World Rabbit Sci. 2014, 22: 279-286
doi:10.4995/wrs.2014.3223

EVALUATION OF CALCIUM SUPERPHOSPHATE AS AN ADDITIVE TO REDUCE GAS EMISSIONS FROM RABBIT MANURE

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Abstract: Techniques to reduce the emission of air pollutants from livestock production are demanded. In this study, the effect of an additive (calcium superphosphate) on gas emissions from rabbit manure was investigated and compared with a control where no additive was used. Calcium superphosphate was applied at a rate of 100 g/m^2 per week in a manure pit during 2 cycles of growing rabbits. Manure samples were collected weekly and then chemically and microbiologically analysed. Gas emissions (ammonia, carbon dioxide, methane and nitrous oxide) were determined in 2 open flux chambers. No differences were observed in gas emissions between the treated and control samples except for ammonia emissions, which were reduced by 33% when the additive was applied (P<0.05). No statistical differences were obtained in the microbial content between control and treatment, as results showed a high variability. Dry matter content and pH were the most influential parameters on the emission of gases from manure. According to these results, the application of calcium superphosphate may be considered as an effective technique to reduce ammonia emission from rabbit manure. The additive may also be potentially effective in other species, but additional research is necessary to investigate its performance.

Key Words: microbiology, gas balance, NH_3, CO_2, N_2O, CH_4.

INTRODUCTION

Rabbit production is typical in some countries in the Mediterranean area, where the environmental impact of livestock production on the atmosphere has still not been widely studied. More specifically, information on the emission level of gases such as ammonia (NH_3) and greenhouse gases (particularly methane, CH_4) from enteric fermentation and manure management in rabbits is scarce (Michl and Hoy, 1996; Calvet *et al.*, 2011). For this reason, mitigation techniques in rabbit production are less developed than in other livestock sectors. Although rabbit production is less important in terms of total meat production than other livestock species (FAO, 2014), characterising mitigation techniques and implementing them at commercial level seems essential to contribute to a more sustainable rabbit production.

Animal production is relatively inefficient in the use of nutrients. Fattening rabbits excrete approximately 60% of the nitrogen intake as urine and faeces (Calvet *et al.*, 2008). Part of the excreted nitrogen is lost as NH_3 gas, with 3 main consequences. Firstly, the NH_3 emitted to the atmosphere contributes to the acidification and eutrophication of the environment (Krupa, 2003). Secondly, nitrogen losses reduce the fertiliser value of manure (Burton and Turner, 2003). Finally, NH_3 accumulation may have serious implications for human and animal health (Roney *et al.*, 2004). NH_3 plays a key role in the nitrogen biogeochemical cycle because it is the intermediate substance between inorganic and organic nitrogen compounds. It is a substrate or product of basic microbial reactions such as ammonification, nitrification, denitrification and biologic fixation of nitrogen (Tate, 2000).

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http://dx.doi.org/10.4995/wrs.2014.3223

Carbon dioxide (CO_2) is produced by the respiration of animals and the aerobic decomposition of their manure. CO_2 emissions have been widely used to estimate ventilation flows in livestock buildings (CIGR, 2002). Finally, CH_4 and nitrous oxide (N_2O) are greenhouse gases with a high global warming potential, with 21 and 310 times the greenhouse effect of CO_2, respectively (Solomon *et al.*, 2007). CH_4 is produced through the anaerobic decomposition of organic matter, whereas N_2O is closely related to the agricultural nitrogen cycle and is produced in the nitrification and denitrification process of manure. The microbial reactions occurring in the manure determine the potential emissions of these gases. Therefore, understanding these microbial processes is essential to establish potential mitigation techniques.

Mitigation techniques, including animal feeding, housing design and manure management, among others, have been investigated in other species. Digestive and manure additives have also been commonly studied in animal production, considering their effect in reducing these emissions. These additives may be effective in several ways, but they mainly reduce NH_3 and odour emission (McCrory and Hobbs, 2001). Acidifying additives, adsorbents and urease inhibitors have also been proved to be effective to reduce NH_3 emissions. However, it is necessary to characterise the dose response of these additives and understand the cross effects on manure properties and the emission of other gases in order to optimise their management in commercial farms.

Calcium superphosphate or calcium dihydrogen phosphate ($Ca(H_2PO_4)_2$) is used in rabbit farms in the Spanish Mediterranean area to improve the indoor environment. Farmers use this compound as a surface drier, and they also report that it avoids the growth of flies and reduces odour. The effects on manure's physical, chemical and biological properties, and therefore gas emissions, are unknown. Thus, the main aim of this work was to evaluate the effect of applying calcium superphosphate powder in manure pits on the physical, chemical and microbiological properties of rabbit manure and gas emissions. A secondary objective was to identify the cause and effect relationships between manure properties and gaseous emissions.

MATERIAL AND METHODS

Housing and animals

The study was conducted at the experimental rabbit farm of the Universitat Politècnica de València (Valencia, Spain) on 2 consecutive cycles of fattening rabbits (*Oryctolagus cuniculus,* Line V; Baselga, 2002), aging from 28 to 63 d. The farm followed a conventional management for fattening rabbits in the Spanish Mediterranean area. Animals were reared in collective cages (80×50 cm and 9 animals per cage) above a manure pit. Manure was completely removed just before each fattening cycle. Animals were fed *ad libitum* using a commercial pelleted rabbit feed. The feed had the following calculated composition: 15.5% crude protein, 90% dry matter (DM), 4.3% fat content, a gross energy of 16.3 MJ/kg and 15.5% crude fibre.

Additive application

Six rabbit cages were monitored in each cycle, 3 of them corresponding to the treated manure (treatment) and the other 3 corresponding to untreated manure (control). Adjacent cages also followed the same treatment during the experiment to avoid boundary interactions due to application of the additive, as well as to cover a larger area in the

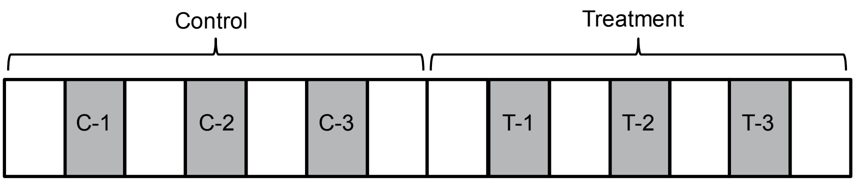

Figure 1: Distribution of the studied cages, inclding controls (C-1 to C-3) and treatment (T-1 to T-3). Four additional cages were used in each case to avoid boundary interactions.

manure pit in order to achieve a better spatial representation. Thus, a total of fourteen cages were included in the experiment (Figure 1). A cross design was used considering the cycle, so the additive was applied during the second cycle in the cages where it was not applied during the first cycle and vice versa.

A calcium dihydrogen phosphate powder (Superfosfato 18%, Agromediterráneo, Murcia, Spain)) was added to the manure pit under the treated cages, whereas the remaining cages were not treated. The additive was applied manually twice weekly (Monday and Friday) at a rate of 50 g/m^2 per application. The calcium superphosphate powder had the following composition: 48% P_2O_5, 28% CaO and 24% SO_3.

Gas emissions

To determine the emission from manure, the dynamic chamber method was used (Estellés *et al.*, 2009). Manure samples were collected weekly using 20×13×13 cm (length×width×height) polymethyl methacrylate (PMMA) boxes. One box was placed in the manure pit below each experimental cage 5 d before emissions measurements and sampling, and accumulated the manure produced by the rabbits during these days. After these 5 d of accumulation, the boxes were removed from the pit for gas emissions measurements, thus achieving 3 treated and 3 untreated samples for gas emission monitoring.

To measure gaseous emissions, 2 identical PMMA chambers were used. Each chamber had a 29×49 cm base and 29 cm height (Figure 2). In each emission test, one treated and one untreated manure box were simultaneously evaluated (one in each chamber). Therefore, 3 emission tests were conducted to evaluate all weekly emission samples. One extraction pump (Silent-pump AC-9902, Resun, Singapore) was used to vent each chamber with a ventilation flow of 3 L/min, which was tested using a flow meter (Yokogawa RAGH, Yokogawa Electric Corporation, Japan). A small fan was used to homogenise the air inside each chamber. Gas concentrations (NH_3, CO_2, N_2O and CH_4) were measured every 2 min using a photo acoustic gas monitor (Innova 1412, Lumasense, Denmark). A multi-point system was used to determine emissions from the 2 chambers alternatively, with a frequency of 2 min per measurement. Gas emissions were calculated following the mass balance equation for non-stationary state:

$$E_i = \frac{F \times (C_{i,t} - C_{i,ext}) \times 3600}{\left(-e^{\frac{-F \times t}{V}} + 1\right) \times S}$$

Where E_i is the emission rate (mg/h m^2) for each gas i, F is the airflow rate in the chamber (m^3/s), $C_{i,t}$ and $C_{i,ext}$ (mg/m^3) are each gas i concentrations measured inside the chamber at time t and entering the chamber, t (s) is the time past from the beginning of the measurement ,V (m^3) is the volume of the chamber and S (m^2) is the surface of the emitting source (sampling box).

Manure characterisation

After measuring the emission rate from each manure sample, a sub-sample was collected from each of them to determine manure properties. These samples were analysed for pH, DM (DM, dehydration at 104°C until constant weight) and total Kjeldahl nitrogen (TKN), according to AOAC (2000) procedures.

Furthermore, from cages C-2 and T-2 (Figure 1) an additional sub-sample was taken and analysed for microbial content. Each sample was refrigerated at 4°C until the start of the analysis 1 h after samples were taken. These manure samples (10 g) were homogenised with standard saline solution (90 mL). Decimal dilutions were poured on different culture media. The analyses that were carried out to estimate the microbiological content were: total viable count were enumerated in Plate Count Agar (PCA, Merck) at 30°C for 48 h, Enterobacteriaceae

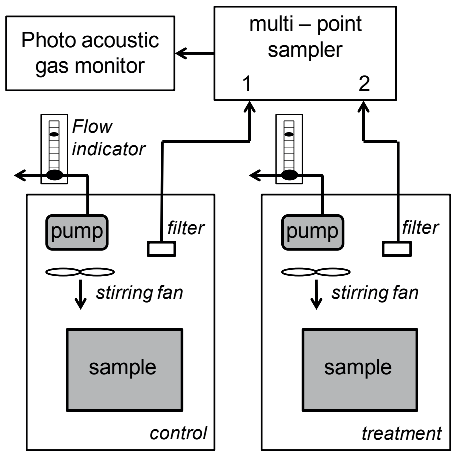

Figure 2: Layout of the gas emission measurement system, with duplicate measurements.

Table 1: Least square means and standard error of the mean (SEM) for manure production, properties and gaseous emissions of NH_3, CO_2, N_2O and CH_4 from treated and untreated (control) manure for the 2 fattening cycles. The total number of measurements (No.) is also indicated.

	No.	Manure production (kg/animal wk)	DM (%)	TKN (% DM)	pH	NH_3 (mg/h/m²)	CO_2 (g/h/m²)	N_2O (mg/h/m²)	CH_4 (mg/h/m²)
Cycle									
1	30	0.85	53.71	1.86	7.61	22.99	9.22	2.34	31.24
2	30	0.94	58.11	1.67	7.66	21.16	9.03	2.12	3.58
Treatment									
Control	30	0.92	54.86	1.76	7.81	26.39	10.81	2.53	16.37
Treated	30	0.87	56.95	1.76	7.46	17.76	7.45	1.93	18.45
SEM		0.10	1.56	0.05	0.13	2.48	1.33	0.27	8.92
Week									
1	12	0.19[a]	72.53[a]	1.65	8.20	22.56	1.22[a]	0.98[a]	6.73
2	12	0.32[a]	65.32[a]	1.70	7.45	14.09	2.69[a]	1.57[a,b]	3.83
3	12	1.26	46.16[b]	1.81	7.47	25.43	11.66[b]	2.41[c]	6.26
4	12	1.08[b]	50.29[b]	1.76	7.53	21.21	10.44[b]	2.30[b,c]	21.52
5	12	1.62[c]	45.22[b]	1.88	7.54	27.10	19.63[c]	3.89[d]	48.72
SEM		1.35	1.82	0.05	0.15	3.57	1.68	0.32	11.60
P-value									
Treatment		0.758	0.552	0.949	0.094	0.032	0.212	0.135	0.873
Week		<0.001	<0.001	0.072	0.229	0.022	<0.001	<0.001	0.060
Cycle		0.613	0.021	0.006	0.774	0.657	0.591	0.311	0.059
T×W		0.976	0.877	0.979	0.850	0.226	0.118	0.357	0.999
T×C		0.524	0.151	0.888	0.805	0.045	0.791	0.773	0.870

Different superscripts in the same row indicate significant difference for week ($P<0.05$).

were determined on Violet Red Bile Glucose Agar (VRBG, Merck), the plates were overlaid before incubation at 37°C for 24 h. Moulds and yeasts were enumerated on Sabouraud (Merck) supplemented with chloramphenicol. Clostridia were determined on SPS agar (Sulfite-Polymixin-Sulfadiazine-A) and ammonification potential was quantified adapting the method proposed for soils based on Nessler's reaction and determined by the most probable number (MPN) technique (Alexander, 1982). All laboratory analyses were performed in duplicate.

Statistical analysis

The effect of the treatment, week and cycle on manure characteristics and emissions were evaluated using the model described below: $X_{ijk}=P_i+T_j+W_k+T_j\times W_k+P_i\times T_j+\varepsilon_{ijk.}$

Where X_{ij} is the measured parameter, P_i represents each of both cycles, T_j is the use of the additive (addition or not addition of calcium superphosphate), W_k is the age of the animals expressed in weeks and ε_{ijk} is the model error. Analyses were performed using Mixed Linear Models Procedure (Proc Mixed) of the SAS System (SAS, 2001). Tukey-Kramer adjustments were used for *post hoc* comparisons.

The effect of manure characteristics on gas emissions was evaluated by a correlation analysis using the CORR procedure of the SAS System (SAS, 2001).

RESULTS

Manure characterisation

The production of manure as well as its properties as a function of animal age, cycle and treatment are reported in Table 1. Manure production increased with animal age ($P<0.01$), but was not affected by the cycle or treatment. DM content of manure was affected by the cycle, showing higher values during the second one ($P<0.05$). This parameter decreased significantly with animal age ($P<0.01$), from over 70% during the first week to about 45% the last week, although no differences were detected between 1st and 2nd wk, or between the 3rd, 4th and 5th wk. No differences were found for DM content between both treatments (P=0.55). Regarding the TKN content (on DM basis), the cycle was the only factor affecting the results (P<0.05), finding a lower TKN content during the second cycle. The effect of animal age (week) on TKN, although not statistically significant, followed an opposite trend compared with DM content, increasing from 1.65% TKN (DM) during the first week to almost 1.90% in the last week. The effect of the treatment was not statistically significant for DM. Finally, the pH of the samples was not significantly affected by any of the studied parameters.

The double interactions between treatment and week of measurement or cycle were not significant for any of these parameters.

Gas emissions

Gas emissions were calculated as a function of the treatment and animal age, as shown in Table 1. NH_3 emissions were the only ones affected by the treatment (P<0.05), showing lower average values for manures treated with calcium superphosphate (32.7% of reduction) compared to the control. Despite finding a significant effect (P<0.05) of the interaction treatment×cycle on NH_3 emissions, the effect of the treatment had the same trend for both cycles. CO_2 and N_2O emissions were only affected by the age of the animals (P<0.05) increasing with the week of the experiment. CH_4 production was not statistically affected by any of the studied factors.

Among the measured manure properties, only a few could be related to gaseous emissions (Table 2). NH_3, CO_2 and N_2O emissions decreased with DM content (P<0.05). pH and NTK content had a strong positive effect on NH_3 (P<0.01) emissions and a negative correlation was found for CH_4 (P<0.05 for pH and P<0.01 for NTK). Manure production (kg) had a strongly significant positive effect on CO_2 and N_2O emissions (P<0.001).

Microbial content

Results of the microbial content analysis are shown in Figure 3. As seen in this figure, a high variability was found in manure's microbial content, and no clear pattern could be identified for any of the determined populations. Considering that only 2 samples (one control and one treatment) were analysed each week, these results must be interpreted as descriptive of the rabbit manure under the pit. Total viable counts were similar for treated and control manure, although the 1st wk in the 2nd cycle presented lower counts than the 1st cycle. This difference was about 2 orders of magnitude during the whole cycle. As regards Enterobacteriaceae counts, they decreased with animal age in cycle 1, whereas they increased in cycle 2. Moulds and yeasts were highly variable, ranging from 10^3 to 10^8 CFU/g. Ammonification potential at 6 d tended to increase with animal age. Finally, Clostridia counts varied from 10^3 to 10^6 UFC/g. However, no differences were obtained between control and treated manure for any of the counts in this study (P>0.05) and no clear patterns could be identified.

DISCUSSION

The treated manure collected under the cages presented a slightly lower pH than the control manure, although not statistically significant, but it did not differ in DM or TKN. That difference could be related to a slight acidifying capacity of the additive. The high urinary alkalinity of rabbit urine (pH about 8) is caused by high concentrations of calcium carbonate, which causes precipitations of these salts (Kiwull-Schöne *et al.*, 2005), and could also attenuate the

Table 2: Correlation matrix between manure characteristics and gaseous emissions. Correlation coefficients and significance are presented for statistically significant relationships.

Parameter	NH_3 (mg/h/m²)	CO_2 (g/h/m²)	N_2O (mg/h/m²)	CH_4 (mg/h/m²)
Dry matter (%)	–0.363; P=0.009	–0.632; P<0.001	–0.450; P<0.001	n.s.
pH	0.535; P<0.001	N.S.	N.S.	–0.284; P=0.044
TKN (% of DM)	0.365; P=0.008	N.S.	N.S.	N.S.
Manure production (kg)	N.S.	0.862; P<0.001	0.569; P <0.001	N.S.

N.S.: not significant (P>0.05).

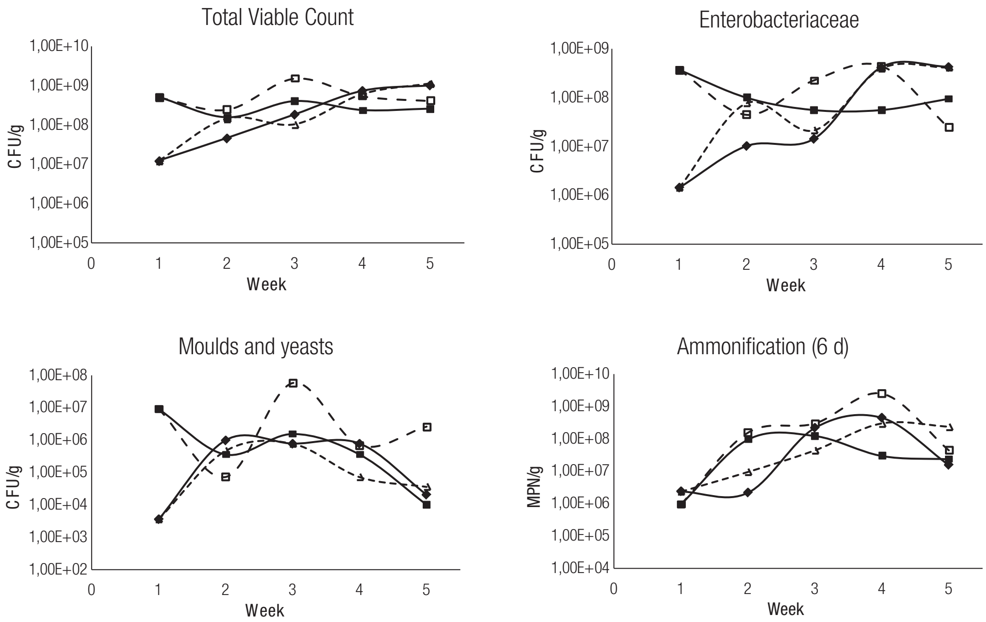

Figure 3: Microbial content (colony forming units, CFU/g) and ammonification potential (most probable number, MPN/g) as a function of rabbit manure as a function of the week of the growing period, and the application of the additive (T) against the control (C) in the 2 studied growing cycles. C-Cycle 1, T-Cycle 1, C-Cycle 2, T-Cycle 2.

acidifying effect of the additive. This effect on pH may affect the ammonium-ammonia equilibrium in the manure, in which the NH_3 is readily available for volatilisation at high pH values. Therefore, any effect in the pH of rabbit manure may affect NH_3 emissions. Although no statistical differences were found for manure pH, it has been reported that pH variations in the range from 7 to 9 strongly affect ammonia emissions as a consequence of the acid-base aqueous equilibrium of this substance (Snoek *et al.*, 2014). Therefore, it should be further confirmed whether the tendency to lower pH values in the treated manure may be the cause of the statistical differences found for NH_3 emissions.

Farmers tend to have the impression that this additive dries surfaces, which could be a potential explanation of changes in gaseous emissions. However, this hypothesis was not observed on manure pits according to our results. Nevertheless, the additive contributed to creating a crust over the manure (although not measured), which may be related to potential changes in manure microbiology and gaseous emissions.

It was expected that manure properties did not differ considerably between both cycles, as the manure pit was exactly in the same conditions before each trial. However, differences in feed intake, water consumption or the environment, which were not registered in this experiment, could originate the changes detected between both cycles in nitrogen or DM content of the manure.

Overall, the amount of microorganisms found in rabbit manure was within the ranges found for other animal species (Espinoza *et al.*, 2009). These analyses, however, could not be compared with previous studies in rabbit production, as microbial analyses in this species have been mainly directed towards digestive and pathogen microorganisms. For example, Carrizo (2003) described the influence of the feed on the caecal microbiology, but not on excreted manure. Given the low number of microbial analyses and the variability of their results, it was not possible to relate the microbial content of manure with gaseous emissions. However, the results obtained in this study can be useful for future studies relating gas emissions with manure microbial content.

As expected, the mass of manure increased with animal age. The reason for this increase is that animals produce more manure when they grow (Calvet *et al.*, 2008). Therefore, at the end of the cycle, higher gas emissions could be expected. An effect on microbial activity is also expected, although this hypothesis could not be proved due to the high variability of microbial analyses.

It is reasonable to accept that water content in manure enhances microbial reactions in manure, and therefore if DM content increases, gas emissions decrease. The direct relationship between pH and NH_3 emission can be explained by the acid and base reaction of dissolved NH_3. In a basic solution, NH_3 is readily available for volatilisation, thus increasing NH_3 formation. Therefore, the reduction in the pH in the treated manure compared to the untreated manure may be directly related with the significant reduction of NH_3 emission.

In general terms, the additive increased the amount of aerobic microorganisms, as well as the facultative anaerobic bacteria, but no significant differences were obtained between treatments. It is reasonable to assume that if the additive affects the emission of certain gases, it may also change the microbial populations in the manure, which are involved in the production of those gases. Nevertheless, a comprehensive characterisation of manure in terms of microbial content is more labour-consuming than only measuring the emission of gases. Therefore, determining the microbial content of the manure may not be an appropriate indicator of the emission level.

The NH_3 reduction efficiency obtained here is comparable to other mitigation techniques reported by UN/ECE (1998) for cattle, pigs or broiler houses. Those techniques are normally based on housing design and have typical reduction potential between 20 and 60% compared to the reference system. Regarding manure additives, Heber *et al.* (2000) reported from 13 to 27% reduction of NH_3 emission in a swine finishing building when using a glyoxal-based commercial additive, and Amon *et al.* (1995) obtained a 26% reduction of NH_3 from a fattening piggery using a *Yucca shidigera* extract. Other additives based on phosphoric acid, aluminium sulphate, ferric sulphate and sodium hydrogen sulphate were also proved to be effective to reduce emissions from poultry manure (DeLaune *et al.*, 2004; Li *et al.*, 2006), reporting reduction potentials over 50% depending on the dose of additive. According to the literature, additives are effective to reduce NH_3, but practical and economic reasons may hinder their use in commercial conditions. The additive tested here, however, has the advantage that it is already in use on some commercial farms, which indicates that its use is technically affordable at a reasonable cost.

CONCLUSIONS

This study assesses the effect of an additive on rabbit manure pits by a multifactorial approach, considering relations among the physical, chemical and microbial properties and gaseous emissions from rabbit manure.

The additive only presented a significant effect on NH_3 emissions, leading to a reduction of emissions of 33% compared with the control. The remaining gases were not affected by the treatment. The emission of gases increased with animal age, according to the greater manure production and accumulation in the pit. Although very high variability was found in microbial analyses, the information obtained may be very useful for future studies, as these parameters have been characterised in this study for the first time. More research is necessary to determine whether the additive is also effective in other animal species.

Acknowledgements: The Spanish Ministry of Science and Innovation (Project GASFARM-2 AGL2008-04125) provided support for this study.

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