

Immunological changes in growing mice fed on diets containing casein or peas (*Pisum sativum* var. Belinda) as the source of protein

BY J. ALFREDO MARTÍNEZ, M. LUISA ESPARZA AND JESÚS LARRALDE

Department of Physiology and Nutrition, University of Navarra, Pamplona, Spain

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The effects of two different sources of protein: peas (*Pisum sativum* var. Belinda) and casein on immunocompetence, nutritional utilization and growth performance have been investigated in recently weaned mice. Feeding these animals on a pea diet resulted in an impairment in growth and significant decreases in the weights of liver, muscle, kidneys and femur, while intestine weights increased. No differences in food consumption were observed, but food conversion efficiency (food intake: weight gain) was increased in pea-fed animals compared with those offered the casein diet. Packed cell volume and serum Fe and Zn levels fell significantly after legume-protein intake, and, by contrast, Cu values increased slightly. Serum albumin levels showed a statistically significant reduction in mice fed on the diet containing peas. However, γ -globulins and immunoglobulin G titres were markedly increased. The characterization of spleen-cell subsets using monoclonal antibodies revealed a significantly higher percentage of T-lymphocytes in the pea group compared with casein-fed animals, while no changes were observed in the proportions of B-lymphocytes and macrophages. *In vitro* mitogenic responses to phytohaemagglutinin, concanavalin A and *Escherichia coli* lipopolysaccharide S were slightly, but not significantly, lower in the pea-fed animals. Our results describe, apparently for the first time in mice, some immunological disturbances after peak intake. These results may lead to a better understanding of the possible role of antigenic proteins in gastrointestinal disorders and the poor individual performance after legume intake.

Pea (*Pisum sativum*): Immune response: Nutritional status: Mice

Protein levels and the amino acid composition of the diet play a role in the maintenance of immune response (Bounous *et al.* 1983; Chandra, 1993; Yamauchi & Suetsuna, 1993). Furthermore, proteins can act as dietary antigens (Perdue & Bienestock, 1991) and the type of protein could affect the immune response through different antigenic or allergic reactions (Le Guen *et al.* 1991; Hankins *et al.* 1992; Lallès *et al.* 1993).

Food-induced allergic responses, especially in young individuals, have been the subject of study in recent years, and alternative protein sources in the treatment of protein allergies have been investigated (Walker, 1992). Moreover, the increasing use of plant proteins in human and animal nutrition has focused great attention on legumes, which are also important sources of other nutrients (Lallès *et al.* 1993). However, the immunogenicity of certain antinutritional factors such as lectins and some legume proteins seems to indicate that the immune response may be involved in digestive disturbances, impaired availability of different nutrients and reduced growth performance (Barratt *et al.* 1979; Grant *et al.* 1983; Lallès *et al.* 1993). These nutritional imbalances and others produced by the phytate, tannin or fibre content, which diminish mineral bioavailability (Larralde & Martínez, 1989; Cashman & Flynn, 1991; Rubio *et al.* 1992), may alter immune responses through complex interactions between nutrition and immunocompetence (Sherman, 1992; Chandra, 1993).

The aim of the present study was to evaluate the immunocompetence in mice receiving diets containing peas (*Pisum sativum* var. Belinda) or casein as the source of protein using different indices of the immune response such as the total serum immunoglobulin G (IgG), the mitogenic response of splenic lymphocytes and the percentage of splenic-cell subsets. The nutritional utilization of different nutrients was also assessed. These studies may be of relevance in populations where legumes represent the staple food, but also for nutritional purposes in immunity or allergy-mediated disorders.

MATERIALS AND METHODS

Animals and diets

Recently weaned male Swiss albino mice (4 weeks old) obtained from Leticia S.A. (Barcelona, Spain) and weighing about 21 g were randomly assigned to two dietary groups of eight animals each. The animals were housed in polypropylene cages with wire-meshed bottoms in a room with constant temperature (20–22°) and with a 12 h light–dark cycle. Feed and water were provided *ad lib.* in feeders specially designed to minimize feed wastage. The diets contained casein (control) or raw pea seeds as the source of protein and were balanced for the remaining nutrients (Table 1). The animals were fed on the same experimental diets for 3 weeks. Mice and feed were weighed daily at the same time (09.00–10.00 hours). Final body and organ weights were assessed at the end of the experimental period as indices of growth performance.

Packed cell volume and serum mineral measurements

Mice were ether-anaesthetized and blood samples were obtained by cardiac puncture. Serum Fe was assayed colorimetrically using ferrozine as the chromogen (Thompson & Motola, 1984), and an Autoanalyser (model Cobas Fara; Roche Diagnostic, Basel, Switzerland). Packed cell volume values, as an index of Fe deficiency, were determined using capillary tubes by centrifugation at 1000 g for 10 min.

Serum Zn and Cu determinations were made by atomic absorption spectroscopy (Perkin-Elmer, model 305 B, Norwalk, CT, USA) with an air–acetylene flame at a wavelength of 213.9 nm for Zn and 324.7 nm for Cu (Smith *et al.* 1985).

Serum protein and IgG determinations

Serum total proteins, albumin and γ -globulins were measured after agarose-gel electrophoresis (Barta & Porciau, 1984). Samples applied to agarose-gel plates were subjected to 100 V for 20 min, then were scanned in a densitometer (Beckman Instruments, Brea, CA, USA) at 600 nm.

Serum levels of IgG were measured by radial immunodiffusion (Hudson & Hay, 1989) using a kit with anti-mouse IgG antiserum contained in agarose gel (Serotec, Oxford, Oxon). After an appropriate dilution, test samples and standards were applied in 5 μ l volumes on different wells of the plates. The incubations were carried out at 22° in moist atmosphere for 72 h. The diameters of the precipitation rings were measured and the concentrations of immunoglobulin in the samples were determined from a calibration curve.

Isolation of mononuclear cells from spleen

Spleens were removed aseptically from mice, weighed and briefly stored in NaCl solution (8.5 g/l) at 0 °C before being washed and minced in the same solution. After removal of residual tissue, mononuclear cells of the cellular suspension were separated by density gradient centrifugation at 400 g for 20 min at room temperature on lymphocyte separation medium (1.083 g/ml; Lymphoprep, Nycomed AS; Böyum, 1983). Mononuclear cells were

Table 1. *Composition of experimental diets (g/kg)*

	Casein diet	Pea diet
Casein*	140	—
Pea (<i>Pisum sativum</i> var. Belinda)	—	600
Sucrose	350	150
Wheat starch	350	150
Olive oil	50	50
Cellulose	50	—
Mineral mix†	45	40
Vitamin mix‡	10	10
Choline chloride	0.6	0.6
Energy content (KJ/kg)	15758	15591
Crude protein (N × 6.25)	12.5	12.5

* Methionine (10 g/kg) was added to the casein diet.

† Harper mixture containing (g/kg): NaCl 130.3, K₂HPO₄ 389.1, MgSO₄·7H₂O 57.3, CaCO₃ 381.4, FeSO₄·7H₂O 27.0, MnSO₄·H₂O 4.0, KI 0.79, CuSO₄·5H₂O 0.5, ZnSO₄·7H₂O 0.12, CoCl₂·6H₂O 0.02.

‡ Harper mixture containing (mg/g): retinol 0.5, cholecalciferol 0.37, tocopherol 3.0, menadione 1.5, *p*-aminobenzoic acid 145, γ -inositol 32.5, nicotinic acid 15.0, calcium pantothenate 5.7, riboflavin 1.5, thiamine 14, pyridoxine 9.5, pteroylmonoglutamic acid 0.5, cyanocobalamin 6.0, biotin 63.0. Lactose as carrier was added to make up to 1 g.

collected at the interface and washed three times with saline solution. The final cell pellet was suspended in RPMI 1640 medium supplemented with foetal calf serum (100 ml/l), 2 mM-L-glutamine, 10 mM-4-(2-hydroxy-ethyl)-1-piperazine ethanesulphonic acid (HEPES), penicillin 100 units/ml, and 100 μ g streptomycin/ml (Gibco, Grand Island, NY, USA). The cells were counted in a haemocytometer and cell viability, as determined by the trypan blue exclusion test, was > 97%.

Analysis of cell surface markers by flow cytometry

Percentage of splenic cells was determined by flow cytometry (Hudson & Hay, 1989). The following rat anti-mouse monoclonal antibodies were purchased from Serotec: anti-Thy 1 (T cells), anti H2-Ia subregion (B cells) and anti-Ly 40 (macrophage/monocyte). The cell pellets (1×10^6) were incubated with 50 μ l of the antibodies for 30 min on ice. After washing twice with phosphate-buffered saline the cell pellets were incubated with 50 μ l fluorescein isothiocyanate (FITC)-labelled anti-rat rabbit IgG. After washing twice with phosphate-buffered saline monoclonal antibodies binding to the cells were analysed with a fluorescence-activated cell sorter (FACScan Flow Cytometer; Becton Dickinson, Erembodegem, Belgium). Fluorescence data were collected with logarithmic amplification. For each sample, data from 10000 volume-gated viable cells were collected. Non-mononuclear cells were gated on the basis of the forward and perpendicular light scatter signal.

Lymphocyte proliferation to mitogen

Cells were resuspended in RPMI supplemented at a concentration of 1×10^6 cells/ml. Blastogenic response of splenocytes to the mitogens phytohaemagglutinin (PHA; Gibco, Grand Island, NY, USA), concanavalin A (Con A; Sigma Chemical Co., St Louis, MO, USA), and *Escherichia coli* lipopolysaccharide S (LPS; Difco, Detroit, MI, USA) was assessed by [methyl-³H]thymidine incorporation, as previously described (Martínez *et al.* 1992). Triplicate cultures were set up in ninety-six-well microplates (Becton Dickinson). Splenocytes (1×10^5 cells in 100 μ l culture medium) were incubated with or without

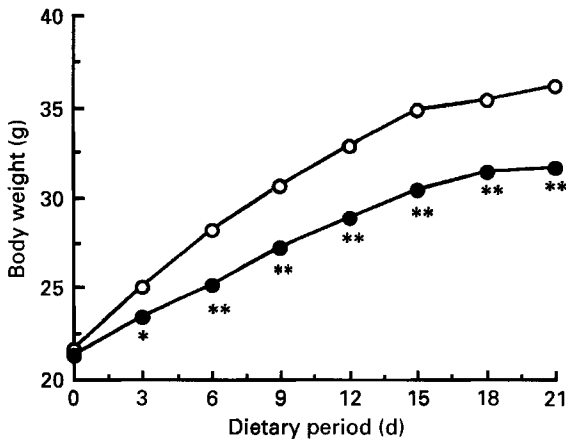


Fig. 1. Body weight gain of mice fed on diets containing casein (○) or pea (*Pisum sativum* var. Belinda, ●) for 21 d. Values are means for eight mice. The analysis showed a significant stunting of growth in mice fed on pea as the source of protein compared with those fed on casein: * $P < 0.05$, ** $P < 0.01$ (Student's t test). For details of diets and procedures, see Table 1 and p. 88.

mitogens at 37°, in an O₂-CO₂ atmosphere (95:5, v/v) for 72 h. Doses of mitogens were 50 µg/ml for PHA, 5 µg/ml for Con A and 100 µg/ml for LPS. At 24 h before cell collection, 3.7×10^4 Bq [methyl-³H]thymidine (Amersham, Bucks) was added to each well. The cultures were then harvested onto fibreglass filter discs and counted in a liquid scintillation counter (LKB 1215). The data are expressed as a stimulation index, defined as a ratio of mean counts/min in stimulated cultures to that in unstimulated cultures of the same cells.

Statistical analysis

The results are presented as the arithmetic means with their standard errors for each casein and pea group. Differences among the means of groups were evaluated using the Student's two-tailed t test and P values ≤ 0.05 were considered to be statistically significant.

RESULTS

Growth performance and feed intake

Mice fed on pea as the source of protein showed a retardation in growth compared with those fed on casein protein (Fig. 1). Although feed intake in the two groups was similar through the experimental trial, feed efficiency ratios, expressed as g intake/g weight gain, were higher in the legume-fed animals ($P < 0.01$). Moreover, liver, gastrocnemius muscle, kidneys and femur weights were smaller in mice fed on the pea diet ($P < 0.01$), while an increase was observed in intestine weights ($P < 0.05$). No statistical differences were found in lymphoid organ weights between the experimental groups (Table 2).

Packed cell volume and serum mineral measurements

Packed cell volume showed a statistically significant decrease in those animals fed on the diet containing peas ($P < 0.01$). Accordingly, serum Fe levels were found to be markedly reduced ($P < 0.01$). Serum Zn levels were also decreased in the legume-fed animals ($P < 0.05$). By contrast, Cu levels were slightly increased, while Fe:Cu and Zn:Cu ratios decreased ($P < 0.05$ and $P < 0.01$ respectively), suggesting an interaction in the uptake of these minerals (Table 3).

Table 2. *Initial and final body weights and organ weights, daily gain and feed-conversion ratio of mice given diets containing casein or peas (Pisum sativum var. Belinda) as the source of protein*†

(Mean values with their standard errors for eight mice per dietary group)

	Casein		Pea		Statistical significance
	Mean	SE	Mean	SE	
Initial body wt (g)	21.9	0.3	21.1	0.3	NS
Final body wt (g)	35.7	0.6	31.6	0.7	**
Daily gain (g/d)	0.67	0.03	0.50	0.04	*
Feed conversion ratio (g/g)‡	16.1	2.6	32.5	3.6	**
Organ wt					
Liver (g)	1.88	0.07	1.36	0.04	**
Gastrocnemius (mg)	336	5.5	283	7.2	**
Kidneys (mg)	532	13.0	449	13.0	**
Intestine (g)	1.50	0.07	1.86	0.11	*
Femur (mg)	130	3.4	103	7.4	**
Thymus (mg)	48	3.6	43	2.7	NS
Spleen (mg)	177	24	173	30	NS

NS, not significant.

* $P < 0.05$, ** $P < 0.01$ (Student's t test).

† For details of diets and procedures, see Table 1 and p. 88.

‡ Food intake/g body weight gain.

Table 3. *Packed cell volume and serum levels of iron, zinc and copper, of mice given diets containing casein or peas (Pisum sativum var. Belinda) as the source of protein*†

(Mean values with their standard errors for eight mice per dietary group)

	Casein		Pea		Statistical significance
	Mean	SE	Mean	SE	
Packed cell volume (%)	46.0	2.1	36.6	2.3	**
Iron (mg/l)	2.71	0.24	1.20	0.27	**
Zinc (mg/l)	1.94	0.19	1.46	0.07	*
Copper (mg/l)	0.78	0.09	1.00	0.06	NS
Iron:copper	3.80	0.6	1.70	0.4	*
Zinc:copper	2.53	0.3	1.5	0.1	**

NS, not significant.

* $P < 0.05$, ** $P < 0.01$ (Student's t test).

† For details of diets and procedures, see Table 1 and p. 88.

Serum protein and IgG determinations

Total serum proteins did not decrease significantly in mice fed on pea as the source of protein compared with casein-fed animals. However, serum albumin levels showed a marked decline ($P < 0.05$), while γ -globulins were increased ($P < 0.01$) in the legume-fed mice (Table 4).

Total serum IgG levels were increased in those animals fed on pea protein ($P < 0.05$), suggesting a systemic humoral immune response (Fig. 2).

Table 4. Total serum protein, albumin and γ -globulins of mice given diets containing casein or peas (*Pisum sativum* var. *Belinda*) as the source of protein†
(Mean values with their standard errors for eight mice per dietary group)

	Casein		Pea		Statistical significance
	Mean	SE	Mean	SE	
Total protein (g/l)	59.6	1.1	57.7	1.5	NS
Albumin (g/l)	34.8	0.5	32.0	1.0	*
γ -Globulin (g/l)	1.40	0.1	2.10	0.2	**

NS, not significant.

* $P < 0.05$, ** $P < 0.01$ (Student's *t* test).

† For details of diets and procedures, see Table 1 and p. 88.

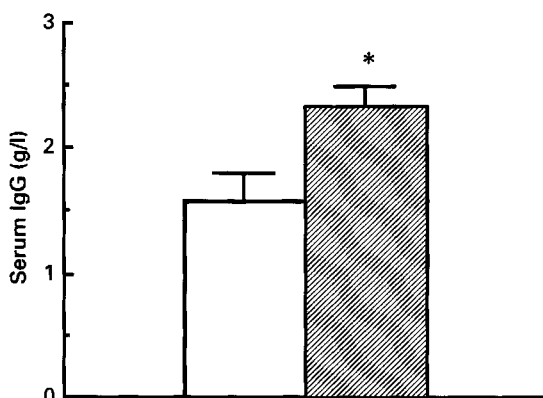


Fig. 2. Serum immunoglobulin G (IgG) levels in mice fed on diets containing casein (□) or pea (*Pisum sativum* var. *Belinda*, ▨) as the source of protein. Values are means for eight mice with their standard errors indicated by vertical bars. The analysis showed that IgG levels were significantly higher in mice fed on pea as the source of protein compared with those fed on casein: * $P < 0.05$ (Student's *t* test). For details of diets and procedures, see Table 1 and p. 88.

Table 5. Splenic-cell subsets (%) of mice given diets containing casein or peas (*Pisum sativum* var. *Belinda*) as the source of protein†
(Mean values with their standard errors for eight mice per dietary group)

	Casein		Pea		Statistical significance
	Mean	SE	Mean	SE	
T lymphocytes (Thy 1 ⁺)	46.9	4.5	66.4	7.1	*
B lymphocytes (H2-Ia ⁺)	34.8	4.0	36.8	7.2	NS
Macrophages (Ly 40 ⁺)	7.7	1.3	8.5	1.7	NS

NS, not significant.

* $P < 0.05$ (Student's *t* test).

† For details of diets and procedures, see Table 1 and pp. 88–89.

Table 6. Mitogenic response (stimulation index, SI) of splenic lymphocytes of mice given diets containing casein or peas (*Pisum sativum* var. *Belinda*) as the source of protein† (Mean values with their standard errors for eight mice per dietary group)

	Casein		Pea		Statistical significance
	Mean	SE	Mean	SE	
PHA (SI)	18.7	6.0	12.2	3.6	NS
Con A (SI)	12.8	4.0	11.2	3.6	NS
LPS (SI)	9.9	5.9	6.7	2.1	NS

PHA, phytohaemagglutinin; Con A, concanavalin A; LPS, lipopolysaccharide; NS, not significant.

† For details of diets and procedures, see Table 1 and pp. 88–90.

Spleen-cell subpopulations and mitogenic response of lymphocytes

No statistically significant differences were observed in the percentage of B-lymphocytes and macrophages after the legume-diet intake. However, an increase was found in the splenic proportion of T-lymphocytes ($P < 0.05$), which could be explained by the immunoregulatory function of T-cells in antibody-dependent humoral response (Table 5).

The proliferative responses to T-cell mitogens (PHA and Con A) and B-cell mitogen (LPS) were slightly depressed in the animals fed on the pea-protein diet (Table 6).

DISCUSSION

There are many studies concerning the influence of dietary protein levels on the development of humoral and cellular immunity (Chandra, 1992, 1993), but only a few reports dealing with the influence of the type and quality of dietary protein on the immune system (Bounous *et al.* 1983; Yamauchi *et al.* 1993). Thus, although legumes are important sources of protein, little is known about the impact of feeding these seeds on the immune response. In this context we have studied a pea seed (*Pisum sativum* var. *Belinda*), which may be used for human nutrition and animal feeding as an important source of protein and other nutrients in different countries (Savage & Deo, 1989).

Mice fed on peas as the source of protein showed a stunting of growth and organ weights at the end of the experimental period, but no changes in feed consumption. These effects could be attributed to a low utilization of different nutrients and the involvement of antinutritional factors (Larralde & Martínez, 1989; Huisman *et al.* 1990). However, small-intestine weights showed a significant increase which some authors have attributed to the potential mitogenic activity of lectins and their capacity to stimulate crypt hyperplasia (Pusztai *et al.* 1986).

Serum Fe and packed cell volume decreased significantly in pea-protein-fed mice. These Fe alterations may involve a decrease in Fe storage, Fe transport and haemoglobin formation (Johnson, 1990). In addition, serum Zn levels, as a valid and useful indicator of the size of the exchangeable Zn pool, showed a significant decrease (King, 1990). The availability of these two minerals from legumes may be reduced by phytates, oxalates, tannins and other organic compounds occurring in legumes (Harland, 1989; Cashman & Flynn, 1991; Rubio *et al.* 1992). This lower bioavailability could have implications for growth, metabolism and immunity (Mengheri *et al.* 1984; Martínez *et al.* 1985; Sherman, 1992). However, serum Cu showed a slight increase in the legume group and the Fe:Cu and Zn:Cu ratios were significantly decreased. This could be explained by interactions among these minerals during absorption. Thus, chelating agents such as phytates and tannins

might decrease Fe and Zn absorption and lead to a reduction in competitive interactions, which could increase Cu absorption (Gordon, 1987).

Serum albumin is the usual index of nutritional status in relation to protein intake (Gibson, 1990). In the present experimental trial, serum levels of this protein were significantly lower in legume-fed mice compared with the casein group. A poor content of S-amino acids reported in these seeds and a reduced nutritional utilization of protein affected by e.g. haemagglutinins and protease inhibitors (Savelkoul *et al.* 1992) could alter plasma protein turnover, which would be linked to a possible situation of subnutrition without external signs, induced by legume intake.

On the other hand, despite the fall in serum albumin levels, serum γ -globulins were markedly increased in mice fed on the diet containing the legume protein. Titres for serum IgG, which accounts for the major proportion of the γ -globulins, were also increased. It is well known that in normal physiological conditions the gut wall prevents the passage of dietary proteins from the intestinal lumen into the blood circulation. However, the chronic exposure of the small intestine to dietary antigens may cause a lesion to the gut mucosa which increases its permeability, allowing the entry of antigens through the impaired host barrier (Perdue & Bienestock, 1991). Thus, the occurrence of antigenic proteins in peas could explain a loss of nutrients as a result of intestinal damage, the decrease in serum albumin levels and the poorer growth performance observed in mice fed on these seeds. Moreover, the rise of IgG titres is consistent with the passage of these antigens through the intestinal barrier.

Several authors have reported that digestive disturbances associated with feeding soya-bean protein may be attributed to immune mechanisms (Barratt *et al.* 1978, 1979). Sissons and co-workers found a positive link between systemic anti-soya antibody titres and the severity of gastrointestinal disorders (Kilshaw & Sissons, 1979; Sissons *et al.* 1989; Sissons & Tolman, 1991). Pre-ruminant calves fed on diets containing high concentrations of soya-bean protein developed long-lasting titres of circulating antibodies specific for soya-bean protein (Barratt *et al.* 1978, 1979). Similarly, early-weaned pigs fed on a diet containing soya-bean protein generated a systemic humoral immune response, which was specific for soya-bean protein (Li *et al.* 1991). Furthermore, young piglets and calves given a raw-pea-based diet developed circulatory antibodies against pea legumin and vicilin (Le Guen *et al.* 1991; Bush *et al.* 1992).

The expression of immune responses to feed antigens by animals depends on the nature of the antigen, the dose as well as the duration of exposure, age, immune status and genetic background of individuals (Crowe & Perdue, 1992; Pollock *et al.* 1994). It is known that the newborn's gastrointestinal tract develops a mucosal barrier against penetration of a great variety and quantity of antigenic substances in food. This transition occurs at a time when systemic and local immunity systems are still immature (Strobel, 1990). Transient humoral and cellular immunodeficiencies have been reported in young piglets or at weaning (Hammerberg *et al.* 1989), while it has been proved that oral tolerance develops in older pigs (Wilson *et al.* 1989). In the present study the mice were recently weaned and, therefore, an immaturity in the immune system could explain the observed systemic humoral immune response.

In sensitized animals, immunological reactions to food can involve different types of mechanism. Although knowledge of the effects of immunologically mediated reactions on gut mucosal morphology and function has progressed rapidly in recent years, it is somewhat scanty and many aspects remain unknown (Strobel, 1990). In the present study the identification of cell subsets by monoclonal antibodies made it possible to identify an increase in the percentage of T-lymphocytes in mice fed on the pea diet. These results suggest a specific role for T-cells, which could be involved in a mucosal-cell-mediated

immune response. The first sign of this reaction is an increase in intra-epithelial lymphocytes followed by an increase in crypt-cell turnover and elongation of the crypt compartment. Depending on the age of the animal, various degrees of villus atrophy have been observed (Strobel, 1990).

On the other hand, the systemic humoral immune response generated by some legume proteins could increase the T-cell subset, which has an immunoregulatory function in antibody-dependent humoral response. Although the percentage of B-lymphocytes did not increase in the pea-fed mice, this may be because a significant increase in B-cells would only occur after polyclonal activation, while the stimulation by some pea antigens would only increase a small percentage of total B-cell clones. No alterations in macrophage:monocyte proportions were found after pea-protein intake, compared with casein protein.

Furthermore, the increase in T-cells could be attributed to a specific effect of some antinutritional factors on the immune system. Thus, lectins when passing from the intestinal lumen into the blood circulation, due to their mitogenic properties, may interact with T-lymphocytes modulating immune responses (Licastro *et al.* 1993). The role of these molecules as immunoenhancing agents in human diseases is being investigated (Wimer, 1990).

In vitro mitogenic assays are performed to study the functional behaviour of lymphocytes and, thus, a reduction in stimulation index may be an index of lymphocyte hyporeactivity (Hudson & Hay, 1989). An impairment in lymphocyte stimulation responses to mitogens as a result of nutritional deficiencies has been reported (Sherman, 1992; Chandra, 1993). Lectins such as PHA and Con A, extracted from legumes, specifically stimulate most T-cell series and T-helper-cells respectively, while B-cell transformation is specifically achieved by the mitogenic agent LPS. In the present study the proliferative responses of splenocytes to PHA, Con A and LPS were lower in mice fed on the legume diet, although no statistical differences were found. Thus, the decrease in the mitogenic response after legume intake could be attributed to the poor utilization of proteins, Fe and Zn observed in this dietary group. Mice fed on other legumes such as faba bean (*Vicia faba*) have also shown a statistically significant decrease in the stimulation index (Martínez *et al.* 1992), which was slightly improved by Zn supplementation (Macarulla *et al.* 1992). Moreover, previous studies in mice fed on a diet containing peas as the source of protein have shown a significant decrease in interleukin-2 (IL-2) activity, which plays a central role in the generation and regulation of the immune response (Larralde *et al.* 1993). Zn-deficient diets have led to impairments in IL-2 activity (Dowd *et al.* 1986) and other immunological variables (Verma *et al.* 1988).

The present study describes a stunting in growth and a low utilization of different nutrients that could be attributed to the occurrence of some antinutritional factors and the poor content of S-amino acids in pea protein. Moreover, the systemic humoral immune response observed and the increase in T-lymphocytes could help to establish a better understanding of the possible role of antigenic proteins in gastrointestinal disorders and the poor individual performance after legume intake.

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REFERENCES

- Barratt, M. E. J., Strachan, P. J. & Porter, P. (1978). Antibody mechanisms implicated in digestive disturbances following ingestion of soya protein in calves and piglets. *Clinical and Experimental Immunology* **31**, 305–312.

- Barratt, M. E. J., Strachan, P. J. & Porter, P. (1979). Immunologically mediated nutritional disturbances associated with soya-protein antigens. *Proceedings of the Nutrition Society* **38**, 143–150.
- Barta, O. & Porciau, S. S. (1984). Electrophoresis. In *Laboratory Techniques of Veterinary Clinical Immunology*, pp. 116–122 [O. Barta, editor]. Springfield, IL: Charles C. Thomas.
- Bounous, G., Létourneau, L. & Kongshavn, P. A. L. (1983). Influence of dietary protein type on the immune system of mice. *Journal of Nutrition* **113**, 1415–1421.
- Bøyum, A. (1983). Isolation of human blood monocytes with Nycodenz. A new non-ionic iodinate gradient medium. *Scandinavian Journal of Immunology* **17**, 429–436.
- Bush, R. S., Toullec, R., Cugant, I. & Guilloteau, P. (1992). Effects of raw pea flour on nutrient digestibility and immune responses in the prerinant calf. *Journal of Dairy Science* **75**, 3539–3552.
- Cashman, K. & Flynn, A. (1991). Effect of tannic acid on iron and zinc absorption from a model food system in sucking rats. *Proceedings of the Nutrition Society* **50**, 185A.
- Chandra, R. K. (1992). Protein–energy malnutrition and immunological responses. *Journal of Nutrition* **122**, 597–600.
- Chandra, R. K. (1993). Nutrition and immunity in serious illness. *Proceedings of the Nutrition Society* **52**, 77–84.
- Crowe, S. E. & Perdue, M. H. (1992). Gastrointestinal food hypersensitivity, basic mechanisms of pathophysiology. *Gastroenterology* **103**, 1075–1095.
- Dowd, P. S., Kelleher, J. & Guillou, P. J. (1986). T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. *British Journal of Nutrition* **55**, 59–69.
- Gibson, R. S. (1990). *Principles of Nutritional Assessment*. New York: Oxford University Press.
- Gordon, D. T. (1987). Interactions among iron, zinc and copper. In *AIN Symposium Proceedings*, pp. 27–31 [O. A. Levander, editor]. Bethesda: American Institute of Nutrition Publications.
- Grant, G., More, L. J., McKenzie, N. H., Stewart, J. C. & Pusztai, A. (1983). A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. *British Journal of Nutrition* **50**, 207–214.
- Hammerberg, C., Schurig, G. G. & Ochs, D. L. (1989). Immunodeficiency in young pigs. *American Journal of Veterinary Research* **50**, 868–874.
- Hankins, C. C., Noland, P. R., Burks, A. W., Connaughton, C., Cockrell, G. & Metz, C. L. (1992). Effect of soy protein ingestion on total and specific immunoglobulin G concentration in neonatal porcine serum measured by enzyme-linked immunosorbent assay. *Journal of Animal Science* **70**, 3096–3101.
- Harland, B. F. (1989). Dietary fibre and mineral bioavailability. *Nutrition Research Reviews* **2**, 133–147.
- Hudson, L. & Hay, F. C. (editors) (1989). *Practical Immunology*. Oxford: Blackwell Scientific Publications.
- Huisman, J., Van der Poel, A. F. B., Van Leeuwen, P. & Verstegen, M. W. A. (1990). Comparison of growth, nitrogen metabolism and organ weights in piglets and rats fed on diets containing *Phaseolus vulgaris* beans. *British Journal of Nutrition* **64**, 743–753.
- Johnson, M. A. (1990). Iron: nutrition monitoring and nutrition status assessment. *Journal of Nutrition* **120**, 1486–1491.
- Kilshaw, P. J. & Sissons, J. W. (1979). Gastrointestinal allergy to soyabean protein in prerinant calves. Antibody production and digestive disturbances in calves fed heated soyabean flour. *Research in Veterinary Science* **27**, 361–365.
- King, J. C. (1990). Assessment of zinc status. *Journal of Nutrition* **120**, 1474–1479.
- Lallès, J. P., Salmon, H., Bakker, N. P. M. & Tolman, G. H. (1993). Effects of dietary antigens on health, performance and immune system of calves and piglets. In *Recent Advances in Antinutritional Factors in Legume Seeds*. EAAP Publication no. 70, pp. 253–270 [A. F. B. Van der Poel, J. Huisman and H. S. Sini, editors]. Wageningen: EAAP.
- Larralde, J., Esparza, M. L. & Martínez, J. A. (1993). Interleukin-2 and T-lymphocytes in mice fed on a legume diet. *Proceedings of the Nutrition Society* **52**, 135A.
- Larralde, J. & Martínez, J. A. (1989). A reappraisal of the nutritional utilization of legumes. *Revista Española de Fisiología* **45** Suppl., 225–232.
- Le Guen, M. P., Tolman, G. M. & Huisman, J. (1991). Antibodies formation against pea proteins in piglets. In *Digestive Physiology in Pigs*, [N. W. A. Verstegen, J. Huisman and L. A. den Hartog, editors]. Wageningen: Pudoc.
- Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., Klemm, R. D., Giesting, D. W., Hancock, J. D., Allee, G. L. & Goodband, R. D. (1991). Measuring suitability of soybean products for early-weaned pigs with immunological criteria. *Journal of Animal Science* **69**, 3299–3307.
- Licastro, F., Davis, L. J. & Morini, M. C. (1993). Lectins and superantigens: membrane interactions of these compounds with T lymphocytes affect immune responses. *International Journal of Biochemistry* **25**, 845–852.
- Macarulla, M. T., Marcos, R., Martínez, J. A. & Larralde, J. (1992). Humoral and cellular mediated immune responses in legume fed mice after zinc supplementation. *Nutrition Research* **12**, 905–914.
- Martínez, J. A., Barcina, Y. & Larralde, J. (1985). Interrelationships between zinc supply and protein source in young and adult rats. *Nutrition Reports International* **32**, 1037–1046.
- Martínez, J. A., Macarulla, M. T., Marcos, R. & Larralde, J. (1992). Nutritional outcome and immunocompetence in mice fed on a diet containing raw field beans (*Vicia faba*, var. minor) as the source of protein. *British Journal of Nutrition* **68**, 493–503.

- Mengheri, E., Bises, G. & Gaetani, S. (1984). Cell mediated immune response in rats fed diets with increasing amount of phytate. *Nutrition Reports International* **32**, 1435-1445.
- Perdue, M. H. & Bienestock, J. (1991). Immunophysiology of the gut. *Current Opinion in Gastroenterology* **7**, 421-431.
- Pollock, J. M., Rowan, T. G., Dixon, J. B. & Carter, S. D. (1994). Level of nutrition and age at weaning: effects on humoral immunity in young calves. *British Journal of Nutrition* **71**, 239-248.
- Pusztai, A., Grant, G. & Oliveira, J. T. A. (1986). Local (gut) and systemic responses to dietary lectins. *IRCS Medical Science* **14**, 205-208.
- Rubio, L. A., Grant, G., Bardocz, S., Dewey, P. & Pusztai, A. (1992). Mineral excretion of rats fed on diets containing faba beans (*Vicia faba* L.) or faba bean fractions. *British Journal of Nutrition* **67**, 295-302.
- Savage, G. P. & Deo, S. (1989). The nutritional value of peas (*Pisum sativum*). A literature review. *Nutrition Abstracts and Reviews (Series A)* **59**, 65-87.
- Savelkoul, F. H. M. G., Van der Poel, A. F. B. & Tamminga, S. (1992). The presence and inactivation of trypsin inhibitors, tannins, lectins and amylase inhibitors in legume seeds during germination. A review. *Plant Foods for Human Nutrition* **42**, 71-85.
- Sherman, A. D. (1992). Zinc, copper, and iron nutriture and immunity. *Journal of Nutrition* **122**, 604-609.
- Sissons, J. W., Pedersen, H. E., Duvaux, C., Heppell, L. M. J. & Turvey, A. (1989). Gut dysfunction and diarrhoea in calves fed antigenic soybean protein. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, pp. 359-363 [J. Huisman, T. F. V. Van der Poel and I. E. Liener, editors]. Wageningen: Pudoc.
- Sissons, J. W. & Tolman, H. (1991). Antinutritional properties of soyabean antigens in calves. In *Toxic Factors in Crop Plants*, pp. 632-685 [J. P. F. D'Mello and C. M. Duffus, editors]. London: Butterworth.
- Smith, J. C. Jr, Holbrook, J. T. & Danford, D. E. (1985). Analysis and evaluation of zinc and copper in human plasma and serum. *Journal of the American College of Nutrition* **4**, 627-638.
- Strobel, S. (1990). Immunologically mediated damage to the intestinal mucosa. *Acta Paediatrica Scandinavica* **365**, Suppl., 46-57.
- Thompson, J. C. & Mottola, A. (1984). Kinetics of the complexation of iron (II) with Ferrozine. *Analytical Chemistry* **56**, 755-758.
- Verma, P. C., Gupta, R. P., Sadana, J. R. & Gupta, R. K. P. (1988). Effect of experimental zinc deficiency and repletion on some immunological variables in guinea-pigs. *British Journal of Nutrition* **59**, 149-154.
- Walker, W. A. (1992). Adverse food reactions in childhood: summary and future directions. *Journal of Pediatrics* **121**, S4-S6.
- Wilson, A. D., Stokes, C. R. & Bourne, F. J. (1989). Effect of age on absorption and immune responses to weaning or introduction of novel dietary antigens in pigs. *Research in Veterinary Science* **46**, 180-186.
- Wimer, B. M. (1990). Characteristics of PHA-L4, the mitogenic isolectin of phytohemagglutinin, as an ideal biologic response modifier. *Molecular Biotherapy* **2**, 4-17.
- Yamauchi, F. & Suetsuna, K. (1993). Immunological effects of dietary peptide derived from soybean protein. *Journal of Nutritional Biochemistry* **4**, 450-457.