A NOVEL IN VITRO POULTRY MODEL TO COMPARISON RELEASE KINETICS OF (UN)PROTECTED BUTYRATE

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Abstract

The gastrointestinal (GIT) segment wherein dietary additives, such as butyrate, are released may influence their observed efficacy. Release kinetics are influenced by pH, gastric and enteric motility, retention time in the different GIT segments and enzymes concentration. Unfortunately, none of the available in vitro poultry model mimics accurately these conditions. As a consequence, a novel in vitro poultry GIT model was developed to compare the release kinetics of commercially available butyrate formulations. Four fat-embedded butyrate matrices originating from three suppliers as well as tributyrin, the triglyceride of butyrate, were evaluated in a three-step (pre-gastric, gastric and enteric) in vitro digestion procedure. Aliquots were drawn at 5 stages of digestion representing the end of the crop, proventriculus-gizzard, duodenum, jejunum and ileum. The percentage of the initial butyrate dose solubilized was calculated for each stage. Significant differences were found among formulations for each digestion stage (P < 0.001). Half of the fat-embedded butyrate matrices showed a sustained release profile, while the other released all their content in the pre-enteric regions. Tributyrin had a release profile targeting enteric segments. Results of this study indicate that despite almost identical visual appearance, release kinetics can vary significantly among butyrate derivatives. Such variations can possibly explain the inconsistency of the bird's growth response to dietary butyrate supplementation as shown in literature.

Keywords: in vitro model, release kinetics, feed additives, butyrate

Introduction

In poultry nutrition, butyrate is used as a feed additive. This short chain fatty acid can be fed as an unprotected salt or in the form of protected derivatives such as butyrate glycerides or butyrate-loaded matrices. Butyrate elicits stimulatory, albeit inconsistent, effects on growth performance of broiler chickens. The GIT segment wherein butyrate is released may modulate its effects given the diversity of cell types and pH conditions encountered throughout the avian GIT, and the differences in microbiota composition in the different gut segments. Release properties of protected derivatives can therefore contribute to the understanding of the mode of action of butyrate. Release kinetics can be determined in vivo by measuring directly the butyrate content in the chyme or by measuring the oxidation of labelled butyrate in respiratory chambers. The first technique doesn’t allow to discriminate between the dietary or fermentative origin of butyrate, while the prohibitive costs of the latter prevents its use when screening numerous formulations. In vitro models are interesting alternatives to the aforementioned techniques as they are quick, cost-effective, and the results are not confounded by fermentation end-products. Release kinetics are being influenced by environmental factors e.g. pH, temperature, enzymatic activity or retention time. Specific conditions encountered in the avian GIT are described in the literature (Long, 1967; Phillips and Fuller, 1983; Zyla et al., 1999; Dendow, 2015), but not in in vitro poultry models. The present work was undertaken to develop a novel in vitro poultry model, using realistic digestion parameters, to compare release kinetics of commercially available butyrate derivatives.

Material and Methods

Tributyrin (T8626, Sigma-Aldrich, Saint-Louis, USA) and 4 commercially available fat-protected butyrate derivatives: Adimix 30 C (Nutri-ad International NV, Belgium), Ding Su (Xiamen Fujian Co., China), Sodium Butyrate 300 and 500 (Vega Pharma Limited, Hangzhou, China) were used in triplicate in the in vitro incubation procedure. The procedure consisted of pre-gastric, gastric and enteric steps. The enteric step had three different durations, resulting in a total of five incubation stages. Approximately 0.25 g of sample was incubated for 35 min in 20 ml of a 1 N tris-HCl buffer solution (pH 5.8) containing 1 g/l NaCl and 0.5 g/l of α-amylase from porcine pancreas (10 U/mg solid; A3176, Sigma-Aldrich). When only the pre-gastric digestion was considered, the procedure was stopped by transferring the tube to ice. Otherwise, incubation proceeded with addition of 2 ml of a 0.085 N HCl solution containing 11 g/l of pepsin from porcine
gastric mucosa (250 U/mg solid; P7000, Sigma-Aldrich). If necessary, pH was adjusted to 2.7 ± 0.05 with drops of 6 N HCl or 6 N NaOH. The second stage of incubation then proceed for 35 mn. The procedure was stopped when only the gastric digestion was considered by transferring the tubes to ice. Otherwise, 4 ml of a 1 N tris-buffer solution (pH 6.5) containing 1.875 g/l pancreatin from porcine pancreas (8 x USP specifications; P7545, Sigma-Aldrich) and 0.7 g/l bile salts (48305, Sigma-Aldrich) was added to the tube. If necessary, pH was adjusted to 6.5 ± 0.05 with drops of 6 N HCl or 6 N NaOH. The incubation was stopped by transferring the tubes to ice 10 mn, 80 mn or 170 mn after addition of pancreatin when duodenal, jejunal or ileal digestion were considered, respectively. A temperature of 40 ± 0.1°C and a stirring rate of 360 rpm were maintained throughout the different stages of incubation. Aliquot were drawn at the end of each digestion stage. Butyrate content was determined for each derivative by incubating ground samples in 20 ml of 1 M HCl solution during 24 hours. Aliquots were analysed for butyrate concentration by gas chromatography. Butyrate yield was expressed as the percentage of the total incubated butyrate dose that was solubilized at a certain time point. Butyrate yields were analysed for each incubation stage with the PROC GLM procedure of SAS® version 9.2 (SAS Institute Inc., Cary, NC, USA), using a significance level $P$ of 0.05. LSD test was carried as a post-hoc test.

**Results**

Table 1 presents the cumulative butyrate release per digestion stage of the assayed butyrate derivatives. For each digestion stage, significant differences were found among the derivatives ($P < 0.001$). Throughout the enteric phases of incubation, pH dropped gradually when tributyrin was incubated, resulting ultimately in a pH significantly lower than the one observed with fat-embedded butyrate matrices ($P < 0.001$).

Table 2. Cumulative butyrate release (%) per incubation stage for different butyrate formulations

<table>
<thead>
<tr>
<th>Butyrate derivative</th>
<th>Crop</th>
<th>Proventriculus -gizzard</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tributyrin</td>
<td>13.4± 1.5</td>
<td>13.9± 2.4</td>
<td>20.1± 2.2</td>
<td>34.0± 4.4</td>
<td>41.2± 2.8</td>
</tr>
<tr>
<td>Adimix 30</td>
<td>57.2± 3.3</td>
<td>77.8± 5.2</td>
<td>82.3± 3.3</td>
<td>89.7± 20</td>
<td>95.6± 6.1</td>
</tr>
<tr>
<td>Ding Su</td>
<td>88.3± 7.4</td>
<td>97.7± 4.1</td>
<td>105.2± 5.6</td>
<td>99.1± 2.1</td>
<td>99.6± 0.2</td>
</tr>
<tr>
<td>Vega 30</td>
<td>66.8± 8.6</td>
<td>87.0± 9.1</td>
<td>100.0± 5.9</td>
<td>107.7± 1.6</td>
<td>112.1± 9.9</td>
</tr>
<tr>
<td>Vega 50</td>
<td>99.2± 2.2</td>
<td>97.8± 1.7</td>
<td>96.5± 2.2</td>
<td>97.8± 1.6</td>
<td>97.1± 0.4</td>
</tr>
</tbody>
</table>

$P$ value <0.0001 <0.0001 <0.0001 <0.0001 <0.0001

Values represent the mean ± standard deviation of three observations per time point. Means with different letters within a column differ significantly at $p < 0.05$. 1Digestion stage 1: 35 min, 2: 70 min, 3: 80 min, 4: 160 min, 5: 250 min.

**Conclusion**

Despite almost identical visual appearance, *in vitro* release profiles can differ significantly among fat-embedded butyrate matrices. In the present study two matrices showed a sustained release profile, with a gradual release of butyrate throughout all the digestion stages. Conversely, two other matrices showed no protection, with a complete release of their content in the first stage of digestion. Differences in release profiles may, in addition to the already described dose, health status and ages factors, be an underlying mechanism explaining the observed discrepancies in the literature concerning the of use fat-embedded butyrate matrices. Tributyrin showed a release profile targeting the enteric region, with a total cumulative yield being lower than the theoretical maximum. This could be due to an inhibitory effect of the butyric acid-induced drop of pH on pancreatic lipase during the enteric steps.

The present *in vitro* model allows to compare release profiles of different butyrate derivatives in quick and inexpensive way, thus meeting the goal of the study. There are, however, a few limitations related to the use of such a model. Avian-specific motility, e.g. gizzard contractions and anti-peristaltic movements of the chyme, are not included in the model. Yields greater than 100% indicates that the butyrate content determination needs to be refined. Also, pH stability can be compromised despite the use of a concentrated buffer when acidic compounds are assessed.
References