Livestock Science 185 (2016) 110–116

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Preparation of synthetic alkane waxes and investigations on their suitability for application as dietary markers in farm animals

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ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form
21 January 2016
Accepted 22 January 2016

Keywords:
External markers
Synthetic alkanes
Alkane waxes
Temperature sensitivity
Bolus labelling accuracy

ABSTRACT

Synthetic alkanes can be applied as external markers to estimate faecal output, digestibility and passage kinetics and are furthermore easy to combine with plant alkanes for the estimation of feed intake. Successful application requires an accurate and uniform labelling of boluses or feedstuffs, which is in turn supported through simplified handling of the markers during preparation. In this study, it was tested whether melting of synthetic alkanes to wax is able to enhance the accuracy and uniformity of subsequent bolus labelling and further simplifies it. The preparation of alkane waxes was performed on model scale using a portion in a ratio of approximately 1:300 to a dosage, which is recommended for administration in large livestock 2 times a day. Additionally, the temperature sensitivity of a range of synthetic alkanes was studied to clarify so far non-explained losses of alkanes, which were observed frequently during the labelling of boluses and feedstuffs or the processing of samples for analysis. Using *n*-octacosane (C28), *n*-dotriacontane (C32) and *n*-hexatriacontane (C36) synthetic alkanes, three single-component waxes (of C28, C32 and C36, respectively), three binary waxes (C28:C32, C28:C36 and C32:C36) and one tertiary mixed wax (C28:C32:C36) were produced with 30 repetitions each. To assess the impact of melting and re-crystallization, the quantity of individual alkanes was determined by gas chromatographic analysis (GCA) in untreated crystals, crystalline mixtures (GCA1) and the finished model waxes (GCA2). Additionally, sub-samples of the waxes were heated for 30 min at 100 °C or freeze-dried for 48 h, respectively, to simulate baking of boluses or freeze-drying as an alternative method for preparation (GCA3). The temperature sensitivity of *n*-tetracosane (C24) to *n*-octatriacontane (C38) even-chain alkanes was studied by thermogravimetric analysis (TGA) with consistently increasing temperature (20 to maximal 600 °C at 10 K/min, TGA1) and under isothermal conditions (180 °C for 20 min, TGA2), respectively. Depending on chain length and thus molecular weight of alkanes, weight reduction by emergence of soot during heating-up started between 176 °C (C24) and 227 °C (C38) and further increased rapidly. Throughout isothermal treatment, weight loss from alkanes was lowest with highest chain length (0.0% for C38) and *vice versa* (23.8% for C24). The originally weighed and via GCA measured quantities of crystalline alkanes did not differ ($P > 0.05$), except for single C36, where the measured quantities were always higher than the weighed ones ($P = 0.019$). The weighed and measured quantities of individual alkanes in single-component and of total alkanes in multi-component waxes were similar with a maximal relative difference of $6.6 \pm 5.5\%$, given as the mean $\pm$ standard deviation among the repetitions of a sample variant. The relative difference between weighed and measured quantities of individual alkanes in multi-component waxes was maximal $47.4 \pm 25.7\%$ and was highly variable. Unexpectedly, the relative difference between weighed and measured quantities was low for C28 ($5.9 \pm 5.8\%$) and C32 ($5.7 \pm 4.3\%$) in their combined binary waxes. The additional treatment (baking or freeze-drying) did not alter the recovery of alkanes from the model waxes. Synthetic alkanes are thermolabile why exposure to high temperature during preparation of boluses or labelling of feedstuffs needs to be assessed critically. Reasons for that might be complex disorders of the conformation of alkane molecules particularly during the melting of alkane mixtures and the apparently incomplete separation following re-crystallization from the melt. This may lead to displacements within waxes, which cannot

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http://dx.doi.org/10.1016/j.livsci.2016.01.018
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be foreseen or quantified, and thus to the loss of their suitability as dietary markers. Alkane recovery from binary waxes of C28 and C32 was unbiased on model scale and whether this can be confirmed on original scale needs to be validated further. For practical use, alkane waxes might nevertheless be beneficial because the handling is easier than that of alkane crystals.

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1. Introduction

Saturated aliphatic hydrocarbons (*n*-alkanes) can be found in the cuticular and epicuticular wax of conventional feed plants and also in other natural waxes. Additionally, they are available as synthetics. In livestock nutrition studies, synthetic alkanes can be applied to estimate the faecal output (Dove and Mayes, 2006), digestibility (Hatt et al., 2001) and passage kinetics (Giráldez et al., 2004; Bulang et al., 2008). Furthermore, they are easy to combine with plant alkanes for a more sophisticated investigation of the intake of feedstuffs of distinct biological origin (Mayes et al., 1986). The suitability of synthetic alkanes to estimate the passage rate of digesta is limited, because they can pass the digestive tract in the solid phase of digesta and likewise in the liquid one (Bulang et al., 2008). The use of markers that are chemically (e.g. plant alkanes and hydrochloric acid-insoluble ash) or at least physically bound to the feed plants (mordants) could overcome this problem but they are usually in very low concentration for a single administration. However, the single administration of a synthetic alkane via bolus followed by repeated sampling of faeces can be applied to determine the faecal excretion curve of this marker, the faecal output and the forage intake of the animal simultaneously (Giráldez et al., 2004). It might be that this approach can have advantage towards bolus administration once or at multiple times per day, which is known to induce diurnal variation of the marker concentration in faeces (Giráldez et al., 2004; Molina et al., 2004). Further work is needed to clarify that issue.

The accurate and uniform labelling of boluses is a basic requirement, regardless of the chosen methodical approach. Using repeated administration of boluses throughout consecutive days, a high uniformity of consecutive dosages may reduce the risk of an additional variation of faecal alkane concentrations. Using the single administration of a bolus, a high uniformity of labelling is required among boluses intended to be applied in a number of animals, because the marker dose rate can be determined from a representative sample of the boluses but not for each animal individually.

Administration methods that can be used to supply external alkanes in ruminant and monogastric livestock involve labelled paper pellets, filters or capsules, labelled feedstuffs (roughages or concentrates) or feed pellets, alkane suspensions or oil-in-water emulsion that need to be offered compulsorily in liquid form and controlled-release devices that are specifically used in ruminants (summarized by Dove and Mayes (2006)). In pigs, alkane-labelled cakes were used as boluses (Mowat et al., 2001). In poultry, synthetic alkanes were mixed into a ground seed mixture and subsequently pelleted (Hatt et al., 2001). For administration in equids specifically, also bread-pieces and other types of biscuits have been used as boluses (Table 1). In most cases, the techniques for preparation of boluses or the labelling of feedstuffs used alkanes that are dissolved to be spread onto the bolus matrix or substrate. According to our experience, this may be accompanied by a higher risk of precipitation and losses of the alkanes in part. Some of the techniques for the labelling of boluses and feedstuffs also involved the exposure to high processing temperatures with the aim to fix the alkanes onto the bolus matrix or substrate (Kuntz et al., 2006; Smith et al., 2007). It is known that plant and other natural alkanes are thermolabile. For example, temperature-dependent losses occurred during oven-drying of faeces samples from sheep that were previously fed with unlabelled or beeswax-labelled diets at 105 °C and 24 h drying time (Elwert et al., 2006). Data of the temperature sensitivity of synthetic alkanes are not available but it is likely that there exists a link to so far non-explained losses of such alkanes during the preparation of boluses and labelled feedstuffs and the further processing of samples for analysis.

We hypothesized that melting synthetic alkanes to wax might enhance the accuracy and the uniformity of subsequent bolus labelling and further simplify it. This is particularly required when multiple alkanes are combined or large quantities of boluses are needed. The aim of the study was to test a procedure of preparing alkane waxes on model scale at a ratio of approximately 1:300 basing upon a dosage of synthetic alkanes, which is, in large livestock, recommended for a daily twice administration (Smith et al., 2007). Additionally, the study was conducted to assess the temperature sensitivity of synthetic alkanes and to clarify how they act when exposed to a processing temperature of 180 °C for a timeframe of 20 min.

Table 1
Methods for the preparation of synthetic alkanes to be applied in equids – literature review.

Preparation method	Alkane	Dosage [mg/d]	Reference
Absorbed into shredded paper and mixed with concentrate (B)	C32, C36	627, 492 (repeated)	Stefanon et al. (1999)
Mixed into concentrate (LF)	C32	n.g. (onetime)	Stevens et al. (2002)
Coated on roughage fibre and compressed into pellets (B)	C32	600 (repeated)	Stevens et al. (2002)
Coated on cellulose, dissolved and mixed with xanthan gum (S)	C32	500 (repeated)	Friend et al. (2004)
Spread onto bread-pieces and microwaved (B)	C32	4000 (two times)	Kuntz et al. (2006)
Dissolved and spread onto biscuits (B)	C32	150 (repeated)	Castelán-Ortega et al. (2007)
Dissolved and sprayed onto shredded paper (B)	C24, C32, C36	914.3–1033.7 (repeated)	Ferreira et al. (2007)
Dissolved, sprayed onto roughage fibre, mixed with gelatine and compressed (B)	C36	308.3 (repeated)	Smith et al. (2007)
Dissolved, pipetted onto Weetabix®-pieces, dried and baked (B)	C32	221.4–454.2 (repeated)	Smith et al. (2007)
Dissolved and sprayed onto concentrate (LF)	C36	337.5 (repeated)	Chavez et al. (2014)

Administration form: B, bolus; LF, labelled feed; S, suspension.
C24, *n*-tetracosane; C32, *n*-dotriacontane; C36, *n*-hexatriacontane; n.g., not given.

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