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Causes of Nitrogen Loss during Animal Manure Analysis

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가축분의 정량과정에서 생기는 질소 손실에 대한 여러 원인

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ABSTRACT : Since nitrogen(N) is a volatile compound affected by many environmental factors, determining the N content of manure tends to be difficult. Upon arrival in the laboratory, the manure should be moist and refrigerated. Manure samples will have variable N contents due to drying temperature, and the presence of soil in the sample will affect N content. Acidification of the sample prevents ammonia volatilization and should be done before drying. It is recommended that manure samples be pretreated with a strong oxidizing agent, $KMnO_4$, followed by digestion under reduced conditions(reduced $Fe-H_2SO_4$), which achieves a complete recovery of both NO_3-N and NO_2-N, without a low recovery of NH_4-N, resulting in a more accurate determination of N content. Accuracy of results for N content determined by recently developed rapid analysis techniques in the field should be tested by comparison with results obtained at laboratories using approved standard methods. Most commonly, the Kjeldahl system is used to determine manure N content. More research is needed on the effects of species, breed, age and individuals on the nutrient contents of manure. The procedures for manure sampling on the farm, shipping and handling of the sample until it reaches the laboratory, and the methods of sampling of the manure at the laboratory must be studied. Development of animal agricultural laboratories where feed, manure, soil, and water are all analyzed by appropriate specialists is needed.

(Key words: animal agricultural laboratory, digestion, Kjeldahl system, manure, nitrogen)

INTRODUCTION

Poultry litter and manures, or livestock manures and urine (hereafter referred to as animal manure or livestock manure) used as feed sources (Ko and Ahn 1987 ; Kim et al., 1993) and added to both pastures and crops as sources of plant nutrients (Kirchmann, 1985 ; Sutton et al., 1987) can also contribute to the pollution of the natural environment by volatilization of ammonia and leaching of nitrate (Pratt et al., 1976). The value of animal manure as a feed or a fertilizer is usually based on its macronutrient content, especially nitrogen(N) (Dou et al., 1996). In most of the European countries, the guidelines on the number of animals maintained per unit area are based on the amount of N produced in the manure (Faassen and Dijk, 1987).

With increasing emphasis on accurate feeding or agronomic land application of nutrients from animal manures, greater demands are being placed on the analytical processes associated with animal manures' characterization. Laboratories typically oven-dry manure prior to N analysis are not accounting for ammonia loss during the process (Nahm, 1992). Sci-

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entists (Kirchmann, 1985 ; Wood and Hall, 1991) reported that significant amounts of N in animal manures can be in volatile forms, making it difficult to quantity the total N content leading to considerable errors in mass balances. Silage or other types of feeds which contain animal manures or animal manures for fertilizers will likely be fed to livestock animal or land applied in the form in which they are sampled. Therefore, it is critical to use values in the report from Laboratories that are based on the "As Received"basis (South Dakota Cooperative Extension Service, 1999).

Animal manures have physiological properties suitable for rapid microbiological growth (Enwezor, 1976), ammonification and loss of N via NH_3 volatilization (Nodar et al., 1990). Because nitrification in animal manure is inhibited due to high biological oxygen demand and limited O_2 availability (Nodar et al., 1990), NH_3 volatilization is enhanced because the NH_3 gas-liquid equilibrium is proportionally related to the concentration of NH_3 in solution and the partial pressure of NH_3 gas in the atmosphere (Witter and Lopez-Real, 1987).

The following section includes information on factors that affect the N content of animal manure as determined by laboratory analysis in order to enable laboratories to determine manure N content as accurately as possible.

FACTORS AFFECTING N CONTENT DURING LABORATORY ANALYSIS

1. Drying Temperature

Dry determination by drying at 105℃ is one of the most widespread methods used in the characterization of manure and loss of volatile compounds during dry matter determination using the mass-balance may lead to considerable error (Derikx et al., 1994). Mass balance has been used in most laboratories, especially in the large-scale manure treatments plants.

To determine the application rates necessary for optimum crop growth and minimal nitrate leaching, estimates of the N content in livestock manure are necessary to protect available N in livestock manure (Bitzer and Sims, 1988).

Parker et al (1959) reported N losses during processing of 11.6 and 17.0% in broiler and layer manure, respectively. Wood and Hall (1991) compared various drying methods and reported N loss during drying ranged from 12 to 15%. Research results of Gale et al.(1991) indicated that oven drying reduced estimates of total N from 5.65 to 4.01% in wet and dry manure, respectively. They showed that from a practical standpoint, the microwave drying at 40℃ might be a more desirable alternative than no drying, or air drying for 10 days, or drying at 40℃ in a forced air laboratory oven for 3days, or drying at 60℃ in a forced air laboratory oven for 3days, drying at 60℃ in a micro-wave oven for 20 minutes. Studies (Parker et al., 1959 ; Tinsley and Nowakowski, 1959) also showed that drying in forced air ovens at temperature near 80℃ decreased the content of total N in poultry manure by much as 17%. These results also show that the ammonia is lost during normal dry-matter determination of untreated manure originating from pigs, cattle, or poultry.

The N content in poultry manure can decrease from the time the manure is excreted to the time of application which influences the manure application rate recommended (Overcash et al., 1975). Giddens and Rao (1975) reported that poultry manure lost 47.6% of the total N upon air-drying for 10 days but only 23.6% after drying in a boiling water bath. They suggested that the difference in N lost is that heat killed the uric acid hydrolyzing microorganisms or that loss of moisture by heating resulted in greater conservation of N than air-drying. It was observed that slow drying of animal manures at low temperature caused greater losses of N than fast drying at higher temperatures (Giddens and Rao, 1975 ; Adriano et al., 1974). The difference in N lost between air dried and oven dried samples suggests that uric acid and urea hydrolyzing microorganisms or extarcellular hydrolases are killed or denatured at higher temperature or that the rapid loss of moisture at high temperature resulted in greater conservation of N than with air drying. Freez-

ing followed by freeze drying inactivates microorganisms and results in loss through decomposition of uric acid (Schefferle, 1965). Research results (Mahimairaja et al., 1990) suggest that the major reduction in total N during drying may be due to the loss the volatile N compounds such as ammonia and amines. This may lead to considerable errors of the dry-matter determination in using mass-balance because an unknown part of the volatile substances is included in the dry-matter determination and attention is focused on ammonia and volatile fatty acid in dry matter results (Derikx et al., 1994).

Total N loss (%) in fresh manures by the modified Kjeldahl method after electric oven drying is presented in Table 1. The magnitude of N losses is a function of drying temperature (Robinson and Sharpley, 1995), drying method of analysis (Overcash et al, 1975) and drying time length (Chao and Kroontje, 1964 ; Westerman et al., 1983). The effects of drying method and length also results in different N values in Table 1. Research results (Wood and Hall, 1991 ; Mahimairaja et al., 1990) demonstrated that maintaining broiler litter in a moist, refrigerated state before chemical analyses was more desirable than drying.

Table 1. Total N loss rate(%) of animal manure as affected by oven drying and different lengths

Types of manure	Total N loss rate time	Oven temperature and drying
Poultry manure	8	
Pig slurry	7	105℃ for 48hrs[1]
Dairy slurry	3	
Horse manure	0	
Sheep manure	4	
Broiler litter		60℃ for 3days[2]
(Hardwood shavings)	12	
Hen manure	5.6 ~ 4.0	66℃ for 48hrs[3]
Broiler manure	11.6	78℃ for 10 hrs[4]
Hen manure	17	

1. Mahimanraja et al. (1990).
2. Woo and Hall (1991).
3. Gale et al. (1991).
4. Parker et al. (1959).

2. Animal Manure Mixed with Soil

Microbial and chemical changes in animal manure as affected by contact with soil have been studied. Manure and litter mixed soil contained greater numbers of bacteria and fungi than manure alone, and total coliform bacteria decreased more rapidly when manure was mixed with soil than when not mixed (Giddens and Rao, 1975). Incorporation of manure into the soil greatly increased the amount of N oxidized to NO_3, and air drying of manure in soil resulted in greater N loss than rapid drying with heat. Litter samples with soil also contain more bacterial counts than fresh poultry droppings (Schefferle, 1965 ; Hallbrook et al., 1951).

Ammonia formation, presumed to be from uric acid, was dependent upon the activity of microorganisms and temperature and humidity of the litter and soil (Ivos et al., 1966), and ammonia emissions in soil manure during composting of animal manure can be significant and decrease the fertilizer value of manure (Kithome et al., 1999).

3. Effect of pH on the N Loss from animal Manure

Acidification of manure is an effective means of reducing volatilization of ammonia from manure during storage, application or drying (Frost et al., 1990 ; ten Have, 1993). Since the behavior of most volatile compounds depends upon pH, these losses may vary sample to sample. It was shown that above pH 8 all ammonia was volatilized and below pH 5 all volatile fatty acids evaporated in pig, cattle and poultry manure (Derikx et al., 1994). They found that total fixation of ammonia was achieved below pH 4, and above pH 10 all volatile fatty acids were fixed in the residue after drying. It has been shown that the pH of broiler litter influences ammonia levels in poultry facilities (Reece et al., 1979). At a litter pH below 7, ammonia release was negligible. As the pH became closer to 7, the ammonia began to be released. To summarize, as the pH of the litter decreases, the ammonia loss also

decreases.

Loss of volatile substances from manure is not a constant factor. Considerable error may result in the laboratory since an unknown part of the volatile substances is included in results of the dry matter determination (Derikx et al., 1994). It is believed that control of ammonia loss during drying processes may provide a more accurate measure of litter N and therefore allow more accurate application of the litter in practical agricultural settings.

Burgess et al. (1998) reported that treatment (small - 10g, large - 100g) of litter samples with $Al_2(SO_4)_3$ (ammonium sulfate) prior to drying resulted in more accurate quantification of N in litter, which can ultimately result in more accurate utilization of litter in agronomic application. Ammonium sulfate and numerous other acidic compounds are also effective in lowering litter pH and reducing ammonia volatilization in commercial broiler houses (Moore et al., 1995). In another report, Moore, Jr. et al. (2000) indicated that ammonium sulfate applications lowered the litter pH, particularly during the first 3 to 4 weeks of each grow out. Reduction in litter pH resulted in less NH_3 volatilization, which led to reduction in atmosphere NH_3 in the ammonium sulfate-treated house.

4. N Loss due to Using Different Chemicals in Processing

Animal manures vary in their total N contents and N forms because of differences in feed, feed conversion by different animal species age of animal bedding materials and water intake (Ministry of Agriculture, Fisheries and Food). The total N content in animal manure may be difficult to quantify since significant amounts of N may be in volatile forms (Kirchmann, 1985).

Kjeldahl digestion is generally used to determine the total N in organic materials such as soils, plant materials and animal manures (Nahm, 1992 ; Bremner and Yeomans, 1988 ; Nahm, 1989)and this is usually referred to as total Kjeldahl N. The organic ammoniacal N makes up most of the Kjeldahl N and measurement of the inorganic forms of N may be done after extraction with 2M KCl solution (Bremner and Mulvaney, 1982). The total N may be calculated by adding the Kjeldahl N to the nitrate $(NO_3)N$ and nitrite $(NO_2)N$ in the KCl extract (Bitzer and Sims, 1988 ; Sims, 1986 ; Tyson and Cabrera., 1993). Chadwick et al.(2000) reported that the organic N fraction varied between manure types and represented from 14% to 99% of the total N content after refluxing 40g (fresh weight)of slurry or manure with 200ml of 2M KCl for 4 hour following the method of Gianello and Bremmer (1986) for organic N and Kjedahl digestion for total N.

Total N may be measured alternatively by pretreating samples with oxidizing and reducing agents to include NO_3-N and NO_2-N during the Kjeldahl digestion. Scientists (Bremner and Yeomans, 1988 ; Haynes, 1980) have studied the effect of some of these oxidizing and reducing agents on NO_3-N and NO_2-N recovery. Mahimairaja et al.(1990) compared these methods for the measurement of Total N in organic materials. They measured total N, Kjeldahl N using a standard micro-Kjeldahl digestion technique (Bremner and Mulvaney, 1982) and inorganic forms of N (NH_4, NO_3 and NO_2) in poultry and animal manures. The pretreatments prior to Kjeldahl digestion for recovering NO_2-N and NO_3-N were: 1) Salicylic acid - thiosulfate (Bremner and Mulvaney, 1982), 2) Aqueous $NO_2S_2O_3$(Dalal et al., 1984), 3) Devard's alloy and H_2SO_4(Liao, 1981), 4) Zinc and acidified (H_2SO_4) solution of $CrK(SO_4)_2$(Pruden et al., 1985), 5) Hydrogen peroxide(H_2O_2) + acidified (H_2SO_4) reduced Fe (Mahimairaja et al., 1990), 6) Alkaline sodium hydrochlorite(NaOCl) + acidified (H_2SO_4) reduced Fe (Mahimairaja et al., 1990), 7) Potassium Permanganate $(KMnO_4)$ + acidified (H_2SO_4) reduced Fe (Bremner and Mulvaney, 1982). Mahimairaja et al.(1990) indicated that the Permanganate method has been found suitable for the analysis of total N and inorganic forms of N in soils, sediments and plant samples. They said that pre-treatment with a strong oxidizing agent, $KMnO_4$, followed by digestion under reduced conditions (reduced Fe-H_2SO_4), achieved a complete recovery of both NO_3-N and NO_2-N without causing a low recovery of NH_4-N (Bremner and Mul-

vaney, 1982).

Since Kjeldahl introduced a method for determining total N, many studies have been made to improve accuracy, shorten digestion time, and improve recovery of N from a wide range of samples with a modification of the conventional Kjeldahl method (Haynes, 1980 ; Dalal et al., 1984 ; Liao, 1981 ; Goh, 1972). However, there is still some disagreement about the effectiveness of these methods in recovering NO_3-N and NO_2-N, as well as other problems with the method (Mahimairaja et al., 1990).

5. N Loss due to in Rapid Analysis and Automated Techniques

For determining the nutrient contents of some animal manures such as dairy and swine slurries, taking samples and sending them to a laboratory may not be the easiest or most accurate method. When slurry is stored, stratification and crusting may make representative sampling questionable without complete agitation. To improve the farmer's ability to properly land apply livestock slurries, a rapid field test to estimate nutrient contents on site immediately prior to application would be useful.

The relationship between N and total solid (TS) has been discussed widely in literaure. Specific gravity (SG) measured with a soil hydrometer correlated well with TS content of slurries and a quadratic relationship with TS for N has been demonstrated for swine and cattle slurries (Tunney, 1979). The slopes of the linear regression equations for N and TS for cattle and swine slurries were found to be different (Chescheir et al., 1986).

Stewart (1968) utilized a water analysist kit which determined NH_3-N content to analyze weak acid extracts of swine slurries in the field. When compared to estimating soluble N through weak acid extraction using Kjeldahl digestion and steam distillation of the extract, the results were very similar (Hoyle and Mattingly, 1954).A device called the "Nitrogen Meter" for estimating nitrogen in manure was introduced in Sweden (AGROS, Ovagen l, S-5333 03 Kallby, Sweden) in 1983. The device consists of a stainless steel reaction chamber with a pressure gauge. In order to oxidize the ammonia to N gas(N_2), the manure is mixed with a strong oxidizing agent (calcium hypochlorite, $CACOCl_2$, 30 ~ 37% available chloride). Oxidation of urea also occurs, but its extent depends on the pH according to the developer of this device. The increase in pressure due to formation of N gas is measured by the pressure gauge and is calibrated to units of N per unit of manure volume.

The Auto-Analyzer which was originally designed for the purpose of clinical chemistry has been adapted to soil analysis (Flannery and Steckel, 1964). Improvements in automated techniques were evaluated by Flannery and Markus (1980). This new analyzing system was critically evaluated by Kane et al., (1981). Markus et al., (1985) introduced the microprocessor-based Auto-Analyzer for analyzing NO_2-N, NO_3-N and NH_4-N in soils. They reported that this new system provided improvement in accuracy and precision of results and has the potential for more rapid chemical determination. For the measurement of total N, they used salicylic acid - thiosulfate ($NO_2S_2O_3$) for pretreatment to recover NO_2-N and NO_3-N in a semimicro-Kjeldahl procedure (Bremner and Mulvaney 1982). These semimicro-Kjeldahl procedures were used by Robinson and Sharpley (1995) for measuring the content of NO_3-N and NH_4-N with colorimeter, and the content of NO_2-N was measured using indophenol blue procedure after being reduced with Cd (Keeney and Nelson, 1982).

To summarize, there have been several new, quick methods developed to measure the N content of manure. It has been reported that these methods should not replace regular laboratory analysis by standard methods (Chescheir et al., 1986). These methods are best used to improve land application rates since they provide a rapid indication in changes in manure slurries when storage facilities are unloaded or they estimate some nutrients when it is not possible to use laboratory analysis.

6. Future Research on N Loss

There are still inadequacies in our knowledge about

the nutrient contents of the manure of each species along with analytical techniques for determining these nutrients, especially the N content. The total N content and N forms vary in animal manures due to differences in feed, feed conversion by different animal species, age of animal, bedding material and water intake (Ministry of Agriculture, Fisheries and Food). For example, urea in bovine uric reacts rapidly with urease in the feces which results in a high pH and loss of NH_3 gas (Lauer et al., 1976) and The total N content of fresh manures varies as follows: poultry>pig>horse = sheep. Whereas poultry manure, pig slurry and dairy slurry samples were composed of both feces and urine (or uric acid), horse and sheep manure samples were composed mainly of feces. This may be one of the reasons for the higher content of total N in the former than in the latter (Mahimairaja et al., 1990). It has been shown that most of the N in fresh animal manure is present mainly as urea or uric acid (Azevedo and Stout, 1974 ; Krogdahl and Dalsgard, 1981). Further research should be done for determining the nutrient content of manure depending on different animal species, age of animal, bedding material, water intake and other factors in the future.

Pretreatment steps that are important in the results of analysis include sampling, sample handling and sample management before analysis. Representative sampling will be very questionable unless there is complete agitation of the manure and slurry storage area. Samples should be transported to the laboratory as soon as possible in proper containers and kept at temperatures below 0℃ (Nahm, 1992). Upon arrival at the laboratory, a portion at the sample should be set aside immediately in case the sample must be analyzed again and the rest of sample must be reduced to a proper size that adequately represents the content of the total sample. there are few reports, however, on proper manure sample pretreatment for each type of manure.

There are two factors that can easily be overlooked by scientists and farmers (Nahm, 1995): 1) very few livestock farmers have any idea which is the best laboratory to use to analyze their samples of animal feed, manure, litter, soil or water and 2)these laboratories must be able to interpret the results for the farmers. It would be beneficial if all of these analytical procedures were handled in one laboratory specializing in animal agriculture with spcialists advising farmers on how best to apply the analytical data (Nahm, 2000).

CONCLUSION

There are many factors to consider when determining the N content of manure. The N content of manure is affected by the drying temperature and length of time, the presence of soil in the sample, the pH of the manure, the chemicals used in the analysis and the use of rapid or automated analytical techniques. N is an important component of manure used as fertilizer or feed, but its volatility makes it an environmental concern as well. Further research is needed on how the N content of manure may be affected by animal breed and age, season and feed or water sources. Appropriate management of the sample from sampling on the farm to analysis in the laboratory should be developed. Establishment of animal agricultural laboratories where feed, manure, soil, water are analyzed in one laboratory and qualified specialists then advise farmers should be a priority in the future research.

적 요

질소(N)는 그 자체가 환경적인 요인에 따라 영향을 받는 휘발성 물질이어서 가축의 분뇨(분에는 반드시 뇨가 묻어 있기 때문에 다음부터는 분이라는 말로 대치한다)중에 함유되어 있는 N의 함량을 정량하기란 무척 어렵다. 우선 가축의 분이 실험실에 도착하면 수분이 있는 상태에서 냉장고에 보관되어야 한다. 분중에 함유되어 있는 N는 건조온도에 따라 함량에 변화를 일으키며 또 시료에 흙이 묻어 있는가에 따라서도 N의 함량 정량에 영향을 받는다. 시료를 산성화 하면 암모니아의 휘발이 방해된다. 따라서 시료를 산성화시킬 때는 건조전에 이루어져야 한다. 분 분석을 위한 이상적인 분 전처리는 강산화제 ($KMnO_4$)로 우선 처리 한후 다시 환원제($Fe-H_2SO_4$)를 처리한 상태에서 시료

를 소화시키면 가장 정량하기 어려운 NH_4-N도 같이 정량이 가능하며 질소의 전체정량이 정확히 이루어질 수 있다. 질소정량의 정확성이 야외에서 이루어질 수 있도록 최근 여러 가지의 약식분석방법이 개발되고 있지만 그 결과는 반드시 공인된 분석방법에 의하여 실험실에서 분석된 결과치와 비교되어야 한다. 실험실에서 일반적으로 질소 정량에 많이 이용되고 있는 방법은 켈달 분석방법이다. 앞으로 가축의 종류나 품종, 나이 또는 개체간에 대한 분의 영양소 함량에 관한 연구가 많이 이루어져야 한다. 또 분에 대한 농장에서의 시료 채취과정, 운반, 및 실험실에 도착한 후 처리 과정 그 다음 실험실에서 분석을 위한 시료채취 과정 등에 대한 연구도 이루어져야 한다. 사료, 분뇨, 토양, 그리고 물을 함께 분석하는 동물농업의 발전을 필요하며 적당한 전문가의 이용이 필요하다.

(색인어 : 가축실험실, 소화, Kjeldahl 시스템, 가축분, 질소)

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