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Assessment of genetically modified prebiotic potato tubers concerning the nutritive value and the fate of DNA

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Introduction

The ability to synthesise high molecular weight fructan as inulin was transferred to potato plants via constitutive expression of the 1-SST (sucrose:sucrose1-fructosyl-transferase) and the 1-FFT (fructan:fructan1-fructosyltransferase) genes of globe artichoke *Cynara scolymus* (Heyer et al., 1999). The fructan pattern of the tubers represents the full inulin spectrum of artichoke roots and the inulin concentration in the dry matter amounts to five percent (Hellwege, 2000). The objective of this study was to analyse the extent to which this modification influences the content of nutrients, their availability to pigs as a model for monogastrics, the content of undesirable substances and the fate of recombinant DNA.

Material and Methods

The tree transgenic potato lines and the non-transformed line (i.e. cv. *Desirée*) were grown under the same field conditions at an experimental station in 2003. The tubers were steamed and ensilaged. The official VDLUFA-methods were applied to analyse the chemical composition. The nutritive value was determined in balance experiments following the GfE-guidelines. The incorporation rate of the isogenic-or transgenic potato silage in the diet was 40 % DM. The same rations were tested in a subsequent feeding trial with 4 pigs per treatment (49 to 78 kg live weight).

After slaughter digesta samples (stomach, duodenum, jejunum, ileum, caecum, colon and rectum) were taken as well as tissue samples from m. long. dorsi., m. glutaeus, thymus, spleen, liver, kidney and kidney fat. For DNA preparation the “Nucleo Spin Food kit” (Macherey-Nagel, Germany) was used. For tracing the DNA derived from the transgenic potato four different PCR-systems were selected.

For all tissue samples a PCR system developed by Laube et al. (2003) was chosen to determine the integrity and quality of the extracted DNA. To identify any inhibition in DNA extracts from the gastro-intestinal tract a sequence was selected from the choloroplast genome. As a single-copy target sequence of the potato genome the metallo-carboxypeptidase inhibitor gene DNA sequence was chosen. To identify the genetic modification in the gastrointestinal tract, a real time PCR system has been developed to target the junction between the Cauliflower mosaic virus (CaMV) 35S promoter and the adjacent *1-sst* gene within the genetic construct.

Results

The content of nutrients, minerals and amino acids analysed for the silage of both potato variants is summarized in Table 1.

Table 1. Chemical composition of potato silage

	Isogenic	Transgenic
DM (%)	21.2	19.5
	Proximates (% of DM)	
OM	94.5	94.2
CP	10.7	10.6
EE	0.6	0.6
CF	2.5	2.8
NfE	80.7	80.2
Starch	67.4	59.9
	Macro-elements (g/kg DM)	
Ca	0.51	0.56
P	2.10	2.20
K	19.60	20.00
Na	0.30	0.73
Mg	0.90	0.84
	Amino acids (g/100 g crude protein)	
Lysine	4.31	4.00
Methionine	1.18	1.39
Cystine	0.95	0.87
Threonine	2.49	2.53

Proximate and mineral contents do not show significant differences . However, the starch content decreased, which is accordance with the result from fresh tubers (13.4vs.15.0%), indicating that the inulin synthesis is limited and does not increase the storage capacity of carbohydrates (Hellwege et al., 2000). The amino acid

profile did also not show meaningful differences in the levels of any of the 16 amino acids measured. However, the alkaloid content of the transgenic tubers was found to be nearly 25% higher than that of the isogenic potatoes.

Table 2. Glycoalkaloid content of isogenic and transgenic potato tubers (mg/kg DM)

	α-Chaconine	α-Solanine	Total alkaloids
Isogenic	524	204	728
Transgenic	652	252	904

The results on apparent digestibility and energetic feeding value as summarized in Table 3 demonstrate no meaningful differences, but the energetic feeding value, which was calculated based on digestible nutrients showed a tendency towards lower values, which was supported by the results of the feeding test (Table 4).

Table 3. Digestibility and energetic feeding value of inulin synthesising potatoes as compared to those of the non-transgenic controls (means ± SD)

	Digestibility of nutrients (%)		
	Isogenic	Transgenic	P
Organic matter	93.9±1.5	93.2±1.2	0.54
Crude protein	76.9±8.3	73.0±10.5	0.60
Ether extract	66.3±16.5	49.9±19.3	0.33
Crude fibre	81.0±6.1	72.6±11.8	0.27
N-free extracts	90.7±7.1	94.2±4.3	0.49
MJ ME/kg DM	14.60±0.90	14.34±0.21	0.66

Table 4. Production efficiency of isogenic and transgenic potato silage

		Isogenic	Transgenic
ME-intake	(MJ/d)	23.19	22.89
Live weight gain	(g/d)	711	668
Energy conversion ratio	(MJ ME/kg)	32.62	33.73

None of the 110 tissue samples from pigs fed isogenic or transgenic potatoes gave a positive result in PCR with the metallo carboxypeptidase inhibitor gene real time PCR system or the system specific for the genetic modification in the transgenic potato. The limit of detection (LOD) of all real time PCR systems used was determined to be at least 10 genome copies corresponding to approximately 25 pg DNA. With all applied control systems no specific genetic alteration could be detected in any sample except from the stomach content (Table 5).

Table 5. PCR results from pigs fed transgenic potato silage

	MY	MCP	CP	Sst-1		MY	MCP	CP	Sst-1
Digesta					Organic tissue				
Stomach	+	+	+	+	M. long. dorsi	+	n.d.	n.d.	n.d
Duodenum	+	+	+	n.d.	M. Glutaeus	+	n.d.	n.d.	n.d.
Jejunum	+	+/-	+/-	n.d.	Thymus	+	n.d.	n.d.	n.d.
Ileum	+	n.d.	n.d.	n.d.	Spleen	+	n.d.	n.d.	n.d.
Caecum	+	n.d.	n.d.	n.d.	Liver	+	n.d.	n.d.	n.d.
Colon	+	n.d.	+	n.d.	Kidney	+	n.d.	n.d.	n.d.
Rectum	+	n.d.	+	n.d.	Kidney fat	+	n.d.	n.d.	n.d.

MY = myostatin gene; MCP = metallo-carboxy-peptidase inhibitor gene; CP = chloroplast-specific sequence (multi-copy); Sst-1 = 35s-promotor – sucrose:sucrose 1-fructosyltransferase gene

Summary

To evaluate silage from transgenic inulin synthesising potatoes as compared to that from the parental cultivar, nutrients, undesirable substances, digestibility and production potential were investigated. The chemical composition showed a decrease of starch (67% vs. 60% in the DM), and an increase of the total alkaloids (728 vs. 904 mg/kg DM). The energetic feeding value was calculated to be 14.6 or 14.3 MJ ME/kg DM for the silage of isogenic or transgenic potatoes. Samples of digesta and tissues were analysed with four real time PCR systems. No plant specific DNA or DNA specific for the genome alteration in the transgenic potato were detected in any organ. In contrast, chloroplast specific DNA was detected in the digesta of duodenum, jejunum, colon and rectum. No evidence for the integration of the foreign DNA into the host genome was observed.

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