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Open access publishing: What is world's best practice?

Open access publishing delivers on the dream of centuries - the free and unfettered access to the written word (and more) to all - everything, everywhere, everyone, and free. When Tim Berners-Lee created the World Wide Web he had in mind an enhanced *Enquire Within Upon Everything* (Berners-Lee, 1999). The book *Enquire Within Upon Everything* was a publishing sensation which was first published in 1856 and there were 1,428,000 copies in print by 1912 (Philp, 1912). The injunction of Philps to "Enquire Within. No Fees to Pay!!" (p.ix), was a subversive idea, to give ready access to information and to bypass the priestly class of gatekeepers of information. The World Wide Web takes Philp's idea to a new level.

"Something serious is at hand" wrote Martin Luther, five centuries ago, in defence of his choice to publish the Bible in colloquial German (cited by Hargreaves, 2002, p.54). The Diet of Worms was never a novel cuisine but rather a court of the Holy Roman Empire which sought to silence Luther, spectacularly unsuccessfully as it turned out. Across the Channel it was a crime to translate the scriptures into English, and punishable by death. William Tyndale devoted his life to translating the books of the Bible from Greek and Hebrew to English, he was hanged, then burned at the stake for his efforts (Bragg, 2011; Hargreaves, 2002; McGrath, 2001). Openness is not a universal value. As Karl Popper pointed out, as the experiences of Julian Assange and Bradley Manning continue to affirm, and as the proponents of GMO labelling recently found, openness has enemies (Goodman, 2013; GreenNet, 2013; Paull, 2012; Popper, 1966).

Open access publishing, one manifestation of openness, can be important for the distribution of niche ideas, subversive ideas, and unpopular ideas. The world's first 'organic' agriculture association went broke publishing its periodical *The Organic Farming Digest* (Paull, 2008b), but had there had been a method of freely distributing the *Digest* then perhaps the Australian Organic Farming and Gardening Society may have persisted beyond 1955?

"There is a big difference between nitrogen and nitrogen" declared Rudolf Steiner (1924, p10). He was not being mystical, and in calling for an agriculture free of synthetic chemicals, he was pointing out that details matter, in that case matters of provenance. There is likewise, a big difference between open access and open access - there are shades of open access, and more than the two shades of Suber (2008).

The *Directory of Open Access Journals* has grown from its beginning in 2003 to now include 9411 journals. The DOAJ defines "open access journals" as those that do "not charge readers or their institutions for access", a condition is "Open Access without delay (e.g. no embargo period)", with "All content freely available", however "Free user registration online is acceptable" (DOAJ, 2013).

Hotels and restaurants use a star system as a shorthand guide to users - the more stars the better - might this be useful for open access? The DOAJ proposes a minimum requirement to qualify as an open access journal, namely, no fee to access. But is there a world's best practice? And best for whom? The issue is considered here from the point of

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view of the clientele - readers and authors. The editor of an open access journal will field incoming questions such as: Is there a charge to authors? Can I put a copy on my institution's website? Who owns the copyright? and Can the paper be reprinted? Four criteria are proposed here which address such questions to generate a star rating for an open access journal - for 'openness' - with a star available for meeting each criterion, to a maximum of four (Table 1).

Table 1. Open access criteria for scoring star ratings.

#	Criteria	Answer
1	There is no barrier to access for the reader	Yes → ★
2	It is free to the author/s	Yes → ★
3	Copyright is retained by the author/s	Yes → ★
4	The paper can be freely distributed under licence	Yes → ★

Using these criteria, the minimum to be included in the DOAJ is a no-star open access journal. With such a journal, the reader relinquishes privacy by registering for access, the author pays a fee, the author relinquishes copyright, and there is no explicit right to freely distribute. To meet the first criterion, 'no barrier to access for the reader', there needs to be no reader registration required, no relinquishment of reader's email address etc., to achieve access.

To meet the second criterion there is no fee to the author. It is a perverse outcome of the rise of open access publishing that there has been a proliferation of author fees - variously called 'submission fees' or 'publishing fees' - with no possibility of any direct financial return. Authors of peer reviewed journal articles have typically not been paid a fee nor received a royalty for their labours, but going from nothing to negative seems an unfortunate innovation.

To meet the third criterion there is no relinquishment of copyright by the author/s. A copyright grab has been the typical model of 'closed access' publishing, and remains common in open access publishing, it is a practice that disadvantage author/s.

To meet the fourth criterion there is a clear statement of distribution rights at the point of publication, such as a Creative Commons license, a generous version of which is CC-BY which allows repurposing and reprinting, with modifications allowed and commercial use allowed (Creative Commons, 2013). This frees the author to upload the paper to an institutional ePrints depository, the paper can be freely distributed to students, the paper can be reprinted and circulated without further permissioning by the author or the journal editor, and it facilitates the republication of a paper such as where a journal article (e.g. Paull, 2007) is republished with attribution as a book chapter (e.g. Paull, 2008a).

The *Journal of Organic Systems* is a free, open access, peer reviewed, international journal dedicated to publishing research pertaining to all aspects of organic food and agriculture and kindred issues, it aspires to achieve world's best practice and it now ticks 'all the boxes' to score a four star open access rating.

Dr. John Paull
Editor-in-chief

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Effects of crop residues and reduced tillage on macrofauna abundance

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Abstract

Conservation agriculture is promoted to safeguard resilient properties of soils and to reclaim degraded arable lands. This is achieved through creating necessary conditions for fauna recolonisation. A study was carried out at Kadoma and Southeast Lowveld of Zimbabwe to assess the effects of conservation agriculture practices on soil macrofauna diversity in the 2008-2009 agricultural season. A randomized complete block design experiment, where four crop residue levels (0t/ha, 2t/ha, 4t/ha and 6t/ha) were replicated four times on un-tilled plots at five sites, was used. Soil fauna found in collected monoliths were identified and quantified. Analysis of variance showed significance ($P<0.001$) in site and treatment effects on both macrofauna abundance and diversity. Reduced tillage with residue cover yielded significantly ($P<0.05$) higher species richness and macrofauna abundance than conventional systems. There was a significant correlation ($r^2=0.767$) between residue amount and species richness. Although there was no apparent consistent relationship between treatment and species richness, diversity and evenness; abundance was in the order 6t/ha>4t/ha>2t/ha>0t/ha>Conventional systems. The major macrofauna groups observed were termites, ants and beetle-larvae. It was concluded that short-term conservation agriculture systems has significantly positive effects on macrofauna species richness and abundance, which are crucial for initiating soil regeneration. The results are discussed in the context of sustainable crop production using conservation agriculture by resource poor farmers.

Key words: Conservation agriculture, residue cover, fauna recolonisation, planting basins, Shannon-Wiener diversity and evenness indices.

Introduction

Conservation Agriculture (CA) is defined as a concept for resource-saving agricultural crop production that strives to achieve acceptable profits together with high and sustained production levels while concurrently conserving the environment (FAO, 2007). It is premised on three key principles, namely, minimum mechanical soil disturbance, permanent soil cover and diverse crop rotations (ACT, 2005; FAO, 2008). Adoption of CA has advanced rapidly in America and Australia, mainly on large mechanized commercial farms (Derpsch, 2005). However, adoption amongst African smallholder farmers, who make up the majority of food producers' on the continent, has been very slow owing to a host of challenges. Considerable areas under smallholder farmer CA were only reported in Ghana (Ekboir *et al*, 2002), Zambia (Haggblade & Tembo, 2003), Tanzania (Shetto & Owenya, 2007) and Zimbabwe (FAO, 2007). CA is adapted to suit specific farmer practices which are heavily dependent on available resources and farmer preferences. Its form varies from country to country and also from farmer to farmer. For example, Zimbabwean smallholder farmers practise a form of CA locally known as conservation farming. It is based on hand-hoe made planting basins and application of soil cover at planting or soon after crop emergence (Harford *et al*, 2009).

Conventional agriculture (Conv) which heavily relies on mouldboard-plough tillage practices is known to be the major driver of soil degradation (Elwell, 1985; Whitlow, 1987) through increased depletion of organic matter and nutrients (Chivenge *et al,* 2007; Gwenzi *et al,* 2009). CA is, therefore, promoted to address challenges posed by conventional agricultural practices. Reduced soil disturbance and application of soil cover provide conditions that favour soil fauna recolonisation of the degraded areas; starting with relatively big-sized organic material primary shredders, followed or accompanied by progressively small-sized fauna groups as the decomposition process becomes more complex. The “early invaders”, the macrofauna group, are generally understood to be important for propagation of pores which give soils their characteristic open structures to facilitate air circulation, water infiltration and root development (Anderson, 1988).

The practice of CA, where zero tillage and soil cover are adopted, has demonstrated significant contributions to increased soil macrofauna. Studies in Zimbabwe by Nhamo (2007) observed significantly higher macrofauna populations in conservation agriculture systems than conventional systems due to improved soil surface micro-climates and organic matter availability on soil surface. These observations are, however, based on long-term CA trials, hence not much is known on whether CA practices would have a significant influence on soil fauna in the short-term. Other long-term CA benefits include reduced soil erosion and improved soil moisture storage (Nyagumbo, 2008). In tropical Brazil (Landers, 2008) and semi-arid Australia (Blank, 2008), CA practice was reported to have aided natural rehabilitation of degraded arable soils despite agriculture being highly mechanized in these two economies. Its potential for degraded soil rejuvenation on African smallholder farms is not well understood, and their practice significantly differs from that in developed countries. In the African smallholder farmer context, CA is highly adapted and promoted to mitigate socio-economic challenges like acute labour shortages and making more efficient use of resources (FAO, 2002).

There is a general lack of empirical data, in developing countries, on the effects of CA practices on macrofauna abundances and diversity; yet the same soil organisms are essential for initiating rehabilitation of degraded soil and maintaining its resilience. Soil organisms carry out a range of important processes for soil health and fertility in both natural ecosystems and agricultural systems. In addition to performing the vital functions in soils, they also make up diversity of life in soil. This soil biodiversity, comprising organisms that spend all or a portion of their life cycles within the soil or on its immediate surface, is an important but poorly understood component of terrestrial ecosystems. This research investigated the impacts of using planting basins with residue cover on soil macrofauna diversity over a single typical Zimbabwean agricultural season.

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Materials and Methods

Site description

The research was carried out on five sites situated in two agro-climatic zones of Zimbabwe; namely agro-climatic zone III and V. Four sites were located 50-km south of Chiredzi town (21° 03' E; 31° 40' S and 450 masl) in the Southeast Lowveld of Zimbabwe (SELZ) which is in agro-climatic zone V (also known as NRV). This region is characterized by low and erratic rainfall averaging less than 450 mm per year. Rainfall is so erratic that 5% of the seasons do not receive any rainfall at all (Morse, 1996). The recommended farming system for this region is extensive cattle and/or game ranching on natural pastures (Vincent and Thomas, 1960). One site was located 20-km south of Kadoma town (18° 21'E; 29° 55' S and 1156 masl) in agro-climatic zone III (also known as NRIII). The recommended farming system for this region is semi-intensive farming based on mixed crop and livestock systems, because rainfall is moderate (averaging 650 to 800 mm per year), but the zone is still prone to severe mid-season dry spells (Vincent and Thomas, 1960).

Chemical and chemical properties of the soils

The physical and chemical properties of the soils from the five sites are depicted in Table 1.

Table 1. Characteristics of soils from different sites before setting up of experiments.

Agro-zone	NR V				NR III
Site No.	1	2	3	4	5
Soil type*	Luvisols	Vertisols	Vertisols	Vertisols	Luvisols
% OC	0.59	1.72	1.22	1.02	1.30
% Clay	8	20	16	15	33
% Silt	4	15	17	14	18
% Sand	88	65	67	71	49
pH ($CaCl_2$)	6.5	7.4	7.8	7.6	5.4
N (ppm)	17	2	8	44	14
P (ppm)	1	2	1	20	3
K (cmol kg^{-1})	0.39	1.28	0.72	0.69	0.30
Ca (cmol kg^{-1})	3.46	11.43	44.74	33.55	9.08
Mg (cmol kg^{-1})	1.48	4.22	4.29	4.87	4.19
Na(cmol kg^{-1})	<0.001	0.40	0.20	0.17	0.04

***Note**: NR = Agro-climatic zone (also known as Natural Region)*
ppm = parts per million
**Soil classification is according to FAO Soil units*

Experimental design and treatments

Before the trials were set up, all the five sites were under low-input rain-fed cropping systems with ox-drawn plough tillage being the base of primary tillage operation; while the same implement together with hand-hoes were used for secondary tillage activities. Sorghum (*Sorghum vulgaris*) is the main grain (food) crop in NRV while maize (*Zea mays*) is the chief crop of NRIII. A randomized complete block design (RCBD) experiment was set up at each site. Reduced-tillage (use of hand-hoes to make planting basins) and soil cover (using previous crop residues) formed the basis of conservation agriculture (CA) treatments while the control was based on conventional ploughing. All CA treatments and the control (Table 2) were replicated four times at each site. Soil cover was applied during planting. On the control plots, previous crop residues were ploughed under during tillage. Sowing was done with the first effective rains at each site, with a target of 38,000 plants ha^{-1} in conservation agriculture systems. Weed management was done by hand in CA systems and hand-hoe weeding in control plots. Basal fertilizer (7% N, 14% P_2O_5, and 7% K_2O) was applied at 400 kg ha^{-1} in NRIII; while top dressing (34.5% N) application was at 200 and 100 kg ha^{-1} for NRIII and NRV respectively.

Table 2. Summary of the different treatments used in the experiments.

Treatment	Explanation
$CA_{0t/ha}$	Hand-hoe made planting basins + no crop residue cover
$CA_{2t/ha}$	Hand-hoe made planting basins + crop residue cover at a rate of 2t/ha
$CA_{4t/ha}$	Hand-hoe made planting basins + crop residue cover at a rate of 4t/ha
$CA_{6t/ha}$	Hand-hoe made planting basins + crop residue cover at a rate of 6t/ha
Conv	Conventional tillage with no soil cover applied; residues were ploughed under

***Note**:* *CA = Conservation Agriculture*
t/ha = tons per hectare

Sampling and analysis of macrofauna

Sampling for macrofauna was done only once at the end of 2008-2009 rainy season using the method of Anderson & Ingram (1993). The soil fauna was collected by sifting through monoliths of size 20cmx20cmx20cm from the plots. The monoliths were collected from 3 randomly selected plots of each treatment on each site. In total, fifteen monoliths were collected at each site. The collected macrofauna was identified and counted. Grouping and counting of individuals in each group was considered since the study was a quick test of the potential impacts of conservation agriculture practice on soil macrofauna abundance. Macrofauna abundance, diversity and evenness indices were computed using the Shannon-Wiener (1963) method and then analysis of variance (ANOVA) was done. The formulae used are:

Macrofauna species richness	**S = number of fauna groups present**
Macrofauna abundance (density)	$N = P/A$
Macrofauna diversity index	$H = -\sum (P_i \ln P_i)$
Macrofauna evenness index	$E = H/\ln S$

Where **P** = fauna population in soil specimen; **A** = surface area of monolith ($20x20cm^2$); P_i = the proportion of individuals in i^{th} order estimated as n_i/N; where n_i is the number of individuals in i^{th} order.

Macrofauna abundance in our case represents the total number of individuals in a sample per unit area. Macrofauna diversity has two components i.e. species richness and evenness. While species richness is indicative of the number of different species present in the sample, the concept of evenness goes beyond that. It quantifies the relative abundances of the different species in the samples. Therefore, evenness can best be regarded a measure of the equality of the individuals in the samples.

Results

Analysis of variance showed significant ($P<0.001$) treatments and sites effects on the results. Correlations between amount of crop residue cover applied in CA systems and abundance (Figure 1) and also species richness were positive.

Effects of reduced-tillage and soil cover on macrofauna abundance

The arthropods identified across the sites were grouped into termites (*Isoptera*), ants (*Hymenoptera*), beetles (*Coleoptera*) and centipedes (*Chilopoda*). The mean abundance of each species at each site and treatment are presented in Tables 3, 4 and 5.

Table 3. The effects of reduced-tillage and soil cover on termite abundance.[1]

Treatments	Site No.				
	Site 1	Site 2	Site 3	Site 4	Site 5
$CA_{0t/ha}$	225 a	263 a	97 a	294 a	394 a
$CA_{2t/ha}$	600 b	637 b	188 b	356 a	806 b
$CA_{4t/ha}$	1269 c	713 c	875 c	1332 b	1331 c
$CA_{6t/ha}$	6463 d	1356 d	6044 d	2644 c	1525 d
Conv	81 e	0 e	25 e	38 e	6 e

Table 4. The effects of reduced-tillage and soil cover on ant abundance.[1]

Treatments	Site No.				
	Site 1	Site 2	Site 3	Site 4	Site 5
$CA_{0t/ha}$	10 a	9 a	13 a	13 a	6 a
$CA_{2t/ha}$	19 b	13 b	13 a	19 a	6 a
$CA_{4t/ha}$	25 c	25 c	25 b	88 b	19 b
$CA_{6t/ha}$	38 c	38 d	44 c	106 b	50 c
Conv	0 d	6 e	13 a	0 c	0 d

Table 5. The effects of reduced-tillage and soil cover on centipede abundance.[1]

Treatments	Site No.				
	Site 1	Site 2	Site 3	Site 4	Site 5
$CA_{0t/ha}$	0 a	25 a	7 a	7 a	6 a
$CA_{2t/ha}$	0 a	25 a	7 a	13 b	13 b
$CA_{4t/ha}$	7 b	25 a	7 a	26 c	13 b
$CA_{6t/ha}$	7 b	56 b	13 b	44 d	25 c
Conv	0 a	0 c	0 c	0 e	0 d

[1] Within a column of a table, reported quantities are significantly different where accompanied by different letters ($p<0.05$).

Table 6. The effects of reduced-tillage and soil cover on beetle-larva abundance.[2]

Treatments	Site No.				
	Site 1	Site 2	Site 3	Site 4	Site 5
$CA_{0t/ha}$	13 a	25 a	25 a	19 a	0 a
$CA_{2t/ha}$	25 b	31 a	44 b	31 b	6 b
$CA_{4t/ha}$	25 b	38 ab	50 b	38 b	13 c
$CA_{6t/ha}$	25 b	44 b	69 c	75 c	19 d
Conv	7 a	6 c	0 d	7 d	0 a

Termites were the most predominant macrofauna group across all the sites. There was a significantly higher macrofauna population in CA systems than conventional practice. This was true for all the groups; i.e. termites, ants, centipedes and beetle-larvae. In the CA systems, abundance increased with increasing amount of crop residues applied. However, the increments were not always significant. Abundance was influenced by amount of residues applied and also the weeds. Weeds from each plot were retained as soil cover during weeding operations. Millipedes (*Diplopoda*), earthworms (*Haplotaxida*), crickets (*Orthoptera*) and mites (*Acarina*) were also observed on a few occasions but their numbers were very low, hence they are not presented in the tables. In general, macrofauna abundance was in the order $CA_{6t/ha}$>$CA_{4t/ha}$>$CA_{2t/ha}$>$CA_{0t/ha}$>Conv on all sites and soil types. Macrofauna abundance in CA systems was significantly correlated (r^2=0.55) to the amount of crop residues applied as soil cover (Figure 1).

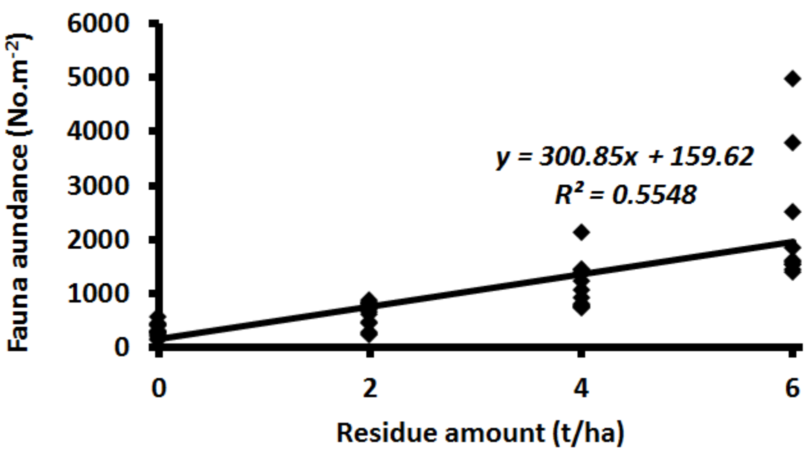

Figure 1. Correlation between amount of residues and macrofauna abundance in CA systems.

Effects of reduced-tillage and soil cover on species richness

The mean numbers of macrofauna groups observed in each treatment for all the five sites are presented in Table 7.

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[2] Within a column of a table, reported quantities are significantly different where accompanied by different letters ($p<0.05$).

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Table 7 Reduced-tillage and soil cover effect on mean number of fauna groups.[3]

Treatments	Site No.				
	Site 1	Site 2	Site 3	Site 4	Site 5
$CA_{0t/ha}$	3 a	3.7 a	4 a	4 a	3 a
$CA_{2t/ha}$	3 a	4 a	4 a	4 a	4 b
$CA_{4t/ha}$	4 b	3.7 a	4 a	4 a	4 b
$CA_{6t/ha}$	3.7 b	4 a	4 a	4 a	4 b
Conv	2 c	2 b	2 b	2 b	1 c

The average number of fauna groups per treatment observed in CA systems was significantly higher than in conventional practice across all sites. Hence, mechanical soil disturbance had a negative influence on species richness. Soil cover did not have a significant influence on the number of fauna groups; however, there was a tendency for increasing number of fauna groups with crop residue amount. Species richness was not influenced by soil type.

Effects of reduced-tillage and soil cover on diversity and evenness indices

Figure 2 shows the relationships between treatment and indices that describe diversity and evenness on the different sites.

Site and treatment effects on macrofauna diversity and evenness were highly significant ($P<0.001$). Both diversity and evenness were influenced by treatments in the same ways at each site, as shown in Figure 2. The figure shows significantly higher ($P<0.05$) diversity and evenness indices for conventional systems (Conv) than CA systems with high levels of crop residue retention (i.e. $CA_{4t/ha}$ and $CA_{6t/ha}$) on sites in SELZ (i.e. sites 1. 2, 3 and 4 in NRV) where sorghum residues were used. In this zone, CA without residues ($CA_{0t/ha}$) also had comparatively higher diversity and evenness indices than CA systems with residue retention. The site in Kadoma (site 5 in NR III), onto which maize residues were retained, deviated from this pattern; instead diversity and evenness indices increased with residue amount. Diversity and evenness indices on this site were significantly higher ($P<0.05$) in $CA_{6t/ha}$ than other CA systems (i.e. $CA_{0t/ha}$, $CA_{2t/ha}$ and $CA_{4t/ha}$) and conventional system (where indices were close to zero).

Although the relationship between crop residue amount on the one hand and diversity and evenness indices, on the other hand, was not consistent across the sites, diversity and evenness were clearly higher in systems with no crop residues ($CA_{0t/ha}$ and Conv) than in CA systems with residues ($CA_{2t/ha}$, $CA_{4t/ha}$ and $CA_{6t/ha}$) where sorghum stover was used. However, the opposite was true for the case where maize stover was applied.

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[3] Within a column of a table, reported quantities are significantly different where accompanied by different letters ($p<0.05$).

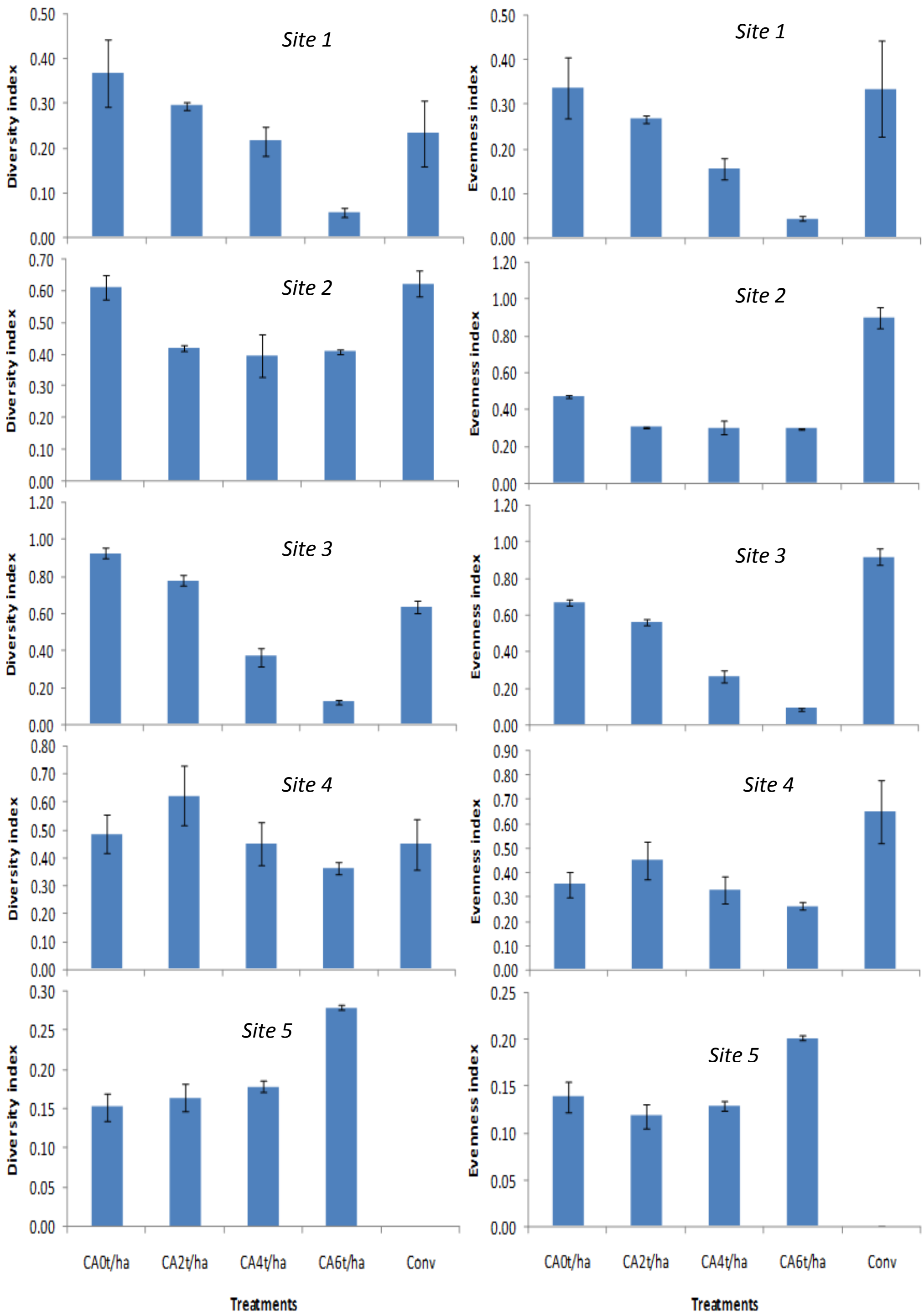

Figure 2. Effects of reduced tillage & soil cover on macrofauna diversity and evenness across sites (Note: error bars = standard errors of means).

Discussion

The most significant macrofauna group found across the sites was termites. Termites are renowned primary shredders of most dry organic materials; hence their populations tend to increase with increasing amounts of organic material applied as soil cover. The beetle-larvae and ant groups were also important in comparison with the rest. The results show that CA has a profound effect on macrofauna density as well as diversity and an increase in macrofauna will aid in the increase in rate of decomposition of organic material. Although Nhamo (2007) observed soil type as an important determinant of fauna groups, soil type did not show a significant effect in this research. Agro-climatic zone did not have any effect on number of fauna groups because all four macrofauna groups were observed on sites in the two agro-climatic zones.

It appears quick and convenient to use the number of fauna groups (species richness) in assessing fauna community situations. Higher species richness would suggest a complex community with a high degree of species interaction; hence that community is capable of supporting higher levels of energy transfer, predation, competition and niche availability than other similar communities that exhibit lower species richness. The result of an improvement in species richness upon adoption of CA clearly demonstrates the importance of reduced tillage in protecting fauna activity and habitat. The addition of organic matter on soil surface further ameliorates soil conditions for better survival and support of more fauna groups. Woltering (2005) observed similar results where soil cover in the form of crop residues increased biological activity. However, the number of fauna groups alone does not give much information about community potential because no account of number of individuals per species or the evenness of individuals within each species is taken; hence it is seldom used for describing community structures. It is only important for initial inferences on condition of a given community. The concept of fauna abundance (or population) is, instead, used to describe the carrying potential for a system.

Fauna abundance is indicative of total number of individuals per unit area which are capable of living within the given environment. If the theory that higher species richness results in higher levels of energy transfer holds, then the same can be applied when fauna abundance is higher. In our study, macrofauna abundance was significantly higher in CA systems than conventional systems. Macrofauna abundance increased with the amount of crop residues applied, in the order $CA_{6t/ha}>CA_{4t/ha}>CA_{2t/ha}>CA_{0t/ha}$. Mutsamba *et al*. (2010) made a similar observation in an assessment of impact of residue amount on termite population. The strong correlation (r^2=0.55) between soil cover and macrofauna abundance suggests increasing capacity to support more soil fauna with additional crop residues. Increased macrofauna abundance would be important in rehabilitation of degraded soils and maintenance of agro-systems prone to serious capping.

It was interesting to note that diversity and evenness were affected in almost the same way by treatments at each site. In the case where sorghum residues were used, diversity and evenness were significantly higher in conventional and CA system without residue retention than CA with residue retention. A possible explanation to the decreasing diversity and evenness indices in our case could be that too high residue cover was detrimental for some fauna species development while favouring development of others. Termites were the most prominent macrofauna group where sorghum residues were used. Further evidence supporting higher diversity and evenness in conventional than CA systems could be that development of some macro-fauna (e.g. mites) are enhanced by tillage and appear to recover from tillage disturbances more rapidly (Reedler et al., 2006 & Wardle 1995). However, our results in the case where maize residues were used agreed with the observations made by Verhulst et al. (2010) who reported an increased species diversity when reduced tillage was combined with residue retention. Therefore, species diversity and evenness appeared to depend on the quality of organic material retained on the soil surface.

Although the relationship between treatments and macrofauna diversity and/or evenness across sites was not consistent, termites became more dominant with soil cover application in the majority of cases. This is indicative of the fact that decomposition was generally in its early stages and the primary shredders (termites) of organic matter were the more active group. It would, therefore, be important to monitor dynamics of fauna diversity and evenness in longer term trials.

Conclusions

Conservation agriculture practices have potential to increase agricultural productivity through better efficiency in utilization of inputs and other resources due to improved soil conditions. The aspects of improved soil structure and fertility which were most pronounced in this study enhance greater environmental sustainability. This study showed that residue rate had significantly positive effects on macrofauna abundance; however the quality of organic material applied is important. Maize residue retention yielded superior macrofauna diversity and evenness indices under conservation agriculture practices than sorghum residue retention. In the short-term, conservation agriculture exhibited potential to attract higher levels of macrofauna and this is important as the initial stage in natural rehabilitation of degraded arable lands. Soil macrofauna are important regulators of decomposition, nutrient cycling, soil organic matter dynamics, and pathways for aeration and water movement as a consequence of their feeding and burrowing activities. Subsequent follow up studies would be important to ascertain soil regeneration in the medium to longer-term. Continuous sampling of fauna during the decomposition cycle would also be important to reduce biases associated with dominance of particular species at certain stages of the process.

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Assessing the training needs of agricultural extension workers about organic farming in the North-Western Himalayas

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Abstract

Agricultural extension organizations worldwide face challenges of professional competence among their employees. Planning, training and management of human resources within extension organizations are essential to increase the capabilities and overall effectiveness of extension personnel. This paper examines the training needs of agricultural extension workers in the state of Himachal Pradesh, India, regarding organic farming. Random sampling was used to select 65 extension personnel of the Himachal Pradesh State Department of Agriculture (HPSDA) from within ten districts of the state. The data are self reported scores collected with a structured instrument in which ten aspects of organic farming were addressed. The results revealed that the majority of extension workers reported medium to high training needs in seven specific areas: bio-dynamic farming, homa farming, bio-rational pest management techniques, biological methods of pest control, bio-fertilizer technology, record keeping/certification standards, and grading/packing and marketing of organic produce. The majority of extension workers reported low or no training needs in the areas of composting/vermicomposting, green manuring/green leaf manuring, and crop rotations. There was no significant relationship between age, educational qualifications, or service experience with identified training needs. To achieve the potential for the uptake and successful implementation of organic farming amongst Himachal Pradesh farmers, the training of HPSDA agricultural extension workers could concentrate on improving their knowledge in the seven identified areas of organic farming skills.

Keywords: Organic agriculture, training needs, extension officers, Himachal Pradesh, India.

Introduction

Agriculture is the back bone of the Indian economy and plays a vital role in the overall development of the nation (DES, 2012). About 70% of India's population, that is 830 million people, reside in rural villages, and agriculture is their primary source of food, fodder and fuel, as well as income to satisfy other needs (Agoramoorthy, 2008; Baruah & Bora, 2008; Singh *et al*., 2007). However, Indian agriculture is facing serious challenges because of its ever-increasing population, limited land and water availability, and

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degradation of natural resources. It is desirable to increase agricultural productivity in a sustainable manner. The excessive use of agro-chemicals over past decades has deteriorated soil health leading to declines of crop yields and produce quality (Yadav, 2011a).

Organic farming is a system involving the use of organic sources for crop nutrition, biological sources for pest and disease management, recycling of farm and animal wastes in order to increase as well as sustain productivity, and could be the most appropriate development path for Indian agriculture. According to Rao (2000) organic farming is an agriculture production system that sustains the demands of production without interrupting the natural eco-system and with little or no dependence on chemical fertilizers and other agricultural chemicals through the increased use of organic matter, bio-fertilizers, reduced tillage, integrated pest management and the adoption of integrated farming systems.

Organic agriculture has grown from 15.8 million hectares to 37.2 milllion hactares worldwide in the course of a decade, and India rates fifth in the world for speed of uptake (Paull, 2011a) and this has occurred with some support from the Indian government. India ranks seventh in the world with 1.2 million hectares of certified organic agriculture, which constitutes about 0.6% of India's total cultivable area (Willer & Kilcher, 2011). India has made substantial progress in organic farming with its national standards of organic production (NSOP) and accreditation widely recognized, including by the European Commission (EC) and the United State Department of Agriculture (USDA) (Wai, 2007; Willer & Kilcher, 2009).

Himachal Pradesh is a hilly northern state of India with the majority of farmers having rain-fed, marginal and small holdings (Singh *et al*., 1998) and with agriculture being their primary source of food and nutritional security. The hill farming systems are organic in nature by default and so they offer vast opportunities for commercialization. The state government has identified organic farming as one of the important areas for agriculture development and have launched a number of programmes for the promotion of organic farming. An organic cluster project of 1200 ha at a cost of 26.7 million rupees (US $490,000) has been launched (Rana, 2011) and the Japan International Cooperation Agency (JICA) has funded a project of 3.21 billion rupees (US$58.7m) described as "crop diversification promotion project" and with organic farming as one of its important elements (www.hpagriculture.com/schemes).

Organic farming has the potential to become an important agri-business among the farmers of the country in general, and marginal farmers of hilly states like Himachal Pradesh in particular, owing to the premium returns from organic produce. However, many organic farmers are not achieving the desired benefits as expected or technically projected by the experts, mainly due to the farmers' lack of technical know-how. This situation has occurred particularly due to the lack of sound technical back-up support, as organic farming is just in its infancy and requires considerable technical knowledge.

A review of the literature indicates variations and deficiencies in knowledge, skills and ability among extension personnel (e.g. Wheeler, 2007, 2008). For the present investigation, the training needs of agricultural extension officers were investigated (a) to identify the training needs of extension personnel and (b) to determine the relationship, if any, between three independent variables (age, educational qualification, and service experience) and training needs.

Materials and Methods

Research design and study area

An exploratory research design was used in the present investigation. The study on training needs of agricultural extension workers of the Himachal Pradesh State Department of Agriculture (HPSDA) about organic farming technology was conducted during the year 2011 in the state of Himachal Pradesh, India (Figure 1). Himachal Pradesh is situated in North-Western Himalayas between 30^{o} 22' 40" N to 33^{o} 12' 40" north latitude and 75^{o} 45' 55" E to 79^{o} 04' 20" east longitude. Out of the 12 districts of the state, a sample of 65 agricultural extension officers across the state (except for two districts, Kinnaur and Lahaul & Spiti) was drawn randomly for the present investigation; the sample size from each participating district is random (Table 1).

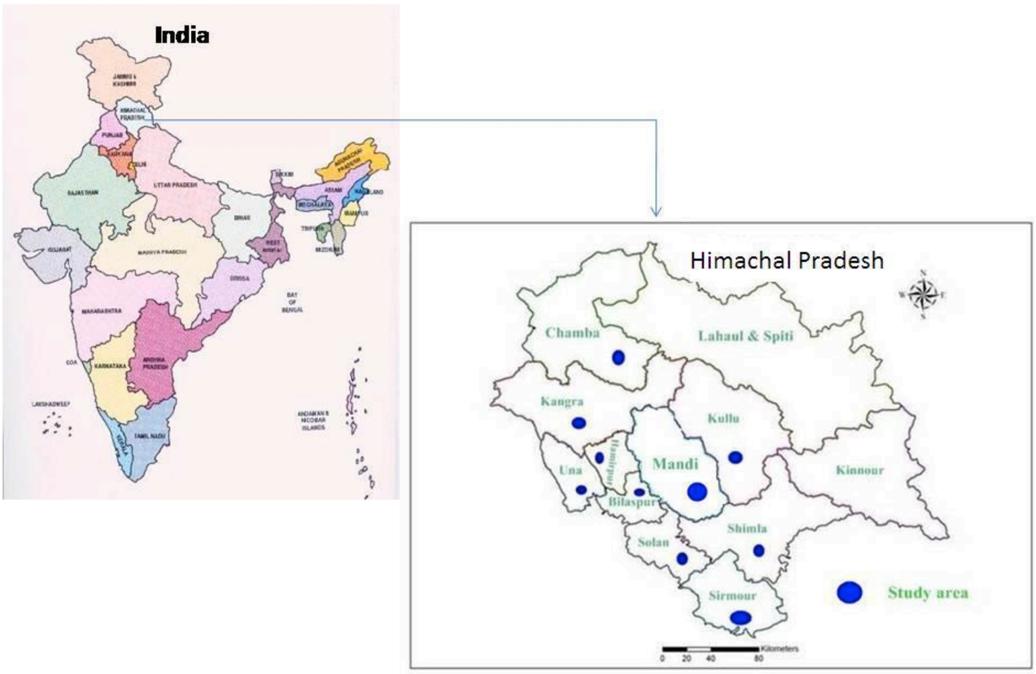

Figure 1. Location of study area.

Table 1. Geographic distribution of sample for assessing the training needs of extension workers of HPSDA.

Districts	Sample size
Kangra	8
Mandi	5
Kullu	7
Hamirpur	7
Chamba	9
Bilaspur	4
Sirmour	8
Una	8
Solan	4
Shimla	5
Total sample	**65**

Tools of the data collection

Training needs were self-assessed and reported, using the perception by self method (Singh 2000). A schedule of ten potential need areas was developed with the aid of literature available on organic farming technologies (Table 3). The schedule was put before the extension workers and were completed based on their self-assessment of their own ability and job need level. The survey instrument included (i) ten questions pertaining to 'Level of ability' which called for a rating by the respondent as 'High', 'Medium' or 'Low' (ii) ten questions on 'Level of need of the job' which also called for a rating 'High', 'Medium' or 'Low' and (iii) three demographic questions. The language of the instrument was the local language of the region as applicable.

The ten identified organic farming need areas for the purposes of the present study were: composting/vermi-composting; green manuring/green leaf manuring; crop rotations; bio-fertilizer technology; bio-dynamic farming; homa farming; biological methods of pest control; bio-rational pest management techniques; record keeping and certification standards; and the grading/packing and marketing of organic produce. The list of ten need areas is not an exhaustive list and there is some overlap between the areas (Table 3).

The so-called green revolution technologies created adverse effects on ecosystems due to the excessive and imbalanced use of synthetic inputs. This situation has led to identifying benign inputs like bio-fertilizers and the use of such natural products to help in safeguarding the soil health as well as quality of products. Bio-fertilizers are an important component in organic farming, they are an alternative to chemical fertilizers, stimulate plant growth, activate soil biologically, restore natural soil fertility, provide protection against drought and some soil borne diseases.

Composting can play an important role in solid waste management programs and can greatly reduce the amount of waste going to landfill and besides it conserves resources, reduces pollution and builds healthy soil. Green manuring is an important component of organic farming as it not only maintains the soil fertility but also conserves soil moisture.

Two specific forms of organic agriculture, bio-dynamic farming and homa farming, were included in the list of ten training topics of the study. Bio-dynamic farming is a form of organic farming that was developed from agricultural lectures of Rudolf Steiner (Paull, 2011b). Homa farming is an Indian practice which derives from the Vedic science of bio-energy denoting the process of removing the toxic conditions of the atmosphere through the agency of fire. This means healing and purifying of the atmosphere with fire as the medium. Homa farming involves the application of agnihotra and homa therapy techniques in organic agriculture. The ash produced by the Hindu ritual agnihotra fire is credited with having fertilizing as well as plant protecting qualities. Agnihotra is reputed to heal the atmosphere, replenish the nutrients necessary for healthy organically grown crops, and to have relevance in pest management (Pathak, 2011; Punam et al., 2011).

Another of the ten knowledge areas identified and included in the study was bio-rational pest management techniques. Bio-rational pesticides are derived from a variety of biological sources, including bacteria, viruses, fungi and protozoa, as well as chemical analogues of naturally occurring biochemicals such as pheromones and insect growth regulators (IGRs). They are considered third-generation pesticides that are

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environmentally sound and closely resemble or are identical to chemicals produced by insects and plants. Most bio-rational insecticides are more effective against some insect pests than others. They are typically target-specific and have little to no impact on non-target organisms (Ware, 1989). As a result of such specificity, proper identification of a target insect pest is essential. Bio-rational insecticides have relatively short residual activity compared with synthetic chemical products, and therefore bio-rational products must be applied when the pest is in its most vulnerable life stage, otherwise, applications may be ineffective (Ware, 1989). Bio-rational insecticides are classified into two distinct groups: biochemical and microbial. Biochemical products include hormones, enzymes, pheromones and natural insect and plant growth regulators. Microbial products originate from biological organisms such as bacteria, fungi, nematodes, protozoa and viruses (Ware, 1989).

Determining job need level

For each need area, respondents were asked to indicate one out of three levels of job need, viz. high, medium or low job need level, with corresponding scores of 3, 2, and 1 applied respectively. High, medium and low job need levels refer to high work need frequency (daily/weekly need), moderate work need frequency (15 days to two months), and low work need frequency (once in two to six months), respectively.

Determining ability level

The ability includes knowledge and skill in a particular aspect of a job. For each need area, respondents were asked to indicate one out of three ability levels viz. low, medium, and high ability with corresponding scores of 1, 2 and 3 applied, respectively. Low, medium, high ability corresponds to ‘many mistakes’, ‘few mistakes’, and ‘no or very few mistakes’ made while performing one’s job, respectively.

Determining Training Need

Depending on the ability level and the job need level, the level of the training need of extension workers was determined by adopting the criteria which appear in Table 2 (Singh, 2000).

Table 2. Criteria for determining of training needs of extension personnel.

Level of need of the job	Level of ability	Training need
High	Low	High training need (HTN)
High	Medium	Moderate training need (MTN)
High	High	Low training need (LTN)
Medium	Low	Moderate training need (MTN)
Medium	Medium	Low training need (LTN)
Medium	High	No training need (NTN)
Low	Low	Moderate training need (MTN)
Low	Medium	No training need (NTN)
Low	High	No training need (NTN)

In the present study 'training needs' was considered as the dependent variable while age, educational qualification, and service experience were taken as independent variables. The data collected were quantified, categorized and tabulated by using statistical tools including frequency counts and percentages to draw conclusions. The effect of independent variables on the dependent variable was studied by using the chi square test of significance.

Results

Content of training needs

The data pertaining to training needs were collated and are presented in Table 3. For three of the need areas (composting, green manuring and crop rotations) the majority of the respondents recorded low to no training needs. For composting/ vermi-composting, 71% of respondents scored a low or no training need (Table 3). For green manuring 54% of respondents reported a low or no training. For crop rotations 77% of respondents scored a low or no training need (Table 3).

For the other seven knowledge areas the majority of respondents scored a medium to high training need. For bio-fertilizer technology, 52% of the respondents reported medium training needs followed by high training need of 22% leading to the conclusion that they possessed inadequate knowledge. For both bio-dynamic farming and homa farming the majority of the respondents scored medium to high training needs (Table 3). Plant protection is a challenging aspect of organic farming. Plant diseases and pests are associated with crop damage and sometimes crop failure, and 51% of extension personnel reported medium training needs followed by high training needs of 26% in biological methods of pest control. For bio-rational pest management techniques, the majority of them reported medium or high training needs, 43% and 37% respectively. Record keeping and certification standards are an important component in organic farming without which the produce can not be sold as certified organic, and the majority of respondents reported a medium (40%) or high (24%) training needs indicating that they possessed inadequate knowledge in this area (Table 3). Without proper grading and packing the best price in the market will not be achieved, and extension workers reported training needs of medium (38%) and high (26%) in this area.

Of the 650 ratings of training needs recorded (65 respondents x 10 training areas), 41.7% of all responses were for a specific 'medium' training need, and this was the most common rating of specific needs. There were 20.6% of responses for a specific 'high' need, 28.9% of responses for a specific 'low' training need, and 8.8% of all responses were for a specific 'no' training need (Table 3).

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Table 3. Training need of extension workers about organic farming components (N=65).

Sr. No	Subject Matter of Training Need	Training Need							
		HIGH		MEDIUM		LOW		NO NEED	
		No. (Frequency)	Percentage (%)	No. (Frequency)	Percentage (%)	No. (Frequency)	Percentage (%)	No. (Frequency)	Percentage (%)
1.	Composting/ vermi-composting	1	1.54	18	27.69	32	49.23	14	21.54
2.	Green manuring/ green leaf manuring	6	9.23	24	36.92	20	30.77	15	23.08
3.	Crop rotations	0	0.00	15	23.08	37	56.92	13	20.00
4.	Bio-Fertilizer technology	14	21.54	34	52.31	15	23.08	2	3.08
5.	Bio-dynamic farming	19	29.23	35	53.85	9	13.85	2	3.08
6.	Homa farming	20	30.77	33	50.77	10	15.38	2	3.08
7.	Biological method of pest control	17	26.15	33	50.77	14	21.54	1	1.54
8.	Bio-rational pest management techniques	24	36.92	28	43.08	12	18.46	1	1.54
9.	Record keeping & Certification standards	16	24.61	26	40.00	18	27.69	5	7.69
10.	Grading/packing & marketing of organic produce	17	26.15	25	38.46	21	32.31	2	3.08
	TOTALS	134	20.62%	271	41.69%	188	28.92%	57	8.77%

Overall extent of training needs

The results pertaining to the extent of training needs of extension workers are presented in Table 4. The overall extent of training need was determined by following the same procedure as earlier adopted by Yadav et al. (2011d & 2012). In this method, the ability level of the respondents were identified for each area as low, medium and high ability level with corresponding scores of 1, 2 and 3 respectively. For each respondent, the scores for all ten areas were added. This was the individual's overall ability level score. The maximum obtainable score for all ten practices was 30 and the minimum 10. The ability level of all the respondents were further categorized as low (a score of 10), medium (11-20 score) or high (21-30 score). Similarly, the job need level of respondents was established for each area as high, medium and low and assigned scores 3, 2 and 1 respectively. Then for each respondent, the scores for all ten areas were added. This was the individual's overall job need level score. The maximum obtainable score for all ten areas was 30 and the minimum 10. These were then categorized as low (a score of 10), medium (11- 20 score) and high (21-30 score). Based on the overall ability level score and the overall job need level score, the overall extent of training need per respondent was established by reapplying the matrix of Table 2 (Singh, 2000).

Using this method, the majority of the respondents scored an overall medium training requirement. The result was that 69% of the respondents were rated as having an overall medium training need and 31% of respondents were rated as having an overall low training need in organic farming (Table 4).

Table 4. Overall extent of training need about organic farming components (N=65).

Extent of training need	No. (Frequency)	Percentage (%)
MEDIUM	45	69.23
LOW	20	30.77

Relationship between independent variables and training needs

The data pertaining to the relationship of the three independent variables to the overall extent of training need is presented in Table 5. The data revealed that all the independent variables, age, education, and professional experience, showed non-significant relationship with the overall extent of training needs of extension workers with chi square values of 4.19, 4.98 and 4.86 respectively (Table 5).

Table 5. Relationship between selected independent variables and overall extent of training need.

Particular	Training need			Chi square Calculated	Chi square Tabulated	Significance level (5%)
	MEDIUM	LOW	Total			
Age						
Young (24-35 years)	17	3	20	4.19	5.99	Non - significant
Middle (36-46 years)	12	5	17			
Old (47-57 years)	16	12	28			
Total	**45**	**20**	**65**			
Education						
Matric	7	3	10	4.98	9.49	Non - significant
Matric+ Diploma	2	4	6			
10+2	6	3	9			
Graduate	13	6	19			
Post Graduate	17	4	21			
Total	**45**	**20**	**65**			
Professional experience						
Up to 10 years	26	7	33	4.86	5.99	Non - significant
11-20 years	5	1	6			
>20 years	14	12	26			
Total	**45**	**20**	**65**			

Discussion

Knowledge is important component of behaviour and plays a major role in the covert and overt behavior of human beings and training is the commonly used method to achieve a direct impact on the knowledge and skills of clients. Grass root extension workers need to be equipped with knowledge of a variety of technological skills which they can transfer through needs based training. This can effect a diffusion of knowledge and innovations to the target groups for the desired outcome of any technology.

In the present study where the majority of respondents scored medium to high training needs for seven of the knowledge areas we speculate that this was probably due to their poor exposure to sources of information and this has had a negative impact on the knowledge level of extension officers in these areas (Wheeler 2008). With the increasing consumer demand for organic, 'natural' and 'green' products, the medium to high training needs of extension workers signifies the importance of inclusion of organic farming components in future training programmes of extension workers.

The respondents reported inadequate knowledge about organic plant protection strategies including biological methods of pest control and bio-rational pest management techniques and the majority of them rated medium to high needs in these areas which could be due to their lack of exposure to appropriate knowledge, skills and awareness. Earlier, Pillegowda *et al.* (1997), Prasad *et al.* (2000) and Yadav *et al.* (2011b) have also reported plant protection as a most important training area in their respective studies.

Record keeping and certification standards are also a very important component of organic farming for registration purposes and for achieving the premium returns for certified organic produce. Similarly, without proper grading and packing the produce will not fetch the best price in the market. In the present study, the majority of respondents rated a medium to high training need in these areas which emphasises their importance.

The majority of the extension workers rated medium to high training needs in most of the nominated areas of organic farming. Precision farming requires specific knowledge and skills (Yadav *et al.* 2011b, 2011c). The overall medium training requirements as rated by the majority of respondents could be due to the lack of exposure to workshops and specific training opportunities. Earlier, Sood (1996), Barman *et al.* (2000) and Yadav *et al.* (2011b, 2011c) also reported overall medium training needs for particular agricultural knowledge areas in their studies.

Organic farming is a new area of agri-business which requires some specific knowledge and skill. However, the selected characteristics of respondents (age, education and service experience) did not show any significant relationship with overall extent of training needs hence the null hypothesis was accepted with the conclusion that these characteristics were not correlated with training need. Earlier, Shrestha (1983), Kaur (1985) and Mahar (1992) also reported non-significant relationships among these variables in their respective studies.

Conclusion

Organic agriculture is emerging as an important income generating oppportunity in the state of Himachal Pradesh. The majority of the extension workers of the HPSDA reported medium to high training needs for seven out of the ten topics of organic farming that were examined. The three exceptions were composting/vermi-composting, green manuring/ green leaf manuring, and crop rotations where the majority of respondents scored a low or no need.

The present study reports a need to upgrade the knowledge and skills of extension workers in seven identified areas of organic farming technology. Therefore, training courses on organic farming coupled with practical demonstrations on important areas including bio-fertilizer technology, bio-dynamic farming, homa farming, biological pest control, bio-rational pest management techniques, record keeping and certification standards, grading/packing and marketing of organic produce should be organized to update the knowledge and skill of the extension workers of HPSDA for the effective transfer of such technology to their clients for harnessing the full potential of organic agriculture.

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The use of desalinated-dried jellyfish and rice bran for controlling weeds and rice yield

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Abstract

To achieve higher rice production, rice-growing countries have used great amounts of synthetic chemical compounds (chemical fertilizers and pesticides) that can have adverse effects on the environment and humans. Organic products and organic farming technologies are friendlier to the environment and more conducive to sustainable agriculture but require different inputs, knowledge and skills. Weed control is one of the major challenges in organic rice cultivation. The present study proposes and tests the use of desalinated-dried jellyfish chips in the development of sustainable rice production. Vast amounts of jellyfishes have been found in the Sea of Japan (Nomura's jellyfish, *Nemopilema nomurai* Kishinouye) and Japan inland sea areas (Water jelly, *Aurelia aurita* (Linne)), and jellyfish populationc can have a negative impact on the fishery industry. In this context, the use of jellyfish in organic agriculture has attracted attention. The present study found that the application of desalinated-dried jellyfish (small pieces of jellyfish which are desalinated and dried) mixed in soil before transplanting can effectively control weeds in rice fields and has a nutrient effect because of the high nitrogen content (12-13%). Desalinated-dried jellyfish has potential as an agricultural material that replaces herbicides and chemical fertilizers. It also contributes to environment-friendly rice production. It was found that both desalinated-dried jellyfish and rice bran effectively controlled rice weeds when mixed in the soil before the transplanting.The grain yields of desalinated-dried jellyfish treatments were consistently higher than the corresponding rice bran treatments. The rice yield from the desalinated-dried jellyfish treatments were comparable to the chemical fertilizer treatment.

Keywords: desalination, jellyfish chip, organic rice, rice bran, weed control and yield, organic agriculture, organic farming, Japan.

Introduction

Rice is highly adaptable to its environment and can be grown in widely different locations and in a variety of climatic environments. However the impacts of chemical fertilizer application on the environment and humans include water pollution, greenhouse gas emissions (N_2O), damage to human health, soil acidification and soil salinization. As a result of these effects, soil productivity, agricultural sustainability and human survivability are negatively impacted (Paull, 2007). A very high level of nitrogenous fertilizer has been applied in Japan (FAO, 2013). The use of herbicides has been accompanied by resistance to herbicides in many countries. One of the major problems with the use of herbicides is that the repeated use of the same herbicide induces the emergence of

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herbicide-tolerant weeds (Diggle *et.al.* 2003). In addition, the overuse of chemicals causes soil exhaustion and environmental pollution. These chemicals are also harmful to human health (Harris & Hill, 2007; Shimbo *et al.,* 2001).

According to the International Federation of Organic Agricultural Movements (IFOAM), the role of organic agriculture, whether in farming, processing, distribution, or consumption, is to sustain and enhance the health of ecosystems and organisms. Thailand is reported as the world's leading organic rice producer country, followed by the Philippines and Italy (Willer & Yussefi, 2007).

Rice is the main food grain in Japan, and Japanese consumers have a strong desire for organic rice grown without the use of, or with a decreased use of, farm chemicals. In Japan, research on the production of organic rice or with a decreased use of farm chemicals was begun in 1988 (Chunjiang & Guiqing, 1999). Organic rice production has been practiced using methods including integrated rice-duck farming (Furuno, 2001; Hossain *et al.* 2005; Hossain *et.al.,* 2007) and using the Japanese rotary weeder, non-woven fabric mulch (Tsuno, 1993; Hossain *et al.* 2009), rice bran, and newspaper mulch (Ueno *et al.,* 1999).

Bran is the hard outer layer of a grain and consists of a combination of aleurone and pericarp. Rice bran has mass amounts of protein, vitamins, minerals and 107 known naturally occurring antioxidants. It contains valuable components such as oil, protein, vitamins and some essential minerals as well as enzymes, microorganisms, natural toxicant constituents, and may also contain harmful contaminants and adulterants (Barber & Barber, 1980). To use rice bran in rice production, the farmer fills the field with water when the seedlings are very young and scatters rice bran on the rice field.

Jellyfish are planktonic marine members of a group of invertebrate animals composed of about 200 described species of the class *Scyphozoa* within the phylum Cnidaria or class Cubozoa. They can be found in every ocean in the world. Jellyfish are an important foodstuff in Asia, especially in China and Japan. The average annual catch of jellyfish between 1988 and 1999 in Southeast Asia was estimated to be about 169,000 metric tons in wet weight, and the worldwide annual catch is approximately 321,000 metric tons (Omori & Nakano, 2001). The jellyfish are reported to have a negative effect on the Japanese fishing industry (http://asiapundit.com/2006/06/12/three-gorges-and-giant-jellyfish/). Nomura's jellyfish, typically found in Japan, measure up to one meter in diameter and can weigh as much as 200 kilograms (http://pinktentacle.com/tag/jellyfish/).

In the present study, we compared the effectiveness of two materials, rice bran and desalinated-dried jellyfish, in controlling weeds in transplanted rice. Other objectives of the study were to determine the effectiveness of rice bran and desalinated-dried jellyfish as sources of nutrients, and ultimately to develop a new organic rice farming system.

Materials and Methods

Analysis

Jellyfish can be found in every ocean in the world and in the present study desalinated-dried jellyfish chips were made from live jellyfish that were killed in a high-concentration mixed-solution of salt and alum and then cut finely and air dried. The desalinated-dried jellyfish and the rice bran were separately ground to a fine powder. Total N and C content in the subsamples were analyzed using an N-C analyzer (NC-80, Sumica Chemical Analysis Service, Japan) (Table 1).

To measure P, K, Ca, Mg, Na, Cu, and Zn contents, the subsamples were digested with HNO_3 and $HClO_4$. After digestion, the filtrate was subjected to P analysis by using the molybdate blue method and the K, Ca, Mg, Na, Cu, and Zn were analysed by atomic absorption spectrometry (AA-6200, Shimadzu Co. Ltd., Japan) (Table 1).

Table 1. Mineral and carbon contents of desalinated-dried jellyfish and rice bran (% of dry weight).

	N	P	K	Ca	Mg	Na	Cu	Zn	C	C/N
Jellyfish	13.05	0.73	0.03	0.07	0.06	2.05	0.00	0.00	41.6	3.2
SE	0.250	0.095	0.003	0.006	0.000	0.073	0.000	0.000	0.681	
Rice bran	2.51	3.04	2.30	0.05	1.03	0.01	0.00	0.00	47.7	19.0
SE	0.082	0.109	0.110	0.004	0.000	0.001	0.000	0.000	1.253	

n=3

The mineral and carbon of desalinated-dried jellyfish and rice bran are shown in Table 1. It was observed that jellyfish is high in nitrogen (13.05%) and that its C/N ratio is very low. On the other hand, rice bran has high quantities of P and K, and the C/N ratio is high (19.0).

Experiment 1: Wagner pot experiment

The experiments were conducted at the Faculty of Agriculture, Ehime University, Japan. In the pots (Wagner size 1/5000a), three seedlings of cultivar Koshihikari were transplanted on May 15, 2007. Desalinated-dried jellyfish (1.25, 2.5 and 5 g/pot) and rice bran (1.25, 2.5 and 5 g/pot) were applied to the pots in the following two ways: (a) as a mixture in the soil before the transplanting (basal application); and (b) as an application on the soil surface just after the transplanting (top application). Thus there were thirteen treatments: 3x2 jellyfish treatments + 3x2 bran treatments + control.

Each treatment was replicated three times. The number of weeds was counted and the total (top plus root) dry weight of the rice plants was measured on July 1, 2007 (47 days after the transplanting). Samples were oven-dried at 85 °C to an almost constant mass (3 days) and weighed (Sugimoto *et al.,* 2005).

Experiment 2: Field tube experiment

Field experiments were conducted at the Faculty of Agriculture, Ehime University, Japan. A tube made from plastic pipe (25 cm in diameter, 30 cm in length) was set in the field so that the treatment condition was contained and protected from the soil situation beyond. Three seedlings were transplanted to the center of each tube on July 12, 2007.

Seven different fertilizer treatments were applied (keeping the applied nitrogen constant) plus there was a control (no application). The eight treatments were:

(1) No application (0-0);
(2) Chemical fertilizer at $10gN/m^2$ mixed in soil just before the transplanting (C10-0);

(3) Desalinated-dried jellyfish at 10gN/m^2 mixed in soil just before the transplanting (J10-0);
(4) Desalinated-dried jellyfish at 5gN/m^2 mixed in soil just before the transplanting and at 5gN/m^2 applied on the soil surface 1 day after the transplanting (J5-J5);
(5) Desalinated-dried jellyfish at 10gN/m^2 applied on the soil surface 1 day after the transplanting (0-J10);
(6) Rice bran at 10g/m^2 mixed in the soil just before the transplanting (R10-0);
(7) Rice bran at 5gN/m^2 mixed in the soil just before the transplanting and at 5gN/m^2 applied on the soil surface 1 day after the transplanting (R5-R5);
(8) Rice bran at 10gN/m^2 applied on the soil surface 1 day after the transplanting (0-R10) (Table 2).

The amount of jellyfish or rice bran applied in the tube was determined by calculation on the basis of the tube size and the amount of nitrogen in the specific product. The amount of desalinated-dried jellyfish was 4.09 g or 2.05 g/tube and that of rice bran was 19.6 g or 9.8 g/tube.

The weight of the chemical fertilizers was 3.77g/tube and chemical fertilizer was applied as $N:P_2O_5:K_2O$=13%:13%:13% (3.77x0.13=0.49 g) (Table 2).

Table 2. Treatment combinations used in the field tube experiment (Experiment 2).

Treatment (Abbreviation)	Kind of fertilizer	Amount of fertilizer (g/tube)		Applied nitrogen	
		Mix in soil	Surface	(g/tube)	(g/m^2)
0-0	Nil	-	-	-	-
C10-0	Chemical Fertilizer	3.77	-	0.49	10
J10-0	Jellyfish	4.09	-	0.49	10
J5-J5	Jellyfish	2.05	2.05	0.49	10
0-J10	Jellyfish	-	4.09	0.49	10
R10-0	Rice bran	19.6	-	0.49	10
R5-R5	Rice bran	9.8	9.8	0.49	10
0-R10	Rice bran	-	19.6	0.49	10

Jellyfish: Desalinated-dried jellyfish

Each treatment was replicated four times. The oxidation-reduction potential was measured at a soil depth of 1-2 cm using a soil oxidation-reduction meter with platinum electrodes (Ehs-120, Fujiwara Seisakusyo, Japan). The number of weeds inside the tube was counted on August 13 (35 days after the transplanting). Plants were harvested on October 12. The grain yield and yield components were measured. The grains were divided into filled grains and unfilled grains using salt water with a specific gravity of 1.06, and the percentage of filled grains was determined. The 1,000-grain weight was measured, and the grain weight was calculated at a moisture content of 15%. Analysis of variance (ANOVA) was performed to statistically analyze the data using a software package (Excel Statistics 2006 for Windows, Social Survey Research Information Co. Ltd., Tokyo, Japan).

Results and discussion

Experiment 1

It was observed that both desalinated-dried jellyfish and rice bran controlled weeds better when mixed in the soil before the transplanting compared to an application on the soil surface just after the transplanting. In both cases, an application amount of 5 g/pot showed better weed control ability than the lower doses of 2.5 and 1.25 g/pot (Figure 1).

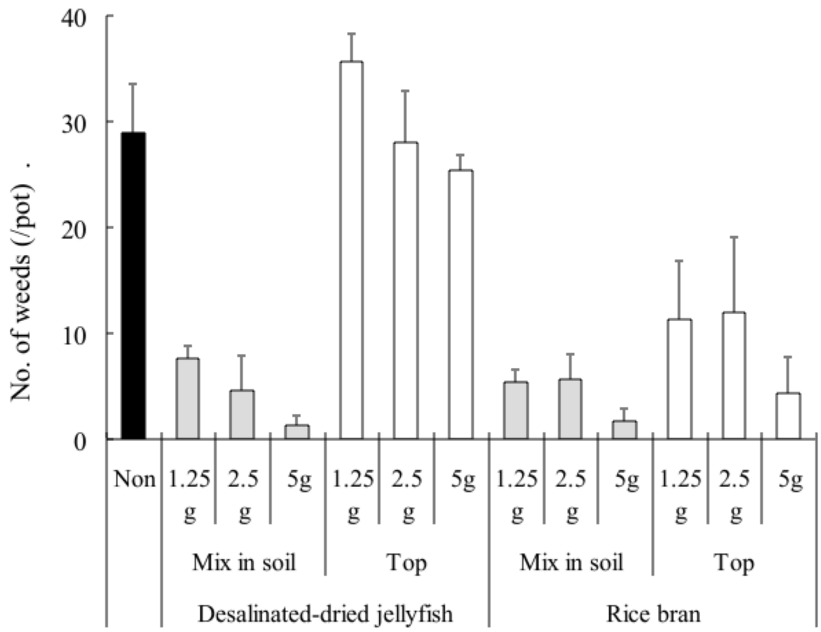

Figure 1. Effects of desalinated-dried jellyfish and rice bran on the number of weeds (Experiment 1).

The total dry weight of rice plants was observed to be much higher in the desalinated-dried jellyfish application plots compared to the rice bran-treated plots, probably due to the higher nitrogen content of jellyfish (Figure 2). The highest dry weight was observed for the jellyfish treatment when mixed in the soil at 5 gN/pot, followed by the top application of jellyfish at 5 gN/pot. The application of rice bran was not effective for improving the growth of the rice plant (Figure 2).

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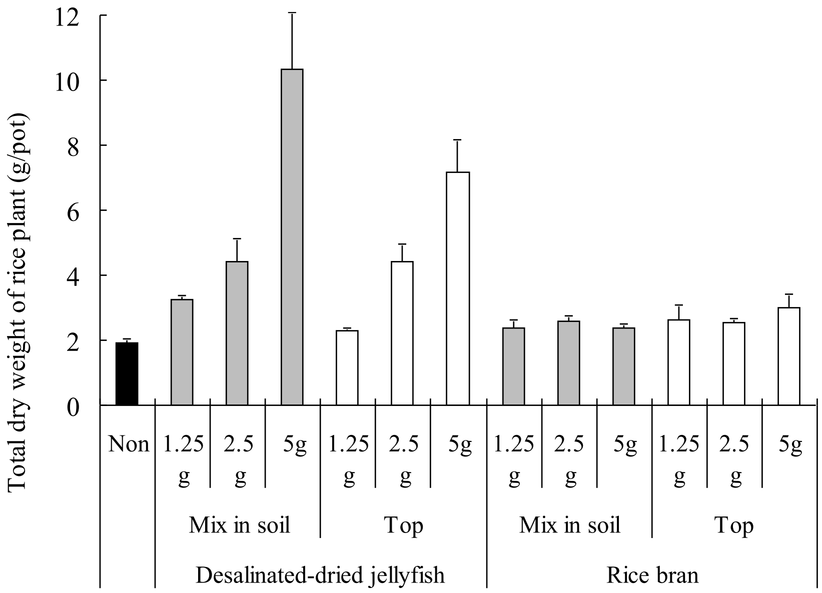

Figure 2. Effects of desalinated-dried jellyfish and rice bran on total dry weight (top+root) of rice plant (Experiment 1).

Experiment 2

The oxidation-reduction potential (Eh) was measured at regular intervals in order to evaluate the weed control ability. The oxidation-reduction potential was found to be lower in the tubes where the treatment was mixed in the soil (J10-0 and R10-0) than in those receiving the top application (0-J10 and 0-R10) (Figure 3).

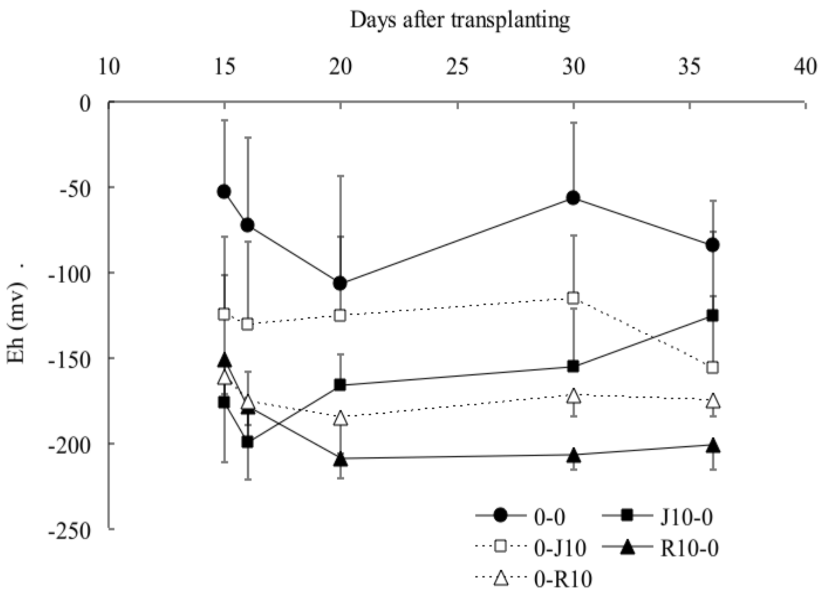

Figure 3. Changes of oxidation-reduction potential (Eh) in the soil (Experiment 2).

It was observed that the application of desalinated-dried jellyfish mixed in the soil (J10-0, J5-J5) effectively controlled rice weeds, while the top application (0-J10) was not effective (Figure 4). The numbers of weeds in the J10-0 and J5-J5 tubes were 18% and 24% of the C10-0 tube, respectively. However, in the 0-J10 tube with the top application, the number of weeds was 76% of that of the C10-0 tube. On the other hand, in the rice bran tubes, although the number of weeds in the soil mixture tube was smaller than the number in the top application tube, the difference between those tubes was not as large as in the desalinated-dried jellyfish tubes. It was taken that, as the Eh value in the soil mixture tube was lower, the number of weeds was smaller as compared with the top application (Figure 3).

The yield and the yield components are shown in Figure 5 and Table 3. The grain yields of desalinated-dried jellyfish treatments were consistently higher than the corresponding rice bran treatments. The yield of J10-0, F5-J5 and 0-J10 were 104%, 94% and 93% of that of C10-0, respectively. The grain yield of the rice bran treatment R10-0 was 49% of that of the chemical fertilizer treatment C10-0. Initial growth was inhibited, the number of panicles was less and the ripening ratio was low in the R10-0. This is considered to result from the Eh reduction value being remarkably low in this treatment (Figure 3).

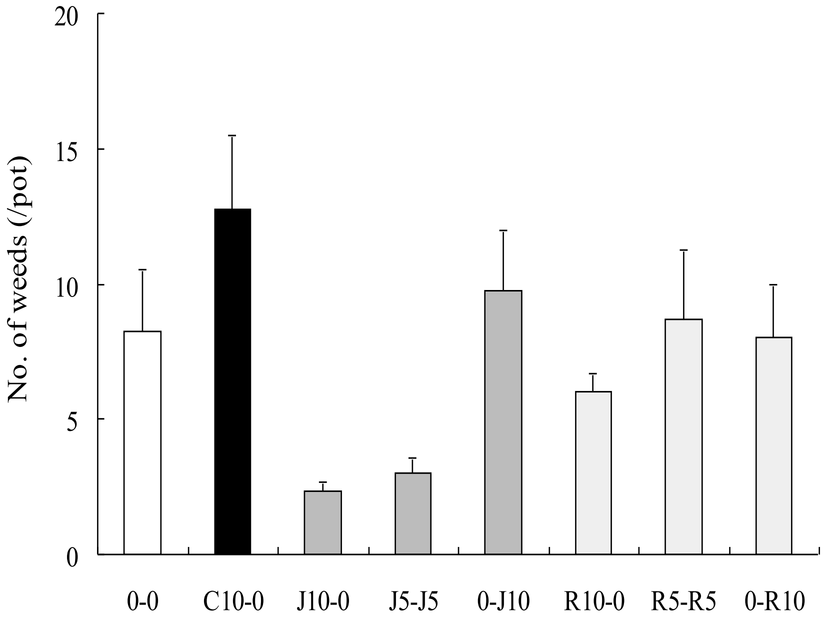

Figure 4. Effects of desalinated-dried jellyfish and rice bran on the number of weeds (Experiment 2).

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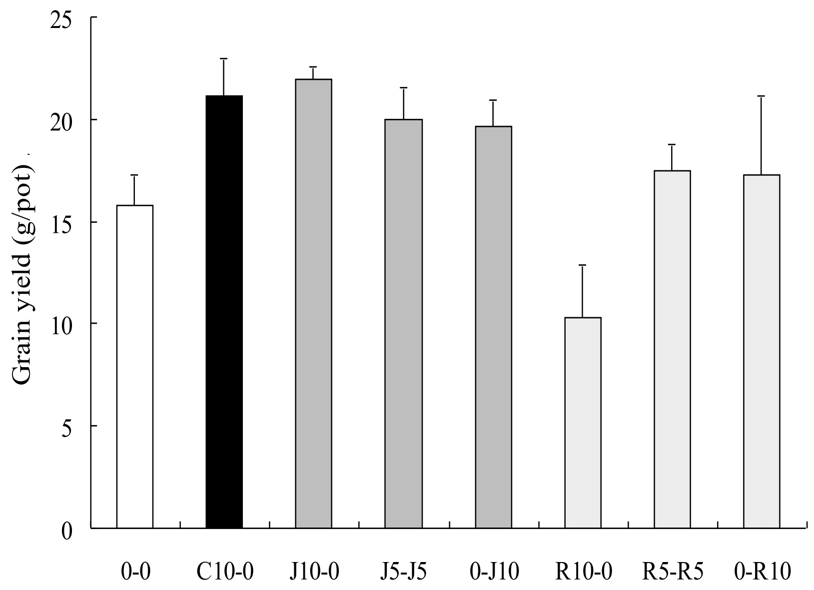

Figure 5. Effects of desalinated-dried jellyfish and rice bran on rice grain yield (Experiment 2).

Table 3. Yield and yield components (Experiment 2).

Treatment	No.of panicles (/hill)	No.of spikelets (/panicle)	No.of spikelets (/hill)	Ripening ratio (%)	1,000 grain weight (g)	Weight of filled grains[a)] (g/hill)	Ratio to C10-0 (%)
0-0	15.3	65	996	71	22.1	15.8 ± 1.5	75
C10-0	23.0	66	1505	64	21.8	21.2 ± 1.8	100
J10-0	22.5	70	1567	66	21.4	21.9 ± 1.3	104
J5-J5	21.3	67	1420	62	22.5	20.0 ± 2.6	94
0-J10	18.8	67	1253	71	22.1	19.6 ± 1.3	93
R10-0	16.8	52	944	39	23.7	10.3 ± 3.8	49
R5-R5	20.8	63	1310	60	22.2	17.5 ± 0.6	83
0-R10	20.8	51	1064	70	23.6	17.3 ± 1.1	82
F value	2.57*	4.25**	1.39ns	3.10*	3.39*	3.19*	

a): Average ± SE. *, **shows significant at P<0.05 and P<0.01 level, and ns is not significant.

It was found that both desalinated-dried jellyfish and rice bran could effectively control rice weeds when mixed in the soil before the transplanting (Figure 1) and that it controlled rice weeds to the maximum at the 10-0 tube treatments (Figure 4). The yield from the desalinated-dried jellyfish treatments were comparable to the chemical fertilizer treatment. The highest yield across the eight treatments was observed in the J10-0 jellyfish treatment, all three jellyfish treatments out yielded the three rice bran treatments (Table 3).

Kuk *et al.* (2000) stated that rice by-products could reduce weed emergence and shoot weight in broadleaf species. The weed population was decreased by the application of rice bran at 5 days after rice transplanting, and the weed occurrence rate decreased by 68% after the application of 3.5Mg ha^{-1} (www.hari.go.kr). Maeda *et al.* (2003) also mentioned that scattering rice bran on the surfaces of fields effectively controlled both the germination and growth of weeds. Paper mulching and feeding by ducks had the highest weed control efficiency (more than 99% of weeds were controlled.) followed by growing *Azolla spp.* and spreading rice bran (Chen, 2006).

Many farmers in Japan spread rice bran and culled soybeans in their rice fields as a form of weed control. Japanese farmers use rice bran (200 g m^{-2}) for weed control and as a fertilizer for transplanted rice, resulting in weed reduction and high-quality grain (http://www.jaec.org/jaec/english/2.pdf). In the present study, although the application of rice bran was observed to provide effective weed control, this is a result not different from previous studies.

As desalinated-dried jellyfish was used here for the first time to control weeds and provide nutrients in rice production, no relevant research on jellyfish is available for comparison. However, different types of fish meal have been found to be effective as an organic fertilizer. Fish powder is commonly used as source of nutrients in organic farming and is a high source of nitrogen. Usually, fish powder is dried with heat and turned into a water-soluble powder.

Conclusions

It can be concluded that the application of desalinated-dried jellyfish, small pieces of jellyfish which are desalinated and dried, mixed in soil before transplanting can effectively control weeds of rice fields and has a nutrient effect, presumed because of the high nitrogen content (10-13%). Desalinated-dried jellyfish has potential as an agricultural material to replace herbicides and chemical fertilizer. It also contributes to environment-friendly rice production. A similar result was observed in the case of rice bran, and it controlled weeds better when mixed in the soil before the transplanting compared to an application on the soil surface just after the transplanting. On the other hand, dry matter production was observed as higher in the jellyfish treatments compared to the rice bran treatments, probably due to higher nitrogen concentration of jellyfish. It can be concluded that the application of desalinated-dried jellyfish mixed in the soil effectively controlled rice weeds rather than top application. But, in the case of rice bran there are only small differences between treatments of mixture with soil or the top application. In the case of the grain yield of the jellyfish treatments, there was little difference observed due to the application method (mixed with soil or top) application, whereas in rice bran, the top application achieved a higher rice yield than the mixed with soil application.

The use of rice bran is an existing practice for weed control in Japan and other rice growing areas as a part of organic rice production, but, as a new product, jellyfish was also used in the present study to control weeds and provide nutrients in rice production, and it was found to have potential to improve organic rice production. It is important to perform further studies of direct-seeding rice (both wet and dry seeding) and transplanted rice to determine the optimal amounts of desalinated-dried jellyfish to be applied and the most effective timing of the application.

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A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet

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Abstract

A significant number of genetically modified (GM) crops have been approved to enter human food and animal feed since 1996, including crops containing several GM genes 'stacked' into the one plant. We randomised and fed isowean pigs (N=168) either a mixed GM soy and GM corn (maize) diet (N=84) or an equivalent non-GM diet (N=84) in a long-term toxicology study of 22.7 weeks (the normal lifespan of a commercial pig from weaning to slaughter). Equal numbers of male and female pigs were present in each group. The GM corn contained double and triple-stacked varieties. Feed intake, weight gain, mortality and blood biochemistry were measured. Organ weights and pathology were determined post-mortem. There were no differences between pigs fed the GM and non-GM diets for feed intake, weight gain, mortality, and routine blood biochemistry measurements. The GM diet was associated with gastric and uterine differences in pigs. GM-fed pigs had uteri that were 25% heavier than non-GM fed pigs (p=0.025). GM-fed pigs had a higher rate of severe stomach inflammation with a rate of 32% of GM-fed pigs compared to 12% of non-GM-fed pigs (p=0.004). The severe stomach inflammation was worse in GM-fed males compared to non-GM fed males by a factor of 4.0 (p=0.041), and GM-fed females compared to non-GM fed females by a factor of 2.2 (p=0.034).

Key words: GMO, GM corn, GM soy, GM animal feed, toxicology, stomach inflammation, uterus weight.

Introduction

Genetically modified (GM) crops have entered human food and animal feed in increasing amounts since they were commercially released into fields in the USA in 1996 (USDA, 2011). The main traits in GM crops to date have been to express proteins for herbicide tolerance (Ht) and insect resistance (Carman, 2004; USDA, 2011). Herbicide tolerant crops are engineered to produce one or more proteins that allow the crop to survive being sprayed with a given herbicide. Insect resistant crops are usually engineered to produce one or more insecticidal proteins that are toxic to target insects. The latter proteins are usually Bt proteins, so named because they are structurally similar to naturally-occurring Cry proteins from a soil bacterium, *Bacillus thuringiensis* (ANZFA, NDb). Hence these crops are also called Bt crops.

Of the GM crops planted in the USA, herbicide-tolerant GM soy has been widely adopted and now constitutes 94% of the soy planted in the USA (USDA, 2011). GM corn varieties have also been widely adopted in the USA (USDA, 2011). They usually contain Ht or Bt traits, or a 'stacked' combination of them (Pioneer Hi-Bred, 2012).

Prior to the release of a new GM crop into the food supply, the developer provides food regulators in many countries with studies it has done on the crop. These studies often include animal feeding studies, even though some regulators, such as Australia's, do not require them (FSANZ, ND; Carman, 2004), while the USA has a voluntary system. Many food regulators do not require any studies to be done on crops containing several "stacked" genes if all the genes in the stack have previously been individually approved for use in the same kind of plant (EFSA, 2010; FSANZ, 2010). Consequently, safety studies on stacked crops are less frequent, even though an analysis of official data (USDA, 2011) indicates that over 37% of GM corn varieties currently planted in the USA are stacked with both Ht and Bt traits.

There have been a number of reviews of the published literature on the safety of GM crops. For example, Flachowsky et al. (2005) and Preston (2005) both conducted reviews and both concluded that GM crops were safe for animals and people to eat. However, many of the feeding studies reviewed used non-mammals (e.g. birds, fish) or animals were fed the crop in a form that humans do not eat (e.g. silage) or only animal production outcomes were measured such as body weight, carcass weight, breast meat yield or milk production, which may not be indicative of potential human health outcomes (Carman, 2004). Only a small proportion of published animal feeding studies have been longer-term toxicological studies where a GM crop was fed to animals that are physiologically comparable to humans, and organs, blood and tissue samples were taken from the animals and examined to assess if the crop caused any adverse effects.

Two recent reviews of these rarer toxicology-type studies have recently been published. Snell et al. (2011) reviewed 12 studies of 90 days or longer duration and concluded that GM plants were nutritionally equivalent to non-GM plants and could be safely used in food and feed. However, once again, most of the studies reviewed used animals that were either not physiologically comparable to humans, or used only small numbers of animals. A broader picture is given in a series of three reviews by Domingo (2000; 2007) and Domingo & Bordonaba (2011). The first two papers concluded that there were few published studies investigating toxicology or health risks, while the third found that most of the more recent studies concentrate on only a few GM crops (soy, corn and rice), ignoring many other GM crops such as potatoes, peas and tomatoes.

Another review of 19 studies of mammals fed GM soy or maize has recently been conducted (Séralini et al., 2011). These authors also reviewed the raw data of some other authors' 90-day feeding studies. They found some evidence for adverse liver and kidney effects from eating some GM crops and concluded that 90-day feeding studies were insufficient to evaluate chronic toxicity of GM crops.

More recently, a highly publicised (e.g. Poulter, 2012), much longer study of two-years' duration on NK603 herbicide-tolerant corn (which contains one of the genes fed in the present study) has been published (Séralini et al. 2012). There were indications of higher death rates, more tumours and liver and kidney pathologies in GM-fed rats.

The aim of the present study was to perform a thorough, long-term toxicology study (for 22.7 weeks, being the normal lifespan of a commercial pig from weaning to slaughter) on pigs in a USA commercial piggery in order to compare the effects of eating either a mixed GM soy and GM corn diet, or an equivalent diet with non-GM ingredients. Pigs in the USA are usually fed a mixed corn and soy diet, containing a high proportion of GM varieties. Even though pigs are physiologically similar to humans, particularly for gastrointestinal observations, very few toxicology studies have been conducted on them for GM crops (Walsh et al., 2012a). In doing this study, we not only used animals that were physiologically similar to humans, but we also weighed and internally examined organs and took blood for biochemical analysis. We further used a large enough sample size (168 pigs, 84 per group) to be able to determine statistical significance for key toxicological outcomes. We also used GM crops that are planted in significant quantities in the USA (Ht soy, and Ht and Bt corn) and hence are commonly eaten by pigs and humans in the USA. We further fed these crops as a mixed diet. Mixed diets commonly occur for pigs and humans. This study therefore reflects the effects of eating GM crops in the 'real world'. To our knowledge, this is the first study of its kind conducted.

Materials and Methods

Animal feed

In accordance with usual commercial USA piggery practice, soy and corn were obtained direct from farmers who had grown it commercially. Different GM corn varieties are usually co-mingled in farm storage. The corn used in this study contained 90% DK 42-88 RR YG PL (a triple stack of NK603, MON863 and MON810 genes) with the remainder being equal quantities of Pannar 5E-900RR (containing NK603), Pannar 4E-705RR/Bt (a double stack of NK603 and MON810) and Producers 5152 RR (containing NK603). Therefore, the GM corn that was used was genetically modified to produce three new proteins. Two were Bt proteins that protected the plant against insect attack, while the third protein provided the plant with tolerance to the herbicide glyphosate (Testbiotech, 2012; Monsanto, 2012).

Because Roundup Ready™ (RR) soy is predominant in the GM soy market, this was used. This crop contains a gene that provides tolerance to the herbicide glyphosate. GM DNA analysis (Genetic ID, Fairfield, Iowa, US) confirmed that the GM corn contained a combination of NK603, MON863 and MON810 genes (expressing the CP4 EPSPS, Cry 3Bb1 and Cry 1Ab proteins respectively), that the RR soy was 100% RR soy (expressing the CP4 EPSPS protein), that the non-GM feed contained a median of 0.4% GM corn and that the non-GM soy contained a median of 1.6% GM soy. Such GM contamination of apparent non-GM material is common in the US.

In a similar way to the GM crops used, non-GM soy and non-GM corn were also obtained direct from farmers who had grown it commercially for human food and animal feed. Isogenic parental varieties of the GM crops, from which the GM crops were developed, were not used because they are generally not commercially available to buy. Furthermore, triple-stacked corn containing all three genes used here was developed

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from conventionally cross-breeding several GM crops, each of which has a non-GM parent, leading to a multiplicity of isogenic parental varieties that would need to be used in combination for a control diet. As the aim of this study was to compare the effects of GM and non-GM varieties present in animal feed and human food in the real world, the soy and corn for the control diet was instead chosen as a mixture of non-GM soy and corn that was destined for animal feed and human food and that came from the same geographical area. The GM soy and corn used in this study have been determined to be compositionally and substantially equivalent to non-GM varieties of soy and corn by government regulators (ANZFA, 2002, NDa, NDb; FSANZ, 2003, 2006) which indicates that there should be no phenotypical variation between the GM and non-GM varieties used in this study that could influence the outcomes measured in this study.

GM and non-GM corn were both ground using the same cleaned equipment, size screen and revolutions per minute to obtain the same particle size. GM and non-GM soy beans were also processed on the same type of cleaned equipment - using Insta-Pro extruders and expellers, rather than being solvent-extracted, in order to preserve the identity of the beans during processing into soybean meal. This process purees the beans and squeezes out most of the oil, leaving a residual oil content of 8%. In the process, the beans are heated to 153^{o}C to 166^{o}C. As pigs grow, they require different amounts of nutrients, so six different sub-diets were progressively used. Soy content decreased from 26.5% to 13.0%, corn increased from 56.4% to 83.8% and protein decreased from 18.3% to 13.3% of the diet (Table 1). Ingredients, including supplements, were those routinely used by the piggery and were the same between groups. The GM and non-GM diets had the same protein, energy, macro- and micro-nutrient contents and only differed in the use of GM or non-GM soy and corn. Pigs were fed on a self-feeding, full-feed basis. The amount of feed consumed by each group was recorded.

Table 1. Details of the six body-weight-specific sub-diets used progressively as pigs grew.

	Sub-diet number					
	1	**2**	**3**	**4**	**5**	**6**
Pig weight (lb)[a]	14-25	25-60	60-90	90-130	130-200	200-260
No. days on diet[b]	39-40	17-18	23-24	24-25	37-38	15-17
Average daily intake (lb)	0.9	2.43	3.45	4.71	6.10	6.78
Protein (%)	18.6	18.0	17.4	16.3	15.2	14.7
Soy (%)[c]	26.5	25.0	23.4	20.4	17.5	16.0
Corn (%)[d]	70.0	71.6	73.2	76.3	79.8	81.3
UN premix (%)[e]	2.5	2.5	—	—	—	—
UG premix (%)[f]	—	—	2.5	2.5	—	—
UF premix (%)[g]	—	—	—	—	2.5	2.5
Boost premix (%)[h]	0.0025	0.0025	0.001	0.0015	0.0015	0.0015
Extra lysine	—	—	0.001	0.0005	—	—
Extra $CaCO_3$ (%)	0.0075	0.0075	0.006	0.006	0.002	0.002
200 mesh bentonite clay (%)	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035

a As the piggery was in the USA, pig diets were changed when pigs reached a certain weight in pounds.

b Because pig handlers were required to keep to usual piggery practices and were blinded as to the GM feeding status of each group of pigs, pigs in each group were changed from one sub-diet to the next according to the body weight of the group. Consequently, one group was often changed to the next sub-diet a day before the other group. While the GM-fed group spent one day longer on a particular diet than the non-GM group for three diets, the non-GM group spent a day longer on a particular diet for the other three diets. Therefore, there was neither a trend nor a difference in the progression of the two groups from one diet to another. Pigs were fed for a total of 158 days if they were slaughtered on the first of the two slaughter days, and 159 days if they were slaughtered on the second slaughter day.

c GM soy went into the GM diets and non-GM soy into the non-GM diets.

d GM corn went into the GM diets and non-GM corn into the non-GM diets.

e Ultra Nursery Plus Premix from Advanced Biological Concepts, Osco, Illinois, containing (as copied directly from the label) guaranteed amounts of 0.5% crude protein, 6.0% lysine, 0.5% crude fat, 3.0% crude fiber 13.0% to 15% calcium, 13.0% phosphorus, 16.0% to 18.0% sodium chloride, 10ppm selenium, 1,500 ppm zinc, 190,000 IU/lb vitamin A, 25,000 IU/lb vitamin D_3 and 800 IU/lb vitamin E. Other ingredients on the label (not quantified), include: copper, iron, zinc, manganese, choline, ascorbic acid, niacin, riboflavin, pantothenic acid, vitamin K, vitamin B_{12}, carotene and iodine.

f Ultra Grower Premix Plus from Advanced Biological Concepts, Osco, Illinois, containing (as copied directly from the label) guaranteed amounts of 0.5% crude protein, 1.0% lysine, 0.5% crude fat, 3.0% crude fiber, 15.0% to 17% calcium, 12.0% phosphorus, 15.0% to 17.0% sodium chloride, 3ppm selenium, 1,500 ppm zinc, 160,000 IU/lb vitamin A, 22,000 IU/lb vitamin D_3 and 800 IU/lb vitamin E. Other ingredients on the label (not quantified) include: copper, iron, zinc, manganese, choline, niacin, riboflavin, pantothenic acid, vitamin K, vitamin B_{12}, carotene and iodine.

g Ultra Finisher Premix Plus from Advanced Biological Concepts, Osco, Illinois, containing (as copied directly from the label) guaranteed amounts of 0.5% crude protein, 3.0% lysine, 0.5% crude fat, 3.0% crude fiber, 18.0% to 20.0% calcium, 10.0% phosphorus, 6.5% to 7.5% sodium chloride, 3ppm selenium, 4,000 ppm zinc, 125,000 IU/lb vitamin A, 20,000 IU/lb vitamin D_3 and 500 IU/lb vitamin E. Other ingredients on the label (not quantified) include: copper, iron, zinc, potassium, magnesium, manganese, choline, ascorbic acid, niacin, riboflavin, pantothenic acid, vitamin K, vitamin B_{12}, carotene and iodine.

h Natural Boost from Advanced Biological Concepts, Osco, Illinois, containing (as copied directly from the label) guaranteed amounts of 10.0% crude protein, 0.005% lysine, 0.005% methionine, 1.0% crude fat, 24.0% crude fiber, 40.0% acid detergent fiber, 0.2% to 0.7% calcium, 0.2% phosphorus, 1.0% to 1.5% sodium chloride, 0.5% potassium, 500ppm copper, 1,500 ppm zinc, 180,000 IU/lb vitamin A, 55,000 IU/lb vitamin D_3 and 500 IU/lb vitamin E. Other ingredients on the label (not quantified) include: iron, zinc, magnesium, manganese, choline, cobalt, ascorbic acid, niacin, riboflavin, pyridoxine HCl, pantothenic acid, biotin, vitamin K, vitamin B_{12}, folic acid, carotene and iodine.

Mycotoxin analyses (Midwest Laboratories Inc, Omaha, Nebraska, US) showed 2.08 ppb total aflatoxins and 3.0 ppm total fumonisins in a pooled sample of the GM feed and no aflatoxins and 1.2 ppm total fumonisins in a pooled sample of the non-GM feed. No other mycotoxins were detected. These levels are well below the USA and EU limits for mycotoxins in pig feed. In addition, according to common industry practice, a mycotoxin binding agent (200 mesh bentonite clay) was added to the diets of young pigs (Table 1).

Animals

Standard commercial Yorkshire-cross piglets were obtained from a commercial farrowing facility as a result of crossing Hampshire Duroc males with Yorkshire Landrace females. All sows were fed the same diet containing some GM ingredients and were impregnated at a similar time to obtain isowean piglets. Male piglets were neutered at three days of age in order to fulfill market requirements for meat free of boar-taint.

Unweaned piglets (N=168; average 24 days of age) were transported to the piggery nursery and randomly placed into pens of 14 each. Pens were then randomly allocated to receive either a GM or non-GM diet. Animals were weighed and then fed their allocated diet as their first solid food. After 32 days, pigs were transported to a different facility for the 'growing and finishing' phase, where they continued on their allocated diets but were housed as 42 pigs per pen with outside access. Throughout, pigs were housed according to usual industry practices, under shelter on concrete floors. They experienced the natural daytime/night-time temperature and light/dark cycle.

Data collected from live pigs

Individual weights were recorded weekly and animals were monitored daily by observers who were blinded to a pig's dietary group. Daily measurements included inside and outside air temperature, air quality, weather conditions, level of activity of pigs around the feeder and the appearance of the feeder itself, the level of activity of the pigs around the water and the appearance of the water, details of any pigs found dead, details of any pigs that were moved away from, or back to, the 'home pen' and the reasons for this (e.g. they were being harassed by other pigs), level of contentment (measured as content, irritable or aggressive), presence of cough or sneeze, the presence of any skin problems (e.g. pale or discoloured skin or the presence of rashes or sores), any eye problems, and the consistency of the stools (normal, some loose or runny stools, lots of loose or runny stools). Blood was taken from the jugular vein of awake pigs according to standard industry methods two days before the first pigs were slaughtered. The blood was taken from a random subset of pigs in the following pattern to prevent any time-related bias: approx. half the pigs in the non-GM-fed group, approx half the pigs in GM-fed group, the remainder of the non-GM-fed group, and the remainder of the GM-fed group. Blood was centrifuged and serum was removed and frozen. Blood biochemical analyses were undertaken by Marshfield Clinic Laboratories, Marshfield, WI, USA, who were blinded to all aspects of the study. The laboratory's reference range for awake three to four month-old Yorkshire cross pigs was used as it was most applicable for this study.

Autopsy procedure

When the pigs were 26 weeks old, they were fasted for 18 hours and transported to a large commercial abattoir where they were slaughtered according to the usual, approved methods of the abattoir on two consecutive days. On each day, approximately equal numbers of GM-fed and non-GM-fed pigs were slaughtered to prevent any temporal between-group bias. Pigs on each day were killed within a few minutes of each other. The internal organs were carefully removed to prevent faecal contamination and placed in individual identified buckets with 2 litres of cold phosphate-buffered saline to quickly chill the organs. Organs were kept under near-freezing conditions until they were examined by two licenced, practicing veterinarians with considerable porcine experience. They were blinded as to which pigs were fed GM feed. To remove any between-inspector bias, one veterinarian examined all the kidneys, hearts, lungs and stomachs while the other examined all the livers, spleens, intestines, uteri and ovaries. Veterinarian comments and organ weights were recorded by the same person to remove any between-person measurement bias or recording bias. Where evisceration resulted in incomplete removal of an organ, veterinarians determined if disease had caused part of an organ to adhere to the chest or abdominal wall and hence remain with the carcass, as well as the nature of that disease. The weights of partial organs were not included in statistical analyses due to the errors they would have produced. Kidney weights were the sum of both kidneys per pig. Ovary weights were the sum of both ovaries per pig except for two GM-fed pigs where one ovary was accidentally removed by the abattoir. Here, the weight of both ovaries was estimated by doubling the weight of the remaining ovary. Intestines could not be weighed or inspected due to the amount of digesta still present in them, even after 18 hours of fasting, so the external surface of the intestines was examined for abnormalities and any intramural, palpable tissue masses. Organ weights were analysed as a percentage of body weights.

In addition to externally examining the organs, veterinarians also examined the interior of every kidney using a single, deep transverse cut, every heart by slicing into both ventricles and both atria, and every lung using at least two deep cuts through the dorsal surface of each lung lobe, and if abnormalities were found, several more cuts to properly identify the abnormality and its extent. Every stomach was examined by cutting it open along the length of its greatest curvature, washing out the contents and inspecting the entire internal surface of the opened-flat stomach, including rugae.

Data analysis

A stomach erosion was defined as an abnormal stomach surface that had a visible area of current inflammation and oedema and where the mucosa was starting to separate (and which could potentially progress to form an ulcer). The length of any ulcer was measured in millimetres. If an ulcer had a clot in it, or showed frank bleeding, it was recorded as a bleeding ulcer. If an ulcer was less than 1 mm in length, it was recorded as a pin-point ulcer, otherwise as a frank ulcer. When calculating the total length of ulceration in each stomach in mm, each pin-point ulcer was numerically rounded to be 1mm in length. Stomach inflammation was scored by the attending, blinded veterinarian as a result of expertise obtained from numerous pig autopsies and a classification system developed as a result of an earlier, preliminary study on pig stomachs. These stomachs were obtained from a random sample of pigs from the same abattoir and came from pigs raised by other commercial pig producers. Inflammation was classified as nil, mild, moderate, or severe based on a combination of the area of current inflammation and level of redness and swelling. Typical examples of each of the four categories of inflammation are shown in Figure 1. For a severe level of inflammation, almost the whole fundus had to be swollen and cherry-red in colour.

Data were analysed using the statistical packages SPSS and EpiInfo. Continuous data were analysed by removing SPSS-identified extreme outliers, being those more than three times the interquartile range away from the lower or upper quartiles. This conservative and well-established approach better tests the nature of the underlying distribution. Data were then tested for normal distribution using the Shapiro-Wilk test. If a normal distribution was found for both dietary groups, data were expressed as means and standard deviations and were analysed using parametric methods (t-test), otherwise data were expressed as medians and ranges and analysed using non-parametric methods (Mann-Whitney U test). Categorical data were analysed using uncorrected chi-squared tests unless an expected cell value was less than five, when Fisher's Exact was used.

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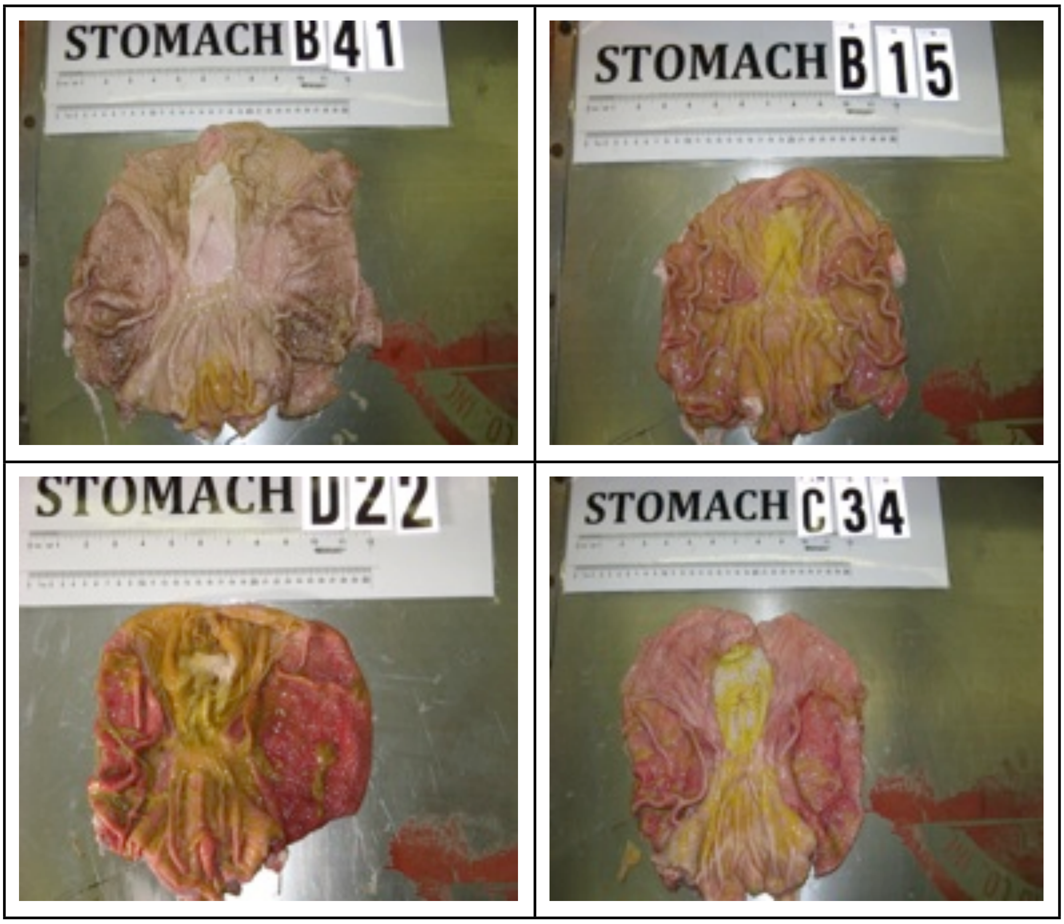

Figure 1. Different levels of stomach inflammation found (clockwise from top left): nil (from a non-GM-fed pig, number B41), mild (from a non-GM-fed pig, number B15), moderate (from a GM-fed pig, number C34) and severe (from a GM-fed pig, number D22).

Results

There were no statistically significant differences in food intake, feed conversion ratios, number or nature of illnesses, number or nature of veterinary interventions, veterinary costs or mortality between the non-GM-fed and GM-fed groups of pigs. Mortalities were 13% and 14% for the non-GM-fed and GM-fed groups respectively, which are within expected rates for US commercial piggeries. All dead pigs were autopsied by blinded veterinarians and deaths were assessed as due to usual commercial piggery-related matters and not to their diets. There was also no difference in body weights between the two dietary groups, initially, during, or at the end of the experiment. Initial weights in kg were : non-GM-fed group: 6.71 + 1.05 (mean + standard deviation); GM-fed group: 6.87 + 0.97. Final weights were: non-GM-fed group: 100.42 + 22.84; GM-fed group: 101.75 + 21.92.

Autopsy results

Organ weights were not statistically different between GM-and non-GM-fed pigs except for uterine weights (Table 2). After removing one extreme outlier, the medians of the non-GM-fed (now N=33) and GM-fed (N=37) groups became 0.084% and 0.105% of the body weight respectively. That is, the median uterus weight of GM-fed pigs, as a proportion of body weight, was 25% higher than that of non-GM-fed pigs, which was statistically significant (p=0.025).

There was no difference in the disease status of organs between the two groups of pigs except for the level of inflammation in the stomachs of the pigs (Table 3, Figure 1). For non-GM-fed pigs, stomach inflammation was concentrated in the mild and moderate range, whereas GM-fed pigs showed much more severe inflammation (p=0.004). GM-fed pigs showed severe stomach inflammation at a rate of 2.6 times that of non-GM-fed pigs (95% confidence interval = 1.29-5.21) (Table 3). This occurred in both male (p=0.041) and female (p=0.034) pigs (Table 4). We found severe stomach inflammation in 22.2% of male pigs fed the GM diet and in 41.7% of female pigs fed the GM diet (compared to 5.6% and 18.9%, respectively, in pigs fed the non-GM diet (Table 4).

Blood biochemistry

Blood biochemistry results are given in Table 5. Aspartate transaminase (AST), potassium and creatine kinase (CK) were not statistically analysed because they were raised substantially in both dietary groups due to the way blood was collected and hence they were unable to reflect any effect of feeding a GM diet. AST and potassium were raised because the collection needle was pushed through muscle, while CK was raised due to the pigs being alert and restrained while blood was taken. While bicarbonate can increase if pigs pant or squeal unduly during blood taking, no pigs recorded a bicarbonate concentration higher than the reference range (Table 6), so this variable was retained in analyses.

To determine if feeding the GM diet was associated with a clinically abnormal biochemistry profile, the proportion of pigs in each dietary group that lay above (or below) the reference (normal) range were then compared (Table 6). No statistically significant differences were found. The means or medians of the biochemical variables were also compared. No significant differences were found (Table 5).

The analyses of several biochemical variables were confounded by the level of haemolysis in the blood sample. Haemolysis can be a problem when taking blood from alert animals, and in non-laboratory settings due to lag times between sampling and centrifuging blood. Haemolysis was reported as nil, mild, moderate or severe by the laboratory. Total bilirubin, urea nitrogen, creatinine, phosphorus, calcium, sodium, chloride, bicarbonate, and anion gap were found to be significantly correlated with the level of haemolysis (results not shown) and hence haemolysis was regarded as a confounder for these variables. Spearman's rho test was used as a measure of the association rather than the Pearson correlation co-efficient as it is less sensitive to outliers and does not assume normality. These biochemical variables then underwent multiple linear regression to control for the effect of haemolysis. As known confounders should be controlled-for, even if they do not appear as actual confounders in initial studies, glucose also underwent this process. No biochemical variable was found to have a significant relationship to the diet with the level of haemolysis controlled-for (results not shown). Consequently, no biochemical differences were found between non-GM-fed and GM-fed pigs. However, the concentration of GGT, which is a measure of liver heath, was 16% lower in GM-fed pigs than non-GM-fed pigs and this result was on the borderline of statistical significance (Table 5).

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Table 2. Organ weights (as a percentage of body weight) - descriptive statistics of raw data and statistical comparisons of extreme outlier-removed data.

	Non-GM-fed						GM-fed						Statistical comparison of dietary groups	
	n[a]	Mean	SD[b]	Median	Min	Max	n[a]	Mean	SD[b]	Median	Min	Max	Test used[c]	p[d]
Kidneys	66	0.32	0.066	0.31	0.19	0.66	68	0.33	0.057	0.32	0.16	0.56	t	0.51
Heart	69	0.40	0.065	0.40	0.27	0.63	69	0.41	0.059	0.40	0.27	0.61	MW	0.79
Liver	71	1.81	0.342	1.77	1.27	3.20	72	1.79	0.348	1.71	1.25	3.16	MW	0.45
Spleen	73	0.16	0.033	0.16	0.11	0.33	71	0.16	0.032	0.15	0.093	0.30	t	0.40
Lung	67	0.91	0.241	0.87	0.58	2.00	68	0.98	0.315	0.94	0.57	2.52	MW	0.20
Stomach	73	0.62	0.130	0.57	0.42	0.99	71	0.64	0.129	0.60	0.44	1.01	MW	0.26
Uterus	34	0.10	0.048	0.086	0.040	0.31	37	0.12	0.053	0.105	0.036	0.244	MW	0.025*
Ovaries	36	0.0085	0.0027	0.0081	0.0040	0.019	36	0.0086	0.0023	0.0084	0.0047	0.014	t	0.38

a An organ was not included in the analysis if adhesions caused only a partial organ to remain with the viscera, due to the errors inclusion would have caused.
b Standard deviation
c After tests for normality, groups were compared by 2-tailed t-test if data from both dietary groups were normally distributed, Mann Whitney U test (MW) otherwise.
d* p<0.05 to 0.01, ** p<0.01 to 0.001, *** p<0.001

Table 3. The proportion of pigs in each dietary group with adverse findings on gross pathology

Organ	Condition	Proportion with condition				Relative risk of condition in GM-fed pigs	95% confidence interval of the relative risk	p[a]
		Non-GM-fed		GM-fed				
		No. N=73	%	No. N=72	%			
Kidney	Any abnormality	0	0.0	0	0.0	—[b]	—[b]	—[b]
Heart	Any abnormality[c]	11	15.1	5	6.9	0.46	0.17-1.26	0.119
Liver	Any abnormality[d]	6	8.2	3	4.2	0.51	0.13-1.95	0.494
Spleen	Any abnormality[e]	3	4.1	2	2.8	0.68	0.12-3.93	1.000
Lung	Pneumonia[f]	42	57.5	43	59.7	1.04	0.79-1.36	0.789
	Fibrous pleuritis or pericarditis	9	12.3	4	5.6	0.45	0.15-1.40	0.153
	Abnormal lymph nodes[g]	13	17.8	16	22.2	1.25	0.65-2.40	0.506
Stomach	Nil inflammation	4	5.4	8	11.1	2.03	0.64-6.44	0.218
	Mild inflammation	31	42.5	23	31.9	0.75	0.49-1.16	0.190
	Moderate inflammation	29	39.7	18	25.0	0.63	0.39-1.03	0.058
	Severe inflammation	9	12.3	23	31.9	2.59	1.29-5.21	0.004**
	Erosion(s)	63	86.3	58	80.6	0.93	0.81-1.08	0.352
	Pin-point ulcer(s)	13	17.8	9	12.5	0.70	0.32-1.54	0.373
	Frank ulcer(s)	15	20.5	17	23.6	1.15	0.62-2.12	0.657
	Bleeding ulcer(s)	0	0.0	2	2.8	—[b]	—[b]	0.245
Intestines	Any abnormality	0	0.0	0	0.0	—[b]	—[b]	—[b]
Uterus	Filled with fluid[h]	0[i]	0.0	2[j]	5.6	—[b]	—[b]	0.493
Ovary	Any abnormality	0[k]	0.0	0[l]	0.0	—[b]	—[b]	—[b]

a Uncorrected chi-square test unless an expected cell value was less than five, when Fisher exact test (2-tailed) was used. * p<0.05 to 0.01, ** p<0.01 to 0.001, *** p<0.001
b No statistic could be calculated because one or more cells contained zeros.
c Adhesions and/or fibrous pericarditis and/or scar tissue.
d Adhesions and/or fibrinous tags and/or the presence of fibrin.
e Adhesions and/or fibrinous tags.
f Consolidating bronchopneumonia of the cranial ventral lung lobe(s) and/or caudal lobe(s).
g Haemorrhagic and/or swollen bronchial lymph node(s).
h When two uteri were removed from neighbouring organs, fluid oozed from them.
i N=36. Of 37 females, one had a congenital defect. It had only the beginnings of a uterine tract and no uterus or ovaries.
j N=36.
k N=36. Of 37 females, one had a congenital defect. It had only the beginnings of a uterine tract and no uterus or ovaries.
l N=35. Of 36 females, one had a uterus but no ovaries, which were removed by accident during slaughter and retained by the slaughterhouse.

Table 4. Stomach inflammation by gender.

Gender	Level of stomach inflammation	Proportion with condition: Non-GM-fed No.[b]	Non-GM-fed %	GM-fed No.[c]	GM-fed %	Relative risk of condition in GM-fed pigs	95% confidence interval of the relative risk	p[a]
Males	Nil	1	2.8	4	11.1	4.00	0.47-34.07	0.357
	Mild	16	44.4	12	33.3	0.75	0.42-1.35	0.334
	Moderate	17	47.2	12	33.3	0.71	0.40-1.26	0.230
	Severe	2	5.6	8	22.2	4.00	0.91-17.56	0.041*
Females	Nil	3	8.1	4	11.1	1.37	0.33-5.70	0.711
	Mild	15	40.5	11	30.6	0.75	0.40-1.41	0.373
	Moderate	12	32.4	6	16.7	0.51	0.22-1.22	0.118
	Severe	7	18.9	15	41.7	2.20	1.02-4.76	0.034*

a Uncorrected chi-square test unless an expected cell value was less than five, when Fisher exact test (2-tailed) was used. * p<0.05 to 0.01, ** p<0.01 to 0.001, *** p<0.001
b N=36 for males, N=37 for females.
c N=36 for males, N=36 for females.

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Table 5. Blood biochemistry descriptive statistics of raw data and statistical comparisons of extreme outlier-removed data.

	Non-GM-fed			GM-fed			Reference range[a]		Statistical comparison of dietary groups	
	N	Median[b] (Mean)	Range[b] (SD)	N	Median[b] (Mean)	Range[b] (SD)	Standard (asleep)[c]	Awake (Yorkshire X)[d]	Test used[e]	p[f]
Glucose (mg/dL)	39	89.0	58 – 109	38	90.5	52 – 111	85 – 150	58.0 – 197.0	MW	0.81
AST[g](U/L)	39	60.0	21 – 2757	38	57.0	12 – 1724	32 – 84	0.0 – 45.0	MW	0.72
Total bilirubin (mg/dL)	39	0.10	0.1 – 0.3	38	0.10	0.1 – 0.3	0.0 – 1.0	0.1 – 0.2	MW	0.76
Cholesterol (mg/dL)	39	100.0	56 – 140	38	100.0	55 – 125	36 – 54	50.0 – 92.0	MW	0.85
Total protein (g/dL)	39	(6.48)	(0.95)	38	(6.63)	(0.91)	7.9 – 8.9	5.1 – 6.9	t	0.16
Albumin (g/dL)	39	4.00	1.7 – 4.7	38	4.10	1.7 – 4.8	1.9 – 3.3	3.0 – 4.4	MW	0.59
Urea nitrogen (mg/dL)	39	11.0	5 – 22	38	12.0	8 – 29	10 – 30	4.3 – 12.7	MW	0.30
Creatinine (mg/dL)	39	0.90	0 – 1	38	0.70	0 – 1	1.0 – 2.7	0.9 – 1.9	MW	0.21
Phosphorus (mg/dL)	39	(9.1)	(1.5)	38	(9.1)	(1.5)	5.3 – 9.6	6.2 – 9.2	t	0.99
Calcium (mg/dL)	39	10.70	5.5 – 11.3	38	10.50	5.1 –12.0	7.1 –11.6	9.1 – 10.8	MW	0.94
Sodium (mmol/L)	37	140.0	98 – 148	37	140.0	98 – 145	135 - 150	132.0–144.0	MW	0.60
Potassium (mmol/L)	38	6.35	4.6 – 13.9	37	6.40	4.3 –16.3	4.4 – 6.7	3.4 – 5.0	MW	0.56
Chloride (mmol/L)	38	97.0	67 – 104	37	98.0	66 – 102	94 – 106	94.0 – 103.0	MW	0.86
Bicarbonate (mmol/L)	39	33.0	19 – 37	38	33.5	18 – 37	18 – 27	28.0 – 37.0	MW	0.44
CK[h] (U/L)	39	2416.0	214 –22500	38	1960.0	10 –22500	61 –1251	264.0–1247.0	MW	0.73
GGT[i](U/L)	39	(35.1)	(18.4)	38	(29.5)	(18.1)	10 – 60	0.0 – 60.0	t	0.05
Anion gap (mmol/L)[j]	37	16.0	12 – 23	37	15.0	11 – 27	–	–	MW	0.61

a From Marshfield Clinic, Marshfield, WI, USA.

b Medians and ranges are reported for non-parametric comparisons, means and standard deviations for parametric comparisons.

c Marshfield Clinic's usual reference range. Pigs were anaesthetised to obtain blood.

d Marshfiled Clinic's reference range for awake, 3-4 month-old Yorkshire cross pigs. This was used as it is much more applicable to this study.

e After tests for normality, groups were compared by two-tailed t-test if data from both dietary groups were normally distributed, Mann Whitney U test (MW) otherwise.

f * $p<0.05$ to 0.01, ** $p<0.01$ to 0.001, *** $p<0.001$

g Aspartate transaminase.

h Creatine kinase.

i Gamma-glutamyl transferase.

j There is no laboratory reference range for anion gap. Sorbitol dehydrogenase results were not given by the lab on this occasion.

Table 6. Biochemical variables compared to the reference range[a] to determine clinical significance.

Biochemical variable	Number (%) above or below reference range			
	Non-GM-fed (N=39)		GM-fed (N=38)	
	Above reference range	Below reference range	Above reference range	Below reference range
Glucose	0 (0)	0 (0)	0 (0)	2 (5)
AST[b]	23 (59)	—[c]	24 (63)	—[c]
Total bilirubin	1(3)	0 (0)	1 (3)	0 (0)
Cholesterol	29 (74)	0 (0)	28 (74)	0 (0)
Total protein	10 (26)	4 (10)	17 (45)	3 (8)
Albumin	7 (18)	5 (13)	3 (8)	5 (13)
Urea nitrogen	10 (26)	0 (0)	16 (42)	0 (0)
Creatine	0 (0)	18 (46)	0 (0)	23 (61)
Phosphorus	12 (31)	2 (5)	16 (42)	1 (3)
Calcium	10 (26)	9 (23)	14 (37)	6 (16)
Sodium	2 (5)[d]	4 (11)[d]	0 (0)[d]	4 (11)[d]
Potassium	34 (89)[e]	0 (0)[e]	36 (97)[d]	0 (0)[d]
Chloride	1 (3)[e]	7 (18)[e]	0 (0)[d]	4 (11)[d]
Bicarbonate	0 (0)	5 (13)	0 (0)	5 (13)
CK[f]	24 (62)	2 (5)	27 (71)	1 (3)
GGT[g]	2 (5)	—[c]	1 (3)	—[c]

a Awake Yorkshire cross pig reference range from Marshfield Clinic, Marshfield, WI, USA. Anion gap has no reference range so was not included in the table.
b Aspartate transaminase.
c It was not possible for a pig to record a concentration below the bottom of the reference range, which was zero.
d N=37.
e N=38.
f Creatine kinase.
g Gamma-glutamyl transferase.

Discussion

In this study, we found that female pigs fed the GM diet had median uterine weights that were 25% greater than non-GM-fed pigs (p=0.025). This result is attributed to the difference in diet as other variables were controlled for, including the presence of mycotoxins, and possible confounders such as infectious diseases, animal husbandry considerations and various forms of bias such as temporal, between-person, measurement or recording bias, as these were all controlled-for. The concentration of mycotoxins in the feed was insignificant, both dietary groups received the same nutrients and care, the care complied with industry standards, and all those doing laboratory analyses and weighing, caring for, slaughtering and doing autopsies on pigs were blinded as to the dietary group of each pig.

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The reported difference in uterine weight warrants further investigation in future studies because such a biologically significant difference in uterine weights may reflect endometrial hyperplasia or carcinoma, endometritis, endometriosis, adenomyosis, inflammation, a thickening of the myometrium, or the presence of polyps. The uteri from two GM-fed pigs were full of fluid compared to nil from non-GM-fed pigs (Table 3) which may be linked to pathology. The link between an increase in uterine weights and GM feeding is supported by other authors (Brasil et al., 2009) who found that GM soy-fed rats had a statistically significant 59% increase in the density of the uterine endometrial glandular epithelium compared to rats fed an equivalent organic soy diet. Further studies should include histology, blood oestrogen, progesterone and cytokine concentrations, and which GM crop(s) and their GM protein products may, or may not, be involved. As this study used neutered males, further studies are required to investigate any potential effect of these crops on male reproduction. Multigenerational reproductive studies should also be considered.

In this study, a diet of GM feed had no effect on stomach erosions or ulceration but had a significant effect on inflammation. Pigs fed the mixed GM soy and GM corn diet showed 2.6 times the rate of severe stomach inflammation compared to non-GM fed pigs. This biologically significant finding was statistically significant (p=0.004). GM-fed male pigs showed severe stomach inflammation at a rate of 4.0 times that of the non GM fed male pigs (p=0.041); and female pigs showed a rate of severe stomach inflammation that was 2.2 the rate of the non-GM fed female pigs (p=0.034).

The pig industry uses finely-ground feed to maximise feed efficiency which can increase inflammation and ulceration of the stomach (Wolf, 2010). We therefore controlled the grind size, removing it as a confounder. Hence our results show that these GM crops were associated with stomach inflammation that was additional to any that may be caused by particle size. The result is attributed to the difference in diet, since the presence of mycotoxins, possible confounders such as infectious diseases, animal husbandry considerations or temporal, between-person, measurement and recording bias were controlled across the two groups.

One explanation for the inflammation results could lie with the Cry 3Bb1 and Cry 1Ab proteins that these GM corn varieties are engineered to produce. They act as insecticides by inducing pore formation and disintegration of the gut tissue (Spok et al., 2007) of certain grubs that attack corn plants. It has been argued that these proteins cannot harm the gastrointestinal tract of mammals because mammals lack the necessary gut environment and receptors (ANZFA, 2000). However, Vazquez-Padron et al. (2000) found six proteins in the mouse small intestine that could bind to a Cry protein (Cry 1Ac). Furthermore, when the Cry protein bound to these proteins, it resulted in hyperpolarisation of the intestine, which is consistent with the formation of cationic channels, as occurs in the insect gut (Vazquez-Padron et al., 2000). In addition, an independent in vivo study found structural changes and hyperplasia in the ileum of mice fed a Cry protein for two weeks (Fares & El-Sayed, 1998). Chowdhury et al. (2003) and Walsh et al. (2012b) found the Cry1Ab protein (which was present in the feed in our study) throughout the digestive tract of pigs. Chowdhury et al. (2003) found the protein (and sections of the gene that codes for it) in the stomach, duodenum, ileum, caecum and rectum of pigs fed Bt11 corn for four weeks, while Walsh et al. (2012b) found the protein in the stomach, caecum and colon of pigs fed MON810 corn for 110 days (they appear not to have looked in the rectum), indicating that this protein is resistant to digestion in pigs. In our study, stomach inflammation may be due to one or both of the Cry proteins fed in the study and future studies may provide answers.

The findings in this study are conservative since the non-GM diet pigs were exposed, albeit minimally, to potential GMO impacts. The presence of small amounts of GM material in the non-GM feed, using out-bred animals, piglets from GM-fed sows, and performing the study in a commercial setting (including the potential exposure of the pigs to any infectious diseases common to US commercial pigs and taking blood on site) could be expected to reduce any differences between the two dietary groups.

We found that our key findings were not reflected in the standard biochemical tests often undertaken by researchers in this area, probably because such tests provide a poor measure of inflammation and matters associated with uterine size. We suggest that the following may be better measures: the red blood cell count and haematocrit to measure anaemia and iron deficiency from possible blood loss, C-reactive protein and white blood cell count to measure inflammation, and oestrogen and progesterone.

In addition, if an autopsy is done at the end of a GM crop feeding experiment, this often involves only a visual inspection of the exterior of organs without weighing them. However by weighing organs we found a significant 25% increase in uterine weights in the GM-fed pigs. Moreover, where organs are weighed in such studies, they are often not examined internally (Carman, 2004) and such an approach would preclude finding the stomach inflammation reported in the present study.

The present study is an observational study of the action of a mixture of GM crops on the health of pigs, versus a comparable non-GM diet. Future work will investigate individual GM crops, will involve histopathology, and will consider mechanisms for reported group differences.

Conclusion

Pigs fed a GMO diet exhibited heavier uteri and a higher rate of severe stomach inflammation than pigs fed a comparable non-GMO diet. Given the widespread use of GMO feed for livestock as well as humans this is a cause for concern. The results indicate that it would be prudent for GM crops that are destined for human food and animal feed, including stacked GM crops, to undergo long-term animal feeding studies preferably before commercial planting, particularly for toxicological and reproductive effects. Humans have a similar gastrointestinal tract to pigs, and these GM crops are widely consumed by people, particularly in the USA, so it would be be prudent to determine if the findings of this study are applicable to humans.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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