

Feeding studies with mice to assess the nutritional quality of NK 603 x MON 810 maize

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Background

- **There is increasing evidence of food effects that are not traceable to the components, but emerge from the interaction of these components (Velimirov et al. 2010).**
- **Therefore nutritional equivalence cannot be assumed from compositional equivalence.**
- **But the nutritional assessment of GM products still heavily relies on this assumption, although there are examples showing different food effects of GM food as compared to the substantially equivalent controls (Spiroux et al. 2009; Kilic and Akay 2008; Malatesta et al. 2002a+b, 2008; Spiekers et al. 2009; Finamore et al. 2008; Séralini et al. 2006; Prescott et al. 2005)**

Hypothesis

Such studies underline the importance to investigate long-term consequences of GM-diets and the potential synergistic effects with ageing, xenobiotics and/or stress conditions.



Multigenerational mouse feeding trials

- The test corn represents the stacked event NK 603 x MON 810 (trade name: DKC 26-79) and contains three gene cassettes, conveying herbicide tolerance and insect resistance.
- The hybrid was produced by traditional breeding of the two genetically modified parental inbred lines derived from maize transformation events NK 603 and MON 810.
- The control maize had a similar genetic background (trade name: DKC 26-75)



Genetic modification

| parent | genetic element | derived from | size | function |
|---------|-----------------|---|--------|--|
| MON 810 | e35S | <i>Cauliflower mosaic virus</i> | 0,32kb | promotor |
| | Zmhsp70 | <i>Zea mais</i> L. | 0,8kb | stabilizes level of gene transcription |
| | Cry1Ab | <i>Bacillus thuringiensis</i> | 3,5kb | Cry1Ab toxin |
| | NOS 3' | <i>In a previous work a truncation event at the 3' end of the cry1(A)b gene leading to the complete loss of the NOS terminator was demonstrated. *</i> | | |

* Rosati A., Bogani P., Santarlasci A., Buiatti M. (2007): Characterisation of 30 transgene insertion site and derived mRNAs in MON810 YieldGard® maize. *Plant Mol Biol* (2008) 67:271–281

| parent | genetic element | derived from | size | function |
|--------------------------|-----------------------|----------------------------------|-------|--|
| NK 603 | | | | |
| 1 st cassette | P-ract1/ ract1 intron | <i>Oryza sativa</i> | 1.4kb | promotor, transcription start site, 1st intron |
| | ctp 2 | <i>Arabidopsis thaliana</i> | 0,2kb | chloroplast transit peptide |
| | cp4-epsps | <i>Agrobacterium sp.</i> | 1.4kb | glyphosate tolerant CP4 EPSPS enzyme |
| | NOS 3` | <i>Agrobacterium tumefaciens</i> | 0,3kb | ends transcription and directs polyadenylation of the mRNA |
| 2 nd cassette | e35S | <i>Cauliflower mosaic virus</i> | 0,6kb | promotor |
| | Zmhsp70 | <i>Zea mais L.</i> | 0,8kb | stabilizes level of gene transcription |
| | ctp 2 | <i>Arabidopsis thaliana</i> | 0,2kb | chloroplast transit peptide |
| | cp4-epsps l214p | <i>Agrobacterium sp</i> | 1,4kb | glyphosate tolerant CP4 EPSPS L214P enzyme |
| | NOS 3` | <i>Agrobacterium tumefaciens</i> | 0,3kb | ends transcription and directs polyadenylation of the mRNA |

Maize Production 2007

(courtesy of R. Martin, Organic Agricultural Centre of Canada in Nova Scotia)

| Variant | DKC 26-75 (Control) | DKC 26-79 (NK603xMON810) |
|------------------|---|---|
| Seed treatment | Poncho 250 (clothianidin) | Poncho 250 (clothianidin) |
| Planting date | June 3, 2007 | June 3, 2007 |
| Planting density | Seeding rate 31500 seeds/acre | Seeding rate 31500 seeds/acre |
| Width of rows | 75 cm | 75 cm |
| Fertilisation | 26t/ha liquid dairy manure 100 kg/ha 30-0-10 at planting 150 kg/ha 18-46-0 with seed 100 kg/ha 34-0-0 top dressed at the 4-5 leaf stage | 26t/ha liquid dairy manure 100 kg/ha 30-0-10 at planting 150 kg/ha 18-46-0 with seed 100 kg/ha 34-0-0 top dressed at the 4-5 leaf stage |
| Crop protection | Primextra II Magnum (3.5l/ha) applied June 15 (S-metolachlor & R-enantiomer at 400 g/L, Atrazine and related active triazines (320 g/L) | Roundup Weather MAX® at 3 l/ha (Glyphosate = 48.8%) July 13 |
| Harvest date | November 14 | November 19 |
| Yield (kg/ha) | 5636 | 6632 |

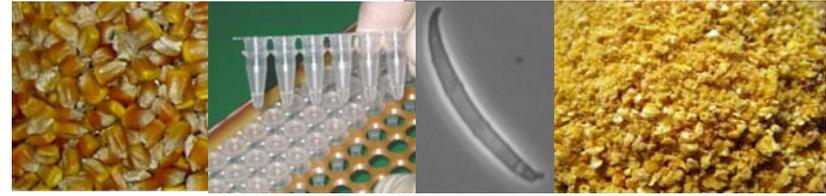
control



GM maize



Diets



- **The test and control maize varieties were substantially equivalent concerning plant contents.**
- **No residual levels of herbicides were found.**
- **The hygienic evaluation showed higher contents of mycotoxins in the GM food, but the concentrations were within the defined limits for animal feed.**
- **A standardized diet for laboratory mice in reproduction with 33 % maize content was chosen.**
- **All diets covered the energy and nutrient requirements of mice in reproduction.**



Mouse strain OF1

Test animals and animal husbandry

- outbred mouse strain (OF1)
- According to the breeders a pup loss up to 10% is normal for this strain.
- The mice were kept in macrolon cages III with an average room temperature of 23°C and a light program for a 12 hour day/night cycle.
- The health status of the mice was checked daily by a veterinarian.
- The cages were furnished with dust-free litter and cleaned weekly.
- Furthermore the mice had mouse houses, soft paper for nesting and paper roles, especially for pups.
- Fresh tap water and test feed were offered *ad libitum*.

Type of animal experiment

- **Although the animal husbandry systems with different feeds are subject of registration only, they were also approved by the ethics committee of the University of Veterinary Medicine of Vienna and the national ethics committee for animal experiments.**



Diets

- In 2005 the control maize was slightly contaminated with the 35S promotor , therefore in the MGS a third diet containing the uncontaminated reference maize variety Sarastro, grown in Austria, was introduced.



Study designs



- **A multigenerational study with maize from 2005:**
the parents of each new generation were randomly chosen from the offspring of the previous generation - 4 generations
- **A modified* reproductive assessment by continuous breeding with maize from 2007:**
the same pairs were allowed to produce litters during a 20 weeks test period - 4 litters

* The modification of the here used test design concerned the keeping of the litters until weaning (3 weeks). In the routine RACB the pups are killed at birth

Preliminary results 1

- Feed uptake and parental weight development showed no consistent trends and were comparable throughout the studies
- The parameters litter size and litter weight were comparable between the groups of the MGS.
- In the 4th generation (MGS) and respectively the 4th litter (mRACB) the number of litters declined, especially in the GM group. The percentage of females with weaned litters was as follows:

MGS:

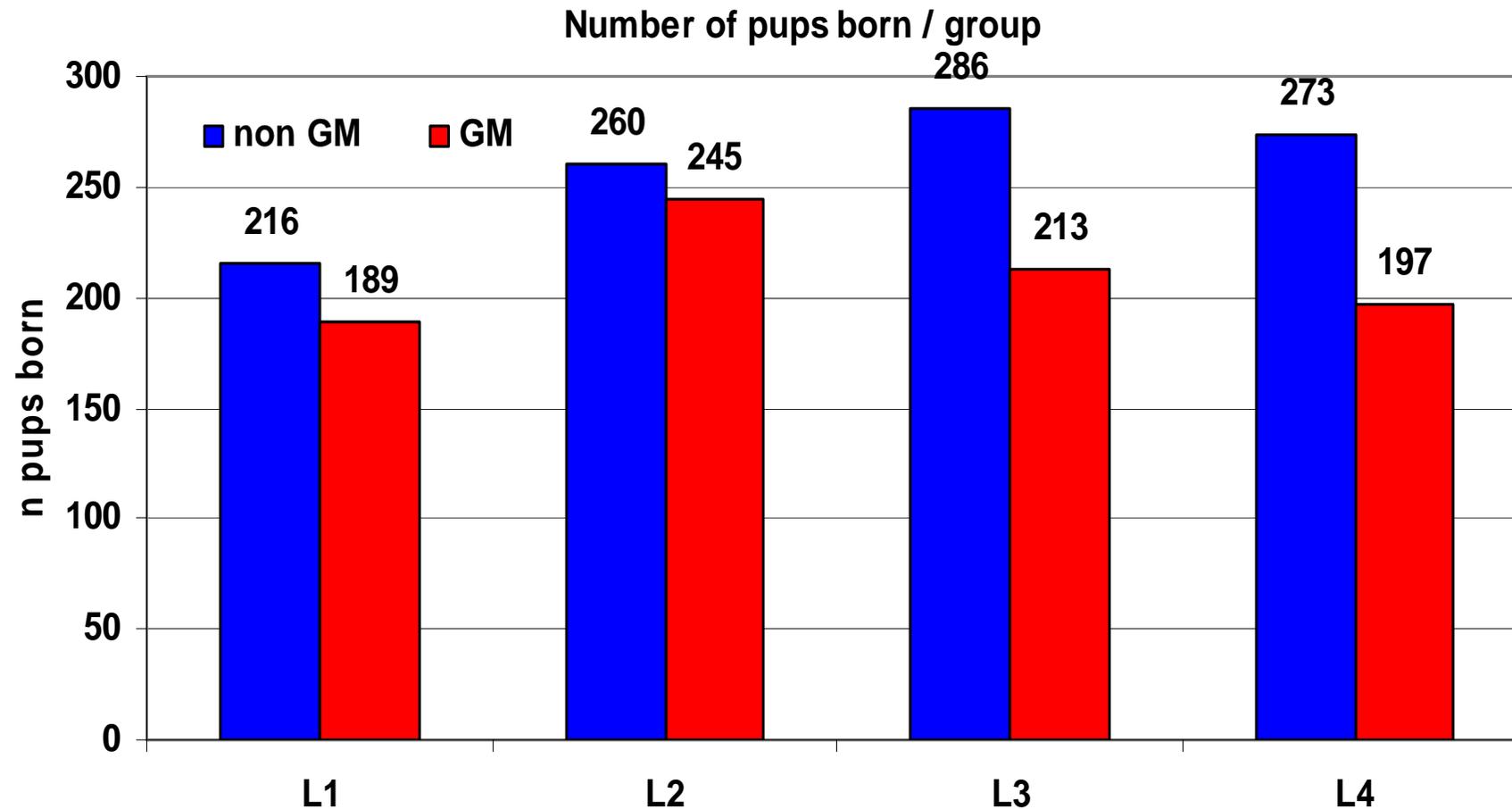
91,0% --- Aref group (presenting the potential base line)
72,7% --- non-GM group
63,6% --- GM group

mRACB:

100% --- non-GM group
82,6% --- GM group

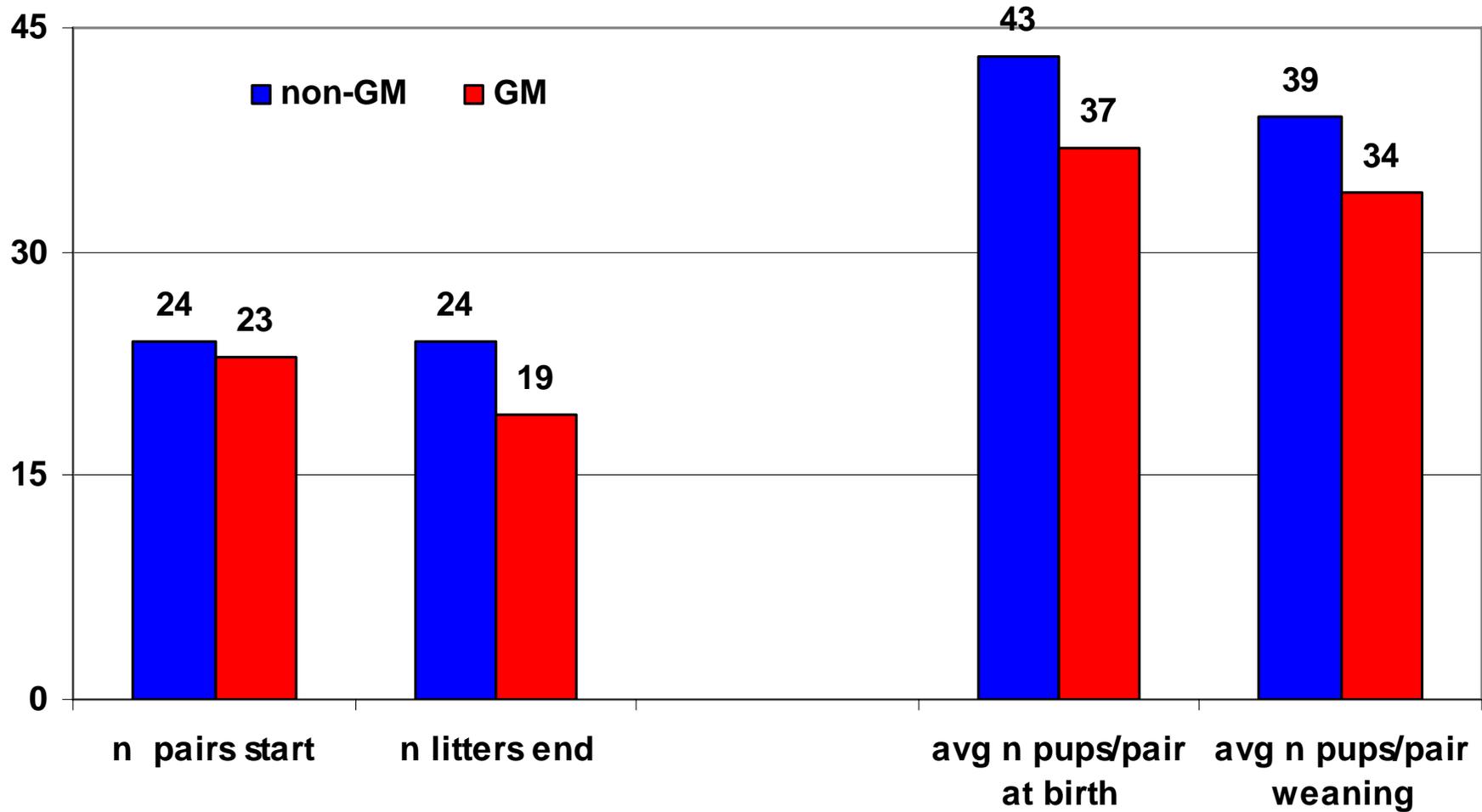


Numerical results mRACB 1

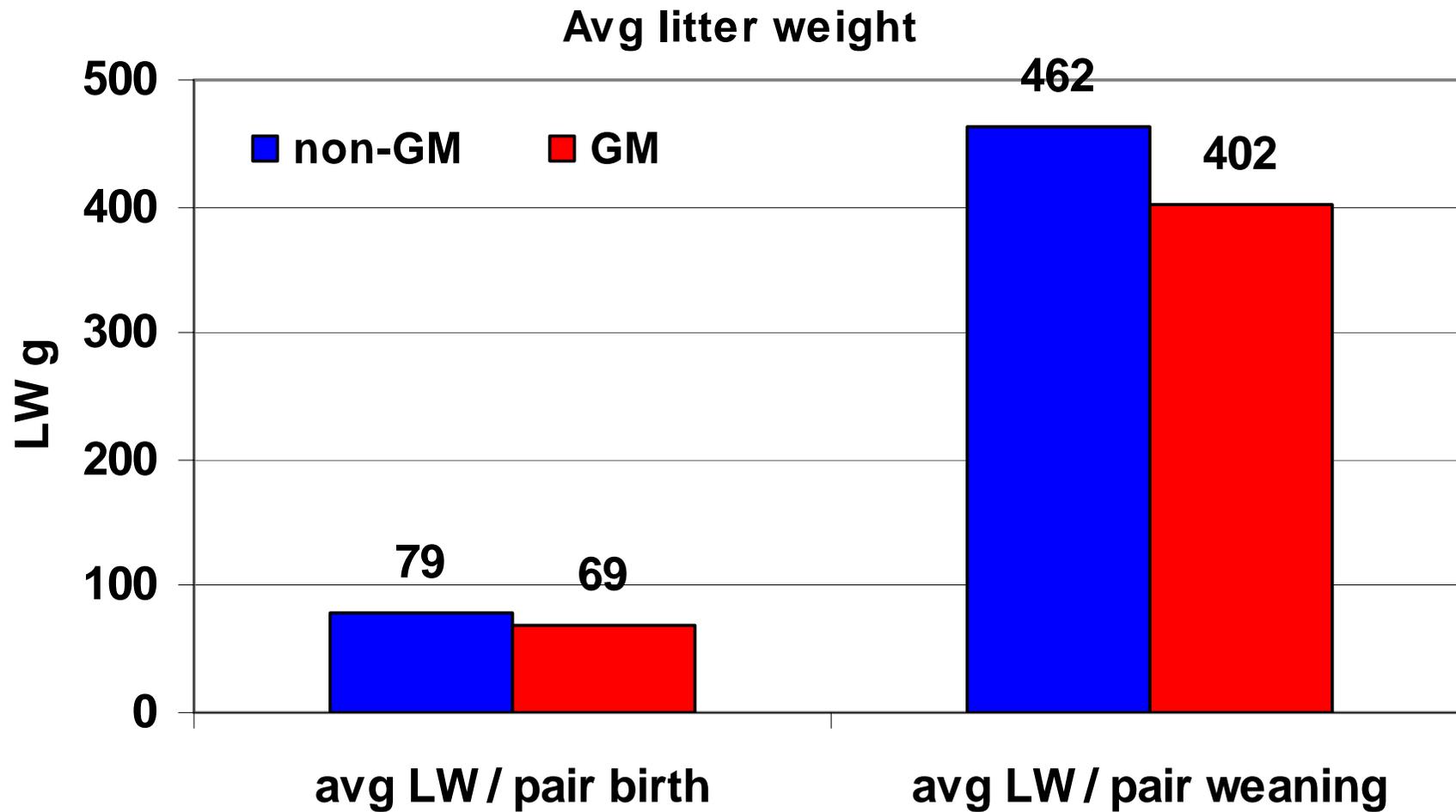


non-GM group: 24 pairs
GM group: 23 pairs

Numerical results mRACB 2



Numerical results mRACB 3





Preliminary results 2

- Thus fewer pups were weaned in the GM groups, which was mainly a consequence of fewer weaned litters, not of litter size which was comparable between the groups and only slightly higher in the non-GM groups.
- But in the assessment of the reproductive performance between differently fed groups the number of deliveries is a basic parameter, in addition to litter size and weight.
- The avg number of pups / pair and the avg litter weights at weaning were higher in the non-GM group of the mRACB.
- The pup loss during lactation was higher in the GM group of the MGS, but lower in the mRACB.



Statistical evaluation



- Only observational preliminary results have been presented
- The results presented in the final report need to be re-evaluated on their level of significance to meet critical reviews concerning the statistical methods used.
- Different statistical approaches are possible and can lead to different conclusions.
- The choice of statistical methods depends on the scientific questions to be answered.
- Thus different specified parameters can be tested as to their statistical significance.
- In the final evaluation all data have to be included and considered.

Conclusions



- So far a final conclusion is still lacking, but whatever statistical analyses might or might not be considered state of the art, the observational numerical evidence of this study is in line with other feeding results indicating nutritional effects of GM food despite substantial equivalence.
- There was an indication that the stacked event NK603 x MON810 maize leads to a reduced reproductive performance as compared to the non-GM line.
- This result should be an incentive to continue investigations in this direction with other GM crops.