Life cycle fitness differences in *Daphnia magna* fed Roundup-Ready soybean or conventional soybean or organic soybean

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Abstract

A life-long feeding study with soybean from different production systems was carried out in the crustacean *Daphnia magna* (water flea), an acknowledged model organism for ecotoxicological studies. Experimental diets were prepared with soybean-meal from different agriculture production systems: i) genetically modified Roundup-Ready soy (Glyphosate-Tolerant), ii) conventional soy, and iii) soy from organic agriculture (agriculture with neither synthetic pesticides nor synthetic fertilizers).

Overall, feed produced from organic soybeans resulted in the highest fitness (higher survival, better growth and fecundity) in the model organism. Animals fed Roundup-Ready soybean consistently performed less well compared to animals fed either conventional or organic soybeans.

We conclude that accumulation of herbicide residues in Roundup-Ready soy and related nutritional differences between the soy types may have caused the observed fitness differences. The results accentuate the need for further research clarifying qualitative aspects, including potential large-scale consequences for food and feed quality, of this dominant crop.

Introduction

Soybean (*Glycine max)* has become a primary ingredient in feed for farmed domesticated animals and for various species of fish and crustacean in aquaculture (Olsen & Hasan 2012). Soybean-meal and soy-oil are widely used to supplement or substitute fish-meal and marine lipids in formulated feeds for aquaculture farming of Atlantic salmon *Salmo salar* (Sagstad *et al.* 2008), rainbow trout *Oncorhynchus mykiss* (Refstie *et al.* 2000; Yang *et al.* 2011), and in aquaculture of crustaceans, such as crayfish (Jones & De Silva 1997), prawn and shrimp (Kumaraguru *et al.* 2005). Methods for soybean-meal preparation in aquaculture feed for crustacean species in general have been known and used for more than two decades, confirming soybean-meal as an alternative protein sources in crustacean diets (Cuzon *et al.* 1994).

The majority of present global soy production is from genetically modified glyphosate-tolerant Roundup-Ready soybean (GM-soy) (James 2010). The remaining quantity of global non-GM soy production is largely from "conventional" industrial farming, upheld through national regulation as well as consumer-group preferences. To some degree higher premium on organic soybeans and costs of stewardship-agreements motivate farmer transition from GM-soy to organic soybean production (McBride & Greene 2009). Despite this, organic production in the USA is still limited to less than 1% of US farmland (Kuminoff & Wossink 2010), thus the leading soy-producing country has to import substantial quantities of organic soy to satisfy an increasing demand (Place *et al.* 2009).

Compositional analyses by industry tend to confirm that GM-soy is compositionally and qualitatively equal (substantially equivalent) to non-modified conventional soy (Lundry *et al.* 2008; McCann *et al.* 2005; Taylor *et al.* 1999). However other studies have shown significant differences in levels of pesticide residues, nutrients and minerals (Bøhn *et al.* 2014; Duke *et al.* 2003; Zobiole *et al.* 2010a). Importantly, interaction from ppm-levels of glyphosate-pesticides present in GM-soy is found to impact plant metabolism and seed composition (Zobiole *et al.* 2010b; Zobiole *et al.* 2011).

GM plant material as feed in farm animals and aquaculture has received particular attention, partly due to potential unintended changes in host plant genome (EFSA 2008; Podevin & Jardin 2012) and due to the potential of the modification itself to induce changes to the plant phenotype which may lead to toxicity and/or allergenicity in animal consumers. The unresolved question whether and how genetic modification of cultivars may affect quality of produced feed and food from such varieties, has led to development of guidelines on animal feeding studies for risk-assessment of GM plants (EFSA 2008; 2010).

Soy is known to contain various bioactive compounds such as phytochemicals with similar effect as mammalian hormones and also anti-nutrients that can negatively influence digestion and metabolism in receiving consumer organisms. Some of these unwanted effects can be avoided by fermentation or heat-treatment, which is common practice in preparation of soybean ingredients for feed production (OECD 2001).

There are distinct regulatory conditions dictating the biological seed-basis and agriculture practice for the farming of soybeans in; 1) organic agriculture, 2) conventional agriculture and 3) agriculture of GM-soy. This implies that there are systematic differences in seed type, soil preparation, fertilizer use and pesticide use. In organic and conventional agriculture, fields are typically plowed, whereas GM-soy has become synonymous with "no-till agriculture" as it represents no need for pre-plant plowing (Trigo *et al.* 2009).

As GM-soy has become dominant in the USA, this agriculture practice confusingly is termed “conventional” in some recent publications (McBride & Greene 2009; Place *et al.* 2009).

Previous studies have demonstrated the feasibility of using full life-cycle feeding-studies in freshwater crustacean *Daphnia magna* to test qualitative aspects of various plant materials in suspended feed (Bøhn *et al.* 2008; 2010). Increasing use of plant-based feed ingredients such as soybean-meal in feed formulations for aquaculture of crustaceans also highlights the relevance of this animal model both as representative of farmed organisms in aquaculture as well as an indicator of ecological effects.

Hypothesis:

H_0: Soybean-meal types from organic, conventional and GM-soy agriculture are substantially equivalent and will thus not produce differences in measurable end-points a) survival, b) growth and c) fecundity in *D. magna*.

Materials and Methods

Materials:

The tested soy was grown on a number of agriculture production sites (farms) in the USA in 2008. Soy material was obtained from individual farmers as 31 individual 3kg samples of whole dehulled beans, harvested at 31 individual fields. Seed type and farming history including pesticide application, residue levels and nutritional composition is documented (Bøhn *et al.* 2014).

3 categories of agricultural practice are defined; 10 samples are from agriculture of GM-soy (genetically modified glyphosate tolerant "Roundup-Ready" soybean, cultivated using fertilizers and pesticides). 10 samples are from "conventional agriculture" (non-transgenic varieties, fertilizers and pesticides applied). 11 samples are from organic agriculture (no artificial fertilizers nor pesticides applied). Test animals for the experiment were a total of 300 juvenile *D. magna* all less than 48hrs old, taken from the mother population (3rd-5th clutch) and randomly assigned to treatments. Animals were kept as independent experimental units in 300 unique-identifier-marked 100-ml borosilicate beaker-glasses (SIMAX type 632 417 010 100, Kavalierglass, Sázava, Czech Republic) for the duration of the experiment, 42 days.

Methods

Soybean-samples from each harvest were shipped to laboratories of GenØk Centre for Biosafety, Tromsø, Norway. Samples were cleaned manually, impurities, hull fragments and other plant material contamination was removed. Standardized subsamples for analysis and feeding tests were prepared by homogenization, weighing and grinding. Material was kept cool during grinding to avoid degradation.

Diets were prepared from the fine ground and dried soy meal as a representative homogenate mixture from standardized subsamples in each category "Organic", "Conventional" and "Roundup-Ready-GM-soy" (n=11, 10 and 10) subsequently filtered through mesh-sizes 1000 um and 600 um metal filters (Retsch GmbH, Haan, Germany). 2 subsamples of 2.400 g (+/- 1 mg) were taken from each category mixture and homogenized into individual 1000 ml flasks containing 600.0 ml of ultrapure water, producing 2 flasks of feed solution for each of the 3 categories of soy. Following homogenization one flask of each category was heat treated by boiling in water bath for 45 minutes. The other feed solution in each category was kept raw. Following cooling and renewed homogenization the feed solutions were labelled, blinded, and individual 10ml aliquots were prepared for freezing in colour-coded 14 ml PP FALCON-tubes (BD-Biosciences, San Jose, California, USA). Feed was kept frozen at -20 ^{o}C until use.

Pilot studies on soy supplements in feed in *Daphnia magna* were initially conducted to explore the suitability of the method. The principal study was subsequently designed with multiple treatment groups to combine the rationale for obtaining i) the highest possible concentration of the feed under testing, (i.e. full-fat soybean-meal only), and ii) nutritional balance (i.e. soy feed in addition to standard green algae diet). We studied soy quality as raw and heated under both these scenarios and also varied the dose of feed ('high' and 'low') in the soy only diets. Thus, in total 18 distinct soymeal diets/treatments (table 1) were tested in the described *D. magna* model for a duration of 42 days, which covers most of the life expectancy of this animal. Experiments were conducted in the laboratories of GenØk - Centre for Biosafety, located at the University of Tromsø, Norway.

Daily feeding doses of 100 ul (low) or 200 ul (high) containing 0.2 or 0.4 mg organic carbon (OC) respectively as soy (table 1), was administered using micropipette. "High-inclusion-rate" diets consisted of soy only, given as high or low dose.

The experiments were performed fully blinded in the laboratory environment. Soy-type was blinded and changing position and order of handling individual animals as well as daily feeding sequence was according to the outcome from MS-EXCEL spreadsheet randomization engine. The feeding experiment included all 18 feed-treatments, with 15 animals in each group. Additionally an algae-fed control group consisting of 30 individual animals was randomly distributed in the setup (table 1). The experimental setup was handled on 9 trays each having 7 rows of treatments each having 5 experimental units consisting of colour coded and numbered individual glasses in each treatment (300 animals in total as 3 rows were blank). Experimental treatments were thus defined combinations of the 3 soy feed types, raw or heat treated, in low or high dosage either as stand-alone diets or combined with supplementary 0.20 mg OC d-1 as *Selenastrum sp.* algal feed in the balanced diets (table 1). Feeding and registration of mortality and fecundity was performed on a daily basis for the 42-day duration of experiment. At days 7, 13, 19, 25, 31 and 37 all surviving animals were microphotographed for subsequent measuring of carapace length (top of head to base of caudal spine) according to standardized procedure using Wayne-Rasband IMAGE-J software (Collins 2007; Dorr *et al.* 2007) calibrated to an AGAR-L4078 scale (Agar Scientific Elektron Technology UK Ltd, Stansted, Essex, UK).

Holding medium for experiment was ADaM artificial lake water (Klüttgen 1994). The physical parameters dissolved oxygen, conductivity and pH were measured regularly and found to be within range of acceptance (OECD 2008). Holding medium was renewed every other day by transferring animals into pre-fed parallel glasses containing fresh medium. Randomization of position for each experimental unit was performed following medium renewal. Data was entered in spreadsheet software Excel and statistical analysis was performed as analysis of means, ANOVA and quantile regression (in SPSS & SYSTAT softwares) and analysis of Cox proportional hazard (in R-software).

Soy meal composition and herbicide residues:

Data on nutrients, pesticide residues and elemental composition for the 31 individual samples of soy used to compose the feed in this study are given in Bøhn et al. (2014), demonstrating consistent differences in soybean composition attributable to farming practice.

The GM-soybean used as feed in this study contained glyphosate (average 3.3 ug g-1) and its main degradation product aminomethylphosphonic acid (AMPA) (average 5.7 ug g-1). Glyphosate or AMPA was not found in conventional or organic soy samples. Fluazifop-P was present in GM-soy (average 0.008 ug g-1). The following pesticide residues were also found; malathion (average 0.002 ug g-1, in conventional soybean) and dieldrine (average 0.0002 ug g-1, in organic soybean)

Results:

Survival

Animals fed combination diets of green algae and soybean-meal (balanced diets):

D. magna fed green algae and soybean-meal in combination had high survival for organic, conventional and GM-soy, with only minor differences attributable to soy types (Fig. 1A and 1B).

Animals fed diets of soybean-meal only (unbalanced diets):

All groups fed soybean-meal only had reduced survival as compared to animals fed combination-diets of soybean-meal and green algae (Fig. 2 a-d). For animals fed raw soybean meal in high doses the mortality was significantly higher in GM-soy fed groups than the two non-GM groups combined (Fig. 2d, p=0.036, coxph-test). Furthermore, the expected median longevity was much lower: 14.5 days versus 35 days.

A relatively high mortality was found in animals fed high doses of soy feed (i.e. both raw and heated soy) (Fig. 2b and 2d). In these groups, the mortality was significantly higher in GM than in non-GM groups (p=0.014, coxph-test), expected median longevity was 14 and 29 days for GM and non-GM fed animals, respectively.

Interestingly, high doses (data pooled from raw and heated) coincided with reduced survival for GM fed animals, with expected median survival time falling from 27 days for low doses to 14 days for high doses, indicating negative effect from feed-ingredient(s) e.g. anti-nutrients or toxins (Fig 2a and 2c versus 2b and 2d). In contrast, for animals fed conventional and organic soy, the median survival time increased from low to high doses from 24 to 30.5 and from 25 to 28 days, respectively. Animals fed soy only showed an overall trend of higher mortality in the GM-fed groups than non-GM fed groups (conventional and organic combined). These differences were not significant (p=0.059, coxph-test). The expected median longevity was 18.5 days for

GM-fed animals and 25 days for non-GM fed animals.

Growth

Animals fed green algae and soybean-meal (balanced diets):

D. magna fed organic soy in addition to algae showed higher growth rates than animals fed conventional and GM-soy. Animals fed GM-soy had lowest growth rates. This phenomenon was consistent for raw as well as heated soy (Fig. 3). Animals fed GM-soy had significantly lower growth rates compared to those fed both conventional and organic soy feed ($p=0.003$ and $p<0.001$, repeated measurement ANOVA, respectively for the combined raw and heated material). Heated soy gave higher growth than raw, for all soy types.

Animals fed soybean-meal only (unbalanced diets):

When animals were fed low doses of raw soy, conventional soy performed best and significantly better than the organic soy ($p=0.041$, repeated measurement ANOVA) (Fig. 4a).
For animals fed high doses of raw soy, the organic soy performed less well than both the conventional and the GM-soy ($p<0.001$ and $p=0.047$, respectively) (Fig. 4b).
For animals fed low doses of heated soy, organic soy performed best and significantly better than the conventional soy ($p<0.021$). Here, animals fed GM-soy also performed significantly better than the conventional soy ($p=0.047$) (Fig. 4c).

For animals fed high doses of heated soy, organic soy performed better than GM-soy and the conventional soy ($p<0.001$ and $p=0.043$, respectively). The conventional soy performed better than the GM-soy ($p<0.001$) (Fig. 4d).

For animals fed soy only, in analyses that combined low and high doses as well as raw and heated soy material, the conventional feed gave the highest growth rate, higher than both organic and GM-soy ($p=0.029$ and $p<0.001$, respectively). The organic soy gave higher growth rates as compared to the GM-soy ($p=0.025$).

Cumulative fecundity

Animals fed green algae and soybean-meal (balanced diets):

When animals were fed a low dose of raw soybean-meal in addition to algae, the organic feed performed much better than both the conventional and the GM-soy types ($p<0.001$ for both, quantile regression) (Fig. 5a).

With heated soymeal, there were no significant differences ($p>0.18$) (Fig. 5b).

Testing across groups

The use of quantile regression made us able to test the slope (central tendency, i.e. the 50 % percentile) of the accumulated fecundity over time, across groups.

In comparisons of animals fed algae/soy combinations (either raw or heated), and correcting in the model for the effect of heating, a higher median slope for accumulative fecundity was found in animals fed organic soy, in comparison to both conventional and GM-soy ($p<0.001$). Conventional soy and GM-soy were not significantly different ($p=0.33$).

Animals fed soybean-meal only (unbalanced diets):

As expected, animals fed soy only did not perform well compared to groups fed algae and soy combinations.

When animals were fed a low dose of raw soybean-meal, the organic soy performed significantly better than both conventional and GM-soy ($p<0.001$ for both, quantile regression) (Fig. 6a).

When animals were fed a high dose of the same raw soybean-meal, the organic soy performed better than the conventional and GM-soy ($p=0.013$ and $p=0.001$, respectively, quantile regression) (fig. 6b). Conventional soy performed better than the GM-soy ($p=0.006$, quantile regression).

When animals were fed a low dose of heated soybean-meal, the GM-soy performed less well than conventional and organic soy ($p<0.001$ for both, quantile regression). The conventional and the organic soy performed similarly (fig. 6c).

When animals were fed a high dose of heated soybean-meal, the conventional soy performed better than both the GM-soy and the organic soy ($p<0.001$ for both, quantile regression) (fig. 6d).

Testing across groups

For animals fed soybean-meal only, with low and high doses pooled, and correcting in the model for the effect of heating, a higher median slope for accumulative fecundity was found for animals fed organic soy than for both conventional and GM-soy ($p=0.003$ and $p<0.001$, respectively, quantile regression). Conventional soy performed better than GM-soy ($p=0.006$, quantile regression).

Discussion

The life-cycle feeding trials in *D. magna* demonstrate significant differences in fitness measured as survival, growth and fecundity, attributable to *soy-type*. These results were consistent for balanced (with algae) and unbalanced soy diets (soy only). Overall, feed produced from organic soybeans resulted in the highest fitness in our model organism, and animals fed Roundup-Ready soy (GM-soy) consistently performed less well, compared to animals fed either conventional or organic soybeans.

Our results show that *D. magna* live, grow and reproduce optimally on full-fat soybean-meal diets produced from *heat treated* soybeans from all three soy types when given in combination with green alga (balanced diets). Interestingly, when testing *raw* soy in balanced diets the organic soy gave significantly improved performance in our test organism as compared to the algae-fed control. In contrast, for the conventional and particularly for the GM-soy, performance was reduced compared to the control, indicating negative effects from toxins or anti-nutrients.

Animals fed unbalanced diets of only soybean-meal had increased mortality and reduced growth and fecundity, demonstrating performance well below the control group for all measured variables. This feed composition is not representative of what aquaculture species would receive from commercially produced feed, though inclusion rates for soybean-meal in farm animal feed have increased from 15-40% (OECD 2001) and inclusion rates of 33-40% soybean meal have been validated in feeding trials with Pacific white shrimp (*Litopenaeus vannamei*) (Amaya *et al.* 2007). Although diets of soy only are unbalanced for aquaculture purpose, such experimental feed has been validated for *D. magna* as ecological indicator (Bøhn *et al.* 2008; 2010). Furthermore, feeding studies with raw and heated soy are complementary. Soy for use as commercial feed is typically heat-treated, whereas raw soy represents an environmentally relevant food-source. Herbivore species feeding in/near monoculture fields are exposed to high proportion of specific plant biomass, thus testing protocols for non-target organisms feeding on e.g. transgenic material use 100 % of the relevant material (Lövei & Arpaia 2005; Lövei *et al.* 2009). In cultivated landscape, streams and ponds allow for diverse aquatic invertebrate species. In such habitats a majority of allochthonous biomass may come from residuals of adjacent monoculture crops (Rosi-Marshall *et al.* 2007; Bøhn *et al.* 2012).

Consistent patterns of fitness differences in *D. magna* are found in balanced and unbalanced diets. For raw soy, organic soy was superior for *D. magna* reproduction whereas GM-soy was inferior. Animals fed high doses of GM soy also showed a markedly increased mortality rate, supporting the observations of toxic or inhibitory effect seen in the balanced diet with algae. Heat-treatment of the feed levelled off the large differences in effects from soy types, but the organic soy still gave the highest growth rates. The effects of heating are complex; soybean contains valuable nutritional elements but also major allergens that potentially affect animal health (Galbas *et al.* 2011; Lemos *et al.* 2004). Heat- and alkali-treatment of soy have beneficial effects for nutritional and digestion properties of soy proteins (Arndt *et al.* 1999), but very high pH and/or temperature should be avoided to maintain nutritional quality (Wu *et al.* 1999). Both quantity and quality of proteins change after heating with a corresponding change in antigenic properties (Galbas *et al.* 2011). For edible oils heating may be harmful (Jaarin *et al.* 2010). Our results indicate positive impact of heating, whereas a study in catfish found no such effect (Evans *et al.* 2005). Since glyphosate is thermally relatively stable, with a degradation point of 199ºC (FAO 2001), heating of soy material is not likely to reduce the levels of glyphosate residues in GM soy.

Effect of farming practice

Documentation of significant qualitative differences in crops, based on these feeding-studies, contribute importantly in discussion of potential effects of farming practice. Here it is important to notice the overall superior performance of animals that had been fed organic soy. A recent review (Smith-Spangler *et al.* 2012) extracted data from 17 studies in human consumers and 223 compositional studies of nutrients and contaminants, and found that there was no strong consistent evidence that organic foods are significantly more nutritious than conventional foods. However, organic foods had significantly lower levels of pesticide residues, which clearly may impact the overall food and feed quality.

Published studies in general only compare compositional elements or feed quality of either organic versus conventional cultivars (Smith-Spangler *et al.* 2012), or transgenic versus conventional cultivars (Flachowsky *et al.* 2005; Pryme & Lembcke, 2003).We have not found any other study that covers produce from all three categories of agricultural practices, as presented here.

The requirement for animal feeding trials to supplement compositional analyses (EFSA 2008; 2010) highlights obvious shortcomings in assessments of plant material intended for food or feed use. Despite this, stand-alone compositional analysis has been used extensively and is still used (Zhou *et al.* 2011).

As GM-soy now constitutes the majority of soy produced globally (James 2010) and is seen to give an overall inferior fitness in our test animals, it is justified to focus on potential effects from this particular soy-type in farm-animal and aquaculture feed.

Bakke-Mckellep *et al.* (2007) found somewhat higher kidney lysozyme and acid phosphatase levels in Atlantic salmon after feeding Roundup-Ready soybeans, but where not able to conclude if this was caused by the genetic modification or other soy-cultivar differences. Residue levels of herbicides were not quantified in the mentioned study, neither in two other feeding-studies in Atlantic salmon which have shown minor differences between Roundup-Ready soy and its near-isogenic conventional soy (Sissener *et al.* 2009a; Sissener *et al.* 2009b). Both these studies used soy material supplied by biotech industry. Sagstad *et al.* (2008) compared organ development, metabolic markers and general health indicators of Atlantic salmon fed diets from GM-soy with fish fed non-GM soy of a near-isogenic parental line. The results demonstrated significant differences in investigated end-point between groups: Fish fed GM-soy diets showed increased feed conversion ratios, decreased protein efficiency ratio, decreased apparent digestibility-coefficients of lipids and dry matter and decreased plasma triacylglycerol levels. Although these indications of histological and metabolical changes in fish liver, spleen and intestine were attributable to agricultural system (GM versus non-GM), a later review of this evidence was inconclusive (Sissener *et al.* 2011)

Zhu et al. (2004) investigated nutritional qualities of meal from conventional vs. Roundup-Ready soybean in a 13-week sub-chronic rat feeding experiment with high inclusion rates (up to 90% soy meal). Despite initial significantly reduced feed intake and reduced growth in 90% GM-soy group (in first week of experiment), no subsequent significant differences in feed intake, body weight or mortality was observed. However, it is indicated the tested GM-soy was not sprayed with glyphosate herbicide (Huang & Xu 2013). Mice fed Roundup-Ready GM-soy showed altered mitochondrial functions and transcription pathways in hepatocytes (Malatesta *et al.* 2002; Malatesta *et al.* 2008b). Subsequently similar responses were confirmed in cultured liver tissue cells exposed to low concentrations of Roundup (Malatesta *et al.* 2008a).

Role of chemical residues and elemental differences

A common weakness of most published feeding studies using herbicide-tolerant crops such as Roundup-Ready soy, is that the tested material is not sprayed by the relevant herbicide (Millstone 1999; Viljoen 2013), and even if sprayed, measurements of herbicide residue levels are often missing.

The causal explanation for the observed fitness differences in our feeding trial is sought in the qualitative aspects of the different types of soy samples used, notably the presence of glyphosate residues as determined in Bøhn et al. (2014). Glyphosate and glyphosate-based herbicides (Roundup) are known to be toxic to *D. magna* by reducing life-expectancy at environmental concentrations of about 1-4 mg/l, and significantly reducing growth and fecundity at a concentration of 0.45 mg/l, i.e. below threshold acceptance levels in the US (0.7-1.0 mg/l) (Cuhra *et al.* 2013; Folmar *et al.* 1979).

Obviously, chemical residues of glyphosate cannot explain the significant differences found in our study in fitness of animals fed diets from organic versus conventional soy, as none of these feeds contained such residues. However, Bøhn et al. (2014) showed that the tested organic soybeans contained more total protein, zinc and a healthier fatty acid composition as compared to the conventional soy.

Relevance of life-long studies

Most feeding studies for risk-assessment of transgenic plant produce are planned and executed over a timespan which is relatively short, compared to the expected life-length of the test animal. However, the actual use of transgenic plants in food and feed represents life-time chronic exposure with relatively low inclusion rates. Studies of such long-time exposure are relevant and unfortunately the published evidence is scarce. The present study in *D. magna* represents a life-cycle study in a short-lived ecological-indicator test-organism. Lasting only 42 days, costs are reduced to a fraction of e.g. a two-year study in mammals. Although testing in both fish and mammals give numerous richer indications such as histological evidence, biochemical evidence and immunological responses, we argue that indications of toxicity and nutritional quality can be harvested from studies in *D. magna* and similar model organisms with shorter generation times.

Studies in progress further aim to investigate the role of glyphosate-residues in Roundup-Ready GM-soy. By feeding diets from individual GM-soy-harvests it will be investigated whether fitness parameters such as survival, growth and fecundity of *D. magna* can be correlated to the levels of glyphosate herbicide residues.

Conclusion

The findings demonstrate that soybean-meal from different agricultural practices significantly influence *D. magna* survival, growth and fecundity, with an overall positive effect from organic soybean and an overall negative effect of Roundup-Ready soybean. This indicates these crop types present significantly different quality of feed. This difference may be attributed to residues of glyphosate herbicide determined to be present in the GM-soy or to other nutritional differences attributable to agriculture practice. Such possible effects of herbicide residues have previously been largely ignored in risk assessment procedures for GM-plants and should be specifically addressed in future feeding trials.

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Figure legends:

Table 1. Experimental treatment types, number of experimental animals and feed dosage (OC = biomass defined as organic carbon, soy = soybean-meal, algae = algae feed, Org. = organic, Conv. = conventional, GM = GM-soy).

Figure 1. Survival of *D. magna* fed diets of soybean-meal with algae (A and B) and of control group fed green-algae only (G).

Figure 2. Survival of *D. magna* fed diets of only soybean-meal (a-d) and of control group fed green-algae only (e).

Figure 3. Body size of *D. magna* at days 7, 13, 19, 25, 31 and 37 after feeding green algae and low doses of raw (dashed lines) or heated (solid lines) soybean-meal of organic (green), conventional (black) or GM-soy (red) origin.

Figure 4. Body size of *D. magna* at days 7, 13, 19, 25, 31 and 37 after feeding low or high doses, raw or heated soybean-meal of organic (green), conventional (black) or GM-soy (red) origin.

Figure 5. Cumulative fecundity for *D. magna* fed raw (a) or heated (b) soybean-meal in addition to green algae. Lines represent regression lines (50 % percentiles) for conventional (black), GM-soy (red) and organic (green) soy.

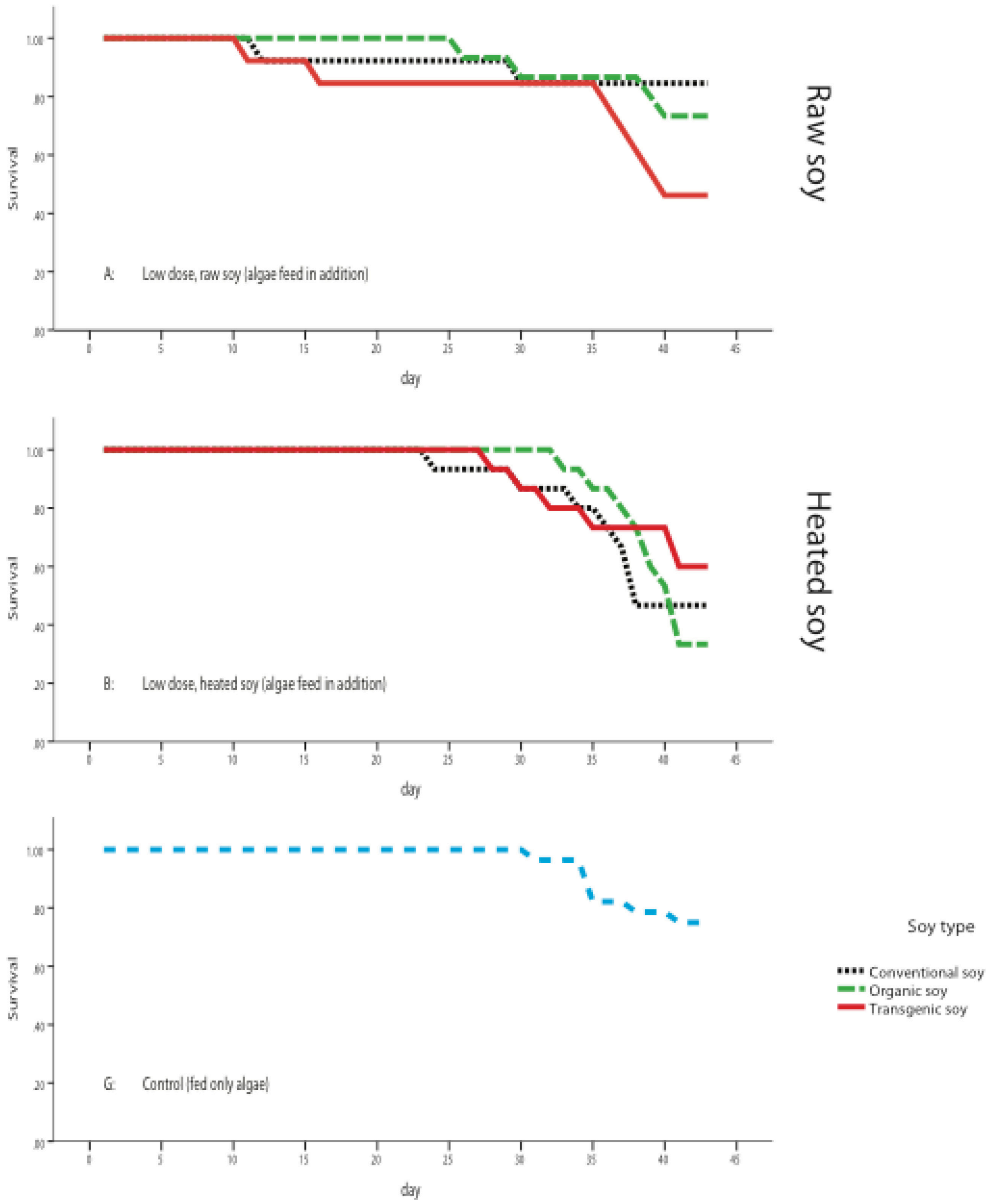
Figure 6. Cumulative fecundity for *D. magna* fed soy raw (a-b) or heated soybean-meal (c-d), in low (a-c) or high (b-d) doses. Lines represent regression lines (50 % percentiles) for conventional (black), GM-soy (red) and organic (green) soy.

Tables:

Treatment	Diet type (mg d-1 OC)	Number of experimental animals and soybean-meal type			
		Org.	Conv.	GM	Control
A	0.1 raw soy + 0.2 algae	15	15	15	
B	0.1 heated soy + 0.2 algae	15	15	15	
C	0.1 raw soy	15	15	15	
D	0.2 raw soy	15	15	15	
E	0.1 heated soy	15	15	15	
F	0.2 heated soy	15	15	15	
G	0.2 algae				30

Table 1. Experimental treatment types, number of experimental animals and feed dosage (OC = biomass defined as organic carbon, soy = soybean-meal, algae = algae feed, Org. = organic, Conv. = conventional, GM = GM-soy).

Figures;



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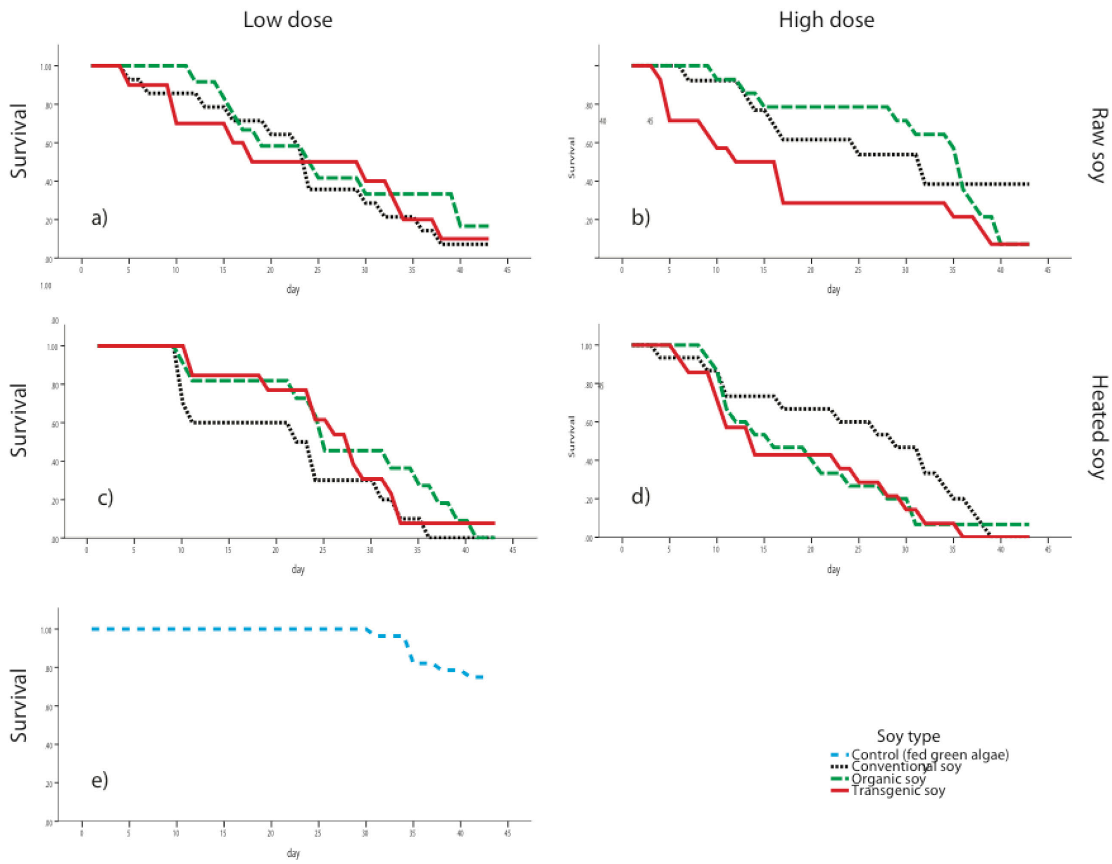
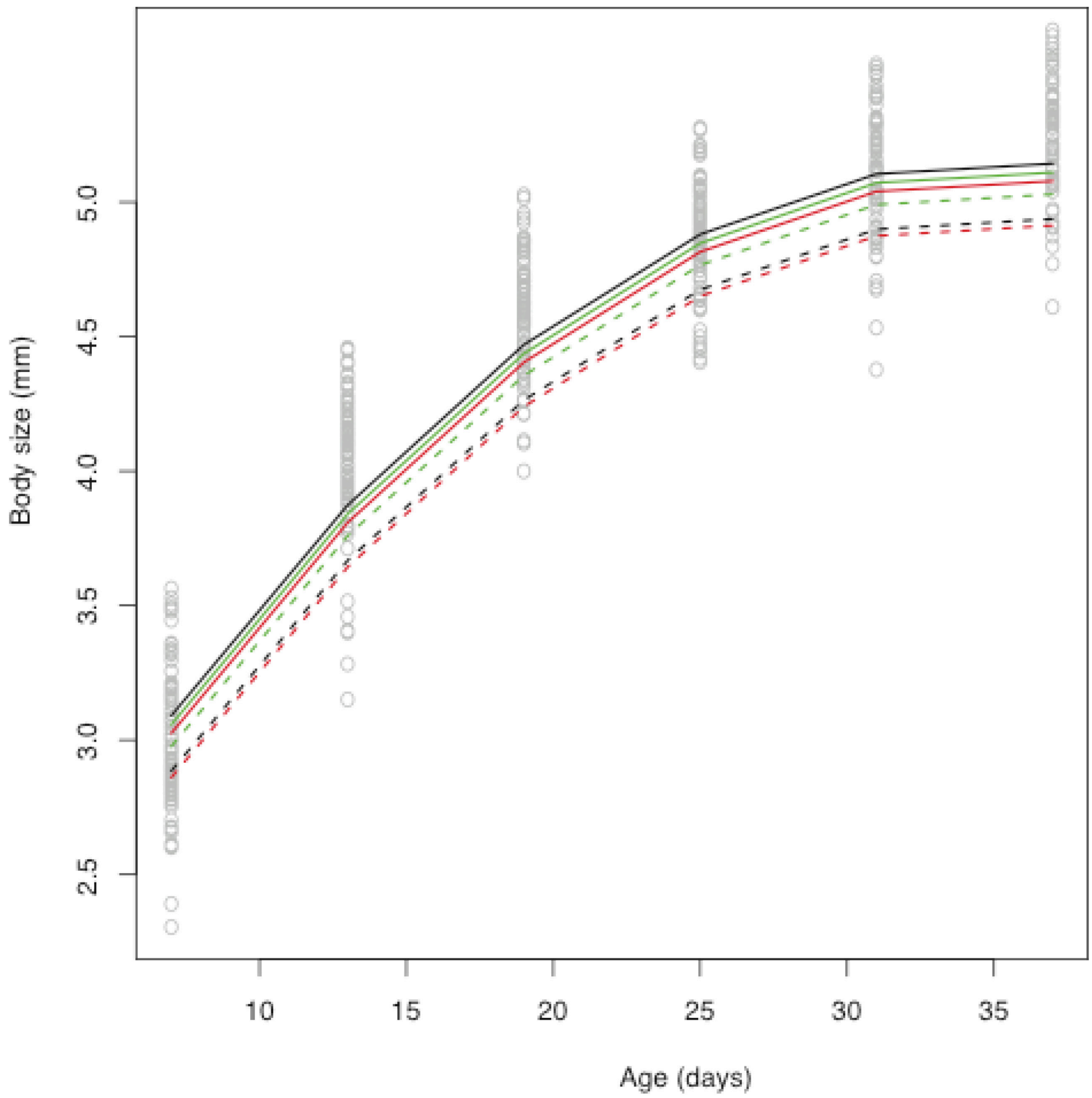


Figure 2. Survival of *D. magna* fed diets of only soybean-meal (a-d) and of control group fed green-algae only (e).



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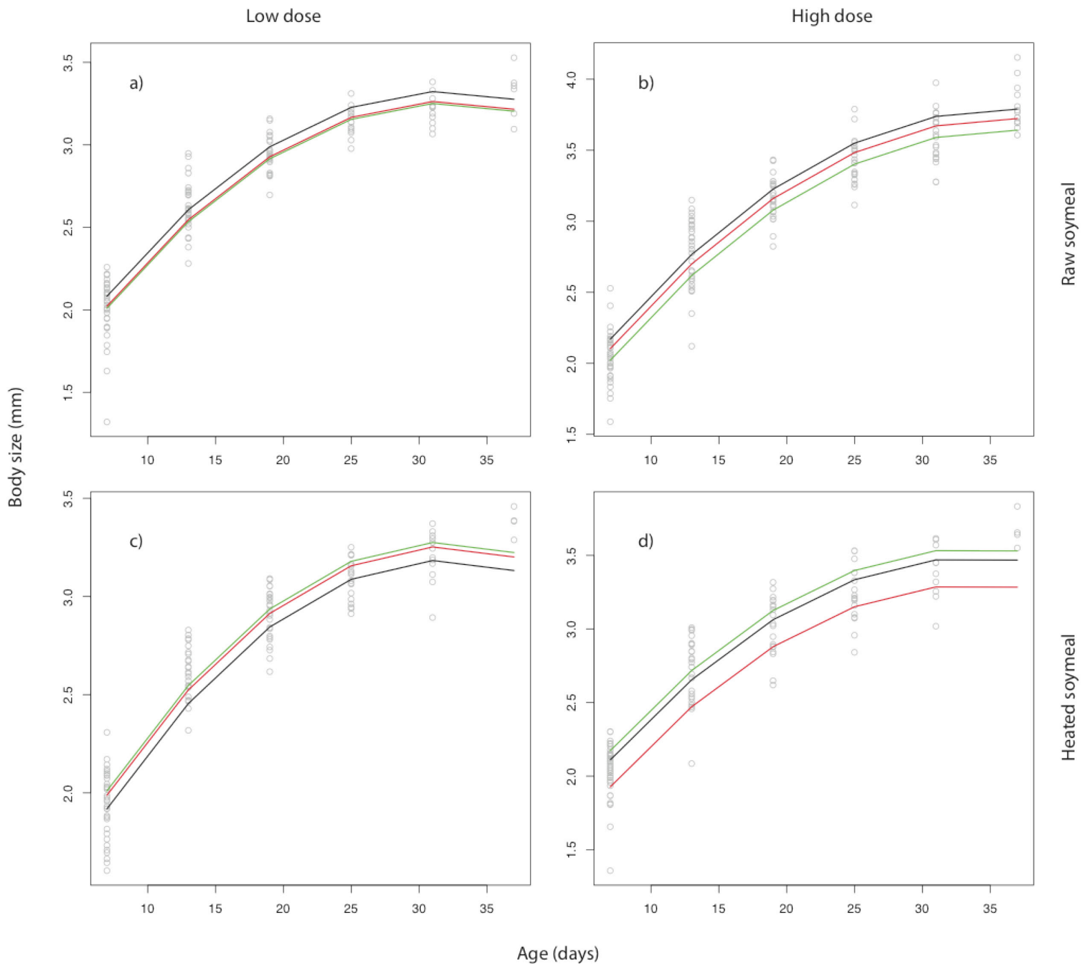

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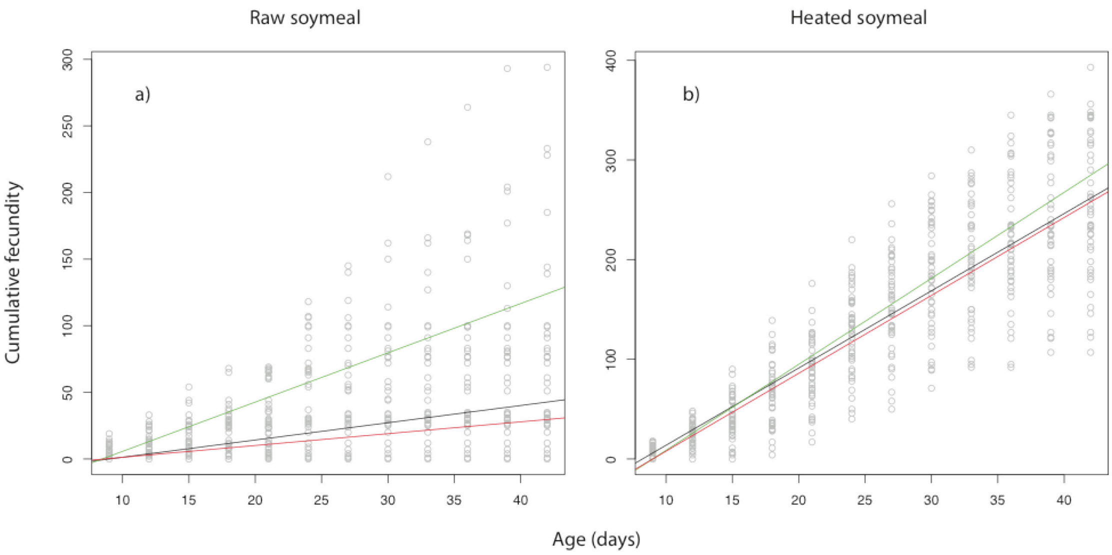

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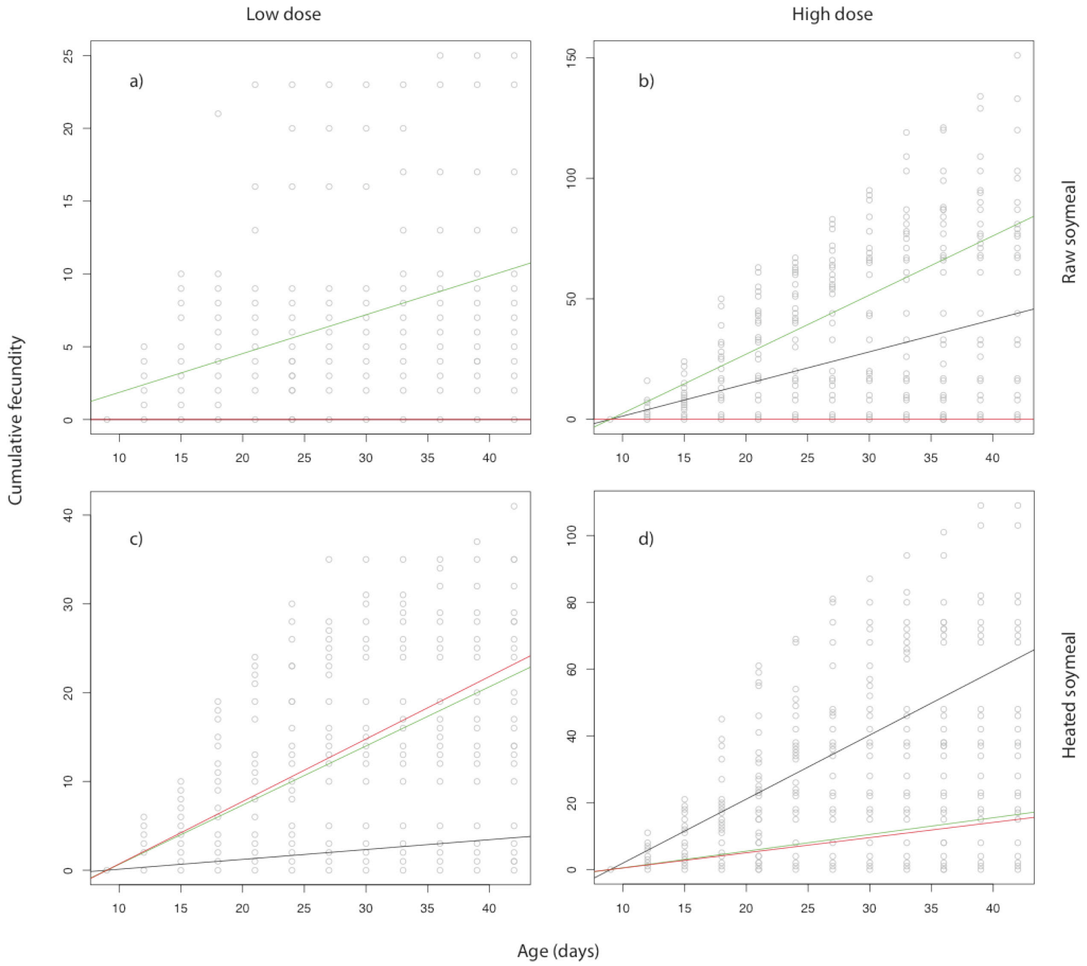

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