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Use of a Protease in Poultry Feed Offers Promising Environmental Benefits

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Abstract: Enzymes are widely used in feed to improve utilization of nutrients and it is generally acknowledged that phytases, by improving phytate phosphorus utilization are an important tool to reduce phosphorus pollution and the environmental problems derived from this such as eutrophication. Lately, the use of proteases as feed enzymes has gained interest. Proteases are added to feed with the purpose of increasing dietary protein hydrolysis and thus enabling improved nitrogen utilization. When animals utilize nitrogen better, there is a possibility to decrease the diet protein content and in turn also reduce the content of nitrogen in manure. The environmental consequences of decreased dietary protein content and reduced nitrogen excretion were investigated in a Life Cycle Assessment (LCA), which considered all steps in broiler production from production of feed ingredients, to the broilers leaving the chicken house, including the use of manure. Wide ranges of environmental impacts were analyzed, covering emissions of nitrous compounds to both air and the aquatic environment. Significant benefits were obtained for all impacts considered. Most important were the benefits related to reduced emissions of ammonia, which help reduce both health risks and environmental impacts, such as acidification and eutrophication. The largest effects were obtained when protease was used as a tool to allow for lower diet protein content. However, even when used in a diet with normal protein level significant benefits were observed. It is illustrated that the use of protease can contribute significantly to current efforts to reduce nitrogen emissions from livestock production.

Key words: Life cycle assessment, environment, protease, feed, poultry

INTRODUCTION

The demand for meat is growing rapidly. World production of poultry has been increasing steadily since the sixties and shows the highest rate of increase, followed by pigs at a substantially lower rate (Raney *et al*., 2009). This means that production of poultry is one of the main drivers for increased production of agricultural feedstock and nitrogen fertilizers. Production of nitrogen fertilizers is very energy demanding. In addition, this production means that non-reactive molecular nitrogen (N_2) is turned into reactive forms of nitrogen compounds (such as NH_3, N_2O, NO_3, NO), which either act as greenhouse gases or cause pollution of atmosphere and water. The pollution issue is further emphasized by the development towards industrial production systems, where return of the manure to agricultural land as fertilizer is becoming increasingly difficult. Overall this means that there is a need to improve feed utilization efficiency and to reduce harmful nitrogen emissions to the environment from broiler production. Using a protease in the feed can help to increase the digestibility of protein by broilers and thus represents a means to address this need.

LCA is a generally accepted method for assessing the environmental impact of different processes throughout the entire life cycle of a product. An LCA study provides a basis for choosing the most environmentally attractive process from a number of alternatives providing the same end product. The purpose of the current study was to investigate quantitatively the environmental importance of using a protease in broiler feed. This was done by comparing the normal process of producing 1 ton broiler (live weight) without the use of a protease with two alternative processes: 1) production of 1 ton broiler where protease is added into the normal process without changing the diet composition and 2) production of 1 ton broiler where protease is used as a tool to allow for a lower protein content in the diet.

This paper presents the results for three environmental impacts; global warming, acidification and eutrophication. Global warming is related to emissions of greenhouse gases to the atmosphere and potential climate change. Acidification is related to emissions of for instance sulfur oxides, ammonia and nitrogen oxides and releasing protons in the atmosphere. The elevated levels of hydrogen ions give rise to acid rain causing damage to the soil as well as the aquatic environment. Plants, animals and microorganisms are influenced by the lower pH and biodiversity is reduced. Damage to buildings and statues (of limestone and marble) may also occur. Eutrophication is related to emissions of nutrients such as phosphate, nitrate and ammonia. When present in the environment in excess amounts, these nutrients will favor growth of some plants over

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others and thus reduce biodiversity. In aquatic environments algal blooms can become so excessive that there is no oxygen left for the animals. The use of environmental impact categories simplifies the compilation of results over the whole life cycle. In addition it takes into consideration that some emissions contribute to several environmental impacts, such as ammonia, which contributes to both acidification and eutrophication. In addition to these environmental effects emissions of ammonia also influence health conditions. The holistic approach of LCA is crucial for environmental optimization of production processes, as it prevents sub-optimization.

MATERIALS AND METHODS

This paper describes an LCA study based on data from a broiler trial published by Freitas *et al*. (2011).

Animal trial: 500 male Cobb broiler chicks were obtained from a commercial hatchery and placed in pens (1.50 x 1.50 m) with 22 birds per pen. Each pen was equipped with 1 tube feeder and 1 bell drinker. Corn, soybean meal and meat and bone meal were used as the main ingredients to formulate the feeds for the individual phases (1 to 7, 8-21, 22-35 and 36-42 d). Dietary treatments resulted from a 2 x 2 x 2 factorial arrangement of 2 dietary protein levels (Normal Protein (NP) or Low Protein (LP)) with a 7% difference, 2 energy levels with a 3% difference and two protease concentrations (0 or 200 ppm [15,000 PROT/kg diet)] added based on guaranteed enzyme concentrations[1]. Live performance measurements were determined at day 21 and day 42. Further details of the trial are described by Freitas *et al*. (2011).

This LCA analysis is based on data from the grower period only (22-42 d) and on broilers receiving the high energy diet. Calculations have shown that using data from the grower period provided similar results as when calculating based on weighted average diet composition for the complete growth period and the final FCR (Oxenboll *et al*., 2011). Diet compositions and corresponding FCR values used for the LCA calculations are shown in Table 1 and 2. Changes in intake of vitamin and mineral premix, choline chloride and kaolin were not included in the calculations; consequently the analysis is more conservative than if all these changes had been included in the calculation.

LCA analysis: The LCA comprises two steps. The first step (the inventory analysis) is a matter of collecting data for inputs and outputs from all the processes influenced by the use of the protease, such as changes in consumption of feed and changes in nitrogen emissions from manure. The outcome of this analysis is an overview of all changes of inputs and outputs caused by use of the protease summarized over the whole life cycle. The second step (the impact assessment analysis) is a matter of translating the change of inputs and outputs to potential environmental effect. As there are more types of emissions giving rise

Table 1: Broiler diets (d 22-42) with normal and low protein level and 200 ppm protease (15,000 PROT/kg) (adopted from Freitas *et al*., 2011)

	Normal Protein Level (NP)			Low Protein Level (LP)		
	d 22-35	d 36-42	Avr d 22-42	d 22-35	d 36-42	Avr d 22-42
Ingredients (g/kg)						
Corn	609.2	628.7	618.95	655.0	671.0	663.00
Soybean meal	270.0	229.3	249.65	232.6	194.7	213.65
Meat and bone meal	66.0	67.0	66.50	67.0	68.0	67.50
Soybean fat	41.2	52.1	46.65	33.8	45.2	39.50
Limestone	1.5	1.5	1.50	1.5	1.5	1.50
Common salt[a]	2.8	2.9	2.85	3.0	3.1	3.05
Na bicarbonate	0.8	0.6	0.70	0.4	0.3	0.35
DL-methionine	2.4	2.0	2.20	1.7	1.3	1.50
L-lysine HCl	2.3	2.0	2.15	1.5	1.4	1.45
L-threonine	0.3	0.4	0.35	0.0	0.0	0.00
Px. Vit and Min[bd]	2.5	2.5		2.5	2.5	
Choline chloride[d]	0.8	0.8		0.8	0.8	
Kaolin[d]	0-0.2	0-0.3		0-0.4	0-0.5	
Protease[c]	0/0.2	0/0.2	0/0.20	0/0.2	0/0.2	0/0.20
ME (kcal/kg)	3,144.0	3,247.0	3,195.00	3,144.0	3,247.0	3,195.00
Analyzed CP (g/kg)	197.0	179.0	188.00	189.0	172.0	180.50

[a]NaCl.

[b]Vitamin, mineral and additive contribution per kg of feed: vitamin A: 9,000 IU; vitamin D3: 2,500 IU; vitamin E: 20 IU; vitamin K3: 2.5 mg; vitamin B1: 1.5 mg; vitamin B2: 6 mg; vitamin B6: 3 mg; pantothenic acid: 1.2 mg; biotin: 0.06 mg; folic acid: 0.8 mg; niacin: 25 mg; vitamin B12: 12 µg; I: 2 mg; Se: 0.25 mg; Cu: 20 mg; Mn: 160 mg; Zn: 100 mg; Fe: 100 mg (all sources as sulphate, except for sodium selenite and calcium iodate); monensim sodium: 100 ppm (1-21 d); salinomycin: 66 ppm (22-40 d).

[d]The protease replaced kaolin.

[d]Not included in the LCA calculation

Table 2: Live performance (day 42) of broilers fed the Normal Protein Diet (NP), the normal protein diet with protease (NP w/protease) or the low protein diet with protease (LP w/protease)

	NP	NP w/protease	LP w/protease
Feed intake (kg)	3.564	3.557	3.534
FCR	1.767	1.744	1.791

to each environmental impact, these are quantified by means of equivalence factors (Hauschild and Wenzel, 1998). Global warming which can be caused by CO_2, N_2O or CH_4 is expressed in CO_2 equivalents, where 1 g of CO_2 corresponds to 1 g CO_2 equivalent, 1 g of CH_4 corresponds to 23 g of CO_2 equivalents and 1 g of N_2O corresponds to 296 g CO_2 equivalents. Eutrophication is caused mainly by nitrate, phosphate and ammonia emissions and is expressed in phosphate equivalents, where 1 g of nitrate corresponds to 0.1 g phosphate equivalent and 1 g of ammonia corresponds to 0.35 g phosphate equivalents. Acidification is in this study mainly caused by ammonia and is expressed in sulfur dioxide equivalents, where 1 g of ammonia corresponds to 1.6 g sulfur dioxide equivalents. The calculation of change of for instance global warming is based on a multiplication of the change in greenhouse gases determined in step one with the relevant equivalence factor. By this transformation, all changes in greenhouse gases are now expressed in CO_2 equivalents and can be summarized.

The LCA assessment was based on the principles of ISO 14040 and 14044 standards (ISO 14040, 2006; ISO 14044, 2006). The practical modeling was performed in the LCA software SimaPro. Equivalence factors were derived from the life cycle impact assessment method called CML baseline 2000 (Institute of Environmental Science, Leiden University). The modeling of production of feed ingredients and transportation was based on data from the EcoInvent database (2009). The calculation of the environmental load of producing the protease was based on data from Novozymes A/S (Bagsvaerd, Denmark).

For the modeling of nitrogen emissions from manure it was assumed that the reduction in nitrogen content of the manure corresponds to the reduction of nitrogen content in the feed. This is equivalent to assuming that the retention of nitrogen (kg N per ton broiler) is the same for the broilers receiving protease as for the control broilers. The calculations of nitrogen emissions were based on the IPCC tier 1 guidelines (Dong *et al.*, 2006; de Klein *et al*., 2006). The calculations cover two steps: a) calculation of change in emissions before application of manure and b) calculation of change in emissions during use of manure. It was assumed that the manure is used as fertilizer, which means that the reduction in nitrogen content has to be compensated with additional production of synthetic nitrogen fertilizer.

The use of the IPCC tier 1 guidelines implies that nitrogen emission calculations are to be considered rough estimates based on average conditions. Sensitivity analyses on all parameters used for calculating nitrogen emissions have been carried out based on the range of uncertainty given by IPCC for each parameter.

The overall changes per 1 ton broilers due to use of protease comprise reductions as well as increases of environmental load. Reductions are obtained in relation to production and transportation of feed ingredients and emissions of nitrogen compounds. Increases in environmental load are related to production of protease and additional fertilizer needed to compensate for the lower nitrogen content of the manure. These changes form the basis for the LCA calculations. Consumption of energy and bedding in the chicken house are not included in the analysis, as these are not influenced by the use of protease.

The complete LCA report (Oxenboll *et al*., 2011) which forms the basis for this paper has been subject to external critical review and has been recognized as meeting the ISO 14040 (2006) and ISO 14044 (2006) standards on LCA. The complete report builds on a wider range of feeding trials and a wider range of environmental impacts have been considered than presented in this paper.

RESULTS

Analysis of the change in emissions of nitrogen from manure due to the use of protease showed that there were savings (negative values) both in NH_3, N_2O and NO_x emissions when using a protease (Fig. 1). The savings in nitrogen emissions were greater when protease was used as a tool to allow for decreased dietary protein level.

Analysis of the change in consumption of feed ingredients differed depending on how the protease was introduced. When protease was introduced into the normal diet (NP w/protease) there was a decreased consumption of all feed ingredients, which was reflected in a decrease in FCR compared to the NP diet. When protease was introduced into the diet along with a reduction in protein content (LP w/protease) there was a decreased consumption of soybean meal and soybean oil but at the same time an increase in consumption of e.g. corn, this was reflected in an increased FCR. However, when summarizing the change in environmental load over the whole life cycle, including both changes in feed consumption and manure emissions, reductions were obtained for all impact categories and for both diets (Fig. 2). In order to estimate the uncertainty, calculations carried out in this study with a wide range of parameters were subjected to sensitivity analyses. Data and assumptions used in this study serve as the base case. Three parameters were found

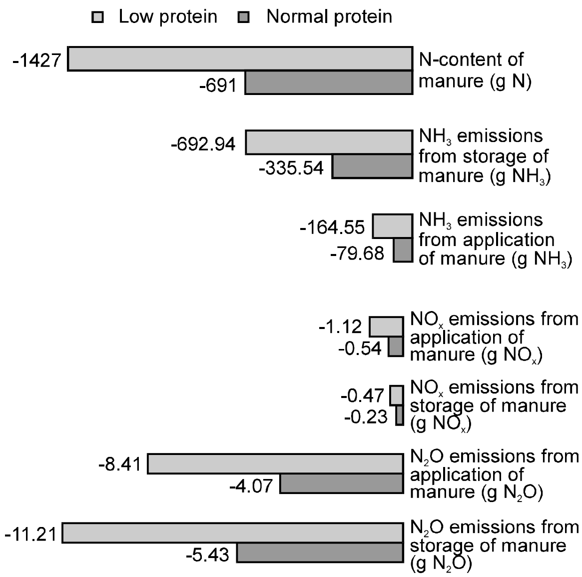

Fig. 1: Changes in N-content emissions per ton broilers induced by adding protease into the normal protein diet or into the low protein diet compared to the normal protein diet without protease

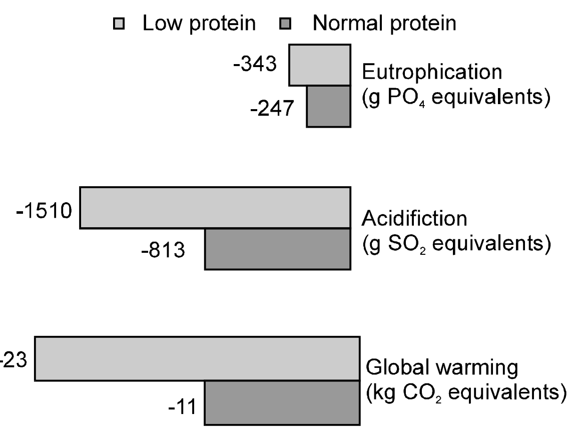

Fig. 2: Changes in eutrophication, acidification and global warming per ton broilers, induced by adding protease into the normal protein diet or into the low protein diet compared to the normal protein diet without protease

to significantly influence the results: Efficiency of crop production (yield relative to input); fraction of nitrogen volatilized from manure before application and IPCC default N_2O emission factors. Based on combined changes of these parameters minimum and maximum reduction scenarios were calculated and compared to the base case calculation (Fig. 3). The results showed that even a combination of worst-case values provided an environmental benefit of using protease. The results

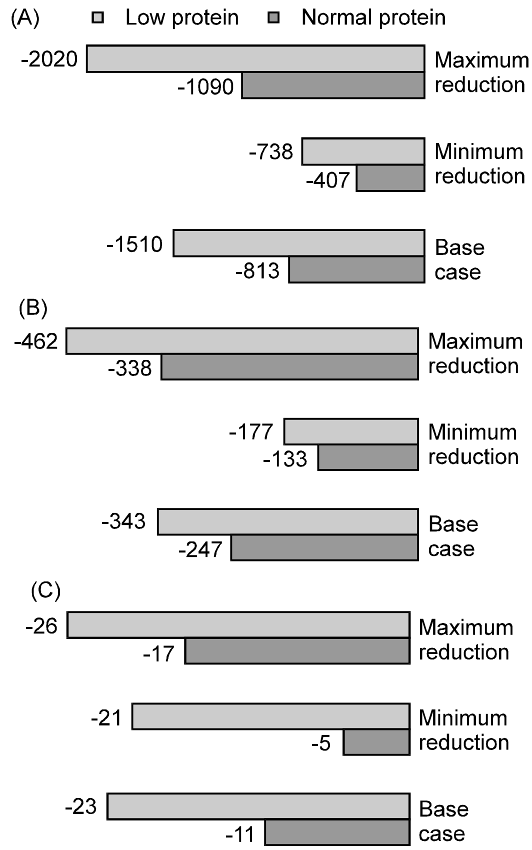

Fig. 3: Calculated minimum, maximum and base case changes in acidification (A), eutrophication (B) and global warming (C) when introducing protease into the normal protein diet or into the low protein diet compared to the normal protein diet without protease

also showed that a combination of best-case values did not dramatically increase the environmental benefits. The calculation of these worst and best case scenarios demonstrates that the results are very robust. The reductions in global warming potential were mainly inflicted by the reduced savings in feed, whereas reduced emissions from manure carried most weight in relation to eutrophication and acidification benefits (data not shown).

DISCUSSION

The change of nitrogen emissions from manure had a significant impact on the overall results, particularly the reductions in eutrophication and acidification. It is difficult to find data from literature on nitrogen emissions from broiler manure which are directly comparable with the results of this study, but a Brazilian study on broiler production reported N_2O emissions of 0.4-0.5 kg and NH_3 emissions of 9-11 kg per ton live weight broilers (da Silva *et al*., 2010). Based on a nitrogen retention of 55%

Table 3: Comparison of different means to reduce emissions of ammonia from poultry production

	Change in NH_3 emissions (ton NH_3)	Change in greenhouse gas emissions (1000 ton CO_2e)
Technology		
Use of protease on top of a normal protein diet (NP w/protease)[a]	-5.300	-173
Use of protease to allow for decreasing the protein content of a diet (LP w/protease)[a]	-11.000	-362
Animal house adoption: Continuous scraping or drying of manure[b]	-75.000	+18.000
100% cover of outdoor storage of manure[b]	-7.500	+400
High use efficiency of manure[b]	-54.000	+200

[a]Current study. The calculation is based on 15.75 million ton broilers produced in EU annually (6.3 billion broilers (FAOSTAT, 2009) of 2.5 kg), NH_3 emission reduction from manure per ton broilers from Fig. 1 and CO_2e emission reduction per ton broiler from Fig. 2.
[b]Leip *et al*. (2010)

in broilers (Bolan *et al*., 2010), the emission calculation methodology of the current study (the IPCC guidelines) provided emissions of 0.2 kg N_2O and 12 kg NH_3 per ton live weight broilers. The reduced emissions of ammonia in broiler houses represent the single most important change in emissions on a nitrogen basis. From a total emission of ammonia of 12 kg per ton broiler the reduction of 691 g ammonia (Fig. 1) obtained if a NP w/protease diet is introduced corresponds to a 6% reduction. When compared to the 25% reduction obtained from substituting built-up litter with new litter (Burns *et al.*, 2011), this improvement may seem minor. However, it should be kept in mind that the current study is very conservative and that the protease technology is likely to be additive to manure handling efforts.

In order to bring into perspective the environmental benefits obtained from the use of protease as a feed additive a number of comparisons have been carried out:

- Comparison with other means to reduce emission of ammonia from production of poultry
- Comparison with ambitions in US to bring down nitrogen emissions from Concentrated Animal Feeding Operations (CAFO)
- Comparison with agricultural contribution to eutrophication and acidification

The first comparison is based on an analysis by Joint Research Center (Leip *et al*., 2010) aiming at evaluating the potential of various means to reduce emissions of ammonia from poultry in Europe. The analysis assumes that all farms implement the technology. The comparison (Table 3) shows that the potential benefits of using protease exceed benefits of manure coverage, whereas the benefits of animal house adoptions and increase in manure use efficiency seem bigger. Nevertheless the benefits of using protease are significant and in contrast to alternative technologies they are not causing an increase in greenhouse gas emissions.

The second comparison builds on the expectations of US Environmental Protection Agency (EPA) upon the introduction in 2008 of new regulations for animal feed lots. EPA estimated that the new regulation comprising all concentrated animal-feeding operations would prevent 50,000 ton of nitrogen from entering the aquatic environment. Based on the current LCA study it has been calculated that the protease driven reduction of nitrogen in broiler manure in US amounts to between 15,000 ton N (NP w/protease) and 30,000 ton N (LP w/protease).[2] This calculation illustrates that the use of protease, even if used only for poultry, could contribute significantly to reaching this ambition.

In the third comparison the reduction of environmental load has been compared with the environmental load of crop production. As an example it has been calculated that the potential reduction of acidification from use of protease for broilers in low protein diets in Brazil corresponds to the acidification from 1.7 million ha of soybeans[3]. With a total soy area of 21.7 million ha this means that the use of protease in Brazilian broiler production is equivalent to an 8% reduction of acidification from soybean production in Brazil. Similar calculations have been carried out for broiler production in the US, where the basis of comparison has been corn production and for broiler production in Europe, where the basis of comparison has been wheat production in France. These calculations confirm the view that the use of protease for broilers represents significant benefits for environmental performance of agricultural production. For acidification the reductions are equivalent to 4-12% reduction of the specific crops.

These comparisons are estimates and only meant to provide an idea about the magnitude of potential impacts of using protease in broiler diets. However, it should be kept in mind that the modeling of this study assumes, that the manure is used as fertilizer and substitutes synthetic fertilizers. This may not be the situation for many industrial broiler production facilities, where the manure is produced in excess amounts and represents a waste problem rather than a convenient source for fertilizers. Under such circumstances the environmental benefits of using a protease are even higher. There will be no fertilizer credit in the calculations and the emissions of damaging nitrogen compounds will be much bigger. If for instance manure is applied in excess amounts to agricultural land, there will also be detrimental emissions of nitrate to the aquatic environment. With this situation in mind the current calculations of the potential environmental benefits of using a protease are judged to be conservative. In other studies (Angel *et al*., 2011; Aureli *et al*., 2010; Fru-Nji *et al*., 2011) protease addition has led to more prominent effects on Feed Conversion Ratio (FCR), which will in turn also lead to larger environmental benefits.

Conclusion: This study has demonstrated that the use of protease as a feed additive offers significant environmental benefits. Most important is the potential to reduce the pollution of water and air with nitrous compounds leading to eutrophication and acidification. Also the potential to reduce the health risks associated with NH_3 emissions in broiler houses are judged to be important. Thus it is illustrated that the use of protease can contribute significantly to current efforts to reduce nitrogen emissions from livestock production. At this point in time proteases are still fairly new on the feed additive market, which means that the use of proteases may not yet be fully optimized. Therefore the results presented in this study only provide a first estimate of the potential environmental benefits of using a protease for production of poultry.

Abbreviations: LCA = Life Cycle Assessment; BWG= Body Weight Gain; FI = Feed Intake; FCR = Feed Conversion Ratio; NP = Normal Protein; LP, Low Protein

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[1]The protease used was RONOZYME® ProAct (DSM Nutritional Products France). One PROT unit is defined as the amount of enzyme that releases 1 µmol of p-nitroaniline from 1 µM of substrate (Suc-Ala-Ala-Pro-Phe-pNA) per minute at pH 9.0 and 37°C.

[2]The calculation is based on

- 21.65 mil ton broilers slaughtered in US annually (8.66 billion broilers (FAOSTAT, 2009) of 2.5 kg).
- Reduction of nitrogen content of manure per ton broiler from Fig. 1.

[3]The calculation is based on

- LCA results from Fig. 2.
- FAOSTAT 2009 data for broiler production and soybean production in Brazil.