Effect of the method of drying on the concentration of sialic acid in poultry excreta

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Abstract

The aim of the study was to investigate the effect of drying method (freeze or oven drying) on the concentration of sialic acid in poultry excreta. Oven dried samples had lower sialic acid concentration compared to freeze dried samples. There was a positive relationship ($r^2 = 0.75$; $P<0.05$) between the sialic acid in both, freeze and oven dried samples. However, the conclusion is that the freeze drying is the better method to prepare excreta samples for sialic acid analysis.

Introduction

Sialic acid is a nine-carbon carboxylated sugar molecule involved in the structure of complex carbohydrates including glycoproteins, glycolipids and keratan sulphate proteoglycan (Nakano et al., 1994). There are many different types of sialic acids spread in all species of vertebrates. The most widely distributed sialic acid is N-acetylneuraminic acid and it is believed to be a biosynthetic precursor of all other types sialic acid (Varki, 1992). Sialic acid is involved in many physiological functions and contributes to cell adhesion, enzyme inhibition, hormonal action, antigenicity and synaptic transmission. Increased concentration of sialic acid is often associated with health problems such as cellular senescence, bacterial infections (e.g. campylobacter), certain pathological conditions and osmotic fragility. Sialic acid has also been used as an indicator of endogenous losses from the gastrointestinal tract of experimental animals (Rutherfurd *et al*, 2002). Recent research showed that gastrointestinal endogenous losses are involved in the mode of action of dietary phytase (Cowieson *et al*., 2004; Pirgozliev *et al.*, 2005), and can explain part of the variation in nutrients digestibility and metabolisable energy. Therefore, the determination of sialic acid in biological samples, e.g. excreta can be used as a valuable scientific tool and provide more information about metabolism and gut health of the animals. Excreta are usually dried in two ways; freeze drying and oven drying. Freeze drying requires a sophisticated and expensive facility that also needs more maintenance and special care compared with ovens. Lots of laboratories are equipped with ovens only and that is the only way to prepare excreta samples for analyses. The aim of this study was to compare the concentration of sialic acid in identical excreta samples which were either freeze or oven dried.

Materials and Methods

Two laboratory experiments were conducted to examine the effect of different drying temperature on the sialic acid concentration in poultry excreta. The first experiment involved two previously freeze-dried excreta samples. Each of the samples was separated into three sub-samples, or six in total. The first two sub-samples remained freeze-dried only, the second two were exposed to 80°C temperature for 48h, and the third two were mixed with distilled water (200 mg + 0.1 ml water in a tube) and exposed to 80°C temperature for 48h. Each sample was replicated five times. Before heat and water treatments were applied, the replicates were weighed (approx. 200mg) in glass tubes on a freeze-dried basis and final results were not corrected for dry matter. Data was analysed statistically as a two by three factorial design.

The second experiment involved five different excreta samples. All samples were mixed with water and thoroughly homogenised. The samples were split in two, or ten in total. Half were freeze-dried (minus 20°C till constant weight) and the remainder were oven dried (80°C

temperature for 48h). Each sample was replicated three times and final results were corrected for dry matter. Data was analysed as a factorial design.
In both experiments the amount of sialic acid was determined as described by Jourdian *et al*. (1971). Statistical analyses were performed using the Genstat VII statistical software package.

Results and Discussion

Although both samples had different ($P<0.001$) sialic acid concentrations, the type of treatment did not affect ($P>0.05$) the concentration of sialic acid in the first experiment (*Table 1*). In the second experiment (*Table 2*) the overall sialic acid concentration was higher ($P<0.05$) in the freeze-dried compared to oven-dried excreta samples. There was a positive relationship ($P=0.038$) between freeze dried and oven dried sialic acid concentrations as follows:

Y (freeze dried) = 0.511 + 0.513 X (oven dried) ($r^2 = 0.75$; SE of observations = 0.0396)

The above equation also suggests that the other 25% of the samples would be predicted erroneously. It is possible that high temperature associated with oven drying can affect/destroy the structure and reduces the amount of measurable sialic acid in the sample. Preparation of the samples by freeze-drying is better than oven drying to allow the estimation of sialic acid. The lack of difference in the first experiment suggests that there is a high variation between samples and probably some chemical reaction occurred during the drying processes.

Table 1. Effect of treatment on sialic acid concentration in excreta (experiment 1)

Treatment / Sample	A	B	Treatment effect
Freeze-dried+T°+water	1.15	0.75	**0.95**
Freeze-dried+T°	1.18	0.79	**0.98**
Freeze-dried only	1.13	0.73	**0.93**
Sample effect	**1.15**	**0.75**	
P<0.001, LSD = 0.070			P=0.436, LSD = 0.086

Table 2. Effect of heat treatment on sialic acid concentration in excreta (experiment 2)

Sample / Treatment	Freeze dried	Oven dried	Treatment effect
C	0.91	0.81	**0.86**
D	1.06	1.03	**1.04**
E	0.89	0.82	**0.85**
F	0.91[a]	0.69[b]	**0.80**
G	0.86[a]	0.69[b]	**0.78**
Sample effect	**0.926**	**0.808**	
P=0.026, LSD = 0.162			P=0.021, LSD = 0.103

Conclusions

Although there was a positive relationship ($P<0.05$) between sialic acid concentrations from freeze and oven dried samples, there was a high variation between samples. It can be concluded that freeze-drying of excreta is the preferred method if sialic acid determination is to be conducted.

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