British Poultry Science Volume 47, Number 2 (April 2006), pp. 103–109

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Reducing gaseous nitrogen loss from stored laying hen manure by the addition of carbohydrates

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Abstract 1. Three separate experiments were conducted to determine if the addition of carbohydrate sources directly to freshly-produced laying hen manure reduced the loss of gaseous nitrogen containing compounds during 7 d of storage.
2. The addition of sucrose (8 g/kg) to the manure resulted in a greater increase in bacterial numbers, a greater loss of carbon and a trend for a reduced loss of nitrogen. Although glucose addition to the manure increased bacterial numbers and tended to increase carbon loss, nitrogen loss increased relative to the control. Addition of straw to the manure did not affect the change in bacterial numbers or rate of nitrogen loss compared to the control treatment.
3. Experiment 2 indicated that, when either sucrose or maltose were mixed (8 g/kg) with laying hen manure, microbial numbers were increased and gaseous nitrogen losses were reduced. A combination of starch plus α-amylase did not affect the characteristics of the manure.
4. Experiment 3 showed that increasing sucrose addition gave a non-linear reduction in nitrogen loss. Minimum nitrogen loss was obtained with 35 g/kg sucrose, though the maximum increase in bacterial numbers occurred at 20 g/kg of added sucrose.

INTRODUCTION

Manure from laying hens can be a valuable agricultural fertiliser, but the location of many layer enterprises and local agricultural nitrogen use regulations may restrict the amount and timing of its application to the land. It is generally the responsibility of the egg producer to store the manure until it is required. Once produced, the manure is stored for varying times within the laying hen house and then may either be stored in a pit within the house or removed to an alternative storage area. The UK edible egg production industry is responsible for producing over one and a half million tonnes of manure each year (Chambers and Smith, 1998). This quantity of manure would initially contain about 60 000 tonnes of nitrogen and up to half of this nitrogen could be lost, mostly as ammonia, during manure storage (Pratt *et al*., 2002). The rate of gaseous nitrogen loss is greatest in the first few days following excretion and up to 10% of the total nitrogen present in freshly-produced poultry excreta can be lost within the first week of storage (Pratt *et al*., 1998). Reducing nitrogen loss from stored laying hen manure would not only reduce environmental pollution but could also reduce the deleterious effects of high ammonia concentrations within poultry houses (Carlile, 1984).

Nitrogen losses from laying hen manure result from the microbial decomposition of uric acid and undigested proteins. The production and release of ammonia from manure is affected by the chemical composition and the storage conditions of the manure. Groot Koerkamp (1994) concluded that nitrogen losses can be reduced by reducing the water content and

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Accepted for publication 12th December 2005.

ISSN 0007-1668(print)/ISSN 1466-1799(online)/06/020103-7 © 2006 British Poultry Science Ltd
DOI: 10.1080/00071660600610500

storage temperature of the manure and reducing the air temperature, relative humidity and the velocity of the air across its surface. However, these measures are often difficult to implement in existing commercial egg production units. The reduction of ammonia emissions is possible using forced air manure drying systems but the application of this technology is not appropriate for some cage systems or other alternative housing systems. Frequent manure removal from the laying house may reduce the ammonia losses directly from the house but may result in higher losses in the alternative storage area, particularly when there are high ambient temperatures. During the removal of droppings from the housing system it is possible to add a beneficial additive in order to reduce ammonia losses from the stored manure. A number of additives have been examined including organic and inorganic acids which lower pH and suppress the rate of microbial degradation, but are highly corrosive, hazardous to stock-workers and equipment and their addition to manure may produce increased chemical content or nutrient loading. Others, such as zeolite or yucca saponin, work by adsorbing the released ammonia, though these are more effective as covers or filters than when incorporated into the manure (Witter and Lopez-Real, 1988).

Ammonia is released during the ammonification stage of the nitrogen cycle by microbial activity (Kruse, 1986). Microbial growth is dependent on a number of environmental factors including temperature, water activity and pH. Growth also depends upon the availability of essential nutrients, especially those used as energy sources and for protein synthesis. If manure is deficient in available carbohydrates, microbes will utilise uric acid, proteins and other nitrogen-containing compounds as energy sources. Nitrogen is a by-product of this process, so ammonia is emitted from the stored manure (Higgins and Burns, 1975). Manure from caged laying hens does not have litter material incorporated, in contrast to most other poultry production systems, so it is relatively deficient in available carbohydrate. There is a need to determine if adding available carbohydrates to freshly-produced caged laying hen manure reduces gaseous nitrogen loss.

The addition of carbohydrates to caged laying hen manure has been investigated primarily in terms of producing stable poultry manure compost. The addition of carbonaceous material to poultry manure increases the C:N ratio, potentially reducing ammonia volatilisation from the decomposing manure. Witter and Lopez-Real (1987) concluded that increasing the C:N ratio of animal manures and slurries by the addition of carbohydrates was highly variable, but overall there were some reductions in nitrogen losses. This reduction in ammonia emission probably occurs as a result of nitrogen being incorporated within the microbial biomass. The availability of the carbon source to the microbial population may determine the magnitude of the nitrogen retained in the biomass. Differences between manure types and the type of carbohydrate used may explain the cause of the variability in nitrogen loss after carbohydrate addition.

The objectives of this work were, firstly to determine if adding carbohydrates to laying hen manure can give significant reductions in gaseous nitrogen losses in stored laying hen manure, secondly to examine whether the type of carbohydrate used gives differences in gaseous nitrogen loss and thirdly to examine the rate of addition of a selected carbohydrate on gaseous nitrogen loss. All three experiments were conducted on freshly-produced caged laying hen manure in the first week of storage, which is the period when the greatest amount of ammonia loss would be expected.

MATERIALS AND METHODS

Experiment 1

The objective was to test the nitrogen loss from stored laying hen manure when one of 4 carbohydrate sources (glucose, sucrose, starch and straw) were incorporated at 8 g/kg fresh weight. The 4 different carbohydrates were examined because of their different resistance to microbial hydrolysis. A fifth control treatment (no carbohydrate addition) was also included.

A single flock of 1400 commercial caged laying hens were fed on a practical, nutritionally-complete wheat and soya bean meal-based laying hen feed and kept in a controlled environment house. The housing system had belt cleaning of the manure, with no drying system. There were three tiers of cages stocked at 625 cm^2/bird. The powered ventilation used a negative pressure, side inlet, ridge extraction system. Excreta produced by the birds were collected for approximately 20 h on metal trays placed on the collection belt. The sample was mixed and 40 representative samples of 500 g were taken. Each sample was mixed and one of the 4 carbohydrates incorporated and then placed on a plastic tray to an average depth of 1 cm. No carbohydrates were added to the control treatment samples. Each treatment was replicated 8 times. The samples were stored in a single environmental chamber (314 m^3) for 7 d at 21°C (±2°C). This chamber did not have continuous relative humidity measurement or control, but good air movement characteristics gave no appreciable variation in humidity around the chamber.

Representative samples that included the whole vertical section of the stored sample were taken at the beginning and end of the experiment and stored at −20°C. Complete vertical sections allowed the non-homogeneous stored manure to be sampled evenly. Samples were thawed and analysed for dry matter (AOAC, 1990), Kjeldahl nitrogen content (Persson, 1996) and pH as described by Pratt *et al*. (2002). Subsamples were freeze dried and analysed for total carbon and sulphur content (Leco SC-144DR, Leco Corporation, St Joseph, MI), electrical conductivity (solution as for pH) and microbial adenoside-triphosphate (ATP) (Luminoskan TL Plus and Quantitative ATP Monitoring kit and ATP Releasing Agent, ThermoLabsystems, Vantaa, Finland). The microbial ATP content was used to calculate the total biomass assuming 1×10^{-15} g microbial ATP corresponds to a single bacterial cell (Lundin, 1999). The changes in the measured variables were compared by a randomised block (position in chamber as blocking factor) analysis of variance with the differences from the control treatment compared by a protected least significant difference test (Snedecor and Cochran, 1989). Any missing data values, in this or subsequent experiments, were estimated by an iterative approach using the method of Healy and Westamacott (1956) and the number of degrees of freedom reduced accordingly.

Experiment 2

The objective was to test the nitrogen loss from stored laying hen manure when one of three carbohydrate sources (sucrose, maltose and starch plus α-amylase (E.C. Number 3.2.1.1.) (1.6 g in 80 ml distilled water, 1.16×10^3 activity units per ml) were incorporated at 8 g/kg. Maltose was selected as a second disaccharide and because it is a major breakdown product of starch hydrolysis. The starch plus α-amylase treatment was selected as a less expensive means of providing disaccharides. A fourth control treatment (no carbohydrate addition) was also included in the experiment.

A single commercial flock of 60 000 caged laying hens were fed a nutritionally-complete wheat and soya-bean meal-based laying hen feed and kept in a controlled environment house. The system used a scraper to move manure to a pit below the cage area. There was no manure drying system. There were 5 tiers of cages stocked at 625 cm^2/bird. Powered ventilation was by positive pressure, ridge inlet, side (of deep-pit) outlet. Approximately 100 kg of freshly produced excreta were collected on metal trays placed on the integral collection boards from the birds over 2 d. The manure was mixed and 2.0-kg aliquots were weighed, mixed with one of the three carbohydrates and then placed on a plastic lined, metal tray to a depth of approximately 2·5 cm. The starch and α-amylase treatment involved adding 40 ml of additional water per kg of manure and this decreased the dry matter content of the starting manure sample. Starch and the α-amylase solution were only combined when mixed into the manure. No carbohydrates were added to the control treatment samples. Each treatment was replicated 12 times. The samples were stored for 7 d in one of 4 environmentally-controlled storage chambers (22 m^3, with insulated concrete floors) that were all kept at 21°C (±1°C) and a relative humidity of 73% (±2%). Ventilation rate, relative humidity and ambient internal and external air temperatures were continuously monitored with data-logging equipment (Farmex, Reading, UK). Four storage chambers were used in order that the large number of samples could all be kept at the same temperature and with the same air movement patterns.

Representative samples that included the whole vertical section of the stored sample were taken at the beginning and end of the experiment and freeze-dried. Laboratory analyses were as described for experiment 1 except that total nitrogen was measured (Leco FP-528, Leco Corporation, St Joseph, MI) instead of Kjeldahl nitrogen. Experiments 2 and 3 used a different nitrogen analysis technique from experiment 1. Samples from experiment 2 were analysed using both techniques and there was no significant difference in the determined results. The Leco nitrogen analyser measures total nitrogen by a combustion method, whilst Kjeldahl nitrogen is a measure of organically bound nitrogen and ammonia. Kjeldahl nitrogen does not measure nitrate or nitrite, but these are not normally present in un-stored manure (Kirchmann and Witter, 1989; Groot Koerkamp, 1994). Water activity was determined using a chilled mirror, dewpoint system (Decagon, Aqualab series 3 supplied by Labcell Ltd, Alton, Hampshire, UK). The changes in the measured variables were compared by a randomised block (room as blocking factor) analysis of variance with the differences from the control treatment compared by a protected least significant difference test (Snedecor and Cochran, 1989).

Experiment 3

The objective of this experiment was to determine the effect on nitrogen loss from stored laying hen manure when different concentrations of sucrose were incorporated at 0, 5, 10, 20, 35, and 50 g/kg.

A single flock of 1400 commercial caged laying hens were fed a practical,

nutritionally-complete wheat and soya-bean meal-based feed and kept in a controlled environment house, as described for experiment 1. Approximately 100 kg of freshly-produced manure was collected directly on the collection belt over 2 d. The manure was mixed and then the different amounts of sucrose were added to replicate 2·0 kg manure samples (6 treatments replicated 8 times). The samples were placed on to plastic lined metal trays to a depth of approximately 2·5 cm and stored in one of 4 environmental chambers for 7 d as described for experiment 2. Representative samples that included the whole vertical section of the stored sample were taken at the beginning and end of the experiment and freeze-dried. Analysis of the samples was as described for experiment 2. The changes in the measured variables were compared using a randomised block (room as blocking factor) analysis of variance with the treatment effects partitioned into their linear and non-linear effects (Mead *et al*., 1993).

RESULTS AND DISCUSSION

The initial manure compositions used in the three experiments (Table 1) were similar to those found by other authors (Nicholson *et al*., 1996). The mean losses of nitrogen in the three experiments were 520, 300, and 240 g/kg, respectively, and were in the same range as the losses measured by Williams *et al*. (1999) and Rotz (2004). The greater nitrogen losses in experiment 1 than in experiments 2 and 3 were probably a consequence of the shorter storage times prior to the start of the experimental storage periods and the smaller sample sizes that were stored. The smaller sample sizes used in experiment 1 had higher surface area to volume ratios, which may have allowed a greater volatilisation. The slight differences in initial dry matters between the three experiments probably had little effect on the overall nitrogen loss, and water activity may have been a more critical factor. The water activities of all the initial samples were in excess of 0·9 which is well above the level needed for bacterial growth (Griffin, 1981). The initial bacteria numbers measured ($1–10 \times 10^4$) were much lower than that reported by Schefferle (1965, 88×10^7) and Halbrook *et al*. (1951, $1–100 \times 10^6$). These authors examined fresh manure whereas the manure in the present study was freeze-dried, so some proportionate loss of viable microbial numbers was expected. The initial bacterial number measured for experiment 1 was higher than for experiments 2 and 3, (probably due to different handling methods of manure samples during analysis) therefore comparison between experiments is not valid.

Table 1. *The mean characteristics of the laying hen manure at the start of the experimental periods*

Characteristic	Experiment 1	Experiment 2	Experiment 3
Dry matter (g/kg)	265·7	302·4	290·4
Nitrogen (g/kg)	13·46	17·90	11·99
Carbon (g/kg)	101·5	104·6	106·6
Sulphur (g/kg)	0·693	0·512	0·628
pH	6·12	6·88	7·07
Electrical conductivity (mS)	2·05	2·59	1·93
Bacterial numbers (no./g)	$10·91 \times 10^4$	$0·84 \times 10^4$	$1·18 \times 10^4$
Water activity	–	0·978	0·974

In experiment 1, addition of sucrose to the manure resulted in a greater increase in bacterial numbers ($P<0·05$), a greater ($P<0·05$) loss of carbon and a trend ($P>0·05$) for reduced loss of nitrogen (Table 2). Addition of starch to the manure gave similar results to sucrose addition, except that bacterial numbers only tended ($P>0·05$) to be greater than the control and carbon loss was not different ($P>0·05$) from the control. However, the addition of glucose to the manure increased ($P<0·05$) nitrogen loss and bacterial numbers and tended ($P>0·05$) to increase

Table 2. *The effect of carbohydrate additions on the changing composition and characteristics of laying hen manure over 7 d of storage (Experiment 1)*

	Water loss (g/kg)	Nitrogen Loss (g/kg)[1]	Carbon loss (g/kg)[1]	Sulphur loss (g/kg)[1]	pH Increase	E.C.[2] Increase (mS)	acterial number increase per g ($\times 10^5$)
Glucose	298*	9·42*	31·3	0·165	1·89*	0·10	12·95*
Sucrose	229	5·73	36·0*	0·150	2·23*	0·27	16·10*
Starch	216	5·58	25·0	0·113	1·83*	0·13	11·45
Straw	86	7·33	23·9	0·103	1·87*	0·04	6·7
Control	176	7·07	23·5	0·102	1·38	0·07	6·18
S.E.M.	35·8	0·776	2·85	0·0356	0·135	0·087	2·305
L.S.D.	103·8	2·289	8·39	0·1031	0·392	0·252	6·677
Statistical significance	$P<0·01$	$P<0·05$	$P<0·05$	N.S.	$P<0·01$	N.S.	$P<0·05$

[1]Data expressed per initial kg of dry matter.
[2]Electrical conductivity.
*Treatment mean significantly different from control treatment ($P<0·05$).

carbon loss relative to the control. Addition of straw to the manure did not affect ($P > 0{\cdot}05$) the change in bacterial numbers or rate of nitrogen loss compared to the control treatment.

The results indicate that some carbohydrate additions to laying hen manure may alter the rapid loss of gaseous nitrogen from newly-stored manure. These differences may be related to the growth of the bacterial population (Miner *et al*., 2000) and how this synchronises with the availability of nitrogenous compounds within the manure. Nitrogen retention within stored manure is increased when microbial biomass formation is maximised (Atkinson *et al*., 1996), but this formation depends not only on an available source of nitrogen but also on a source of available energy being simultaneously available. Laying hen manure has a high nitrogen content but, because hens are fed highly-digestible diets, it has a low available carbohydrate content. The addition of sucrose to the manure probably gave a suitably available source of energy for the microbes to use and so they were able to proliferate. Surprisingly, the addition of glucose gave an increase ($P < 0{\cdot}05$) in nitrogen loss. Microbial numbers were increased ($P < 0{\cdot}05$) with this treatment and there tended ($P > 0{\cdot}05$) to be an increased carbon loss. The glucose may have been metabolised too rapidly by the microbes and so this source of energy may not have synchronised with the ability of the microbes to incorporate nitrogen into biomass. The straw incorporation had no effect on nitrogen loss or microbial numbers. Bacterial degradation of the high cellulose content of straw is slow and probably did not occur fast enough to release a significant amount of available energy within the short storage period.

In experiment 2, the results showed that there was an increase ($P < 0{\cdot}05$) in bacterial numbers and a reduced nitrogen loss ($P < 0{\cdot}05$) when either sucrose or maltose was mixed with stored laying hen manure (Table 3). The starch plus α-amylase treatment did not give any differences ($P > 0{\cdot}05$) in the storage characteristics of the manure. This may be as a result of the additional water supplied when adding the enzyme (Table 3). Reece *et al*. (1979) and Parkhurst *et al*. (1974) examined manure additives and attributed increased nitrogen losses in some treatments to them being in aqueous form.

In experiment 3, the increasing sucrose additions gave a non-linear reduction in nitrogen loss ($P < 0{\cdot}01$, Table 4). The lowest rate of nitrogen loss was obtained with 35 g/kg sucrose although the maximum increase in bacterial numbers was obtained at 20 g/kg of added sucrose. Increasing sucrose gave linear changes in the rate of change of pH ($P < 0{\cdot}001$) and the level of water activity ($P < 0{\cdot}01$) in the manure samples over the 7-d storage period.

In conclusion, the results of these experiments have shown that when disaccharides were mixed with freshly-produced laying manure bacterial numbers were increased and nitrogen loss to the atmosphere (most likely in the form of ammonia) was decreased. The effects of sucrose addition were consistent over three separate experiments. The rate of sucrose addition was important and 35 g/kg of added sucrose minimised nitrogen loss by 80% over the 7-d storage period. This compares favourably to additives such Yucca saponin that frequently do not give statistically-significant effects, though a number of studies have reported reductions in ammonia loss of 30–40% (Davidson, 1991). In practical situations, sucrose could be mixed with the manure on its removal from the house prior to further storage.

Sucrose is an expensive ingredient to add to manure solely to decrease ammonia loss and its potential commercial viability would depend upon whether financial penalties start to be

Table 3. *The effect of carbohydrate additions on the changing composition and characteristics of laying hen manure over 7 d of storage (Experiment 2)*

	Water loss (g/kg)	Nitrogen loss (g/kg)[1]	Carbon loss (g/kg)[1]	Sulphur loss (g/kg)[1,2]	pH increase	E.C. Increase (mS)	Bacterial number increase per g($\times 10^4$)	Water activity decrease ($\times 10^{-5}$)[3]
Sucrose	155	3·73*	17·5	0·074	0·91*	0·174	10·32*	5·9
Maltose	154	3·85*	15·3	−0·009	1·06	0·266	10·37*	5·1
Starch and -amylase	143	7·05	18·6	0·092	1·28	0·178	4·99	0·9
Control	141	6·65	19·6	0·056	1·31	0·064	3·79	2·6
S.E.M.	9·0	0·738	1·83	0·0335	0·107	0·0763	1·361	1·29
L.S.D.	25·6	2·109	5·242	0·0958	0·306	0·2192	3·886	3·68
Statistical significance	N.S.	$P < 0{\cdot}01$	N.S.	N.S.	$P < 0{\cdot}05$	N.S.	$P < 0{\cdot}01$	$P < 0{\cdot}05$

[1]Data expressed per initial kg of dry matter.
[2]Negative loss represents a measured increase in the variable.
[3]Water activity (A_w) = Equilibrium relative humidity/100.
*Treatment mean significantly different from control treatment ($P < 0{\cdot}05$).

Table 4. *The effect of added sucrose on the changing composition and characteristics of laying hen manure over 7 d of storage (Experiment 3)*

Added Sucrose (g/kg)	Water loss (g/kg)	Nitrogen loss (g/kg)[1]	Carbon loss (g/kg)[1]	Sulphur loss (g/kg)[1]	pH Increase[2]	E.C. Increase[2] (mS)	Total bacterial increase per g ($\times 10^5$)	Water activity decrease ($\times 10^{-3}$)[3]
0	144	5·06	19·6	0·154	1·066	−0·007	3·80	8·5
5	151	3·83	15·3	0·063	0·920	−0·064	7·90	6·1
10	172	3·29	16·3	0·008	0·697	0·002	11·78	1·9
20	169	2·30	16·8	0·005	0·340	0·095	12·17	3·4
35	144	0·99	13·8	0·031	−0·138	0·331	10·46	0·9
50	136	1·50	16·6	0·021	−0·208	0·423	10·04	−2·1
S.E.M.	14·9	0·523	3·20	0·0485	0·1523	0·0637	1·910	2·49
Statistical significance								
Linear	N.S.	$P<0·001$	N.S.	N.S.	$P<0·001$	$P<0·001$	N.S.	$P<0·01$
Quadratic	N.S.	$P<0·01$	N.S.	N.S.	N.S.	N.S.	$P<0·05$	N.S.
Cubic	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Deviations	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[1]Data expressed per initial kg of dry matter.
[2]Negative increase represents a decrease in the variable.
[3]Water activity (A_w) = Equilibrium relative humidity/100.

levied to poultry producers for environmental pollution. Carbohydrates are a non-toxic and non-polluting ingredient that could have a potential use in reducing gaseous nitrogen emissions. A carbohydrate additive would also have to suppress the long-term emission of gaseous nitrogen to the atmosphere from stored manure so would probably use a mixture of ingredients including cheaper, but less available, types of carbohydrates. The present study has demonstrated that disaccharides could be used in these products to suppress the early peak of ammonia loss from stored manure.

ACKNOWLEDGEMENTS

We wish to thank Mrs Jan Pemberton for her invaluable advice and technical support throughout this work.

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