Leinonen I, Williams AG. [Effects of dietary protease on nitrogen emissions from broiler production: a holistic comparison using Life Cycle Assessment](#). *Journal of the Science of Food and Agriculture* 2015, 95(15), 3041-3046

Copyright:

This is the peer reviewed version of the following article: Leinonen I, Williams AG. [Effects of dietary protease on nitrogen emissions from broiler production: a holistic comparison using Life Cycle Assessment](#). *Journal of the Science of Food and Agriculture* 2015, 95(15), 3041-3046, which has been published in final form at <http://dx.doi.org/10.1002/jsfa.7202> . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

DOI link to article:

<http://dx.doi.org/10.1002/jsfa.7202>

Date deposited:

02/12/2015

Embargo release date:

24 April 2016

This work is licensed under a [Creative Commons Attribution-NonCommercial 3.0 Unported License](#)

1 **EFFECTS OF DIETARY PROTEASE ON NITROGEN EMISSIONS FROM**
2 **BROILER PRODUCTION: A HOLISTIC COMPARISON USING LIFE CYCLE**
3 **ASSESSMENT***

4
5
6 **Ilkka Leinonen^a and Adrian G Williams^b**

7
8
9 ^aSchool of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon
10 Tyne, NE1 7RU, UK

11
12 ^bSchool of Energy, Environment and Agri-Food, Cranfield University, Bedford, MK43 0AL,
13 UK

14
15
16 Corresponding author: Ilkka Leinonen, e-mail: ilkka.leinonen@newcastle.ac.uk

17
18
19 Running title: Effect of dietary protease on nitrogen emissions from broiler production

20

* This paper was presented at the 18th Nitrogen Workshop, 30 June – 3 July 2014, Lisbon, Portugal

ABSTRACT

BACKGROUND

The aim of the study was to quantify the effects of the use of a protease “RONOZYME® ProAct” in broiler feed on the environmental impacts of broiler and broiler feed production chains. This was done by using a Life Cycle Assessment (LCA) modelling approach with data from trials using both standard soya-based broiler diets and reduced protein diets with added protease.

RESULTS

The results for the feed production chain showed that there was a reduction in all environmental impact categories, when protease was used in the diets. The biggest reduction occurred in the category of Global Warming Potential, mainly as a result of decreased CO₂ emissions from land use changes related to soya production. In the results for the broiler production chain, there were relatively bigger reductions in Eutrophication Potential and especially in Acidification Potential, mainly as a result of reduced feed protein content and subsequent nitrogen emissions from housing and manure management.

CONCLUSIONS

The use of protease in the broiler diets reduced the environmental impacts of both the feed production and broiler production. The latter is mainly through reduced ammonia emissions, which has substantial benefit *per se* in poultry industry.

Keywords

Acidification Potential, Ammonia emissions, Broiler production, Global Warming Potential, Life Cycle Assessment, Protease

INTRODUCTION

Agricultural production and especially livestock systems have generally been considered to have various negative environmental impacts, including nutrient leaching and a significant contribution to global warming.¹ Amongst different livestock production systems, poultry production has been found to be relatively “environmentally friendly”.² Despite for example relative low greenhouse gas emissions, poultry systems have, however, some features that require special attention in terms of their environmental impacts. These particularly include nitrogen emissions, for example in the forms of ammonia emissions to air, nitrous oxide emissions that contribute to global warming, and nitrate leaching. These emissions can occur at all stages of the poultry production chain, including growing the feed crops, housing the birds and finally managing the manure. Some can have relatively local effects, e.g. ammonia can have harmful effects close to poultry buildings, while other emissions cause global effects, e.g. nitrous oxide.

A systematic, quantitative approach is needed to evaluate the environmental impacts in complex agricultural systems and especially in livestock production where the environmental burdens related to feed production, processing and transport, together with the emissions from animal housing and manure managements should be taken into account. A method called Life Cycle Assessment (LCA) has been found to be a suitable approach for such research problems, as it is designed to account for all burdens occurring during the whole production and consumption cycle, starting from raw material extraction through to the disposal of end products.^{3,4} This method is also able to quantify multiple categories of environmental impacts, e.g. acidification and eutrophication, in addition to the more widely considered “carbon footprint”, which is the only focus of some other environmental impacts assessment frameworks. In LCA, impacts are quantified in relation to the functional unit, which is a defined output of the production system.

The composition of feed has been found to have a major effect on the environmental impacts of broiler production, as it contributes both to the emission arising from the feed production chain and from the broiler housing and subsequent manure management.⁵ One suggested method to reduce such emissions is to reduce the required amount of dietary protein (and the nitrogen content) of the feed by adding a specific enzyme, protease, to the feed, aiming to improve the protein utilization of the animals.⁶ The aim of the present study was to quantify the effects of the use of a protease “RONOZYME® ProAct” in broiler diets on the environmental impacts, especially those affected by the nitrogen emissions, in the context of standard indoor broiler production in the UK. This was done by using a Life Cycle Assessment (LCA) modelling approach for different scenarios of broiler production where diets either (a) without protease with standard protein content, or (b) with protease combined with reduced protein content were applied.

MATERIAL AND METHODS

The approach taken in the current study was Life Cycle Assessment based on systems modelling of agricultural production as described by Williams et al.⁷⁻⁹ and Leinonen et al.^{10,11} This included both structural models of the industry and mechanistic process models that were unified in the systems approach so that changes in one area caused consistent interactions elsewhere. This approach was applied to both feed crop and animal production. The systems modelled in this study included crop production, non-crop nutrient production, feed processing, breeding, broiler production (including farm energy and water use and gaseous emissions from housing), and manure and general waste management.

The structural model for the broiler system calculated all of the inputs required to produce the functional unit, taking into account breeding overheads, mortalities and productivity levels. It also calculated the outputs, both useful (broilers) and unwanted (e.g. wastes and mortalities). In the model, changes in the proportion of any activity must result in changes to the proportions of

others to keep producing the desired amount of output. Establishing how much of each activity was required was found by solving linear equations that described the relationships that linked the activities together.

The model calculated the nitrogen, phosphorus and potassium contents of the manure according to the mass balance principle, i.e. the nutrients retained in the animal body were subtracted from the total amount of nutrients obtained from the feed. For the purpose of the study, it was assumed that all manure was used for soil improvement as a fertiliser.

A separate sub-model for arable production was used to quantify the environmental impacts of the main feed ingredients, with main features as in Williams et al.⁹ All major crops used for production of broiler feed were modelled. For the crops produced overseas (e.g. soya, maize and sunflower), the production was modelled based as closely as possible on local techniques. Transport burdens for importing overseas crops and burdens from processing the feed were also included.

According to a widely accepted carbon footprinting method, PAS 2050,⁴ the direct greenhouse gas (GHG) emissions resulting from recent land use changes (LUC) for crop production must be included in the carbon footprint (i.e. the sum of the GHG emissions per unit of mass of the product) of each crop. Most GHG emissions from LUC are as CO₂ from loss of soil and biomass carbon. “Recent LUC” is defined as having occurred during the last 20 years in PAS 2050.⁴ This time period is arbitrary, given the non-linear rates of change in soil carbon, but is considered to be a pragmatic value that covers the largest change and the time during which the change is nearly linear. In this study, the LUC emissions were mainly related to soya production and arise from changes from forest or pasture (natural or managed) to arable land. The pasture is mainly cerrado in Brazil and pampa in Argentina, which are the main origin countries of soya used in the UK.¹²

PAS 2050:2011 also states that “Where the country of production is known, but the former land use is not known, the GHG emissions arising from land use change shall be the estimate of average emissions from the land use change for that crop in that country”. In this study, a weighted average for soya was derived from an analysis of land use and crop production statistics of the United Nations Food and Agricultural Organization.¹² The data were analysed to estimate to rates of change of types of land use and the degree of interchange between crops, taking into account that some soya expansion is actually from existing arable land. This was used to estimate the proportions of soya grown on mature arable land and on that converted from pasture, forest or other land in Brazil and Argentina. LUC emissions were amortised over 20 years for each unit area of land, as in Audsley et al.¹³ Annual rates of LUC emissions taken from PAS 2050⁴ were combined with the national yields of soya reported by FAO¹² to obtain LUC emissions per unit mass of soya. As the overall result of the analysis, the LUC effect of 1.3 kg CO₂ was included in the GHG emissions arising from production of 1 kg of soya meal used for broiler feed in the UK.

In order to model the emissions from the manure, the current study followed the principles of Audsley et al.¹⁴ and Williams et al.,^{7,9} taking a long-term approach to agriculture, for example ensuring that nitrogen emissions and uptake from manure are accounted for on an infinite time horizon. This differs from the shorter term methods that are often applied in less mechanistic carbon footprinting approaches⁴. Poultry manure is a source of direct gaseous emissions of ammonia (NH₃), nitrous oxide (N₂O) and to a lesser extent methane (CH₄), which occur during housing, storage and land-spreading and were quantified with a separate manure sub-model. Emissions of NH₃, N₂O and CH₄ arising from excreta during housing were calculated following the methods of Williams et al.,⁷ which are based on UK national inventories.¹⁵⁻¹⁸ For example, a nitrogen (N) flow approach is built into the NH₃ inventory, so that N ingested must be rigorously partitioned through mass balances into N in production and excreted N, which provides the quantities of N to which volatilised N is related. The emissions of N₂O and CH₄ are “unofficial” Tier 2 methods in that UK-specific emission factors for emissions from housing are

used in this study, but these have not yet been incorporated into the UK GHG inventor for agricultures, which is undergoing major revisions (www.ghgplatform.org.uk). Manure management also uses energy and these burdens were debited against the broilers (along with burdens from direct gaseous emissions). In the model, all of the nutrients applied to the soil as manure were accounted for as either crop products or as losses to the environment.¹⁹ The benefits of plant nutrients (nitrogen, phosphorus and potassium) remaining in soil after land application were credited to broiler production by offsetting the need to apply fertiliser as described by Sandars et al.¹⁹ and Williams et al.⁷

The production system in this study was considered to represent typical UK broiler production as described by Leinonen et al.¹⁰ In such a farm the total number of birds per house was 28 500 and the stocking density 20.4 birds/m². Energy consumption at the broiler farms for heating (0.19 litres liquefied petroleum gas per bird), lighting and ventilation (total electricity use 0.19 kWh per bird), together with information on material consumption such as the type and amount of bedding (0.17 kg wood shaving per bird), were based on average data from typical farms, provided by the UK broiler industry.¹⁰ Also the information about broiler breeding and hatcheries was obtained from the industry.¹⁰ The data on the production of the protease came directly from the manufacturer. Other non-crop feed ingredients were assumed to be analogues of L-lysine, which is provided in diets as a pure amino acid. It is usually manufactured by a fermentation process using *Corynebacterium glutamicum*. Given that most other biologically-based non-crop ingredients are produced in this generic way, the assumption was considered to be reasonable. In addition, the inclusion rate on enzymes is much lower than pure amino acids. Hence any error introduced by the analogue assumption will be very small indeed. Additional data, such as the life cycle inventories of agricultural buildings and machinery, came from Williams et al.⁷

All experimental data on the effects of the dietary protease were provided by the industry or obtained from literature. The information on the composition of the broiler feed and the

performance (including feed consumption) of the birds within each feeding programme came from seven separate trials, the details of some of which were published earlier.²⁰⁻²³ In all these trials, two alternative feeding programmes, based either on standard soya-based diets or on low protein diets with protease supplement were applied in broiler production. The protease was added to the feeds based on the guaranteed analysis (75,000 PROT units/g) of the commercial product. Enzyme preparations (Ronozyme ProAct CT) were obtained from Novozymes A/S (Bagsvaard, Denmark). The protease used in this study was a commercial enzyme produced by submerged fermentation of *Bacillus licheniformis* containing transcribed genes from *Nocardia* *prasinus*. Enzyme activity for this protease is measured in PROT units, with 1 unit defined as the amount of enzyme that releases 1 µmol of *p*-nitroaniline from 1 µM of substrate (Suc-Ala-Ala-Pro-Phe-*p*-nitroaniline) per minute at pH 9.0 and 37°C.

The overview of each of the trial is shown in Table 1. Details of the diets (usually 3-4 successive phase diets during the production cycle) are shown in the Supplementary Information for trials 1-6, and in Angel et al.²³ for the trial 7.

The Life Cycle Assessment was carried out separately for two alternative systems:

1. Feed production chain included growing of feed crops, production of additives, processing of ingredients and mixtures, transport of the ingredients, production of fertilizers etc. The system boundary was the feed mill gate and the functional unit 1000 kg broiler feed, calculated on the basis of the relative amount of each phase diet consumed in each trial. The results of all trials 1-7 were applied in this analysis.

2. Broiler production chain quantified all impacts related to feed production (as above) and included also energy use in housing the broilers, emissions from broiler house and storage and land spreading of the manure, broiler breeder production, hatching etc. The functional unit was 1000 kg expected broiler carcass weight at the farm gate. The expected carcass weight is the body weight excluding feathers, heads, necks, feet and internal organs. In this study a constant

relationship between the carcass weight and live weight of the bird was assumed, based on the data from the UK broiler industry.¹⁰ As the trial 7 considered only the starter phase of the bird growth cycle and therefore did not produce finished broilers, this trial was excluded from this analysis. An overview of the modelling of the broiler production chain, including the use of the data, is shown in the Figure 1.

As an output of the LCA model, the calculated emissions were aggregated into environmentally functional groups. Nitrogen emissions have an important contribution to the following groups:

Global Warming Potential (GWP) is a measure of the greenhouse gas emissions to the atmosphere, and was calculated here using a timescale of 100 years. The main sources of GWP are carbon dioxide (CO₂) from fossil fuel and land use changes, nitrous oxide (N₂O) and methane (CH₄). GWP was quantified as CO₂ equivalent: with a 100 year timescale 1 kg CH₄ and N₂O are equivalent to 25 and 298 kg CO₂ respectively. The sum of GWP per functional unit is also known as the “carbon footprint”.

Eutrophication Potential (EP) is used to assess the over-supply (or unnatural fertilisation) of nutrients as a result of nutrients reaching water systems by leaching, run-off or atmospheric deposition. EP was calculated using the method of the Institute of Environmental Sciences (CML) at Leiden University (<http://www.leidenuniv.nl/interfac/cml/ssp/index.html>). The main sources are nitrate (NO₃⁻) and phosphate (PO₄³⁻) leaching to water and ammonia (NH₃) emissions to air. EP was quantified in terms of phosphate equivalents: 1 kg NO₃-N and NH₃-N are equivalent to 0.44 and 0.43 kg PO₄³⁻, respectively.

Acidification Potential (AP) is mainly an indicator of potential reduction of soil pH (and causing damage to some building materials, like limestone). AP was also calculated using the method of the Institute of Environmental Sciences (CML) at Leiden University The main source

is ammonia emissions, together with sulphur dioxide (SO₂) from fossil fuel combustion. AP was quantified in terms of SO₂ equivalents: 1 kg NH₃-N is equivalent to 2.3 kg SO₂.

RESULTS

The results for the environmental impacts arising from the feed production chain are presented in Table 2, where in all categories the impacts arising from the protease-supplemented diets are presented as a percentage of the soya-based (control) diets. The results show that there is a reduction in all environmental impact categories, per mass unit of feed, when protease is used in the diets. The main reason for this is the reduction of the amount of soya used in the diets. The biggest reduction occurs in the category of Global Warming Potential (up to 12 % reduction with an average of 5 %). This is mainly caused by decreased CO₂ emissions from land use changes related to soya production and lower emissions from the transport of the soya. With the Eutrophication Potential, there were only small differences between the diets, while the Acidification Potential showed bigger reduction. This resulted from less use of soya, but the reduction is mainly from the transport step, which emits relatively large amounts of SO₂ from marine fuels.

Although the environmental impacts of production of the protease were relatively high per unit of the product (e.g. GWP 6.5 kg CO₂ equivalent per kg of protease), the amount applied in the broiler diets was so small that the protease inclusion itself had only a minimal effect per unit of broiler feed.

In Table 3, the results for the environmental impacts arising from the whole broiler production chain are presented. Again, in order to make the results comparable, the impacts arising from production with the protease diets are presented as a percentage of the impacts with the control diets. For these results, the absolute values of the impacts can be seen in the Supporting Information. In the results, there were relatively bigger reductions in Eutrophication Potential

and especially in Acidification Potential, compared to the feed production chain alone. For Acidification Potential, the maximum reduction was 9 % and the average reduction 5 %. The reason for this is that a major part of these impacts arises from the emissions from housing and manure management. When protease was used in the diets, the crude protein content of the feed was reduced, reducing the amount of nitrogen in manure. This reduced the emission of ammonia, affecting both the Acidification and Eutrophication Potentials, and leaching of nitrate, affecting the Eutrophication Potential.

Ammonia emissions from housing and manure were the biggest single source of emissions contributing to the Acidification Potential in broiler production chain. In general, ammonia emissions are considered to be problematic in poultry production and a subject of regulation in the case of large broiler production units. Therefore, a substantial benefit of reducing the protein content of the diet is the reduction of ammonia emissions *per se*. This is presented in Table 4, which shows the absolute values of the ammonia emissions to air (kg NH₃ / 1000 kg broiler carcass at farm gate) for each of the trial considered in this study, both for the standard soya based diet and for the alternative low protein diet with protease supplementation.

DISCUSSION

The main differences in the broiler feeding programmes with and without protease were the amount of soya used in the diets. In general, the amount of soya bean meal removed from the diet when protease was applied was relatively small, which set the limits for the magnitude of the resulting reduction of GWP. However, there was one exception: the protease-supplemented diet in the trial 7,²³ which considered the starter phase of the broiler production only, had a big reduction of soya (from 29 % to 23 % on the weight basis),²³ and as a result, there was a substantial reduction of the GWP arising from feed production (by 12%). However, it should be noted that as not containing the whole production cycle, the results of this trial are not directly comparable with the other studies.

A large part of the reduction of the GWP observed in this study is a result of reduced greenhouse gas emissions arising from land use changes. These emissions should be allocated to production of certain crops, including South American soya, unless the exact origin of the crop is known and certified to be “mature” agricultural land, as specified for in PAS 2050⁴. However, it should be noted that currently there is no single generally accepted method how to account for the greenhouse gas emissions related to land use changes.^{24,25} In this study, a method recommended by PAS 2050 was used, but alternative methods could produce strongly differing results when different diets containing varying amount of soya are compared. For example, it should be noted that there is a more recent version of the PAS 2050 specification²⁶, where in some cases it is recommended that from two optional accounting methods, the one producing the “highest calculated value” of GHG emissions should be used in the assessment. This approach was not used in this study because it was considered that systematically selecting a higher value from two options would violate the Completeness and Consistency principles of the PAS 2050 guidelines and lead to overestimation of the emissions, as discussed by Leinonen et al.²⁴ Also, the calculations of the environmental impacts of soya meal were based on the current mixtures of sources of soya bean and soya meal in the UK. If more soya was sourced from Brazil rather than Argentina, this would increase the overall GWP of the soy meal, and this would also increase the relative reduction of this impact when protease is applied in the diet.

Amongst the other environmental impacts associated with the feed production, in the Eutrophication Potential, there were only small differences between the diets, while the Acidification shows bigger reduction, again as a result of lower requirements of soya. Amongst all the feeding programmes analysed, the biggest reductions occurred in the study by Angel et al.²³ where more soya is removed from the diets that in other studies.

In the results of the analysis of the whole broiler production chain (Table 3), in Global Warming Potential the reductions in environmental impacts were smaller than in the case of the feed production chain. The reason for this is that in broiler production there are greenhouse gas emissions arising from other sources than feed and manure (where the N₂O emissions are affected by the nitrogen content of the diet). The additional sources include for example the farm fuel and electricity use, and these are not expected to be affected by the differences in the diets. In contrast, there were relatively bigger reductions in Eutrophication Potential and especially in the Acidification Potential in the overall broiler production, compared to the feed production chain alone. The reason for this is that a major part of these impacts arises from the emissions from housing and manure management. When protease was used in the diets, the crude protein content of the feed was reduced, which automatically reduces the amount of nitrogen excreted by the birds even if the nitrogen retention remains constant. This effect reduced the emission of ammonia (presented in Table 4), which affects both the Acidification and Eutrophication Potentials, and leaching of nitrate, affecting the Eutrophication Potential, and also has indirect effects on Global Warming Potential by contributing to the emission of nitrous oxide.

In general, the use of protease systematically reduced all environmental impacts considered in most of the trials analysed in this study. The only exception was the trial 6, where the birds with the low protein diet had a worse performance (higher feed consumption per unit of live weight and higher mortality) than the control birds (Details of the bird performance in all trials are shown in Supplementary Information). This trial differed from the others in the respect that rapeseed meal (and to a lesser extent sunflower meal) was used as a major protein source in addition to soya bean meal, which was the main protein source in other experiments. Whether the effect on performance was related to the enzyme specificity for the protein structure *per se* of these materials or rather that the protein in such materials is simply less accessible to the protease due to the complex cell wall structure is unclear. However, it should be also noted that in this trial the reduction in the amino acid content in the low protein diet was larger than in

some others. This may also have had effect on the bird performance. As a result of reduced performance with the low-protein diet in this trial, the impacts in all categories were higher with the protease diet compared to the control treatment. This demonstrates the fact that although the reduction of soya has potential to reduce the environmental impacts, it should be done in a way that has no negative effect on bird performance. Otherwise the beneficial effects of such diet changes can be easily lost.

In order to compare statistically the environmental impacts for example between different production methods, the uncertainty related to the estimates of the impact categories should also be taken into account. In earlier LCA studies on poultry production the uncertainty has been estimated using Monte-Carlo simulations^{10,11}, or with analytical error propagation²⁷. These approaches would require quantitative estimates of the uncertainties of the model inputs, which were not available for all the data applied in this study, so any formal uncertainty analysis was not carried out. However, it should be noted that the uncertainty analysis should focus on those model variables the uncertainty of which is expected to vary between the systems in consideration, i.e. the “alpha” uncertainties.^{10,11,27} In all the trials analysed in this study, both treatments were carried out in a similar environment, so differences could be expected only in the feed composition and in the animal performance. The feed composition was well specified, so minimal uncertainty was expected to occur in this quantity. Furthermore, the origin of the feed ingredients was the same for both treatments, with only their relative amounts varying. As a result, the main findings of the environmental benefits of protease can be expected to contain only minimal uncertainty when the feed production chain is considered. In the broiler production chain, the differences in bird performance remain the only potential major source of the “alpha” uncertainty. It can therefore be concluded that the predicted environmental benefits with protease will be achieved with high certainty unless there are any negative effects on animal performance. In most of the original trials the data of which was used in this study, such effects were not observed.

The magnitude of the reduction of environmental impacts in this study was similar or higher compared to other studies aiming to reduce the soya in broiler diets. For example, Leinonen et al.⁵ found that when using “realistic” inclusion rates of peas replacing part of soya as a protein source in broiler diets, the GWP of broiler production could be reduced by about 4%, i.e. with a similar magnitude as observed in many trials analysed in this study. With other European protein sources (beans, sunflower) in diets, the reduction was smaller or did not exist⁵. However, it should be noted that in those studies the performance of the birds was assumed to remain unchanged; if, in reality, the alternative protein crops have negative effects e.g. on the growth, the environmental benefits can be expected to even smaller. Similarly, other studies have also found a limited potential of alternative protein crops in reduction of the impacts of livestock production.^{28, 29} This indicates that when aiming to improve the environmental performance of livestock, the use of protease in feed is a promising option, either as used alone or combined with other dietary alterations, together with other changes in animal husbandry.

Amongst different methods aiming to reduce the ammonia emissions arising from poultry production, a nutritional solution can be considered to be the most practical as it requires no changing in building design or use of retrofitted scrubbers. Reduced ammonia emissions should also improve air quality for both birds and workers. In broiler production, the main motivation for using protease is reduction of feeding costs, as protein is one of the most expensive components in diets. The results of this study suggest that with protease supplement, as far as there are no negative effects on bird performance, this economic benefit can also be combined with environmental improvements.

CONCLUSIONS

The use of protease in the broiler diets and the resulting reduction of the use of soya reduced the environmental impacts of both the feed production (mainly Global Warming Potential) and

broiler production (mainly Acidification Potential) chains. The latter is mainly through reduced ammonia emissions, which has a range of environmental benefits.

ACKNOWLEDGEMENTS

This study was funded by the DSM Nutritional Products.

REFERENCES

1. Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M and De Haan C, *Livestock's long shadow: environmental issues and options*. FAO, Rome, Italy (2006)
2. de Vries M, and de Boer IJM, Comparing environmental impacts for livestock products: A review of life cycle assessments. *Livestock Sci* **28**: 1-11 (2010)
3. BSI, *Environmental management: Life cycle assessment - Principles and framework*. http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=37456 [4 December 2014]
4. BSI, *Specification for the assessment of the life cycle greenhouse gas emissions of goods and services. PAS 2050:2011*. <http://www.bsigroup.com/Standards-and-Publications/How-we-can-help-you/Professional-Standards-Service/PAS-2050/> [4 December 2014]
5. Leinonen I, Williams AG, Waller AH and Kyriazakis I, Comparing the environmental impacts of alternative protein crops in poultry diets: the consequences of uncertainty. *Agr Syst* **121**: 33-42 (2013)

6. Oxenboll KM, Pontoppidan K and Fru-Nji F, Use of a Protease in Poultry Feed Offers Promising Environmental Benefits. *Int J Poult Sci* **10**: 842-848 (2011)
7. Williams AG, Audsley E and Sandars DL, *Determining the environmental burdens and resource use in the production of agricultural and horticultural commodities*. Main Report. Defra Research Project IS0205. <http://preview.tinyurl.com/Cranfield-IS0205> [4 December 2014]
8. Williams AG, Audsley E and Sandars DL, Environmental burdens of livestock production systems derived from life cycle assessment (LCA) in *41st University of Nottingham Feed Conference*, Sutton Bonington, Nottingham, ed. by Garnsworthy P, Nottingham University Press, Nottingham, England, pp. 171-200 (2007)
9. Williams AG, Audsley E and Sandars DL, Environmental burdens of producing bread wheat, oilseed rape and potatoes in England and Wales using simulation and system modelling. *Int J LCA* **15**: 855-868 (2010)
10. Leinonen I, Williams AG, Wiseman J, Guy J and Kyriazakis I, Predicting the environmental impacts of chicken systems in the UK through a Life Cycle Assessment: broiler production systems. *Poult Sci* **91**: 8-25 (2012)
11. Leinonen I, Williams AG, Wiseman J, Guy J and Kyriazakis I, Predicting the environmental impacts of chicken systems in the UK through a Life Cycle Assessment: egg production systems. *Poult Sci* **91**: 26-40 (2012)
12. FAO, *Food and Agriculture Organization of the United Nations*. <http://faostat.fao.org> [4 December 2014]

13. Audsley E, Angus A, Chatterton J, Graves A, Morris J, Murphy-Bokern D, Pearn KR, Sandars DL and Williams AG, *Food, land and greenhouse gases. The effect of changes in UK food consumption on land requirements and greenhouse gas emissions*. A report prepared for the United Kingdom's Government's Committee on Climate Change. Cranfield University (2010)
14. Audsley E, Alber S, Clift R, Cowell S, Crettaz P, Gaillard G, Hausheer J, Jolliett O, Kleijn R, Mortensen B, Pearce D, Roger E, Teulon H, Weidema B and van Zeijts H, *Harmonisation of environmental life cycle assessment for agriculture*. Final Report, Concerted Action AIR3-CT94-2028, European Commission, DG VI Agriculture, Brussels (1997)
15. Chadwick DR, Sneath RW, Phillips VR and Pain BF, A UK inventory of nitrous oxide emissions from farmed livestock. *Atmos Environ* **3**: 3345-3354 (1999)
16. Intergovernmental Panel on Climate Change, *IPCC Guidelines for National Greenhouse Gas Inventories*. (2006 Guidelines) <http://www.ipcc-nggip.iges.or.jp/> [5 December 2014]
17. Misselbrook TH, Chadwick DR, Smith KA and Williams J, *Inventory of ammonia emissions from UK agriculture 2007*. DEFRA Contract AC0112, Inventory Submission Report (2008)
18. Sneddon S, Brophy N, Li Y, MacCarthy J, Martinez C, Murrells T, Passant N, Thomas J, Thistlethwaite G, Tsagatakis I, Walker H, Thomson A and Cardenas L, *Greenhouse gas inventories for England, Scotland, Wales and Northern Ireland: 1990 – 2008*. AEAT/ENV/R/2669 Issue 1 (2008)
19. Sandars DL, Audsley E, Canete C, Cumby TR, Scotford IM and Williams AG, Environmental benefits of livestock manure management practices and technology by Life Cycle Assessment. *Biosystems Eng* **84**: 267-281 (2003)

- 492
- 493 20. Olukosi OA, Beeson L, Pirgozliev V and Smith A, Supplementation of a mono-component
- 494 *Bacillus licheniformis* protease improved growth performance and selectively improved nutrient
- 495 digestibility in broilers. *Proceedings of WPSA (UK) Branch Annual meeting, 2013*, Nottingham,
- 496 UK (2013)
- 497
- 498 21. Leleu S, Vandeweghe A, Smith A and Maertens L, The effect of Ronozyme® ProAct on the
- 499 performances, feather dirtiness, and foot pad and hock dermatitis of broilers. *Proceedings of the*
- 500 *XXIV World's Poultry Congress. Vol. 68 Supplement 1*, Bahia : World's Poultry Science
- 501 Association (2012)
- 502
- 503 22. Gornowicz E, Effect of mono-component serine protease and multi-component
- 504 carbohydrase complex supplementation on performance of broiler chickens fed low protein
- 505 diets. *Proceedings of XXV International Poultry Symposium PB WPSA*, 2 April 2013, Zegrze
- 506 near Warsaw (2013)
- 507
- 508 23. Angel CR, Saylor W, Vieira SL and Ward N, Effects of a monocomponent protease on
- 509 performance and protein utilization in 7- to 22-day-old broiler chickens. *Poult Sci* **90**: 2281–
- 510 2286 (2011)
- 511
- 512 24. Leinonen I, Williams AG and Kyriazakis I, Evaluating methods to account for the
- 513 greenhouse gas emissions from Land Use Changes in agricultural LCA. *Proceedings of LCA*
- 514 *Food 2014*. 8-10 October 2014, San Francisco, USA (2014). In press.
- 515
- 516 25. Williams AG, Dominguez H and Leinonen I, A simple approach to land use change
- 517 emissions for global crop commodities reflecting demand. *Proceedings of LCA Food 2014*. 8-10
- 518 October 2014, San Francisco, USA (2014). In press.
- 519

26. BSI, *Assessment of life cycle greenhouse gas emissions from horticultural products. PAS 2050-1:2012*. <http://shop.bsigroup.com/Browse-By-Subject/Environmental-Management-and-Sustainability/PAS-2050/PAS-2050-1/> [20 January 2015]
27. Leinonen I, Williams AG and Kyriazakis I, Comparing UK turkey production systems using analytical error propagation in uncertainty analysis. *Proceedings of LCA Food 2014*. 8-10 October 2014, San Francisco, USA (2014). In press.
28. Baumgartner DU, de Baan L and Nemecek T, *European Grain Legumes - Environment-Friendly Animal Feed? Life Cycle Assessment of Pork, Chicken Meat, Egg, and Milk Production. Grain Legumes Integrated Project*. Final Report WP2.2, Environmental Analysis of the Feed Chain. Agroscope Reckenholz-Tänikon Research Station ART, Zürich, 106 p (2008)
29. Nguyen TTH, Bouvarel I, Ponchant P and van der Werf HMG, Using environmental constraints to formulate low-impact poultry feeds. *J Cleaner Prod* **28**: 215-224 (2012)

Table 1. The overview of the feeding trials applied in this study.

Trial	Final age of birds (d)	Slaughter weight (kg)	Number of phase diets	Source of data
Trial 1.	42	3.1 – 3.2	3	Olukosi et al. ²⁰
Trial 2.	39	2.3 – 2.4	3	Leleu et al. ²¹
Trial 3.	42	2.4 – 2.5	3	Gornowicz ²²
Trial 4.	42	2.4 – 2.5	3	Unpublished data*
Trial 5.	34	2.0	3	Unpublished data*
Trial 6.	40	2.3 – 2.4	4	Unpublished data*
Trial 7.	22	N/A	1	Angel et al. ²³

*Data provided by DSM Nutritional Products

Table 2. The relative environmental impacts Global Warming Potential (GWP), Eutrophication Potential (EP) and Acidification Potential (AP) arising from production of the low-protein feed with protease supplement compared to production of standard soya-based feed, per mass unit of feed at feed mill gate.

	GWP	EP	AP
Trial 1.	97%	100%	98%
Trial 2.	97%	98%	96%
Trial 3.	95%	100%	97%
Trial 4.	94%	99%	96%
Trial 5.	97%	100%	98%
Trial 6	97%	99%	98%
Trial 7.	88%	96%	94%
Average	95%	99%	97%

Table 3. The relative environmental impacts Global Warming Potential (GWP), Eutrophication Potential (EP) and Acidification Potential (AP) arising from broiler production using the low-protein feed with protease supplement compared to production with standard soya-based feed, per mass unit of expected carcass weight at farm gate.

	GWP	EP	AP
Trial 1.	96%	94%	93%
Trial 2.	98%	96%	95%
Trial 3.	96%	93%	91%
Trial 4.	96%	94%	92%
Trial 5.	98%	97%	95%
Trial 6.	102%	105%	105%
Average	98%	97%	95%

Table 4. Emissions of ammonia (NH₃) to air with the standard soya-based feed and a low-protein feed with protease supplement, kg NH₃ per 1000 kg expected broiler carcass at farm gate.

Trial	Standard ^a	Protease ^b
Trial 1.	26.9	24.8
Trial 2.	25.7	24.3
Trial 3.	23.8	21.5
Trial 4.	25.7	23.6
Trial 5.	23.0	21.9
Trial 6.	29.0	30.6

^a standard soya-based diet

^b low-protein diet with protease supplement

Figure 1. Outline of the broiler production modelling system applied in this study.