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Assessment of the potential of digestibility-improving enzymes to reduce greenhouse gas emissions from broiler production

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ABSTRACT

The objective of this study was to examine the potential of digestibility-improving enzymes to reduce greenhouse gas (GHG) emissions from commercial broiler production. The enzyme product which was examined is a combination of xylanase (X), α -amylase (A), and protease (P) developed by Danisco Animal Nutrition (DuPont Industrial Biosciences). XAP facilitates higher inclusion rates in the diet of cheaper and possibly more environmentally friendly feed ingredients that have a lower nutritional value. XAP can be used for corn–soybean based diets comprising up to 12% by-products. Two scenarios were compared: one included XAP whereas the other scenario did not include XAP. The potential of XAP to reduce GHG emissions was documented through a GHG assessment based on Life Cycle Assessment principles. Consequential modelling was applied including indirect land use changes (ILUC) and direct land use (LU). The findings showed that XAP facilitated savings in GHG emissions from broiler production in the order of 90 g CO₂ eq. per FU. It corresponded to a 5–9% reduction of GHG emissions from broiler production. The sensitivity analysis showed that the results varied substantially, but in all analyses the GHG emissions were reduced. The two most important parameters were: assumptions about the actual changes in the feed formulation and the modelling of ILUC. The two parameters can significantly influence the estimated improvement potential.

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1. Introduction

Food production generates around one third of all human induced greenhouse gas (GHG) emissions (Foley et al., 2011). Emissions resulting from food production and the continuous growth in population and affluence represent a major environmental challenge for the future. The livestock sector contributes significantly to this challenge. Activities associated with the livestock sector contribute around 18% of the total anthropogenic GHG emissions (FAO, 2006). However, the livestock sector also provides livelihoods for many poor people. It is a major contributor to the agricultural economy, employs around 1.3 billion people and animal products constitute a large and important part of the human diet, comprising one third of humanity's protein intake (FAO, 2006).

When considering the environmental implications of the livestock sector in combination with the increasing demand for animal

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products, it becomes evident that new solutions must be found. Various solutions that reduce the environmental impacts of livestock already exist, such as intensifying technologies that make optimal use of both land and resources in the production of both livestock and feed for livestock. One of these technologies is enzymes, which are already applied to the livestock sector to some extent. However, a large unutilised potential still remains (FAO, 2006). This study focuses on enzymes and strives to assess the potential of digestibility-improving enzymes to reduce GHG emissions.

Enzymes are part of the majority of all chemical reactions in living cells. They help reduce reaction time without being part of the reaction. When digestibility-improving enzymes are applied in animal feed, they help break down parts of the diet that the animal's digestive system cannot effectively break down itself. As a result, nutrients previously unavailable to the animal are now released from the diet. Furthermore, digestibility-improving enzymes can help reduce the anti-nutrient effect of certain components in the diet by breaking down the anti-nutritional substances (e.g., arabinoxylans, trypsin inhibitors and phytate) (Barletta, 2011).

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The enzymes can provide an improved growth performance for a specific diet due to the improvement in nutrient availability. However, enzymes are most often commercially applied in ways that makes them allow for inclusion of a higher ratio of cheaper feed ingredients with a lower nutritional value in feeds, while achieving the same growth performance through the assignment of nutrient values (matrix values) to the enzymes themselves in the feed formulation (Barletta, 2011).

Previous studies have documented the environmental advantages of applying enzymes in animal feed. The studies by Nielsen and Wenzel (2006) and Nielsen et al. (2007) examined the environmental benefits of using phytase and xylanase, respectively, in pig diets, and the study by Oxenbøll et al. (2011) examined the environmental benefits of using protease in broiler diets. The present study will examine the possible reductions in GHG emissions resulting from the use of enzymes in commercial broiler diets. The study will focus on a specific enzyme product, Axtra[®] XAP (developed by Danisco Animal Nutrition, a part of DuPont Industrial Biosciences), which consists of a combination of three enzyme activities: $2000 \text{ U}^1/\text{g}$ xylanase (fibre degrading), 200 U^2/g amylase (starch degrading) and 4000 U^3/g protease (protein degrading). The enzyme product, hereafter referred to as XAP, is intended for corn-soybean based diets that are comprised of up to 12% dried distillers grain with solubles (DDGS) or other by-products (such as wheat or rice by-products) (Romero et al., 2013).

2. Material and methods

Broiler feed for commercial production can be composed of various raw materials that are selected on the basis of factors such as geographical location, rearing methods and individual feed ingredient prices. The broiler feed must meet the nutritional requirements of the broiler chicken at the lowest price possible. Typically, the feed formulations are optimised using computer modelling in order to obtain the required nutrient value at the lowest possible price, which is termed "least cost formulation". In the study, two feed formulations were composed; one without XAP and one with XAP, using a computer software tool (Format Singlemix software, version, Format International, Woking, UK) that ensures the lowest feed cost while still providing the necessary nutrition for the broiler. As XAP is presently commercialised in the US and South Africa, the two feed formulations are economically optimised according to US feed prices in 2011.

The potential of XAP to reduce GHG emissions is assessed through a GHG assessment based on Life Cycle Assessment (LCA) principles. Consequential modelling (Weidema, 2003) is applied, and indirect land use changes (ILUC) and direct land use (LU) are included. There is no commonly agreed upon model to calculate ILUC, although various models exist. For this study, the approach used to calculate the effect of ILUC is developed by Schmidt et al. (2012). Version two of the model is applied in the present study, as it is the most current version published. The impact from the ILUC model changes in the different versions, but the modelling principles remain the same. The main principles in the model are that the current use of land reflects the current demand for land, and, moreover, that changes in demand for land will result in



Fig. 1. Illustration of the functional unit. The study assesses the change in the feed formulation when XAP is included in the feed formulation as well as the consequences of this change when rearing the broilers.

changes in land use (Schmidt et al., 2012). The importance of ILUC for this study is evaluated in a sensitivity analysis.

The GHG assessment is modelled using the program SimaPro version 7.2 and predominantly follows the ISO 14040 and 14044 standards. The IPPC 2007 GWP 100a impact method is used (Hischier et al., 2007; IPCC, 2007). Uncertainty in data and critical assumptions are examined by means of a sensitivity analysis.

Changes in methane and nitrous oxide emissions caused by the inclusion of XAP from the broiler manure and the end-use of the manure as an organic fertiliser are calculated according to the Intergovernmental Panel on Climate Change's (IPCC) guidelines (De Klein et al., 2006; Dong et al., 2006). Enteric fermentation from the broiler is omitted, as no standard exists for poultry in the guidelines and, furthermore, because enteric fermentation from poultry is limited (Mikkelsen et al., 2011).

2.1. Goal and scope

The objective of the study is to examine the commercial application of XAP as it might provide a truer picture of the savings XAP could facilitate when applied in the broiler industry. XAP increases the digestibility of key nutrients in the feed (e.g. energy and amino acids); thereby, increasing the broiler's utilisation of the nutrients in the feed. Typically, XAP will be used to reduce feed costs. Therefore, when XAP is included in the feed formulation using an assigned nutrient matrix to account for the digestibility improvements it provides, it is used to include a higher amount of cheaper feed ingredients that have a lower nutritional value without compromising the broiler performance. The study assesses the changes in GHG emissions when XAP is included in a commercial broiler diet compared to a scenario in which XAP is not included in a commercial broiler diet. As the GHG assessment is comparative, identical life cycle stages and processes in the two scenarios can be omitted. Only GHG emissions are assessed in the study; however, changes in other impact categories might be expected when applying XAP to the diet. Such impact categories could include: eutrophication, acidification, nutrient enrichment, photochemical smog formation, fossil energy and agricultural land use. In future studies, the effect of XAP on these impact categories should be examined to ensure that reductions in GHG emissions do not result in increased impacts in other categories.

 $^{^1}$ One xylanase unit of activity liberates 0.5 μ mol of reducing sugar (expressed as xylose equivalents) in 1 min under the conditions of the assay.

² One Thermostable Amylase Unit (TAU) is the quantity of enzyme converting 1.0 mg of starch (100% of dry matter) per minute in standardised conditions.

 $^{^{3}\,}$ One protease unit liberates 1 μmol of tyrosine per minute under the conditions of the assay.

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Fig. 2. System boundary including marginal mechanisms. The dotted and full lines illustrate the changes in demand for different products between Scenario one and Scenario two. The dotted line illustrates a decrease in demand for the product and the full line illustrates an increase in demand for the product in Scenario two compared to Scenario one.

2.1.1. The functional unit

The functional unit (FU) of the study is a certain but unspecified quantity of broiler meat. The study provides an assessment of the changes in GHG emissions when a switch is made from using 1.8 kg broiler feed without XAP to using 1.8 kg broiler feed with XAP. Fig. 1 provides an illustration of the FU and the changes in the feed formulation assessed in the study. The FU is inspired by the study conducted by Nielsen et al. (2007). The amount of broiler meat produced is not quantified, as it will depend on a number of factors, such as rearing methods and broiler breed. However, to make the study more easily comprehensible, the FU equals approximately 1 kg live broiler.

2.1.2. System boundary

The broilers will be reared in the exact same way in the two scenarios with the exception of the following points: the addition of XAP, the changes XAP facilitates in the feed formulation and the resultant changes in manure composition. Thus, downstream and upstream processes of the broiler production can be omitted from the assessment. Further, many processes involved in broiler production can be omitted from the assessment as the same amount of broilers is reared in the two scenarios. The two feed formulations will differ due to the inclusion of XAP, and therefore the feed production is included in the system boundary (Fig. 2). Changes in the feed formulations in the two scenarios will result in changes in the emissions emitted from the broilers and the manure emitted from

the broilers, and, as a result, part of the broiler production is also included in the system boundary. Changes in the manure emitted from the broiler will affect the later use of the manure as a nitrogen fertiliser, and therefore manure use is also included in the system. Several other processes are included in the system boundary. This is due to the consequential modelling approach, which includes marginal mechanisms within the system boundary. This is further explained in Section 3.1.

2.1.3. Data collection and treatment

The data on the improvement potential of XAP in terms of digestibility and performance benefits are summarised in Tables 1 and 2. The feed formulations were provided by Danisco Animal Nutrition and reflect how XAP can be used in the US broiler sector. It should be noted that feed formulations will change according to market prices of individual feed ingredients, e.g. as the price of one grain type increases, the least cost formulations tools may replace it with another cheaper grain. Therefore, the feed formulations may have many different combinations of raw materials depending on feedstock prices. The feed formulations used for the GHG assessment were formulated using commercial feed ingredient prices from the U.S. in September 2011.

The LCA databases used in the study are the Danisco database (Dalgaard and Schmidt, 2011), the Ecoinvent database (Ecoinvent, 2007), and the LCA food DK database (Nielsen et al., 2003). The data used from the Danisco database is on soybean meal and corn. The data on soybean meal is in line with the study by Dalgaard et al. (2008). The data on corn is based on the process "corn, at farm/kg/ US" from Ecoinvent v.2.2 (Ecoinvent, 2007) and altered according to consequential modelling principles. The majority of all the feed ingredients are based on data from the Danisco database (97% of the total weight). The data used from the Danisco database is consequential, whereas the data used from the Ecoinvent database is based on average data using allocation. Data from the LCA food DK database is market-based, including marginal mechanisms and system expansion to deal with multi-output processes. Data from the Danisco database can include ILUC, whereas data from the Ecoinvent database and the LCA food DK database cannot. As the majority of data used derived from the same database, data and methodological consistency were assessed. In the data collection process, the marginal use and the marginal supplier of different feed ingredients were identified.

2.2. Feed formulations

The effects of using XAP were assessed in a number of feed trials made in collaboration with research institutions in different parts of the world (Tables 1 and 2) (Gilbert et al., 2011; Romero and Ravindran, 2011; Romero et al., 2012a,b, 2013). Between 144 and 1800 broilers were included in each feed trial. The results showed a reduction in the amount of feed used to rear the same amount of broiler meat of 3–5.5%, depending on the feed formulation.

The results of the digestibility improvements seen in the feeding trials were modelled to determine the average nutrient improvements when using the product. Commercially this then allows nutrient values to be assigned to the XAP product for use in least cost formulation software. Least cost formulation is used routinely in industry and works by formulating the lowest cost diet that will meet all nutritional requirements of the animal. Consequently, by assigning nutrient values to the enzyme product, the least cost formulation software will reformulate the feed, accounting for these contributions. For example, when an energy value is assigned to an enzyme product, the software will take the most expensive energy source out of the diet as a consequence of the enzyme being there (e.g. oil which is expensive will be reduced or taken out of the

Table 1

Ileal nitrogen (N) digestibility, total tract nitrogen retention, and apparent metabolisable energy corrected for nitrogen (AME_n) as influenced by supplementation of carbohydrases without or with protease in each of six broiler trials with 21 day broilers.

Trial	Enzyme ^a	Apparent ileal N digestibility (g/g N)	Total tract N retention (g/kg feed DM)	AME _n (MJ/kg feed DM)
1 (Romero et al., 2013)	Control XA XAP P value	0.806b 0.817ab 0.829a 0.012	23.3 23.5 23.9 0.21	3026b 3054a 3062a 0.035
2 (Romero et al., 2013)	Control XA XAP P value	0.793 0.810 0.812 0.18	23.5b 24.3a 24.4a 0.045	3031 3064 3062 <i>NS</i>
3 (Romero et al., 2013)	Control XA XAP P value	0.822 0.821 0.833 <i>NS</i>	18.8b 20.0ab 20.4a 0.06	2976b 3102a 3148a <0.001
4 (Romero et al., 2013)	Control XA XAP P value	0.780 0.784 0.800 0.43	21.4b 20.9b 22.3a 0.002	3026c 3109b 3160a <0.001
5 (Gilbert et al., 2011)	Control XAP P value	-	-	2731a 2890b <0.05
6 (Gilbert et al., 2011)	Control XAP P value	-	-	2801 2892 NS

a.b.c Means with no common superscripts within column and sub-grouping are different at P < 0.05. NS = P > 0.05.

^a Negative control diets were supplemented with an enzyme complex containing xylanase and amylase (XA), or one containing xylanase, amylase and protease (XAP).

formulation) resulting in a cheaper feed. The addition of XAP also allows nutritionists to add in larger amounts of cheaper raw materials without adversely effecting animal performance due to the increase in anti-nutritional factors. As feed is the largest contributor to costs of commercial poultry production, enzymes are routinely used in this way to reduce feed costs without compromising animal performance. Scenario 2, illustrated in Table 3, was produced with

Table 2 Performance effects of XAP in four 42 day performance studies.

Trial	Enzyme ^a	Bodyweight gain (g/bird)	Feed conversion (FCR) (g feed/g gain)
1 (Romero and Ravindran, 2011) ^a	Control XAP P value	3159a 3286b <0.01	1.638 1.596 0.06
2 (Gilbert et al., 2011) ^b	Control	2702	1.87a
	XAP	2783	1.79b
	P value	<0.10	<0.05
3 (Gilbert et al., 2011) ^b	Control	2676a	1.88a
	XAP	2815b	1.79b
	P value	<0.05	<0.05
4 (Romero et al., 2012a) ^c	Control (Corn)	3378a	1.60ab
	XAP (Corn)	3352ab	1.57bc
	Control (Mixed)	3286b	1.63a
	XAP (Mixed)	3344ab	1.54c
	P value	<0.05	<0.05

a.b.c means with no common superscripts within column and sup-grouping are different at P < 0.005.

Diets were reduced by 100 kcal ME/kg.

^b Diets were reduced by 85 kcal.

^c 2 diet types used either corn based or mixed diets (corn/DDGS based), NS = P > 0.05.

Total

nutrient values assigned to the XAP (as explained above) to reduce feed cost without compromising broiler growth. This approach was selected because this is how XAP is usually used in the industry. The feed formulations used in Scenario one and Scenario two are presented in Table 3.

The improvement in digestibility and performance obtained by applying XAP is used to reduce the amount of expensive ingredients such as corn, sovbean meal, meat and bone meal and pig and poultry fats in the feed and to increase the amount of cheaper DDGS in the feed. This provided an economic benefit of 4.04 \$ per ton of feed, including all costs of the feed ingredients (also including XAP and phytase). The economic benefit corresponds to a 1.3% reduction in feed price when adding XAP to the feed formulation. It was not possible to apply the full benefits for XAP, as a minimum level of fat is required in the feed formulations to ensure that the pellet quality is maintained (to ensure that the pellet can stick together). Hence, in Scenario two there is, if including the digestibility improvement facilitated by the enzyme, an excess amount of energy compared to Scenario one, corresponding to 1.5%. It would be expected that this excess amount of energy would result in additional performance improvements for the broiler. However, the excess energy is not accounted for in the study, and therefore, the GHG assessment represents a conservative estimate of the potential of XAP.

3. Life cycle inventory

3.1. Modelling of the feed ingredients

Fig. 2 provides an overview of the main feed ingredients and the marginal mechanisms included in the system boundary. All feed ingredients in Table 3 have been included in the study. Life cycle inventory (LCI) data did not exist for L-lysin, DL-Methionine, vita-mins/minerals, and L-threonine, and they are modelled as proxies (Ecoinvent, 2007).

Corn is not considered a constraint product and is modelled as corn produced in the US based on data from Dalgaard and Schmidt (2011). In Scenario two, the amount of corn in the diet decreases, resulting in less demand for corn production compared to Scenario one. Soybean meal is not considered a constraint product either (Dalgaard et al., 2008), and it is therefore modelled as soybean meal produced in Brazil as it is identified as the marginal soybean meal (Dalgaard and Schmidt, 2011). In Scenario two, less soybean meal is needed in the feed formulation compared to Scenario one, reducing the demand for soybean meal.

DDGS is considered a constraint by-product from the bioethanol production and is predominately used in livestock feed (Saunders and Rosentrater, 2009). An increased demand for DDGS will not lead to an increased production of DDGS, as this is determined by the demand for ethanol, instead it will result in an increased production of the marginal feed energy and the marginal feed protein. The marginal feed energy is identified as barley (Schmidt and Dalgaard, 2012) and the marginal feed protein is identified as soybean meal (Dalgaard et al., 2008). Data on soybean meal and barley is based on Dalgaard and Schmidt (2011). In Scenario two, an increased ratio of DDGS is used compared to Scenario one resulting in less DDGS available for the rest of the animal feed production, and this results in an increased demand for barley and soybean meal.

Meat and bone meal is also considered a constraint by-product. The majority of rendered protein products in the US and Canada are used as animal feed (Jekanowski, 2011). In Scenario two, the amount of meat and bone meal is decreased compared to Scenario one. This will result in more meat and bone meal available for the rest of the animal feed production, thereby decreasing the demand for the marginal feed energy (barley) and the marginal feed protein (soybean meal).

Pig and poultry fat is used as the fat source but it could be any other suitable source of fats and greases. Rendered fats and greases are also considered a constraint by-product and have several uses, the main ones being as a feed ingredient, in the oleochemical industry and as a biofuel. The livestock industry is the largest user of rendered fats and greases, but the biodiesel production industry is the fastest growing market for rendered fats and greases (National Renders Organisation, 2009a,b). The livestock industry is used as the marginal use of rendered fats and greases. In Scenario two, less pig and poultry fats are needed compared to Scenario one, making more pig and poultry fat available to the rest of the animal feed

Table 3

Feed formulations provided by DuPont (Danisco Animal Nutrition) according to the functional unit. The column "total change" indicates the difference between Scenario one and Scenario two. A minus indicates a decrease from Scenario one to Scenario two and a plus indicates an increase from Scenario one to Scenario two. The nutrient composition of the two feed formulations is also provided, including the improvement in digestibility facilitated by XAP in Scenario two.

			-	
Raw materials	Scenario	Scenario two	Total change (g)	Incl. in model
	one feed	feed	(Scenario two – Scenario one)	
	formulation	formulation		
	without XAP (g/FU)	with XAP (g/FU)		
Corn	1172	1163	-9.000	Yes
DDGS	180.0	216.0	36.00	Yes
Phytase	0.3600	0.3600	0	Yes
Soybean meal	347.2	334.3	-12.90	Yes
Meat and bone meal	52.91	35.38	-17.53	Yes
L-Lysine (HCL)	5.733	6.293	0.560	Yes
DL-Methionine	4.167	4.192	0.025	Yes
NaCl	5.983	7.011	1.028	Yes
Limestone	15.22	19.93	4.710	Yes
Dicalcium phosphate	1.364	5.139	3.775	Yes
Vitamins/minerals	1.800	1.800	0	Yes
XAP	0.000	0.900	0.900	Yes
Pig and poultry fats	11.94	4.500	-7.440	Yes
L-threonine	1.517	1.316	-0.201	Yes
Total	1800	1800	0	
Nutrient composition				
Metabolisable	3075	3123	+48	
energy (kcal/kg)				
Total protein %	18.5	18.5	0	

Table 4

Methane emissions from the broiler sheds, the manure storage and the change in methane emissions from Scenario one to Scenario two per functional unit, as a consequence of the inclusion of XAP in the feed formulation.

	Scenario one	Scenario two	Change (Scenario two –
	(g CH ₄ /FU)	(g CH ₄ /FU)	Scenario one) (g CH ₄)
Methane emissions	0.579	0.571	-0.008

production and decreasing the demand for the marginal feed energy (barley).

In Scenario two, there is an increased demand for both supplements and enzymes compared to Scenario one. The supplements are modelled using ecoinvent processes (Ecoinvent, 2007). Phytase is modelled using a fixed GHG emissions rate of 5.0 kg CO₂ per kg enzyme produced (Dalgaard and Schmidt, 2011). XAP is modelled based on production formulas and represents 90–100% of the actual ingredients and materials. Data for energy and material use from the production of XAP derives from the enzyme factories' production records. Data on the ingredient production is from Ecoinvent (2007), LCA food database (Nielsen et al., 2003), suppliers and literature.

3.2. Modelling of changes in methane and nitrogen oxide emissions from manure and its use as organic fertilisers

The increased digestibility of the feed ingredients which XAP facilitates results in less methane emissions from the manure in Scenario two compared to Scenario one from both the broiler sheds and the manure storage.

Changes in the methane (CH₄) emissions from the manure management are calculated based on the IPCC Guidelines (Dong et al., 2006) and applying the following assumptions: gross energy intake is calculated based on the two feed formulations (Table 3) and is converted from metabolisable energy into gross energy using a 1.38 ratio between the apparent metabolisable energy and the gross energy (Board on Agriculture, 1994), urinary energy is 7.5% of the gross energy intake (Board on Agriculture, 1994) and ash content in the poultry litter is 167 g per dry matter content. The results are presented in Table 4.

XAP facilitates less nitrogen in the manure, due to changes in the feed formulation and the broiler's increased nitrogen retention.

This leads to decreased nitrous oxide (N₂O) emissions from the broiler sheds, the manure storage and from the fields where the manure is eventually used as fertiliser. Changes in the N₂O emissions from the manure management and the later use of the manure as a fertiliser are calculated based on a nitrogen balance (see Table 5) and the IPCC guideline (De Klein et al., 2006). The nitrogen input derives from the feed input (Table 3) and from the bedding material. The feed formulation in Scenario two contains less nitrogen than in Scenario one, due to the changes in the feed formulation. Part of the nitrogen from the feed intake is retained in the broiler. It was assessed, based on the feed trials, that the broiler retains 58.2% of the nitrogen in the feed formulation without XAP and 65.2% of the nitrogen in the feed formulation with XAP (Romero et al., 2013). This implies that less nitrogen is present in the manure management system in Scenario two compared to Scenario one.

In the manure system, part of the nitrogen evaporates as direct N₂O emissions or as indirect NH₃-N and NO_x-N emissions, part of which also ends as N₂O emissions. As the manure contains less nitrogen in Scenario two compared to Scenario one, the N2O emissions from the manure management system are equivalently reduced. After removal from the manure management system, the manure is used as fertiliser. When the manure is applied to the soils, part of the nitrogen evaporates as direct N₂O emissions, indirect NH₃-N and NO_x-N emissions, part of which also ends as N₂O emissions, and part of the nitrogen is lost through leaking. Again N₂O emissions from the managed soils are reduced in Scenario two compared to Scenario one due to reduced nitrogen content in the manure. However, when the manure contains less nitrogen it then substitutes less marginal nitrogen fertiliser, and the demand for the marginal nitrogen fertiliser increases. Ammonium nitrate is assumed to be the marginal nitrogen fertiliser (Sonesson et al., 2009). Inorganic nitrogen fertiliser is modelled as ammonium nitrate, and it is assumed that poultry manure offsets the use of inorganic nitrogen by 70% (Ministry of Food, Agriculture and Fisheries of Denmark, 2012).

4. Results

As it is a comparative GHG assessment, the results are presented as the difference between Scenario one and Scenario two. The

Table 5

Nitrogen balance for Scenario one and Scenario two per functional unit and the changes in the nitrogen balance in Scenario one minus Scenario two.

	Scenario one (g N/FU)	Scenario two (g N/FU)	Change (g N/FU)	Comments
N in feed	55.1	54.2	0.9	Amount of crude protein in the diet (Table 3)
N in bedding material	0.425	0.425	0	(Poulsen et al., 2001)
Total N input	55.5	54.6	0.9	SUM
Retained in broiler	32.1	35.3	-3.2	Nitrogen retention 58.2% without XAP and 65.2% with XAP (Romero et al., 2013)
Manure management system				
N in manure	23.5	19.3	4.2	Nitrogen input minus the amount of nitrogen retained in the broiler.
N ₂ O-N losses due to direct emissions	0.0230	0.0189	0.0041	0.1% of nitrogen evaporates as N_2O (Dong et al., 2006)
NH ₃ -N losses due to indirect	9.22	7.54	1.68	40% of nitrogen evaporates as NH_3-N and NO_x-N and 1% of NH_3-N and NO_x-N
emissions volatilisation				that volatilises ends as N ₂ O (Dong et al., 2006)
N ₂ O-N losses due to indirect	0	0	0	No leaching
emissions leaching				
Managed soils				
N applied to the soil	14.2	11.7	2.50	Total nitrogen input minus nitrogen lost in the manure management system
N losses due to direct emissions	0.142	0.117	0.025	1% of nitrogen evaporates as N_2O (De Klein et al., 2006).
N losses due to indirect	2.85	2.34	0.51	20% of the nitrogen volatilises as NH ₃ -N and NO _x -N and 1% of NH ₃ -N and NO _x -N that what illuse as $M = 0.000$
emissions volatilisation	4.27	2.52	0.75	that volatilises ends as N_2O (De Kieln et al., 2006).
N losses due to indirect	4.27	3.52	0.75	30% of the hitrogen applied is lost through leaching and 0.75% of the hitrogen lost
Untake by plants	6.97	5 74	1 2 2	The difference between what is lost and what is applied to the soil. This assumption is a
	0.37	5.74	1.25	simplification.

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Fig. 3. Changes in GHG emissions caused by the use of XAP in Scenario two compared to Scenario one per FU. A negative value indicates a decrease in GHG emissions and a positive value indicates an increase in GHG emissions in Scenario two compared to Scenario one. The black column shows the total savings caused by the use of XAP.

results are presented in Fig. 3, and the total reduction in GHG emissions per FU when applying XAP is in the order of 90 g CO_2 eq. per FU (the black column).

The main driver for reductions in GHG emissions in Scenario two compared to Scenario one is changes in the feed formulation. The use of XAP in the feed formulation results in decreased GHG emissions from pig and poultry fat, meat and bone meal, soybean meal and corn, and increased GHG emissions from DDGS, the supplements and XAP. GHG emissions from the supplements increase by approximately 6 g CO₂ eq. per FU in Scenario two compared to Scenario one. The use of XAP also results in an increase of about 1 g CO₂ eq. per FU in Scenario two compared to Scenario one. Hence, the impact from producing XAP comprises less than 1% of the total improvement potential of XAP. GHG emissions from manure management and storage are reduced by approximately 10 g CO₂ eq. per FU in Scenario two compared to Scenario one. GHG emissions from the use of the manure as a crop fertiliser are also reduced, as a consequence of the lower nitrogen content in the manure. However, when less organic nitrogen is emitted by the broiler to be used as fertiliser then less inorganic nitrogen fertiliser is displaced, resulting in a total increase of around 9 g CO₂ eq. per FU in Scenario two compared to Scenario one.

4.1. Sensitivity analysis

Different choices were made during the project, both in terms of methodology use and data collection. These choices are examined in the sensitivity analysis (Table 6).

As mentioned, it was not possible to apply the full benefits of XAP in Scenario two due to the need for a minimum fat content to maintain pellet quality (to ensure that the pellet can stick together). Therefore, two additional feed formulations are made (without XAP and with XAP) where no restrictions are made on the fat content, allowing for the full benefits of XAP to be applied. The purpose of this analysis is to examine the implication of the assumption that a

minimum fat content is required to maintain the pellet quality. Furthermore, it provides an indication of XAP potential to reduce GHG emissions, if the challenges regarding the pellet quality could be resolved. The alternative feed formulations resulted in a reduction of 345 g CO_2 per FU when applying XAP corresponding to almost three times as large savings in GHG emissions compared to the main results. This indicates that the feed formulations in the study might be conservative or that a large unutilised potential for further improvement with XAP exists.

The second most significant parameter in the sensitivity analysis is the modelling of ILUC. An extreme case was evaluated in the sensitivity analysis, which showed that if the burdens for ILUC were negligible, the use of XAP in the feed formulation resulted in savings of only 6 g CO₂ eq. This shows that the contribution from ILUC is significant, according to the applied model (1.23 kg CO₂ eq. emitted per kg Carbon of net primary productivity (NPP_o)). Key aspects of uncertainty associated with the ILUC model are discussed in Schmidt et al. (2012). Factors specific to this study, which contribute to the amount of ILUC, are also associated with uncertainty, such as the selection of marginal products and the

Table 6

Overview of the results of the sensitivity analysis. The savings in GHG emissions when XAP is included compared to when XAP is not included is presented in the first column along with the relative change in relation to the default scenarios (Scenario one minus Scenario two).

	Changes in GHG emissions g CO ₂ eq. per FU	Change relative to the default scenarios in %
Default scenarios	-90	
No minimum fat content	345	281
	-6	_93
Marginal use of rendered fats	-43	-52
Replacement of organic fertiliser	-80	-12

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quantification of NPP_0 for these products. While uncertain, the impacts from ILUC have shown to be significant.

Some indicate that the marginal use of rendered fats is as animal feed, however, others indicate that it is as biodiesel. Hence, the significance of changing the marginal use of rendered fat to energy is assessed in the sensitivity analysis. In line with Nielsen et al. (2007), it is assessed that animal fats displace an equivalent quantity of fuel oil, that the energy value of fat and fuel oil is alike and that the emissions are similar. Thereby, the only difference included in the GHG assessment is that the burning of fossil fuel oil contributes to global warming, which animal fat does not, as it is considered CO₂ neutral. The calculation shows that when changing the marginal use of rendered fats reductions of 43 g CO₂ eq. per FU would be reached when XAP was used compared to when it was not. The reductions correspond to a decrease in the improvement potential of XAP by 52% compared to the default scenarios (Scenario one minus Scenario two).

The least important assumption evaluated in the sensitivity analysis proved to be the ratio to which inorganic nitrogen replaces organic fertiliser. The use of inorganic nitrogen fertiliser is considered to be more effective than using manure as a nitrogen fertiliser, thus less inorganic nitrogen fertiliser is needed to replace the nitrogen in the organic fertiliser (Ministry of Food, Agriculture and Fisheries of Denmark, 2012). Hence, to examine the assumption that 0.70 kg nitrogen in inorganic fertiliser replaces 1 kg nitrogen in manure, the sensitivity analysis assessed the assumption that 1 kg inorganic nitrogen fertiliser replaces 1 kg nitrogen from the manure. The calculations showed that this would result in reductions of 70 g CO_2 eq. per FU when using XAP in the feed formulation, corresponding to a 12% reduction of the improvement potential of XAP compared to the default scenarios.

Based on the sensitivity analysis, it must be concluded that there are considerable uncertainties connected with both the methodological choices made and the data collection. However, all sensitivity analyses resulted in a reduction in GHG emissions when XAP was included in the feed formulation compared to when it was not.

5. Discussion

To evaluate the savings which XAP facilitates it is compared to the total GHG emissions from the production of broiler meat. In total, four studies are examined (Cederberg et al., 2009; Nielsen et al., 2011, 2003; Williams et al., 2009). The results from these studies differ considerably from approximately 1.9 kg CO₂ per kg bone free meat produced in Cederberg et al. (2009) to approximately 3.4 kg CO₂ per kg bone free meat produced in Williams et al. (2009). It is estimated that carcass weight makes up 70% of live weight and bone free meat makes up 77% of the carcass weight. The calculations show that the savings in GHG emissions facilitated by XAP in broiler production are around 5–9%.

To assess the validity of the results, a comparison with existing studies on digestibility-improving enzymes in animal feed is made. The study by Nielsen et al. (2007), on the use of xylanase in Danish pig production, documented savings of around 5% in GHG emissions depending on the feed prices. The assessment of protease used in poultry feed (Oxenbøll et al., 2011) showed reduction of 11 kg and 23 kg CO₂ eq. per ton broiler corresponding to 0.6-1.1% and 1.2-2.3% total reduction in GHG emission from broiler production, respectively, applying the same assumptions as previously. Hence, the results of this study are in accordance with existing studies. However, these results should not be used to compare the performance of the enzyme products, as the studies are too different to justify such a comparison.

The current study does not assess whether or not reductions in GHG emissions facilitated by XAP will lead to trade-offs with other

impact categories. Other studies, however, have examined additional impact categories. The studies by Oxenbøll et al. (2011) and Nielsen et al. (2007) have included additional impact categories such as: eutrophication, acidification, nutrient enrichment, photochemical smog formation, fossil energy and agricultural land use. The two studies showed some correlation between reductions in GHG emissions and reductions in other impact categories with some exemptions. However, the studies are very diverse and it only indicates that there may be some correlation.

6. Conclusions

The study showed that XAP has the potential to reduce GHG emissions from broiler production in the order of 90 g CO₂ eq. per FU. Comparing the potential savings in GHG emissions documented in the present GHG assessment to the total GHG emissions from the production of 1 kg bone free broiler meat showed that XAP has the potential to reduce the impact from broiler rearing by approximately 5–9%. The results of the sensitivity analysis showed that both methodological choices and choices made in the data collection significantly affected the GHG saving potential of XAP. However, in all the analyses conducted, the use of XAP in the feed formulation resulted in reductions in GHG emissions ranging from 6 to 345 g CO₂ eq. per FU. The two most important parameters were assumptions about the actual changes in the feed formulation and the exclusion of ILUC. Finally, it can be concluded that the use of XAP has a potential to reduce GHG emissions from broiler production, despite the uncertainties detected in the sensitivity analysis.

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