Livestock Science 185 (2016) 43–49

Contents lists available at ScienceDirect

Livestock Science

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

Bolus matrix for administration of dietary markers in horses

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ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form
12 January 2016
Accepted 15 January 2016

Keywords:
External markers
Bolus matrix
Preparation
Acceptance
Mastication
Storage stability

ABSTRACT

External markers are useful or required to estimate forage intake, digestibility, faecal output and passage rate in horses. Oral administration is challenging because of horses' high sensitivity and selection skills. A suitable bolus matrix should ensure high and consistent acceptance. Boluses were investigated with and without labelling. Synthetic alkane wax, embedded in filter paper or contained in hypromellose capsules, was used as a test marker. Boluses were baked (100 °C, 30 min) or freeze-dried. The freeze-dried boluses varied in size (1.5, 2, or 3 cm Ø) and drying time (6, 12, 24, or 48 h). In two tests (AT1 and AT2), acceptance by the horses was assessed with scores between 1 (complete intake) and 4 (refusal). In AT1, marginal rejection of the marker was recorded, whereas the following tests were performed with placebos only. In two bending tests (BT1 and BT2), the force required to break the boluses (FL, flexural load) was determined because this may affect acceptance. Pre-selected variants were stored for 4 weeks in closed boxes under controlled conditions (20 °C and light for 16 h/d, 16 °C and night for 8 h/d, and 65% relative humidity) and were subsequently analysed for residual moisture (RM) and spoilage-indicating microbes. In baked boluses, the alkanes were partly found outside of the inner matrices. This was not evident in the freeze-dried variants. Acceptance of the labelled boluses (scores $\leq 1.7 \pm 0.18$, AT1), baked placebos (scores $\leq 2.2 \pm 0.35$, AT1) and the freeze-dried placebos (scores $\leq 1.1 \pm 0.31$, AT2) was consistently high. This was explained by the BT, with a mean FL of 202 ± 16.5 N for the baked (BT1) and up to 257 ± 22.5 N for the freeze-dried placebos (BT2) being obtained, which was close to the masticatory forces in horses. However, when the boluses distinctly exceeded a size of 3 cm Ø, this size probably led to increased FL (BT1), thereby depressing the acceptance of the dried placebos (AT1). The results indicated that the adaption to suitable boluses can lead to increased acceptance. Limiting the drying time to at most 24 h seemed justified, especially for the smaller boluses. After 6 and 12 h, the RM was $7.5 \pm 0.52\%$ (1.5 cm Ø) and $5.7 \pm 0.52\%$ (2 cm Ø), which make the risk of microbial spoilage appear low. Tested boluses were unspoiled for up to 1 month after preparation. It was suggested that the variable sizes of the boluses may enable the use of various marker dosages and, incidentally, also use in different target animals. A freeze-dried matrix is likewise open to use with other thermolabile markers or substances. We recommend the pre-administration of placebos prior to marker administration to ensure high acceptance of the labelled boluses.

1. Introduction

External markers such as chromium oxide (Takagi et al., 2002), lanthanides (Miyaji et al., 2014) or wax and synthetic alkanes (Elwert and Dove, 2006; Ferreira et al., 2007) can be used as indicators in nutrition studies with ruminant and monogastric livestock. If direct measurement is impossible, markers provide important information for the estimation of feed intake when coupled with plant-inert markers (Mayes et al., 1986), feed and nutrient digestibility (Takagi et al., 2002), faecal output (Giráldez et al., 2004) and digesta kinetics (Bulang et al., 2008). Markers that are incompletely bound to plant material have the disadvantage that they do not completely label the solid phase of digesta (Bulang et al., 2008). This can predominantly affect estimates of passage parameters. This problem can be overcome by a single administration of real plant markers such as alkanes or hydrochloric acid-insoluble ash. Then, intake and digestibility can be estimated

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http://dx.doi.org/10.1016/j.livsci.2016.01.010
1871-1413/

simultaneously (Giráldez et al., 2004). However, plant marker concentrations are commonly too low for a single administration. So far, the application of boluses with unbound or mordanted markers is therefore still the best method of choice.

Marker administration in horses is challenging because of horses' particularly high sensitivity and selection skills. Markers offered via bread pieces (Kuntz et al., 2006), biscuits (Castelán-Ortega et al., 2007; Smith et al., 2007), pellets (Stevens et al., 2002) and capsules may easily be regurgitated, separated, or ingested incompletely, which is likewise reported for markers mixed into concentrated feed (Smith et al., 2007). Invasive methods, such as controlled release devices (Dove et al., 1991) and compulsory marker administration (Marais et al., 1996; Friend et al., 2004), are either not useable in horses, undesirable, or unfeasible with free-ranging animals.

We hypothesized that, for application in horses, a suitable bolus matrix ensures broadly consistent and high acceptance for ingestion and resists microbial spoilage over a sufficient period of time.

The size of the boluses, preparation and dry matter (DM) content may influence the required strain for mastication. This is surmised to be one main factor affecting acceptance in horses. This study was carried out stepwise to test acceptance of the different types of boluses, which varied in size (1.5, 2 and 3 cm diameter, Ø) and preparation (baking or freeze-drying). Both unlabelled boluses (placebo, P) and those labelled with wax of mixed synthetic alkanes as the test markers (verum, V) were investigated in horses in at least one of two acceptance tests (AT). In addition, unlabelled bolus types were subjected to bending tests (BT) to obtain an indication of the horses' required masticatory strain. The most suitable variants were subsequently stored under standardized conditions and tested with regard to resistance to microbial spoilage.

2. Materials and methods

2.1. Bolus preparation

The outer matrix shell of all types of boluses was made of oat flakes (fine grade), sugar beet syrup and wheat flour of food quality, mixed by weight at 1:0.8:0.6, and a small quantity of water. The analysed chemical composition is given in Table 1. Test markers were made of synthetic *n*-octacosane (C28), *n*-dotriacontane (C32) and *n*-hexatriacontane (C36) alkanes (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The tertiary mixtures were melted at 80 °C, re-crystallized to wax at ambient temperature and subsequently chopped by hand. Alkane wax fragments were weighed either into smooth laboratory filter paper (LFP, 1 piece per bolus, grade 388, 7 cm Ø, Munktell & Filtrak GmbH, Bärenstein, Germany) or hypromellose (HPMC) capsules (1 piece per bolus, size 000, Silvaco A/S, Dah Feng, Taiwan) as the inner matrix shell using a dosage that was recommended for administration in horses 2 times a day (in total: 450 mg per bolus; 150 mg of each alkane per bolus; Smith et al., 2007). The parts and ingredients of the matrix did not contain measurable quantities of target alkanes. The boluses were prepared by baking (30 min at 100 °C) or freeze-drying for a defined duration (specified below). The following bolus types were formed: V1: V with filter paper, 3 cm Ø, baked; V2: V with filter paper, 3 cm Ø, freeze-dried (48 h); V3: V with HPMC capsule, 3 cm Ø, baked; V4: V with HPMC capsule, 3 cm Ø, freeze-dried (48 h); P1: P, 3 cm Ø, baked; and P2: P, 3 cm Ø, freeze-dried (48 h). In addition, the freeze-dried P boluses were graded by size (1.5, 2 and 3 cm Ø) and drying time (6, 12, 24 and 48 h), which led to 12 possible combinations. After pre-selection (explained below), the P boluses were graded as follows: P3: 1.5 cm Ø, freeze-dried (24 h); P4: 2 cm Ø, freeze-dried (24 h); and P5: 3 cm Ø, freeze-dried (24 h).

Table 1
Analysed chemical composition of the outer matrix shell of boluses.

	P1	P2
Dry matter (DM) [g/kg]	882	937
Crude ash [g/kg DM]	13	12
Crude protein [g/kg DM]	100	98
Acid ether extract [g/kg DM]	30	37
Acid detergent fibre [g/kg DM]	35	39
Starch [g/kg DM]	433	427
Sugar [g/kg DM]	248	233
Gross energy [MJ/kg DM]	18.5	18.1

P1-bulk sample: 5 × 20%-aliquots, P2-bulk sample: 5 × 20%-aliquots.
P1, placebo, baked (30 min, 100 °C), 3 cm Ø; P2, placebo, freeze-dried (48 h), 3 cm Ø.

2.2. Chemical analyses

The freeze-dried boluses were pre-chopped by hand. Before analysis, the baked boluses were freeze-dried (48 h) to achieve a pulverisable condition. The inner shell of the matrix in the V-type boluses, which included an alkane dosage, was separated manually from the outer one. The outer shell of the matrix was pre-milled with water-cooling, separated into parts and milled under liquid nitrogen at a constant −196 °C using a Retsch® CryoMill (Retsch® GmbH, Haan, Germany; settings: 25 ml cup, 14 mm steel ball, and 5 min per iteration). This allowed sufficient homogenization of the remaining alkanes and the matrix but avoided the high processing temperature, which is known to affect alkane recovery in sample material. Dried P samples and matrix pastry ingredients were ground to pass through a 0.5 mm sieve in a standard laboratory sample mill.

Dry matter determination and crude nutrient analyses of the outer matrix shell were performed according to official methods (VDLUFA, 2012, method no. 3.1: DM, 4.1.1: crude protein, 5.1.1 B: acid ether extract, 6.5.2: acid detergent fibre, 7.1.2: sugar and 8.1: crude ash) using a FOSS 2300 Kjeltec™ Analyser Unit for nitrogen determination and a FOSS Tecator™ Soxtec™ 1047 Hydrolysing Unit and a Soxtec™ HT 1043 Extraction Unit for acid ether extract analysis (FOSS GmbH, Rellingen, Germany). The gross energy was ascertained by bomb calorimetry using a C7000 Oxygen Bomb Calorimeter (IKA® Werke, Staufen, Germany). Starch was determined enzymatically referring to the amyloglucosidase method (VDLUFA, 2012, method no. 7.2.5).

Alkane extraction from the matrices of the V samples and gas chromatographic analysis were performed according to Elwert et al. (2004). A Shimadzu GC-2010 FID unit (Shimadzu Corporation, Kyoto, Japan) with an on-column injection and an Rtx®-1 w/ Integra-Guard™-column (Restek Corporation, Bellefonte, PA, USA) were used.

2.3. Testing procedures

The tests were carried out in the order AT1, BT1, BT2, AT2 and storage test (ST). In the AT1, the P (P1 and P2) and V-type boluses (V1, V2 and V4) were tested simultaneously. The V3-type boluses had already been excluded during the preparation process because baking led to visible losses of the marker alkanes at the bolus surface. Rejection of the marker dosage itself has rarely been recorded in AT1. Therefore, acceptance was assumed to be mainly influenced by properties of the bolus matrix. Consequently, only P variants were subjected to the following tests. Bending test 1 was performed to obtain an explanation for the poor acceptance of P2 compared with P1 in AT1. Upon the basis of the results of AT1 and