Livestock Science 188 (2016) 153–158

Contents lists available at ScienceDirect

Livestock Science

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

The effect of alfalfa saponins on the contractility of bovine isolated abomasum and duodenum preparations

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ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form
18 April 2016
Accepted 23 April 2016

Keywords:
Alfalfa saponins
Abomasum
Duodenum
Contractility
Cow

ABSTRACT

Saponins, a vast group of secondary plant metabolites, occur naturally in plants that are commonly present in ruminants' diet or plants that are used as phytogenic feed additives. They have gained particular attention among ruminant nutritionists due to numerous health benefits resulting in improved animal performance and feed conversion efficiency. Despite the broad knowledge of saponins' effects of on cattle, little is known about their impact on gut motility. Therefore, the effect of two structurally divergent alfalfa saponins on the contractility of abomasum and duodenum smooth muscle was examined.

The study was conducted on tissues obtained from routinely slaughtered, healthy cows. The experiments were carried out on longitudinal and circular smooth muscle preparations under isometric conditions. The effect of hederagenin and medicagenic acid in a concentration range of 0.001–100 μM was verified in a non-cumulative manner on acetylcholine-precontracted smooth muscle specimens. The results are expressed as percentage of the control contraction induced by acetylcholine.

Generally, both triterpenoid saponins caused significant enhancement of acetylcholine-induced contraction of abomasum and duodenum preparations. Hederagenin generated dose-dependently significant increase of acetylcholine-evoked contraction of abomasum strips. The strongest contraction caused by hederagenin in a concentration of 100 μM was observed in abomasal circular smooth muscle and amounted to 184.1 ± 15.5% of the control treatment. Similarly, in case of duodenum hederagenin increased smooth muscle contractility dose-dependently. Hederagenin applied in the highest tested concentration doubled the force of acetylcholine-induced contraction in duodenal longitudinal smooth muscle. Medicagenic acid caused a remarkable increase of abomasum but not duodenum smooth muscle contractility. The contractile effect of medicagenic acid was observed in a concentration range of 0.001–10 μM. The highest contractions of abomasal longitudinal and circular smooth muscle generated by medicagenic acid exceeded 150% of the control treatment with acetylcholine. All reactions caused by hederagenin and medicagenic acid were reversible and did not abolish the spontaneous motoric activity of specimens.

Taking into consideration the physicochemical properties of saponins it is postulated that the contractile effect of hederagenin and medicagenic acid results from their impact on the fluidity or plasticity of the membrane and subsequently the ion flow through cell membrane. The increased ability of cells to depolarize can explain the enhancement of the response to acetylcholine. The reversibility of induced contractions, preserved spontaneous contractility and remained reactivity of smooth muscle after saponin treatment indicate that the effect caused by alfalfa saponin does not result from cell membrane damage.

1. Introduction

In the last decade, there has been growing interest among ruminant nutritionists and breeders to incorporate various bioactive phytochemicals, including saponins, for improving rumen metabolism and the general efficiency of ruminants production. The significance of phytogenic feed additives increased additionally after the implementation of EU Regulation No 1831/2003 which banned completely the use of antibiotic feed additives in livestock.

Saponins occur naturally as glycosides or free aglycones and are widely distributed in higher plants and plant-based food and fodder. The diet of ruminants includes several plants rich in

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http://dx.doi.org/10.1016/j.livsci.2016.04.017
1871-1413/

saponins, predominantly alfalfa and clovers that are a valuable source of protein for temperate climates (Cheeke, 1971). Besides, saponins become main active constituents of medicinal herbs and dietary supplements in various animal species, including cattle (Cheeke, 2000; Cieślak et al., 2013). Growing interest in the use of saponins as phytogenic feed additives results from their numerous biological activities, *e.g.* immunostimulatory, anti-inflammatory, hepatoprotective, antibacterial, antiviral, antifungal and anti-parasitic properties (Francis et al., 2002; Sprag et al., 2004). In recent years, there have been sufficient *in vitro* and *in vivo* evidence of methane production inhibiting activity (Holtshausen et al., 2009; Patra and Yu, 2013), anti-protozoal effects of saponins (Lovett et al., 2006; Lu and Jorgensen, 1987; Sanosto et al., 2007) and their ability to modify rumen fermentation *via* direct influence on the composition and activity of rumen protozoa, bacteria, fungi and archea (Patra and Saxena, 2009; Wang et al., 2000). Besides, it is postulated that saponins may improve ruminant performance, especially in animals on a roughage-based diet (Lu and Jorgensen, 1987; Patra and Saxena, 2009). Moreover, although saponin supplementation does not improve milk yield and quality, it reduces somatic cell count in milk (Holtshausen et al., 2009).

Data of saponins bioavailability and their distribution in ruminants is very limited (Mathison et al., 1999) since most of pharmacokinetic studies have been performed on monogastric species, mostly rodents (Yu et al., 2012). Nevertheless, the transient character of anti-protozoal activity of saponins has been explained by partial degradation of saponins by rumen bacterial population (Teferedegne et al., 1999). There is some evidence that saponins undergo deglycosylation in the rumen (Newbold et al., 1997; Wang et al., 1998) and then a part of released sapogenins is oxidized or reduced. Eventually, only free aglycones or their metabolites are absorbed in the duodenum, jejunum and ileum (Meagher et al., 2001).

Although there are quite a lot of studies of the effects of saponins on ruminant tissues and metabolism, there is little known about their impact on gastrointestinal motility. The existing data reveals opposing – myorelaxant and contractile – effects of lucerne saponins on sheep rumen and intestine motility (Klita et al., 1996; Lindahl et al., 1957). According to our knowledge, there are no studies in the field of nutritional toxicology that evaluate the effect of triterpene saponins, in particular alfalfa saponins (Fig. 1), present in ruminant diet on abomasal and duodenal motility, though their impaired motility is one of the prerequisite for abomasal displacement (AD) in cows. Thus, the aim of the present research was to estimate the effect of hederagenin (HED) and medicagenic acid (MA) on bovine isolated abomasum and duodenum smooth muscle preparations.

2. Materials and methods

2.1. Chemicals

Acetylcholine chloride (ACh), dimethylsulfondioxide (DMSO) (Sigma Chemicals Co, St. Louis, USA), hederagenin (ChromaDex, Irvine, Canada), medicagenic acid (PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany) CaCl2 (Merck, Darmstadt, Germany), NaH2PO4 (Fluka Chemie, AG, Buchs, Switzerland), all other salts needed for the preparation of the incubation media: NaCl, KCl, MgSO4, NaHCO3 and glucose (Avantor Performance Materials, Gliwice, Poland) were used for preparing the experiments. Hederagenin and medicagenic acid were dissolved in 0.5% DMSO. Modified Krebs-Henseleit solution (M K-HS) containing: NaCl (123.76 mM), KCl (5 mM), CaCl2 (2.5 mM), MgSO4 (1.156 mM), NaHCO3 (14.5 mM), KH2PO4 (2.75 mM) and glucose (12.5 mM) was employed as an incubation medium. Heating up to 37 °C and bubbling with carbogen (95% O2 + 5% CO2) guaranteed a stable pH value that ranged from 7.35 to 7.45.

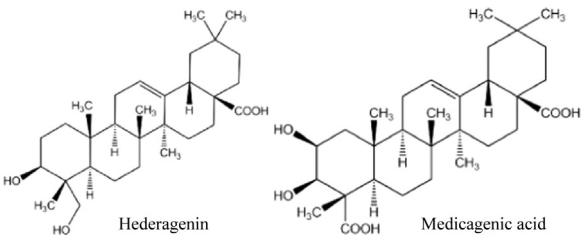

Fig. 1. Molecular structure of triterpenoid saponins used in the study.

2.2. Tissue collection and preparation

The tissues were collected from twelve healthy adult dairy cows that underwent routine slaughtering. The abomasal and duodenal tissues were harvested within 20–30 min after stunning of the cattle. Whole thickness muscle preparations with the mucosa were taken from the pyloric antrum of the abomasum, approximately 20 cm adoral and 30 cm aboral to the pylorus. The pylorus was dissected and discarded. The obtained tissues were immersed in cooled (4 °C) M K-HS and transported to the laboratory (ca. 1 h). Upon arrival to the laboratory, the samples were dissected, firstly from adipose tissue and adventitia, and latter from mucosa and submucosa. The remaining tissue was then cut parallel to the longitudinal muscle fibers or parallel to the circular fibers with a final size of 5 mm width and 20–25 mm length. Final samples obtained included abomasal circular (AC), abomasal longitudinal (AL), duodenal circular (DC) and duodenal longitudinal (DL) smooth muscle strips. One animal served as a donor of one AC, one AL, one DC and one DL specimen.

2.3. Registration of smooth muscle activity

The obtained smooth muscle specimens were suspended in separate organ bath chambers of 5 mL volume (Organ Schuler Bath, Hugo Sachs Elektronik, Germany) filled with 5 mL heated MK-HS constantly bubbled with carbogen. The tissues were clamped distally to hooks and proximally attached to an isometric force transducer (F30, type 372, Hugo Sachs Elektronik, Germany). All experiments were carried out under a load of 0.01 N. The isometric force transducer was linked to an analogue-digital registration set (PowerLab, ADInstruments, Australia) by a bridge amplifier (DBA, type 660, Hugo Sachs Elektronik, Germany). Recordings of the isolated muscle strips' motoric activity were then registered by LabChart v7.0 program. All the calculations and analysis were completed by LabChart Reader v8.1.1 program and Excel (MS Office XP Professional).

2.4. Design of experiments

Each experiment began with 75 min of preincubation. Throughout this preincubation period the chambers were washed out every 15–20 min with fresh warm M K-HS. The initial 45 min of preincubation occurred without tension, and was followed by a 15-minute period with 0.005 N tension. Ultimately, another 15 min with 0.01 N tension occurred. Next, the reference substance (acetylcholine, 10 μM, dissolved in M K-HS) was added several times till the evoked reaction stabilized. Only specimens that reacted adequately, *i.e.* with a clear contractile reaction, to ACh treatments and exhibited the spontaneous contractility were utilized in further parts of experiment. Next, all strips were treated with ACh dissolved in DMSO (0.5%) in order to verify their response to the solvent. The reaction of preparations to ACh