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Spatially differentiated examination of biodiversity in LCA (Life Cycle Assessment) on national scale exemplified by biofuels

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Abstract

Purpose The critically discussed effects on biodiversity due to the intensive cultivation of energy crops are largely lacking in the LCAs of biofuels due to basic methodological deficits. This was the starting point to develop a new methodology for impact assessment on biodiversity. The central research question to be clarified was: how can the spatial references, which are necessary for the assessment of biodiversity, be included in the usually spatially unspecific LCA.

Methods Prevalent methods for both LCA and environmental planning were investigated with regard to interfaces and integration capabilities as well as differences. Drawing on this analysis a theoretical and methodological approach was developed which integrates the spatially specific procedure of environmental planning into the product-specific approach of the LCA. The available geodata and statistical data base for the assessment was examined for Germany and the method adapted to the data situation in Germany.

Results and discussion The resulting methodological approach is explained and tested. It is compatible with LCA principles and leads to product-specific quantitative results. The method can be applied on a higher geographic level (Germany) but, nevertheless, makes reference to spatial characteristics of biodiversity on different scales. This is achieved by combining area specific data about biodiversity with statistical data about pressure factors on the regional level. The procedure is demonstrated by the example of bio-ethanol from wheat. An overall view of the various indicators yields a "Biodiversity profile" for the considered fuel and a specific bio-fuel target. This approach leads the way for an integration of spatial knowledge about biodiversity into the spatially non-specific LCA.

Keywords: Biodiversity, Land Use, Geographic information system (GIS), Environmental planning, Biofuels, Methodology, Spatial indicators, Agricultural products

Zusammenfassung

Räumlich differenzierte Untersuchung von Biodiversität in Ökobilanzen auf nationaler Ebene am Beispiel biogener Kraftstoffe

Ziel Die kritisch diskutierten Wirkungen auf die biologische Vielfalt durch den intensiven Anbau von Energiepflanzen fehlen in Ökobilanzen von Biokraftstoffen weitgehend aufgrund methodischer Defizite. Diese waren Anlass zur Erarbeitung eines neuen methodischen Ansatzes zur Wirkungsabschätzung für Biodiversität. Als zentrale Forschungsfrage galt es dabei zu klären, wie die für die Beurteilung von Biodiversitätsveränderungen zwingend erforderlichen räumlichen Bezüge in die meist räumlich unspezifischen Ökobilanzen einbezogen werden können.

Methoden Die Methodenspektren der Ökobilanz und der Umweltplanung wurden systematisch in Hinblick auf Schnittstellen untersucht. Auf dieser Analyse aufbauend wurde ein Methodenansatz entwickelt, der das raumbezogene Vorgehen der Umweltplanung mit dem produktbezogenen Vorgehen der Ökobilanz verbindet. Die verfügbaren Datengrundlagen wurden für Deutschland geprüft.

Ergebnisse und Diskussion Es wurde ein mit der Ökobilanz kompatibler Methodenansatz entwickelt und getestet, der zu produktspezifischen quantitativen Ergebnissen führt. Die Methode kann auf einer höheren räumlichen Ebene angewendet werden, ohne flächenkonkret vorzugehen und stellt dennoch den für die Biodiversität notwendigen Bezug zu räumlichen Ausprägungen her. Am Beispiel Bioethanol aus Weizen wird das Vorgehen erläutert. In der Gesamtschau aller Indikatoren ergibt sich ein typisches „Biodiversitätsprofil" für den betrachteten Kraftstoff. Das methodisch neue Vorgehen zeigt damit im Ansatz einen Weg auf, wie eine Transformation von räumlichem Wissen über Biodiversität in die räumlich unspezifische Ökobilanz gelingen kann.

Schlüsselworte: Biodiversität, Landnutzung, Geografisches Informationssystem (GIS), Umweltplanung, Biokraftstoffe, Methodologie, räumliche Indikatoren, Agrarprodukte

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1 Introduction

The potential environmental impacts of products are usually assessed with the help of an LCA according to the ISO standards 14040/44 (ISO, 2006a; b). An LCA aims to represent the "ecological backpack" of a product along its entire life cycle in consistent units so that individual product paths can be compared (Kanning, 2001). Many studies exist which investigate the possible effects of biofuels and other biomass products on the environment (e.g. Zah et al., 2007; Bernesson, 2004; Kaltschmitt and Reinhardt, 1997; Malça and Freire, 2011). For example the emissions from the use of biofuels in internal combustion engines are considered with regard to different parameters (Krahl et al., 2009; Munack et al., 2009). However, the critically discussed effects on biological diversity due to the intensive cultivation of energy crops are largely lacking in the studies (Urban et al., 2007). The UNEP study "Assessing biofuels" (Bringezu et al., 2009) gives an overview of the research and emphasizes that biodiversity is generally lacking and that LCAs need to be extended by spatial parameters. Forecasts and assessments for the loss of biodiversity are dependent on scale and region (Sala et al., 2000; 2009; Dauber et al., 2010).

Because so far no accepted method is available for accounting the impacts on biodiversity in LCA (Milà i Canals et al., 2007; Klöpffer and Grahl, 2009), political decisions, which are based on LCA, are made on an incomplete information basis. Previous approaches either operate, as is customary in LCA, without considering or processing area-specific information or are restricted to local, place-based applications where site specific data are available (Schweinle, 2000; Haas et al., 2000; Geier, 1999). Spatially non-specific methods use general pressure-related characterization factors for land use e.g., the hemeroby - an indicator for the degree of anthropogenic influence on the ecosystem (Brentrup et al., 2002). Others are based on typical species numbers and -compositions of land uses (Koellner and Scholz, 2008; Koellner, 2003; De Schryver et al., 2010). In particular, there is a methodological deficit concerning the inclusion of impacts on biodiversity in the life cycle stage 'agricultural cultivation.'

An area-specific approach would be needed for assessing biodiversity on high decision levels, e.g., for calculating the effects that the introduction of different biofuels (or biofuel quotas) would have on biodiversity in Germany. The impact of energy crop cultivation differs depending on the sensitivity of nature and landscape. This is disregarded when a pure accounting of the cropland is performed. However, a spatially explicit approach is not feasible at the national level, because the specific future cultivation areas of energy crops are unknown.

Against the background of this dilemma and the basic deficits with regard to content and methodologies, the objective of the research presented in this paper is to develop an approach for the integration of biodiversity into LCAs on a national scale, which is sensitive to the requirement of spatial differentiation as well as suitable for LCA accounting. The research is a combination of a methodical development (how to integrate biodiversity in LCA) and a case study (biofuels in Germany with its impacts on biodiversity) based on data that are available on the national level in Germany. The principal research question to be solved is, how spatial references, which are necessary for the assessment of biodiversity, can be included in spatially superordinated LCAs. A possible approach is to use methodological knowledge of spatial environmental planning (e.g. von Haaren et al., 2004; Fürst and Scholles, 2008; Selman, 2000; Marsh, 2010) which was created to assess concrete spatial situations and adapt methods suitable for the structure and accounting procedures of LCA. In this paper we propose a methodological approach for accounting the impact of different biofuel targets on biodiversity (Section 2), as well as its operationalization with indicators based on nationally available data in Germany, and then test this approach (Section 3). The research concentrates on impacts on a national scale (Germany), the impact category land use (and here only impacts on biodiversity), and the life cycle stage agricultural cultivation.

2 Methodology

2.1 Basic concept

In order to design an approach that can be applied without knowledge of concrete sites, and nevertheless makes reference to spatial characteristics, spatially differentiated data and summarized impacts on different spatial scales are used. The starting point for a spatially differentiated impact assessment is a given quantity target (biofuel quota) and the spatial distribution in scenarios using a geographic information system (GIS) (Urban et. al., 2008, see also Geyer et al., 2010). In particular, the procedure is characterized by the following features:

1. In the goal and scope definition, a quantity target for the selected product needs to be defined.
2. Representative model cultures are the basis for the operationalization of the polluter side in the inventory analysis with agricultural statistical data at the national level (pressure indicators).
3. The affected side (the ecosystem) in the impact assessment is operationalized by state and impact indicators

for three ranges of impacts: impacts on the cropland itself, impacts on the (standardised) nearest surroundings of the plots and impacts on landscape scale (including functional impacts on areas beyond).
4. The indicators are shown spatially differentiated by using a GIS.
5. By scenario modelling, changes in biodiversity, compared to the status quo, are assessed in two variations (worst case, spatially optimized), which can serve as a basis for LCA-based decisions.

The key precondition for the integration of biodiversity information into the LCA (in our approach) is the operationalization of the indicators that must always lead to calculable values on the county level. By the existing relation of the indicator results to the required additional acreage for biofuel production, which is the cause for the change, a conversion is possible to a per hectare value. This allows the calculation of indicator values per functional unit, which are necessary for a characterization method. Many LCAs use a hectare of agricultural area as the functional unit (so do we), but a conversion to other functional units, such as passenger kilometers, is also possible (Zah et al., 2007).

2.2 Selection of a product (biofuel) and analysis of the current land use

The first step consisted in the selection of the biofuel ethanol from wheat, because the example application should be made with a common culture (with good data availability). Since the cultivation of wheat for bioethanol differs only slightly from the one of conventional wheat (Mentz, 2006), agricultural data that characterize existing wheat cultivation can be used. As the quantity target, a share of 5.75 % bioethanol in petrol sales in Germany was defined (former EU goal for the year 2010, defined in the Directive 2003/30/EC). This goal equals about 1.8 million tons of bioethanol, which require about 1 million hectares of land for growing (approximately 6 % of the agricultural area of Germany) (Schmitz, 2006). For the determination of space requirements in future calculations it would be possible to take different yield values for wheat in the various counties instead of an average value.

As a second step, model cultures were designed in order to characterize the pressure coming from wheat and other existing agricultural land uses. For the inventory analysis the data from all main agricultural uses is required (not only those of wheat for bioethanol), to show the impact of displacements between the cultures through the cultivation of biomass. On the basis of statistical data from the main field crops and grassland on a county level (simplified term for 437 NUTS-3 spatial units in Germany; NUTS is the abbreviation for Nomenclature of Territorial Units for Statistics; NUTS-3, in particular, designates small regions) the relevant data for model cultures were compiled (share of area, area sizes, yields, fertilizer and pesticide use). As county-specific values these data represent properties of the different typical cultures (wheat, barley, etc.) for the respective district (therefore no differentiation in varieties is useful). The model cultures in the districts are linked with spatial data in the GIS. Thus various spatial differentiations in the GIS can be performed, depending on the needs of the indicator system and scenarios. Aggregation of more detailed areas specific data is always done on the NUTS-3 level as the least differentiated spatial unit used.

2.3 Outlining of land use scenarios

To estimate the impacts of land use change through the cultivation of energy crops in the determined area size, two different spatial scenarios have been designed. The difference between the scenarios demonstrates the possible range of habitat impairment and associated flora and fauna changes. Baseline is the current wheat distribution in Germany. The allocation of the additional wheat areas is done in two steps:

1. In an initial distribution, equal for both scenarios, the additional areas needed for wheat production are allocated to the districts according to agronomic conditions (indicated by the present share of wheat). So each county gets allocated a certain quantity of wheat. As a crop ecology limit a maximal concentration of 66 % wheat per county is implemented, because this is the maximum concentration for wheat cultivation in the crop rotations (KTBL, 2009).

2. In the "spatially optimized" scenario the assumption for the distribution of additional wheat production areas *within* the counties is that no ploughing up of grassland areas and use of fallows takes place. More areas, which have been identified as important for biodiversity (3.1.1), are also excluded from a possible use for energy crop cultivation in this scenario. Altogether this means that the areas needed for wheat production are distributed in a way which is spatially optimized for biodiversity protection. In contrast, the "worst case" scenario claims preferentially these areas with a high risk of damaging biodiversity. For this purpose, shifts in crop cultivation are assumed in the counties corresponding with the agronomic potential. For example, wheat could be grown in recent winter barley areas, winter barley could move to previous grassland (assuming ploughing up in the worst case scenario), etc. Crops with low requirements on soil fertility are forced out by wheat and would shift to areas more valuable for biodiversity. Nevertheless the wheat would be the cause of this shift of cultivation.

2.4 Impact assessment of biomass cultivation on different spatial scales by indicators

The impacts of energy crop cultivation on biodiversity within the two scenario options are described and evaluated on the basis of proxies. In principle, different reference systems are possible (previous, no or alternative uses) (Jungk and Reinhardt, 2000). We chose the status quo of agricultural land use as suitable reference system. This state does not represent an undisturbed natural biodiversity but is characterized by different biotopes and species according to the site as well as land use intensity. The present land use effects are not attributed to biofuels. Only the changes caused by the cultivation of energy crops for biofuels are ascribed to them (similarly Kaltschmitt and Reinhardt, 1997; Jeanneret et al., 2009). The definition of agricultural land follows Corine Land Cover 2000 (CLC). This broad definition comprises arable land, permanent crops, pastures and heterogeneous agricultural areas including natural areas (Bossard et al., 2000). The biodiversity of this agricultural land is very different, depending on habitat type and connectivity as well as the intensity of use (Dauber et al., 2010; Stachow et al., 2003). The changes for biodiversity through altered land use due to energy crop production can be substantial, particularly through use of previously only extensively used agricultural land, if grassland is converted into cropland, if landscape structures like hedgerows are destroyed or impaired or if the input of fertilizer, pesticides and the intensity of tillage operations increases.

With the support of a GIS, regionalized ratings are carried out for all of Germany. Because of the size of areas there is a need for an extensive formalization of the valuations. Impacts on biodiversity are considered on three scale levels: at the landscape level, in the direct vicinity of the cropland, and on the cropland itself. Reference areas for the evaluation are the NUTS-3 areas (in Germany: counties). The LCA should account the impacts on all spatial scales in a summarized form for each NUTS unit.

In our methodology the *landscape level* represents all supra-local effects that are beyond the scope of field / habitat and its immediate surroundings. At the landscape scale effects become visible which are possibly quantitatively negligible on the field or farm level. In this context, a principal advantage of the approach using national spatial data becomes clear, because changes and impairments due to a reduction of landscape diversity (e.g. national decline of grassland or nationally rare biotope types as well as narrowing of crop rotations) are recognizable. An approach that focuses only on the accounting for individual areas couldn´t reflect these important changes. The use of areas which are valuable for biodiversity is mentioned in the literature as the most serious threat from biomass cultivation (SRU, 2007; Dauber et al., 2010; EEA, 2007). This is because of the fact that, in this case, not only already intensive agriculture is modified, but the effect of biomass cultivation extends to land that has been extensively used or set aside so far and shows a high habitat quality. An indicator reflects this claiming of land (3.1.1). Furthermore, changes in crops that – individually – don't have significant effects can lead to a homogenization of cropping patterns at the landscape level with negative impacts on biodiversity. On this impact level an indicator has been found and operationalized which shows the changes in proportion and distribution of the different land use types (3.1.2).

In the immediate *surroundings of the cropland* in particular small structures and edge habitats could be affected by energy crop cultivation. Small structures and habitats such as small copses, hedges or hems are especially important under biodiversity considerations. By the changing land uses in the scenarios, the changes in the proportions of small biotopes at different biofuel targets can be estimated and compared with assessment criteria from the literature.

The impacts on the *cropland* itself were first indexed by a site-independent assessment of the crops in terms of their respective typical effects on biodiversity. This was based on literature reviews. In addition, a rating of the reduction in habitat quality of agricultural land was derived on the basis of indicators for management intensity (fertilization, crop protection) (3.1.3). Supplemental to the assessment of crops and management intensity the method provides a way to aggregate these indicators in combination with site conditions in order to integrate the different sensitivities of agricultural habitats. "Situation types" can be formed which are evaluated for their combination of crop type, management intensity and bio-geographical situation. The habitat development potential can be used to obtain a combination with local properties that can lead to a differentiated statement for habitat suitability.

2.5 Data availability

The optimal approach would start with the assumption that sufficient data would be available about the conditions of biodiversity at different scales. Unfortunately, such comprehensive data is not available for the Federal Republic of Germany. Somewhat problematic is the fragmentation of the German environmental monitoring, since it has lead to a confusing and incomplete data situation (SRU, 2004). So far, Germany has no nationwide monitoring program for biodiversity in cropland and grassland (Oppermann et al., 2009). Since there are neither homogeneous and detailed data at the federal level about biotope quality and size, nor on the distribution of populations of wild species in the "normal" cultural landscape (in opposite to

protected areas, where detailed data are available), one has to resort to a mosaic of different sources of information in order to move closer to the required information. Spatially differentiated data sets are available for some agricultural data at the county level due to agricultural statistics (Statistische Ämter des Bundes und der Länder, 2008; Institut für Betriebswirtschaft, 1996). It is therefore necessary to use, as a reference point, these administrative units as an auxiliary means for the description of the use of natural influences on the natural-areas level and farm level. The spatial location of the administrative boundaries of the districts is done with the corresponding data of the Federal Agency for Cartography and Geodesy (BKG, 2007). For the regionalized analysis of the use of plant protection, data exist at the network for the determination of pesticide use in different, agriculturally relevant natural areas of Germany (NEPTUN) (Rossberg et al., 2002).

The data from Corine Land Cover 2000 (CLC) (UBA, 2004) have been used as a substitute for homogeneous geo-referenced data on biodiversity and agricultural areas at the landscape level and biotope level (including field). These data are the single homogeneous, area-covering and geo-referenced data, with which at least positioning of the agricultural land is possible nation-wide. A uniform data base for Germany that simultaneously locates and spatially differentiates the agricultural land and its crop-specific data, does not currently exist. Therefore, the data of agricultural statistics at the county level had to be brought together with data from Corine Land Cover to enable a GIS platform for a nationwide consolidation of data. Furthermore, the Natura 2000 network provides information on the rare or vulnerable areas for all of Europe in usable data quality (BfN, 2009). The data basis for regionalized small biotopes of the landscape is a directory of the Julius Kühn-Institut (JKI, 2004). It lists, for the Federal Republic, the proportion of small biotopes in agricultural areas.

3 Results

3.1 Operationalization of indicators and scenario based impact assessment

The necessary data were collected for Germany, the scenarios were designed and the indicators were realized and implemented in different degrees (Urban et al., 2011). For three of the indicators proposed in Section 2.4, a concrete operationalization was developed. The most influential of these (cf. 2.4), the indicator "Claiming of land that is particularly valuable with respect to biodiversity" was tested in a GIS application for all of Germany (3.1.1). The indicators "Cultivation proportion of agricultural crops" (3.1.2) and "Nitrogen fertilizer" (3.1.3) were tested exemplarily for one county to demonstrate the procedure.

3.1.1 Indicator: "Claiming of land that is particularly valuable with respect to biodiversity"

Following the "high nature value farmland indicator" (HNV indicator) (EEA, 2004) the following areas can be defined as valuable agricultural land for biodiversity:

- CLC Category 242: Complex cultivation patterns
- CLC Category 243: Land principally occupied by agriculture, with significant areas of natural vegetation
- Natura 2000 sites, which lie within the agricultural area.

As a result for Germany 4,976,219 hectares of valuable agricultural land have been identified by means of the GIS, which correspond to 23.2 % of total agricultural land (Figure 1).

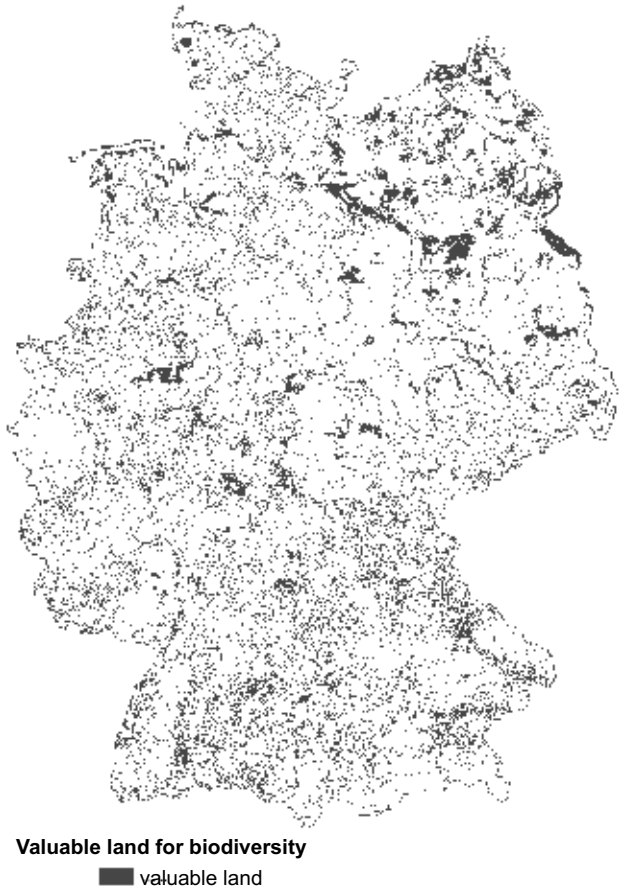

Figure 1:
Agricultural areas valuable for biodiversity in Germany

For the calculation of the indicator, the valuable agricultural land is determined in the GIS separately for each county in absolute and percentage size and compared with the

Table 1:
Calculation example for the claiming of land valuable for biodiversity in the fictional Counties A, B and C

County	Agricultural area (AA) (ha)	Additional wheat area (ha)	Valuable areas (ha)	Valuable areas (% of AA)	Worst case occupancy (ha)	Worst case conservation (ha)	Worst case % of occupied valuable areas in county	Spatially optimized % of occupied valuable areas in county
A	1000	115	50	5	50	0	100	0
B	1000	59	70	7	59	11	84	0
C	1000	7	30	3	7	23	23	0
total	3000	181	150			34		

respective additionally required land for wheat. The following sample calculation based on simplified numbers in hypothetical counties is used to clarify the procedure.

In County A, 115 hectares of area are needed because of the additional wheat cultivation for ethanol (volume allocation based on arable conditions done in the scenarios). Fifty hectares of farmland in the county have high value for biodiversity, and in the worst case scenario this area will fully be occupied by shifts within the county. In County B, 59 hectares of land required for wheat are accompanied by 70 hectares of valuable land. In the worst case, 59 hectares of valuable land are claimed and 11 hectares remain untouched. In the spatially optimized scenario, all valuable areas will remain in their valuable state for biodiversity. Therefore for the indicator "Claiming of land that is particularly valuable with respect to biodiversity" is assumed, that the spatially optimized scenario has no impact on biodiversity since none of the valuable land is affected (0 % in the last column of Table 1).

In the simplified example, in both the baseline and the spatially optimized scenario 150 hectares of 3000 hectares are rated as valuable; the indicator value for Germany is therefore 0.05 (5 % of the agricultural land). In the worst case scenario, only 34 hectares of 3000 hectares remain valuable; the indicator value amounts then to 0.0113 (1.13 % of the land remains in its precious status). Thus a deterioration in the amount of 0.0387 can be observed. The indicator calculated as shown, reflects the valuable land per hectare agricultural land and can be calculated both county-based as well as for Germany as a whole.

Accordingly, the individual values for the indicator in the counties are based, on the one hand, on the additional wheat area depending on the agricultural conditions, and on the other hand, they are based on the existing land valuable for biodiversity. The values were determined and calculated in GIS for all counties of Germany. Figue 2 shows the county-related deterioration of the indicator when switching from the spatially optimized scenario to the worst-case scenario. For the whole of Germany, the indicator value for both the baseline and the spatially optimized scenario is 0.232, according to the 23.2 % valuable agricultural land that was found (Figure 1). In the worst case scenario, the value is 0.189, according to 18.9 % valuable land. Thus a deterioration of 0.043 is observed and the valuable land will decrease by 4.3 percentage points (absolute: 925,108 hectares).

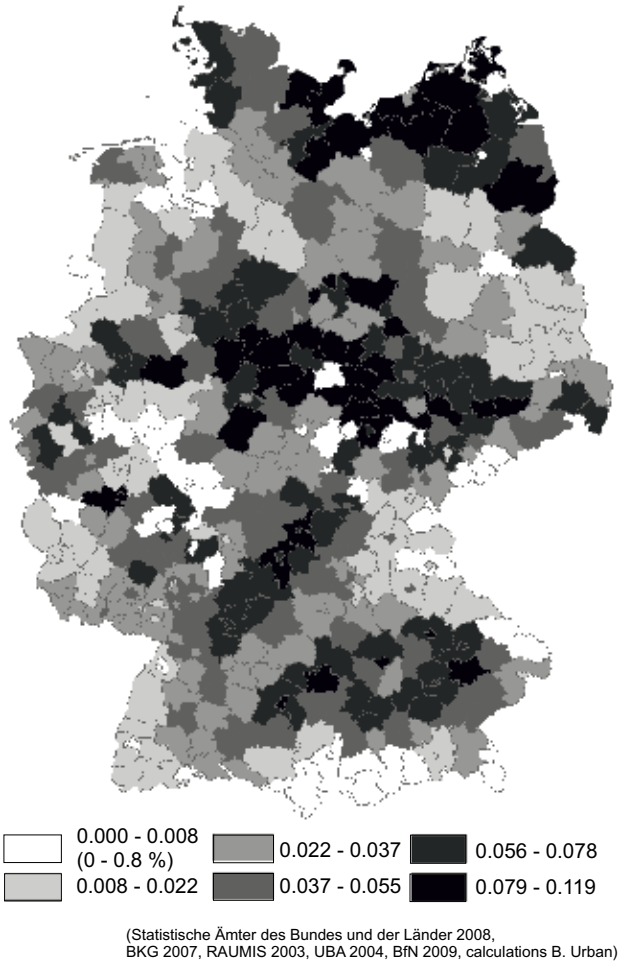

Figure 2:
Worsening of the indicator "for the biodiversity valuable land per hectare agricultural area" from the spatially optimized scenario to the worst case scenario in the counties of Germany

As a first step in assessing the validity of the results, a test is performed for this indicator. It examines the extent to which the areas considered (on the basis of Corine Land Cover) really represent the valuable biotopes which are important for biodiversity. For this purpose, the results obtained nationwide were checked by random sampling with the more accurate data that is available at local and regional level in some cases, but does not or does not homogenously exist for the whole of Germany. The results of both information bases were compared. Compared with the selective biotope mapping in Lower Saxony, a coverage level of 62 % was found for the areas considered. A comparison with the mapped biotope types of very high importance in the more detailed regional landscape plan of the county Verden resulted in a coverage rate of 51 % valuable land identified in the project. This plausibility check reveals that about 38 % to 49 % of the valuable biotopes are not found with the available data on the national level.

3.1.2 Indicator: "Cultivation proportion of agricultural crops"

This indicator is operationalized with a modified Shannon-Weaver index, which describes the cultivation proportion of agricultural crops (following Wetterich and Koepke, 2003). The valuation standard is basically the diversity in agricultural crops. A high index value stands for high crop diversity. It is assumed that a higher diversity of agricultural crops on landscape scale is better for biodiversity than a homogenous agricultural landscape with monocultures (Haber, 2008; Glemnitz et al., 2008). Piorr and Lehmann (2003) have calculated the baseline for the counties in Germany and found 2.83 as a maximal value. Crop rotation is not considered since the evaluation does not take place on the farm level. At the county level, the crop composition remains the same, only the individual areas are changing. With the Shannon-Weaver index, the change in the diversity of agricultural uses in the two scenarios compared to baseline are ascertained:

$$H = -\sum_{i=1}^{s} p_i \cdot \ln p_i \qquad (1)$$

H Shannon-Weaver Index
s Number of crops
p_i Proportion of the i-th crop

Table 2 shows for example the determination of the Shannon-Weaver index for the baseline and the worst case scenario in the county of Hildesheim. The indicator value shows that the diversity of use has decreased (-0.28) in the county.

Table 2:
Calculation example of the Shannon-Weaver Index in the county of Hildesheim (data source: Statistische Ämter des Bundes und der Länder, 2008; RAUMIS, 2003)

County of Hildesheim	Baseline (%)	Worst case scenario (%)	Difference (%)	H Baseline	H Scenario	Difference H
Grassland	6	5	-1			
Wheat	51	63	12			
Rye	0.2	0.1	-0.1			
Winter barley	7	6	-1			
Spring barley	1	0.4	-0.6			
Oat	1	0.4	-0.6			
Triticale	0.2	0.1	-0.1	1.52	1.24	-0.28
Potato	1	1	0			
Sugar beet	22	17	-5			
Rape	3	2	-1			
Maize	0.4	0.3	-0.1			
Fallow	8	4	-4			

3.1.3 Indicator: "Nitrogen fertilizer"

The relationship between nitrogen fertilization and decrease of the typical plant and animal species in agricultural ecosystems is well known (e.g. Flade et al., 2003). Using information provided by various authors, Vogel (2009) derived grades of nitrogen fertilizer application for arable land and grassland, which were used for the indicator assessment (Tables 3, 4).

Table 3:
Rule for evaluation of N-fertilizer levels in cropland (after Schumacher, 1995b; modified in Vogel, 2009)

N-fertilizer (kg/ha)	Grades of impact	Utilization adjusted to nature conservation
> 170	5	very small
100 to 170	4	small
50 to 100	3	medium
0 to 50	2	high
0	1	very high

Table 4:
Rule for evaluation of N-fertilizer levels in grassland (after Schumacher, 1995a; b; modified in Vogel, 2009)

N-fertilizer (kg/ha)	Grades of impact	Utilization adjusted to nature conservation
> 230	5	very small
150 to 230	4	small
50 to 150	3	medium
0 to 50	2	high
0	1	very high

The county-specific fertilizer data for the various crops were described for the model cultures. For each county, crop-specific fertilizer data of the original state are compared with the corresponding values of the scenarios due to the changing land uses. Table 5 shows as an example the calculation for the baseline in the county of Hildesheim. The average level of impact per hectare of agricultural land in Hildesheim is calculated as follows:

$$286{,}616 : 67{,}522 = 4.24 \qquad (2)$$

The difference to the scenario values can show the relative change of the indicator in the county by the additional wheat cultivation.

Table 5:
Calculation of the evaluation of the nitrogen fertilizer levels for the baseline in Hildesheim (data source: Statistische Ämter des Bundes und der Länder, 2008; RAUMIS, 2003)

County of Hildesheim	N-fertilizer kg/ha	Grade of impact	Baseline (ha)	Area (ha) x grade of impact
Grassland	119.3	3	3798	11394
Wheat	196.7	5	34859	174295
Rye	137.8	4	104	416
Winter barley	155.5	4	4839	19356
Spring barley	82.8	3	382	1146
Oat	82.2	3	378	1134
Potato	129.7	4	732	2928
Sugar beet	154.7	4	14850	59400
Rape	189.5	5	2046	10230
Maize	143	4	261	1044
Fallow	0	1	5273	5273
total			67522	286616

3.2 Linking of the indicators with the impact assessment of LCA

For the implemented example indicator (3.1.1) the following calculation arises:

$$\frac{0.043}{1 \cdot 10^{6}\ \text{ha}} = 4.3 \cdot 10^{-8}\ \text{ha}^{-1} \qquad (3)$$

The change of the indicator value of *0.043* (reduction of valuable land for biodiversity) is related to the additional wheat area of 1 million hectares (*1 × 10⁶ ha)*, which is the cause for the change of the indicator. For the given biofuel target the indicator value for valuable agricultural land is deteriorating, under the described assumptions, by *4.3 × 10⁻⁸ per hectare* grown wheat. So it results in an indicator value (*4.3 × 10⁻⁸*) per functional unit (ha grown wheat) that can basically be used similarly to other impact categories. No valuable land is claimed in the spatially optimized scenario and the result for this indicator is thus a value of zero. It should be noted that no proportional calculation is possible in the sense that, for example, a doubling of the additional acreage results in twice the loss of valuable land. The results are valid only for the predetermined quantity target (to the discussion of non-linearity see Geyer et al., 2010). The calculations for the other indicators operate analogously. For instance the Shannon-Weaver Index could be calculated for all counties and also related to the 1 million hectares of wheat.

An aggregation of the various indicators into an overall indicator was not performed. Rather, the various indicators result in an overall view of a "Biodiversity profile" for the considered fuel with a specific biofuel target. This "profile" could be compared with the results of another biofuel.

4 Discussion

The essential goal of a methodical linking of the LCA with spatial methods of environmental planning for the assessment of biodiversity could be achieved (in terms of "path-finding"). An approach was designed that follows some methods of environmental planning with comprehensive statistical data and impact indicators, by which the polluter (pressure) and affected side (state and impact) are operationalized. No area-specific overlays are performed (because the concrete sites are not known). However, a Germany-wide spatial differentiation can be obtained through the conversion of spatial characteristics into percentages at the county level and by the use of scenarios. An advantage of the method lies in the fact that it allows different spatial levels (cropland, plot vicinity and landscape) to be combined by reference to the functional unit and the treat-

ment of model cultures. The effects on the different spatial levels are projected by the proposed approach on a single spatial scale (county as an assessment object).

In principle it is possible to aggregate these values for the different scales to one biodiversity value to facilitate integration into superordinated LCIA methods. However, in consequence the relevant information from the measurement of individual indicators would be lost. Therefore the method only results in non-aggregated indicator values (similarly the methods of Oberholzer et al., 2006 for soil or Schweinle, 2000 for land use) that could be compared in a biodiversity profile in addition to the usual LCA.

In sum, abstract statements for the LCA can be formulated at national level which have the necessary spatially diverse background for biodiversity and include in particular impacts on the landscape scale. The method is basically leading the way. However, it has to be improved, especially with regard to better input data and in consequence differentiated indicators. The examination of the results for the indicator "Claiming of land that is particularly valuable with respect to biodiversity", for example, makes clear that for this indicator, due to data gaps, the actual effects in the worst case scenario are still greatly underestimated (see 3.1.1: a large percentage of valuable biotopes were not found). Thus only a rough estimate is possible with the national data available in Germany. To obtain better results, uniformly classified and assessed biotope maps on the national level are particularly necessary. With this data much more accurate results could be calculated. A most urgent goal is the inclusion of data about habitat connectivity. Such spatially differentiated results for habitat networks were recently produced for the national level in Germany (Fuchs et al., 2010). For legal reasons these (GIS-) data were not available for this research. However, they could be integrated if provided.

Another option for further development of the method is the scenario construction. The focus of the approach presented here is on impact assessment on the basis of a probable crop distribution from a cultivation point of view. This approach could be improved by an area-specific land use distribution modelling using more parameters. For the future this would be a starting point for the coupling with agro-economic models. Different approaches of spatially explicit modelling of biomass production should be tested (Hellmann and Verburg, 2008; 2010; Lovett et al., 2009). Furthermore, the method and particularly the indicators were so far chosen for agricultural land uses. An application to other forms of land use (e.g. forest) is in principle imaginable. Currently only agricultural products can be compared, because the indicators do not reflect the impact of forestry and other land uses. Thus a fuel derived from grain cannot yet be compared with one made of wood. The indicator "claiming of land that is particularly valuable with respect to biodiversity" (3.1.1) could be extended to other land uses if further valuable areas for biodiversity (apart from agricultural land) are included in the calculation. In addition, for forestry, the indicators "amount of deadwood" or "tree species diversity" may be suitable.

A transferability to other product systems, especially other agricultural products and other geographical areas is in principle possible. For other products the scenarios and indicators have to be calculated with the data of the corresponding product. The data availability has to be examined for this purpose and for other geographical regions. The data of CLC are available for all of Europe. The problem is the availability of data on valuable land for biodiversity.

5 Conclusions

The sensitivity and value of biodiversity can only be calculated with spatial relationships. Only if concrete production sites are known, can concrete accounting be done. For all other cases the necessary spatial reference has to be integrated into LCA by proxies. A suitable – though still incomplete - approach has been demonstrated in the research presented here.

The attempt to integrate the complex issue of biodiversity into the LCA also provoked some questions as to the suitability of the categories of the LCA (look also to the discussion in Curran et al., 2011; Finnveden et al., 2009). The impact category "land use" is intended to encompass such diverse impacts as nature conservation as a whole, including biodiversity, soil protection, erosion and other environmental effects. The attempt to cover the issue of biodiversity in LCA by methods environmental planning revealed, that there is a difference between pressure categories like eutrophication, which are (implicitly) considered to be generally harmful in LCA and impact categories like impairment of biodiversity which result from the combination of a potentially harmful pressure with a vulnerable /sensitive acceptor biotope or species. This difference should be better expressed in future LCAs.

A simple integration of the presented approach into existing LCIA methods is in the current state of the proposed method not possible. Further research would be necessary for this purpose. In the present state the proposed biodiversity assessment would be in addition to the usual approach of LCA. A complex and detailed process is necessary for the assessment of biodiversity that cannot be easily integrated in LCA like CO_2-equivalents.

Finally, the approach could be of interest for further developments of other spatially influenced environmental impacts (e.g. soil, water) in LCA. The proposed spatially differentiated approach could be transferred with adjusted indicators. As also indirect land use changes are considered it should be investigated whether the basic approach

can be applied to the much-discussed indirect land use change in the impact category climate change. A global analysis of individual areas, which uses are displaced from others, is hard to imagine. In contrast, a regional approach of displacements as shown here would be quite plausible.

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Dual inoculation with *Pseudomonas fluorescens* and arbuscular mycorrhizal fungi increases phosphorus uptake of maize and faba bean from rock phosphate

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Abstract

The process of combining microbial inoculants is a promising method to effectively improve nutrient availability in soil. In two pot experiments the single and combined application of *Pseudomonas fluorescens* (PF, strain DR54) and arbuscular mycorrhizal fungi (AMF) were evaluated in terms of their effects on phosphorus (P) nutrition of plants supplied with rock phosphate (RP). Faba bean and maize plants were cultivated for 55 days in the first and 45 days in the second experiment under semi-controlled conditions in Mitscherlich pots containing 6 kg of a loamy sand. Five treatments were evaluated for each crop including a control without any application (Con), RP, RP+PF, RP+AMF, and RP+PF+AMF. After plant harvest nutrient concentration in plant tissue (P, N, Mg, K), contents of plant available P (P_{dl}) and pH values of soil were analysed. Beans were more efficient than maize in using P from rock phosphate. Highest P uptakes of plants and values of P_{dl} in soil were found in the combined RP+PF+AMF treatment. Elevated P_{dl} and lower pH values compared to the control after sole AMF inoculation indicate a direct P mobilization by AM fungi.

Keywords: AMF, Pseudomonas, rock phosphate, phosphorus mobilization

Zusammenfassung

Eine kombinierte Ausbringung von *Pseudomonas fluorescens* und Mykorrhiza-Pilzen führt bei Mais und Bohne zur Erhöhung der P-Aufnahme aus Rohphosphaten

Ein kombinierter Einsatz von Mikroorganismen ist eine effektive Methode um die Verfügbarkeit von Nährstoffen im Boden zu erhöhen. In 2 Gefäßversuchen wurde die Einzel- und Kombinationswirkung von *Pseudomonas fluorescens* (PF, Stamm DR54) sowie von arbuskulären Mykorrhiza-Pilzen (AMF) in Bezug auf die Phosphor (P) – Aufnahme von Pflanzen nach Zufuhr von Rohphosphat (RP) untersucht. Faba-Bohnen und Mais wurden für 55 Tage im ersten und 45 Tage im zweiten Experiment in Gefäßen mit 6 kg lehmigen Sandboden unter teil-kontrollierten Bedingungen kultiviert. Fünf Varianten wurden etabliert: RP, RP+PF, RP+AMF and RP+PF+AMF sowie eine Kontrolle (con) ohne Zufuhr von Mikroorganismen oder RP. Nach der Ernte der Pflanzen wurden deren Nährstoffkonzentration (P, N, Mg, K) sowie der pflanzenverfügbare P-Gehalt (P_{dl}) und der pH-Wert des Bodens bestimmt. Die Bohne war bezüglich der Nutzung von P aus Rohphosphat effektiver als der Mais. Der größte Anstieg der P-Aufnahmen der Pflanzen und des P_{dl}-Gehaltes des Bodens wurde in der Variante RP+PF+AMF ermittelt. Auch nach einer Einzelbeimpfung mit AMF stieg der P_{dl}-Gehalt des Bodens an verbunden mit einer Verringerung des pH-Wertes, was auf eine direkte P-Mobilisierung durch die AMF hindeutet.

Schlüsselwörter: AMF, Pseudomonas, Rohphosphat, Phosphormobilisierung

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Introduction

The direct application of rock phosphate (RP) as a fertilizer is an easy and comparatively cheap way to add P to soils. However, the solubility of phosphorus (P) from phosphate rock is low. Vassilev et al. (2001) reported that the amount of P released from directly applied RP may be too low to provide sufficient P for crop needs.

On the other hand, plants have different strategies to mobilize P and may take up P from less soluble sources (Eichler-Löbermann et al., 2008). Legumes can be particularly well-suited for the use of RP. Mnkeni et al. (2000) reported that legumes are more efficient than cereals at utilizing P from RP. Adams and Pate (1992) found positive results for lupin and Ae et al. (1990) for pigeon pea. The enhanced solubilization of RP was mainly due to excretion of organic acids and the subsequent mechanisms of acidification, chelation, and exchange reactions (Reyes et al., 2001; Dakora and Phillips, 2002).

Like plants microorganisms can also improve the availability of sparingly soluble inorganic and organic phosphates by releasing substances such as organic ions, enzymes and protons. Using the microbial effects for plant production can be done by the management of existing soil microbial populations to optimize their capacity to mobilize P (Oberson et al., 2001; Khan et al., 2010) or by the application of specific microbial inoculants (Mäder et al., 2011; Krey et al., 2011). Although attempts to improve the plant nutrition using microbial inoculants has focused mainly on fungi (Wakelin et al., 2004; Relwani et al., 2008), the application of rhizobacteria has also been proposed as a component of sustainable nutrient management systems (Taurian et al., 2010). Bacteria belonging to the genera *Bacillus* and *Pseudomonas* were often found to be very efficient in liberating orthophosphate from organic and inorganic sources (Tye et al., 2002; Richardson et al., 2011).

Arbuscular mycorrhizal fungi (AMF) colonize most agricultural crops and also play an important role in P supply to plants in P deficient farming systems. The importance of AMF in P supply may be comparable to that of root hairs. Their hyphae can extend further from roots than the root hairs, which resulted in a higher soil volume that a colonized root can explore (Smith and Read, 2008). Mainly for plants with short root hairs, the AMF colonization has advantages, as it was shown for *Phaseolus* beans (Miguel, 2004).

Although the major P effect of AMF colonization is due to an increase in spatial P availability, studies have also shown a biochemical mobilization by AMF. This resulted in a better respond of plants colonized with AMF to the application of insoluble phosphate forms in comparison with non-mycorrhizal roots (Medina et al., 2006). Furthermore, AMF can protect plants against toxic elements (e.g. Zn, Cd, and Mn) by accumulation of these in their hyphae (Clark and Zeto, 2000) and may enhance plants' tolerance against pathogen by competing with pathogenic microorganisms (Turk et al., 2006). For Faba bean root colonization by indigenous AMF increased vegetative growth and seed yield in addition to improving nodulation (Mathur and Vyas, 2000).

The development of **multifunctional microbial inoculants** is a promising method to increase the positive effects of microorganisms. This can be based on more than one effect of the single organism or on a combination of organisms (Vassileva et al., 2010). Bacterial and fungal populations can interact in the rhizosphere and stimulate plant growth and improve nutrient availability very effectively (Zaidi et al., 2003; Toljander et al., 2007). Additive effects between AMF and plant growth-promoting bacteria were observed, e.g., after the combined application of AMF and Pseudomonas species (Gamalero et al., 2004) or *Bacillus circulans* (Singh and Kapoor, 1999).

The main objectives of this study were to evaluate rock phosphate as a P source for maize (*Zea mays*) and bean (*Vicia faba*) in combination with single or dual application of *Pseudomonas fluorescens* (PF) and AMF. The following hypothesis was tested: Combined inoculations with P solubilizing bacteria and AMF can increase the fertilizing effects of rock phosphate.

In order to test this hypothesis under semi-controlled conditions, two outdoor pot experiments were established with naturally P poor soil. The *P. fluorescens* DR54 strain was chosen due to its effects on soil P pools in pot experiments.

Materials and methods

Experimental setup

Two pot experiments were conducted in a greenhouse in 2008. The soil utilized was loamy sand originating from the upper soil layer (0 to 30 cm) of a field experiment close to Rostock (North Germany, soil characteristics are given in Table 1). The dominant soil type on the field site was a Stagnic Cambisol. The trials were carried out in a complete randomized design with four replications using Mitscherlich pots containing 6 kg air-dried and sieved (10 mm) soil. Maize (*Zea mays*) and faba bean (*Vicia faba*) were cultivated in both experiments in June and August and harvested 55 and 45 days respectively after sowing. Five treatments were arranged for each crop including controls without any application (Con), rock phosphate (RP), RP+*Pseudomonas fluorescens* (RP+PF), RP+mycorrhizal inoculum (RP+AMF) and RP+PF+AMF. Eight seedlings were sown in each pot and thinned to four plants after germination.

Before sowing, 2 g of RP powder and 25 ml of mycorrhizal inoculum were added to the respective treatments at a depth of 10 cm. The RP contained 13.1 % of P (30 % P_2O_5) and came from Kola Peninsula, Russia. The extractability of P in the RP powder was 6.7 % in water, and 57.3 % in ammonium citrate, whereas the pH value was 8.4. The commercial mycorrhizal product consisted of *Glomus etunicatum*, *Glomus intraradices* and *Glomus claroideum* with a spore number of 10^5 per l litre. The carrier material was expanded clay with a grain size of 2 to 4 mm and a pH of 7.5. Per pot 25 ml of this product was used. The PF-DR54 inoculum was prepared by growing in liquid R2A medium (Difco) at 25 °C for 36 h, suspended in 0.1 M $MgSO_4$ buffer, washed twice and re-suspended in distilled water at about 10^8 cfu per ml. After appearance of the second leaf the PF suspension was applied to the respective treatments (10^8 cfu per plant). The pots were irrigated with distilled water. In order to consider the possible effect of experimental time, the plants in the first experiment (June-Exp) were harvested after 55 days, the plants in the second (August-Exp) after 45 days. The plants were cut above ground, oven-dried at 60 °C for 72 h, weighed and ground for chemical analyses. At harvest soil samples were taken and dried for further analyses.

Table 1:
Characteristics of the soil used for planting

Texture	P mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	OM %	pH
sandy loam	48.2	61.0	234	2.80	6.86

Plant and soil analyses

Shoot-biomass P concentrations were measured after dry-ashing using the vanadad-molybdate method (Page et al., 1982). Shoot biomass N concentrations were measured by using the Kjeldahl digestion method modified according to Jones et al. (1991). Nutrient uptake per pot was calculated by multiplying shoot biomass (g) with shoot nutrient concentration (mg g^{-1}). The organic matter content in soil was determined after ashing in an oven at 550 °C. Soil contents of double lactate soluble P, K and Mg (P_{dl}, K_{dl}, Mg_{dl}), as well as pH ($CaCl_2$) were measured according to Blume et al. (2000). Phosphates extracted with double lactate solution represent the plant available P (Schachtschabel and Beyme, 1980). K_{dl} and Mg_{dl} concentrations were measured with a flame? photometer. P shoot biomass and P_{dl} were measured with a spectral photometer at 430 nm.

Statistical procedure

Data were subjected to the GLM (General Linear Model) procedure of SPSS version 15. Treatment effects were tested by one-way analysis of variance followed by Duncan's multiple range test used for multiple comparisons. Mean differences were considered significantly different at $p < 0.05$.

Results and discussion

In both experiments the **yield** of maize and beans significantly increased when RP was combined with *P. fluorescens* and AMF together, whereas no yield effect was found after the RP addition only compared to the control. Single application of either AMF or Pseudomonas with RP only resulted in higher yields for beans in the June experiment, when the plants were harvested at the end of flowering stadium.

Table 2:
DM yields of maize and beans (g per pot) in pot experiments (sowing in June and August 2008)

Treatment	Maize				Beans			
	June		August		June		August	
Con	55.5	a	38.9	a	29.6	a	18.8	a
RP	57.6	a	40.6	ab	31.6	ab	19.6	a
RP+PF	59.4	ab	40.4	ab	33	b	20.1	a
RP+AMF	57.9	a	40.9	ab	33.8	b	20	a
PR+PF+AMF	63.5	b	42.5	b	34.4	b	21.6	b

Different letters within a column indicate significant differences between the treatments; $p < 0.05$ (Duncan), Con = control, RP = rock phosphate, PF = *Pseudomonas fluorescens*, AMF = arbuscular mycorrhizal fungi

With regard to the **P uptake**, for maize significantly higher values compared to the control were only found in the combined RP+PF+AMF treatment. For beans higher treatment effects were observed. In the June experiment P uptake by beans was raised when RP was combined with dual microorganism inoculation (RP+PF+AMF) as well as with one inoculation alone (RP+PF and RP+AMF). The RP without AMF or PF resulted in 10 % higher P uptake compared to the control, but this difference was not significant. In the August experiment however, all RP treatments resulted in higher P uptakes of beans compared to the control. This strongly indicates that beans were more efficient in using P from rock phosphate than maize. These results are in accordance with other studies emphasising

the high potential of P solubilization of legumes as stated in the introduction chapter. Missing effects of the applied *Pseudomonas* strain on maize yield are in accordance with a previous pot experiment by Krey et al. (2011). In both experiments we found higher contents of available P in soil after application of *P. fluorescens* (see below), but the yield and P uptake by maize did not increase. Although the initial soil P content was suboptimal according to the German soil classification, it can be expected that stronger yield effects of the applied *P. fluorescens* strain might have occurred in even lower soil P contents. This supposition is also supported by a strong plant-growth promotion after inoculation of maize with *Pseudomonas alcaligenes*, *Bacillus polymyxa*, and *Mycobacterium phlei* to a nutrient deficient Calcisol in a greenhouse pot experiment by Egamberdiyeva (2007). Also for AMF better results were found when the plant available P in soil was very low which went together with high percentages of root colonization (Graham and Abbott, 2000).

Positive influences of both microorganism treatments together indicate the increased effectiveness of combined inoculation regarding the nutrient availability and plant growth, which was also shown in a study of Boer et al. (2005). Babana and Antoun (2006) found that strains of *Pseudomonas* sp. act both as plant growth promoting bacteria and mycorrhizal-helper bacteria resulting in comparable high yields of wheat plants after RP application compared to a treatment with high soluble P fertilizer.

Similar results as those for P were found for the **other nutrients**. The N uptake of maize and beans increased in relation to the control when both microorganisms together were applied (160 % and 122 %, respectively). The K and Mg uptake of both plants were also promoted when microorganisms (single or dual inoculation) were added to the soil. In particular the K uptake of maize was enhanced by microorganism application. The increase in nutrient uptake was partially higher than the increase in yields, which can be explained by higher availability of nutrients in the soil and therefore higher nutrient concentrations in plant tissue (data not shown). Also in other studies higher responses on nutrient supply were found regarding plant nutrient uptake than regarding plant yield (Eichler et al., 2004).

Table 3:
Nutrient uptake of maize and bean (mg per pot) in pot experiments (sowing in June and sowing in August 2008)

Experiment	Treatment	P uptake	N uptake	K uptake	Mg uptake
			Maize		
	Con	72.4 a	294 a	656.0 a	154 a
	RP	77.7 a	299 ab	700.8 ab	155 ab
June	RP+PF	81.3 a	339 bc	759.4 b	167 ab
	RP+AMF	78.5 a	339 bc	724.4 ab	164 ab
	PR+PF+AMF	81.3 a	362 c	752.6 b	177 b
	Con	85.7 a	371 a	956.8 a	84.1 a
	RP	93.9 a	377 a	1004 ab	85.7 a
August	RP+PF	94.9 a	448 a	1150 b	93.6 ab
	RP+AMF	95.5 a	460 a	1141 b	93.4 ab
	PR+PF+AMF	87.7 a	606 b	1460 c	108 b
		P uptake	N uptake	K uptake	Mg uptake
			Faba bean		
	Con	38.8 a	668 a	230 a	126 a
	RP	42.5 ab	674 a	246 ab	133 a
June	RP+PF	48.3 bc	793 b	273 b	138 a
	RP+AMF	49.7 bc	761 b	279 bc	141 a
	PR+PF+AMF	50.5 c	815 b	310 c	141 a
	Con	36.6 a	603 a	281 a	81.8 a
	RP	42.2 b	672 ab	286 a	84.7 a
August	RP+PF	45.1 b	690 b	295 a	87.6 ab
	RP+AMF	42.4 b	685 b	295 a	87.8 ab
	PR+PF+AMF	44.5 b	738 b	321 a	99.0 b

Different letters within a column indicate significant differences between the treatments; $p < 0.05$ (Duncan), Con = control, RP = rock phosphate, PF = *Pseudomonas fluorescens*, AMF = arbuscular mycorrhizal fungi

As expected, due to the longer vegetation time and higher sunlight intensity the plant yields of the June experiment were higher than the yields of the second experiment in August.

Positive effects of microorganisms were observed regarding the **soil values**. In the combined treatment RP+PF+AMF the highest P_{dl} values were found, regardless of the high P removal by plants in this variant. Application of RP together with only one inoculant resulted in higher P values mainly in the first experiment with the longer experimental time. Probably the second experiment was too short to make the microorganism effect visible. RP as a fertilizer is known to dissolve slowly and needs a long time to release P available for plants. Truong et al. (1978) studied changes in the estimated relative agronomic effectiveness (RAE) of RP with time. The RAE coefficients changed considerably between 1 and 4 months for most RP.

Table 4:
Plant available P contents in soil (Pdl mg per kg) and pH values in pot experiments (sowing in June and sowing in August 2008)

Experiment	Treatment	Maize		Faba bean	
		P_{dl} in soil (mg kg^{-1})	pH	P_{dl} in soil (mg kg^{-1})	pH
June	Con	42.8 a	6.93 c	46.1 a	6.93 c
	RP	45.9 ab	6.82 bc	51.8 b	6.93 c
	RP+PF	49.9 bc	6.08 a	53.7 b	6.87 c
	RP+AMF	52.8 c	6.56 b	56.4 b	6.57 b
	PR+PF+AMF	51.6 c	5.98 a	55.3 b	6.06 a
August	Con	47.0 a	6.94 b	48.6 a	6.93 b
	RP	50.2 a	6.93 b	50.8 ab	6.90 b
	RP+PF	53.4 a	6.56 b	53.8 ab	6.68 b
	RP+AMF	51.5 a	6.49 ab	54.5 ab	6.54 ab
	PR+PF+AMF	53.5 a	6.08 a	55.6 b	6.19 a

Different letters within a column indicate significant differences between the treatments; $p < 0.05$ (Duncan), Con = control, RP = rock phosphate, PF = *Pseudomonas fluorescens*, AMF = arbuscular mycorrhizal fungi

On average, for beans slightly higher soil P_{dl} values were measured compared to maize which is in relation to the lower P removal by bean plants.

Generally the pH was lower in the treatments with the microorganism inoculation and may have resulted in a higher release of orthophosphate ions from RP. The lowering effect on pH by microorganisms and following an increasing availability of P is mainly due to excretion of organic acids and protons (Bünemann et al., 2004; Stevenson, 2005). Maliha et al. (2004) found that pH of bacterial broth dropped significantly (5.96 to 2.75) compared to the control.

Between the two cultivated plants only slight differences in soil reaction were measured. Therefore, the solubilizing effect of beans on RP cannot be explained by the pH values. However, the pH might have been lower in the direct rhizosphere zone, where organic acids and protons are released.

Conclusion

Combined inoculation of bacterial and fungal populations can improve nutrient availability and plant growth very effectively. According to our results, a combination of *P. fluorescens* and AMF fungi of the genus *Glomus* can improve the P nutrition from RP more effectively than single applications of one of the microorganism preparations. Generally, both microorganism treatments showed the same efficacy. Higher P_{dl} and lower pH values in AMF treatments compared to the control suggest a direct P mobilization resulting in the best effects for combined inoculation.

The partly diverse effects of the microorganism application on plant and soil parameters underline the complexity of the system. Field experiments are necessary to test the efficacy of the inoculants under more practical conditions.

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Enteric methane emissions from German pigs

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Abstract

Methane emissions from enteric fermentation of pigs are object of emission reporting. Hitherto they were treated as part of the energy balance of pigs, in accordance with IPCC guidance documents. They were calculated from the gross energy intake rate and a constant methane conversion ratio. Meanwhile numerous experimental data on methane emissions from enteric fermentation is available in Germany and abroad; the results are compiled in this work. These results also allow for a description of transformation processes in the hind gut and a subsequent establishment of models that relate emissions to feed and performance data.

The model by Kirchgeßner et al. (1995) is based on German experimental data and reflects typical national diet compositions. It is used to quantify typical emissions and methane conversion ratios. The results agree with other experimental findings at home and abroad.

The application of the model results in emission rates that fall below those calculated with the IPCC standard procedures by about one fifth.

Keywords: methane, emission, model, enteric fermentation, pigs

Zusammenfassung

Methan-Emissionen aus der Verdauung bei deutschen Schweinen

Die Methan-Emissionen aus der Verdauung bei Schweinen sind Gegenstand der Emissionsberichterstattung. Sie wurden bisher nach Vorgaben des IPCC-Regelwerks als Bestandteil der Energiebilanzen von Schweinen aus der Gesamtenergie-Aufnahmerate und einem (festen) Methan-Umwandlungsfaktor berechnet. Mittlerweile liegen zahlreiche experimentelle Untersuchungen aus dem In- und Ausland zu Methan-Emissionen aus der Verdauung vor, deren Ergebnisse in diesem Beitrag zusammengefasst sind. Aus den Ergebnissen dieser Messungen lassen sich in Kenntnis der Umsetzungsprozesse im Enddarm Modelle ableiten, die eine Quantifizierung der Emissionen aus Fütterungs- und Leistungsdaten erlauben.

Das aus deutschen Daten abgeleitete Modell von Kirchgeßner et al. (1995) dient dazu, aus den aus Umfragen erhaltenen Futterzusammensetzungen in Deutschland typische Emissionen und Methan-Umwandlungsraten berechnen. Die mit dem Modell erhaltenen Ergebnisse stimmen mit anderen experimentellen Befunden aus dem In- und Ausland überein.

Im Mittel werden danach Emissionsraten errechnet, die um ein Fünftel unter den nach dem derzeit gültigen IPCC-Standardverfahren berechneten liegen.

Schlüsselwörter: Methan, Emission, Modell, Verdauung, Schweine

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Introduction

Methane (CH_4) is a greenhouse gas and air pollutant. International conventions require that its emissions be quantified and reported. [1] In animal production, CH_4 emissions originate from enteric fermentation and from manure storage. In Central Europe these emissions are dominated by the emissions from enteric fermentation of cattle. Emissions from enteric fermentation of pigs are of minor importance albeit not negligible (German data for 2010: estimated emissions from enteric fermentation in pig production 26.3 Gg a^{-1} CH_4, total CH_4 emissions from agriculture 1,231 Gg a^{-1} CH_4; Haenel et al., 2012).

At present, emission reporting makes use of the IPCC (1996) guidance document that relates CH_4 emissions from enteric fermentation to the gross energy (GE) intake using a default methane conversion rate (MCR) of 0.6 % (expressed as energy loss; IPCC, 1996, Table A-4). It is unclear whether this tool is adequate for the environmental valuation of changes in feeding practices, as GE may differ with feed composition – feed intake is governed by metabolizable energy (ME). In contrast to IPCC (1996), IPCC (2006), Table 10.12, entirely omits pigs as sources of methane from enteric fermentation.

This work develops a national approach to quantify emissions from enteric fermentation in pig production for the purpose of emission reporting, using experimental data and modelled data based on German national diet compositions. It concludes the series of publications that revised the treatment of emissions from pig production in the national agricultural emission inventory. [2]

1 Experimental data – an overview

1.1 Measurement technique

CH_4 emissions can be measured using respiratory chambers (see eg Kirchgeßner et al., 2008, pg 151f) where animals are kept for a few hours up to a few days. Care is taken that faeces are removed before they can contribute to relevant emissions. Gas exchange in these chambers is recorded continuously. However, during their stay in the chamber the animals lack social contact. Furthermore, their physical activity is restricted. Normally, the release of CH_4 is related to standing up (Jørgensen et al., 2011), so the intestinal activity pigs within a respiration chamber may be abnormal. However, there is experimental evidence that the overall energy balance is not changed significantly (Gray and McCracken, 1980), so it is also likely that the CH_4 emission rates are not greatly affected by the lack of physical activity.

1.2 Measured methane emission rates

Several European groups measured CH_4 emission rates from non-lactating sows and growing pigs. In most cases the measurements were part of feeding experiments, including variations of the share of fibre fed. In these cases, emission rates for the respective control group only are considered in the following Tables 1 to 3. In the literature, emission rates are presented as mass, volume or energy contents of CH_4 emitted per animal per day. If performance data are mentioned in the publications, an attempt is made to relate emissions to the GE intake rate. The following relations are used for conversion:

Conversion of digestible energy

$$GE = \frac{DE}{X_{\mathrm{DE}}} \qquad (1)$$

where

GE gross energy intake rate (in MJ animal^{-1} d^{-1})
DE digestible energy intake rate (in MJ animal^{-1} d^{-1})
X_{DE} digestibility of energy (default X_{DE} = 0.866 MJ MJ^{-1}; Müller and Kirchgeßner, 1983a)

Conversion of metabolizable energy

$$GE = \frac{ME}{X_{\mathrm{ME}}} \qquad (2)$$

where

GE gross energy intake rate (in MJ animal^{-1} d^{-1})
ME metabolizable energy intake rate (in MJ animal^{-1} d^{-1})
X_{ME} metabolizability of energy (as a rule provided in the literature) (in MJ MJ^{-1})

Conversion of net energy

$$GE = \frac{NE}{X_{\mathrm{NE}}} \qquad (3)$$

where

GE gross energy intake rate (in MJ animal^{-1} d^{-1})
NE net energy intake rate (in MJ animal^{-1} d^{-1})

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[1] For air pollutants, reporting was required within the Geneva Convention on Long-Range Transboundary Air Pollution until 2002. Since then reporting has been mandatory within the United Nations Framework Convention on Climate Change only.

[2] Redistribution of animal numbers (Haenel et al., 2011a), update of energy requirements (Haenel et al., 2011b), feed composition (Dämmgen et al., 2011a), volatile solids excretion (Dämmgen et al., 2011b, nitrogen excretion (Dämmgen et al., 2010), methane emission from storage (Dämmgen et al., 2012), basic ammonia emission factors and amounts of bedding (Eurich-Menden et al., 2011).

X_{NE} ratio of net to gross energy (default X_{NE} = 0.53 MJ MJ^{-1})

Conversion of volume to mass

The density ρ_{CH4} of CH_4 is 0.716 kg m^{-3} at standard conditions. (German standard DIN 1343 uses a standard temperature $T_{n,DIN}$ = 273.15 K and a standard pressure of 1013 hPa. Gas densities (ρ) can then be adjusted using the relation $T_{n,1}/T_{n,2} = \rho_2/\rho_1$.)

Conversion of mass to energy

The energy content η_{CH4} of CH_4 is 55.65 MJ kg^{-1}.

Units frequently used to describe CH_4 emission rates and relate them to animal or feed properties

- EV_{CH4} denotes the volume of CH_4 emitted per animal and per unit of time.
- $EV_{CH4}{}^*$ (specific volume) relates the volume of CH_4 emitted to the metabolic weight of the animal.
- EM_{CH4} is the mass of CH_4 emitted per animal and per unit of time.
- EE_{CH4} is the energy equivalent of the CH_4 released per animal and per unit of time.
- $EE_{CH4}{}^*$ is the specific energy equivalent of the CH_4 released per unit of metabolic animal weight and per unit of time.

The methane conversion ratio MCR is the fraction of gross energy taken in that is converted to CH_4 in the hind gut. [3]

$$MCR = \frac{EE_{CH4}}{GE} \quad (4)$$

where

MCR methane conversion ratio (in kJ MJ^{-1})
EE_{CH4} energy equivalent of methane released (in kJ animal^{-1} d^{-1})
GE gross energy intake rate (in MJ animal^{-1} d^{-1})

Table 1:
Methane emissions from sows (for the symbols used see explanations above)

reported entity	reported value	unit	live weight (LW) kg animal^{-1}	notes	MCR kJ MJ^{-1}	reference
MCR	0.7	% GE		early gestation	7	Beyer et al. (1994)
MCR	0.4	% GE		late gestation	4	Beyer et al. (1994)
MCR_{DE}	0.7	% DE	205	lactating	5.3	Jakobsen et al. (2005)
MCR_{DE}	1.31	% DE	225		10.1	Jørgensen (2007)
MCR_{DE}	0.8	% DE			6.7	Jørgensen et al. (2001)
MCR_{DE}	0.7	% DE	210		5.5	Jørgensen et al. (2007)
MCR	0.74	% GE	203		7.4	Kirchgeßner and Müller (1981)
MCR	0.94	% GE	187		9.4	Kirchgeßner et al. (1987)
MCR_{DE}	0.88	% DE	235		6.6	le Goff et al. (2002b)
EE_{CH4}	0.3	MJ d^{-1}	190		9.2	Müller and Kirchgeßner (1983a)
MCR_{DE}	0.6	% DE	201	increased straw	5.2	Müller and Kirchgeßner (1983b)
EE_{CH4}	0.25	MJ animal^{-1} d^{-1}			7.6	Müller and Kirchgeßner (1985a)
MCR_{DE}	0.8	% DE	184		6.9	Müller and Kirchgeßner (1985b)
MCR_{DE}	0.8	% DE	239		6.9	Noblet and Le Goff (2001)
SE [A]	0.21	MJ (kg DM)$^{-1}$	208		11.4	Noblet et al. (1993)
MCR_{DE}	1.2	% DE	290		9.8	Olesen et al. (2001)
MCR_{DE}	0.85	% DE	260	low fibre	7.4	Ramonet et al. (2000)
MCR_{DE}	3.36	% DE	260	high fibre	29	Ramonet et al. (2000)
MCR_{DE}	0.8	% DE	183	high protein	6.7	Theil et al. (2002)
MCR_{DE}	0.5	% DE	175	low protein	4.2	Theil et al. (2002)
MCR_{DE}	0.73	% DE	206		6.4	Theil et al. (2004), Jørgensen et al. (2011)

[A] SE: specific emission (see unit)

[3] The IPCC terminology uses the symbol Y_m (IPCC: methane conversion factor) (in MJ MJ^{-1}) for the methane conversion ratio MCR (used in this work). IPCC also call the ratio with which CH_4 is formed within manure storage a methane conversion factor (in %) and apply the symbol MCF.

In some cases, the methane conversion ratio is not related to gross energy. Instead, MCR_{DE}, is used to describe the fraction of digestible energy taken in that is converted to CH_4 in the hind gut.

$$MCR_{DE} = \frac{EE_{CH4}}{DE} \quad (5)$$

where

MCR_{DE} methane conversion ratio for digestible energy (in kJ MJ^{-1})
EE_{CH4} energy equivalent of methane released (in kJ $animal^{-1}$ d^{-1})
DE digestible energy intake rate (in MJ $animal^{-1}$ d^{-1})

The live weight LW (in kg $animal^{-1}$) is used to characterize the animals.

1.2.1 Sows

Measurements on sows were published almost only for non-lactating animals (Table 1). It is customary to feed sows in this time span a diet that is high in fibre. Hence, the emissions listed in Table 1 cannot be extrapolated to annual emission rates.

1.2.2 Piglets

As sows milk does not contain fibre or polysaccharides, suckling piglets should not produce CH_4. However, milk replacer does contain fibre which results in small CH_4 emission rates (Table 2).

Table 2:
Methane emissions from piglets fed on milk replacer (for the symbols used see explanations above)

reported entity	reported value	unit	live weight (LW) kg $animal^{-1}$	MCR kJ MJ^{-1}	reference
EV_{CH4}	0.13	l $animal^{-1}$ d^{-1}	4.9	1.2	Theil et al. (2007), Jørgensen et al. (2011)

1.2.3 Growing pigs

Growing pigs comprise both weaners and finishing pigs. However, the measurements available only deal with finishing pigs. As a rule, animal weights and weight gains are reported (Table 3).

1.3 Résumé

The data collated originate from measurements in Germany, Denmark, The Netherlands, France, Italy as well as from The USA, Canada and China. Care was taken that the results obtained for the control groups were extracted from the articles. In some cases it was difficult to identify the control.

The number of animals in the respective experiments varied from "a few" to "many". Hence the calculation of *weighted* mean values of MCR was impossible. However, the calculation of *arithmetic* mean values may be used to support results obtained from modelling.

For **sows**, only 1 result describes lactating animals (Jacobsen et al., 2005). The 30 values obtained for gestating sows can be reduced to a arithmetic mean MCR of about 7.0 kJ MJ^{-1}. If one keeps in mind that diets for gestating sows are richer in fibre than those for lactating sows, and that the energy intake with feeds during the lactation phase is about one third of the overall GE intake (Haenel et al., 2011b), then a weighted mean MCR of less than 7.0 kJ MJ^{-1} results.

$$MCR_{sow,\,mean} = \frac{2 \cdot MCR_{sow,\,gest} + 1 \cdot MCR_{sow,\,lact}}{3} \quad (6)$$

Data listed in Table 1 suggest an MCR of about 6.5 kJ MJ^{-1}.

Only one reference could be found for **suckling piglets**. The MCR of 1.2 kJ MJ^{-1} can be ignored considering the low GE intake of these animals and the small share of milk replacer fed in addition to sows' milk.

The majority of data sets describe **growing pigs**. 3 papers refer to animals in the subcategory of weaners (LW < 35 kg $animal^{-1}$) for which an MCR of about 3 kJ MJ^{-1} can be identified. For finishing pigs, a mean MCR of about 4.5 kJ MJ^{-1} can be calculated; here, the results published by Atakora et al. (2011) are considered outliers and omitted from this and subsequent calculations.

Experiments with varying animal weights (*ceteris paribus*) showed that MCR increases with animal weight (e.g. Christensen and Thorbek, 1987; Noblet and Shi, 1994). A regression analysis leads to the same conclusion, although the scatter is considerable (Figure 1).

MCR also increases with the fibre content of the diet (e.g. Jensen and Jørgensen, 1994) and with increasing protein content (e.g. Theil et al., 2002).

The IPCC (1996) default MCR exceeds almost all values derived from measurements.

Table 3:
Methane emissions from growing pigs

reported entity	reported value	unit	live weight (LW) kg animal^{-1}	notes	MCR kJ MJ^{-1}	reference
EM_{CH4}	17.0	g animal^{-1} d^{-1}	81	very low protein, barley	25.3	Atakora et al. (2011)
EM_{CH4}	17.6	g animal^{-1} d^{-1}	81	medium protein, barley	27.3	Atakora et al. (2011)
EM_{CH4}	23.2	g animal^{-1} d^{-1}	75	high protein, barley	36.3	Atakora et al. (2011)
EM_{CH4}	23.9	g animal^{-1} d^{-1}	63	medium protein, maize	39.4	Atakora et al. (2011)
EM_{CH4}	25.4	g animal^{-1} d^{-1}	63	high protein, barley	42.1	Atakora et al. (2011)
MCR_{DE}	0.4	% DE	80		3.5	Barea et al. (2010)
EV_{CH4}	1.7 to 8.5	l animal^{-1} d^{-1}	20 to 25	feed varied	5.4	Christensen and Thorbek (1987)
EV_{CH4}	12.2 to 8.5	l animal^{-1} d^{-1}	35 to 110	feed varied	5.2 to 11.0	Christensen and Thorbek (1987)
MCR	0.45	% GE	85		4.5	Galassi et al. (2004)
EV_{CH4}	1.4	l animal^{-1} d^{-1}	112 to 132	low fibre	0.8	Jensen and Jørgensen (1994)
EV_{CH4}	12.5	l animal^{-1} d^{-1}	112 to 132	high fibre	7.1	Jensen and Jørgensen (1994)
MCR	0.4 to 0.5	% GE	30 to 125		4 to 5	Jentsch and Hofmann (1977)
MCR	0.05	% GE	13 to 28		0.5	Jentsch et al. (1991)
MCR	0.44	% GE	28 to 63		4.4	Jentsch et al. (1991)
EM_{CH4}	1.13	g animal^{-1} d^{-1}	60		2.8	Ji et al. (2011)
EM_{CH4}	2.01	g animal^{-1} d^{-1}	90		3.4	Ji et al. (2011)
MCR_{DE}	0.49	% DE	65		4.2	Jørgensen (2007)
MCR_{DE}	0.2	% DE	20	low fibre	1.9	Jørgensen et al. (1996a)
MCR_{DE}	1.1	% DE	20	high fibre	9.0	Jørgensen et al. (1996a)
MCR_{DE}	0.51	% DE	20		4.0	Jørgensen et al. (1996b)
MCR_{DE}	0.8	% DE	60 to 115		6.7	Jørgensen et al. (2007)
MCR_{DE}	0.4	% DE	35		3.1	Jørgensen et al. (2001)
MCR_{DE}	0.80	% DE	60 to 115		6.7	Jørgensen et al. (2007)
MCR_{DE}	0.70	% DE	65.2		6.1	Le Bellego et al. (2001)
MCR_{DE}	0,46	% DE	65.9		4.1	Le Bellego et al. (2001)
MCR_{DE}	0,60	% DE	65.5		5.3	Le Bellego et al. (2001)
MCR_{DE}	0,50	% DE	65.2		4.3	Le Bellego et al. (2001)
MCR_{DE}	0.20	% DE	41		1.8	le Goff et al. (2002a)
MCR_{DE}	0.24	% DE	76		2.2	le Goff et al. (2002a)
MCR_{DE}	0.41	% DE	43		3.5	Noblet and Shi (1994)
MCR_{DE}	0.44	% DE	48		3.8	Noblet and Shi (1994)
MCR_{DE}	0.60	% DE	100		5.2	Noblet and Shi (1994)
EE_{CH4}	0.14	MJ animal^{-1} d^{-1}	75 to 90		4.3	Schneider and Menke (1982)
EV_{CH4}*	0.242	l kg - 0.75 d^{-1}	48		8.5	Schrama et al. (1996)
EE_{CH4}*	4.9	kJ kg - 0.75 d^{-1}	54		3.9	Schrama et al. (1998)
EE_{CH4}*	6.2	kJ kg - 0.75 d^{-1}	46		4.8	Schrama et al. (2003)
MCR_{DE}	0.29	% DE	33 to 60		2.8	Wang et al. (2004)

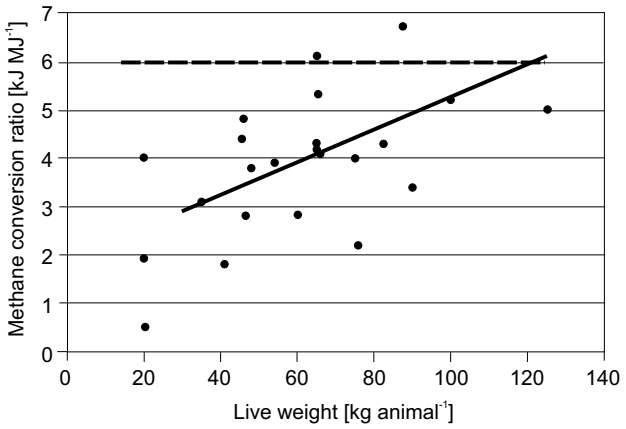

Figure 1:

Methane conversion ratios of growing pigs as a function of live weight using those data sets from Table 3 where weights and MCR were provided. Full line: regression for pigs with 30 kg animal^{-1} ≤ LW ≤ 125 kg animal^{-1}; R^2 = 0.34. Dotted line: default MCR in IPCC (1996)

2 Modelling methane formation in pigs

Modelling should allow for the weighing of effects and side effects of potential reduction measures, in particular the influence of diet composition and feeding practices on emissions. However, this requires a more mechanistic approach relating emissions to animal performance and feed constituents than provided in IPCC (1996).

2.1 Methane formation in the digestive system

The formation of CH_4 in the digestive system (enteric fermentation) of pigs is mainly centred in the hind gut (colon) (see Jensen and Jørgensen, 1994). Here, bacterial action degrades those organic species that passed the digestive tract undigested, mainly cellulose, hemicellulose and pectin which are summed up as bacterially fermentable substrates (BFS). Bacterial action converts these substrates to volatile fatty acids, CH_4 and carbon dioxide (see Figure 2). The fatty acids play an important role in the energy supply of pigs (Kirchgeßner et al., 1987; Dierick et al., 1989; Noblet and Le Goff, 2001). In experiments with sows, about half the cellulose and about 90 % of the sugar (xylose), starch [4] and cellulose (pectin) as well as the protein casein that were applied to the animals intracaecally were degraded in the hind gut (Kreuzer et al., 1991a, b). Despite the efforts described e.g. in Kirchgeßner et al. (1987, 1991) or Noblet (2007), Jørgensen et al. (2011) state "However, information on how dietary composition and intrinsic animal factors influence gas production in pigs is rather limited."

2.2 Relating methane emission rates to feed intake, diet composition and animal performance

A number of relationships have been developed to predict enteric CH_4 emission to dietary or animal characteristics. In this section, we briefly review these relationships. Note that Equations 8 to 11 return the CH_4 emission per feeding day. To calculate the annual emission per place, the so-called emission factor EF_{CH4}, it is necessary to take account of the duration of any period during which the place is empty (e.g. for cleaning).

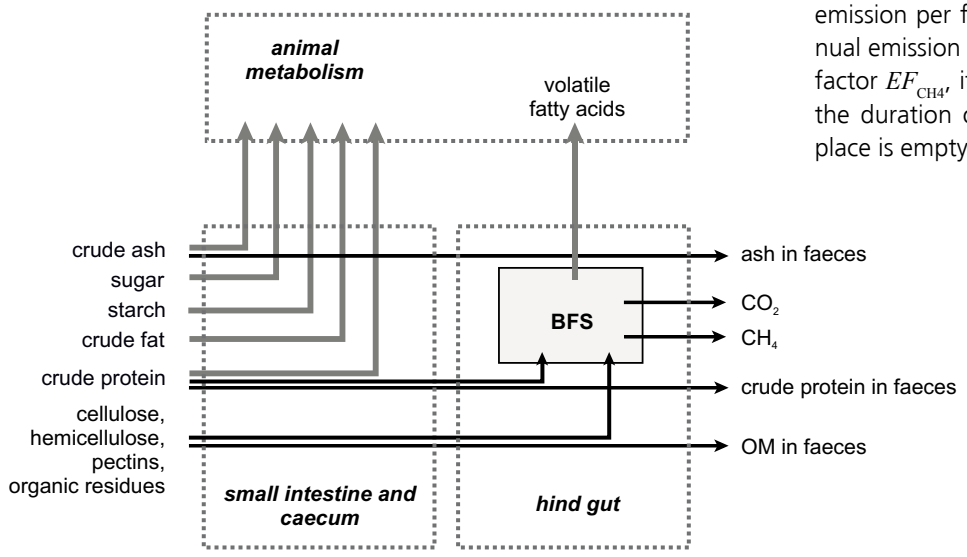

Figure 2:

Pathways of major feed constituents through the gut of pigs. Gray arrows: share of constituents that can be resorbed. Wide black arrows: bacterially fermentable substrates; narrow black arrows: matter that is neither resorbable nor bacterially fermentable.

[4] Sugars and starch will normally be digested in the small intestine and caecum (see Figure 2).

The relation between emission factor (emission per place per year) and emission rate (emission per animal per day) is as follows:

$$EF_{\mathrm{CH4}} = E_{\mathrm{CH4}} \cdot t_{\mathrm{lifespan}} \cdot n_{\mathrm{round}} \quad (7)$$

where

EF_{CH4} CH_4 emission factor (in kg place^{-1} a^{-1} CH_4)
E_{CH4} CH_4 emission rate (in kg animal^{-1} d^{-1} CH_4)
t_{lifespan} duration of lifespan in a subcategory (in d)
n_{round} number of animal rounds (in animal place^{-1} a^{-1})

2.2.1 Model 1: methane formation rate and BFS supply rate

From respiration chamber measurements Kirchgeßner et al. (1991) deduced functions describing emission rates for growing pigs and for sows.

$$E_{\mathrm{CH4,1}} = a_1 + b_1 \cdot m_{\mathrm{BFS}} = a_1 + b_1 \cdot DM \cdot \eta_{\mathrm{BFS}} \quad (8)$$

where

$E_{\mathrm{CH4,1}}$ CH_4 emission rate obtained with model 1 (in kg animal^{-1} d^{-1} CH_4)
a_1 constant (in kg animal^{-1} d^{-1})
b_1 coefficient (in kg kg^{-1} CH_4)
m_{BFS} rate of BFS available for fermentation (in kg animal^{-1} d^{-1})
DM dry matter intake rate (in kg animal^{-1} d^{-1})
η_{BFS} mean BFS content of feed (dry matter) (in kg kg^{-1})

Both constant and coefficient vary between growing pigs and sows, for sows also with the mean BFS content η_{FBS}. Kirchgeßner et al. (1991) propose to differentiate between three cases:

growing pigs:
in any case a_1 = 0.00000 kg animal^{-1} d^{-1};
b_1 = 0.020 kg kg^{-1}

sows:
if $\eta_{\mathrm{BFS}} < 0.08$ kg kg^{-1} then a_1 = 0.00000 kg animal^{-1} d^{-1};
b_1 = 0.020 kg kg^{-1}
if $\eta_{\mathrm{BFS}} \geq 0.08$ kg kg^{-1} then a_1 = 0.00285 kg animal^{-1} d^{-1};
b_1 = 0.013 kg kg^{-1}

Boars (mature males for reproduction) are treated like sows.

The comparatively low regression coefficients R^2 of about 0.6 can be explained by variations in the individual digestion processes, including variations in the composition of the bacterial populations.

2.2.2 Models 2 and 3: methane formation rate and supply rate of fermented dietary fibre (FDF)

Model 2 is defined by an equation deduced by Noblet et al. (2004) (as quoted in Noblet, 2007):

$$E_{\mathrm{CH4,2}} = a_2 \cdot \frac{m_{\mathrm{FDF}}}{\eta_{\mathrm{CH4}}} \quad (9)$$

where

$E_{\mathrm{CH4,2}}$ CH_4 emission rate obtained with model 2 (in kg animal^{-1} d^{-1} CH_4)
a_2 coefficient (growing pigs: a_2 = 0.67 MJ (kg FDF)$^{-1}$; sows: a_2 = 1.33 MJ (kg FDF)$^{-1}$)
m_{FDF} supply rate of fermented dietary fibre (FDF) (in kg animal^{-1} d^{-1})
η_{CH4} energy content of CH_4 (η_{CH4} = 55.65 MJ kg^{-1})

A similar approach was published in Jørgensen (2011). It is used as model 3:

$$E_{\mathrm{CH4,3}}{}^{*} = a_3 + b_3 \cdot m_{\mathrm{FDF}} \quad (10)$$

where

$E_{\mathrm{CH4,3}}{}^{*}$ CH_4 emission rate obtained with model 3 (in l animal^{-1} d^{-1} CH_4)
a_3 constant (growing pigs: a_3 = 0.440 l animal^{-1} d^{-1} CH_4; sows: a_3 = 0.626 l animal^{-1} d^{-1} CH_4)
b_3 coefficient (growing pigs: b_3 = 0.0206 l (kg FDF)$^{-1}$; sows: b_3 = 0.00894 l (kg FDF)$^{-1}$)
m_{FDF} supply rate of fermented dietary fibre (FDF) (in kg animal^{-1} d^{-1})

2.2.3 Model 4: methane formation rate, feed intake and animal weight

If the diet composition is kept constant, CH_4 emissions vary with dry matter (DM) intake. This again is related to animal weight or metabolic weight. An equation is provided in Jørgensen (2011, Equation 3):

$$E_{\mathrm{CH4,4}}{}^{*} = a_4 + b_4 \cdot w \quad (11)$$

where

$E_{\mathrm{CH4,4}}{}^{*}$ CH_4 emission rate obtained with model 4 (in l animal^{-1} d^{-1} CH_4)
a_4 constant (a_4 = 1.01 l animal^{-1} d^{-1} CH_4)
b_4 coefficient (b_4 = 0.0107 l (kg LW)$^{-1}$ d^{-1})
w live weight of the animal (in kg animal^{-1})

2.2.4 IPCC (1996) approach: methane conversion ratio and gross energy intake

If feed composition is about constant, then fibre and feed intake rates are proportional to the gross energy (*GE*) intake rate of the animals. This simplification is used by IPCC (1996) without any further differentiation of animal subcategories to deduce the emission per place and year. The constant is called the methane conversion ratio (*MCR*) in the IPCC nomenclature.

$$EF_{\mathrm{CH4,\,IPCC}} = \frac{MCR \cdot GE}{\eta_{\mathrm{CH4}}} \tag{12}$$

where

$EF_{\mathrm{CH4,\,IPCC}}$ CH_4 emission factor obtained with the IPCC (1996) approach (in kg place^{-1} a^{-1} CH_4)
MCR coefficient (MCR = 6 kJ MJ^{-1})
GE gross energy intake (in MJ place^{-1} a^{-1})
η_{CH4} energy content of CH_4 (η_{CH4} = 55.65 MJ kg^{-1})

3 The recommended methodology: application of the Kirchgeßner approach to the German dataset of feed intake rates and diet composition

Based on the brief review above, we conclude that since the formation of CH_4 in the hind gut is mechanistically related to the availability of BFS and that any approach that relates emissions to this entity should be preferred over others. In Germany, the fraction of bacterially fermentable substrates in diet constituents is a standard entity listed among feed properties. Furthermore, low but nevertheless satisfactory correlations were established experimentally in Germany (see Kirchgeßner et al., 1991, and literature cited therein). Keeping in mind that the conversion of undigested carbohydrates in the hind gut also depends on the state of health of the animal and the microbial population in the gut, we nevertheless consider that the most promising method should relate the formation of methane to the undigested fibre available.

3.1 The methodology

The methodology is based on the equation reported by Kirchgeßner et al. (1991) which relates the rate of CH_4 emissions to the rate of BFS supplied to the hind gut (see Equation 7).

The rate of BFS supplied in the diet is calculated from the diet composition using Equation (13) (see Kirchgeßner et al., 2008, pg 169).

$$\begin{aligned} m_{\mathrm{BFS,\,i}} &= \\ &\eta_{\mathrm{BFS,\,i}} \cdot m_{\mathrm{DM,\,i}} = \\ &m_{\mathrm{OM,\,i}} \cdot x_{\mathrm{D,\,OM,\,i}} - m_{\mathrm{XP,\,i}} \cdot x_{\mathrm{D,\,XP,\,i}} - \\ &m_{\mathrm{XF,\,i}} \cdot x_{\mathrm{D,\,XF,\,i}} - \left(m_{\mathrm{st,\,i}} + m_{\mathrm{su,\,i}}\right) \end{aligned} \tag{13}$$

where

$m_{\mathrm{BFS,\,i}}$ rate of BFS available for fermentation in a feed constituent i (in kg animal^{-1} d^{-1})
$\eta_{\mathrm{BFS,\,i}}$ BFS content of a feed constituent i (in kg kg^{-1})
$m_{\mathrm{DM,\,i}}$ intake rate of dry matter with a feed constituent i (in kg animal^{-1} d^{-1})
$m_{\mathrm{OM,\,i}}$ intake rate of organic matter with a feed constituent i (in kg animal^{-1} d^{-1})
$x_{\mathrm{D,\,OM,\,i}}$ digestibility of organic matter in feed constituent i (in kg kg^{-1})
$m_{\mathrm{XP,\,i}}$ intake rate of crude protein with a feed constituent i (in kg animal^{-1} d^{-1})
$x_{\mathrm{D,\,XP,\,i}}$ digestibility of crude protein in feed constituent i (in kg kg^{-1})
$m_{\mathrm{XF,\,i}}$ intake rate of crude fat with a feed constituent i (in kg animal^{-1} d^{-1})
$x_{\mathrm{D,\,XF,\,i}}$ digestibility of crude fat in feed constituent i (in kg kg^{-1})
$m_{\mathrm{st,\,i}}$ intake rate of starch with a feed constituent i (in kg animal^{-1} d^{-1})
$m_{\mathrm{su,\,i}}$ intake rate of sugars with a feed constituent i (in kg animal^{-1} d^{-1})

BFS contents of single feed constituents $\eta_{\mathrm{BFS,\,i}}$ are obtained from a modified Weender analysis (cf Kirchgeßner et al, 2008, pg 22 f) and are produced as a matter of routine in German feed analysis.

The BFS content of a diet is the weighted mean of the BFS contents of its constituents:

$$\eta_{\mathrm{BFS}} = \sum\left(\eta_{\mathrm{BFS,\,i}} \cdot x_{\mathrm{i}}\right) \tag{14}$$

where

η_{BFS} mean BFS content of a diet (in kg kg^{-1})
$\eta_{\mathrm{BFS,\,i}}$ BFS content of feed constituent i (in kg kg^{-1})
x_{i} mass fraction of feed constituent i in the diet (in kg kg^{-1})

and

$$\sum x_{\mathrm{i}} = 1 \tag{15}$$

The dry matter (DM) intake rate results from the calculation of energy requirements. In Germany these are obtained from the metabolizable energy (*ME*) requirements that are calculated according to Flachowsky et al. (2006) as described in Haenel et al. (2011b).

3.2 Livestock properties

The methodology distinguishes between the following subcategories of pigs:

Sows and their litter are treated together. Energy requirements are calculated for two gravidity phases, for the lactating period and for the period between weaning and covering. The number of piglets raised, their final weight and the mean weight of the sow are taken into account.

Suckling-pigs are supplied with energy and nutrients via the sow's milk only. This milk does not contain fibre. Hence its BFS content is zero.

Weaners and finishing pigs are treated in a similar manner. Their weights and weight gains are considered as drivers for energy requirements. For details see Haenel et al. (2011b).

Boars are those mature males that are used for reproduction. The only driver in the energy demand calculations is their weight.

For an investigation in the effectiveness of phase feeding on enteric CH_4 emissions, **standard animals** were used as in Dämmgen et al. (2011b). They have properties as follows: The standard sows used in this work have a mean weight of 200 kg animal^{-1}. No weight gain is considered. 23 piglets are raised per sow per year and weaned at a weight of 8.5 kg animal^{-1}. Standard weaners have a mean weight gain of 410 g animal^{-1} d^{-1} and a final weight of 28.5 kg animal^{-1}. The number of production cycles per year takes a service and disinfection period of 8 d round^{-1} into account. Standard finishers are assumed to have a mean weight gain of 750 g animal^{-1} d^{-1} and a final weight of 110 kg animal^{-1}. Service and disinfection periods are variable. Standard boars have a mean weight of 180 kg animal^{-1}. A weight gain is not taken into account. Boars are fed sows' feed.

3.3 Composition of diets and feeding regimes in German pig production

A survey was made in 2010 and 2011 to assess the regional variation in pig feeding. As described in Dämmgen et al. (2011b), feeding experts were asked to provide typical diet compositions for the German federal states. Niedersachsen (Lower Saxony) with its high pig populations was subdivided in 11 territorial units that were uniform with respect to pig feeding. In all, 288 diets were provided, 86 for sows, 66 for weaners and 122 for finishing pigs. For sows and weaners, single and two phase feeding were investigated. Feeds for finishing pigs allowed for the consideration of single, two and three phase feeding. No special feeds for boars were reported; they are fed sow feed. In Niedersachsen, diets with reduced nitrogen contents (RAM feed [5]) are fed to some extent. The census data includes the number of animal rounds per year for each German federal state and each year from 1990 to 2009.

3.4 BFS contents of diet constituents

BFS contents of feed constituents can be extracted from the literature. For the diet constituents mentioned by the experts, the contents are listed in Table 4.

Table 4:
BFS contents of feed constituents in pig production. This Table supplements Table 1 in Dämmgen et al. (2011b).

Feed constituent		η_{BFS} kg kg^{-1}	source[1]
green meal	Grünmehl	0.270	[2]
wheat	Weizen	0.043	[1]
triticale	Triticale	0.096	[3]
rye	Roggen	0.066	[1]
barley	Gerste	0.071	[1]
oat	Hafer	0.075	[1]
CCM	CCM	0.060	[1]
maize	Mais	0.052	[1]
maize flakes	Maisflocken	0.052	[1]
millet	Hirse	0.024	[6]
linseed	Leinsamen	0.210	[3]
potato peel	Kartoffelschalen	0.167	[3]
potato chips	Kartoffelchips	0.107	[3]
cassava root meal	Maniokmehl	0.129	[1]
sugar beet pulp	Trockenschnitzel	0.664	[1]
sugar beet pulp with molasses	Melasseschnitzel	0.506	[1]
bakery waste	Backabfälle	0.159	[1]
wheat bran	Weizenkleie	0.191	[1]
rye bran	Roggenkleie	0.280	[1]
oat flakes	Haferflocken	0.079	[1]
oat bran	Haferschälkleie	0.132	[1]
wheat gluten feed	Weizenkleber	0.038	[1]
maize gluten feed	Maiskleberfutter	0.241	[1]
distillers dried grains with solubles	Weizenschlempe	0.239	[4]
maize starch	Maisstärke	0.000	[5]
maize germs	Malzkeime	0.200	[1]
apple pomace	Apfeltrester	0.260	[2]
molasses	Melasse	0.084	[1]
peanut oil	Erdnussöl	0.000	
soya oil	Sojaöl	0.000	
rape seed oil	Rapsöl	0.000	
sunflower oil	Sonnenblumenöl	0.000	

[5] RAM: Rohprotein-angepasste Mischung: mixture adjusted to crude protein demands

Continuation of Table 4:

Feed constituent		η_{BFS} kg kg^{-1}	source[1]
sugar	Zucker	0.000	[1]
peas	Erbsen	0.090	[1]
faba bean	Ackerbohne	0.079	[1]
soya bean	Sojabohne	0.152	[1]
soya protein	Sojaeiweißkonzentrat	0.000	[5]
linseed expeller	Leinexpeller	0.302	[1]
rape seed expeller	Rapsexpeller	0.213	[1]
soy pulp	Sojaschalen	0.419	[3]
rape seed extraction meal	Rapsextraktionsschrot	0.215	[1]
sunflower extraction meal	Sonnenblumen-extraktionsschrot	0.143	[6]
soya bean extraction meal 48 % XP	Sojaextraktionsschrot 48 %, getoastet	0.157	[1]
soya bean extraction meal 44 % XP	Sojaextraktionsschrot 44 %, getoastet	0.189	[1]
potato protein	Kartoffeleiweiß	0.088	[1]
sweet whey	Molke, Süß-, frisch	0.018	[1]
acid whey	Molke, Sauer-, frisch	0.095	[1]
whey protein	Molkeneiweiß, frisch	0.000	[5]
skimmed milk powder	Milchprodukte (Magermilchpulver)	0.053	[1]
whey concentrate	Molke, Süß-, getrocknet	0.003	[1]
cows' milk	Kuhmilch (Vollmilch)	0.000	[1]
fish meal 64 % XP	Fischmehl 64 % RP	0.001	[1]
yeast	Bierhefe, Weinhefe (Vinasse)	0.306	[1]
corn steep	Maisquellwasser	0.000	
fish oil	Fischöl	0.000	
lignocellulose	Lignocellulose	0.730	[2]
rice gluten feed	Reiskleber	0.038	[1]
palm butter	Pflanzenfett	0.000	
formic acid	Ameisensäure	0.000	
propionic acid	Propionsäure	0.000	
calcium phosphate	Calciumphosphat	0.000	
lime (calcium carbonate)	kohlensaurer Kalk	0.000	
sodium bicarbonate	Natriumhydrogencarbonat	0.000	
salt	Viehsalz	0.000	

[1] Sources: [1] Kirchgeßner (2004) pp 571-578; [2] Lindermayer et al. (2009), pg 134; [3] LfL (undated); [4] Lindermayer (undated); [5] calculations using Equation (12) and data provided in Beyer et al. (2004); [6] DLG (undated)

Most data in Table 4 is taken from Kirchgeßner (2004). Some feed constituents could be extracted from lists published by LfL (Lindermayer, 2009, undated: LfL, undated) or the DLG data base Futtermittel.Net.

Properties of corn steep could be deduced from information provided by the manufacturer (Beuker, undated).

Some less frequently used feed constituents were replaced by similar constituents due to missing or inconsistent data:

- maize flakes by maize
- potato chips by steamed potatoes
- soya protein by soya beans
- soya pulp by legume seed hulls
- bakery waste by wheat second flour
- fish oil by fish juice
- rice gluten feed by wheat gluten feed

η_{BFS} for all oils and fats was set zero, as they do not contain fibre or carbohydrates. The fibre content of corn steep is very low, which justifies setting η_{BFS} to zero.

3.5 Back-calculation of gross energy related methane conversion ratio

It is good practice within emission reporting to indicate MCR according to Equation (16).

$$MCR_{i,j} = \frac{EF_{CH4,i,j} \cdot \eta_{CH4}}{GE_{i,j}} \quad (16)$$

where

$MCR_{i,j}$ methane conversion ratio for subcategory i in region j (in MJ MJ^{-1})
$EF_{CH4,i,j}$ CH_4 emission factor for subcategory i in region j (in kg place^{-1} a^{-1} CH_4)
η_{CH4} energy content of methane (η_{CH4} = 55.65 MJ (kg CH_4)$^{-1}$)
$GE_{i,j}$ gross energy intake rate for subcategory i in region j (in MJ place^{-1} a^{-1})

The German inventory uses the metabolizable energy (ME) requirements to derive feed intake. Both ME and GE contents of the diets are variables. Hence the conversion of ME to GE intake rates varies with diets. The energy equivalent of CH_4 is constant.

$$GE_{i,j} = ME_{i,j} \cdot \frac{\eta_{GE,i,j} \cdot \eta_{CH4}}{\eta_{ME,ij}} \quad (17)$$

where

$GE_{i,j}$ gross energy intake rate per place for subcategory i in region j (in MJ place^{-1} a^{-1})
$ME_{i,j}$ intake rate of metabolizable energy for subcategory i in region j (in MJ place^{-1} a^{-1})
$\eta_{GE,i,j}$ gross energy content of diet for subcategory i in region j (in MJ kg^{-1})
η_{CH4} energy content of methane (η_{CH4} = 55.65 MJ (kg CH_4)$^{-1}$)
$\eta_{ME,i,j}$ metabolizable energy content of diet for subcategory i in region j (in MJ kg^{-1})

The national overall MCR for pigs is calculated as weighted mean for sows (with litter), weaners, finishing pigs and boars.

4 Results

4.1 National mean methane emission factors and methane conversion ratios

For the years 1994, 2001 and 2007, full census data (animal numbers and farm structure survey data) were available. For the feeding regimes put into practice in the respective region and year, the CH_4 emission factors (emissions per place and year) and CH_4 conversion ratios (MCR) were calculated for the four standard animals, see Chapter 3.2. The results are presented in Tables 5 and 7. Table 5 shows that the application of modelled MCR for specific diets results in elevated emission rates for sows and boars and reduced emission rates for weaners and finishing pigs as compared to default MCR (previous calculations). No significant variation between the years can be observed for sows and weaners. The reduction of emission rates for finishing pigs is attributed to an increased use of phase feeding (see below).

Phase feeding has become standard during the past decades. For sows, the diet during lactation is particularly rich in ME and poor in fibre, the diet in the non-lactating phase is rich in fibre with comparatively lower ME contents. It is normal to feed weaners two different diets with a change at a weight of about 15 kg animal^{-1}. Boars are fed the same feeds as sows. In some regions, farmers prefer to feed the lactation diet, in others the non-lactating diet.

Phase feeding and the introduction of N-reduced diets have increased significantly for finishing pigs (see Dämmgen et al., 2011b). The mean properties of the diets reported (non-weighted means) exhibit a trend. As shown in Table 6, the step from two phases to three phases clearly reduces the CH_4 emission rate per place as well as MCR. The effect of N reduced diets may be adverse; the data shown are ambiguous.

Table 7 illustrates that MCR (i.e. the fraction of GE that is lost with CH_4) is constant with time for all four standard animals.

4.2 Regional variation of methane emission factors and methane conversion ratios

The national mean MCR listed in Table 7 follow from regionally diverse data that reflect regionally different diet composition and feeding regimes. Table 8 shows the variability of EF_{CH4} and MCR in the regions considered. For boars, the large differences result from the two diet types used. Small EF_{CH4} and MCR are associated with the use of lactation diets.

Table 8 also highlights the value of regional data if emission reduction measures are to be taken.

The modelled methane emission rates agree satisfactorily with those estimated from experiments, see Chapter 1.3.

Table 5:
Methane emission factors EF_{CH4}, annual national means (values for 1994, 2001 and 2007 according to Chapters 3.1 to 3.4)

Table 6:
Finishing pigs, methane emission factors EF_{CH4} and methane conversion ratios MCR as a function of feeding strategy (2007 data set) (non-weighted means) (duration of service time: 5 d round^{-1})

Table 7:
Methane conversion ratios MCR, annual national means

	1994	2001	2007	previous calculations		unit
				modified [A]	inventory [B]	
sows (including suckling piglets)	6.3	6.4	6.4	6.0	6.0	kJ MJ^{-1}
weaners	4.3	4.4	4.4	6.0	6.0	kJ MJ^{-1}
finishing pigs	4.6	4.7	4.6	6.0	6.0	kJ MJ^{-1}
boars	7.1	7.1	7.0	6.0	6.0	kJ MJ^{-1}
average pigs [C]	4.6	4.8	4.7	6.0	6.0	kJ MJ^{-1}

[A] modified diets and feeding strategies as in Dämmgen et al. (2011b), but use of IPCC (1996) default MCR of 6 kJ MJ-1.
[B] using the methodology described in Rösemann et al. (2011) with constant diet composition and feeding strategies
[C] weighted mean taking animal populations (Rösemann et al., 2011) into account

Table 8:
Regional variation of methane emission factors EF_{CH4} and methane conversion ratios MCR (results for 2007)

Region	EF_{CH4} kg place^{-1} a^{-1}				MCR kJ MJ^{-1}			
	sows[A]	weaners	finishers	boars	sows[A]	weaners	finishers	boars
01	2.50	0.30	0.96	2.49	7.2	4.2	4.8	8.6
02	2.48	0.36	0.99	2.24	7.1	5.0	5.1	7.6
03	2.56	0.29	0.94	2.48	7.3	4.2	4.8	8.2
04	2.53	0.31	0.86	2.38	7.0	4.4	4.4	7.9
05	2.68	0.32	0.94	1.98	7.7	4.4	4.9	7.0
06	2.33	0.34	0.92	2.14	6.8	4.7	4.7	7.4
07	2.47	0.30	0.98	2.13	7.1	4.3	4.9	7.4
08	2.24	--- [B]	0.71	1.16	6.5	--- [B]	3.7	4.2
09	2.74	0.30	0.91	2.13	7.9	4.2	4.6	7.4
10	2.37	0.29	0.76	1.29	6.9	4.0	3.9	4.5
11	2.74	0.30	0.96	2.13	7.9	4.2	4.8	7.4
12	2.65	0.30	0.83	1.34	7.5	4.2	4.0	4.6
13	2.34	0.31	0.95	1.30	6.8	4.4	4.8	4.6
14	2.51	0.30	1.01	2.53	7.2	4.2	5.1	8.8
15	--- [B]	0.35	0.91	2.00	--- [B]	4.8	4.6	7.0
16	2.61	0.30	0.98	2.41	7.5	4.3	4.9	7.9
17	2.45	0.31	0.98	2.14	7.0	4.4	5.0	7.2
18	2.05	0.28	0.88	1.29	5.8	3.9	4.4	8.2
19	2.73	0.30	1.05	2.32	7.8	4.2	5.3	7.5
20	2.40	0.28	0.90	1.28	6.9	4.0	4.6	4.5
minimum	2.05	0.28	1.05	2.53	5.8	3.9	3.7	4.2
maximum	2.74	0.36	0.71	1.16	7.9	5.0	5.3	8.8
mean[C]	2.49	0.31	0.93	1.90	7.1	4.4	4.6	7.1

[A] including suckling pigs
[B] value omitted as outlier
[C] weighted mean taking animal populations (Rösemann et al., 2011) into account

5 Conclusions

Even though CH_4 emissions from pigs' enteric fermentation do not form a key category in the emission inventory, they can now be treated with a state of the art methodology. The experimental data available and the model approaches deduced from them allow for a detailed treatment.

As the effort to perform a Tier 3 approach as described here may not be justified elsewhere, a set of adequate MCR can be provided at least for Northwest European conditions. The following values are proposed (see Table 8):

- sows with litter 7.1 kJ MJ^{-1}
- weaners 4.4 kJ MJ^{-1}
- finishing pigs 4.6 kJ MJ^{-1}
- boars for reproduction 7.1 kJ MJ^{-1}

If no differentiation between subcategories is possible, a MCR of 5 kJ MJ^{-1} is considered adequate. (This mean is depending on the respective shares of animal subcategories. The German weighted mean for 2010 amounts to 4.8 kJ MJ^{-1}.)

For Germany and 2010, the application of the MCR listed in Table 8 results in an emission reduction of about 15 %, i.e. about 4.0 Gg a^{-1} CH_4 or 100 Gg a^{-1} CO_2 equivalents [6].

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Quantitative and qualitative overview and assessment of literature on animal health in organic farming between 1991 and 2011 – Part I: general and cattle

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Abstract

On the subject of "animal health in organic farming" an evaluative review and assessment of publications has been conducted. This overview is intended to provide recommendations for future research to optimize the organic farming. The literature search covers the period from 1991 to 2011. Articles from international and national magazines, some with a peer-review process, other periodicals and reports have been collected. A total of recorded 569 publications (thereof 33 % reviewed) related to animal health in organic farming were found. This included articles on general topics (42 publications), on cattle (211), on pigs (181), on poultry (100), on small ruminants (25) and on other species (10). A majority of the studies deals with "status quo - representations" (n = 222). Particularly comparative studies within organic production systems are rare (n = 86). Less than half of the investigations carried out a comparison with the aid of a control group or cohort (n = 205). Of 417 studies which originated from organic or comparable farming systems only 103 were published reviewed. In addition, there is a lack of research to examine a practical approach (n = 124). In organic cattle farming frequently the subject of mastitis has been edited. Problems such as lameness, metabolic disorders or calves' diseases were studied subordinately. The problems concerning animal health in organic as in conventional farming are comparable. In general, an implementation-oriented, but at the same time high-quality scientific research is needed to optimize the conditions for animal health and animal welfare in organic farming.

Keywords: production animal, animal husbandry, animal welfare, ecological farming, organic livestock production, organic cattle, Cattle, organic agriculture, animal health

Zusammenfassung

Quantitative und qualitative Übersicht und Bewertung der Literatur über Tiergesundheit in der ökologischen Tierhaltung zwischen 1991 und 2011 – Teil 1: Allgemein und Rinder

Dazu ist eine bewertende Zusammenfassung und Einschätzung der Veröffentlichungen durchgeführt worden. Dieser Überblick soll Empfehlungen für die zukünftige Forschung zur Optimierung des ökologischen Landbaus aussprechen. Die Literaturrecherche umfasste den Zeitraum von 1991 bis 2011. Artikel aus internationalen und nationalen Zeitschriften, aus Berichten sowie Abschlussarbeiten wurden dafür zusammengetragen. Insgesamt wurden 569 Veröffentlichungen (davon 33 % reviewed) mit Bezug zur Tiergesundheit im ökologischen Landbau erfasst, davon zu allgemeinen Themen (42 Artikel), zu Rindern (211), Schweinen (181), Geflügel (100), Kleinen Wiederkäuern (25) und sonstigen Tierarten (10). Ein Großteil der Untersuchungen beschäftigen sich mit Status quo - Darstellungen (n = 222). Besonders vergleichende Studien innerhalb ökologischer Produktionssysteme sind eher selten (n = 86). Weniger als die Hälfte der Studien führte einen Vergleich mit Hilfe einer Kontrollgruppe oder Kohorte durch (n = 205). Von 417 Untersuchungen, die aus ökologischen oder vergleichbaren Haltungssystemen stammten, wurden nur 103 reviewed publiziert. Außerdem mangelt es an Forschungsprojekten, die praktische Lösungsansätze untersuchen (n = 124). Für die ökologische Rinderhaltung wurde gehäuft das Thema Mastitis bearbeitet. Probleme wie Lahmheit, Stoffwechselerkrankung oder Kälberkrankheit wurden eher nachrangig untersucht. Die Probleme bei der Gesundheit in der ökologischen wie in der konventionellen Tierhaltung sind vergleichbar. Eine umsetzungsorientierte, aber dabei anerkannt hochwertige wissenschaftliche Forschung ist notwendig, um die Bedingungen für Tiergesundheit und Tiergerechtheit auch im ökologischen Landbau zu optimieren.

Schlüsselwörter: biologische Nutztierhaltung, ökologische Nutztierhaltung, Tiergesundheit, ökologische Rinderhaltung, Tierwohlbefinden, Rind, ökologischer Landbau

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Introduction

A major criterion for the quality of organic animal husbandry should be exemplary good animal health as part of animal welfare. This goal is one of the challenges of the future to demarcate organic from conventional animal husbandry und must be a response to increasingly critical consumer consciousness. At present a non-professional image of an idealized animal husbandry is still frequently used as a figurehead of organic agriculture. The allegedly greater animal well-being serves as an argument to purchase organic food of animal origin.

The valid European-wide regulations (see box) serve as the basis for animal husbandry oriented to the principles of animal fairness and the well-being of the domestic livestock. Animal health is to be assured by preventive measures.

Some authors doubt that animal health in organic farming is actually better than in conventional agriculture (Ruegg, 2009; Sundrum et al., 2004). Multiple studies on some aspects of organic animal husbandry and several overviews were undertaken in recent years, but generally accepted indications of better animal health could not be found.

Certain scopes of action, in accordance with organic guidelines, could promote animal health: e.g., increased movement could induce a longer life span and support easier births by ensuring a higher level of fitness of the cow; and increased well-being could cause a better fertility (intermediate calf times, culling rates, born farrows per sow and year) or could improve social behaviour (feather pecking, cannibalism).

Council regularisation (EC) NO 834/2007 and Commission regularisation (EC) NO 889/2008

[laying down detailed rules for the implementation of Council regularisation (EC) NO 834/2007] define the rules, which are European-wide binding for all organic animal husbandry. Some excerpts are represented here.

EC No. 834: *(highlighting of words by authors)*

Reasons:

(17) Organic stock farming should *respect high animal welfare standards … animal-health management should be based on disease prevention. …*

Article 3

Organic production shall pursue the following general objectives:

… aim at producing a wide variety of foods and other agricultural products that respond to consumers' demand for goods produced by the **use of *processes that do not harm*** the environment, human health, plant health or ***animal health*** and welfare.

Article 5:

… the *maintenance of animal health* by encouraging the natural immunological defense of the animal, as well as the selection of appropriate breeds and husbandry practices;

… the observance of a *high level of animal welfare* respecting species-specific needs;

Article 14

... husbandry practices, including stocking densities, and housing conditions shall ensure that the developmental, *physiological and ethological needs of animals are met;*

… *any suffering, including mutilation, shall be kept to a minimum* during the entire life of the animal, including at the time of slaughter

EC No. 889: *(highlighting of words by authors):*

Reasons:

(10) … specific housing conditions should serve ***a high level of animal welfare, which is a priority in organic livestock farming*** …

(16) *Animal-health management should mainly be based on prevention of disease. …*

Articles 10 to 18:

… *minimum surface* for indoor and outdoor areas …

… At least half of the indoor surface area … shall be solid, that is, *not of slatted or of grid* construction.

… dry bedding strewn with *litter material* shall be provided

… Operations such as attaching *elastic bands to the tails of sheep, tail-docking, cutting of teeth, trimming of beaks and dehorning* shall not be carried out routinely in organic farming. …

Mutilation such as clipping the wings of queen bees is prohibited.

Article 24:

… animals become sick or injured they shall be *treated immediately, …*

Phytotherapeutic, homeopathic products, trace elements and products listed in Annex V, part 3 and in Annex VI, part 1.1. shall be *used in preference* to chemically-synthesized allopathic veterinary treatment or antibiotics, *provided that their therapeutic effect is effective* for the species of animal, and the condition for which the treatment is intended.

If the use of measures referred to in paragraph 1 and 2 is not effective in combating illness or injury, and if treatment is essential *to avoid suffering or distress of the animal*, chemically-synthesized allopathic veterinary medicinal products or antibiotics may be used under the responsibility of a veterinarian.

However, there are also questionable regulations which could hamper good animal health: e.g., the danger of injury and infection for the animal rises with the use of an outdoor run and pasture going. In this case, increased pain, suffering and unsatisfactory animal health can be connected to the regulations as well as less food security for the final product. This could result in the necessity for more veterinary medicinal products and more disinfectants. A second example is the restriction on the number of treatments [(EC) No 889, Article 24 (4)] to only one treatment during the whole life of fattening pigs and broiler. This could influence the therapeutic efforts of farmers, followed by a negative impact on health and welfare of livestock (Koopmann et al., 2011). The priority of complementary medicine could negatively affect the timely (and thus on the disease process adapted quantity of veterinary drugs) administration of effective, chemical-synthetic medicines.

For the advancement of organic animal husbandry a detailed and systematic review and evaluation of the available appropriate announcements and publications, both in the scientific and in the non-scientific range, are necessary. These review-data should promote some justified recommendations for future research, which should support the optimisation of organic animal husbandry. This overview will focus on general aspects and the health of cattle (Part I) and on the health of pigs, poultry and others (Part II).

Methods

Firstly a literature search was done to investigate literature on animal health in organic farming. The priority laid on the main species cattle, pigs and poultry. After checking all the abstracts, on the second step, a subsample of papers was assessed completely. Selection criteria are described below.

The search included international and national journals with a peer review procedure, publications of other journals and literature of special databases on organic farming as well as diploma and doctoral theses. The literature search was conducted between July 2011 and December 2011 using the databases Medline (PubMed), ISI Web of Science and Organic Eprints. These are the major databases in the area of veterinary medicine and animal health. Access to PubMed and Organic Eprints, a database especially on organic farming, are free of charge and both are therefore usable regardless of this research project. Only articles published between January 1991 and December 2011 were included. Journal articles which were not published in English, German, French, Dutch or Danish were excluded.

Factor considered in the search on animal health in organic farming included the search terms: animal species (*cattle, dairy, swine, pig, pigs, sow, poultry, chicken, hens, laying hens, fowl, and animal*); animal housing and health (*complementary therapies, animal husbandry, animal disease, animal welfare, housing, health, animal health and position*) and organic farming. Additionally special search terms were used: *organic agriculture, organic system, organic, biological, biologically, ecological, nonconventional, extensive, low input, sustainable, agriculture research, lameness, pasture, grazing, mortality, risk factors, productivity, outdoor and enrichment*. The literature review was not limited to only organic studies. Articles on alternative or extensive farming were added as well.

The so-called grey literature (master and doctoral thesis and similar) were determined, in addition, in cooperation with German universities and advanced technical colleges. The universities and institutes were selected on the basis of their focus on agriculture and animal health.

The literature was managed using EndNote© (version X4; Thomson Reuters). An MS-Access© – database (Microsoft Office Access; 2003) was created from these data.

To ease the search in the database it is possible to sort the results according to different selection criteria. Categories for assessment, containing qualitative and content aspects, were added into this database.

The qualitative assessment considers the scientific excellence of the study and the source given. Case reports and personal opinions which do not underpin their knowledge with scientific studies are of low quality. Scientific studies, particularly those which have been carried out using a control group, posses a higher level of evidence. Articles published in peer reviewed journals belong to the group with the best quality. After a short overview, we decided that it was not useful to implement criteria like impact factor of journal, quality of statistics and number of replicates additionally. Furthermore, it was determined whether the article contains a status quo analysis or a comparison study of the different management forms (e.g. conventional or ecological).

The content based assessment considers articles with selection criteria like "species", "production systems"(e.g. milk, eggs, meat) or "disease". Subcategories were classified for certain illnesses. E.g. the category "microbiology" included articles which examine bacterial, protozoal as well as viral illnesses. An additional indexing occurs in the ecological status of the study farm (Yes, No), whether a solution attempt is offered (Yes, No) and whether future research needs are found (Yes, No).

Finally, the articles were classified by their estimated importance for concrete realization in organic husbandry. Articles which major content focuses on animal health in organic farming were included into the first category. The second category provided articles which include relevant aspects on animal health in organic farming, but with small deficiencies (e.g. the article contains two research

aims and only one is relevant to animal health, animal health in alternative farming for example on pasture is described). Studies which describe only aspects of the animal health in farms similar to organic farming were sorted into the category three.

93 articles with focus on practical aspects of organic farming and/or declared research needs were read and evaluated. 53 of those have been published in peer-reviewed journals). Finally a small evaluation-text was added to the Access © – database as well.

General Results

A total of 569 publications on animal health in organic farming were included in the Access© -database. The results provide an overview of the literature on animal health in organic farming. 423 articles care directly for farmer's practice, 127 are more concerning theoretical knowledge, 19 may interest the consumer. The number of articles on the specific species and on organic farming is shown in Table 1. Articles describing at least one organic farm were defined as an „organic" article. Accordingly articles describing, e.g., comparative studies on conventional and organic farms are counted in this category. As the search was limited not only to organic studies, articles on alternative or extensive farming were added as well. Studies which were not classified as organic could still provide valuable knowledge for animal health in organic farming as well. These studies were, e.g., on the animal health at pasture, stables with outlet or housings with particular high level of animal welfare.

Table 1:
Number of organic articles

Species	Number of articles	
	Total	Thereof "organic"
General	42	40 (95 %)
Cattle	211	156 (74 %)
Pig	181	117 (65 %)
Poultry	100	76 (76 %)
Small ruminants	25	21 (84 %)
Other	10	7 (70 %)
Total	569	417 (73 %)

The origin of the studies on only organic farming was included in Table 2. The database includes national and international literature for which the results could be relevant for German conditions. Nearly half of articles came from Germany (46 %), followed by Scandinavia (16 %) and 8 % from Switzerland and the Netherlands, respectively.

One reason could be a bias because "grey" literature from German universities was included.

Regarding the relative importance of the German market for organic food, the small part of German research publications is interesting. Austrian research on organic husbandry is probably not smaller as the Swiss one, but generates nearly half of searchable papers.

Most articles are originally written in English (n = 287) or German (n = 271).

Table 2:
Origin organic articles

Country	Organic articles	Percent
Germany	192	46 %
Denmark	43	10 %
Netherlands	32	8 %
Switzerland	28	7 %
United Kingdom	22	5 %
Schweden	20	5 %
Austria	17	4 %
more than one country	33	8 %
others	30	7 %
Total	417	100 %

However, not only the number of the studies is important, but also the quality of source (Table 3). Journals with a „peer review" procedure should ensure high quality. 33 % of retrieved articles met that criterion. Only 18 % of the studies done on organic farms are published with high quality.

Table 3:
Quality of the literature source

Source Quality	Number of articles (thereof "organic")	Percent (thereof "organic")
Peer-Review	186 (103)	33 % (18 %)
Scientific	375 (311)	66 % (55 %)
Other	8 (3)	1 % (0,5 %)
Total	569 (417)	100 % (73,5 %)

The second category „Scientific" included scientific conference contributions (which could be sometimes a personal opinion), final reports and scientific works as for example theses. This category includes the greatest amount of literature. Conference contributions are often a short description of a study and therefore sometimes insufficient. Final works, like theses, are mostly accessible in libraries and less often found in online databases. Therefore, their propagation and knowledge transfer is more

difficult. The category "Other" contains publications in non-scientific technical journals.

This result indicates that more studies on organic farming should have a high scientific quality which allows to publish reviewed.

The procedures of the study itself were assessed as well. For our assessment it was important whether farming systems have been compared (Table 4). Possible differences were examined between alternative and conventional systems and between organic systems. Comparative studies in-between organic farms could be important to improve the animal health. However, this category was rare in the available literature (15 %). Most of studies describe the present state. Their results recognise problems, but no solutions or improvements can be examined.

Table 4:
Type of the studies

Study procedure	Number of articles (thereof "organic")	Percent (thereof "organic")
Status quo analysis	222 (174)	39 % (30,5 %)
Comparison conventional x conventional (alternative)	69 (0)	12 % (0 %)
Comparison conventional x organic	75 (73)	13 % (13 %)
Comparison organic x organic	86 (86)	15 % (15 %)
no classification	117 (84)	21 % (15 %)
Total	569 (417)	100 % (73,5 %)

Table 5:
Quality of Studies

Study quality	Number of articles (thereof "organic")	Percent (thereof "organic")
Meta-analysis	3 (2)	1 % (0,5 %)
Review	41 (26)	7 % (5 %)
study with control group	205 (139)	36 % (24 %)
study without control group	211 (153)	37 % (27 %)
Case report	14 (12)	2 % (2 %)
Personal opinion	21 (15)	4 % (3 %)
no classification	74 (70)	13 % (12 %)
Total	569 (417)	100 % (73,5 %)

The quality of study-design could be very different (Table 5). Studies which compare different groups with each other have a high likelihood of valid results. The groups can either based on given circumstances (farms with stable or pasture are compared) or be determined by the test protocol (two different strategies of treatment are examined on a farm). Only 24 % of studies on organic farms belong to this category. Sometimes case reports and personal opinions which are of low quality as they do not underpin their knowledge with scientific studies, are published as „Scientific" – paper, e.g. in a conference report.

In the future it will be most important to have results from comparative studies of high quality, to clarify free of doubt the advantages of organic farming system and the best organic practice available for the farmer.

Some publications propose a way to resolve problems (n = 124). 74 out of those are done on organic farms.

59 articles describe a successful solution for the focussed problem. The main topics of these publications are specific illnesses and conditions of husbandry with 47 % (n = 28), respectively. Species in these articles are represented as pigs 41 % (n = 24), cattle 30 % (n = 18), and poultry 29 % (n = 17).

296 (52 %) of all articles may have a high relevance and importance, 149 (26 %) may have a good relevance for solutions in organic animal health problems.

A version of the created Access© -database will be accessibly free of charge at the website of the vTI.

Results of literature search on health of cattle

78 (37 %) of the 211 publications concerning cattle were published in a scientifically reviewed journal. A predominant part of 81 % (n = 170) from all retrieved articles deals with health problems of dairy cows. Only 9 % of the articles concern with young cattle and 5 % with mother cows. Following the search order, the main focus of retrieved publications lay on the investigation of "illnesses" (62 %, n = 130). The remaining articles focus aspects of "housing" (26 %, n = 55), improvement of "performance" (9 %, n = 18) or "genetics" (3 %, n = 7).

Key aspects of publications on "illnesses" are presented in Table 6.

Table 6:
Illnesses of cattle differentiated by main aspects

Main aspect	Number of publications (n)
udder health	56
disease of locomotive system	14
parasites	12
metabolism disorder	11
problems of reproduction	8
other infections	7
without classification	13
others	9
Total	130

Review of selected articles on health of cattle

In the following section the main conclusions of the retrieved articles on health of bovine livestock are drawn.

The combination of an expanding organic dairy production and limited and inconsistent valuable publications on animal health means that additional studies are an urgent issue. The most important health issue in dairy cows in the western world is poor udder health, often expressed as mastitis (Fall et al., 2009). Many studies have been conducted on udder health in organic husbandry. Similar to conventional management, in organic herds most cows were culled due to poor udder health (Ahlman et al., 2011). Still, studies clarify that the incidence of clinical mastitis treated by a veterinarian were lower in organic herds than in conventional herds (Bennedsgaard et al., 2003; Hamilton et al., 2006), but often organic farmers do not use the same criteria to detect and treat clinical mastitis (Ruegg, 2009). However, substantial variations exist both between and within farm types (Langford et al., 2009). On the other hand, one study indicates that the same udder health status was achieved in organic or conventional management types, which could imply that organic farmers perform a better udder health management as they achieved this status with restrictive antibiotic policy (Fall et al., 2009). But it could be as well the result of either a truly better udder health or lack of treatment and poor bookkeeping routines (Fall et al., 2009; Hamilton et al., 2006; Garmo et al., 2010). In general, the evaluation of veterinary treatments has been described as a questionable indicator of herd health, because of different treatment thresholds for different farmers and diseases (Bennedsgaard et al., 2003). No differences were found when investigating the time /number of veterinary-treated cases of mastitis between organically and conventional managed cows (Fall et al., 2009).

In the future high quality studies should be performed, e.g., with a control group. They should focus as well on determining animal health with clinical and bacteriological examinations. Furthermore, the complete use of antibiotics and other medications should be monitored. Antibiotics were regularly used for dry-cow management in 65 % of all study farms, although the regulations for organic dairy production prohibit the application of these substances for other than curative reasons (Busato et al., 2000). This leads to the conclusion that especially investigations of wise dry cow management in organic farming without setting back animal health should be carried out.

More high quality research is needed to further elucidate the mechanisms and the associations between high levels of concentrates and udder disease in dairy cows (Hamilton et al., 2006).

Furthermore, little data is available supporting the clinical efficacy of alternative treatments (Ruegg, 2009). Organic farmers reported varied success rates for alternative treatments and many farmers were gathering the information on homeopathic remedies and other alternatives from sales representatives (Langford et al., 2009). On the other hand Klocke et al. (2010) indicate a way of implementing "Comparative Alternative Medicine" in routine treatment processes. Anyhow, there is no indication that a presumably lower use of antibiotics in organic dairy farms has resulted in frequency of antibiotic resistance considerably different from conventional farms (Busato et al., 2000a, Roesch et al., 2006). In addition, measures to postpone the spread of resistance against antimicrobials should be examined in organic farming as well.

No differences were found in general health and longevity between organically managed cows and conventionally managed cows (Fall et al., 2008). This similarity between organic and non-organic farms in most respects indicates that cow housing and health on organic dairy farms is neither compromised by the regulations, nor considerably better than on non-organic farms (Langford et al., 2009).

The available literature shows a low amount of studies about animal health of calves, young stock and of mother cows. Future studies in these areas would be important to recognise and avoid problems in these production systems. Sometimes housing of young stock in organic farming could be improved. Meanly inadequate equipment and barns could cause poor hygiene (Hamilton et al., 2002). Organic producers reported more frequently than conventional farmers that they had observed poor weight gain and/or diarrhoea in their calves in the first grazing season, probably due to parasite infections (Svensson et al., 2000). Furthermore, future research could lie as well in the problem of cross suckling of calves in relation to group housing (Georg et al., 2004).

Overall, the prevalence of hock lesions and hock swellings was less on organic as compared with nonorganic farms (Rutherford et al., 2008). Additionally, cows housed on straw and under organic management have the lowest chance of being lame (Rutherford et al., 2009; Barker et al., 2010). The studies showed substantial variation between the top performing and the poorest herds, within any farm type. This indicates that organic certification, although it has a beneficial impact, does not always necessarily ensure cow comfort (Rutherford et al., 2008). Further efforts are needed to develop new, and to optimize existing housing systems so that they meet the animal welfare requirements in organic dairy farms (Busato et al., 2000b). Additionally, breed can be a variable to reduce lameness, as pure HF-herds show higher lameness prevalence (Barker et al., 2010). Further studies to monitor breeds which may be better adapted on organic farming conditions could be interesting.

In organic farming parasites were common, even if not always clinically evident (Hamilton et al., 2002). Svensson

et al (2000) showed in a questionnaire study that there were no differences between organically and conventionally raised cattle, in terms of the presence of parasite-induced damages at slaughter. Better knowledge about the effects of strategic nutritional supplementation on the level of parasitism in young cattle needs to be attained through further experimentation (Höglund et al., 2001). Future studies should focus on pasture grazing from the perspective of the parasite load on cattle, including targeted treatment systems to reduce the amount of deworming drugs and to postpone the spread of anthelmintic resistant worm populations in organic farming (Höglund et al., 2009; Kleinschmidt et al., 2010)

Bidokhti et al. (2009) suggest that organic farm management may be effective in reducing virus infections relative to conventional farming methods. However, further studies will be required to substantiate this hypothesis. Furthermore, possible risk factors at organic farms, e.g., passenger traffic and less efficient disinfection should be analysed, as e.g., no disinfectant effective against *Coccidia spp.* oocysts is allowed in organic farming (Koopmann et al., 2011).

However, organic farming is to demonstrate an improved animal health and welfare compared to mean conventional farms. That's why research is needed about the possibilities of management or housing to improve animal health (e.g., mastitis, lameness) in organic farming. Additionally, necessary requirements should be available before a farm gets certified for organic production, where animal welfare is expected to be a top priority (Hamilton et al., 2002). However, the assessment of animal health is a difficult task in the absence of obvious standard health indicators (Fall et al., 2009). There is a lack of implementable and standardized methodology to measure animal welfare especially in larger populations (Busato et al., 2000b). To measure wellbeing by indicators the Welfare Quality®-project gives advises (Welfare Quality® consortium, 2009). It lacks until now the implementation of proposed indicators into the daily work of advice- and control bodies and the financial appreciation of animal welfare. Therefore, it is an important task for the future to develop high quality scientific studies which define objective indicators to perceive and quantify animal health in organic farming.

Conclusion and Outlook

High-quality studies in organic husbandry are rare, mainly comparative studies with control groups within the organic system are essential to perform in the future.

To achieve no bias of the results, data should be raised increasingly on the basis of scientific investigations and in a scientifically controlled environment. Status quo analyses matter to receive actual data from farms and to recognise the existing problems. Nevertheless, universally valid statements concerning the future improvement of animal health in organic husbandry are hard to be pulled out of these data because of the bias caused by a great amount of diversities between the different farms. Even as some of these differences may be measurable some are not e.g. the personal traits of the farmers. Studies to investigate solution attempts are lacking as well.

The research needed on health in organic cattle farming may arise from gaps of knowledge on:

- Improvement of management and housing
- Prevention and treatment of infections
- Parasite's prevention and treatment
- Lameness and respiratory illness
- Studies on young stock and feeder cattle as well as mother cows
- Studies on hornless cattle

In general, similar problems on animal health exist for both organic farming and conventional farming. Beside the high influence of individual management skills of the owners, organic husbandry has to have an outstanding good status of animal health as a basic goal. The realization of that goal would exclude all possibilities of doubts and meet the consumer's perceptions. Finally, objective indicators especially on good animal health and welfare are needed to implement quantitative criteria which could help all those farmers who realise completely an outstanding good status of animal health, in example by financial support.

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The authors have no financial or personal relationships with other people or institutions that could have influenced or biased the content of this review.

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Quantitative and qualitative overview and assessment of literature on animal health in organic farming between 1991 and 2011 – Part II: pigs, poultry, others

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Abstract

On the subject of "animal health in organic farming" an evaluative review and assessment of searchable publications has been conducted. This overview is intended to provide recommendations for future research to optimize the organic farming. General results are described in Part I (Simoneit et al., 2012): 569 publications (thereof 33 % reviewed) related to animal health in organic farming were found. This included articles on general topics (42 publications), on cattle (211), on pigs (181), on poultry (100), on small ruminants (25) and on other species (10). In organic pig farming the health of fattening pigs, sows and piglets together account for only 57 % of articles. Mortality of piglets and poor body condition of sows after weaning, parasites and other difficulties tributary to free-range-system are described with priority. The remaining publications attend to general problems. Articles concerning health in poultry farming attend mostly to layers (54 %) and to broilers (26 %). E.g., feather pecking and hygienic problems due to the free-range system are described. Health especially in organic small ruminants and other minor species is described rarely. The problems concerning animal health in organic as in conventional farming are comparable. In general, an implementation-oriented, but at the same time high-quality scientific research is needed to optimize the conditions for animal health and animal welfare in organic farming.

Keywords: production animal, animal husbandry, animal welfare, ecological farming, organic livestock production, organic pigs, pigs, organic poultry, poultry, organic agriculture, animal health

Zusammenfassung

Quantitative und qualitative Übersicht und Bewertung der Literatur über Tiergesundheit in der ökologischen Tierhaltung zwischen 1991 und 2011 – Teil 2: Schweine, Geflügel, andere

Dazu ist eine bewertende Zusammenfassung und Einschätzung der recherchierbaren Veröffentlichungen durchgeführt worden. Dieser Überblick soll Empfehlungen für die zukünftige Forschung zur Optimierung des ökologischen Landbaus aussprechen. Allgemeine Ergebnisse sind in Teil I (Simoneit et al., 2012) beschrieben: Insgesamt wurden 569 Veröffentlichungen (davon 33 % reviewed) mit Bezug zur Tiergesundheit im ökologischen Landbau erfasst, davon zu allgemeinen Themen (42 Artikel), zu Rindern (211), Schweinen (181), Geflügel (100), Kleinen Wiederkäuern (25) und sonstigen Tierarten (10). Krankheiten von ökologisch gehaltenen Mastschweinen, Sauen und Ferkeln werden in insgesamt 57 % der Artikel genannt. Ferkelsterblichkeit und mangelhafte Kondition der Sauen beim Absetzen, Parasiten und andere Probleme, die durch das Auslaufsystem begründet sind, werden vorrangig beschrieben. Artikel über die Gesundheit in ökologischen Geflügelhaltungen beschäftigen sich hauptsächlich mit Legehennen (54 %) und Masthähnchen (26 %). Federpicken und Hygieneprobleme im Auslauf werden beschrieben. Gesundheitliche Probleme speziell bei ökologisch gehaltenen, kleinen Wiederkäuern und anderen Tierarten werden selten beschrieben. Die Probleme bei der Tiergesundheit in der ökologischen wie in der konventionellen Schweine- und Geflügelhaltung sind vergleichbar. Allgemein ist eine umsetzungsorientierte, aber dabei qualitativ hochwertige wissenschaftliche Forschung notwendig, um die Bedingungen für Tiergesundheit und Tiergerechtheit auch im ökologischen Landbau zu optimieren.

Schlüsselwörter: biologische Nutztierhaltung, ökologische Nutztierhaltung, Tiergesundheit, ökologische Schweinehaltung, ökologische Geflügelhaltung, Schweine, Geflügel, Tierwohlbefinden, ökologischer Landbau

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Introduction

A literature search and assessment was performed to achieve an overview on the published knowledge about animal health in organic farming in the period between 1991 and 2011. A number of 596 articles were retrieved; thereof 186 were published in reviewed journals. Detailed introduction, methods, general results and results on cattle are previously published in Part I of this review (Simoneit et al., 2012). The objective here is the presentation of results on pigs, poultry and other species.

Results of literature search on health of pigs

31 % (n = 56) of the 181 publications retrieved concerning pigs were published in a scientifically reviewed journal. A part of 29 % (n = 52) from all retrieved articles occupies with health problems in fattening pigs, 16 % (n = 30) with piglets and 12 % (n = 21) with sows. A high amount of publications was not assigned to a certain production system (43 %, n = 78).

Following the search order, the main focus of retrieved publications lay on "illnesses" (57 %, n = 104). 36 % (n = 65) concerns with "housing".

Within the articles concerning illnesses the "parasites" (27 %, n = 28) and "infection diseases" (20 %, n = 21) were predominate subcategories. Problems of the "digestion system" (n = 5) and of the "respiratory system" (n = 4) are described less frequently. Items like "reproductive system", "metabolism disorders", and "lameness" included the fewest number of publications. Most articles were not to assign to a certain disease (30 %, n = 31).

Review of selected articles on health of pigs

In the following section the main conclusions of the retrieved articles on health of swine livestock are drawn.

Published research results in organic pig production based on special organic conditions and on surveys with a satisfactory number of observations are scarce (Bonde et al., 2004).

In piglet production deficits exist in studies on housing, hygiene and health management (Werner et al., 2008). The increased losses of piglets in organic farms compared to conventional pig farms must be seen as an indicator for lower animal health status (Dietze et al., 2007; Bonde et al., 2006). Further studies on the influence of litter size on piglet mortality (Prunier et al., 2010) and on the reduction of this problem, e.g., the development of a special breeding program, should be done.

The incidence of poor body condition at weaning, especially in young sows, has to be reduced in organic sow herds. However, this cannot exclusively be explained by the later weaning in organic farming (Kongsted et al., 2009). Results on influences of this lower body condition and their conclusions are needed. In swine production the financial pressure is obvious and drives the management decisions. The needs of an animal-welfare-based organic pig meat production should be scientifically evaluated including all financial implications.

Due to the free-range and litter systems, high endoparasite infection levels are often found in organic pig production (Carstensen et al., 2002; Roepstorff et al., 2011; Giessen et al., 2007). Even the simple presence of endoparasites induces a reduction in body weight (Kipper et al., 2011). This result should concern organic farming as well. Important research needs exist in the prevention of the parasite load in organic farming. Possible risk factors, e.g. of the environment or management, should be analysed. Animal husbandry following the 'all in / all out' concept, enabling an effective hygiene regime, was rare in organic farming (Dietze et al., 2007). However, no significant connection could be determined between hygiene management and the load with endoparasites (Werner et al., 2011; Werner et al., 2009). Therefore, there is a need in organic farming to develop new strategies for endoparasite control (Roepstorff et al., 2011). The usefulness of developing risk scores and the actual presence of infectious parasites in meat has to be reviewed (Kijlstra et al., 2007). The risk of transmission of endoparasites from wild animals to free-range pigs should be studied (Giessen et al., 2007) and we have to keep in mind, that especially *Trichinella spp.* in wild boar is increasing (via rats to farm pigs). Furthermore, many studies have been performed on the mechanisms behind the biological control of flies, but it is still largely unknown whether these methods pose threats to farm animal health or food safety (Meerburg et al., 2007). Leirs et al. (2004) provided a number of suggestions for preventative rodent control which need to be established in specific and controlled experiments. Difficulties exist as some organic farmers are reluctant towards stringent rodent control using rodenticides and/or traps since they consider these animals an integral part of the farm ecosystem (Kijlstra et al., 2008). Additionally, future research of other factors which may influence the transmission of parasites in outdoor housing such as pasture design (e.g., trees) or weather conditions should be carried out (Thomsen et al., 2001). Further research is needed to overcome a potential infection risk from contaminated soils by soil treatments such as ploughing (Jensen et al., 2006 a, b). In general, infection with parasites remains a major problem in organic pig farming and special studies to solve these problems are still missing.

Outdoor pig production may imply increased exposure to microbiological pathogens as well as a risk of attracting emerging diseases of zoonotic importance.

In housing on straw with outdoor access more lameness and illnesses of the respiratory systems as in conventional farming were found (Badertscher et al., 2002). Another study reveals no major differences between conventional and organic farming (Werner et al., 2008). Furthermore, the prevalence of leg disorders and other clinical diseases in outdoor herds might be under-estimated due to difficulties in diagnosing the problems in extensive production systems (Bonde et al., 2004). Better monitoring systems are needed in order to improve animal welfare in organic pig farming (Bonde et al., 2006). The routine control of risk factors at regular intervals to prevent lameness may be an option (Bonde et al., 2004). Other options are breeding for disease resistance and a good locomotive foundation or, in general, choosing more robust breeds in the production system (Bonde et al., 2004; Badertscher et al., 2002). Research for the development of animal-adapted production facilities better suited to control infectious diseases is needed as well as optimisation in feeding, hygiene and animal handling in relation to animal health and welfare (Bonde et al., 2006). Furthermore, alternative treatments against a wide range of disorders have no scientific justification (Hameenoja, 2001). Therefore, the need for further research on alternative prevention and treatment is evident.

Results of literature search on health of poultry

The literature search on animal health in organic poultry farming retrieved 100 articles, thereof were 52 % reviewed published. In the sum of all retrieved articles the production system "laying hens" was dominant (54 %). A part of 26 % occupies with "broiler", 6 % with "turkeys". 14 publications were not assigned to a certain production system. 20 articles attend to the item "feather pecking".

The main focuses of the publications lay in the area "illnesses" (48 %) and were followed by disorders caused by "housing" (31 %) or "genetics" (17 %).

Within the articles which concern illnesses "infectious diseases" (46 %, n = 22) were found most often. Further articles focus, for example, on "parasites" (n = 9).

Review of selected articles on health of poultry

In the following section the main conclusions of the retrieved articles on health of poultry are drawn.

Respective to the importance of poultry farming there is only a limited number of scientific publications related to animal health and welfare (Berg, 2001).

Contradictory results exist on the influence of housing systems on *Salmonella spp.* prevalence in organic chickens (Bailey et al., 2005; Van Hoorebeke et al., 2011). Still the true underlying mechanisms causing the prevalence of *Salmonella* in organic farming remains unknown, because hen breed, condition of the equipment, disease status of the flock, rodent and insect load, etc., all contribute to the complexity of this issue (Van Hoorebeke et al., 2011). *Campylobacter* is highly prevalent in both organic and conventional poultry production systems (Luangtongkum et al., 2006). No evidence is provided that the free-range environment represents a major source of contamination which indicates that other potential sources should be examined (Colles et al., 2008). Future studies should develop alternative strategies to decontaminate the free range (Colles et al., 2008). There is a need for information about biosecurity, disease detection and disease prevention on organic poultry farms (Berg, 2001; Allen et al., 2011). However, the pathogens of organically raised poultry and turkeys seemed to be more susceptible to antimicrobials (Luangtongkum et al., 2006; Cui et al., 2005).

It is questionable if the prolonged withdrawal periods after pharmaceutical treatment in organic farming, which could lead to a possible under-treatment of diseases, followed by unnecessary suffering, is an issue in organic poultry as well (Berg, 2001). Similar problems may arise due to the fact that only a single treatment is allowed in organic broiler husbandry (Koopmann et al., 2011).

Mortality in laying hens in organic farming is high (Hegelund et al., 2006). Cannibalism is one of the causes of death in laying hens housed in free range systems (Fossum et al., 2009). Furthermore, a high proportion of feather pecking exists (Berg, 2001). Jones et al. (2002) explored enrichment devices to reduce feather pecking, but research is needed on whether this would reduce inter-bird pecking among herds known to contain feather peckers as well. Further studies on the type of feeding equipment, air quality, light and sound quality, as well as the role of changes between rearing and laying environment to reduce feather pecking would be valuable (Drake et al., 2010). It would be very interesting as well to monitor the amount of case-by-case exceptional permissions for beck-trimming in organic layers, as this could be an indicator for animal management which respects successfully animal needs.

Contradictory results to the foot health in organic poultry production exist (Berg, 2001; Pagazaurtundua et al., 2006). Further studies should review the influence of the breed, slaughter age and weight of birds on foot pad dermatitis (Pagazaurtundua et al., 2006). In general, it should be investigated what management tools are required to improve animal welfare in organic poultry farming (Hegelund et al., 2006).

Furthermore, future studies should examine factors which affect the hens' use of the run to create a more attractive environment (Hegelund et al., 2006; 2005). Alternative methods of sanitation procedures for the run should be developed as well (Berg, 2001).

Parasite infections present a major problem in the production of organic chickens (Thamsborg et al., 1999; Swinkels et al., 2007; Jansson et al., 2010; Hegelund et al., 2006). However, the significance of these infections in terms of disease and production losses on organic farms has not been assessed (Thamsborg et al., 1999). Future studies should be carried out to obtain a dataset on biosecurity measures (Jansson et al., 2010). Thamsborg et al. (1999) described some procedures for parasite control without a heavy reliance on antiparasitic drugs, but future studies are needed to confirm their efficacy under practical farming conditions.

Further on, aspects of adequate organic feeding while maintaining animal health (Hörning et al., 2003) and the effects of the extended growing period (Berg, 2001) should be examined. Future research should review the practical use of slow growing breeds and possible subsequent problems. Additionally, many studies have been conducted that have led to a variety of possible animal welfare indicators, but not all are ready to use (Bokkers et al., 2009). Therefore, the development of objective indicators to capture animal welfare in poultry is of great importance.

Studies on animal health in turkeys and water fowl are rare and should therefore be carried out increasingly.

Results of literature search on health of small ruminants and other species

Apart from the main search focus on the species cattle, pig and poultry, 25 articles were retrieved on health of sheep (n = 8), goats (n = 9) and small ruminants in general (n = 8). Thereof the production systems "milk" (52 %, n = 13), "landscape conservation" (16 %, n = 4), and "meat" (12 %, n = 3) are represented. The main health problems depend on parasites (n = 13).

Further on, 10 articles about other species as bees, fish and rabbits were found.

Review of selected articles on health of small ruminants and other species

In the following section the main conclusions of the retrieved articles on health of small ruminant livestock and other species are drawn.

Parasitic nematodes are a major threat to the health and welfare of small ruminants (Rahmann et al., 2007; Ganter et al., 2005). Future research needs exist above all in alternative prevention and the treatment of parasites. For example, the need to identify the role of dietary protein on the resilience and resistance of growing kids is evident (Hoste et al., 2005).

Very little literature is available on the health of lambs. A balanced feeding for small ruminants especially in the fattening period needs to be reviewed (Humann-Ziehank et al., 2006). The development of adequate housing of horned goats and studies which matter on the breeding of genetically hornless breeds should be the subject of further investigations (Waiblinger et al., 2011). Additionally, studies on alternative treatments as well as studies to improve animal health in the areas of infectious illnesses, illnesses of the digestive and respiratory system are important (Humann-Ziehank et al., 2006).

Publications on other species in organic farming (n = 10) such as rabbits (n = 5), bees (n = 3) and fish (n = 2) were rare. In contrast to all other species, the main focus was on "housing." Further research on these species is needed. Especially studies on a balanced feeding of rabbits and breeds adapted to organic farming should be carried out (Mergili et al., 2010). In fish a research need exists on alternative animal feed for carnivore fishes, developing methods to protect fish against predators and on alternative prevention and treatment strategies (Bergleiter et al., 2004).

Conclusion and Outlook

High-quality studies on organic swine and poultry husbandry are rare, mainly comparative studies with control groups within the organic system are essential to perform in the future.

As mentioned in Part I of this review data should be raised increasingly on the basis of scientific investigations and in a controlled environment. Status quo analyses matter to receive actual data from farms and to recognise the existing problems. Nevertheless, universally valid statements concerning the future improvement of animal health in organic husbandry are hard to be pulled out of these data because of the bias caused by a great amount of diversities between the different farms. Even as some of these differences may be measurable some are not e.g. the personal traits of the farmers.Studies to investigate solution attempts are lacking as well.

The research needed on pig-, poultry- and minor species-health in organic farming arises from the gaps of knowledge on the following questions:

- Improvement of management and housing
- Prevention and treatment of infections
- Parasite prevention and treatment
- Lameness and illnesses of the respiratory system
- Mortality of piglets
- Studies on organic turkeys and water fowls as well as fish and other minor species
- Conversion of slow growing breeds in organic poultry
- Studies on hornless goats.

In general, similar problems on animal health exist for both organic and conventional farming. Beside the high influence of the individual management skills of the owners, organic husbandry has to have an outstanding good status of animal health as a basic goal. The realization of that goal would exclude all possibilities of doubts and meet the consumer's perceptions. Finally, objective indicators especially on good animal health and welfare are needed to implement quantitative criteria which could help those farmers who realise completely an outstanding good status of animal health, in example by financial support.

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Influence of Vitamin B_{12} and Cobalt on performance of laying hens

Ingrid Halle and Mohammad Ebrahem*

Summary

The effect of Vitamin B_{12} and Cobalt (Co) on laying performance, egg composition, and organ mass and haematocrit was studied in laying hens. A total of 216 laying hens were allocated to 8 dietary treatment groups (0/5/10/20 µg vitamin B_{12} per kg, 0.6 mg Co, 0.6 mg Co + 5 µg B_{12}, 0.6 mg Co + 10 µg B_{12}, 0.6 mg Co + 20 µg B_{12}) and fed for 13 laying months (364 days). The daily feed intake per hen was significantly increased in the mean value of the total laying period of the 5 µg B_{12}-Group compared to the other B_{12}-Groups and the Control. The laying intensity was ranged between 90 to 93 % and was not different between groups. The egg weight and the daily egg mass production were significantly increased with supplementation of vitamin B_{12} (all groups) and B_{12} x Co. Feed conversion was significantly improved in the group with 5 µg B_{12} per kg feed and also the combination B_{12} x Co. The supplementation of 5 to 20 µg B_{12} to the diet significantly increased the final hen body weight. Co alone without B_{12} in the diet significantly increased the mass of the eggs and did not change the other laying parameters. Supplementation of diets with vitamin B_{12} or Co or both additives didn't directly improve the egg composition and the yolk colour. The results of slaughtering showed that supplementation of 20 µg B_{12} the liver mass decreased significantly and the haematocrit content increased significantly compared to the Control and the 0.6 mg Co-Group. The supplementation of 0.6 mg Co significantly increased the spleen mass. The results allow the conclusion that a supplementation of 5 µg vitamin B_{12} per kg laying hen feed was sufficient to compensate the deficiencies on feed intake and laying performance. An additional supplementation of Co to feed does not have additional advantages for the birds.

Keywords: Vitamin B_{12}, Cobalt, laying hen, laying performance, egg composition, haematocrit

Zusammenfassung

Einfluss von Vitamin B_{12} und Kobalt auf die Leistungsentwicklung von Legehennen

Der Einfluss der Supplementierung von Vitamin B_{12} und Kobalt (Co) auf die Leistungsentwicklung, die Eizusammensetzung, die Organgewichte und den Anteil an Hämatokrit am Volumen des Blutes wurde an Legehennen untersucht. Im Versuch über 13 Legemonate (364 Tage) wurden 216 Hennen (LB) in 8 Versuchsgruppen (0/5/10/20 µg Vitamin B_{12} per kg, 0,6 mg Co, 0,6 mg Co + 5 µg B_{12}, 0,6 mg Co + 10 µg B_{12}, 0,6 mg Co + 20 µg B_{12}) eingeteilt. Der tägliche Futterverzehr der Hennen der Gruppe mit 5 µg Vitamin B_{12} per kg war signifikant höher im Vergleich zu den weiteren B_{12}-Gruppen und der Kontrolle. Die Legeintensität von 90 bis 93 % war zwischen den Gruppen gleich. Sowohl das Eigewicht als auch die tägliche Eimasseproduktion verbesserten sich statistisch gesichert durch die Supplementierung von Vitamin B_{12} oder der Kombination aus B_{12} + Co ins Futter. Die Zugabe von 5 bis 20 µg Vitamin B_{12} hatte einen signifikant positiven Einfluss auf die Lebendmasse am Versuchsende. Die alleinige Zugabe von Co ins Futter erhöhte gesichert das Eigewicht und veränderte nicht die weiteren Legeparameter. Die Eizusammensetzung und die Dotterfarbe wurden weder durch die Zugabe von B_{12} noch Co verändert. Die Ergebnisse der Ausschlachtung zeigten, dass durch die Zugabe von 20 µg Vitamin B_{12} das Lebergewicht signifikant reduziert und der Gehalt an Hämatokrit gesichert höher war im Vergleich zu Hennen der Kontrolle und der 0,6 mg Co Gruppe. Die Supplementierung von 0,6 mg Co erhöht signifikant das Gewicht der Milz. Die Ergebnisse lassen die Schlussfolgerung zu, dass eine Supplementierung von 5 µg Vitamin B_{12} pro kg Hennenfutter ausreichend war und eine zusätzliche Co Supplementierung keinen additiven Einfluss hat.

Schlüsselworte: Vitamin B_{12}, Kobalt, Legehenne, Legeleistung, Eizusammensetzung, Hämatokrit

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Vitamin B_{12} belongs to a specific group of Cobalt containing corrinoids with biological activity in humans and animals. The term vitamin B_{12} is restricted to recommended biochemical nomenclature for a form of cobalamin. Cobalamin does not occur in plants, it is synthesized in nature by certain bacteria, fungi and algae (Green, 2005). Microbes in the rumen of ruminants incorporate Co into a corrin ring to form vitamin B_{12}. Poultry obtain their B_{12} either pre-formed in feed or indirectly by ingesting faeces. Vitamin B_{12} is available commercially as cyanocobalamin for addition to feed. Vitamin B_{12} is an essential component of several enzyme systems that carry out a number of very basic metabolic functions in the animal's body (McDowell, 1989). This vitamin plays a central role in normal functions of the brain and nervous system, in the homocystein metabolism, in the blood function, energy metabolism, cell division and function of the immune system (EFSA 2009 a, 2010). 4 µg per kg feed are indicated by the NRC (1994) and 9 µg B_{12} per kg by the GfE (1999) as the requirement of laying hens.

There is no evidence that monogastric animals (pigs and poultry) require Cobalt (Co) other than through vitamin B_{12}. Consequently, there is no need for any Co supplementation to the feed for these animals (EFSA, 2009 b). Although Co is not necessary for the nutrition of laying hens, it is often added into the vitamin-mineral premix.

The objective of this study was to determine the effect of a Vitamin B_{12}-free diet supplemented with Vitamin B_{12} or/and Cobalt on laying hen performance.

Material and Methods

A total of 216 laying hens (LB) were used in the feeding study. The hens were allocated to 8 groups of 26 hens each (Table 1). The hens were kept individually in a cage battery. The study commenced when the hens were 22 weeks old and continued until the 13th laying month. The basal diet was Vitamin B_{12}-free and contained Co from only natural sources (wheat, barley, corn, soya bean meal). The microbiological analyses (LUFA Speyer, LUFA SP 22013) of the control diet yielded a value of 8 µg B_{12} per kg. This value resulted from microbiological contamination of the feedstuffs. The basal diet was supplemented with 0/5/10/20 µg B_{12} per kg, 0.6 mg Co, 0.6 mg Co + 5 µg B_{12}, 0.6 mg Co + 10 µg B_{12} and 0.6 mg Co + 20 µg B_{12} per kg (Table 2). Each hen was offered the respective diet and water ad libitum. The number of laid eggs was recorded daily and the individual feed consumption was recorded monthly per hen. Each month the collected eggs were weighed four times within two weeks. In the 3rd, 6th and 10th laying month all eggs laid over 3 consecutive days were collected and the yolk weight, albumen weight and shell weight were measured. The color of the egg yolk was examined with the Roche-Fan. Body weight of hens was recorded at the start and at the end of the trial.

Table 1:
Trial design

Group	B_{12} µg/kg	Co mg/kg
1	0	0
2	5	0
3	10	0
4	20	0
5	0	0.6
6	5	0.6
7	10	0.6
8	20	0.6

Table 2:
Ingredient composition, analysed and calculated nutrients in the diet (g/kg)

Ingredient	Diet
Wheat	547.0
Barley	100.0
Corn	50.0
Soya bean meal	175.0
Soya oil	20.0
Di-calcium-phosphate	12.7
Calcium carbonate	79.5
Sodium chloride	3.3
DL-Methionine	1.1
L-Lysine-HCl	1.4
Premix [1]	10.0
Vitamin $_{B12}$ [2], µg/kg	0/5/10/20
Co [2], mg/kg	0/0.6
Dry matter [3]	894
Crude protein [3]	161
ME, MJ/kg [4]	11.36
Lysine [5]	8.5
Methionine + Cystine [5]	6.6
Cobalt, µg/kg [6]	34
Vitamin B_{12}, µg/kg [7]	8

[1] Vitamin- mineral premix provided per kg of diet: Fe, 40 mg; Cu, 10 mg; Zn, 80 mg; Mn, 100 mg; Se, 0.25 mg; I, 1.2 mg; vitamin A, 10000 IE, vitamin D_3, 2500 IE; vitamin E, 20 mg; vitamin K_3, 4 mg; thiamine, 2.5 mg; riboflavin, 7 mg; pyridoxine, 4 mg; nicotinic acid, 40 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 25 µg; choline chloride, 400 mg
[2] Cobalt carbonat – 5 % Co; Cyanocobalamin Product – 0.1 % vitamin B_{12}
[3] Analysed values
[4] Calculated values (WPSA; 1985)
[5] Calculated values
[6] Control group calculated values (Souci et al., 2008)
[7] Control group analysed values

Eight hens of the Control, 20 µg B_{12}-Group, 0.6 mg Co-Group and 0.6 mg Co + 20 µg B_{12}-Group each were slaughtered at the end of the trial to determine hematocrit, expressed as a percentage of the total blood volume and organ weights. Blood was collected into heparinized tubes from the neck vessels of the hens. Hematocrit was determined by using heparinized capillaries for blood sampling after 8 minutes of centrifugation at 13,000 RPM in a micro-hematocrit centrifuge.

Data were analyzed with a two-way ANOVA: yijk = µ + Vi + Cj + VCij + eijk, with yijk = observation, µ = general mean, Vi = vitamin B_{12} (0, 5, 10 and 20 µg/kg), Cj = Cobalt (0 and 0.6 mg/kg), interaction VCij and eijk = error term (random). Multiple comparisons of means were carried out using the Student-Newman-Keuls Test ($P \leq 0.05$). The statistical analyses were performed by the SAS software package (Version 9.1).

Results

In total 5 hens died apparently without relevance to the treatment. The laying performance of the hens is shown in Table 3, 4 and 5. The daily feed intake per hen was significantly increased ($P \leq 0.05$) over the total laying period of the 5 µg B_{12}-Group compared to the other B_{12}-Groups and the Control (Table 4). The laying intensity of the hens was range between 90 % and 93 % in mean of the 13th laying month and was not different between groups. The egg weight and the daily egg mass production were significantly increased with supplementation of vitamin B_{12} (all groups) and B_{12} x Co in the feed. Feed conversion was significantly improved in the group with 5 µg B_{12} per kg feed and also the combination B_{12} x Co. The supplementation of 5 µg to 20 µg B_{12} to the diet significantly increased the final body weight of hens (Table 4). Co alone without vitamin B_{12} in the diet significantly increased the mass of the eggs and did not change the other laying parameters. Supplementation of diets with vitamin B_{12} or Co or both additives didn't directly improve the egg composition or the yolk colour (Table 6). The results of slaughtering showed that with supplementation of 20 µg B_{12} the liver mass significantly decreased and the haematocrit significantly increased compared to the Control and 0.6 mg Co-Group (Table 7). The supplementation of 0.6 mg Co significantly increased the spleen mass.

Table 3:
Performance of laying hens (Means, SD, P-value)

Treatments		Feed intake g/hen/day	Laying intensity %	Egg weight g/egg	Egg mass production g/hen/day	Feed : egg mass production kg/kg	Body weight, g/hen	
B_{12} µg/kg	Co mg/kg						Start	End
0	0	109.4 ± 12.8	92.2 ± 8.9	61.9 ± 4.8	57.2 ± 7.1	1.923 ± 0.17	1637 ± 133	1741 ± 226
5	0	109.2 ± 9.2	93.0 ± 8.6	62.9 ± 5.2	58.3 ± 6.0	1.883 ± 0.17	1637 ± 114	1845 ± 125
10	0	111.5 ± 10.9	92.2 ± 10.4	63.0 ± 6.5	58.0 ± 8.0	1.948 ± 0.25	1637 ± 137	1884 ± 193
20	0	110.7 ± 12.6	91.9 ± 9.8	62.3 ± 5.7	57.0 ± 6.9	1.961 ± 0.30	1637 ± 185	1922 ± 255
0	0.6	108.1 ± 12.7	90.3 ± 11.5	61.9 ± 4.4	55.7 ± 7.4	1.963 ± 0.29	1637 ± 148	1778 ± 164
5	0.6	109.4 ± 12.2	91.9 ± 9.0	63.4 ± 6.2	58.1 ± 6.8	1.893 ± 0.17	1637 ± 134	1828 ± 168
10	0.6	112.5 ± 9.6	92.0 ± 10.5	63.9 ± 5.4	58.7 ± 7.5	1.947 ± 0.30	1637 ± 122	1876 ± 160
20	0.6	109.0 ± 9.6	92.6 ± 11.0	63.2 ± 4.7	58.6 ± 7.9	1.892 ± 0.32	1637 ± 111	1878 ± 138
ANOVA, P-value								
B_{12}		0.001	0.13	0.001	0.001	0.001	1.0	0.001
Co		0.31	0.12	0.006	0.63	0.62	1.0	0.76
B_{12} x Co		0.10	0.059	0.34	0.001	0.001	1.0	0.73

Table 4:
Influence of vitamin B_{12} on performance of laying hens (Means)

Vitamin B_{12}, µg/kg	0	5	10	20
Feed intake, g/hen/day	108.8 b	109.3 b	112.0 a	109.8 b
Laying intensity, %	91.3	92.4	92.1	92.3
Egg weight, g/egg	61.9 b	63.1 a	63.5 a	62.8 a
Egg mass production, g/hen/day	56.5 b	58.2 a	58.3 a	57.8 a
Feed : egg mass production, kg/kg	1.943 a	1.888 b	1.947 a	1.927 a
Final body weight, g/hen	1759 b	1836 a	1880 a	1900 a

Table 5:
Influence of Co on performance of laying hens (Means)

Co, mg/kg	0	0.60
Feed intake, g/hen/day	110.2	109.7
Laying intensity, %	92.3	91.7
Egg weight, g/egg	62.5 b	63.1 a
Egg mass production, g/hen/day	57.6	57.8
Feed to egg mass production, kg/kg	1.928	1.924
Final body weight, g/hen	1847	1839

Discussion

The effect of Vitamin B_{12} and Cobalt on laying performance, egg composition, and organ mass and haematocrit was studied in laying hens. The available data on the requirement of Vitamin B_{12} in laying hens is very limited.

In the present study the main sign of prolonged vitamin B_{12} deficiency in laying hens was a reduced mean egg weight and body weight of hens at the end of the laying period. As a result the egg weight of these hens (0 µg B_{12} supplemented per kg feed) was 2 % less, and the body weight was 4 % less and the feed conversion was increased by 3 % compared to hens with 5 µg B_{12} supplemented to the diet.

Table 6:
Egg composition (n = 163 to 181/ eggs/group, from three collection periods) (Means, SD, P-value)

Treatments		Egg weight	Yolk	Albumen	Shell	Yolk colour
B_{12} µg/kg	Co mg/kg	g/egg	%	%	%	Roche fan
0	0	62.4 ± 4.6	27.8 ± 2.0	60.0 ± 2.4	12.2 ± 1.0	12.7 ± 0.6
5	0	63.3 ± 4.5	27.6 ± 1.9	60.3 ± 2.3	12.1 ± 0.9	12.6 ± 0.6
10	0	63.3 ± 6.4	28.1 ± 2.1	59.2 ± 2.5	12.6 ± 1.1	12.6 ± 0.6
20	0	62.6 ± 5.6	27.8 ± 2.3	60.0 ± 2.7	12.2 ± 0.9	12.8 ± 0.6
0	0.6	62.4 ± 4.2	28.2 ± 2.0	59.3 ± 2.4	12.4 ± 1.2	12.8 ± 0.5
5	0.6	63.3 ± 5.9	28.0 ± 2.4	59.5 ± 2.8	12.4 ± 0.9	12.7 ± 0.6
10	0.6	64.7 ± 5.2	27.9 ± 2.5	59.9 ± 2.8	12.2 ± 1.0	13.0 ± 0.6
20	0.6	63.4 ± 4.6	27.6 ± 2.4	60.1 ± 2.8	12.2 ± 1.1	12.5 ± 0.8
ANOVA, P-value						
B_{12}		0.001	0.24	0.05	0.048	0.002
Co		0.048	0.38	0.24	0.28	0.26
B_{12} x Co		0.21	0.06	0.001	0.001	0.001

Table 7:
Organ mass, breast meat mass (g/hen) and hematocrit (%) in blood at the end of the trial (n = 8) (Means, SD, P-value)

Treatments		Heart	Liver	Spleen	Breast meat	Hematocrit
B_{12} µg/kg	Co mg/kg					
0	0	6.8 ± 0.7	34.4 ± 4.9	1.3 ± 0.4	132.0 ± 24.5	25.5 ± 3.3
20	0	6.8 ± 0.9	28.8 ± 6.8	1.4 ± 0.2	144.2 ± 22.5	28.1 ± 1.9
0	0.6	6.8 ± 1.0	39.0 ± 8.4	1.8 ± 0.4	140.6± 22.2	26.6 ± 2.3
20	0.6	7.2 ± 0.8	32.7 ± 3.2	1.8 ± 0.4	140.6 ± 19.3	28.0 ± 2.3
ANOVA, P-value						
B_{12}		0.55	0.01	0.71	0.44	0.03
Co		0.66	0.06	0.004	0.75	0.57
B_{12} x Co		0.45	0.89	0.46	0.44	0.53

Our laying hens originated from a parent flock with feeding according to the nutritional requirements of breeder hens and were also fed throughout the rearing period of chickens in accordance with nutrient requirements. Therefore it can be assumed that the storage of vitamin B_{12} into the hen body had an optimum B_{12} depot at the start of the laying period.

Scott et al. (1982) observed that two to five months would be needed to deplete hens of vitamin B_{12} stores to such an extent that progeny would hatch with low vitamin B_{12} reserves. On the question of vitamin B_{12} storage in the egg, Robel (1983) established that changes in vitamin levels (also in vitamin B_{12}) deposited in the eggs are related to the aging process of the turkey breeder hen. Souires and Naber (1992) concluded from the results of a study of vitamin profiles of eggs – which concentrations of vitamin B_{12} between 1.3 and 2.9 µg/100 g egg yolks are found, and for this reason, 8 µg vitamin B_{12} per kg diet appeared to be needed to support maximum hatchability and egg weight.

In the present study a vitamin B_{12} content of 8 µg per kg was analyzed in the complete control diet, this value resulted from microbiological contaminations of feed stuffs. But this content was not adequate for the laying hens of the Control to produce eggs with the same egg weight as compared to B_{12}-supplemented groups. A supplementation of 5 µg vitamin B_{12} per kg diet contributed to significantly higher egg weight, daily egg mass production and improved feed conversion. The results do not allow any clear conclusion, if this result is the outcome of the B_{12} content from microbiological contamination plus the supplemented 5 µg vitamin B_{12} per kg diet or this is exclusively the result from the supplemented 5 µg vitamin B_{12} per kg diet.

In studies with laying hens Bunchasak and Kachana (2009) measured no effect of B_{12} supplementation on feed intake or laying intensity, this is comparable with the own results. From a series of hen studies, Keshavarz (2003) concluded that certain manipulations of the combination of methionine, choline, folic acid, and vitamin B_{12} have the potential to reduce egg weight and improve shell quality without affecting egg production during the latter stages of the laying period.

In this study the supplementation of B_{12} significantly increased the body weight of the laying hens at the end of the trial. Studies on broilers showed that a deficiency of B_{12} in growing chickens results in reduced weight gain and feed intake (Halle et al., 2011), along with poor feathering and nervous disorders (Leeson and Summers, 2001). While a deficiency may lead to perosis, this is probably a secondary effect due to a dietary deficiency of methionine, choline or betaine as sources of methyl groups. Vitamin B_{12} may alleviate the perosis condition due to its effect on the synthesis of methyl groups. Further clinical signs reported in poultry are anaemia, gizzard erosion, and fatty infiltration of heart, liver and kidneys. A deficiency of Vitamin B_{12} in laying hens also resulted in a significantly higher liver mass. The measured haematocrit in the B_{12} supplemented hen groups was higher and was in agreement with the literature (Mehner and Hartfiel, 1983, 21 to 55 %).

The supplementation of Co alone without vitamin B_{12} in the diets in the present study did not change daily feed intake or laying intensity but significantly improved egg weight. The body weight of the hens at the end of the trial was not changed in the group with Co supplementation compared to Control. In studies with pigs it was observed that vitamin B_{12} – deficiency signs, including an accumulation of serum homocysteine, can be attenuated by Nickel and Co, although the mode of action seems to differ (Stangl et al., 2000). Previous studies have established that Co ions induce a series of metabolic changes in experimental animals. Co supplementation only improved homocysteine accretion in serum, whereas the vitamin B_{12} status remained completely unchanged (Goncharevskaia et al., 1985; Rosenberg and Kappas, 1989; Zhang et al., 1998). These findings indicate that there is no improvement in the vitamin B_{12} status and protein synthesis of the meat after supplementation of Co.

Furthermore it cannot be excluded that hens in the present study obtained a part of their vitamin B_{12} requirement more indirectly by ingesting faeces in the litter. The faeces bacterial flora is an important source of vitamins for coprophagic animals. The supplementation of Co to laying hen diets supported the vitamin B_{12} synthesis of bacteria in the digestive tract.

The combined supplementation of vitamin B_{12} and Co in the laying hen diets did not provide recognizable advantages relating to feed intake or laying performance.

It can be concluded from these observations that a supplementation of 5 µg vitamin B_{12} per kg laying hen feed was sufficient to compensate the deficiencies on feed intake and laying performance. The results allow the conclusion that an additional supplementation of Co to feed does not have additional advantages for the birds.

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Can compensatory growth contribute to reduce the so-called protein gap in organic pig fattening?

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Abstract

The lack of organic feed with high quality amino acid pattern (protein gap) makes it more difficult to achieve Lysine-energy-ratios in 100 % organic grower diets required for high yielding growing-finishing pigs. The resultant marginal Lysine supply threatens to reduce body protein synthesis. Hence, the objective of our study was to investigate whether a moderate oversupply of Lysine in the finisher period after a marginal Lysine supply in the grower period will result in compensatory growth (CG). 48 gilts and 48 barrows were equally divided into four groups with different Lysine-energy-ratios (g Lysine per MJ Metabolisable Energy [ME]) in the grower diet (grodi) and in the finisher diet (findi): (**i**) Group_1 – experimental group: 0.69 (grodi)/0.69 (findi); (**ii**) Group_2 – negative control group: 0.69 (grodi)/0.59 (findi); (**iii**) Group_3 – positive control group: 0.89 (grodi)/0.69 (findi); (**iv**) Group_4 – regular control group: 0.89 (grodi)/0.59 (findi). N-retention (as criterion for body protein gain) was measured for a random sample of barrows, and growth performance, carcass quality, and meat quality were recorded for all pigs. N-retention indicated compensatory body protein gain for Group_1 barrows. All groups were similar in meat quality and growth performance for the whole fattening period but Group_1 pigs showed CG for daily weight gain and daily feed intake in the finishing period. Group_1 and Group_2 pigs showed only slightly, non-significant lower lean meat percentages than Group_3 and Group_4 pigs, this is taken to be at least partial CG. It is concluded that CG mechanisms help to reduce the protein gap in organic pig fattening, facilitating the implementation of 100 % organic feeding.

Keywords: organic pig fattening; compensatory growth; N-retention; growth performance; carcass quality; meat quality

Zusammenfassung

Kann kompensatorisches Wachstum einen Beitrag zur Entschärfung der sogenannten Proteinlücke in der ökologischen Schweinemast leisten?

Der Mangel an Öko-Futtermitteln mit einem hochwertigen Aminosäurenmuster (Proteinlücke) erschwert die Einstellung des für Hochleistungstiere notwendigen Lysin-Energie-Verhältnisses im Vormastfutter ausschließlich ökologischer Herkunft und droht den körpereigenen Proteinansatz zu vermindern. Daher wurde geprüft, ob der negative Effekt einer marginalen Lysinversorgung in der Anfangsmast durch eine moderate Überversorgung in der Endmast kompensiert werden kann. 48 weibliche und 48 kastrierte männliche Mastschweine wurden gleichmäßig auf vier Gruppen mit unterschiedlichen Lysin-Energie-Verhältnissen (g Lysin pro MJ Umsetzbare Energie) in der Anfangsmastration (AM) und in der Endmastration (EM) aufgeteilt: (**i**) Gruppe_1 – Versuch: 0,69 (AM)/0,69 (EM); (**ii**) Gruppe_2 – Negativkontrolle: 0,69 (AM)/0,59 (EM); (**iii**) Gruppe_3 – Positivkontrolle: 0,89 (AM)/0,69 (EM); (**iv**) Gruppe_4 - reguläre Kontrolle: 0,89 (AM)/0,59 (EM). Erfasst wurden: N-Retention (Stichprobe der Kastraten), Mastleistung, Schlachtkörper- und Fleischqualität (sämtliche Schweine). Die N-Retention belegte einen kompensatorischen Proteinansatz bei den Gruppe_1-Kastraten. Die vier Gruppen unterschieden sich nicht in der Fleischqualität und Mastleistung in der Gesamtmastperiode. Aber Gruppe_1 zeigte bei der Tageszunahme und Futteraufnahme in der Endmast kompensatorische Wachstumseffekte. Der Muskelfleischanteil war in den Gruppen 1 und 2 auf gleichem Niveau nur geringfügig niedriger als bei den auch auf gleichem Niveau liegenden Gruppen 3 und 4, was ein zumindest teilweiser kompensatorischer Effekt ist. Es wird geschlussfolgert, dass kompensatorische Wachstumsmechanismen die Proteinlücke in der ökologischen Schweinemast entschärfen und damit die Implementierung der 100 % Biofütterung erleichtern können.

*Schlüsselwörter***:** *Ökologische Schweinemast; kompensatorisches Wachstum; N-Retention; Mastleistung; Schlachtkörperqualität; Fleischqualität*

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Introduction

Also in *organic* pig fattening carcass quality significantly influences farmers' economic success. Corresponding market-driven lean meat contents predominantly depend on the choice of a suitable genotype and an adequate diet (Weißmann, 2011). Nutrition-related body protein synthesis is primarily determined by the ratio of feed energy to essential amino acids (EAAs) in the diet – in particular Lysine, mostly the first limiting amino acid – which in turn depends on available protein feeds (Szabo et al., 2001). Organic monogastric feeding is characterised by the so-called protein gap, a lack of feedstuffs of organic origin with a high quality amino acid pattern (Zollitsch, 2007). Hence, it is particularly difficult to achieve the DLG-required (Deutsche Landwirtschafts-Gesellschaft) Lysine-energy-ratio for high yielding growing-finishing pigs in the grower period with possible negative impact on growth performance, carcass quality, and resulting profits. Usually two solution strategies are combined (Wlcek & Zollitsch, 2004): (**i**) high amount of protein feeds in the diet to achieve the required amino acid contents via high crude protein contents but with possible negative environmental impact and (**ii**) use of non-organic high quality protein feeds (in Germany usually potato protein) based on the legal exceptions within the EU-Eco-regulations 834/2007 (EU-Kommission, 2007) and 889/2008 (EU-Kommission, 2008) which will expire in 2014 (EU-Kommission, 2012). Both strategies with their inherent shortcomings have to be negatively valued with regard to the reliability and the future profile of organic agriculture (Niggli, 2005; Rahmann et al., 2009).

In this context the biological principle of compensatory growth could be of interest. Compensatory growth is a phenomenon observed in livestock and describes the animals' ability to recover from a previous period of nutritional restriction during the realimentation period (Molnar, 1995). Restriction and realimentation occur in terms of limited versus free access to feed (e.g. Therkildsen et al., 2004) or in terms of marginal versus improved diet quality (e.g. Yang et al., 2008). The compensatory effect is caused by an increased feed intake (e.g. Critser et al., 1995) and/or by an improved feed conversion reflecting better metabolic nutrient utilisation (e.g. Oksbjerg et al., 2002). The compensation rate can be complete (e.g. Fabian et al., 2004) or partial (e.g. Wahlstrom & Libal, 1983) and mostly depends on the severity of the restrictive period. The metabolic processes behind compensatory growth are not yet completely understood (Hornick et al., 2000).

Against that background, an organic feeding trial was set up to clarify whether reduced body protein gain in the grower period (due to a lower Lysine-energy-ratio in the grower diet because of the non-use of increased dietary crude protein and the non-use of non-organic protein feeds) can be *compensated* by a slightly enhanced Lysine-energy-ratio in the finisher period compared to the DLG recommendations (DLG, 1991; GfE, 2008).

Methods

Test design

The feeding trial was divided into the actual fattening trial and a therein embedded nitrogen (N) balance trial with a limited number of barrows from the fattening trial.

Four different groups were set up with differing ratios of dietary Metabolisable Energy (ME) and dietary Lysine (g Lysine per MJ ME) in the grower diet (grodi) and in the finisher diet (findi) (Table 1): (**i**) Group_1: 0.69 (grodi)/0.69 (findi) – experimental group for the confirmation of compensatory growth, i.e. body protein synthesis, with a Lysine undersupply in the grower diet and a slight Lysine oversupply in the finisher diet in the form of a one-phase diet; (**ii**) Group_2: 0.69 (grodi)/0.59 (findi) – negative control group in the form of a Lysine undersupply in the grower and a regular supply in the finisher diet, so that the missing enhanced Lysine supply in the finisher diet would not initiate compensatory protein gain; (**iii**) Group_3: 0.89 (grodi)/0.69 (findi) – positive control group with the expectation that an oversupply of Lysine in the finisher diet would not result in an additional body protein synthesis; (**iv**) Group_4: 0.89 (grodi)/0.59 (findi) – regular control group with a regular Lysine-ME-ratio in the grower and in the finisher diet corresponding to the German feeding recommendations of the DLG (1991).

Table 1:
Experimental design

	Group_1	Group_2	Group_3	Group_4
Number of intended animals (barrows + gilts)	24 (12 + 12)	24 (12 + 12)	24 (12 + 12)	24 (12 + 12)
Lysine-energy-ratio [g Lysine/MJ Metabolisable Energy] in the …				
grower diet	0.69	0.69	0.89	0.89
finisher diet	0.69	0.59	0.69	0.59
Labelling	Experiment	Negative control	Positive control	Regular control
Diet in the …				
grower period	Feed_2	Feed_2	Feed_1	Feed_1
finisher period	Feed_2	Feed_3	Feed_2	Feed_3

Feed

Table 1 illustrates that the three differing Lysine-ME-ratios of 0.89, 0.69 and 0.59 in the grower and finisher periods were achieved by feeding three different feeds. Table 2 describes the corresponding feed ingredients and the analysed feed nutrients according to the German VDLUFA-standard (Naumann & Bassler, 1993). The diets consisted of feed ingredients of 100 % organic origin except for potato protein in Feed_1 which is necessary to achieve the high Lysine content and which is inherent to the subject of the study. Table 2 points out the good congruence of the intended and analysed Lysine-ME-ratios which is of extreme interest for the goals of the study. Inexplicably, the targeted contents of the sulphur-containing amino acids (g Methionine + g Cystine per g Lysine) of 0.59, 0.62 and 0.66 in Feed_1, Feed_2 and Feed_3, respectively, were not exactly achieved (Table 2) which cannot be explained. However the congruence to the DLG-recommendations is good enough in all three feeds to exclude negative impacts on the subject of the paper. This is also the case for Threonine with a target level of 0.68, 0.68 and 0.69 g Threonine per g Lysine in Feed_1, Feed_2 and Feed_3 respectively (Table 2) (DLG, 1991). Dietary energy supply in the three feeds is in accordance with the German recommendations for growing-finishing pigs with intended 900 g average daily weight gain (DLG, 1991).

Table 2:
Characteristics of the three feeds used in the experiment

	Feed_1	Feed_2	Feed_3
Feed ingredients			
Winter barley [g/kg]	362	382	416
Triticale [g/kg]	150	210	240
Field peas + field beans [g/kg]	200	200	185
Soybean cake [g/kg]	80	90	50
Rapeseed cake [g/kg]	75	50	40
Wheat bran [g/kg]	55	40	40
Potato protein, conventional origin [g/kg]	50	- -	- -
Mineral feed* [g/kg]	20	20	20
$CaCO_3$ [g/kg]	8	8	9
Feed composition (referring to dry matter = DM)			
Metabolisable energy, ME [MJ/kg DM]	14.24	14.21	14.16
Crude protein [g/kg DM]	212	168	148
Crude fat [g/kg DM]	44	40	37
Crude fibre [g/kg DM]	54	52	51
Nitrogen-free extract [g/kg DM]	632	681	709
Starch [g/kg DM]	447	503	512
Sugar [g/kg DM]	41	43	41
Lysine [g/kg DM]	12.66	9.54	8.44
Methionine + Cystine [g/kg DM]	6.57	5.65	4.89
Threonine [g/kg DM]	7.23	6.56	5.25
Lysine - energy - ratio [g Lysine/MJ ME]	0.89	0.67	0.60
Methionine + Cystine - Lysine - ratio [g Methionine + Cystine/g Lysine]	0.52	0.58	0.58
Threonine - Lysine - ratio [g Threonine/g Lysine]	0.57	0.69	0.62

* Commercial supplement containing minerals, trace elements and vitamins (contains per kg: 210 g Ca; 70 g P; 60 g Na; 10 g Mg; 500,000 I.E. Vit. A; 50,000 I.E. Vit. D3; 2,500 mg Vit. E; 60 mg Vit. B1; 185 mg Vit. B2; 150 mg Vit. B6; 1,200 mcg Vit. B12; 120 mg Vit. K3; 1,250 mg Vit. C; 750 mg Nicotinic acid; 450 mg Ca-Pantothenate; 30 mg Folic acid; 4,000 mcg Biotin; 10,000 mg Choline Chloride; 4,500 mg Fe; 400 mg Cu; 2,130 mg Mn; 2,670 mg Zn; 53 mg I; 10.5 mg Se; 6.6 mg)

Animals

The trial consisted of 48 gilts and 48 barrows randomly assigned to four groups considering equal distribution of sex and of body weight except pedigree. All animals came from the research farm of the Institute of Organic Farming in Trenthorst with known dams and sires. The dams' genetic origin are Schaumann®-sows, i.e. DE*DL-crossbreds (DE = Deutsches Edelschwein, German Large Wight; DL = Deutsche Landrasse, German Landrace) and replacement sows by criss-crossing the Schaumann®-sow with DE or DL respectively. Two individual Piétrain*Duroc-crossbred terminal sires were used via artificial insemination and natural service respectively. Four gilts were lost due to diseases of the respiratory and the musculoskeletal system without relation to the experimental approach. Hence, Group_1, Group_2, Group_3 and Group_4 ultimately consisted of 22, 23, 24 and 23 recorded and analysed pigs respectively.

Experimentation

The trial was performed at the pig fattening facility of the Institute of Animal Nutrition in Braunschweig. The fattening trial started in December 2008 and consisted of two runs of 54 and 42 animals respectively, at intervals of four weeks.

The animals were individually housed in a Danish housing system with individual trough feeding and without straw bedding. All animals were individually identified by ear tags on the occasion of initial weighing and grouping at the pig fattening facility for complete individual data recording concerning growth performance, carcass and meat quality.

The fattening period ranged from about 28 kg to about 116 kg live weight. The change between the grower period and the finisher period was projected at an animal's live weight between 75 kg and 80 kg. Slaughtering took place in the Institute's own abattoir usually on Mondays when the animals had reached > 109.5 kg live weight in the previous week. Subsequent to a resting period of 40 min at the abattoir the animals were slaughtered after electrical stunning. Table 3 shows the corresponding data. Feed was offered daily *ad libitum*.

The feed-, faeces-, and urine-based N-balances in individual metabolism crates with individual feeding and separated collection of faeces and urine were in accordance with the guidelines of the German Gesellschaft für Ernährungsphysiologie – Society of Nutrition Physiology – (GfE, 2005). A total of 24 barrows were involved (4 groups x 2 runs x 3 barrows per group and run) always with the same individuals in the grower and in the finisher period. Two animals in Group_4 had to be withdrawn as they failed to accept the metabolism crates at the end of the collection period in the finisher period in Run 1 and Run 2. Hence Group_4 consisted of only four recorded animals, whereas the other three groups consisted of the intended six barrows per group. The balance period was divided into a collection period of seven days and a previous period of 4 days to adapt the animals to the metabolism crates and to replace the ad libitum feeding with twice daily restricted feeding. This kind of feeding is necessary to (**i**) avoid feed losses and (**ii**) to achieve daily a nearly equal digestion process. The balance trials started with a mean live weight of 42.5 ± 2.7 kg (grower period) and 83.6 ± 5.5 kg (finisher period).

Table 3:
Number, mean live weight, mean carcass weight, and mean amino acid consumption of the recorded pigs (standard deviation)

	Group_1 Experiment	Group_2 Negative control	Group_3 Positive control	Group_4 Regular control
Barrows + gilts [n]	12 + 10	12 + 11	12 + 12	12 + 11
Live weight [kg] at the …				
start of the trial	28.2 (1.8)	28.7 (2.2)	28.2 (1.5)	29.5 (2.7)
end of the grower period	76.8 (5.6)	76.1 (5.0)	77.5 (5.3)	81.2 (6.7)
end of the trial	115.9 (3.5)	116.8 (3.9)	117.0 (4.2)	117.3 (4.4)
Carcass weight, warm [kg]	91.0 (2.8)	91.5 (2.6)	91.6 (2.9)	91.3 (2.9)
Consumption [g/animal] during the whole fattening period of …				
Lysine	2458 (130)	2305 (200)	2853 (251)	2691 (236)
Methionine+Cystine	1416 (75)	1331 (115)	1546 (136)	1451 (124)
Threonine	1690 (89)	1517 (134)	1762 (155)	1583 (136)

Data collection

The pigs were individually weighed at the outset and at the end of the trial on the day of slaughtering with intermediate weekly weighing. The daily weight gain of each animal was calculated for the grower, finisher and whole fattening period respectively. The daily feed intake was calculated from the weekly offered feed and the rest in the trough as weighed. Total consumption of the relevant amino acids Lysine, Methionine+Cystine and Threonine (Table 3) was derived from animals' mean feed intake and respective amino acid contents of the diets (Table 2). Feed conversion ratio is the relation between feed intake and live weight gain and presented for the grower, finisher and whole fattening period.

Warm carcass weight was recorded in order to calculate dressing percentage based on the final live weight. The day following slaughtering, the chilled right carcass half was used for measuring muscle and fat area and five different fat thicknesses (for its locations compare Results: Table 7) following the guideline for on-station testing of growth performance, carcass quality, and meat quality of the German Board for Performance Testing and Estimation of Breeding Value of the Pig (ZDS, 2007). Lean-to-fat ratio is the quotient of fat area and muscle area. Lean meat content is calculated with the "Bonner Formula" (ZDS, 2007), which uses the above mentioned fat area, muscle area, and five fat thickness measurements; lean meat percentage in the belly is calculated with the "Gruber Formula" taking muscle area, fat area, and two fat thickness measurements into account (ZDS, 2007).

The measuring of the physical meat quality traits *electrical conductivity* (EC) and *pH-value* (for its locations compare Results: Table 8) followed the above-mentioned pro-

cedure of data collection of the federal standard of the German testing stations (ZDS, 2007). In addition, the intramuscular fat content (IMF) of the *Musculus longissimus dorsi* (13th rib) was estimated by Near-Infrared-Reflectance (NIR) Spectroscopy (FOSS NIRS-System 6500) for a random sample of 50 % of the total number of animals equally divided between the four groups, the two runs and both sexes. NIR-calibration was additionally validated for half of the random sample by wet chemistry technique (modified §64-method without hydrochloric acid digestion, Lebensmittel- und Futtermittelgesetzbuch as amended on July 24, 2009).

Sampling and analysis of the N-balances were in accordance with the methodology of the German Society of Nutrition Physiology (GfE, 2005).

Statistical analysis

The data of growth performance, carcass quality and meat quality were statistically analysed with the analysis of variance procedure GLM of SAS Version 9.2 (SAS, 2008). The general linear model included the fixed effects of feeding group (FG: Group_1 (0.69-grodi/0.69-findi), Group_2 (0.69-grodi/0.59-findi), Group_3 (0.89-grodi/0.69-findi), Group_4 (0.89-grodi/0.59-findi)), sex (SEX: barrows or gilts), interaction FG*SEX, and run (RUN: first or second trial run). Terminal sires and breed of dams were also tested as fixed effects and removed from the model due to non-significance. In addition the live weight at the start of the trial (LWS), the live weight at the change from the grower to the finisher period (LWC) and the live weight at the end of the trial (LWE) for growth performance traits, and carcass weight (CW) for carcass traits and IMF were included as covariates in the model. The LSQ-Means were statistically compared using the Tukey-Kramer-Test at 5 % significance level. N-balance data of the barrows are presented as means incl. standard deviation and the Tukey-Kramer-Test was used for multiple mean comparisons between groups and feeds respectively, at 5 % significance level.

Results

N-balance trial

The live weight (LW) data were raised to 0.67 to eliminate the influence of LW on N-retention as well as possible (Gebhardt, 1966). Further, all measured data were divided by $LW_{kg}^{0.67}$ of the respective animal. So it is possible to use

Table 4a:

Effect of the three feeds used in the trial on mean N-consumption and mean N-retention of the balanced barrows during the 7-day N-collection periods, joined for the grower and the finisher period (standard deviation)

Lysine-ME-ratio	Feed_1 0.89	Feed_2 0.69	Feed_3 0.59
Barrows [n]	11	24	10
Live weight (LW), [$kg^{0.67}$]	12.35^{c} (0.68)	15.93^{b} (3.82)	19.17^{a} (0.77)
N*-consumption [g N/day and $LW_{kg}^{0.67}$]	3.08^{a} (0.27)	2.65^{b} (0,28)	2.28^{c} (0.09)
N*-retention [g N / day and LW_{kg}0.67]	1.52^{a} (0.24)	1.18^{b} (0.17)	0.95^{c} (0.10)
N*-retention [g N/kg weight gain and $LW_{kg}^{0.67}$]	2.35^{a} (0.78)	1.70^{b} (0.66)	1.04^{c} (0.30)

$^{a, b, c}$ Means with different letters within a row differ significantly for $P < 0.05$ (Tukey-Kramer-Test)
* N = Nitrogen

Table 4b:

Mean N-consumption and mean N-retention of the balanced barrows during the 7-day N-collection periods, joined for the grower and the finisher period (standard deviation)

Number of barrows [n]	Group_1 Experiment 12	Group_2 Negative control 12	Group_3 Positive control 12	Group_4 Regular control 9
Live weight (LW), [$kg^{0.67}$]	16.30 (3.78)	15.38 (3.79)	15.67 (3.87)	15.73 (3.62)
N*-consumption [g N/day and $LW_{kg}^{0.67}$]	2.68 (0.31)	2.50 (0.31)	2.84 (0.34)	2.68 (0.48)
N*-retention [g N/day and $LW_{kg}^{0.67}$]	1.22 (0.18)	1.13 (0.19)	1.31 (0.33)	1.20 (0.35)
N*-retention [g N/kg weight gain and $LW_{kg}^{0.67}$]	1.62 (0.54)	1.66 (0.54)	1.89 (0.98)	1.66 (0.62)

Means without letters within row differ NOT significantly (Tukey-Kramer-Test, $P < 0.05$)
* N = Nitrogen

all balance data, independently of the period (grower or finisher), to achieve the results shown in Tables 4a and 4b.

The N-balance results in Table 4a refer to the three different feeds. All differences between feeds are significant. Feed_1 with the highest quality (Lysine-ME-ratio 0.89) generated the best results and poor Feed_3 (Lysine-ME ratio 0.59) caused the lowest N-retention.

In Table 4b the results of the N-balances refer to the four feeding groups. The lack of significances of N-retention between groups indicates its independence from the different feeding strategies.

Feeding trial

The significances of the effects of the statistical model on growth, carcass and meat traits are summarised in Table 5. The interaction between group and sex was non-significant for all traits, so the following results concerning growth performance, carcass, and meat quality for groups are presented in each case as LSQ-Mean (LSQM) over both sexes (overall non-significance of group*sex-interaction is not presented due to better clarity of Table 5).

In Table 5 it can be seen that daily weight gain as well as feed intake are affected by the different feeding strategies only in the finisher period, the last mentioned also by Sex. Feed and energy conversion ratios are not influenced by feeding group and sex, in contrast to the conversion ratios of Lysine, Methionine+Cystine, and Threonine due to the intended experimental design, i.e. the huge variation of AA-contents in the three feeds. The striking, highly significant effect of the run (first run better than the second, results not presented) cannot be explained. Concerning carcass quality the different dietary Lysine-ME-quotients only affect muscle area which is directly associated with body protein gain, i.e. N-retention. The visible effect of sex on lean meat and fat associated carcass criteria is in accordance with biological principles (Latorre et al., 2003). No meat quality characteristics are significantly influenced by the different feeding strategies.

Table 6 illustrates growth performance traits. Whereas weight gain in the grower and in the whole fattening period is not influenced by the different feeding strategies, Group_1 animals in the finisher period show a significantly enhanced daily weight gain of nearly 10 % compared to Group_3 and Group_4 animals, whereas Group_2 pigs represent a medium position with 6 % higher weight gain than Group_3 and Group_4 pigs. These findings obviously correspond with feed intake. Group_1 animals have a significantly increased intake of 11 % in the finisher period compared to Group_3 and Group_4 animals and again, Group_2 pigs represent a medium position with 6 %. Feed and feed energy conversion ratios remain unaffected by the feeding strategies. Amino acid conversion ratios correspond to amino acid consumption (compare Table 3).

Table 5:
Significance levels of the fixed effects on growth performance, carcass quality, and meat quality traits

	FEEDING GROUP (FG 1 – 4)	SEX (barrows or gilts)	RUN (1st or 2nd)
Daily weight gain in the …			
grower period	ns	*	ns
finisher period	*	ns	*
whole period	ns	ns	ns
Daily feed intake in the …			
grower period	ns	ns	**
finisher period	**	*	ns
whole period	ns	ns	*
Feed conversion ratio in the …			
grower period	ns	ns	***
finisher period	ns	ns	***
whole period	ns	ns	***
Energy conversion ratio in the …			
grower period	ns	ns	***
finisher period	ns	ns	***
whole period	ns	ns	***
Amino acid conversion ratio for …			
Lysine	***	ns	***
Methionine+Cystine	***	ns	***
Threonine	***	ns	***
Dressing percentage	ns	ns	ns
Back fat thickness, hind	ns	ns	ns
Back fat thickness, mid	ns	ns	ns
Back fat thickness, fore	ns	ns	ns
Lateral fat size	ns	**	ns
Fat size B	ns	*	ns
Muscle area	**	**	*
Fat area	ns	*	ns
Lean-fat ratio	ns	**	ns
Lean meat content	ns	*	ns
Lean in belly	ns	**	ns
pH_1	ns	ns	ns
Electrical conductivity	ns	ns	ns
pH_24	ns	ns	ns
Intramuscular fat content	ns	*	ns

ns: not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
FG*SEX-Interaction not presented due to complete non-significance

Table 6:
Growth performance traits of pigs fed with diets of different Lysine-energy-ratios in the grower and finisher period (LSQM ± SE)

Number of animals [n]	Group_1 Experiment 22	Group_2 Negative control 23	Group_3 Positive control 24	Group_4 Regular control 23
Daily weight gain [g / animal] in the …				
grower period	908 ± 7	917 ± 7	915 ± 6	903 ± 7
finisher period	999[a] ± 22	971[ab] ± 22	918[b] ± 21	911[b] ± 24
whole period	943 ± 13	927 ± 12	912 ± 12	927 ± 13
Daily feed intake [g / animal] in the …				
grower period	2.88 ± 0.05	2.86 ± 0.04	2.80 ± 0.04	2.81 ± 0.05
finisher period	3.38[a] ± 0.07	3.24[ab] ± 0.07	3.06[b] ± 0.07	3.03[b] ± 0.08
whole period	3.07 ± 0.05	2.98 ± 0.05	2.90 ± 0.05	2.97 ± 0.05
Feed conversion ratio [kg feed/kg weight gain] in the …				
grower period	3.17 ± 0.05	3.12 ± 0.05	3.06 ± 0.05	3.11 ± 0.05
finisher period	3.38 ± 0.04	3.35 ± 0.04	3.34 ± 0.04	3.34 ± 0.05
whole period	3.26 ± 0.04	3.23 ± 0.04	3.19 ± 0.04	3.21 ± 0.04
Energy conversion ratio [MJ Metabolisable Energy/kg weight gain] in the …			±	
grower period	40.50 ± 0.63	39.94 ± 0.62	39.30 ± 0.60	39.97 ± 0.66
finisher period	43.26 ± 0.55	42.56 ± 0.55	42.68 ± 0.53	42.41 ± 0.59
whole period	41.64 ± 0.53	41.15 ± 0.51	40.84 ± 0.50	41.03 ± 0.53
Amino acid conversion ratio [g … /kg weight gain] for …			±	
Lysine	28.0[b] ± 0.4	26.1[c] ± 0.4	32.2[a] ± 0.4	31.3[a] ± 0.4
Methionine+Cystine	16.1[b] ± 0.2	15.1[c] ± 0.2	17.5[a] ± 0.2	16.9[ab] ± 0.2
Threonine	19.2[ab] ± 0.2	17.2[c] ± 0.2	19.9[a] ± 0.2	18.5[b] ± 0.3

[a, b, c] LSQM with different letters within a row differ significantly for P < 0.05 (Tukey-Kramer-Test)

Table 7:
Carcass quality traits of pigs fed with diets of different Lysine-energy-ratios in the grower and finisher period (LSQM ± SE)

Number of animals [n]	Group_1 Experiment 22	Group_2 Negative control 23	Group_3 Positive control 24	Group_4 Regular control 23
Dressing percentage [%]	78.1 ± 0.3	78.3 ± 0.3	78.2 ± 0.3	78.3 ± 0.3
Back fat thickness [cm] …				
hind (thinnest location above *M. gluteus medius*)	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
mid (thinnest location above *M. long. dorsi*)	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
fore (thickest location at withers)	3.8 ± 0.1	3.9 ± 0.1	3.7 ± 0.1	3.8 ± 0.1
Lateral fat thickness, ventral end of *M. latissimus dorsi*, 13th rib [cm]	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	3.1 ± 0.1
Fat thickness B, thinnest location lateral of *M. long. dorsi*, 13th rib [cm]	1.5 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
Muscle area, *M. long. dorsi*, 13th rib [cm^2]	45.5[ab] ± 0.8	45.2[b] ± 0.7	48.0[a] ± 0.7	47.9[ab] ± 0.8
Fat area, *M. long. dorsi*, 13th rib [cm^2]	18.1 ± 0.7	18.0 ± 0.6	17.3 ± 0.6	16.5 ± 0.7
Lean-fat-ratio [fat area / muscle area]	0.40 ± 0.02	0.40 ± 0.02	0.36 ± 0.02	0.35 ± 0.02
Lean meat in the carcass [%]	55.1 ± 0.6	55.0 ± 0.5	56.4 ± 0.5	56.4 ± 0.6
Lean meat in the belly [%]	54.9 ± 0.6	54.6 ± 0.6	55.9 ± 0.6	56.4 ± 0.6

[a, b] LSQM with different letters within a row differ significantly for P < 0.05 (Tukey-Kramer-Test)

Table 8:
Meat quality traits of pigs fed with diets of different Lysine-energy-ratios in the grower and finisher period (LSQM ± SE)

Number of animals [n]	Group_1 Experiment 22	Group_2 Negative control 23	Group_ 3 Positive control 24	Group_4 Regular control 23
pH_1, 40 min *post mortem*, 13th/14th rib, *M. long. dorsi*	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1
Electrical conductivity, 24 h *post mortem*, 13th/14th rib, *M. long. dorsi* [mS/cm]	5.1 ± 0.5	4.5 ± 0.4	4.7 ± 0.4	4.2 ± 0.5
pH_24, 24 h *post mortem*, 13th rib, *M. long. dorsi*	5.7 ± 0.03	5.7 ± 0.03	5.7 ± 0.03	5.7 ± 0.03
Intramuscular fat*, 13th rib, *M. long. dorsi* [%]	1.5 ± 0.2	1.9 ± 0.2	1.6 ± 0.2	1.5 ± 0.2

LSQM without letters within a row differ NOT significantly (Tukey-Kramer-Test, $P < 0.05$)
* Random sample of 12 animals (6 castrates + 6 females) in each group

Table 7 illustrates carcass quality traits. Dressing percentage with slightly above 78 % remains unaffected by the four different feeding strategies. Also all body fat associated traits are statistically independent from the differing feeding groups with a slight tendency to lower fat measurements for Group_3 and Group_4 carcasses of pigs fed with improved dietary Lysine-ME-ratio. Muscle area of Group_2 carcasses significantly amounts to only about 94 % of Group_3 pigs' muscle area; whereas the differences between the other groups are non-significant. Group_3 and Group_4 carcasses have insignificantly improved lean-to-fat-ratios compared to Group_1 and Group_2 carcasses; as a result, Group_3 and Group_4 carcasses have somewhat higher lean meat contents than Group_1 and Group_2 carcasses. However in both cases differences are not significant between the respective pairs of groups: about 11 % for lean-to-fat-ratio, and about 2 % for carcass lean. Also lean meat content in the belly has only an insignificant difference of about 3 % between the highest (Group_4) and the lowest (Group_2) roughly corresponding to carcass lean gradation.

Table 8 shows meat quality traits. Physical meat quality criteria, like pH and EC, as well as intramuscular fat content (IMF) as a chemical meat quality trait are not significantly affected by the differing Lysine-ME-ratios in the grower and finisher diets of the four feeding groups. However Group_2 pigs have a non-significantly higher IMF of at least 24 % compared to the mean IMF of the remaining three groups.

Discussion

Lowest N-retention of Feed_3 (Table 4a) is due to the undersupply with EAAs compared to the German recommendations (DLG, 1991; GfE, 2008). This result was to be expected. Data of N-retention concerning day and $LW_{kg}^{0.67}$ in Table 4b explicitly point out the influence of feed quality and the influence of the application of the different feeds during pigs' growth period, i.e. in the grower and in the finisher period. Pigs of the negative control group (Group_2) – both fattening periods with poor feed quality – show non-significantly lowest N-retention (1.13 g per day and $LW_{kg}^{0.67}$). Group_3 pigs of the positive control group (with high feed quality in the starter and in the finisher period) exhibit non-significantly highest N-retention (1.31g per day and $LW_{kg}^{0.67}$). However there are no N-retention differences between Group_1 pigs (experimental group) with 1.22 g per day and $LW_{kg}^{0.67}$ and Group_4 pigs (regular control group) with 1.20 g per day and $LW_{kg}^{0.67}$ although only Group_4 pigs received a diet in the grower period according to the German recommendations (DLG, 1991; GfE, 2008). The slightly higher feed quality in the finisher period for Group_1 pigs (0.69 Lysine-ME-ratio vs. 0.59 Lysine-ME-ratio for Group_4 pigs) seems to compensate the lower N-retention of Feed_2 compared to Feed_1 (Table 4a) in the grower period. This fact is denoted as "compensatory growth" – according to other studies (De Greef et al., 1992; Therkildsen et al., 2004; Whang et al., 2003) which connect compensatory growth with enhanced muscle protein synthesis (= N-retention). The results of the N-balance studies are supported by the growth performance data of Table 6 summarized under "whole period" due to the lack of significances between the four feeding strategies.

Concerning carcass quality traits (Table 7) there is obviously a lack of clear confirmation of a significant carcass-related compensatory effect due to the insignificant decline of meat associated measurements and the simultaneous insignificant increase of fat associated measurements related to carcass graduation (Table 7). This overall tendency is in accordance with Critser et al. (1995) but in conflict with findings of Fabian et al., (2002), Therkildsen et al. (2004) and Skiba (2010).

The lack of variability of the adipose traits in terms of only small differences (Table 7) can be explained by the fact that body fat synthesis is largely independent of the amino acid supply, if simultaneous feed energy supply does not greatly exceed energy demand (Apple et al., 2004), as

it is the case in the present study (compare Table 2 and Methods: Feed). At the same time, the Lysine-ME-ratio is of eminent importance for body protein synthesis (Moehn et al., 2000), leading to a pronounced decline in muscle area of Group_1 and Group_2 pigs (Table 7). Lean meat content of the carcasses reflects the gradation of muscle area (Table 7). In this context it is conspicuous that the differences of lean meat contents between groups are not statistically confirmed in contrast to the above mentioned muscle areas. The lack of significance could be explained by carcass classification procedure. The Bonner Formula for carcass lean estimation uses a regression equation based on muscle area, fat area, three back fat thickness measurements and two lateral fat thickness measurements, as presented in Table 7. The clear predominance of six only slightly shifting adipose traits reduces the effect of declining muscle areas, obviously leading to an only minor numerical graduation in carcass leanness. Similarly, the lean-to-fat-ratio gradation shows the same trend (Table 7).

The question concerning muscle area and lean meat content is whether there is a compensatory effect for Group_1 (and Group_2) pigs or not. Comparing the declining Lysine consumption in Group_3, Group_4, Group_1 and Group_2 pigs following 1 **:** 0.94 **:** 0.84 **:** 0.81 (calculated after Table 3) with its respective muscle area decline of 1 **:** 0.99 **:** 0.95 **:** 0.94 (calculated after Table 7) and carcass lean decline of 1 **:** 1 **:** 0.98 **:** 0.98 (calculated after Table 7) a compensatory effect seems to be visible for Group_1 and Group_2 growing-finishing pigs. Concerning lean meat content, the lack of significance of the differences with simultaneously rigorous interpretation could suggest nearly complete compensation. This is also true when gilts and barrows are considered separately. Only Group_1 barrows with 53.8 % carcass lean differ significantly ($p < 0.05$) from Group_3 gilts with 57.2 % carcass lean, whereas all other differences between group and sex are within a non-significant range from 54.6 % to 56.9 % (data not presented). The same trends apply for muscle area (data not presented). This is in contrast to Martinez-Ramirez et al. (2008b) who report that the greater the intensive genetically determined protein synthesis capacity of the growing-finishing pig, the more pronounced the effect of compensatory protein growth, e.g., intact males are superior to castrated barrows. The same effect could be expected when gilts and barrows are compared according to their well known differing body protein synthesis capacity (Wood et al., 2004). On the other hand, the decline in muscle area and carcass lean is so obvious that compensation is at most partial, as reported by Millet et al. (2006). All things considered, complete negotiation of any compensatory effect concerning muscle area and lean meat content should be denied. And finally, considering muscle area, lean meat content, and N-retention, it can be maintained that the Lysine oversupply of Group_3 animals does indeed not cause significantly enhanced body protein gain, as mentioned in the characterisation of the four groups (compare Methods: Test design).

The enhanced daily weight gain in the finisher period of Group_1 (and Group_2) pigs is attended by an increased feed intake (Table 6), which is a compensatory strategy (Critser et al. 1995) and which is also reported by Millet et al. (2006). The significantly sex-related influence on feed intake (Table 5) is mainly due to an increased feed intake of Group_1 barrows (3.6 ± 0.1 kg/d) compared to 2.9 - 3.2 (S.E. ± 0.1) kg/d for both, barrows and gilts in Group_3 and Group_4 (data not presented). Together with the slightly increased feed intake of Group_2 pigs, the theory reviewed by Whittemore et al. (2001) that pigs "eat by energy and protein" could be supported. So, under lower energy content and limiting amino acids (here Lysine) in the diet, pigs increased their voluntary feed intake (King et al. 2000). The unaffected feed conversion ratio (Table 6) does not support the compensatory growth effect by metabolically improved nutrient utilisation, as mentioned by Kristensen et al. (2004).

The overall weight gain level of the pigs (Table 6) is high and feed and energy conversion ratios represent an improved organic standard compared to conventional pig fattening (Millet et al., 2004). The respective conversion levels reflect ad libitum offered feed. Furthermore it can be seen that the different Lysine-ME-ratios between the groups do not influence daily weight gain in the grower and whole fattening period as well as feed and energy conversion ratios in all periods. The development of these traits is primarily influenced by the dietary energy supply (Moehn et al., 2000). Therefore the respective results are not surprising because dietary energy supply was according to need compared to DLG-recommendations (DLG, 1991; GfE, 2008), as mentioned in Table 2 (compare Methods: Feed). In contrast, the ratio of the essential amino acids (in particular relating to the first limiting amino acid Lysine) to the energy content in the diet is responsible for the body's own protein and fat synthesis (Moehn et al., 2000). The chosen Lysine-ME-ratios in the four feeding groups – together with the optimised ratios of the further limiting amino acids to each other (Table 2) – obviously did not result in augmented adiposis (Table 7), otherwise daily weight gain, feed and energy conversion ratios (Table 6) would have been negatively affected (Kapelanski et al., 2001).

As expected, physical meat quality criteria (pH and electrical conductivity) are unaffected by the four feeding strategies (Table 8), because its variability is based on the genetic origin or on the environmental pre-slaughter conditions but not on the diet fed (Fischer, 2001). The values above 6.0 for pH_1 (measured about 40 minutes post mortem) and beneath 6.0 mS/cm for electrical conductivity (mea-

sured 24 hours post mortem) indicate the absence of PSE (pale, soft, exudative) meat quality aberrance (Weißmann & Honikel, 1998). The pH_24 values (measured 24 hours post mortem) indicate lacking DFD (dark, firm, dry) meat quality defect. Also intramuscular fat content does not differ significantly between the four feeding strategies (Table 8). The range from 1.5 % to 2.0 % approximately corresponds to the expectations resulting from the level of lean meat in the carcass (Table 7). The highest ($P > 0.05$) intramuscular fat content of the pigs of the negative control group (Table 8) corresponds with its lowest ($P > 0.05$) N-retention (Table 4b) and with the poor characteristic of its lean meat and body fat associated carcass traits (Table 7).

Conclusions

The study demonstrated that diets of 100 % organic origin with moderate crude protein content result in decreased Lysine-ME-ratios in the grower diet. Independently from a slight over-supply or demand-covering Lysine-ME-ratio in the finisher period, economically important traits were insignificantly enhanced (daily weight gain), more or less unaffected (feed conversion ratios), and insignificantly reduced (lean meat content of the carcass) compared to finisher diets according to or exceeding German Lysine-ME-ratio recommendations. It is concluded that at least a certain degree of compensatory growth takes place. The results indicate that a 100 % organic-based feeding of growing-finishing pigs is slightly problematical for carcass quality, but not problematical for growth performance or meat quality. Hence, the current market situation with the predominance of lean meat which determines the price situation must be taken into account; in particular, farmers' marketing partners have to adjust their carcass quality-related standards and payment system to the potential of the desired organic fattening system. In conclusion, 100 % organic-based feeding in pig fattening is a challenge which can be met, not least due to the presence of compensatory growth mechanisms which help to reduce the protein gap in organic monogastric feeding.

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Effects of rare earth elements (REE) supplementation to diets on the health and performance of male and female pre-ruminant calves and growing female calves

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Abstract

Two feeding trials with pre-ruminant and growing Holstein calves were carried out in order to investigate the effect of rare earth elements (REE) on feed intake and performance parameter. In the first experiment, applying a complete two by two factorial design, 87 calves (45 female and 42 male) with a mean age of 9.6 ± 1.8 days were randomly assigned to one of two treatments (Con or REE). The animals received milk replacer either with or without 200 mg/kg REE-citrate containing mainly cerium (57.9 %), lanthanum (34.0 %) and praseodymium (6.5 %) as well as concentrate and grass hay over a period of 44 days. The feed intake and performance parameter were not significantly and interactively affected by sex and REE supplementation. However, the supplemented animals consumed numerically less concentrate (13.5 %) and grass hay (26.3 %) compared to control animals which led to a slightly reduced live weight gain (496 g/day in REE group vs 525 g/day in control group). In the second experiment, a total of 47 growing female calves (average initial live weight of 108 ± 9 kg) were divided into four treatment groups (n = 11 or 12 per group): one control group and three REE-treated groups fed a supplement of 100, 200 and 300 mg REE-citrate per kg dry matter (DM). The calves were offered grass silage, grass hay and concentrate. The experiment was terminated when the calves reached a live weight of approximately 182 kg. The feed intake, live weight gain, feed-to-gain ratio and ME-to-gain ratio were not significantly influenced by increasing REE-citrate supplementation. Also, a numerically tendency towards reduced feed intake and live weight gain could be observed for the group fed with the highest amounts of REE. Thus, it can be concluded that REE-citrate are not suited to improve the performance of calves.

Keywords: pre-ruminant, female calves, rare earth elements; growth performance

Zusammenfassung

Einfluss des Einsatzes von seltenen Erden in der Fütterung von männlichen und weiblichen Kälbern und Jungrindern auf Gesundheits- und Leistungsparameter

Zur Bestimmung des Einflusses Seltener Erden (REE) in der Ernährung von Kälbern und Jungrindern auf deren Futteraufnahme und verschiedene Gesundheits- und Leistungsparameter wurden zwei Versuche durchgeführt. Im ersten Experiment erhielten 87 Kälber (45 weiblich und 42 männlich) über einen Zeitraum von 42 Tagen beginnend mit einem mittleren Alter von 9.6 ± 1.8 Tagen Milchaustauscher mit oder ohne Zulage von 200 mg/kg REE-Zitrat, Kraftfutter und Heu. Das REE-Zitrat enthielt hauptsächlich Cer (57.9 %), Lanthan (34.0 %) und Praseodym (6.5 %). Die REE-Supplementierung führte zu keiner signifikanten Beeinflussung der Futteraufnahme und der Leistungsparameter. Dennoch nahmen die mit REE supplementierten Tiere 13.5 % weniger Kraftfutter und 26.3 % weniger Heu auf, was zu einer geringfügig reduzierten Lebendmassezunahme von 496 g/Tag in der REE-Gruppe gegenüber 525 g/Tag in Kontrollgruppe führte. Im zweiten Experiment wurden 47 Jungrinder mit einer mittleren Lebendmasse von 108 ± 9 kg in vier Gruppen mit 11 oder 12 Tieren aufgeteilt. Neben einer nicht supplementierten Kontrollgruppe wurden drei Versuchsgruppen mit 100, 200 und 300 mg REE-Zitrat je kg Trockenmasse supplementiert. Die Tiere erhielten bis zum Versuchsende mit einer Lebendmasse etwa 182 kg Grassilage, Heu und Kraftfutter. Die Futteraufnahme, die Lebendmassezunahme und die Futterverwertung wurden nicht durch die REE-Supplementation beeinflusst, wobei ein Trend zu einer geringeren Futteraufnahme und einer verminderten Lebendmassezunahme in der am höchsten supplementierten Gruppe beobachtet wurde. Unter den in den Versuchen vorliegenden Bedingungen führte die Zulage von Seltenen Erden nicht zu einer verbesserten Leistung der Tiere.

Schlüsselwörter: nicht ruminierend, weibliche Kälber, Seltene Erden, Wachstumsleistung

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1 Introduction

In China, it was discovered that rare earth elements (REE) could stimulate the growth of agricultural products and food producing animals, hence they have been used successfully for decades in farms as fertilizer and as feed additive, mainly containing lanthanum, cerium, praseodymium and neodymium (Redling, 2006). Based upon this information, trials were performed under European production conditions with the aim to find new substances in order to replace antibiotics as growth promoters. Also, in several Western feeding studies it could be shown that dietary supplementation of REE had positive effect on both weight gain and feed conversion rate of pigs and poultry (He and Rambeck, 2000; He et al., 2001; He et al., 2010). In a Swiss study, Kessler (2004) showed, that supplementation of 200 mg REE-citrate in diet of pigs significantly improved daily live weight gain by 8.8 % and significantly decreased feed-to-gain ratio by 3.6 %. In this study, it was particularly interesting and has not been described before that the effect in female pigs was roughly twice as high as in castrated pigs. Up to now, only a limited number of investigations on ruminants at different genders and periods of growth, including rearing, growing and fattening are available in the literature and the obtained results of REE are controversial. Schwabe et al. (2009) observed after a supplementation of REE to diet for 32 weeks adverse effects on live weight gain, feed-to-gain ratio and metabolizable energy-to-gain ratio of fattening bulls (initial live weight 245 kg), while Miller (2006) found no effect on live weight gain after an application of REE on growing male calves (initial live weight 83 kg, average age 44 days) for 10 weeks. In contrast to that, positive effects on performance due to REE supplementation were observed by Meyer et al. (2006) on pre-ruminant female Holstein calves. Based upon these findings, it might be possible that age and gender play a role regarding efficiency of REE. Therefore, the objective of these two feeding studies was to investigate the effect of REE on feed intake and growth performance focused on pre-ruminant female and male calves as well as on growing female Holstein calves.

2 Material and methods

2.1 Treatments, experimental design and animals

Two feeding experiments were conducted at the Experimental Station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI) in Braunschweig, Germany.

In feeding experiment 1, a total of 87 calves (42 male and 45 female) of the "German Holstein breed" from the milk cattle herd FLI-Braunschweig were used. They were born between October 2006 and March 2007 and separated from their dams one day after birth, and moved into individual straw-bedded calf hutches (87 x 175 cm) in an adjacent thermally non-regulated stable. In the first seven days of life, each calf received six litres of colostrum per day and then milk replacer (manufacturer, Norlac GmbH, Zeven), divided into two equally-sized portions, in the morning and afternoon from teat buckets. After 9.6 ± 1.8 days, the calves were randomly assigned to one of two treatments (Con or REE) for 44 days and kept separately in two identical pens with straw bedding in the same building. Each calf started individually with the trial depending on its day of birth. After transfer to the group pens, the calves were fed 600 g milk replacer at a concentration of 100 g/L water per animal and day up to day 43 and 200 g per calf on day 44 supplemented either with or without 200 mg REE-citrate/kg milk replacer via a computer-controlled milk feeder. The supplement (Lancer® 500, Zehentmayer AG Berg, Switzerland) consists of 50 % wheat starch and 50 % lanthanoide, whereby the lanthanoide (REE-citrate) itself consisted of 25.3 % rare earth elements and 74.7 % citrate, respectively. The REE-citrate was a mixture containing organic citrate compounds of 57.9 % cerium, 34.0 % lanthanum, 6.5 % praseodymium and 1.6 % of other rare earth elements. In addition to milk replacer, the calves had free access to grass hay and concentrate up to 1 kg per calf and day by using concentrate feeder from the beginning of the trial. Water was continuously provided by bowl drinkers. The composition of the milk replacer and the concentrate is presented in Table 1 and 2. Each calf was equipped with an ear transponder recording continuously the daily individual feed intake of milk replacer and concentrate, however, the hay intake was measured daily per group. The live weight [LW] of calves was measured at the start and end of the study with cattle scales.

Table 1:
Composition of the milk replacer [g/kg] used in the milk feeding period

Group	Control	REE 200
Components		
Skim milk powder	505	505
Whey powder	289	289
Palm-coconut-soya oil	173	173
Wheat starch	30	29.6
Mineral and vitamin premix*	3	3
Lancer®500†	-	0.4

Notes: *per kg mineral and vitamin premix: 8 g Ca; 7.5 g P; 100 mg Fe; 8 mg Cu; 100 mg Zn; 50 000 IU vitamin A; 3.300 IU vitamin D_3; 80 mg vitamin E; 150 mg vitamin C; 8 mg vitamin B_1; 7 mg vitamin B_2; 4 mg vitamin B_6; 50 mcg vitamin B_{12}; 8 mg vitamin K_3; 150 mcg Biotin; †Lancer®500 consists of 50 % wheat starch and 50 % lanthanoide

Table 2:
Composition of the concentrates [g/kg] used in the milk feeding period (MFP) and growing period (GP)

Group		A	B	C	D
	Con/REE	Control	REE 100	REE 200	REE 300
Period	MFP	GP	GP	GP	GP
Components					
Soy bean meal	300	300	300	300	300
Oats	305	305	305	305	305
Barley	180	180	180	180	180
Wheat	170	170	169.7	169.4	169.1
Soybean oil	15	15	15	15	15
Calcium carbonate	10	10	10	10	10
Mineral and vitamin premix*	20	20	20	20	20
Lancer®500†	0	0	0.3	0.6	0.9

Notes: *per kg mineral and vitamin premix: 160 g Ca; 100 g Na; 80 g P; 30 g Mg; 1000 mg Fe; 800 mg Cu; 4000 mg Mn; 6000 mg Zn; 50 mg I; 50 mg Se; 30 mg Co; 800 000 IU vitamin A; 80 000 IU vitamin D_3; 100 mg vitamin E; †Lancer®500 consists of 50 % wheat starch and 50 % lanthanoide

The feeding experiment 2 was carried out with 47 female German Holstein calves and started with a stepwise inclusion of animals according to the calving of the FLI-milk cattle herd. At the beginning of the trial the calves had an average initial LW of 108 ± 9 kg and an average age of 14 ± 0.4 weeks. They were randomly assigned to one of four treatment groups with 11 or 12 animals each designated as groups A, B, C and D with an intended REE-citrate supplementation of 0, 100, 200 and 300 mg/kg dry matter in the diet, respectively, over the complete trial period. During this time, the calves were housed in an unheated and non-insulated stable and were kept in group boxes according to their feeding group. The group pens with the dimensions 5.20 x 7.40 m were equipped with a slatted floor and partly covered with straw bedding. Grass silage and grass hay were available for *ad libitum* intake via computer controlled troughs (Type RIC, manufacturer: Insentec B.V., Marknesse, The Netherlands) and water via bowl drinkers. Additionally, each calf had access to 2.0 kg of concentrate per day from the concentrate station (Type AWS HF, manufacturer: Insentec B.V., Marknesse, The Netherlands), which was supplemented with or without REE. The precise composition of the concentrates is summarised in Table 2. The ration was formulated to meet the nutritional requirements for growing calves as recommended by the German Society of Nutrition Physiology (GfE, 2001). The daily intake of concentrate and roughage was recorded continuously by using an automated feeding system (Insentec B.V., Marknesse, The Netherlands) and ear transponder for each calf. The individual LW was measured automatically when calves entered the concentrate feeding station. The experiment was terminated when the individual calves reached a LW of approximately 182 kg.

2.2 Sample collection, preparation and analysis

Representative samples of the milk replacer, concentrate and grass hay were collected once, while grass silage samples were taken twice a week and pooled over approximately four weeks. During the collection period, the grass silage samples were stored at -19 °C and then dried at 60 °C for 72 h. For proximate analyses, all samples were ground to pass though a sieve with 1 mm pore size and homogenized. The composition of the feedstuffs (dry matter (DM), crude ash, crude protein, crude fat, crude fibre, acid detergent fibre and neutral detergent fibre) was determined according to the methods of the Association of German Agricultural Analysis and Research Centres (Seibold & Barth, 1976 to 1997) in the laboratory of the Institute of Animal Nutrition, Braunschweig. Additionally, the REE concentrations in feedstuffs and REE-citrate mixture (Lancer® 500) were determined by inductively coupled plasma-mass-spectrometry (Thermo ICP-MS X Series) according to the international standard DIN EN ISO 17294-2: 2005-02 in the laboratory of the Food GmbH Analytik–Consulting in Jena, Germany.

2.3 Calculations and Statistics

The metabolizable energy (ME) concentration of the diets was calculated by using the equation number 1.1.2 of the Society of Nutrition Physiology (GfE, 2001). The digestibilities of feedstuffs (milk replacer, concentrate and grass hay) were taken from the tabular values for each ingredient (DLG, 1997), whereas the ME concentration of grass silage was estimated by using the nutrient digestibilities from the standardised digestibility trial with four adult wethers according to the procedures as described by (GfE, 1991). The daily LW gain for both trials was calculated as the difference between the recorded live weight at the start and the end of the two experiments, divided by the number of days.

The performance parameters of experiment 1 were analyzed by a complete two by two factorial design of analysis of variance (ANOVA) according to the following model:

$$Y_{ijk} \quad \mu + a_i + b_j + axb(_{ij}) + e_{ijk} \qquad (1)$$

where

Y_{ijk} k^{th} observation related to the REE concentration i and sex j
μ overall mean

a_i effect of dietary inclusion of REE (0; 200 mg REE-citrate/kg diet)
b_j effect of the sex
$axb_{(ij)}$ interactions between REE concentration and sex
e_{ijk} error term.

The performance parameters of experiment 2 were analyzed according to a one-way factorial design of analysis of variance (ANOVA) corrected for initial LW using the following model:

$$Y_{ij} = \mu + a_i + \beta_{y.x}(x_{ij} - \bar{x}\ ..) + e_{ij} \quad (2)$$

where

Y_{ij} tested parameter of the calf"j" fed diet type "i"
μ overall mean
a_i effect of dietary inclusion of REE (0, 100, 200 and 300 mg REE-citrate/kg diet)
$\beta_{y.x}$ regression coefficient of y on x
x_{ij} co-variable
$\bar{x}$ mean value for x
e_{ij} error term

Results of AVONA are presented in the tables either as arithmetic means (Experiment 1) or as least square means (LSmeans; Experiment 2) and the pooled standard errors of means (PSEM). In case of significant treatment effects the Tukey-Test for multiple comparisons was used to identify significant mean value differences ($p < 0.05$).

The Statistica for Windows™ operating system (StatSoft Inc. 2007, Version 10.0) was used for all statistical evaluations.

3 Results

3.1 Composition and REE concentrations of feedstuffs used in experiment 1 and 2

The contents of crude nutrients and the REE (pure elements) concentrations of the analyzed feedstuffs milk replacer, concentrate and roughage as well as the Lancer 500® are summarised in Tables 3 and 4. The nutrient composition of the two milk replacers was almost equal and only very small differences in crude nutrients could be detected between control and supplemented concentrates (Tables 3 and 4). Negligible amounts of REE were detected in the unsupplemented milk replacer, whereas 9.5 and 17.0 mg total REE/kg DM were measured in the concentrate of experiment 1 and in the unsupplemented control concentrate of experiment 2. In the supplemented concentrates, total REE concentrations of 43, 90, 124 mg/kg DM were detected and were increased as intended by formulation. Also grass hay and grass silage, which were used as roughage contained low levels of REE (Tables 3 and 4).

Table 3:

Dry matter, nutrient composition and energy concentration of the milk replacer, concentrates and grass hay as well as the rare earth element (REE) contents in the milk feeding period

	Milk replacer				
REE-citrate supplementation [mg/kg DM]	0	200	Concentrate	Grass hay	REE-Citrate
Dry matter [g/kg]	961	961	883	863	
Crude nutrients [g/kg DM]					
Crude ash	78	77	64	62	
Crude protein	212	216	234	85	
Crude fat	141	142	42	15	
Crude fibre	-	-	67	303	
Acid detergent fibre	-	-	84	318	
Neutral detergent fibre	-	-	200	604	
Metabolizable energy [MJ/kg DM]	15.9	15.9	12.6	9.0	
Pure rare earth elements [mg/kg DM]					
Lanthanum	0.1	24	2	0.3	42 243
Cerium	0.1	41	4	0.6	73 395
Praseodymium	0.0	5	0.5	0.1	8 641
Neodymium	0.0	0.02	1.7	0.1	23
Remaining REE*	0.0	1	1.3	0.1	1 977
Total rare earth elements	0.2	71.02	9.5	1.2	126 279

Notes: *Without the element Scandium

Table 4:
Dry matter, nutrient composition and energy concentration of the concentrates, grass silage and grass hay as well as the rare earth element (REE) contents in the growing period

REE-citrate supplementation [mg/kg DM]	Concentrates				Grass silage	Grass hay	REE-citrate
	0	100	200	300			
Dry matter [g/kg]	889	894	890	889	342	870	
Crude Nutrients [g/kg DM]							
Crude ash	64	64	65	62	100	64	
Crude protein	223	216	210	213	150	95	
Crude fat	43	43	42	42	36	17	
Crude fibre	69	71	69	70	281	306	
Acid detergent fibre	88	89	87	89	308	323	
Neutral detergent fibre	207	216	213	212	520	609	
Metabolizable energy [MJ/kg DM]	12.6	12.6	12.6	12.6	9.5	9.0	
Pure rare earth elements [mg/kg DM]							
Lanthanum	5	13	29	41	0.5	0.2	42 243
Cerium	9	23	51	71	0.9	0.4	73 395
Praseodymium	1	3	6	9	0.1	0.1	8 641
Neodymium	1	2	2	1	0.2	0.1	23
Remaining REE*	1	2	2	2	0.2	0.1	1 977
Total rare earth elements	17	43	90	124	1.9	0.9	126 279

Notes: *Without the element Scandium

3.2 Performance parameters

3.2.1 Experiment 1

In the REE group, one male calf died on the 4th day of trial for unknown reasons and was not included in the statistical analysis. Furthermore, in both groups some calves were treated due to digestive and respiratory problems. The performance parameters of the pre-ruminant calves over the milk feeding period are shown in Table 5. The mean daily REE-citrate intake of the REE group was 111 mg/d or 123 mg/kg total DM. The initial and final weight was similar between the control and REE group and no interaction was observed between group and sex. However, a significant sex effect was observed at the start and the end of the experiment, as the female calves showed lower live weights than the male calves (Table 5). The mean intake of milk replacer and concentrate was not significantly affected by the REE supplementation, although numerically differences could be determined in the concentrate intake. The supplemented animals consumed 42 g/d (13.5 %) less concentrate than the control animals. With regard to the single group means it becomes obvious that this fact mainly result from the supplemented male calves (Table 5). Furthermore, the REE group showed a lower grass hay intake (26.3 %) compared to the control group, but no statistical analysis could be performed because the feed consumption was recorded daily per group. This depression in feed intake was reflected in the daily weight gain of the calves (496 g/day in REE group vs 525 g/day in control group). As a consequence of the lower feed intake the ME- and crude protein intake was influenced in a similar manner. The feed-to-gain ratio and ME-to-gain ratio was similar between the two groups (Table 5).

3.2.2 Experiment 2

The growth experiment 2 comprised the LW range between 108 ± 9 kg and 182 ± 14 kg and lasted approximately 11 weeks in total. During this time, no calf died and no health problems were observed. The average actual intake of REE-citrate/kg DM amounted to 0; 74 ± 7; 149 ± 13 and 225 ± 15 mg for the Group A, B, C, and D, respectively, and differed distinctly from the planned intake. The grass silage, grass hay and concentrate intake was not different among treatments. Although not statistically significant, the calves of Group D (300 mg REE-citrate/kg DM) consumed less grass silage (2.9 %) and grass hay (12.1 %) than the calves of the control group (Table 6). Hence, the total feed intake was the lowest for Group D with 4.02 kg DM/d followed by the Group C with 4.06 kg and Group B with 4.10 kg daily DM intake. The control

Table 5:

Effects of rare earth elements (REE) supplemented feed for calves on feed intake, live weight as well as live weight gain, feed-to-gain ratio and ME-to-gain ratio during the milk feeding period (Experiment 1)*

Group	Sex	Number of calves/ group	Feed intake [g DM/day]				Nutrient intake	Energy intake	Pure REE		Initial weight	Final weight	Live weight gain	Feed-to-gain ratio	Metabolizable energy – to gain ratio
			Milk replacer	Concentrates	Grass hay	Total feed intake	Crude protein [g/d]	Metabolisable energy [MJ/d]	[mg/kg DM]	[mg/kg LW/d]	[kg]	[kg]	[g/d]	[kg/kg]	[MJ/kg]
Control	.....	43	558	311	137	1006	204	14.0	3.1	0.05	43.4	66.5	525	2.00	27.9
REE	.....	44	557	269	101	927	193	13.2	45.9	0.8	43.7	65.5	496	1.94	27.6
.....	female	45	558	295							41.1	63.3	504		
.....	male	42	556	283							46.1	68.9	518		
Control	female	22	560	300							40.8	62.9	502		
Control	male	21	555	321							46.2	70.4	549		
REE	female	23	557	291							41.4	63.7	506		
REE	male	21	557	245							46.1	67.5	486		
AVOVA (probability)															
Group			0.838	0.172	-‡	-	-	-		-	0.807	0.570	0.270	-	-
Sex			0.362	0.688	-	-	-	-		-	< 0.001	0.003	0.621	-	-
Group x Sex			0.383	0.291	-	-	-	-		-	0.756	0.380	0.212	-	-
PSEM			2	22	-	-	-	-		-	0.9	1.3	19	-	-

..... denotes that these effects were pooled
*Values are given as means and pooled standard errors of means (PSEM)
‡ No statistical analysis was carried out because the grass hay intake was measured daily per group and the probability of difference could not be calculated

Table 6:

Effects of rare earth elements (REE) supplemented feed for female calves on feed intake, crude protein intake, ME intake, live weight gain, feed-to-gain ratio and ME-to-gain ratio during the growing period from 108 kg to 182 kg body weight. (Experiment 2)*

Group	A	B	C	D	ANOVA (probabilities)			
REE-citrate [mg/kg DM]	0	100	200	300	PSEM	REE	Linear	Quadratic
Female calves/group	12	11	12	12				
Feed intake [kg DM/d]								
Grass silage	1.74	1.80	1.72	1.69	0.10	0.906	0.596	0.670
Grass hay	0.66	0.58	0.56	0.58	0.07	0.733	0.408	0.482
Concentrate	1.72	1.72	1.78	1.75	0.03	0.342	0.214	0.559
Total feed intake	4.12	4.10	4.06	4.02	0.10	0.857	0.387	0.938
Crude protein intake [g/d]	688	681	685	669	13	0.720	0.364	0.699
ME# intake [MJ/d]	44.2	44.0	43.7	43.3	0.9	0.901	0.458	0.883
Live weight gain [g/d]	994	997	991	972	17	0.739	0.352	0.525
Feed-to-gain ratio [kg/kg]	4.18	4.13	4.11	4.15	0.10	0.966	0.818	0.666
ME-to-gain ratio [MJ/kg]	44.8	44.3	44.3	44.7	1.0	0.981	0.970	0.678
REE-citrate [mg/kg DM]	0^{d}	74^{c}	149^{b}	225^{a}	3.0	< 0.001	< 0.001	0.715
Pure REE [mg/kg DM]	8.0^{d}	18.5^{c}	40.5^{b}	55.3^{a}	0.8	< 0.001	< 0.001	0.016
Pure REE intake [mg/kg LW/d]	0.2^{d}	0.5^{c}	1.1^{b}	1.6^{a}	0.0	< 0.001	< 0.001	0.004

Notes: *Values are given as least square means (LSmeans) and pooled standard errors of means (PSEM)
#Metabolizable Energy
Values with different superscripts within a row are significantly different ($p < 0.05$)

group (A) showed the highest total feed intake but these observed differences were not significant (Table 6). Similar results were observed for the crude protein and metabolizable energy intake. The averaged daily weight gain over all treatment groups amounted to 988 ± 77 g and was in normal ranges for female calves of this age. However, due to reduced feed intake the calves of Group D showed a slightly but not significantly lower daily weight gain than the other groups. The calculated feed-to-gain ratio and ME-to-gain ratio also remained unaffected by the dietary treatments.

4 Discussion

These two experiments were conducted to test whether age and gender play a role regarding efficiency of REE on feed intake and performance parameter. The nutrient composition and energy content of the milk replacer and concentrates, as shown in Table 3 and 4, were comparable between dietary treatments. As already extensively discussed by Schwabe et al. (2011), REE are occurring ubiquitously, therefore they were also detected in roughage and concentrate used in experiment 1 and 2 of the control group.

The health state of calves was not impaired by the REE application as reported by different research groups in feeding experiments with broilers (He et al., 2010) pigs (Förster et al., 2008), calves (Miller, 2006) and fattening bulls (Schwabe et al., 2011), before. In experiment 1, diarrhoea and respiratory diseases were diagnosed and treated, but a relation to REE supplementation was not observed. These diseases are the two most common diseases that occur in young calves.

The birth weight of male calves was higher than that of female calves (data not shown). This result is similar to reports of various authors (Kertz et al., 1997; Junge et al., 2003), who also found verifiable differences of similar magnitude among different gender. For this reason, the male calves were 10.5 % and 8.1 % heavier at the beginning and the end of the first trial (Table 5). With regard to the age of the piglets, some of the preliminary studies suggested, that the effect of performance enhancement of REE is better feasible in newly weaned than in older animals under the optimized conditions of European piglet production (He and Rambeck, 2000; Knebel, 2004; Kraatz et al., 2006). To our knowledge, Meyer et al. (2006) are the sole authors, whom up to now studying the influence of dietary REE supplementation (200 mg REE-citrate/kg milk replacer) on feed intake and performance of female pre-ruminant calves for a period of 6 weeks. Following this study, we used in the experiment 1, the same feed, dose and additives, however with both gender and a higher number of animals. We observed, that the REE treated calves consumed numerically lower concentrate (42 g/d), which mainly resulted from the male calves, however, no interaction between group and sex was observed ($p = 0.291$; Table 5). Furthermore, the supplementation of REE reduced grass hay intake, resulting in lower total DMI in REE group (-7.9 %) versus control group. However, the individual grass hay intake could not be recorded for technical reasons. This finding is supported by the results of the above mentioned authors (Meyer et al., 2006), who also found a decreased total DMI of -7.8 % as a result of reduced hay intake (-44 %), while no differences were observed either in the milk replacer intake (553 g/d in the control group versus 560 g/d) nor in the concentrate intake (156 g/d in the control group versus 161 g/d). In a more recent long-term study with growing bulls Schwabe et al. (2011) reported a significant linear decrease in the grass hay intake from 0.55 to 0.31 kg/d during the growing period (119 to 180 kg LW) as well as in the maize silage intake from 6.09 to 5.44 kg/d during the fattening period (180 to 556 kg LW) after the supplementation of 0, 100, 200 and 300 mg REE-citrate/kg diet. In the present experiment, the increasing REE-citrate supplementation to the diet of growing female calves had no significant effect on the feed intake but a numerally reduction in the feed intake was also detectable (Table 6). The reason for the often observed reduction in the feed intake of cattle, as described above, induced by REE supplementation with the exception of the investigation of Schwabe et al. (2009) is unclear. However, it is well known that REE have many similarities to the element calcium in size, bonding as well as in coordination geometry and donor atom preference which enables them to compete with the calcium ions (Evans, 1990; Redling, 2006). On this basis, it was described that REE can replace calcium ions at superficial membranes sites and prevented calcium ions uptake to less accessible calcium ions sites in smooth muscles and the muscle contraction is thereby inhibited (Weiss and Goodman, 1969; Weiss, 1974; Triggle and Triggle, 1976; Redling, 2006). This in turn can alter the gastrointestinal motility which may decrease the passage rate and therefore affect the feed intake. A second explanation may be an effect on nervous system as feed intake and gastrointestinal motility are controlled via the vegetative nervous system. Since previous studies have demonstrated that lanthanoide showed a variety of actions on nerve cells and most of them are related to the interaction with Ca^{2+} involved transport processes such as inhibition of calcium binding to synaptosomal membranes (Basu et al., 1982) and inhibition of calcium-dependent neurotransmitter release (Przywara et al., 1992). It was also observed that lanthanum can influence the neural release of serotonin (Stefano et al., 1980).

The slightly decrease in feed intake of the REE treated calves was reflected in the live weight gain in both ex-

periments. In experiment 1 the LW gain was 5.5 % and in experiment 2 up to 2.2 % lower compared to the control groups. In contrast, Meyer et al. (2006) observed in spite of a decreased total feed intake a higher average daily LW gain of 14.6 % ($p > 0.05$) in supplemented female pre-ruminant calves. However, our findings are in agreement with Miller (2006), who also found a numerical decrease in LW gain when fattening bulls (83 kg initial LW, an average age of 44 d) were fed with 200 mg REE-citrate/kg over a period of 10 weeks. A recent study by Schwabe et al. (2011) showed similar results, while Schwabe et al. (2009) could even determine a significant decrease in live weight gain of 7 % and an increase in feed-to-gain ratio and ME-to-gain ratio of 10 % when REE-citrate was fed at a concentration of 200 mg/kg DM to fattening bulls in a LW range of 245 to 558 kg. In both experiments of the present study, the feed-to-gain ratio and ME-to-gain ratio were not affected by dietary treatments which indicated that the observed decrease in the weight gain of REE calves could primarily be attributed to reduced voluntary feed intake.

5 Conclusion

In the present feeding trials the use of REE-citrate as feed additive in rations for pre-ruminant and growing calves caused a numerically reduced feed intake, which resulted in a decreased live weight gain.

Based on this inefficiency of REE-citrate under the current experimental conditions it may be concluded that its use as growth promoters in calf diets cannot be recommended.

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Erprobung des Echtzeit-Ortungssystems Ubisense zur Erfassung der Aufenthaltsdauer von Ziegenlämmern in Futterhecken

Sophia Bender, Gracia Ude und Heiko Georg*

Zusammenfassung

Um Tierverhalten auf der Weide untersuchen zu können, wurde das Echtzeit-Ortungssystem Ubisense 7000 erstmals unter Freilandbedingungen und mobil zur Erfassung und Ortung von 30 Ziegenlämmern erprobt. Zum einen sollte die Frage geklärt werden, ob sich das Ortungssystem Ubisense 7000 in Bezug auf Anbringung am Tier, Datenerfassung sowie Wetterfestigkeit für den Einsatz im Freiland eignet, zum anderen sollte die Aufenthaltsdauer von Lämmern in unterschiedlich gepflegten Futterhecken untersucht werden. Die Ergebnisse zeigen, dass die Futterhecke im Versuchszeitraum Herbst nur tagsüber genutzt wurde, während einzelne Weidephasen auch nachts stattfanden. Die Datenaufnahme war nach Anpassung von Technik an Futterhecke und Tier erfolgreich.

Schlüsselwörter: Echtzeit-Ortungssystem, Ziegenlämmer, Futterhecke

Abstract

Feasibility test of the real-time location system Ubisense to measure browsing of fodder hedges with goat kids

To assess animal behaviour of goat kids on pasture, a real-time location system, Ubisense 7000, was installed mobile and outdoors. One major goal of the study was the feasibility of the location system regarding fixation at animals, collecting data and outdoor durability. The other goal was to analyse the durations of stay that goat kids spent in three differently maintained fodder hedges. Results show that grazing periods happen even during the night, whereas fodder hedges were used during daytime only. Data were successfully collected after adaptation of technique to fodder hedges and animals.

Keywords: Real-time-location system, goat kids, fodder hedges

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Einleitung

Eine artgerechte Haltung von Ziegen sollte einen regelmäßigen Weidegang vorzugsweise mit Heckennutzung einschließen, da natürliche Neugier, Bewegungsdrang und Sozialverhalten oft besser befriedigt werden können als im Stall (Deinhofer, 2009); als fakultative Buschbeweider können Ziegen bis zu 60 % ihres Futterbedarfs über Gehölze decken (Rahmann, 2000).

Eine Positionsbestimmung in großräumigen Freilanduntersuchungen erfolgt über Satellitennavigation, die in der Tierhaltung auch angewendet wird (Umstatter, 2011). Die Ortung von Nutztieren in ihrer Stallumgebung hingegen gestaltet sich selbst für bereits industriell erprobte Verfahren aufgrund von Abschattungen, Metallen in der Stalleinrichtung und dem rauen Umgebungsklima schwieriger und muss angepasst werden (Gygax et al., 2007).

Zur Untersuchung des Tierverhaltens von Ziegenlämmern auf der Weide wurde das Echtzeit-Ortungssystem Ubisense 7000 erstmals unter Freilandbedingungen und mobil erprobt.

Literatur

Status quo Verhaltensforschung

Die Verhaltensforschung in Ställen erfolgt zumeist immer noch über Direktbeobachtungen oder Kameraaufzeichnungen, die später aufwendig aufgearbeitet und ausgewertet werden müssen.

Technische Hilfen sind z. B. bei Rindern Pedometer, die sich für die Aufzeichnung von Aktivität, Liegen und Temperatur, aber nicht für eine Positionsbestimmung eignen. Im Schweinebereich untersuchten Hessel et al. (2008) eine stationäre Antenne zur simultanen Einzeltiererkennung im Futtertrog von Ferkeln, die mit High-Frequency-Transpondern gekennzeichnet waren und erreichten eine Lesequote von rund 98 %. Burose und Zähner (2008) entwickelten eine stationäre Antenne zur automatischen Identifikation von Schweinen, deren Lesequote je nach Antenne (ein- und zweidimensional, Kunststoffmatten, kleine Leseantennen) und Transpondertyp (Anti-Kollisionstransponder und ISO-Transponder) zwischen 52 und 98 % lag.

Ortungssysteme zur automatisierten Erfassung von Verhaltensdaten werden seit einigen Jahren in Rinderställen (zur Arbeitserleichterung) zur Unterstützung des Herdenmanagements, wie z. B. der Brunstbeobachtung eingesetzt. Da sich GPS-basierte Systeme in Stallgebäuden durch die Abschattung der Satellitensignale nicht für eine genaue Positionsbestimmung eignen, wird bei Ortungssystemen zumeist eine eigene Infrastruktur mit Sensoren und Identifikationseinheiten aufgebaut. Hierbei kommen verschiedene Ortungsprinzipen zum Einsatz: Das Triangulationsprinzip, die Signallaufzeiterfassung oder Kombinationen von beiden (Georg et al., 2011).

Roth (2005) weist darauf hin, dass in der Regel hochpräzise Systeme auf kleinere Areale beschränkt sind und eine höhere Genauigkeit auch höheren Aufwand sowie höhere Kosten nach sich zieht. Doch die Anforderungen an ein Ortungssystem variieren mit dem Anwendungszweck. Als praxistaugliche Systeme für die Tierhaltung gelten Entwicklungen der Firmen Abatec und Ubisense. Bei beiden erfolgt die Positionsbestimmung über die Erfassung von Signallaufzeitunterschieden zwischen den mobilen und den fest installierten Stationen, bei Ubisense Series 7000 zusätzlich über Messungen des Einfallswinkels des Transpondersignals (Beyer et al., 2009).

Mithilfe des Systems Cowdetect, das auf Ubisense-Komponenten basiert, konnten auf sechs Praxisbetrieben Bewegungsaktivität und die aktuelle Position aller Tiere bzw. eines beliebigen Tieres in Echtzeit angezeigt werden. Wenn z. B. ein Tier unter besonderer Beobachtung steht oder sich auffällig zeigt und im Computer entsprechend farblich markiert wurde, kann es jederzeit zügig ausfindig gemacht und betrachtet werden und eine Separation entfällt. Dies kommt den Herdentieren in ihrem Verhalten entgegen. Vor allem Betriebe mit größeren Beständen bekunden nach Søgaard (2010) reges Interesse an diesem Ortungssystem.

Ziegen als fakultative Buschbeweider

Als fakultative Buschbeweider können Ziegen bis zu 60 % ihres Futterbedarfs über Gehölze decken (Rahmann, 2000) und fressen bevorzugt zuerst Blätter und Gehölze und erst später Gräser und Kräuter (Zingg und Kull, 2006). Neben der Eignung von Gehölzen als Futtergrundlage mit vergleichbaren Nährstoffgehalten üblicher Futterarten (Rahmann, 2004) wirkt das Gehölzfutter auch entzündungshemmend (Gerbsäuren), anregend und Parasiten reduzierend (Blausäureglykosid) (Machatschek, 2005).

Eine Untersuchung von Ude et al. (2011) zur Beäsung von Futterhecken, die dem im folgenden Artikel vorgestellten Versuch zur Ortung vorangegangen war, ergab bedingt durch fakultative Bipedie, nach einer Beweidungsdauer von zehn Tagen Fraßhorizonte von 1,50 m bis 2,0 m, sowie einen Blattverbiss von 80 bis 90 % innerhalb von fünf Beweidungstagen. Ein Gehölzverbiss zeigte sich schon innerhalb der ersten 24 h an den dünnen und mittleren Ästen. Nach 48 Stunden lag der Anteil an unberührten dünnen Gehölzen nur noch bei 30 %, bei den mittleren Gehölzen wurde dieser Wert nach sechs Tagen beobachtet. Auch die Stämme wurden bereits zwischen dem ersten und zweiten Tag beäst, so dass nach vier Tagen 50 % der Stämme Verbissschäden zeigten. Eine Fallstudie von Zingg und Kull (2006) mit 941 m² Waldweide und vier

Ziegen zeigte durch die Beweidung erhebliche Veränderungen in der Krautschicht; große Bäume ab Stangenholz wurden nicht geschädigt, jedoch wurde eine Verjüngung des Baumbestandes verhindert.

Eine Möglichkeit der Nutzung der Funktion des fakultativen Buschbeweiders ist der Einsatz von Ziegen im Naturschutz, der auf der einen Seite der Biotoppflege aufgrund der sehr guten Verbissleistung dient und auf der anderen Seite eine zusätzliche Einkommensquelle im Vertragsnaturschutz bietet (Rahmann, 2008).

Ziel der Untersuchung

Das Ortungssystem Ubisense 7000 wurde erstmals bei Ziegenlämmern und unter Freilandbedingungen mit Futterheckennutzung eingesetzt. Es ergaben sich folgende Fragestellungen:

- Eignet sich Ubisense 7000 für eine Ortung auf einer Weide bei Heckennutzung?
- Ist eine tierindividuelle Erfassung möglich?
- Eignet sich das System als zukünftige Managementhilfe?
- Kann dieses Verfahren eine aufwändige Verhaltensbeobachtung ersetzen?
- Wie werden die drei unterschiedlich gepflegten Futterhecken von den Ziegenlämmern angenommen?
- Zu welchen Tages- und Nachtzeiten nutzen die Lämmer die Hecke?

Material und Methode

Bei dem im Folgenden dargestellten Versuch handelte es sich um ein Teilprojekt eines Forschungsprojektes, das mit 120 Ziegenlämmern der Rasse Bunte Deutsche Edelziege am Thünen-Institut für Ökologischen Landbau in Trenthorst/Wulmenau im Jahr 2010 durchgeführt wurde. Dieses Forschungsprojekt beschäftigte sich mit der elektronischen Tierkennzeichnung bei Ziegenlämmern in Form von verschiedenen Ohrmarkentypen und Injektaten zu zwei Applikationszeitpunkten und deren Abheilung nach der Applikation sowie mit deren Verlustraten bei systematischer Beweidung von Gehölzen und Weideflächen bei Nutzung von Knotengittern und Litzen.

Das Teilprojekt mit dem Ortungssystem Ubisense 7000 wurde mit 60 Ziegenlämmern der Rasse Bunte Deutsche Edelziege im August und September 2010 im Rahmen der Diplomarbeit von Bender (2010) durchgeführt. Dargestellt werden im Folgenden die Daten von 30 Ziegenlämmern.

Lämmer

Der Versuch wurde mit 30 männlichen, z. T. sterilisierten Ziegenlämmern der Rasse Bunte Deutsche Edelziege durchgeführt. Die Lämmer waren zum Zeitpunkt des Ortungsversuchs zwischen sieben und neun Monaten alt und wogen bei Versuchsende durchschnittlich 29 kg.

Auf den Versuchsparzellen stand den Lämmern als Unterstand eine Hütte zur Verfügung. Die Wasserversorgung wurde über eine mobile Schwimmertränke gewährleistet. In einem Kraal in Holztrögen wurden die Lämmer zweimal täglich pro Lamm mit 150 g Weizenschrot, das mit 8 g Mineralfutter und 8 g Bierhefe angereichert wurde, gefüttert. Zusätzlich stand jeder Gruppe ein Leckeimer zur Mineralstoff- und Spurenelementversorgung zur freien Verfügung.

Futterhecken

An den Ziegenstall grenzt eine ca. 25 ha große Grünlandfläche. Durch den Treibeweg, der diese Fläche nahezu halbiert, und durch das Pflanzen von sieben parallel angeordneten Futterhecken im Jahr 2001 gliedert sie sich seitdem in 8 Weideflächen mit einer Größe von ca. 2,8 bis 3,5 ha (Abbildung 1). Zum Zeitpunkt der Pflanzung waren die Gehölze zwischen 30 bis 60 cm hoch. Die Bepflanzung erfolgte in drei Reihen mit einem Reihenabstand von 1,5 m und einem Pflanzenabstand von 75 cm. Es wurden rund 30 heimische Gehölzarten gepflanzt (z. B. verschiedene Weidenarten, Brombeere, Haselnuss, Schwarzdorn, Wildapfel, Zitterpappel, Feldahorn). Die Futterhecken haben eine Länge zwischen 100 und 265 Metern.

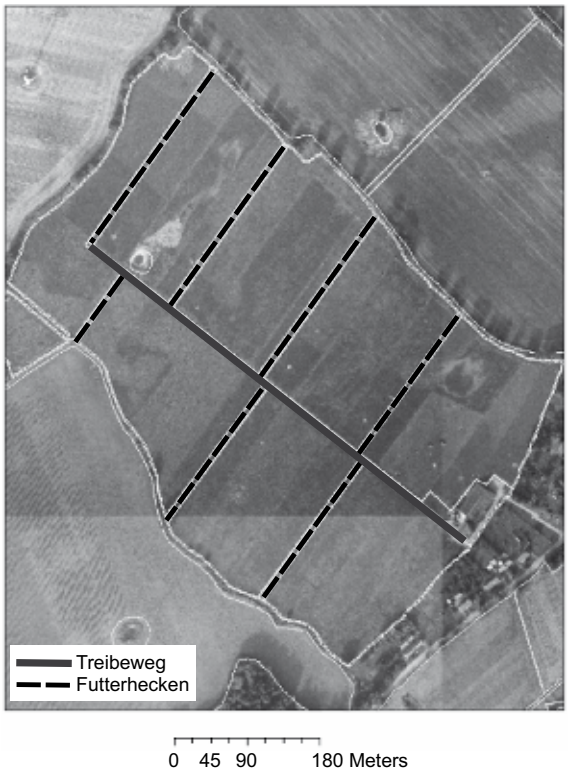

Abbildung 1:
Luftbild der Weidefläche Kornsahl mit eingezeichneten Futterhecken und Treibeweg

Drei der sieben Hecken wurden 2008 von den Ziegen beäst und anschließend bodennah (ca. 5 bis 10 cm hoch) abgesägt, d. h. „auf den Stock gesetzt". 2009 wurde eine weitere Hecke auf diese Weise genutzt und gepflegt.

Die Bonituren von Ude et al. (2011) von vergleichbaren Parzellen zeigten Wuchshöhen bei zwei bis 2010 noch nicht auf den Stock gesetzten alten Hecken je nach Gehölz zwischen ca. 2,50 m (Schwarzdorn, Heckenrose, rote Heckenkirsche) und 6,5 bis 7,5 m (Silberweide, Salweide, Bruchweide). Die Wuchshöhen der in 2008 auf den Stock gesetzten jungen Hecke betrugen 0,8 m (Hainbuche) bis 2,8 und 4,3 m (Weiden). In der 2009 auf den Stock gesetzten ganz jungen Hecke waren maximale Wuchshöhen von 2,5 bis 2,7 m (Silberweide und Bruchweide) und geringe Wuchshöhen von 0,8 bis 1,1 m (Linde, Schneeball, Salweide) ermittelt worden.

In den Versuch zur Ortung wurden alle drei unterschiedlich gepflegten Hecken einbezogen, d. h.:

Variante „ganz junge Hecke": 2009 von Ziegen beäst und anschließend auf den Stock gesetzt (Abbildung 2)

- Variante „junge Hecke": Im Jahr 2008 von Ziegen beäst und auf den Stock gesetzt (Abbildung 3)
- Variante „alte Hecke": Bis zum Jahr 2010 noch nicht gepflegt (Abbildung 4).

Abbildung 2:
Beäsen der ganz jungen Hecke

Abbildung 3:
Beäsen der jungen Hecke

Abbildung 4:
Beäsen der alten Hecke

Technik

Das verwendete Ubisense Series 7000 Real-Time-Ortungssystem arbeitet zur Feststellung der Position der aktiven Transponder, *Tags* genannt, im Ultrabreitband (Ultra Wideband, kurz: UWB, Frequenzbereich 6 bis 8,5 GHz). UWB-Signale können keine Metalle oder Flüssigkeiten durchdringen, weshalb je nach räumlichen Gegebenheiten eine entsprechende Anzahl und Anordnung der Sensoren nötig ist. Mit der Ultrabreitband-Technologie lassen sich aber Daten über kurze Distanzen mit hohen Übertragungsraten bei geringem Stromverbrauch kabellos austauschen.

Auf der UWB-Frequenz werden von den Sensoren des Ubisense-Systems die von den Tags ausgesandten Signale zur Positionsbestimmung ausgewertet. Die Tags senden auf Anforderung der Sensoren ihr Signal, daraufhin findet deren Positionsbestimmung über Laufzeitdifferenz TDoA (Time Difference of Arrival) sowie über die Winkelbestimmung (Angle of Arrival, kurz: AoA) statt. Bei diesem Verfahren ermitteln mindestens zwei Basisstationen, deren Entfernung zueinander bekannt ist, den Eingangswinkel eines Transpondersignals, zusätzlich senden sie Signale auf einer Geraden in einem bestimmten Winkel zum Boden aus. Aus den Schnittpunkten dieser Geraden errechnet der PC die Position des Transponders.

Die Genauigkeit der Messung kann nach Herstellerangaben bis zu 15 cm betragen. Zur genauen Ortung sind mindestens zwei Sensoren notwendig; eine komplette Zelle des Ubisense-Systems besteht aus 4 Zellen. Die Steuerung der Tags erfolgt über einen 2,4-GHz-Kanal. Tags können damit auch individuell abgeschaltet werden.

Die Kommunikation der Sensoren untereinander erfolgt durch eine Ethernet-Schnittstelle und einen Netzwerk-Switch, der die Sensoren durch PoE (Power over Ethernet) auch mit der notwendigen Betriebsspannung versorgt. Zur

Zeitsynchronisation der Sensoren wird der synchronisierende Master-Sensor durch Timing-Kabel mit den als Slave definierten Sensoren verbunden.

Die Software enthält ein sogenanntes Location Engine Configuration-Modul zur Einbindung des Echtzeit-Ortungssystems in das Netzwerk, Definition von Sensoren und Sensor-Zellen, Konfiguration und Kalibrierung von Sensoren, Registrierung von Tags, sowie das Modul Site Manager zur Einbindung von Grundrissen in den Ortungsbereich, Definition von zu ortenden Personen, Tieren oder Gegenständen und die Definition und Zuordnung bestimmter Zonen.

Im Modul Map werden Events visualisiert, wenn ein Tag eine bestimmte Zone erreicht (Speichern des Zeitstempels für Entry/Exit in einer Zone).

Die verwendeten Tags hatten die Bezeichnung Compact Ubitags, mit den Abmessungen 38 x 39 x 16,5 mm und einem Gewicht von etwa 25 g.

Versuchsaufbau

Parzellen

Zunächst wurden die Weideparzellen im Bereich vor der Hecke mit DGPS (Differential GPS mit Real Time Kinematik, AgGPS 332, Trimble Navigation Ltd.) auf die Größe 50 m x 70 m vermessen. Hinter der Hecke, die sich in allen Varianten am nördlichen Ende der Parzelle befand, wurden zusätzliche 2,50 m berücksichtigt, damit bei der Heckenpflege mit der Motorsäge die umfallenden Bäume den Zaun möglichst wenig gefährdeten. Als Zaunmaterial wurden herkömmliche Litzen und Weidepfähle verwendet; d. h. es wurde ein rechteckiger Bereich eingezäunt, der 70 m lang (parallel zur Hecke) und 50 m breit war, zuzüglich Heckenbreite und 2,50 m Fallraum.

Abbildung 5 verdeutlicht das Schema des Versuchsaufbaus eines untersuchten Weidestücks mit Hecke, Hütte, Position der sechs Sensoren, Netzwerk-Switch außen sowie im Container, Netzwerkkabel und Stromversorgung.

Sensoren

Für die Weidefläche von 3 500 m² wurden 6 Sensoren benötigt.

Ausgehend von der Herstellerempfehlung wurden die Sensoren in ca. 4 m Höhe angebracht, mit einem Mindestabstand von 10 m und einem Maximalabstand von 50 m zum nächsten Sensor. Dazu wurden die Sensoren an „Dreibeinen" befestigt (Abbildung 6) und mit einer Wasserwaage horizontal ausgerichtet. Der Fußpunkt wurde mit einem Maurerlot erfasst und die Höhe anhand der Schnurlänge ermittelt. Der Fußpunkt wurde mit DGPS mit

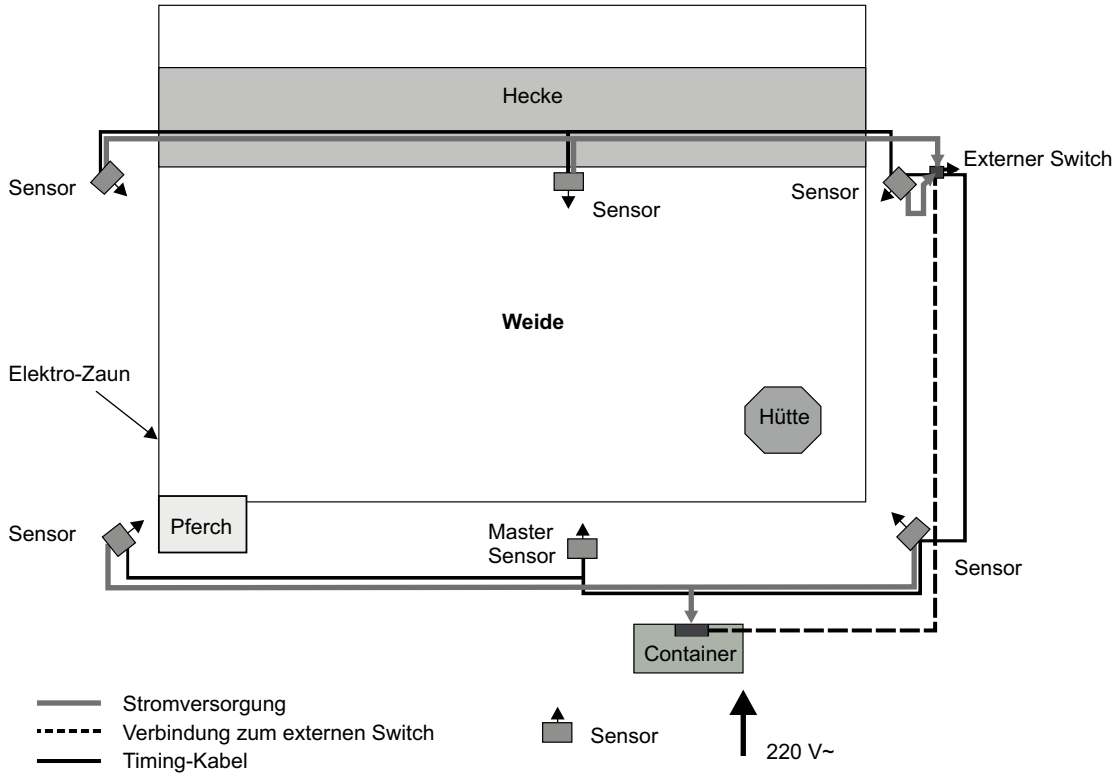

Abbildung 5:
Schematischer Versuchsaufbau

einer Genauigkeit von 2 cm eingemessen und zusammen mit der Höhenangabe im Koordinatensystem der Ortungssoftware eingetragen, damit eine gegenseitige Kalibrierung der Sensoren möglich war.

Abbildung 6:
Dreibein mit Sensor

Kalibrierung der Sensoren

Zur Kalibrierung wurde die Position sieben fester Punkte in der Messfläche, deren Position ebenfalls mit DGPS eingemessen wurde, mithilfe eines Tags gemessen. Aufgrund der dem System bekannten Koordinaten erfolgt durch die Software eine automatische Kalibrierung der Sensoren.

Tags

Die Sender bzw. Tags wurden geschützt vor Verbiss in einer kleinen Dose an Gurten verschraubt, die im Rückenbereich der Tiere gepolstert wurde (Eigenkonstruktion). Jeder Gurt erhielt eine eigene Nummer, so dass ein Gurt einem Lamm zugeordnet werden konnte.

Damit die Signalübertragung zwischen den Tags und den Sensoren möglichst ungehindert war, wurden die Gurte täglich mehrmals kontrolliert. Um den störenden Einfluss so gering wie möglich zu halten, geschah dies in der Regel bei den Routine-Kraftfuttergaben. Die Gurte wurden ggf. zurechtgerückt, so dass der Tag gen Himmel zeigte, bzw. wurden bei Verlust wieder angelegt. Während der Kraftfuttergaben im Kraal stellte dies keinen für den Versuch relevanten Einfluss dar.

An den Tagen, an denen eine Aufnahme stattfand, wurden alle 30 Lämmer mit einem Gurt ausgestattet, so dass alle Lämmer gleichzeitig erfasst werden konnten (Abbildung 7).

Abbildung 7:
Lämmer mit Gurten und Tags

Datenerfassung

Zur Beantwortung der Versuchsfrage, wie lange sich die Lämmer in der Hecke aufhalten, wurden verschiedene Ereigniszonen eingerichtet: Weide, Kraal, Hütte, Futterhecke und Vorzone Futterhecke.

Der SpatialEventLogger ist ein zusätzliches Programm-Modul, das gestartet wird, um Tiere in bestimmten Alarmzonen oder Bereichen zu lokalisieren. Es zeichnet den Zeitpunkt des Ereignisses auf, an dem ein Transponder in eine Zone hinein bzw. wieder hinaus bewegt wird. So entsteht für jeden Transponder eine Liste mit Zeitstempeln für das Eintreten und Verlassen der definierten Zonen. Das Programm-Modul Multicell-Logger speichert für jeden Tag Zeit und Ortskoordinaten (Gauß-Krüger-Koordinaten) im Sekundenabstand. Aus diesen Datensätzen lassen sich zurückgelegte Wegstrecken, Ruhe- sowie Aktivitätsparameter berechnen.

Zusätzlich zum Ortungssystem wurden die Aktivitäten der Lämmer mit Kameras (Panasonic, Modell WV-BP 102) und einem Digitalrekorder (Everfocus EDR-810-H) aufgezeichnet.

Die Aufenthaltsdauer wurde in allen drei unterschiedlich gepflegten Hecken erfasst: Der Versuch in der ganz jungen Hecke, d. h. 2009 auf den Stock gesetzt, dauerte

vom 15.08.2010 bis 23.08.2010. Der Versuchszeitraum in der jungen Hecke, d. h. 2008 auf den Stock gesetzt, erfolgte vom 27.08.2010 bis zum 04.09.2010. Die Variante alte Hecke, d. h. bis zu dem Zeitpunkt noch nicht gepflegt, umfasste den Zeitraum vom 08.09.2010 bis zum 18.09.2010 (Tabelle 1).

In den Varianten ganz junge Hecke und junge Hecke wurden die Lämmeraktivitäten am ersten, zweiten und siebten Tag nach dem Umweiden sowie an Tag acht, also am ersten Tag, nachdem die Hecke auf den Stock gesetzt wurde, mit dem Ortungssystem erfasst. In der Variante alte Hecke lief die Ortung während der Tage eins, zwei und neun nach dem Lämmerauftrieb, sowie ebenfalls am ersten Tag, nachdem die Hecke auf den Stock gesetzt wurde – hier der zehnte Tag.

Die Datenerfassung begann jeweils morgens gegen acht Uhr. Zu diesem Zeitpunkt wurden die Lämmer im Kraal mit Kraftfutter gefüttert, so dass die Gurte mit geringem Stör-Einfluss kontrolliert werden konnten.

Tabelle 1:
Varianten und Grunddaten

Variante	Datum Datenerfassung	Uhrzeit	Versuchstag	Anzahl Lämmer
ganz junge Hecke	15.08.2010 - 16.08.2010	8:13 - 7:58	1	30
	16.08.2010 - 17.08.2010	8:00 - 8:00	2	29
	21.08.2010 - 22.08.2010	8:00 - 8:00	7	29
	22.08.2010 - 23.08.2010	8:00 - 8:00	8	29
junge Hecke	27.08.2010 - 28.08.2010	8:46 - 8:46	1	29
	28.08.2010 - 29.08.2010	8:46 - 8:46	2	29
	02.09.2010 - 03.09.2010	7:59 - 7:59	7	29
	03.09.2010 - 04.09.2010	7:59 - 7:59	8	29
alte Hecke	08.09.2010 - 09.09.2010	8:00 - 8:00	1	28
	09.09.2010 - 10.09.2010	8:00 - 8:00	2	28
	16.09.2010 - 17.09.2010	8:00 - 8:00	9	28
	17.09.2010 - 18.09.2010	8:00 - 8:00	10	27

Datenauswertung

Die Event- und Multicell-Logger-Daten, die im csv-Format vorlagen, wurden zunächst in Microsoft-Excel eingelesen. Hier erfolgte eine manuelle Datenbereinigung, da z. T. ein Datensatz nicht aus einem Enter (einem Eintritt in eine Zone) und einem Exit (dem Austritt aus dieser Zone) bestand, sondern z. B. aus zwei Eintrittszeiten. Diese Datenbereinigung wurde für jedes Tier durchgeführt.

Nach der Datenbereinigung wurden die Daten in SAS 9.2 importiert. Je Tier und Versuchstag wurden die Summen pro Stunde und Ereignis (Aufenthalt Hecke, Hütte, Weide) gebildet. Anschließend wurden die Daten mittels deskriptiver Statistik ausgewertet, indem von allen 30 Lämmern bzw. allen auswertbaren Tags die Summen, Mittelwerte oder Häufigkeiten je Versuchstag, Stunde, Variante und Ereignis gebildet wurden.

Ergebnisse

Eignung von Ubisense 7000 für eine Ortung auf der Weide mit Heckennutzung

Ubisense Series 7000 arbeitet auf Basis von Ultrabreitband. UWB-Signale können keine Metalle oder Flüssigkeiten durchdringen. Für den Versuch bedeutete dieses, dass die Signale die Futterhecke nicht überwinden konnten. Deshalb wurde die Versuchsparzelle so konzipiert, dass sich die Futterhecke am Rand der Versuchsparzelle befand. Ein Eintritt in die Ereigniszone Fresszone bedeutete gleichzeitig das Verlassen des Aufnahmebereichs. Zur Überprüfung der Daten wurde entlang der Fresszone Futterhecke noch eine Vorzone Hecke eingerichtet, die die Lämmer passieren mussten, um in die Hecke zu gelangen.

Für den Versuch wurden Dreibein-Stative aus Stahlrohren konstruiert, an denen die Sensoren befestigt werden konnten. Vor der Witterung wurden die Sensoren und Kabelverbindungen mit Plastikfolien geschützt. Von jedem Slave-Sensor führte jeweils ein Netzwerkkabel zum Master (Zeitsynchronisation) und zum Switch (Stromversorgung), der Master-Sensor war über eben solche Kabel mit dem Switch verbunden. Die Kabel wurden nach Möglichkeit außerhalb des Tierbereichs verlegt, um Verbiss durch die Lämmer zu verhindern. Da sich dadurch die Kabellänge erhöhte, mussten im Versuch die verwendeten 50-m-Kabel mit Kupplungen verlängert werden. Die Kupplungen wurden ebenso wie die Sensoren mit Plastikfolien geschützt, allerdings musste auf Schwitzwasser oder Regen geachtet werden, da die Kabel auf der Weide verlegt wurden.

Die tierindividuelle Erfassung der 30 Ziegenlämmer mit den Tags war erfolgreich. Die verwendeten Gurte bestanden aus einem etwa 2 cm breiten Gummiband mit verstellbarem Plastikverschluss. Im Rahmen einiger Vorversuche wurden die Tiere an die Gurte gewöhnt. Einige Lämmer schienen den Gurt zunächst als Belästigung zu empfinden, was sich aber bald legte. Während der Versuchszeit zeigten die Lämmer keine Anzeichen einer Störung durch die Gurte.

Fehlende Daten durch Gurtverluste traten auf und wurden im Protokoll mit Datum, Uhrzeit und Fundort festgehalten, so dass die Daten für die Auswertung bereinigt werden konnten. Bei bis zu drei Lämmern pro Aufnahmetag konnten die Daten nicht verwendet werden, da nicht genug Aufnahmematerial vorhanden war bzw. dieses deutliche Fehler aufwies (Tabelle 1).

Aufenthaltsdauern der Ziegenlämmer in den Futterhecken

Dargestellt wird im Folgenden die Nutzung der unterschiedlich gepflegten Futterhecken durch die Lämmer, die durch das Ortungssystem Ubisense 7000 erfasst wurde. Die Aufenthaltszeiten in der Hütte oder auf der Weide werden nicht im Einzelnen betrachtet. Die Daten zeigen, dass die Hütte im Versuchszeitraum überwiegend nachts und bei Regen auch tagsüber genutzt wurde, während ein Aufenthalt auf der Weide hauptsächlich tagsüber aufgezeichnet wurde.

Nutzung der ganz jungen Futterhecke

In Tabelle 2 dargestellt ist je Versuchstag die absolute Anzahl an Besuchen in der Futterhecke pro Stunde und die Anzahl an Lämmern, die die Hecke jeweils genutzt haben.

Bei Versuchstag 1 handelte es sich um den ersten Tag nach dem Umweiden auf eine neue Fläche. Am Versuchstag 2 wurden die darauf folgenden 24 h betrachtet. Versuchstag 7 war der siebte Tag in der Futterhecke und bei Versuchstag 8 wurde morgens die Hecke auf den Stock gesetzt.

Bei der ganz jungen Hecke wurden am ersten Versuchstag innerhalb von 24 Stunden 2855 Besuche registriert. An den Tagen zwei und sieben fanden mit einer Anzahl von 1740 und 1948 weniger Besuche statt. Die niedrigste Anzahl an Besuchen wurde am achten Versuchstag mit 1598 Besuchen registriert.

An jedem Versuchstag gab es zwischen 5 Uhr und 20 Uhr drei bis vier Stunden, an denen vermehrt Besuche zu verzeichnen waren. Zwischen 21 Uhr und 5 Uhr wurde die Futterhecke nicht aufgesucht.

Die Anzahl an Lämmern in der Futterhecke je Stunde zeigt bis auf wenige Ausnahmen, dass zwischen 5 Uhr und 20 Uhr zwischen 75 und 100 % der Herde in der Futterhecke registriert wurden.

Die Abbildung 8 stellt die mittlere Aufenthaltsdauer der Lämmer in der ganz jungen Hecke je Stunde an den vier Versuchstagen dar. Die mittlere Aufenthaltsdauer je Stunde

Tabelle 2:
Anzahl Heckenbesuche und Anzahl Lämmer in der Hecke je Stunde für die einzelnen Versuchstage in der ganz jungen Hecke

	Tag 1		Tag 2		Tag 7		Tag 8	
Stunde [h]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]
8					78	27	17	11
9	537	30	109	25	108	27	150	28
10	191	28	259	29	9	4	145	29
11	120	25			94	24	129	28
12	20	19	46	25	76	21	117	28
13	423	27	191	28	97	22	229	29
14			106	28	299	27	60	15
15	32	20	205	29	205	29	120	28
16	446	28	262	29	78	22	223	28
17	488	29	2	2	337	28	72	23
18	51	14	126	28	177	27	71	23
19	101	25	28	16	181	27	264	29
20	120	27			29	17		
21								
22								
23								
0								
1								
2								
3								
4								
5	49	23			28	14		
6	230	28	66	25	126	23	1	1
7	47	19	340	27	26	16		
Σ	2855 [n/d]		1740 [n/d]		1948 [n/d]		1598 [n/d]	

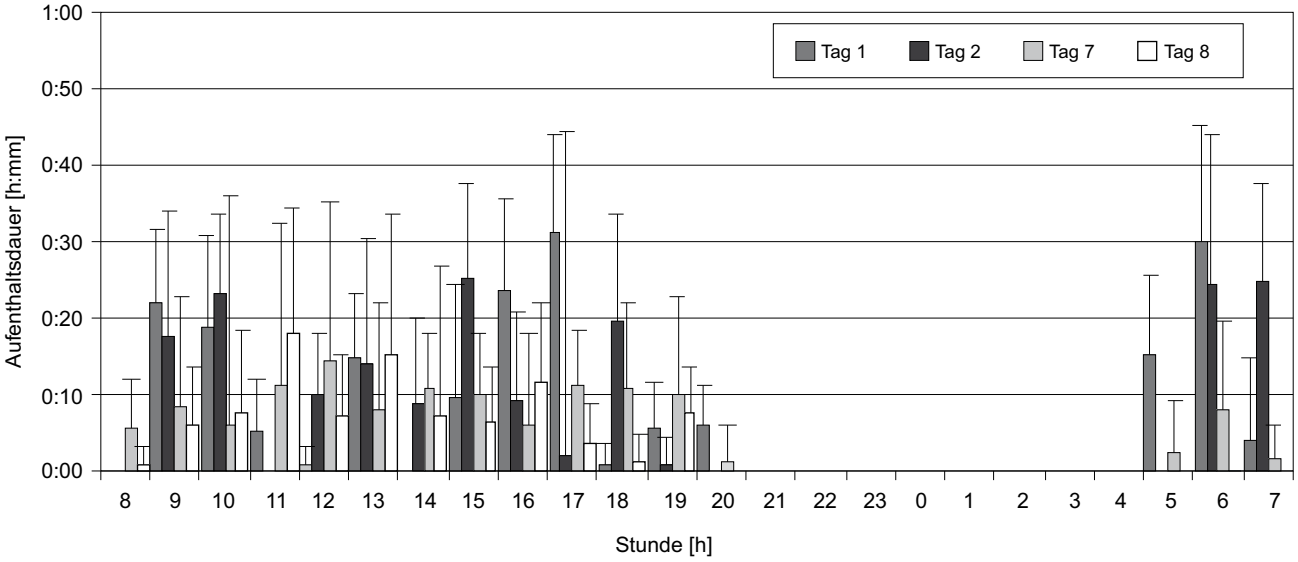

Abbildung 8:
Mittlere Aufenthaltsdauer pro Stunde der Lämmer in der ganz jungen Hecke für die einzelnen Versuchstage

Tabelle 3:
Anzahl Heckenbesuche und Anzahl Lämmer in der Hecke je Stunde für die einzelnen Versuchstage in der jungen Hecke

	Tag 1		Tag 2		Tag 7		Tag 8	
Stunde [h]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]
8	48	18	83	25	12	11	2	2
9	38	19	33	14	12	11		
10	2	2	28	17	26	17	13	9
11	64	21	163	29	23	18	15	11
12	30	18	9	9	14	14	8	8
13	2	2	1	1	34	18	17	13
14					18	15	6	6
15	10	9	42	18	30	19	43	23
16	45	21	74	27	9	9	31	17
17	4	4	38	20	51	24	8	8
18	1	1	152	29	66	26	62	27
19	70	21	69	22	48	23	27	15
20	72	27	62	23	9	9	24	17
21	16	15	2	2			5	5
22			2	2			3	3
23							2	2
0								
1								
2								
3								
4	28	4	1	1	7	7		
5	49	4			6	6		
6	70	11	22	15	17	15	5	4
7	45	20	125	28	16	13	14	8
Σ	594 [n/d]		906 [n/d]		398 [n/d]		285 [n/d]	

lag an den ersten beiden Versuchstagen zwischen 1 min und 31 min, im Mittel zwischen 13 bis 15 min. Hingegen reduzierte sich der mittlere Aufenthalt auf 1 bis 18 min, im Mittel auf 7 min an den Versuchstagen sieben und acht.

Aufenthalte in der Hütte, hier nicht grafisch dargestellt, konnten über den gesamten Tag verteilt dokumentiert werden, doch die meisten erfolgten zwischen 20 Uhr und 5 Uhr morgens.

Die durchschnittlichen Tagestemperaturen lagen vom 15.08 bis 23.08.2010 nach Lübecker Daten bei 17,8 bis 19,7 °C. Es fielen zwischen 0 (21.08.2010) und 5 mm Niederschlag. Die Sonnenscheindauer lag bei einer bis sechs Stunden pro Tag.

Aufenthaltszeiten junge Hecke

Bei der jungen Hecke wurden am ersten Versuchstag innerhalb von 24 Stunden lediglich 594 Besuche registriert (Tabelle 3). Am zweiten Versuchstag hingegen wurden 906 Besuche aufgezeichnet. An den Tagen sieben und acht reduzierte sich die Anzahl an Besuchen auf 398 (Tag 7) und 285 (Tag 8).

Auch bei der jungen Hecke gab es an den Versuchstagen Stunden, an denen vermehrt Besuche zu verzeichnen waren. Die Hecke wurde zwischen 22 Uhr bzw. Mitternacht und 4 Uhr morgens nicht aufgesucht.

Die Anzahl an Lämmern in der Futterhecke je Stunde lag lediglich an 5 bis 11 Stunden pro Tag bei mehr als 50 % der Herde.

Die Abbildung 9 stellt die mittlere Aufenthaltsdauer der Lämmer in der jungen Hecke je Stunde für die einzelnen Versuchstage dar.

Die mittlere Aufenthaltsdauer je Stunde lag an den ersten beiden Versuchstagen zwischen 1 min und 27 min, im Mittel bei fünf bis sechs Minuten. Der Aufenthalt erhöhte sich auf bis zu 35 min an Versuchstag sieben, im Mittel 15 min, und reduzierte sich auf 18 min, im Mittel 9 min, an Versuchstag acht (Abbildung 9).

Hier nicht grafisch dargstellt ist, dass die Lämmer am ersten Versuchstag auch tagsüber mit Aufenthaltsdauern von bis zu 55 min pro Stunde in der Hütte registriert worden sind. Auch am zweiten Versuchstag waren die Lämmer in jeder Stunde auch in der Hütte. Den dritten und vierten Aufnahmetag verbrachten die Lämmer sowohl tagsüber als auch nachts zumeist auf der Weide.

Die durchschnittlichen Tagestemperaturen lagen im Versuchszeitraum bei 10,8 bis 13,5 °C. Es fielen vom 27.08. bis 29.08. rund 30 mm Niederschlag, 20 mm am ersten Versuchstag. Die Sonnenscheindauer betrug am ersten Tag null Stunden, am zweiten bis vierten Versuchstag lag sie zwischen fünf und sieben Stunden pro Tag.

Aufenthaltszeiten alte Hecke

Bei der alten Hecke wurden am ersten und zweiten Versuchstag innerhalb von 24 Stunden lediglich 289 bis 302 Besuche registriert. An Tag 9 erhöhte sich dieses auf 434 Besuche. Die niedrigste Anzahl an Besuchen wurde wiederum am letzten Versuchstag mit 185 Besuchen registriert.

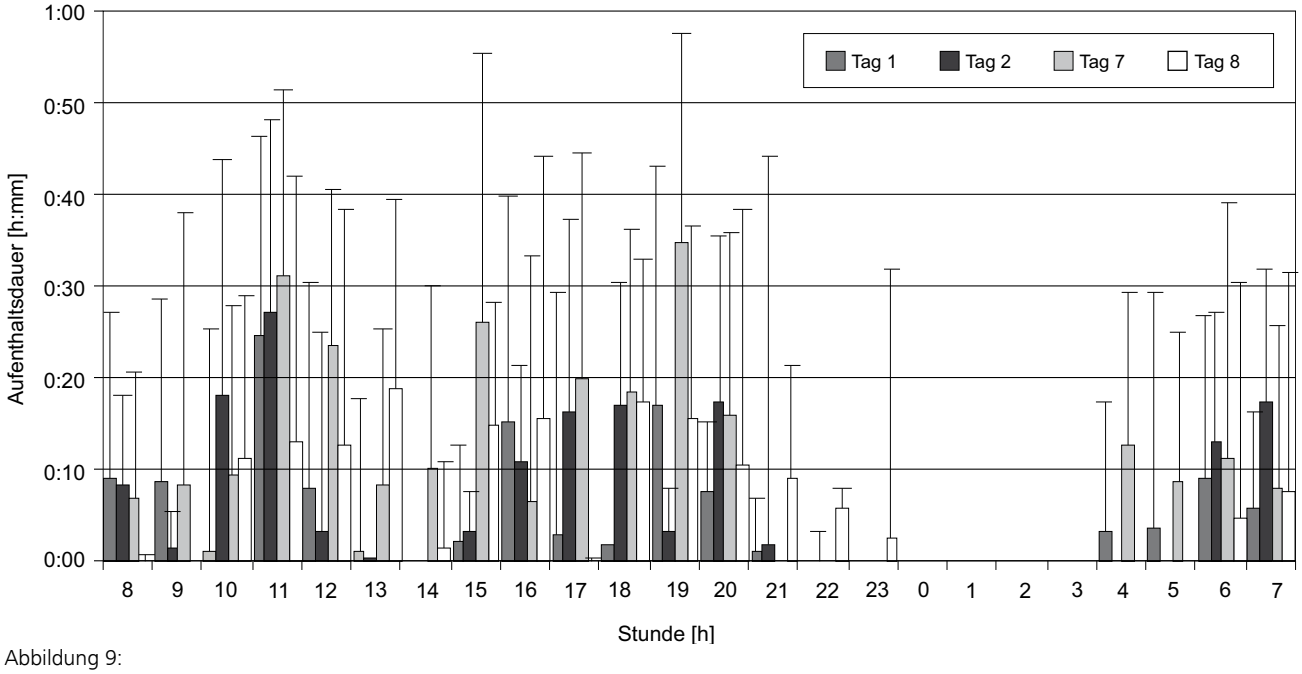

Abbildung 9:
Mittlere Aufenthaltsdauer pro Stunde der Lämmer in der jungen Hecke für die einzelnen Versuchstage

Tabelle 4:
Anzahl Heckenbesuche und Anzahl Lämmer in der Hecke je Stunde für die einzelnen Versuchstage in der alten Hecke

	Tag 1		Tag 2		Tag 9		Tag 10	
Stunde [h]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]	Anzahl Besuche [n/h]	Anzahl Lämmer [n]
8	40	22	12	9	6	6	7	5
9	10	7	1	1			10	8
10			24	15	35	13	10	9
11			53	19	86	18	33	16
12	68	21	20	13	112	22	3	3
13	35	12			61	19	28	18
14			5	3	52	15	4	4
15	1	1	66	15			10	10
16	17	10					1	1
17	37	18	11	9	13	9	32	16
18	46	18	40	16	39	8	36	8
19	6	6	61	23	24	13	2	1
20			2	2				
21			2	2				
22			1	1				
23								
0								
1								
2								
3								
4								
5								
6					2	2	3	3
7	29	17	4	1	4	4	6	4
Σ	289 [n/d]		302 [n/d]		434 [n/d]		185 [n/d]	

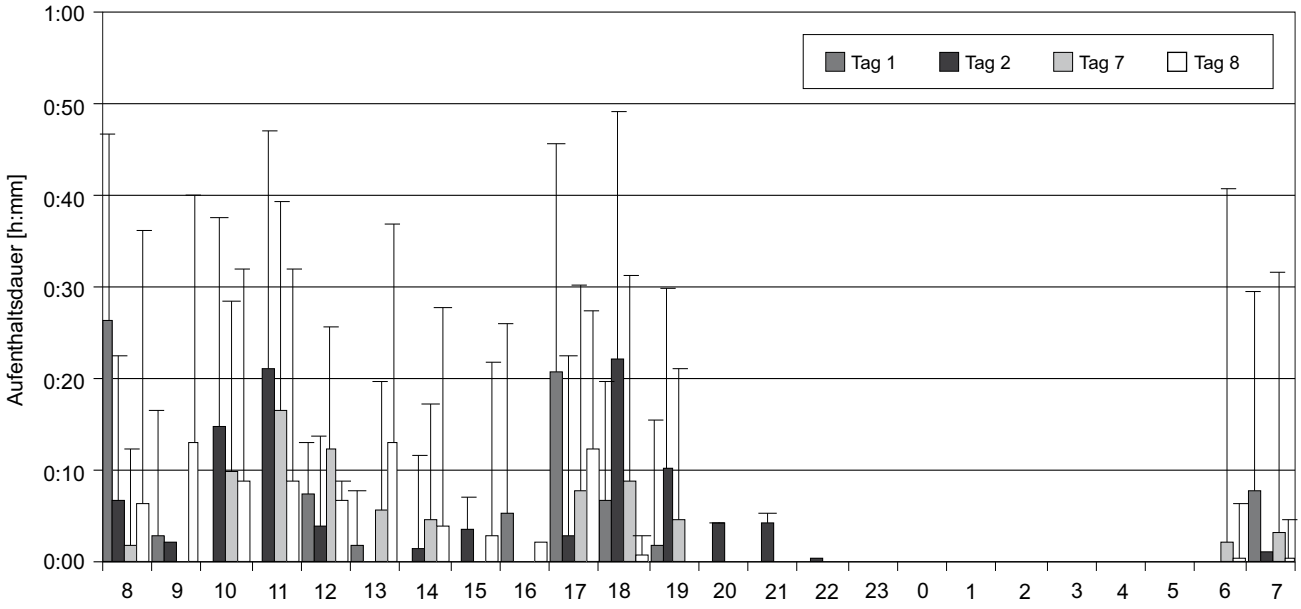

Abbildung 10:
Mittlere Aufenthaltsdauer pro Stunde der Lämmer in der alten Hecke für die einzelnen Versuchstage

Zwischen 22 Uhr und 6 Uhr wurde die Futterhecke nicht aufgesucht (Tabelle 4).

Die Anzahl an Lämmern in der Futterhecke je Stunde zeigt, dass zu keiner Stunde alle Lämmer in der Hecke anzutreffen waren. Die Anzahl an Lämmern in der Futterhecke je Stunde lag lediglich an drei bis fünf Stunden pro Tag bei mehr als 50 % der Herde (Tabelle 4).

Die Abbildung 10 stellt die mittlere Aufenthaltsdauer der Lämmer in der alten Hecke je Stunde und Versuchstag dar. Die mittlere Aufenthaltsdauer je Stunde lag an den ersten beiden Versuchstagen zwischen 1 min und 26 min, im Mittel bei drei bis vier Minuten. Die Aufenthaltsdauer an den Versuchstagen neun und zehn reduzierte sich auf 13 bis 16 min, im Mittel auf drei Minuten.

An den ersten drei Versuchstagen wurden zu nahezu jeder Stunde Aufenthalte in der Hütte registriert mit Dauern zumeist zwischen 10 und 30 min. Am vierten Versuchstag wurde die Hütte tagsüber so gut wie nicht bzw. kaum aufgesucht, nachts lagen die Aufenthaltsdauern zwischen 30 bis 55 min pro Stunde.

Die durchschnittlichen Tagestemperaturen lagen im Versuchszeitraum bei 9,8 bis 14,7 °C. Am ersten Versuchstag fiel kein Niederschlag, am zweiten Versuchstag rund 8 mm und am dritten Versuchstag 6,4 mm, am vierten 1 mm. Die Sonnenscheindauer lag zwischen null Stunden (Tag zwei), ansonsten zwischen 2,7 bis 4,8 h pro Tag.

Diskussion

Eignung von Ubisense 7000 zur Erfassung von Verhaltensdaten

Nach einer Aussage von Platen et al. (2007) kann Elektronikeinsatz als Mittel zur Optimierung im Bereich Precision Dairy Farming genutzt werden.

Dabei hängt der Erfolg sowohl von der Nutzung eines geeigneten Systems als auch vom Anwender selbst ab. Das Einmessen der Fläche erfolgt sowohl beim GPS als auch beim lokalen Verfahren manuell. Damit gehen auch ein gewisses Technikinteresse des Anwenders sowie eine umfassende Einweisung in das System einher. Da das System erstmalig so eingesetzt wurde, war der gesamte Versuch ein ständiger Lernprozess. In der Praxis wird Ubisense in der Regel von geschulten Technikern aufgebaut und installiert. Der Anwender erhält Gelegenheiten, sich in Lehrgängen in das System einzuarbeiten und den Kundendienst vor Ort zu nutzen.

In einer weiteren Untersuchung von Georg et al. (2011), in welcher dieses System in einen Milchviehstall installiert wurde, konnte das Ortungssystem bereits innerhalb von sieben Arbeitstagen eingerichtet werden. In diesem Zeitraum wurde neben der Installation und Anpassung auch die Kalibrierung des Systems durchgeführt und eine Software zur Erfassung programmiert.

Nach Søgaard (2010) und Florczak (2010) konnte das Ergebnis der Brunstbeobachtung verbessert werden. Die Untersuchung von Georg et al. (2011) zeigte, dass die Genauigkeit des Systems für die 2D-Ortung der Tiere und die Zuordnung zu Funktionsbereichen ausreichend war, nicht jedoch für weitergehende Auswertungen wie Aufsprungverhalten oder Geschwindigkeitsbestimmung. Dieses sei jedoch mit einer optimierten Anbringung der Sensoren denkbar.

Bei der eigenen Untersuchung zur Bestimmung der Aufenthaltsdauer in der Futterhecke konnte diese Aufenthaltsdauer nur indirekt bestimmt werden, weil die UWB-Signale die Futterhecke nicht durchdringen konnten. Für eine direkte Bestimmung hätte auf der anderen Seite der Futterhecke auch eine Zelle installiert werden müssen, da den Lämmern hinter der Hecke noch der Fallraum als Aufenthaltsort zur Verfügung stand. Dieser wurde wie die Hecken nicht vom System erfasst.

Nach Georg et al. (2011) konnten Teilaufgaben der aufwändigen Videoauswertung wie Aufenthaltszeiten in bestimmten Funktionsbereichen durch den Einsatz von Ubisense 7000 hinreichend genau erledigt werden.

Für eine Untersuchung auf der Weide sollte eine Wetterstation zur Verfügung stehen, um Sonnenscheindauer, Niederschlag und Außentemperatur mit einbeziehen zu können. In Kombination mit einer Videoüberwachungsanlage zur Erfassung von Umgebungseinflüssen könnte das System auch in Forschungsfragen gute Untersuchungsergebnisse liefern. Die Möglichkeit der direkten Umsetzung in Grafiken, wie es bei CowDetect geschieht, und die sofortige Umrechnung und Nutzbarkeit der Daten könnte aufwendige Verhaltensanalysen vereinfachen.

Aufenthalte in der Futterhecke

Durch das Ortungssystem konnte beobachtet werden, dass sich die Lämmer in den Nachtstunden nicht in der Hecke, sondern eher in der Hütte oder auch auf der Weide aufhielten. Dieses kann aber auch daran liegen, dass die Daten erst ab Ende August erfasst werden konnten. Die Technik konnte erst im Laufe der Weidesaison an die Lämmer und die Weide angepasst werden, daher beziehen sich diese Daten nur auf den Herbst. Während der Sommermonate wurde tagsüber und auch bei der abendlichen Tierkontrolle beobachtet, dass die Lämmer die Hecke auch zum Ruhen attraktiv fanden.

Nach Rahmann (2010) und Korn et al. (2007), nehmen Ziegen Laub und Gehölze gern in größeren Mengen auf und sind zur Landschaftspflege und Vorbeugung von Verbuschung geeignet. Dieses zeigte auch die Untersuchung von Ude et al. (2011), in welcher die drei dargestellten Futterhecken beäst wurden. Innerhalb von kürzester Zeit zeigte sich Blatt- und Rindenverbiss. Die Attraktivität der

Futterhecke kann aus der Anzahl an Besuchen in der Futterhecke oder auch der Anzahl an Lämmern in der Hecke abgeleitet werden. Allerdings scheinen die beiden 2008 und 2009 gepflegten Hecken interessanter als die bis zum Versuch noch nicht gepflegte Hecke. Dieses kann mit dem höheren Biomasseanteil im Fraßhorizont und den schmackhafteren Trieben des Wiederaustriebs zusammen hängen.

Geringe Aufenthaltszeiten in der Hecke lassen sich insgesamt durch die häufig feuchte Witterung, die Ziegen nicht mögen, und zum Teil auch durch die niedrigen Temperaturen erklären. Außerdem fielen an jeweils dem Versuchstag, an dem die Hecke auf den Stock gesetzt worden war, die abgesägten Bäumstämme, die eine Länge von bis zu 8 m hatten, aus der Fresszone in den Weidebereich hinein, so dass die Lämmer während des Äsens nicht der Hecke zuzuordnen waren (alte Hecke). Die beiden bereits gepflegten Hecken hingegen konnten aufgrund der fakultativen Bipedie schon vor dem Absägen zum großen Teil komplett beäst werden.

Sommermessungen sollten nachgeholt werden.

Fazit

Das Ortungssystem Ubisense 7000 kann Positionen tierindividuell in Echtzeit erfassen, so dass durch das Einrichten von Zonen Funktionsbereiche zugeordnet und Aufenthaltszeiten mit geringem Aufwand ausgewertet werden können. Eine Systemanpassung an das jeweilige Haltungssystem ist erforderlich.

Die Ziegenlämmer haben die Futterhecken auch bei herbstlicher Witterung genutzt. Dabei waren die beiden Hecken mit jüngerem Aufwuchs attraktiver.

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Cryopreservation of semen from genetic resource chicken lines

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Abstract

The objective of the present study was to establish an effective semen freezing procedure to set up a cryobank for chicken breeds and genetic lines of special interest. The fowls used in the experiments originated from White Leghorn lines maintained as conservation flocks. In this study, different protocols were evaluated in terms of differences in the fertilization success. 1 A) Comparison of slow and fast freezing procedures using a cryoprotectant containing Mix 2 (1 part 6.5 % Dimethyl formamide + 2 parts 6.5 % Methyl acetamide). The slow freezing procedure in a programmable freezer included two steps from 0 °C to -35 °C with a freezing rate of -3 °C/min, and from -35 °C to -130 °C with a freezing rate of -50 °C/min. Fast freezing was carried out in a box by exposing the straws to liquid nitrogen vapour at 4 to 4.5 cm above liquid nitrogen surface (-40 °C/min). 1 B) Test of insemination procedures with one or two straws with semen frozen slowly with Mix 2, and 2) comparing three cryoprotectants Mix 1 (1 part 6.5 % DMF + 1 part 6.5 % MA), Mix 2 and MA only (6.5 %). The base extender was the trehalose containing HS1-diluent to Hanzawa et al. (2006). The cryoprotectant-diluted semen was packaged in 0.25 ml plastic straws after dilution to a standardized number of spermatozoa (300 million/straw). All samples were thawed in a water bath at 4 °C. The females were inseminated intravaginal with a dose of 300 million (1 straw) or 600 million sperm (2 straws). The respective results were: the slow freezing procedure was superior to fast freezing and resulted in 49.3 % compared to 11.5 % fertility. There was no significant fertility difference between inseminations with one (43.6 %) and two straws (52.1 %). The fertility of both Mix 1 (77.3 %) and Mix 2 (81.1 %) showed higher fertilization rate than MA only (39.8 %). In conclusion, the slow freezing procedure with Mix 2 media is an applicable method for establishing a sperm cryobank in chicken genetic resource populations.

Keywords: Chicken, semen, cryopreservation, insemination, cryobank, ex situ conservation

Zusammenfassung

Kryokonservierung von Hahnensperma von Genreservelinien

Ziel der vorliegenden Untersuchungen war die Etablierung einer Einfriermethode für Hahnensperma, um eine Spermabank für genetisch wertvolle Linien aufbauen zu können. Für die Experimente wurden eigene Genreservelinien verwendet, die seit Jahren am Institut in situ gehalten werden. Folgende Versuche wurden durchgeführt: 1 A) Vergleich von langsamer und schneller Einfrierrate unter Verwendung des Gefrierschutzmittels Mix 2 (1 Teil 6,5 % Dimethylformamid + 2 Teile 6,5 % Methylacetamid); 1 B) Vergleich von zwei Besamungsdosen mit Mix 2 tiefgefrorenem Sperma; 2) Vergleich der Kryoprotektiva Mix 1 (1 Teil 6,5 % DMF + 1 Teil 6,5 % MA), Mix 2 und MA (6,5 %). Als Basisverdünner wurde der Trehalose haltige Verdünner nach Hanzawa et al. (2006) eingesetzt. Das Sperma wurde in 0,25 ml Straws abgefüllt, je Straw 300 Millionen Spermien. Das langsame Einfrieren erfolgte in einem Einfrierautomaten in zwei Schritten. Von 0 °C bis -35 °C fiel die Temperatur um -3 °C/min und von -35 °C bis -130 °C um -50 °C/min. Beim schnellen Einfrieren wurden die Proben in einer Styroporkiste im Stickstoffdampf 4 bis 4,5 cm über dem flüssigen Stickstoff eingefroren. Die Einfrierrate betrug -40 °C/min. Die Proben wurden im Wasserbad bei 4 °C aufgetaut. An jeden Einfrierversuch schloß sich ein Besamungsversuch an. Die Hennen wurden intravaginal mit dem Inhalt von einem bzw. von zwei Straws besamt. Folgende Ergebnisse wurden erzielt: 1 A) Das langsam eingefrorene Sperma befruchtete signifikant besser als das schnell eingefrorene (Befruchtungsrate von 49,3 zu 11,5 %); 1 B. Die Besamung mit zwei Straws erhöhte im Vergleich mit einem Straw die Befruchtungsrate nicht signifikant (52,1 zu 43,6 %); 2) Den höchsten Anteil befruchteter Eier erzielte Mix 2 (81,1 %), gefolgt von Mix 1 (77,3 %) und MA (39,8 %). Somit erwies sich das Mix 2-Medium in Verbindung mit langsamem Einfrieren als gut geeignet, eine Spermabank für genetische Ressourcen aufzubauen.

Schlüsselworte: Kryokonservierung, Hahnensperma, Genbank, Ex situ Erhaltung

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Introduction

Ex situ preservation of endangered breeds is a crucial part of measures to preserve genetic diversity in chickens as in other farm animal species. Up to now, cryopreservation of semen represents the only efficient method for the ex-situ management of avian genetic resources. So far, three national avian germplasm cryobank programs exist operating in North America, The Netherlands and France (Blesbois, 2006; Blesbois et al., 2008). All of them store mainly semen and blood samples. Compared to livestock species, chicken semen in general seems to react differently in the freezing process. This is obviously due to some unique physiological characteristics (Long, 2006). A good post-thaw survival rate of rooster semen is obtained with glycerol as cryoprotectant. However, glycerol acts as a contraceptive and should not exceed 0.7 % (v/v; 0.1 M) prior to insemination (Long and Kulkarni, 2004). Other successful techniques to freeze chicken semen use dimethylformamide (DMF), dimethylacetamide (DMA) or N-methylacetamide (MA) as cryoprotectants in connection with both fast and slow freezing rates (Tselutin et al., 1999; Woelders et al., 2006; Hanzawa et al., 2006; Schramm, 2008; Sasaki et al., 2010).

The aim of the present study was to establish an efficient semen cryopreservation system as a methodological prerequisite to create a semen cryobank for conservation of chicken resource populations in Germany.

Material and methods

Housing and Environment

The experimental design is shown in Table 1. Three chicken lines of White Leghorn (R11, M11, and R22) background were used for semen collection and females of line R22 for the insemination trials. The lines have been maintained at the Institute of Farm Animal Genetics Neustadt-Mariensee of FLI as genetic resources for many years. Hatching eggs of these lines were brought to the former Institute of Small Animal Breeding in Celle in 1965. More details about the origin of these lines have been reported elsewhere (Hartmann, 1997). For more than 20 generations, populations have been managed as closed breeding populations composed of 10 sire families, each made up by 1 sire and 10 dams. In every generation, male offspring have systematically been rotated across families via artificial insemination with no selection, and a generation interval of one year. The animals were kept in individual cages, and provided with a commercial layer feed (Agravis Mischfutter Ostwestfalen-Lippe GmbH) and water ad libitum. During the period of the study, the animals received 14 h of light per day starting at three o'clock in the morning. Roosters and hens were 28 to 36 weeks old at the beginning of the experiments.

Table 1:
Experimental design

	Experiment 1		Experiment 2
	A	B	
Cryoprotective agents (CPA)	Mix 2	Mix 2	MA, Mix 1, Mix 2
Freezing method	fast or slow	slow	slow
Insemination dose	1 straw	1 or 2 straws	1 straw
Roosters			
hatching date	28-10-2009		
genetic lines	R22, R 11, M11		
number	11		
Semen collection period	June 2010	August 2010	February 2011
Hens			
hatching date	28-10-2009		15-12-2011
genetic line	R22		
number	49	44	34
Insemination period	July 2010	September 2010	July 2011

Experiment 1 A. Comparison of fast and slow freezing

Ejaculates of 11 White Leghorn roosters of three lines (R11, M11, and R22) were pooled and diluted 1:2 at room temperature with HS1 extender. The extender was prepared as described by Hanzawa et al. (2006): Sodium glutamate (H_2O) 1.2 g, potassium acetate (anhydrous) 0.3 g, trehalose 3.8 g, glucose (anhydrous) 0.2 g, N,N-Bis(2-hydroxyethyl)-2-aminoethansulfonic acid (BES) 0.5 g, Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bis-tris) 0.5 g, gentamicin sulfate 0.001 g per 100 ml distilled water, osmolarity 360 mOsm/kg, pH 6.8. After 60 minutes at 5 °C, ejaculates were further diluted by mixing one part of prediluted semen with one part of freezing extender Mix 2. The composition of the cryodiluents is shown in Table 2. The diluted sperm contained 6.5 % cryoprotectant in the final concentration. The semen was diluted to a standardized number of 1200 million spermatozoa per ml before freezing. Consequently, in each 0.25 ml straw 300 million sperm were included. For slow freezing procedure, a programmable freezer (Ice Cube 14S, Minitube, Tiefenbach, Germany) was used. The start temperature was 0 °C, followed by a 2-step freezing method with a cooling rate of -3 °C/min until -35 °C in the first step, and cooling rate of -50 °C/min until -130 °C. Fast freezing was carried out in a box by exposing the semen samples to liquid nitrogen vapour at 4 to 4.5 cm above the surface of liquid nitrogen. In this one-step freezing procedure the

temperature declined with -40 °C/min. Afterwards semen samples frozen with both procedures were placed in liquid nitrogen for long-term storage.

Table 2:
Composition of rooster sperm cryodiluents (ml)

	MA-diluent	DMF-diluent
Base diluent HS-1	87	87
Methyl acetamide (MA)	13	
Dimethyl formamide (DMF)		13

Mix 1- diluent: mixing one part MA-diluent with one part DMF-diluent
Mix 2- diluent: mixing two parts MA-diluent with one part DMF-diluent

Experiment 1 B. Comparison of one and two straws for insemination

The same roosters as in experiment 1 A were used (11 ejaculates per pool). Collection of semen, processing and examination were as described in experiment 1 A, and only the slow freezing method was used because freezing fast had achieved a low fertilization result. The same females as in experiment 1 A were inseminated with either one or two straws.

Experiment 2. Comparison of different cryoprotectants

The same roosters were used as in experiment 1 A. Semen collection and processing as well as slow freezing procedure were carried out as described in experiment 1 A. Three different freezing extenders, Mix 1, Mix 2 and MA, were used (composition see Table 2). The final concentration of all cryoprotectants was in each case 6.5 %.

Semen evaluation

Sperm cell concentration was determined by the NucleoCounter SP-100 system (ChemoMetec, Denmark). The fresh and frozen-thawed semen was evaluated for motility and morphological alterations. The straws were thawed in 4 °C water bath for 1 min. The motility characteristics of spermatozoa were evaluated by means of CASA (computer-assisted sperm analysis) with a Hamilton Thorne Biosciences-IVOS (Beverly, USA) and 4- Leja analysis chambers of a thickness of 20 µm (Minitube, Tiefenbach, Germany). The instrument's setting was adapted from Klimowicz et al. (2008); only the temperature of analysis was increased from 24 °C to 37 °C. Fresh and thawed semen were diluted 1:200 and 1:50, respectively, in HS1 medium before measurement. The parameters measured were: percentage of total motile sperm (MOT%), percentage of progressive motile sperm (ProgMot%), average path velocity (VAP, µm/s), progressive velocity (VSL µm/s), curvilinear velocity (VCL, µm/s), amplitude of lateral head displacement (ALH, µm), beat cross frequency (BCF, Hz), straightness of track (STR %, ratio of VSL/VAP) and linearity of track (LIN %, ratio of VSL/VCL). For the morphological examination, sperm cells were fixed in formol citrate solution and microscopically evaluated using a 100x oil immersion objective (Alkan et al., 2002). In each preparation, 100 spermatozoa were counted, and the percentage of defect types was calculated.

Fertilization trials

The fertilization ability of spermatozoa was tested by intravaginal artificial insemination (AI). Hens of the line R22 were used. Each hen was inseminated three times (day 1, 4 and 8) with the content of one (Exp. 1 A/2) or either one or two straws (Exp. 1 B). The semen was thawed in a water bath at 4 °C and was immediately used for insemination. Hens inseminated with fresh spermatozoa served as controls. Insemination started at 11 o'clock in the morning. Eggs were collected daily, beginning at the second day after the first AI, and continued for 10 d. Eggs were stored at 14 °C up to the end of the collection period, and subsequently placed together into an incubator. Fertility was checked at 7th day of incubation by candling. Fertility rate was calculated as ratio of eggs fertilized and eggs laid during the 10 d period of collection.

Statistical analyses

Statistical analyses were performed using SigmaStat 2.0 (Jandel Scientific, Erkrath, Germany). Semen quality traits were analyzed by One-way ANOVA. In the case of a statistically significant difference, all pair wise comparisons were carried out using Tukey procedure. If distribution of data significantly deviated from normal distribution, the Kruskal-Wallis ANOVA on Ranks was carried out. In this case, differences of pair wise comparisons were tested using the Dunn's method. Chi-square tests were applied to test the influence of freezing rate (slow or fast), insemination dose (1 or 2 straws) and the kind of the cryoprotectant (Mix 1, Mix 2, MA) on fertility.

Results

The fertility data are summarized in Table 3. In the first experiment (part A) a significantly higher fertility with slowly frozen (49.3 %) than with fast frozen (11.5 %) semen was found. The semen had been taken only two times to check quality after thawing. It was very similar between the slowly frozen and the fast frozen samples (Table 4). Merely at the second semen collection, the qual-

ity of the semen was lower after fast freezing. In particular, the portion of motile sperm and their speed were lower than in slowly frozen and thawed samples.

Table 3:
Fertilization rates with fresh and frozen-thawed semen

Exp. No.	No. of hens	No. of eggs			Straws/ AI	Semen processing	Freeze rate
		incu-bated	fertile	% fertile			
1 A	10	79	74	93.7[a]	-	Fresh, diluted	-
	20	148	73	49.3[b]	1	Frozen with Mix 2	slow
	19	148	17	11.5[c]	1	Frozen with Mix 2	fast
1 B	5	38	34	89.5[a]	-	Fresh, diluted	-
	19	133	58	43.6[b]	1	Frozen with Mix 2	slow
	20	146	76	52.1[b]	2	Frozen with Mix 2	slow
2	34	374	365	97.6[a]	-	Fresh, diluted	-
	12	97	75	77.3[b]	1	Frozen with Mix 1	slow
	11	90	73	81.1[b]	1	Frozen with Mix 2	slow
	11	93	37	39.8[c]	1	Frozen with MA	slow

Within experiment, different superscripts indicate significant differences ($P < 0.05$)

In experiment 1 B, we tested whether the fertility rate can be improved by increasing the insemination dose. Although the percentage of fertile eggs was slightly higher after the insemination with two straws (52.1 %) instead of one (43.6 %), the differences were statistically not significant (Table 3). The semen quality before and after freezing is shown in Table 5. The frozen-thawed sperm showed a significantly lower portion of total and progressively motile spermatozoa, lower velocity parameters as well as a higher percentage of sperm with morphological modifications in comparison with the fresh sperm. Only parameters amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN) were hardly changed after freezing.

Table 4:
Quality parameters of fresh and frozen-thawed rooster semen in experiment 1 A

Sperm Parameters	Fresh semen		Frozen-thawed semen			
	21-06-2010	23-06-2010	21-06-2010		23-06-2010	
			slow	fast	slow	fast
% motile	74	75.3	49.3	49	46	35.5
% ProgMot	47	40.7	27.7	27	21	14
VAP (µm/s)	81.1	72.6	73.5	69	68.4	60.8
VSL (µm/s)	67.7	57.3	58.6	55.1	52.7	46.6
VCL (µm/s)	120.7	115.5	115.8	111.4	112.6	104.2
ALH (µm)	4.8	4.9	5.4	5.4	5.6	5.2
BCF (Hz)	30.8	33.5	31.4	31.1	33.7	34.2
STR (%)	80	76.3	76.7	77	74	74.3
LIN (%)	53	48.3	49.7	49	45	43.5
% abnormal	21	18	44	45	24	40

% motile, percentage of motile sperm; % ProgMot, percentage of progressive motile sperm; VAP, velocity average path; VSL, velocity straight line; VCL, velocity curve line; ALH, average lateral head displacement; BCF, beat-cross frequency; STR, straightness ((VSL/VAP)x100); LIN, linearity ((VSL/VCL)x100); % abnormal, percentage of morphologically abnormal sperm

Table 5:
Quality parameters of fresh and frozen-thawed rooster semen (mean ± SD) in experiment 1 B

Sperm Parameters	Experiment 1 B		Test
	Fresh semen	Mix 2 frozen	
% motile	81.3 ± 2.3[a]	49.8 ± 4.5[b]	t- Test
% ProgMot	53.3 ± 3.9[a]	29.3 ± 3.5[b]	t- Test
VAP (µm/s)	89.8 ± 5.1[a]	74.0 ± 4.0[b]	t- Test
VSL (µm/s)	74.2 ± 4.8[a]	61.1 ± 4.0[b]	t- Test
VCL (µm/s)	131.4 ± 3.9[a]	112.1 ± 3.3[b]	t- Test
ALH (µm)	5.3 ± 0.2[a]	5.0 ± 0.1[a]	MW*
BCF (Hz)	31.4 ± 0.9[a]	31.0 ± 1.1[a]	t- Test
STR (%)	79.2 ± 1.4[a]	79.1 ± 0.9[a]	t- Test
LIN (%)	54.8 ± 2.7[a]	52.9 ± 1.9[a]	t- Test
% abnormal	13.0 ± 1.0[a]	39.2 ± 13.0[b]	t- Test

Different superscripts indicate significant differences ($P < 0.05$)
*Mann-Whitney-Rank Sum Test
% motile, percentage of motile sperm; % ProgMot, percentage of progressive motile sperm; VAP, velocity average path; VSL, velocity straight line; VCL, velocity curve line; ALH, average lateral head displacement; BCF, beat-cross frequency; STR, straightness ((VSL/VAP)x100); LIN, linearity ((VSL/VCL)x100);
% abnormal, percentage of morphologically abnormal sperm

In the second experiment, very good fertilization results (Table 3) were achieved with frozen sperm in both Mix 1 (77.3 %) and Mix 2 (81.1 %). Hens inseminated with fresh sperm diluted in HS1 produced 97.6 % fertility. The fertilization result at application using MA as cryodiluent

was worst (39.8 %). The post-thaw quality of MA frozen semen was also notably lower than with the other two cryoprotectants (Table 6). Particularly, the portions of total and progressively motile sperm as well as the speed of the sperm were significantly less in MA frozen samples. Figure 1 shows the portion of daily-fertilized eggs after insemination with Mix 2 frozen-thawed semen. Collecting hatching eggs started one day after first insemination. On this day, the portion of fertile eggs was still low between 15 and 30 % and increased gradually thereafter. The course of fertility rates over time emphasised the outstanding result of the experiment 2. The fertilization rate of this experiment remained on a very high level till the end of the testing period. In the other experiments, the fertilization rate decreased between the inseminations and increased after that again.

Table 6:
Quality parameters of fresh and frozen-thawed rooster semen (mean ± SD) in experiment 2

Sperm Parameters	Experiment 2				Test
	Fresh semen	MA frozen	Mix 1 frozen	Mix 2 frozen	
% motile	81.3 ± 6.4^{a}	32.7 ± 3.9^{b}	47.7 ± 6.2^{c}	44.3 ± 5.6^{c}	Tukey
% ProgMot	58.4 ± 7.8^{a}	15.8 ± 1.8^{b}	26.4 ± 4.4^{a,b}	24.8 ± 2.1^{a,b}	Dunn's Method
VAP (µm/s)	97.3 ± 9.7^{a}	60.8 ± 3.7^{b}	75.6 ± 3.5^{c}	73.6 ± 2.7^{c}	Tukey
VSL (µm/s)	84.0 ± 11.2^{a}	50.5 ± 3.0^{b}	61.8 ± 2.6^{a,b}	59.9 ± 2.2^{a,b}	Dunn's Method
VCL (µm/s)	132.4 ± 6.3^{a}	97.0 ± 4.1^{b}	113.9 ± 3.9^{c}	112.7 ± 2.4^{c}	Tukey
ALH (µm)	5.0 ± 0.3^{a}	4.7 ± 0.5^{a}	5.1 ± 0.3^{a}	5.0 ± 0.3a	F- Test
BCF (Hz)	30.9 ± 0.9^{a}	31.8 ± 0.7^{a}	31.8 ± 0.7^{a}	32.0 ± 0.9^{a}	F- Test
STR (%)	82.5 ± 3.0^{a}	79.6 ± 1.2^{a,b}	78.1 ± 1.0^{b}	77.7 ± 1.2^{b}	Tukey
LIN (%)	61.0 ± 5.1^{a}	50.9 ± 2.1^{b}	51.8 ± 1.2^{b}	51.4 ± 1.2^{b}	Tukey
% abnormal	21.0 ± 6.0^{a}	45.0 ± 5.0^{b}	35.4 ± 10.0^{b,c}	31.5 ± 8.6^{a,c}	Tukey

Different superscripts indicate significant differences (P<0.05)
% motile, percentage of motile sperm; % ProgMot, percentage of progressive motile sperm; VAP, velocity average path; VSL, velocity straight line; VCL, velocity curve line; ALH, average lateral head displacement; BCF, beat-cross frequency; STR, straightness ((VSL/VAP)x100); LIN, linearity ((VSL/VCL)x100);
% abnormal, percentage of morphologically abnormal sperm

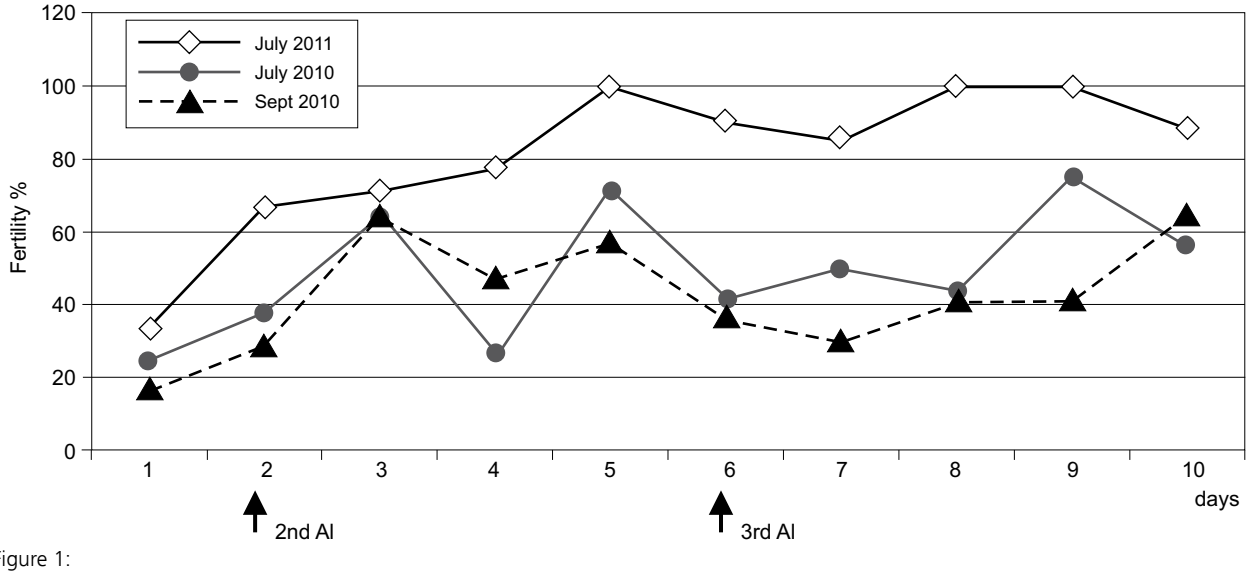

Figure 1:
Daily fertility after inseminations with frozen-thawed semen in Mix 2 (Day 1 = second day after first insemination)

Discussion

In previous studies, we applied a fast freezing method to cryopreserve rooster sperm. As cryoprotectants DMA and DMF were used in final concentrations of 6.5 %. Fertilization success, however, was very unsatisfying with a rate of fertilized eggs of 10 %. Fast freezing with a mixture of DMF and MA (Mix 2) improved fertilization results, but were still modest (29.9 %, Ehling, unpublished data). Therefore, first the effects of a fast and slow freezing rate on the fertility results by using of Mix 2 were examined in the present study (experiment 1A). According to our former study, the fast freezing protocol led to a rather low fertility success, although in vitro semen quality after thawing did not differ between slow and fast freezing procedure (CASA values and morphology). Other functional tests of sperm and examination of the acrosome integrity would give deeper insight into differences like in the examinations of Santiago-Moreno et al. (2011). A low fertility was also observed in other studies, using DMA as cryoprotectant and a fast freezing protocol (Blesbois et al., 2007; Baguio et al., 2008; Santiago-Moreno et al., 2011). Santiago-Moreno et al. (2011) examined three different freezing rates (slow-one step, medium-2 step and rapid) which led to different semen qualities, but had no significant effect on fertility rate. The medium-rate 2-step method (-7 °C/min from 5 °C to -35 °C, -60 °C/min from -35 °C to -140 °C) achieved the best fertility of 40.7 % and preserved best the motility and the acrosome and membrane integrity. Similar good fertilization results were found with a slow freezing method and the use of DMA or DMF (Lake and Ravie, 1984; Schramm, 1991; Chalah et al., 1999; Schramm, 2008). There are numerous studies reporting very good fertility rates with cryoprotectants of the amide group, straws and fast freezing (Woelders et al., 2006; Hanzawa et al., 2006; Purdy et al., 2009; Sasaki et al., 2010). However, the best fertility rates were obtained with semen frozen rapidly as pellets with DMA (Tselutin et al., 1999; Chalah et al., 1999; Woelders et al., 2006). Different procedures are not directly comparable as they are affected by various factors and may also be dependent on genotypes of chickens. In our hands, however, the slow freezing protocol worked well, and better than a fast freezing procedure.

Experiment 1 B, based on two straws per insemination compared to one, resulted only in a slightly higher fertilization result. Bielefeld (1985) also reported that doubling of insemination dose to 360 million sperm did not lead to a significant increase in the fertilization rate. Brillard and McDaniel (1986) concluded that the changes in semen doses modify duration rather than maximum levels of fertilization. The FAO guidelines for the Management of Small Populations at Risk (FAO, 1998) recommend a total dose of 600 million rooster sperm per insemination. The insemination volume should be approximately 60 to 100 µl. In our experiments the insemination dose was 200 µl, what corresponds to the content of one straw. The higher insemination volume (2 straws) in experiment 1 B possibly led to a partial sperm loss by reflow.

In the second experiment, MA was tested as a cryoprotectant. Hanzawa et al. (2006) and Sasaki et al. (2010) obtained higher fertility with MA (60.8 %) than with DMF (47.6 %), DMA (32.9 %) or DMSO (41.3 %). However, in the present experiment the fertilization rate was only moderate (39.8 %) when using MA as single cryoprotectant. In contrast, in our experiment the combination of MA and DMF led to very high fertilization results of approximately 80 %. The altered mixing ratio of DMF and MA in Mix 1 did not produce a higher fertility in the fertilization test. In all experiments, the same group of roosters was used. Their semen was inseminated in experiment 1 A and B into the identical group of hens, for experiment 2 another set females was used. These new hens had a high laying performance at the time of inseminations with cryopreserved semen. This might have contributed to the high fertilization success in this experiment.

In the experiment 2, the sperm had the same quality than in the other tests. The age of the animals was possibly of influence. The roosters were older (already 16 months) than in the experiment 1 A (8 months) and B (10 months). Long et al. (2010) accounted for an influence of rooster's age on the hydrolyzing ability of frozen-thawed sperm among elite layer lines. Sperm from 12 month old males hydrolyzed more holes in the inner perivitelline membrane than sperm from six month old males. Whether the age of the genetic pool roosters really influences the fertility results has to be verified in other experiments.

The movement of spermatozoa is one of the important parameters of semen quality. Motility assessment depends on the structural and functional integrity of spermatozoa and allows prediction of the fertilizing potential of semen (Froman, 2007; Blesbois et al., 2008). In the present study sperm quality was assessed by motility parameters, objectively measured by means of CASA. Most of CASA parameters were affected by cryopreservation. Primarily, the percentage of motile and progressively motile spermatozoa was reduced in approximately 50 % of the cryopreserved samples, when compared with fresh samples. Additionally the post-thaw sperm showed a significant decrease of their velocity. Blesbois et al. (2008), Sontakke et al. (2004), Fungfuang et al. (2009), Lemoine et al. (2011) and Santiago-Moreno et al. (2011) also reported such changes following freezing and thawing of chicken sperm.

Cryopreservation significantly affected the percentage of morphologically normal spermatozoa. In the present examination, percentage of abnormal sperm is, however,

fundamentally less than in studies by Schramm (1991; 2008) and Fungfuang et al. (2009).

In conclusion, best fertility results of 80 % were achieved using a mixture of DMF and MA in combination with a 2-phase slow freezing procedure. These results suggest that this protocol can be used to establish a cryobank for conserving chicken genetic resource populations for an unlimited period of time.

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