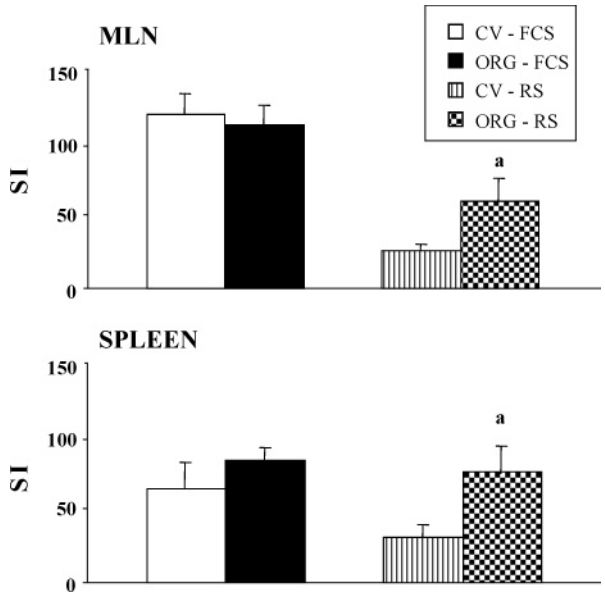

Figure 2. Stimulation index (SI) of lymphocytes of mesenteric lymph nodes (MLN) and spleen of protein-energy-malnourished rats fed a diet containing conventional (CV) or organic (ORG) wheat. Cells were cultured in medium containing fetal calf serum (FCS) or rat serium (RS). SI indicates the ratio of cpm of ConA stimulated/cpm unstimulated cells. Data are the means ± SD of at least 10 rats for each group. "a" for ORG-RS versus CV-RS: $P < 0.01$ in spleen; $P < 0.05$ in MLN.

medium containing FCS, as a standard serum, and also in medium containing the corresponding RS, to mimic the in vivo conditions. The results are also reported as stimulation index (SI), which is the following ratio: cpm of [^{3}H]thymidine incorporation of ConA-stimulated lymphocytes/cpm of [^{3}H]thymidine incorporation of unstimulated lymphocytes (**Figures 1** and **2**).

As shown in **Table 3**, when culture medium contained FCS, the proliferative response to ConA of both MLN and spleen of WN-ORG rats did not differ from that of WN-CV rats. When the cells were cultured in medium containing RS, the proliferative capacity was lower than that obtained with medium containing FCS, but no difference was seen in either MLN or spleen lymphocyte proliferation between WN-ORG and WN-CV rats. **Figure 1** shows that the values of SI with FCS or RS were in the same range and that the SI of WN-ORG rats did not differ from that of WN-CV rats. In **Table 4** and **Figure 2** the proliferative response of lymphocytes of PEM rats is shown. When FCS was used, no significant differences were seen between PEM-ORG and PEM-CV. When RS was used, the proliferation of unstimulated cells of MLN was higher in PEM-CV than in PEM-ORG and, vice versa, the proliferation of stimulated cells was lower in PEM-CV than in PEM-ORG, although not significantly (**Table 4**). The SI of both MLN and spleen lymphocytes of PEM-ORG rats did not differ from that of PEM-CV rats when the medium contained FCS (**Figure 2**). When the medium contained RS, the SI of both MLN and spleen lymphocytes of PEM-ORG rats did not differ from that of PEM-ORG and PEM-CV rats when cultured with FCS. However, the SI of PEM-CV rats was significantly lower than that of PEM-ORG rats.

Table 5. Effect of Ochratoxin A (OTA) and Deoxynivalenol (DON) Treatment on Acute-Phase Proteins[a]

	amount[b] (%)	
serum protein	A + OTA	A + DON
albumin	43 ± 2	
TTR	25 ± 2	39 ± 5
RBP	103 ± 8	
transferrin	173 ± 25	
ceruloplasmin	251 ± 36	268 ± 31

[a] Proteins were fractionated on SDS-PAGE and transferred onto nitrocellulose and the acute phase proteins immunostained. Bands relative to the individual proteins were scanned and quantified by densitometry. [b] Percentage of each protein of A + OTA/A or A + DON/A rats, respectively. Data are the means ± SD of at least 10 rats for each group.

Acute-Phase Response. To analyze the liver acute-phase response after organic or conventional wheat consumption, the following acute phase proteins synthesized and secreted by the liver were analyzed in serum: albumin and TTR as negative reactants, transferrin and ceruloplasmin as positive reactants, and RBP as control protein, which is supposed to remain unchanged during the acute-phase reaction in rats (*31*). First, the effect of OTA and DON treatment on these proteins was checked, by giving to the animals an amount of these toxins corresponding to an amount that can be assumed with the diet containing contaminated wheat (*20*, *44*). The amount of OTA used, 1 μg/day given for 21 days to animals weighing 200 g, is much lower than the amount used in experiments in which histopathological changes in renal or liver tissue are induced. Indeed, nephrotoxicity and other pathological effects are obtained with amounts that range from 0.3 to 10 mg/kg of body weight administered from 1 to 30 days (*45*, *46*). The amount of DON used (10 μg/day) was lower than that shown to induce immunosuppression or other damages (*25*, *26*).

In **Table 5** the results of A + OTA and A rats are shown. The densitometric quantitation of the individual proteins is reported as a percentage of the amount of each protein in A + OTA rats/amount of the same protein in A rats, equal to 100. The results show that the amount of RBP did not change, and TTR and albumin decreased, whereas transferrin and ceruloplasmin increased. In A + DON rats, ceruloplasmin and TTR were measured as positive and negative acute-phase reactants, respectively (not shown), and the respective percentages did

Table 6. Effect of Diet Containing Organic (ORG) or Conventional (CV) Wheat on Acute-Phase Proteins in Well-Nourished Rats[a]

serum protein	amount[b] (%)
albumin	102 ± 15
TTR	115 ± 20
RBP	99 ± 11
transferrin	101 ± 17
ceruloplasmin	97 ± 12

[a] Proteins were fractionated on SDS-PAGE and transferred onto nitrocellulose and the acute-phase proteins immunostained. Bands relative to the individual proteins were scanned and quantified by densitometry. [b] Percentage of each protein of WN-ORG/WN-CV rats. Data are the means ± SD of at least 10 rats for each group.

Table 7. Effect of Diet Containing Organic (ORG) or Conventional (CV) Wheat on Acute-Phase Proteins in Protein-Energy-Malnourished (PEM) Rats[a]

serum protein	amount[b] (%)
albumin	99 ± 14
TTR	103 ± 13
RBP	107 ± 9
transferrin	95 ± 10
ceruloplasmin	120 ± 15

[a] Proteins were fractionated on SDS-PAGE and transferred onto nitrocellulose and the acute-phase proteins immunostained. Bands relative to the individual proteins were scanned and quantified by densitometry. [b] Percentage of each protein of PEM-ORG/PEM-CV rats. Data are the means ± SD of at least 10 rats for each group.

not differ from those of A + OTA rats. The acute-phase proteins of WN and PEM rats are reported in **Tables 6** and **7**, respectively. The results are expressed as a percentage of the amount of each protein in WN-ORG or PEM-ORG rats/amount of the same protein in WN-CV or PEM-CV rats, respectively. The results indicate that the amount of serum albumin, TTR, RBP, transferrin, and ceruloplasmin did not change after organic wheat consumption in both WN and PEM rats, as compared to conventional wheat.

DISCUSSION

Several studies have measured the amount of OTA and DON present in organic or conventional wheat to evaluate the health risk associated with wheat consumption (*20*, *47*−*49*). On the other hand, no sufficient data are available to exclude that long-term consumption of contaminated cereal-based food can affect some fundamental cell function, and no data on immune response and liver function in different physiological conditions have been published. There is also a possibility that toxic substances, other than OTA and DON, such as pesticide residues or their metabolites, may be present in cereal grains and that a synergistic effect may also occur (*49*−*51*). Moreover, heavy metals such as lead or cadmium can be present in cereals, and an amount of lead exceeding the recommended maximum level was found in conventional wheat (*49*).

On the basis of these considerations, in this study we have used lymphocyte proliferation and liver acute-phase reaction of rats as two sensitive functional assays to evaluate the safety of organically grown wheat compared to that of conventionally grown wheat after long-term consumption. We have evaluated lymphocyte proliferation of both intestinal and peripheral sites, which represent the essential sites for immune response. In fact, the first defense against noxious antigens and pathogens is represented by the lymphocytes of the gut-associated lymphoid tissue (GALT), which is the largest immunological organ, comprising specialized lymphoid nodules (Peyer's patches), intraepithelial and lamina propria lymphocytes, and mesenteric lymph nodes (*32*). Several toxic compounds at certain concentrations may affect the lymphocyte proliferative capacity, making them unable to provide the necessary defense (*14*, *24*), and may elicit the liver acute-phase reaction (*31*, *32*). Thus, through the assays used in this study we have evaluated whether toxins, or their metabolites or other unknown contaminants that have not been detected by chemical analysis, were present in the two samples of wheat and whether these contaminants induced damage on some fundamental cell function. Furthermore, by using rats at weaning and in protein-energy malnutrition, we have taken into account the fact that in more vulnerable conditions the defense response of cells can be less efficient and the toxic effect stronger than in normal physiological conditions (*33*−*37*).

By using the model described, we have found that the organic wheat sample tested is as safe as, or even safer, than the conventional one. One piece of evidence for this comes from the data on the proliferative capacity of lymphocytes of WN-ORG rats, which is similar to that of WN-CV rats. Moreover, even when the immune response was less efficient, as in PEM, organic wheat did not affect the proliferative capacity of lymphocytes. Finally, when lymphocytes of PEM rats were cultured in a medium in which FCS was replaced by the corresponding RS to better mimic the in vivo lymphocyte activity, the capacity to respond to a mitogen stimulation was lower in PEM-CV than in PEM-ORG rats, suggesting that one or more contaminants were present in conventional and not in organic wheat, affecting the lymphocyte proliferative capacity of PEM-CV as compared to PEM-ORG rats. The fact that there was no difference in SI between PEM-ORG and PEM-CV rats when FCS was used may be due to the higher content of nutrients and other factors in FCS than in RS, rendering the cells able to exert their activity against noxious agents.

Further evidence for the safety of the organic and conventional wheat used in this study derives from the absence of acute-phase reaction in WN and PEM rats, indicating that no toxins were present in the two sources of wheat at least in an amount that can induce the reaction.

Several studies have already shown that DON, one of the most frequent contaminants of wheat, can either suppress or stimulate immune functions depending on dose, exposure frequency, and timing of administration (*26*). In vitro studies have reported a reduction of lymphocyte proliferation by high doses of DON (*52*). Treatment of mice with a single high dose of DON (from 1 to 25 mg/kg of body weight) or subchronic oral exposure to DON (from 0.5 to 5 mg/kg of body weight) for short OLINIT-term induced an increase of pro-inflammatory cytokine expression in serum, in spleen, and in Peyer's patches lymphocytes (*53*, *54*). After long-term treatment with different doses of DON (from 2 to 25 mg of DON/kg of diet), an elevation of serum immunoglobulin A was found. The amount of DON in wheat is usually up to 1 mg/kg of diet (*20*). Considering that contaminants other than DON can be present in wheat and that a synergistic effect of contaminants could take place, an immunomodulatory activity can be found when DON is present at doses lower than those described above. In the present study, the amount of DON in the diet containing the eight pooled organic wheat samples was lower than that reported to affect immune response. In agreement with this, no alteration was found on immune response induced by organic wheat, even in a vulnerable condition, further suggesting that

no other contaminants were present in the organic wheat used in this study.

Despite several studies on dose and time effect of DON and OTA, data were lacking on the effect of these toxins on acute-phase proteins. Thus, we have checked whether an amount of these toxins corresponding to a dietary intake of OTA and DON at level that can be found in wheat elicited an acute-phase reaction. The results demonstrate that in the tested conditions the synthesis of acute-phase proteins was modified without any effect on food consumption and total serum protein content.

In conclusion, the results of the pilot experiment using samples of organic and conventional wheat indicate no higher risk of introducing toxic compounds with organic compared to conventional wheat that could affect fundamental cell functions, such as gut mucosal immune response and liver function, even in a condition of protein-energy malnutrition that induces a greater susceptibility to the effects of toxic substances. Moreover, the data of proliferative capacity of PEM rats when RS was used, showing an adverse effect of conventional food despite the lower content of DON, further support the idea that it is advisable to associate functional with chemical assays in the evaluation of food health risk. The last consideration is that the model utilized in this study is not restricted to wheat safety, but it can be more generally used for the evaluation of possible risk for health associated with the consumption of several contaminated foods.

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