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Assessing the tolerance of weanling pigs to a model feed flavour in the diet

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ABSTRACT

A tolerance trial was carried out to evaluate the safety of feeding multifold doses of a model flavour containing a mixture of 20 chemically defined flavouring compounds to crossbred weanling piglets (Duroc male × Landrace/Large White female). Animals were weaned at 28 days and assigned to one of five treatments: a control (no flavour) or diets containing the model flavour at levels of 1×, 3×, 10× or 30× the typical commercial inclusion rate of 500 mg/kg feed. A pre-starter diet was fed to piglets from 28 to 42 days of age and a starter diet from 42 to 70 days of age. Parameters measured included average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio FCR), haematology, serum biochemistry, general health and mortality. The model flavour was well tolerated by the piglets at all four dosage levels. No differences in ADFI or FCR were observed between treatment groups during the pre-starter/starter periods or for the whole trial. The ADG of piglets in receiving 3× the normal dosage was less ($P < 0.05$) than that of the control animals, but not different from the other dosage groups. The group of piglets with the lower ADG had a higher incidence of diarrhoea than the other groups. Differences observed among treatments for the haematology and serum biochemistry parameters analysed were limited to piglets fed 3x the normal dosage. The extraction method developed to quantify the different compounds of the model flavour using routine GC/MS RTL is described. The importance of monitoring flavour content of the constituents present in the mixture and reducing losses due to volatility is highlighted. Results obtained from the trial demonstrate the feasibility of tolerance testing in target animal species using flavour mixtures.

1. Introduction

Feed flavourings can be added to animal diets or drinking water to stimulate intake during pre/postpartum transition periods (Shah et al., 2004), lactation (Thomas et al., 2007; Wang et al., 2014), weaning (Montoro et al., 2011; Adeleye et al.,

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

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2014) and stressful periods (Jacela et al., 2010). Flavourings are formulations of additives or compounds that combine taste and smell (aroma); aroma blends consist of a mixture of several single flavourings. The set of feed additives approved for use in aroma formulations in the European Union (EU) consists of several hundreds of compounds (EU, 2014). Due to a change in EU procedure, these feed flavourings must now be individually reassessed and separately authorised (EC, 2008).

Tolerance trials provide a limited evaluation of short-term toxicity of a feed additive to the target animals and establish a margin of safety, if the additive is consumed at higher doses than recommended (EFSA, 2011a). The feed flavour industry currently has two options for assessing the safety of a flavouring compound. The first option is to determine the tolerance level of each compound for the intended target animals; a rigorous approach that meets the EU procedure for evaluating feed additives. However, applying this procedure to the hundreds of different flavouring compounds requiring reauthorization is not practicable. Moreover, a tolerance trial requires a minimum of three groups (control, use-level and tolerance), thereby requiring a substantial number of experimental animals. The second option, employed in absence of data from dedicated experimental studies, determines the tolerance level of a compound with a conservative estimate derived from studies on laboratory animals, such as rats (EFSA, 2012a,b). A weakness of this approach is that it can underestimate the actual tolerance levels in target animals (Hashemipour et al., 2013; Starčević et al., 2014; Haselmeyer et al., 2014). Most importantly, neither of these two options assesses target animal safety under representative conditions, namely that flavouring substances are added to commercial feed products as mixtures, rather than single compounds. A more applicable approach would be one that confirms animal safety under conditions of actual use.

Tolerance trials with feed additive blends containing few flavouring compounds, sometimes associated with organic acids, have been carried out with pigs and poultry (EFSA, 2010, 2011b, 2012c, 2014, 2015). The study reported in the present paper investigates the procedure of tolerance testing using many different substances in model flavour mixtures. The purpose of the conducting the study was to demonstrate a viable alternative option for assessing feed flavours that meets EU testing standards. The study objective was to evaluate the safety of feeding multifold doses of an aroma mixture composed of 20 chemically defined flavouring compounds to weaned piglets. A method developed for quantifying flavouring compounds in feed is also described.

2. Materials and methods

2.1. Animals, experimental design and diets

A total of 480 crossbred (Duroc male × Landrace/Large White female) piglets (half males and half females) weaned at 28 days of age and weighing an average 7 kg were used in the study. The piglets were randomly allocated to 40 pens (12 animals per pen balanced by sex and weight) located in two nursery rooms (20 pens per room). Each pen was 6.05 m^2 in size with a fully slatted floor and contained one feed hopper and two nipple drinkers. The rooms were illuminated by a combination skylights and artificial light (used only to compensate for day length during feeding). Ventilation of the rooms was controlled by a single, variable-speed fan linked to temperature sensors located in the building. The temperature inside the building was approximately 28 °C at the start of the trial and for the first week post-weaning. Thereafter, the temperature was reduced by 1.5 °C weekly until a temperature of 22 °C was reached and then maintained until the end of the trial. The study was carried out in accordance with the standards set forth in Directive 2010/63/EU regarding the protection of animals used for scientific purposes (EU, 2010).

Two isonutritive mash diets (pre-starter and starter) were prepared to meet or exceed the nutrient requirements for pigs recommended by NRC (2012). Both diets were void of any growth promoter or antibiotics, or of any other flavour mixture or sweetener (Table 1). Results of a nutritional analysis showed that all feeds were in accordance with the diets (data not shown).

2.2. Model feed flavour

A red fruit model flavour (Flavour 3168Z) containing a mixture of 20 chemically defined flavouring compounds was formulated to imitate the flavour of red fruits (strawberry, cherry and raspberry) (Table 2). The model flavour was designed to be added to the diets of weanling pigs at a typical commercial inclusion rate of 500 mg/kg feed.

2.3. Tolerance testing

Tolerance testing of the model flavour was done for both diets using a completely randomised design with five experimental treatments; a control with no flavour (T1) and four treatments (T2-T5) containing the model flavour at 1×, 3×, 10× or 30× of the typical commercial use level, respectively. Each treatment was replicated eight times, thus each of the 40 pens formed an experimental unit. In order to minimize cross contamination, the treatment mixtures were manufactured beginning with the control (T1) and then in the following order, T2-T5, increasing the dose of the model flavour, respectively. The model flavour was premixed with 15 kg ground corn before addition to the final feed treatment mix for each diet to assure homogeneity and reduce dust formation. The piglets received the pre-starter diet from 28 to 42 days of age and the starter

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