TOXICITY STUDIES OF CRUCIFERIN-RICH AND NAPIN-RICH CANOLA PROTEIN ISOLATES

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Abstract

The objective of the studies was to evaluate the safety of both a cruciferin-rich canola protein isolate (Puratein®) and a napin-rich canola protein isolate (Supertein™) when fed as a protein source at various dietary levels to rats for a period of 13-weeks. The cruciferin-rich and napin-rich canola protein isolates were obtained by protein extraction from canola meal using a proprietary process. The no observed adverse effect level (NOAEL) for both protein isolates was the highest fed level of 20% which as a mean daily intake for 90 days was equivalent to 12.5 g/Kg BW/day for males and 15.0 g/Kg BW/day for females for napin-rich canola protein isolate and to 11.2 g/KgBW for males and 14.1 g/Kg BW/day for females for cruciferin-rich canola protein isolate. Under the conditions of the studies cruciferin-rich canola protein isolate and napin-rich canola protein isolate were considered safe.

Introduction

Canola meal is currently almost exclusively used as animal feed. However, a recent study (Bos et al. 2006) suggests that canola protein has a very high metabolic utilization in humans. A proprietary process developed by Burcon NutraScience Corp. has made possible the production of canola protein isolates suitable for human consumption. This process results in two canola protein isolates, one of which being rich in cruciferin (commercially know as Puratein®) and the other one being rich in napin (commercially known as Supertein™). Both of these protein isolates have distinctive ingredient functionality properties which make them interesting for a variety of foods. For example, Puratein® has remarkable emulsifying/binding characteristics and Supertein™ is completely soluble and can be added to transparent acidic beverages.

Only a few toxicological feeding studies are available which address the safety of canola protein products (Loew et al., 1976; Plass et al., 1992). However, toxicological data of canola protein products manufactured by current technologies is limited. Since these canola proteins are targeted for human consumption, their toxicological evaluation becomes imperative. Therefore, the objective of the present study was to evaluate the safety of cruciferin-rich and napin-rich canola protein isolates.

Materials & Methods

The study was conducted as described in detail by Mejia et al., 2009. Sprague Dawley [Crl:CD (SD)] rats (Charles River Laboratories, Raleigh, NC) were fed ad libitum with an AIN-93 G based protein-free diet (Purina Test Diet 5T6X, PMI Nutrition International) containing respectively 5%, 10% and 20% (w/w) Puratein® and Supertein™ (test articles) or 20% (w/w) vitamin-free casein (control article) for 90 days. Protein levels were adjusted to 18% with vitamin-free casein in all groups. Body weights, food consumption, locomotor activity and behavioral and clinical pathology parameters were recorded at various study intervals, followed by macroscopic examination, measurement of organ weights and microscopic examination at study termination.

Supertein™ and Puratein® were produced using a proprietary process including extraction of the protein with a salt solution. The extracted protein was then subjected to ultrafiltration followed by a dilution step which caused the separation of the cruciferin fraction and the napin fraction. The cruciferin fraction was removed in the form of a protein micellar mass and was spray dried to form Puratein®. The supernatant from the dilution containing the napin fraction was further processed and finally spray dried to generate Supertein™.

The proteins were analyzed for their chemical composition and microbiological characteristics as well as for other substances of toxicological concern like glucosinolates, erucic acid or phytic acid.

Results & Discussion

As can be seen in Table 1, both canola protein products were protein isolates. Potential contaminants were either not detected or not of toxicological concern.

Table 1: Chemical composition, microbiological and potential contaminants analysis of both canola protein products.

	94.9	86.7
Fat (%)	0.22	0.29
Ash (%)	3.36	3.96
Moisture (%)	7.30	7.10
Carbohydrates (%)	0.22	0.94
Total Glucosinolates* (µmol/g)	1.22	0.80
Erucic Acid (µg/g)	<25	<25
Phytic Acid (%)	0.32	3.34
Total Phenolic Acids** (%)	0.40	0.26
Heavy Metals (Cd, Pb, Hg, As) (ppm)	0.08	0.04
Lead	ND	ND
Standard Plate Count (cfu/g)	2000	600
Salmonella	Negative	Negative
E. coli	Negative	Negative
S. aureus	Negative	Negative

* Includes levels of 15 different identified glucosinolates
** Sum of Sinapic (>90%), p-coumaric and ferulic acids. Others were not detected
ND: Not Detected

There was no Puratein® or Supertein™ related mortality or moribundity noted during the study. There were no test article related effects on clinical observations, ophthalmic examinations, functional observational battery parameters, motor activity, clinical pathology parameters, macroscopic or microscopic findings for Supertein™ or Puratein®.

A slightly higher absolute (males only) and relative to body weight thyroid/parathyroid weight ratio were noted in the 20% Puratein®-treated males and females. However, there were no corresponding histopathology findings.

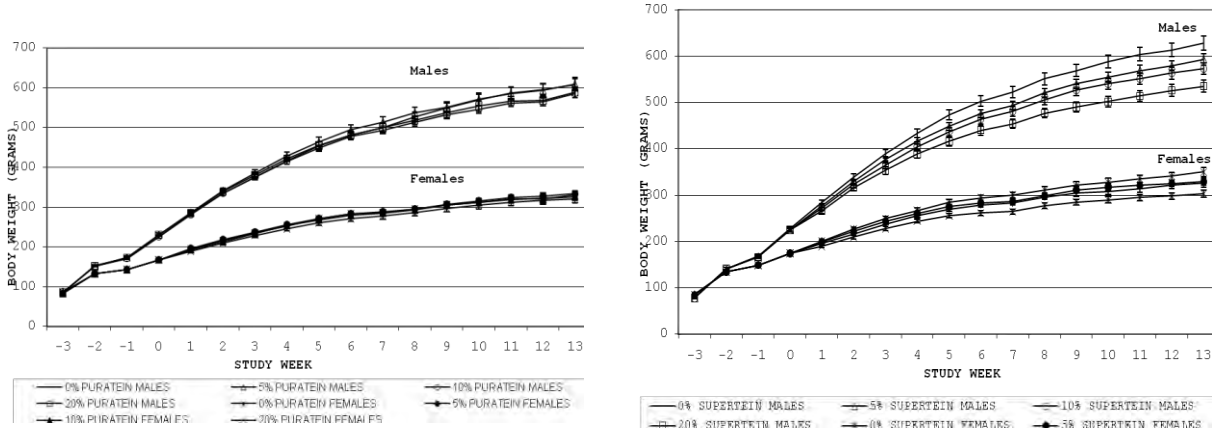

Figure 1: Body weight for male and female rats throughout the period of study (left: Puratein, right: Supertein). Feeding of test articles at dietary levels of 0%, 5%, 10% and 20% started at week 0.

Table 2. Weekly Food Consumption (g/kg BW/day) during the Period of Study for Male and Female Rats

Studied Week	Dietary Level of Test Article Puratein®				Dietary Level of Test Article Supertein™			
	0%	5%	10%	20%	0%	5%	10%	20%
Males								
0-1	97 ± 5.6†	92 ± 7.6*	90 ± 5.3**	92 ± 6.7*	27± 3†	25 ± 2	24 ± 3*	23 ±
1-2	83 ± 5.3	82 ± 5.8	80 ± 3.8	81 ± 4.3	27 ± 3	26 ± 3	25 ± 3*	25 ± 3*
2-3	74 ± 3.2	74 ± 8.7	74 ± 4.6	67 ± 10.1*	28 ± 3	27 ± 2	26 ± 3*	26 ± 3*
3-4	68 ± 5.3	65 ± 4.0	67 ± 3.3	66 ± 4.7	30 ± 3	28 ± 2	27 ± 3*	27 ± 3*
4-5	60±5.7	60±3.9	61±4.2	60±3.1	29 ± 3	26 ±	26 ±	25 ±
5-6	57±5.2	55±3.8	56±3.2	54±4.8	29 ± 3	27 ± 3*	26 ±	25 ±
6-7	50±5.0	49±3.4	49±3.8	51±4.2	26 ± 3	25 ± 2	24 ± 3	24 ± 2
7-8	52±5.8	50±3.6	51±3.3	49±5.6	28 ± 3	27 ± 2	27 ± 2	26 ± 2*
8-9	49±3.6	46± 5.3	46±5.4	46±6.4	27 ± 4	27 ± 3	26 ± 3	24 ± 3*
9-10	46±2.9	44±2.6	45±2.5	47±3.7	29 ± 5	26 ± 2*	26 ±	26 ±
10-11	44±3.3	43±2.9	43±4.5	41±4.3	28 ± 3	26 ± 3	26 ± 3	26 ± 3
11-12	42±3.2	40±4.0	42±4.6	43±5.6	27 ± 3	26 ± 3	26 ± 2	26 ± 4
12-13	40±2.9	41±4.1	40±7.9	36±8.1	27 ± 3	26 ± 3	25 ± 3	25 ± 3
Females								
0-1	101±10.9	101±9.9	97±9.0	96±9.8	20 ± 3	20 ± 2	19 ± 2	17 ±
1-2	92±11.4	86±8.3	89±10.7	90±8.3	20 ± 3	19 ± 3	18 ± 2*	18 ±
2-3	90±9.3	87±12.3	88±12.6	87±10.3	22 ± 3	21 ± 3	21 ± 3	20 ± 2
3-4	81±13.8	79±8.5	80±8.2	81±10.0	22 ± 3	21 ± 3	21 ± 3	21 ± 2
4-5	80±10.2	73±6.5	73±8.4	76±10.4	22 ± 2	21 ± 3	20 ± 2	20 ± 3
5-6	73±9.3	66±6.1	66±6.7	68±6.1	20 ± 3	19 ± 2	19 ± 2	18 ±
6-7	64±12.8	58±7.4	63±11.2	64±8.4	19 ± 3	18 ± 2	17 ± 2	17 ± 3
7-8	65±10.9	63±8.3	64±6.6	67±7.2	20 ± 3	20 ± 2	20 ± 3	20 ± 3
8-9	62±9.0	60±7.4	62±4.8	62±5.0	21 ± 3	20 ± 3	19± 3	19 ± 2
9-10	60±8.0	57±5.9	57±4.3	61±6.9	21 ± 3	20 ± 3	19 ± 2*	19 ± 2
10-11	57±8.4	56±5.3	56±7.5	58±6.9	20 ± 3	19 ± 3	18 ± 2	19 ± 2
11-12	54±7.2	54±4.4	54±4.8	57±7.6	20 ± 3	19 ± 3	19 ± 3	18 ± 2
12-13	53±10.9	50±4.6	50±6.2	52±8.6	20 ± 4	19 ± 3	18 ± 3	18 ± 2

† X ± S.D.
* p <0.05 (Dunnett's Test)
** p <0.01 (Dunnett's Test)

Male and female rats fed Supertein™ experienced lower body weight gains throughout the study, in comparison to control animals (Figure 1). The cumulative mean body weight gains were significantly lower than controls ($p < 0.05$) in the 10% Supertein™-treated males and 20% Supertein™-treated males and females starting the first week of the study. As a result, at the end of the study, compared to the control group, mean body weights were 8.8% and 14.8% lower for male animals in the 10 and 20% Supertein™-treated groups, respectively. Similarly, for the 20% Supertein™ treated females, the mean body weights were 13.4% lower than control values. Despite this lower growth, the body weights of the Supertein™-treated animals were within the historical control range for rats of similar age and strain. As can be seen in Table 2, rats fed diets containing 10% and 20% Supertein™ consumed less food than the control group. This was particularly noticeable in the early weeks of the study. The lower food consumption corresponded to the lower body weight gain seen throughout the study. A separate palatability and preference study (data not shown) suggested that the lower food consumption was due to a lower palatability of the test article. For rats fed the Puratein® diets food consumption and body weights were not affected.

Conclusion

There were no test article related effects on clinical observations, functional observational battery parameters, motor activity, clinical pathology parameters, or ophthalmic examinations for cruciferin-rich or napin-rich canola protein isolates. The no observed adverse effect level (NOAEL) for both protein isolates was the highest fed level of 20% which as a mean daily intake for 90 days was equivalent to 12.5 g/Kg BW/day for males and 15.0 g/Kg BW/day for females for napin-rich canola protein isolate and to 11.2 g/KgBW for males and 14.1 g/Kg BW/day for females for cruciferin-rich canola protein isolate. Under the conditions of the studies cruciferin-rich canola protein isolate (Puratein®) and napin-rich canola protein isolate (Supertein™) were considered safe.

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