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Nutritional quality assessment of two potato intraspecific somatic hybrid lines

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RESEARCH ARTICLE

Abstract

The nutritional quality of new potatoes from intraspecific somatic hybrids called CN1 and CN2 was investigated and compared to that of the conventional Nicola and BF15 varieties. The chemical composition of tubers was determined to ascertain substantial equivalence. No significant difference in ash and mineral levels was noticed between all the potato samples. However, some differences were observed in dry matter, starch, soluble sugar, protein and lipid contents between hybrid potatoes and commercial varieties, but all values were within the normal ranges reported in the literature. The hybrid potatoes as well as the Nicola and BF15 varieties were then separately added to rat diet at a level of 30% (w/w). Animals were divided into five groups of 5 rats each. The rat group fed the standard diet served as control. The responses of rats fed diets containing hybrid potatoes were compared to those fed conventional potato varieties. Overall health, weight gain, food consumption and digestibility, aspects and relative weight of organs were comparable between rats fed with hybrid and with commercial potatoes. The results obtained show that the nutritional quality of the CN1 and CN2 potatoes is similar to that of commercial varieties.

Keywords: *Solanum tuberosum*, tuber composition, food value

1. Introduction

Potato (*Solanum tuberosum* L.) is a major crop in many parts of the world. It has wide food versatility and a full complement of nutrients (Burlingame *et al.*, 2009). Because its genetic basis is rather restricted, several strategies are currently being undertaken for the genetic improvement of cultivated genotypes including widely used somatic hybridisation and genetic transformation.

Somatic hybridisation carries considerable potential for the future since, unlike transgenesis, it is more efficient in transferring polygenic traits of disease resistance than transgenesis. This technique can be based on the use of wild *Solanum* species as a unique source of useful genes to enlarge the cultivated gene pool (Simko *et al.*, 2007). However, intraspecific hybridisation can give rise to a functional heterozygosity resulting from parental genome recombination events leading to the improvement of disease resistance (Cooper-Bland *et al.*, 1994, 1996; Rasmussen *et al.*, 1998). Somatic hybrid lines can thus be considered as new cultivars if they are in agreement with required agronomic criteria.

In a previous study, intraspecific somatic hybrids (CN) were produced by protoplast fusion between dihaploid potato lines of the Nicola and Cardinal varieties (Nouri-Ellouz *et al.*, 2006). Moreover, interspecific somatic hybrids were produced by protoplast fusion between a dihaploid potato line of the BF15 variety and the wild potato species *Solanum berthaultii* (Bidani *et al.*, 2007). These hybrid lines showed improved resistance to *Pythium aphanidermatum* and potato virus Y (Nouri-Ellouz *et al.*, 2006, 2016). Such resistance traits can be of great interest to increase potato crop production. However, consumers are aware of the potential of biotechnological methods applied to modify crop plants used as food for humans and feed for livestock. Somatic hybridisation, as well as breeding technologies and genetic engineering techniques, can cause the appearance of undesired traits and/or changes in the chemical composition of newly produced genotypes (Esposito *et al.*, 2002; Väänänen *et al.*, 2005). Even though these somatic hybrids originating from sexually compatible parents are not considered genetically modified material (EFSA-GMO, 2012), a precautionary study of the nutritional quality of an interspecific somatic hybrid was conducted in a previous report (Nouri-Ellouz *et al.*, 2015). The present study focuses on the CN intraspecific somatic hybrids. Their nutritional quality was assessed to ensure nutritional equivalence with commercial varieties. The experiments envisaged were based on recommendations used to establish substantial and nutritional equivalence for transgenic crops although these hybrid lines are excluded from the GMO legislation (Codex, 2003; EFSA-GMO, 2008, 2012; Parrot *et al.*, 2010). The assessment of substantial equivalence and the *in vivo* evaluation of nutritional quality of the intraspecific somatic hybrids were performed and are described here in relation to conventional varieties.

2. Materials and methods

Potato lines

The CN1 and CN2 intraspecific somatic hybrid lines were produced by protoplast fusion of dihaploid Cardinal and Nicola potato lines (Nouri-Ellouz *et al.*, 2006). These hybrid lines as well as the commercial Nicola and BF15 varieties were multiplied *in vitro* by subculturing nodal shoot segments on MS basal medium (Murashige and Skoog, 1962) supplemented with vitamins (Morel and Wetmore, 1951), sucrose (30 g/l) and solidified by addition of agar (8 g/l). Cultures were performed in a growth chamber at 20 °C, with a day length of 12 h under 62 μE/m^{2}s light intensity. Rooted plantlets from the different lines were transferred to a greenhouse for tuber production. The culture covered the period of February-May in 2011. The harvested minitubers were used for field cultures conducted in the period from October 2011 to January 2012 using conventional agronomic practices (http://www.ctpt.com.tn). Tubers from the second generation were analysed. The Nicola and BF15 varieties were used as controls.

Analytical assays

Autoclaved (121 °C, 20 min) field-produced tubers were dried and then subjected to the analyses of dry matter, ash, protein, fat, soluble sugars, starch and mineral parameters. These values were determined in triplicate. The dry material was measured using the method described by Väänänen *et al.* (2005). For ash, the sample was ignited at 550 °C for 4 h in an electric furnace and quantified (Rogan *et al.*, 2000). Total nitrogen and proteins were determined by the Kjeldahl method. Proteins were calculated from the total nitrogen using N×6.25 (Hashimoto *et al.*, 1999a). Fat was extracted from samples in petroleum ether. The extracts were evaporated, dried and quantified as fat (Rogan *et al.* 2000). Soluble sugars were determined as described by Dubois *et al.* (1956). Starch content was evaluated by an enzymatic colourimetric method (Khabou *et al.*, 1996). Mineral (calcium, potassium, phosphorus, sodium, magnesium, iron, manganese and zinc) contents were determined after acid hydrolysis (Bidani *et al.*, 2007).

Animals and diets

The study was conducted on twenty-five adult female rats of the Wistar strain from a Tunisian breeding centre (SIPHAT, Ben Arous, Tunisia). Water and diet were provided to rats *ad libitum*. The standard diet (ALCO CGV2; STPA, Nabeul, Tunisia) was primarily composed of 48.81% starch, 7.15% soluble sugars, 9.1% proteins and 1.27% lipids. The ground maize grain that accounts for 54% in the standard diet was composed of 77.9% starch, 2.4% soluble sugars, 2.9% proteins and 2.3% fats. After acclimatisation to the laboratory conditions, the animals were randomly divided into five groups of five rats each, housed in cages and maintained under standard temperature conditions (22±3 °C) and humidity (50±10%) with 12 h light/day. They were treated as follows: group 1, the control group, received the standard diet; groups 2 and 3 were fed with tubers of the Nicola and BF15 varieties respectively added to the commercial diet in the proportion of 30% (w/w) as an alternative primary source of starch, replacing a part of the ground maize grain supplementation; groups 4 and 5 were fed with either CN1 or CN2 hybrid tubers added in the same proportion as those of groups 2 and 3. All tubers were autoclaved, dried and ground.

The total calories of diets were assessed as recommended by the United States Department of Agriculture (USDA, 1975). The energy of potato diets was adjusted to the same energy value by the addition of casein (Sigma, Saint Quentin Fallavier, France) and maize starch (Vanoise; GIAS, Sfax, Tunisia) to reach the energy value of the standard diet (Table S1). The protein contents of the diets containing hybrid potatoes had been adjusted to the protein proportion in the diet with Nicola tubers according to recommendations reported by Zdunczyk *et al.* (2005).

The protocol of the animal experiments was performed according to the general guidelines for the use and care of living animals during scientific investigations (Council of the European Communities, 1986). It was approved by the Ethical Committee of our university.

Animal growth and digestibility analyses

The body weights of the rats of all the groups were measured daily for 30 days. The daily feed intake, and the macronutrient digestibility were assessed as reported by Ennouri *et al.* (2006). The food coefficient ratio (FCR), the protein efficiency ratio (PER) and the apparent digestibility coefficient (ADC) were determined as described by Zdunczyk *et al.* (2005).

Blood biochemical and haematological analyses

At the end of the feeding period (30 days), all rats were euthanised by cervical decapitation to avoid stress. Blood samples were collected and stabilised using heparin for blood biochemical analyses (Makni *et al.*, 2008). Plasma samples were obtained by centrifugation at 2,500×*g* for 10 min. Different biochemical parameters were measured: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase, total protein, total bilirubin, blood urea nitrogen (BUN), creatinine, glucose, cholesterol, triglycerides, sodium, potassium, chlorine, calcium and phosphate. All analyses in blood plasma were performed on an Abbott Architect ci 4100 Clinical Chemistry analyser (Abbott, Wiesbaden, Germany).

The following haematology parameters were assessed in blood samples treated with K3 EDTA anticoagulant (FL Medical, Torreglia, Italy): white blood cells, lymphocytes, red blood cells, platelets, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) using a Sysmex KX-21N Haematology analyser (Sysmex, Norderstedt, Germany).

Histopathological studies

After decapitation of the rats, liver, kidneys and spleen were excised and weighed. Samples from these organs were fixed in buffered formalin solution and embedded in paraffin (Makni *et al.*, 2008). Sections of 4-5 µm were stained with haematoxylin-eosin for liver and kidney examination. Spleen sections were stained using Perls colouration. Organ injuries were evaluated on organ sections using the scores reported by Momma *et al.* (2000) where a scale was used for none (0), slight (1), mild (2), moderate (3) and severe (4) abnormalities. This study was conducted with the aid of pathologists in a hospital (CHU Habib Bourguiba, Sfax, Tunisia).

Statistical analysis

Mean values were obtained by averaging independent measurements and data are expressed as means ± standard deviation. The data were analysed statistically by the one way ANOVA test using the statistical software GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Differences were considered significant at $P<0.05$.

3. Results

Morphological characters of hybrid tubers

The hybrid tubers were basically distinguished from the commercial Nicola and BF15 potatoes by their pink skin colour, intermediate between the red and the yellow skin of the parental Cardinal and Nicola tubers, respectively. As for the commercial tubers, the hybrid tubers presented a yellow flesh and regular shape with shallow eyes.

Compositional analysis of tubers

To estimate the nutritional value of hybrid potatoes the chemical composition of hybrid tubers was analysed and compared to the Nicola and BF15 potato varieties. The hybrid lines exhibited higher dry matter contents than those of commercial potatoes (Table 1). However, lower protein and soluble sugar contents were measured in hybrid tubers compared to the BF15 and Nicola varieties, but they remain within the potato average (Gravoueille, 1996; Langkilde *et al.*, 2012).

The level of the starch content, the major component of potato dry matter was higher in the hybrid lines than in the commercial varieties. Significant differences in fat contents were observed between the CN1 and CN2 hybrid potatoes. Although a lower fat content was measured in CN1 tubers as also in commercial Nicola potatoes, CN2 tubers displayed the highest fat level. No significant differences in ash and mineral contents were observed between hybrid lines and commercial varieties.

Feeding study on rats

The study was conducted on rats fed diets containing 30% (w/w) of hybrid or control potatoes (Table S1). Throughout the study (30 days), no apparent adverse effects on animal behaviour were observed. No noticeable changes were observed in their behaviour and activity during the course of the study.

Effect of diets on feed intake and body weight

The differences observed in feed intake and body weight gain (Table 2) were not significant between the groups. However, a slight increase in the FCR was observed in rats fed with CN2 hybrid tubers (group 5). The difference observed in the digestibility of the diet, the apparent digestibility coefficient and the PER between the different rat groups also remained insignificant. These data confirm nutritional equivalences between the different diets.

Table 1. Chemical composition of autoclaved and dried potato tubers (on a dry matter basis).[1]

Parameter	Commercial varieties	Hybrid lines		
	BF15	Nicola	CN1	CN2
Dry matter (%)[2]	17.66±2.25[b]	19.44±2.28[b]	25.07±0.20[a]	20.90±1.20[b]
Starch (%)	58.74±1.68[c]	60.88±1.74[c]	76.24±0.87[a]	67.35±1.09[b]
Protein (%)	6.03±0.40[a]	6.19±0.58[a]	3.80±0.06[c]	4.69±0.05[b]
Lipid (%)	0.68±0.12[b]	0.23±0.10[c]	0.21±0.08[c]	1.45±0.30[a]
Soluble sugar (%)	6.28±0.98[a]	6.99±0.74[a]	3.06±0.77[b]	3.41±0.10[b]
Ash (%)	6.30±0.92	6.89±0.42	7.54±0.50	6.70±0.01
Mg (g/kg)	1.940±0.064	1.874±0.009	2.116±0.092	1.555±0.122
Fe (g/kg)	0.021±0.008	0.028±0.006	0.014±0.001	0.048±0.018
Na (g/kg)	1.309±0.059	2.012±0.582	1.510±0.022	1.017±0.202
Ca (g/kg)	0.875±0.036	0.901±0.080	0.940±0.064	0.970±0.055
Zn (g/kg)	0.022±0.004	0.016±0.001	0.022±0.007	0.016±0.002

[1] Each value is presented as the mean ± standard deviation. The absence of superscripts on the same line indicates the absence of significant differences ($P<0.05$). Values with different superscripts on the same line indicate a significant difference between potato lines.
[2] On a fresh matter basis.

Table 2. Diet intake, body weight gain and digestibility in rats fed different diets with or without potatoes.[1]

Parameter	Group 1, standard diet (control)	Group 2, diet with BF15	Group 3, diet with Nicola	Group 4, diet with CN1	Group 5, diet with CN2
Diet intake (g/rat/day)	11.37±1.37	11.64±2.19	11.52±2.58	11.97±3.28	11.05±2.68
Faeces (g/rat/day)	2.28±0.42	2.55±0.72	2.19±0.44	2.66±1.19	2.17±0.45
Digestibility (%)[2]	79.68±4.37	77.83±5.79	79.61±7.97	76.90±8.43	78.65±8.93
Initial body weight (g)	144.60±11.82	144.20±21.59	142.40±16.86	142.80±12.15	149.60±6.46
Final body weight (g)	175.40±14.84	181.40±21.04	175.40±24.09	180.80±11.21	173.00±8.63
Body weight gain (g/rat/day)	1.02±0.26	1.24±0.12	1.10±0.29	1.26±0.22	0.78±0.13
FCR[3]	11.08±1.33[b]	9.39±1.76[b]	10.48±2.35[b]	9.46±2.60[b]	14.16±3.44[a]
PER[4]	0.77±0.19	0.80±0.08	0.79±0.21	0.85±0.15	0.59±0.10
N intake (mg/rat/day)	212.79±23.38	246.85±22.10	222.57±39.36	238.33±94.70	209.25±52.61
N in faeces (mg/rat/day)	53.24±9.48	55.49±4.35	45.77±8.23	63.93±29.34	46.04±11.05
ADC (%)[5]	74.63±5.76	77.40±2.44	78.90±5.33	72.59±5.91	76.81±7.71

[1] Each value is presented as the mean ± SD. The absence of superscripts on the same line indicates the absence of significant differences ($P<0.05$). Values with different superscripts on the same line indicate a significant difference between rat groups.
[2] Digestibility = ((diet intake – faeces) / diet intake) × 100.
[3] FCR = food coefficient ratio, g diet intake/g body weight gain.
[4] PER = protein efficiency ratio, g body weight gain/g protein intake.
[5] ADC = apparent digestibility coefficient, ((N intake – N faeces)/ N intake) × 100.

Biochemical and haematological analyses

The effect of supplemented hybrid potatoes in rat diets on blood parameters was investigated at the end of the treatment (30 days) and compared to the effect of conventional varieties (Tables 3 and 4). No significant differences were revealed between the different rat groups in glucose, total cholesterol, triglyceride, creatinine and protein levels as well as in AST and ALP enzyme activities (Table 3). Total bilirubin was not detected in all rat groups. Only a slight significant decrease in the ALT activities was observed in rats fed the diet supplemented with CN2

Table 3. Blood biochemical parameters in rats fed different diets with or without potatoes.[1]

Parameter[2]	Group 1, standard diet (control)	Group 2, diet with BF15	Group 3, diet with Nicola	Group 4, diet with CN1	Group 5, diet with CN2
AST (IU/l)	240±52.24	250.40±62.62	304.60±54.95	252.40±60.93	208.40±61.45
ALT (IU/l)	68.40±6.87[a]	68.00±8.88[a]	67.80±11.73[a]	60.60±2.60[ab]	49.60±6.80[b]
ALP (IU/l)	231.20±37.43	180.60±26.12	175.40±46.50	208.20±26.80	165.80±71.11
LDH (IU/l)	1,291±348	1,605±562	1,680±547	1,443±317	1,158±575
Total protein (g/dl)	6.56±0.23	6.60±0.20	6.44±0.89	6.76±0.35	6.8±0.55
Glucose (mg/dl)	118.21±4.86	111.00±8.50	114.24±11.43	127.94±13.30	118.93±7.75
Total cholesterol (mg/dl)	69.50±3.86	57.91±10.57	50.96±15.77	59.46±8.88	68.72±11.05
Triglycerides (mg/dl)	72.39±14.60	65.84±14.19	78.76±18.24	89.21±34.55	60.71±8.95
Creatinine (mg/dl)	0.56±0.02	0.58±0.03	0.55±0.08	0.55±0.03	0.60±0.05
BUN (mg/dl)	19.77±1.52[ab]	20.21±3.11[ab]	17.36±4.01[b]	20.66±2.71[ab]	25.03± 3.51[a]
Na (mmol/l)	139.40±2.07	138.20±0.83	136.20±13.59	138.40±5.36	140.20±1.92
K (mmol/l)	5.94±0.39	6.54±0.53	6.18±0.83	6.40±0.20	6.88±1.32
Cl (mmol/l)	99.20±1.78	98.40±1.14	97.00±8.51	99.20±4.20	102.80±1.30
Ca (mg/dl)	9.91±0.29	9.50±0.47	9.57±0.90	9.97±0.41	9.67±0.98
P (mg/dl)	5.84±0.43[bc]	6.52±0.40[b]	6.25±1.05[b]	7.88±0.56[a]	5.01±0.58[c]

[1] Each value is presented as the mean ± SD. The absence of superscripts on the same line indicates the absence of significant differences (*P*<0.05). Values with different superscripts on the same line indicate a significant difference between rat groups.

[2] ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; LDH = lactate dehydrogenase; P = phosphate.

Table 4. Haematological parameters in rats fed different diets with or without potatoes.[1]

Parameter[2]	Group 1, standard diet(control)	Group 2, diet with BF15	Group 3, diet with Nicola	Group 4, diet with CN1	Group 5, diet with CN2
RBC ($\times 10^6$/µl)	6.62±0.27	6.59±0.28	6.61±0.62	6.87±0.16	7.15±0.43
HCT (%)	36.88±1.83	37.32±2.07	37.46±3.20	38.12 ±1.28	39.08±2.38
HGB (g/dl)	12.40±0.56	12.54±0.58	12.52±0.74	12.90±0.31	12.60±0.57
MCV (fl)	55.70±1.33	56.62±1.23	56.70±1.45	55.50±1.42	54.62±0.25
MCH (pg)	18.70±0.46[a]	18.92±0.21[a]	19.00±0.89[a]	18.78±0.55[a]	17.62±0.40[b]
MCHC (g/dl)	33.64±0.38[a]	33.46±0.40[a]	33.48±0.97[a]	33.84±0.61[a]	32.24±0.79[b]
WBC ($\times 10^3$/µl)	9.92±1.57	10.18±2.33	9.36±3.07	13.08±2.24	10.38±2.00
LYM ($\times 10^3$/µl)	7.56±1.40	7.54±1.86	7.06±2.83	10.40±1.22	7.62±1.59
PLT ($\times 10^3$/µl)	1,017±87	953±66	935±90	1,064±71	1,029±60

[1] Each value is presented as the mean ± standard deviation. The absence of superscripts on the same line indicates the absence of significant differences (*P*<0.05). Values with different superscripts on the same line indicate a significant difference between rat groups.

[2] HCT = haematocrit; HGB = haemoglobin; LYM = lymphocytes; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; PLT = platelets; RBC = red blood cells; WBC = white blood cells.

potatoes (group 5) compared to rats fed the diet with commercial varieties (groups 2 and 3) or with standard diet (group 1). The data obtained for BUN also showed some difference between the CN2 hybrid and the commercial potato diets. Indeed, the rats fed with CN2 tubers (group 5) exhibited a significant increase in this parameter when compared to rats fed Nicola potato (group 3). Nevertheless, the BUN value remained at the same level as that observed in the control rats (group 1) and the other treated rats (groups 2 and 4). A difference between hybrid potato diets was also revealed in mineral contents of the plasma rats. Supplementation with CN2 potato in the diet led to a significant decrease in phosphate (group 5) while an increase in this parameter was measured in rats fed with CN1 potato (group 4) compared to those fed with BF15 (group 2) and Nicola tubers (group 3). However, the plasma phosphate level of rats fed with either standard (group 1) or CN2 supplemented diet (group 5) did not show any significant difference.

The results of the haematological parameter analyses (Table 4) indicate that there were no significant differences between the rat groups fed with potatoes (groups 2, 3, 4 and 5) and the control group (group 1) for most of the parameters. Only a slight decrease in MCH and MCHC was observed in rats fed with CN2 hybrid potatoes (group 5).

Organ weights and histopathological analyses

Liver, kidney and spleen weights are presented in Table 5. Although some significant differences were observed in the absolute liver and kidney weights between rat groups fed with potatoes (groups 2, 3, 4 and 5), no significant differences in absolute and relative organ weights were observed between the rats fed with hybrid potatoes (groups 4 and 5) and the control group (group 1).

The histopathological examination did not reveal any severe abnormalities between the different groups (Table 6). Moreover, no modifications in the kidney tissues were observed. The incidence of microscopic changes observed in the liver and spleen of the different rat groups was of slight to mild severity. The findings in rats fed hybrid potatoes (groups 4 and 5) were in general randomly distributed among all the groups and were of the type commonly observed in control rats (group 1) and/or in the rat groups fed commercial potatoes (groups 2 and 3). Minor increases were detected in the incidence of specific chronic inflammation in the spleen and vascular dilatation and hepatocyte necrosis in the liver of rats receiving CN1 hybrid potatoes (group 4) when compared to the groups fed commercial tubers (groups 2 and 3). In contrast, a small degree in the incidence of specific inflammatory sites in the liver of the group fed with Nicola tubers (group 3) was observed while none of these abnormalities was detected in the organs of rats fed with hybrid potatoes (groups 4 and 5). These data illustrate the minor variability in the incidence of common microscopic changes that occurs in the rats of this strain.

4. Discussion

The nutritional value of intraspecific potato somatic hybrids (CN) was assessed in the present study. Phenotypic traits of hybrid tubers were first determined as they are of primary importance, requested for novel varieties prior to availability on the market and for the identification and certification of novel varieties. The hybrid potatoes have morphological characteristics that fall within the expected range of natural variability in potato species.

The nutritional quality assessment of intraspecific somatic hybrid potatoes examined here was based on the approach envisaged for transgenic plants (Codex, 2003; EFSA-GMO, 2008; Parrott *et al.*, 2010). It includes a comparative concept of substantial equivalence between novel potatoes and conventional varieties. Indeed, because of their history of safe use, it is generally acknowledged that traditional food products may serve as baseline for comparison (Herman and Price, 2013). The basic idea is that food from novel varieties should be at least as safe as the traditional products they may replace in the diet. In the present study, both the Nicola parent and the BF15 variety were used as controls to reveal variations related with natural variability from the conventional breeding system, but also to compare the parental Nicola variety with the CN1 and CN2 hybrid lines. In determining substantial equivalence, food compositional analyses are important elements. The chemical composition

Table 5. Absolute weights (AW, g) and relative weights (RW, g/100 g body weight) of organs of rats fed different diets with or without potatoes.[1]

Parameter		Group 1, standard diet (control)	Group 2, diet with BF15	Group 3, diet with Nicola	Group 4, diet with CN1	Group 5, diet with CN2
Liver	AW	6.13±0.42[ab]	6.50±1.03[a]	6.78±1.06[a]	6.85±0.65[a]	5.07±0.42[b]
	RW	3.51±0.30	3.62±0.71	3.98±1.18	3.81±0.54	2.94±0.31
Kidney	AW	1.14±0.10[b]	1.36±0.20[ab]	1.52±0.28[a]	1.35±0.14[ab]	1.26±0.10[ab]
	RW	0.65±0.07	0.69±0.08	0.87±0.16	0.75±0.10	0.73±0.06
Spleen	AW	0.44±0.06	0.41±0.05	0.48±0.05	0.43±0.10	0.43±0.12
	RW	0.25±0.05	0.25±0.06	0.28±0.06	0.23±0.05	0.25±0.07

[1] Each value is presented as the mean ± standard deviation. The absence of superscripts on the same line indicates the absence of significant differences (*P*<0.05). Values with different superscripts on the same line indicate a significant difference between rat groups.

Table 6. Histopathological findings in organs of rats fed diets with or without potatoes.[1]

Histopathology		Group 1, standard diet (control)	Group 2, diet with BF15	Group 3, diet with Nicola	Group 4, diet with CN1	Group 5, diet with CN2
Liver	Congestion (vascular dilatation)	1	1	0-1	2	0
	Acute inflammation	0	0	0	0	0
	Portal chronic inflammation	1	1	1	1	0-1
	Lobular chronic inflammation	1	1	0	1	0-1
	Specific inflammation (microgranuloma)	0	0	1	0	0
	Steatosis	0	0	0	0	0
	Necrosis of hepatocytes	0	1	0	1-2	0
Kidneys	Focal chronic Inflammation	0	0	0	0	0
	Tubular atrophy	0	0	0	0	0
	Tubular necrosis	0	0	0	0	0
	Glomerular sclerosis	0	0	0	0	0
Spleen	Haemosiderin pigmentation	1	1-2	2	1-2	2
	Specific chronic inflammation	0	0	0	1	0
	Hyperplasia of the red pulp	0	0	0	0	0

[1] Results are given as the scoring of abnormalities for each organ on a scale where 0= none, 1= slight, 2= mild, 3= moderate and 4= severe abnormalities.

of hybrid tubers and commercial BF15 and parental Nicola potatoes was determined. Although small significant differences were observed between commercial and hybrid tubers with regard to dry matter, starch, protein, lipid and soluble sugar contents, these variations can be accepted for potato use since all values remained in agreement with literature data (Gravoueille, 1996; Langkilde *et al.*, 2012). Moreover, performance with reference to dry matter content and starch in the hybrid potatoes can be of great interest. Indeed, high amounts of dry matter in tubers such as CN1 hybrid tubers are more suitable for the production of chips (Elfnesh *et al.*, 2011; Gravoueille, 1996). The CN1 and CN2 hybrid tubers can even serve as major sources of starch for food and application in a wide range of industries (Šimková *et al.*, 2013).

A substantial equivalence study cannot be considered as a safety assessment in itself; rather it represents the starting point used to structure the safety assessment of novel products. A feeding study can provide supplementary indications to ensure safe conditions since it is considered as a major step in nutritional safety assessment (EFSA-GMO, 2008). In a first attempt tubers from potato hybrid lines and conventional varieties were added to the rat diet and a feeding study of these components was conducted for one month. Body weight gain and food consumption were monitored in the feeding study since they were demonstrated to be sensitive indicators of overall animal health (Domingo and Giné Bordonaba, 2011). General animal well-being was also supported by the comparable in-life nutritional performance response to variables such as digestibility, FCR, PER and ADC between the treated and control animal groups (Appenzeller *et al.*, 2009; Zdunczyk *et al.*, 2005). The data indicated no significant differences for most of these parameters between animals fed CN1 and CN2 hybrid potatoes (groups 4 and 5) and those fed diets containing commercial tubers (groups 2 and 3) and control rat group (group 1). Only a minor increase of the FCR parameter was measured with the CN2 hybrid diet. This might have been due to the lower body weight gain in this rat group (group 5; 0.78±0.13 g/rat/day) compared to the other groups although not statistically significant. Variations in this parameter were routinely observed between rats fed different potato varieties. This can be related to differences in chemical composition such as amino acid composition of proteins between tubers of the different potato lines and between diets when isocaloric diets were adjusted and protein amounts were balanced between the diet containing the CN2 hybrid line and the diet with tubers of the Nicola variety (Zdunczyk *et al.*, 2005). Analyses of the data from haematology, blood biochemistry and organ systems were commonly reported to evaluate the nutritional value of diets (Domingo and Giné Bordonaba, 2011). The small decrease observed with the CN2 hybrid potato diet compared to commercial potato and standard diets in MCH and MCHC parameters cannot be considered since all the values are within the normal reference intervals reported for MCH (17-29 pg) and MCHC (32-45 g/dl) by Awounfack *et al.* (2016) in this rat strain (Wistar strain). A minor decrease in blood plasma ALT enzyme activity was also observed in rats fed the diet with CN2 potatoes (group 5) compared to control rats (group 1). This cannot be considered as relevant to adverse effects since increase in blood plasma ALT activity is specific of hepatic damage (Wittwer and Bohmwald, 1986).

Organs such as liver, kidney and spleen were examined in all rats since they are widely analysed to investigate the nutritional quality and safety of foods (Appenzeller *et al.*, 2009; Domingo and Giné Bordonaba, 2011; Hammond *et al.*, 2006; Hashimoto *et al.*, 1999b; Momma *et al.*, 2000). Indeed, liver and kidney are well known major detoxifying organs. Minor histopathological findings observed in the liver of rats fed CN1 hybrid tubers (group 4) were considered accidental since they were sporadically found in other rat groups without relevance to any clinical manifestations and blood biochemical parameters. Assessment of macroscopic and microscopic abnormalities in other organs such as spleen was also reported to confirm the nutritional quality of foods. Minor findings in spleen with histopathology analysis of rats fed the diet with hybrid CN1 cannot be considered significant since no obvious changes in morphology and weight of this organ were observed. Indeed, this is supported by the normal haematological parameters since spleen is well known to be involved in haematopoietic homeostasis.

The histopathological analyses confirm that the effect of hybrid diets was not significantly different from those of diets with conventional varieties. Differences observed between diets with the BF15 and Nicola varieties can be related to natural variability from the conventional breeding system (Batista and Oliveira, 2010). These significant outcomes demonstrate the sensitivity of the feeding test.

In conclusion, the present study suggests that the CN1 and CN2 hybrid potatoes supplemented in the proportion of 30% (w/w) to the diet have equivalent nutritional qualities as commercialised potatoes. A safety margin was provided in the animal feeding test since the consumption of hybrid potatoes by rats far exceeds the potential human dietary consumption per kg of body weight (when considering the consumption rate of 31.5 kg/person/year; FAO, 2009). These data, combined with previous results on an interspecific somatic hybrid (Nouri-Ellouz *et al.*, 2015), suggest that somatic hybridisation had no negative effect on the nutritional value of potatoes. However, long-term and multigenerational animal feeding trials (EFSA-GMO, 2008; OECD, 1998; Snell *et al.*, 2012) should be conducted to confirm the nutritional safety of the hybrid lines.

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Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/QAS2016.1034.

Table S1. Chemical composition of diets with or without potatoes.

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