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ORIGINAL PAPER

Alternative tannin sources in post-weaning piglets: impact of *Lythrum salicaria* and *Bistorta officinalis* on health and performance

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ABSTRACT. Tannins have attracted increasing interest as dietary components for the prevention of post-weaning diarrhoea in piglets. The objective of this study was to investigate the effects of dietary supplementation with an extract or plant material derived from *Lythrum salicaria* L. and *Bistorta officinalis.* Eighty weaned piglets (40 males and 40 females) were randomly divided into five treatment groups: control (CON), *L. salicaria* extract (LSE), *B. officinalis* extract (BOE), *L. salicaria* plant material (LSP), and *B. officinalis* plant material (BOP). Treatment diets were formulated to provide an equal tannin supplementation level (2 g/kg feed). Animals were slaughtered on days 13 and 14 of the trial. Intestinal digesta, faeces and tissue samples (duodenum, mid jejunum) were collected to analyse pH, ileal and total tract apparent nutrient digestibility (AID and ATTD), and histomorphological parameters. No significant differences were observed in performance and most histomorphological traits. Inclusion of LSP and BOP lowered AID of proline in comparison to CON ($P = 0.044$). The LSP group had the lowest arginine ($P = 0.003$) and histidine ATTD ($P = 0.004$). Both the LSP and BOP groups had reduced ATTD of glutamic acid ($P = 0.003$) and proline ($P < 0.001$), while BOP additionally showed the lowest proline ATTD. In conclusion, *L. salicaria* and *B. officinalis* supplementation did not adversely affect piglet performance, although reductions in the digestibility of several amino acids were recorded. These moderate effects suggest that tannins from *L. salicaria* and *B. officinalis* can be considered safe for piglet diets at the tested inclusion levels.

Introduction

Weaning represents a critical phase in the life of a piglet and is frequently associated with dysbiosis, gastrointestinal disorders, and diarrhoea due to stress and the abrupt transition from milk to solid feed (Lallès et al., 2007; Gresse et al., 2017). Tannins have received increasing attention in recent years as a potential strategy to prevent post-weaning diarrhoea (Girard and Bee, 2020), particularly since antimicrobial growth promoters have been banned in the European Union under Regulation (EC) No. 1831/2003. Tannins are specialised plant metabolites with documented antiviral, antifungal,

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antibacterial and antioxidant properties (Girard and Bee, 2020) and are classified into two major groups: hydrolysable tannins and condensed tannins, which differ in chemical structure, stability, and biological behaviour (Scalbert, 1991). Hydrolysable tannins are esters of a polyol, usually glucose, with phenolic acids such as gallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins) (Scalbert, 1991). In contrast, condensed tannins, commonly termed proanthocyanidins, are polymers of flavan3ol units like catechin and epicatechin. Unlike hydrolysable tannins, they are linked by carbon–carbon bonds and are therefore resistant to hydrolysis (Hagermann, 2002). Extracts from the European chestnut (*Castanea sativa* Mill.) and American quebracho (*Schinopsis* spp.) trees are frequently used tannin sources in swine nutrition. Chestnut extract contains hydrolysable tannins, while quebracho extract provides condensed tannins (Caprarulo et al., 2021). The aerial parts of *Lythrum salicaria* L. have long been utilised in European traditional medicine to treat diarrhoea in humans and animals and contain a wide range of chemical compounds, with hydrolysable tannins as the dominant group (Piwowarski et al., 2015). Another tannin-rich plant material, the rhizome of *Bistorta officinalis (*synonym: *Polygonum bistorta (*L.*))* has been traditionally applied in similar gastrointestinal conditions; however, it contains different type of tannins-namely condensed tannins (Pawłowska et al., 2020). To our knowledge, no *in vivo* trials have examined the effects of extracts obtained from these plants, or the corresponding plant material, in diets for post-weaning piglets. Previous experiments with other tannins sources supplemented with diets for weaned piglets have shown that, despite health benefits, they can negatively affect nutrient digestibility. Tannins are considered to exert antinutritive effects by forming complexes, particularly with feed proteins in the digesta or with digestive enzymes (Ma et al., 2024). Condensed tannins can reduce the digestibility of proteins, starch, and fats by interfering with the activity of amylase, lipase, proteases and other enzymes (Hassan et al., 2020). In addition to proteins, tannins can also bind directly to polysaccharides (Girard and Bee, 2020). These phenolic compounds also exert an antinutritive effect by reducing feed palatability (Hassan et al., 2020), which is related to their interaction with bitter taste receptors and with proteins in saliva (Girard and Bee, 2020).

The inclusion of chestnut extract at 4.5 g/kg significantly improved the feed conversion ratio (FCR) in weaner piglets, whereas lower doses resulted in values comparable to the control group (Biagi et al., 2010). Weaner piglets fed diets with 0, 0.2, or 0.3% condensed tannins from the quebracho tree showed no significant differences in growth performance between treatment groups; diarrhoea was not observed in the 0.3% group, while the other groups had an incidence of 12.5% (Ma et al., 2021). Dietary inclusion of tannic acid from gallnut at 1.5 and 3 g/kg resulted in final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) values comparable to those of the control group. In the group receiving 3 g/kg, duodenal and jejunal villus height were significantly increased, whereas jejunal and ileal crypt depths were reduced compared to the control (Deng et al., 2024).

Based on this evidence, it was hypothesised that extracts and plant material from *L. salicaria* (aerial parts) and *B. officinalis* (rhizomes) could support intestinal function in the early post-weaning period in piglets.

The objective of the study was to assess the effects of these extracts and the respective plant materials on post-weaning piglets with respect to performance, histomorphological parameters, digesta pH, and apparent ileal and total tract nutrient digestibility.

Material and methods

Plant materials and extract preparation

Lythrum salicaria L. was purchased from NANGA Przemysław Figura (Złotów, Poland), and rhizomes of *Bistorta officinalis* were obtained from Zakład Konfekcjonowania Ziół FLOS (Mokrsko, Poland). The plant materials were validated by microscopic evaluation of dried samples, according to the relevant procedures described in a European Pharmacopoeia monograph (European Pharmacopoeia, 2019) Voucher specimens (No. LSHU2022 for *Lythrum salicaria* and No. BRU2022 for *Bistorta officinalis*) were deposited in the Department of Pharmaceutical Biology, Medical University of Warsaw, Poland.

The extract from *L. salicaria* was prepared according to the method described by Piwowarski and Kiss (2013). Briefly, dried, cut plant material (approx. 4 kg) was extracted threefold with water over 30 min in an ultrasonic bath (40 °C). The combined extracts were subsequently freeze-dried, yielding 550 g of dry *L. salicaria* extract.

The *B. officinalis* extract was prepared according to the method described by Pawłowska et al. (2020). Briefly, dried, cut plant material (~3.5 kg) was extracted four time with water in an ultrasonic bath at room temperature. The resulting extract was freeze-dried, yielding 700 g of dry *B. officinalis* extract. Both extracts were prepared at the Department of Pharmaceutical Biology, Medical University of Warsaw (Warsaw, Poland).

Phytochemical standardisation of plant materials and extracts

Quantitative analysis – total polyphenol, and total tannin content

Total polyphenol content (TPC) was determined using the colorimetric Folin-Ciocalteu assay (McDonald et al., 2001). Extracts were dissolved in methanol:water (1:1, v/v) to a final concentration of 1 mg/ml. An aliquot of 40 µl from each extract was transferred to a 96-well microplate and added to 105 µl of 10% Folin–Ciocalteu (Sigma, USA) reagent and 85 µl of 1 M sodium carbonate solution. The mixtures were incubated at 45 °C for 15 min. Absorbance was measured at 765 nm using a microplate spectrophotometer (Agilent BioTek Synergy H4, USA). Gallic acid was used as the standard for calibration, and results were expressed as mg of gallic acid equivalents per gram of dry sample (mg GAE/g dry weight). Data are reported as means ± standard deviation from three independent replicates.

A modified Folin–Ciocalteu assay with polyvinylpolypyrrolidone (PVPP) was employed to quantify total tannin content (TTC) by removing free polyphenols (Makkar et al., 1993). Samples were prepared in deionised water at a concentration of 2 mg/ml and mixed with a 10% PVPP solution. After vortexing, the mixtures were cooled at 4 °C for 15 min and centrifuged at 3000 × g for 10 min at 4 °C. The resulting pellet contained polyphenols bound to PVPP, while unbound tannins remained in the supernatant. The supernatants were subsequently diluted with methanol (1:1, v/v), and the TPC content was determined according to the procedure described above.

Qualitative LC-MS

Chromatographic and mass spectrometric analyses were performed using an ultra-high-performance liquid chromatography system (Dionex, Leipzig, Germany) equipped with a diode array detector and coupled to an AmaZon SL ion trap mass spectrometer (UHPLC-DAD-MSn) via an electrospray ionisation interface (Bruker Daltonik GmbH, Bremen, Germany). UV–Vis spectra were recorded in the range of 200–450 nm. The mass spectrometer was operated under the following conditions: nebuliser pressure of 40 psi, drying gas flow rate of 9 l/min, nitrogen gas temperature of 134 °C, and capillary voltage of 4.5 kV. Mass spectra were acquired over a scan range of 70–2200 m/z. The mobile phase consisted of solvent A (H_2O/formic acid (100:0.1; v/v) and solvent B (acetonitrile/formic acid (100:0.1; v/v). Separation was carried out on a reversed-phase column (Kinetex XB-C18 Phenomex, Torrance, California, USA; 150 mm × 3.0 mm, 2.6 µm particle size) maintained at 25 °C. The gradient elution programme, with a constant flow rate of 0.3 ml/min, was as follows:

- or *L. salicaria:* 0–20 min, 1–10% B; 20–30 min, 10–20% B; 30–35 min, 25% B; 35–60 min 60% B (Piwowarski and Kiss, 2013)
- for *B. officinalis*: 0–30 min, 1-20% B; 30–40 min, 20–50% B; 40–50 min, 50–75% B; 50–60 min, 75–100% B (Pawłowska et al., 2020)

Dry extracts of *L. salicaria* and *B. officinalis* were prepared at a concentration of 10 mg/ml. The *L. salicaria* extract was dissolved in deionised water, and *B. officinalis* extract was dissolved in methanol:water (1:1 v/v). Both solutions were filtered through a 0.45 µm PVDF or PET syringe filter prior to analysis. The injection volume was 5 µl.

Ethical approval

The animal trial was conducted at the Institute of Animal Nutrition, Freie Universität Berlin, in accordance with national animal protection law. The study was authorised by the State Office for Health and Social Affairs (LaGeSo, STN 022/23 and STN 035/23) in Berlin, Germany. Health checks were performed twice daily by qualified staff. Animals were housed and managed in compliance with European Union Directive (2010/63/EU) on the protection of animals used for scientific purposes.

Animals, housing and husbandry

For the trial, 80 piglets of the German Landrace breed were randomly assigned to five feeding groups: control (CON; 7 males and 9 females), *L. salicaria* extract (LSE; 8 males and 8 females), *B. officinalis* extract (BOE; 9 males and 7 females), *L. salicaria* plant material (LSP; 7 males and 9 females), and *B. officinalis* plant material (BOP; 9 males and 7 females). The allocation ensured similar body weight in each pen. The suckling period before weaning lasted 24 ± 2 days. At the start of the trial, piglets had an average body weight of 7.74 ± 0.98 kg. Piglets were housed in pairs in eight pens per treatment with slatted floors. During the trial, the temperature in the pig facility was gradually reduced from 32 to 24.4 °C. The lighting regimen consisted of 16 h of light followed by 8 h of darkness. CON diet was formulated to meet or exceed the nutrient requirements of weaned piglets (GfE, 2006). Experimental groups received the basal diet supplemented with tannin-rich extracts or plant materials. Specifically, the LSE group was fed a diet containing 0.75% dried extract of *L. salicaria* (aerial parts), while the BOE group received 0.95% dried extract of *B. officinalis* (rhizomes). The LSP group was fed a diet with 4.98% dried *L. salicaria* (aerial parts), and the BOP group received 4.74% dried *B. officinalis* (rhizomes). The plant material in LSP and BOP were milled (MOD.EMC50 Magico, AMA S.p.A., San Martino in Rio, Italy) to a particle size of 1 mm, prior to inclusion in the diets. The additional tannin content in the experimental diets was 2 g per kg of feed. Diets were isocaloric, provided as mash, and offered *ad libitum* (Table 1). Celite was included as an indigestible marker for digestibility analysis. Piglets had *ad libitum* access to water.

Table 1. Ingredient composition of experimental diets (% as-fed basis)

	CON	LSE	BOE	LSP	BOP
Ingredients					
wheat	33.5	33.6	33.6	30.2	34.2
corn	32.0	32.0	32.0	34.2	30.4
HP Soybean meal	11.4	11.4	11.4	10.9	10.4
skim milk powder	10.0	10.0	10.0	10.0	10.0
rapeseed oil	3.34	3.34	3.34	3.40	2.56
calcium carbonate	1.30	1.30	1.30	1.18	1.15
monocalcium phosphate	1.00	1.00	1.00	1.00	1.00
vitamin-mineral premix[1]	1.20	1.20	1.20	1.20	1.20
cellulose	2.27	2.25	2.30	0.08	1.16
L-lysine	0.77	0.77	0.77	0.79	0.80
D-/L-methionine	0.40	0.40	0.40	0.40	0.41
threonine	0.49	0.49	0.49	0.50	0.51
tryptophane	0.20	0.20	0.20	0.20	0.20
Lythrum salicaria extract		0.75			
Bistorta officinalis extract			0.95		
Lythrum salicaria plant material				4.98	
Bistorta officinalis plant material					4.74
tixosil	1.10	0.35	0.09	0.03	0.22
celite	1.00	1.00	1.00	1.00	1.00

CON – control group, LSE – *Lythrum salicaria* extract group, BOE – *Bistorta officinalis* extract group, LSP – *Lythrum salicaria* plant group, BOP – *Bistorta officinalis* plant group; [1] contained per kg of premix: IU: vit. A 600 000, vit. D_3 120 000; mg: vit. E (tocopherol acetate) 8 000, vit. K_3 300, vit. B_1 250, vit. B_2 (riboflavin) 250, vit. B_6 (pyridoxin-HCl) 400, niacinamide 2500, folic acid 100, pantothenic acid (Ca d-pantothenate) 1000, choline (chloride) 80 000, mg iron (iron- (II)-sulfate monohydrate) 5 000, zinc (zinc sulfate) 5 000, manganese (manganese-(II)-oxide) 6 000, copper (copper-(II)-sulfate pentahydrate) 1 000, iodine (calcium-iodate) 45, selenium (sodium selenite) 35; g: sodium (NaCl) 130, magnesium (magnesium sulfate) 55; µg: vit. B_{12} (cyanocobalamin) 2 000, biotin 25 000

Performance and sampling

Piglets were fed the experimental diets for 13–14 days (dissection on days 13 and 14 of the trial). During this period, BW was recorded weekly, and ADFI, ADG, and FCR were calculated for each week. At the end of the trial, eight piglets per group were euthanised after anaesthesia with 20 mg ketamine-hydrochloride/kg BW (100 mg/ml; Ursotamin®, Serumwerk Bernburg AG, Germany) and 2 mg azaperon/kg BW (40 mg/ml; Stresnil®, Elanco GmbH, Germany), followed by intracardial injection of 4 ml T61 (tetracaine hydrochloride, 5 mg/ml, mebezonium iodide, 50 mg/ml, and embutramide, 200 mg/ml; Hoechst Veterinär, Germany) on two consecutive days. The pH values of digesta was measured in the stomach (proximal, distal, and mixed digesta), duodenum, proximal jejunum, distal jejunum, ileum, caecum, colon and in faeces using an electronic pH meter (SevenExcellence, Mettler-Toledo GmbH, Greifensee, Switzerland). The stomach was separated into three sections using two brackets and the pH electrode was inserted via the cardia or the pylorus (Table S1). For nutrient digestibility analysis, ileal and faecal samples were stored at −20 °C. Tissue samples from the duodenum and mid-jejunum were carefully rinsed with a 0.9% NaCl solution and then fixed in buffered formaldehyde (ROTI® Histofix 4.5%, Carl Roth GmbH + Co.KG, Karlsruhe, Germany).

Analytical methods

Feed and plant material were milled (ZM 200, Retsch GmbH, Haan, Germany) to a particle size of 0.5 mm. Feed, plant material and dried extracts were analysed according to the official standard methods of the Association of German Agricultural Analytics and Research Institutes (VDLUFA, 1976): 3.1 dry matter (DM), 8.1 crude ash, 5.1.1 ether extract, 7.2.1 starch (Tables 2, S2). The crude protein (CP) content was determined according to the Dumas method with regard to the Makro N-determination (VDLUFA, 1976; 4.1.2, Vario Max CN V7.3.9, Elementar Analysensysteme GmbH, Hanau, Germany). Fibre fractions – amylasetreated organic neutral detergent fibre, acid detergent organic fibre, and lignin, were analysed using an Ankom 2000 automated Fibre Analyzer (NY, USA) according to VDLUFA (1976; 6.5.1, 6.5.2, and 6.5.3). Crude fibre was determined according to AOCS method Ba 6a-05 (AOCS, 2017) using the Ankom 2000 system with ash correction. Total dietary fibre was analysed with a commercial enzymatic assay kit (Megazyme, K-TDF, Megazyme, Ireland) to determine total, insoluble, and soluble dietary fibre fractions. Sugar content in the extracts was analysed according to Commission Regulation (EC) No 152/2009 (Annex III J: 2009-01) by AGROLAB LUFA GmbH (Kiel, Germany). Moreover, the sugar profile was determined by an ion exchange chromatography with pulsed amperometric detection (gradient pump GP 40 and electrochemical detector ED 40; Dionex GmbH, Idstein Germany; Thermo Fisher Scientific Inc., Waltham, MA, USA) using a Dionex CarboPac™ PA-100 guard column and analytical column.

Calcium (Ca), sodium (Na) and potassium (K) were determined by an atomic absorption spectrometry (ContrAA 800, Analytic Jena AG, Jena, Germany) after dry ashing and acidification with HCl. Samples were diluted with a caesium chloride-lanthanum chloride buffer solution (Merck KGaA, Darmstadt, Germany). Phosphorus content in the feed was determined with the Vanadate-molybdate method (Gericke and Kurmies, 1952) using spectrophotometric (Ultospec 2100 pro, Amersham Biosciences Europe GmbH, Germany) measurements at 436 nm.

Celite, which was used as an indigestible marker, was analysed according to the VDLUFA method 8.2.

Amino acids were determined by ion-exchange chromatography using an amino acid analyser (Biochrom 30 Plus AA Analyzer, Biochrom Ltd., UK) with a lithium precolumn (5.503.601; Laborservice Onken, Gründau, Germany) and a lithium separation column (5.503.600; Laborservice Onken, Gründau, Germany). The samples were processed according to VDLUFA (1976) as briefly described below. For the determination of methionine and cysteine, samples were pre-treated for 24 h with H_2O_2 and formic acid to protect amino acids from oxidation. The oxidation reaction was stopped with sodium disulphide. Samples were then hydrolysed with 6 M

Table 2. Analysed nutrient composition of the experimental diets

	CON	LSE	BOE	LSP	BOP
Dry matter (DM), g/kg	904	896	894	895	891
Nutrient content, g/kg DM					
crude ash	72.8	63.8	61.1	60.1	62.1
crude protein	199	193	197	188	192
ether extract	52.1	51.3	49.5	54.8	43.7
crude fibre	40.4	44.0	42.5	43.5	38.0
starch	445	485	474	474	478
neutral detergent fibre	138	128	129	127	139
acid detergent fibre	56.4	52.5	54.5	61.5	60.4
acid detergent lignin	13.3	10.3	10.3	11.8	15.6
total dietary fibre	149	130	133	133	142
insoluble dietary fibre	137	115	105	121	125
soluble dietary fibre	7.01	14.6	27.9	11.9	16.7
Ca	8.92	7.79	8.82	9.35	8.98
total phosphorus	6.66	6.06	6.15	5.95	6.20
K	6.36	6.34	6.65	6.57	6.39
Na	2.18	2.10	1.89	1.93	2.10
Indispensable amino acids					
lysine	14.2	14.9	14.8	15.4	15.0
methionine	7.86	7.52	7.34	7.44	7.49
threonine	10.9	10.6	10.9	11.2	11.2
tryptophane	3.21	3.47	3.28	3.14	3.29
arginine	9.33	9.19	9.60	8.32	9.54
histidine	3.94	4.18	4.15	4.00	4.41
isoleucine	6.99	7.05	7.01	6.83	7.12
leucine	13.3	14.0	13.9	13.9	14.2
phenylalanine	7.76	7.61	7.82	7.80	7.91
valine	8.48	8.27	8.61	8.53	8.73
Dispensable amino acids					
alanine	6.71	7.01	7.16	7.33	7.28
aspartic acid	3.88	3.71	3.62	4.01	3.71
cysteine	3.22	3.12	3.23	3.47	3.42
glutamic acid	35.1	36.3	36.7	34.2	36.0
glycine	5.86	5.82	5.94	5.90	6.13
proline	11.5	13.2	11.7	11.5	12.4
serine	8.16	8.25	8.33	8.17	8.33
tyrosine	5.20	5.32	4.84	5.25	5.50
Metabolizable energy, calculated, MJ/ kg DM	15.2	15.2	15.3	15.3	15.5

CON – control group, LSE – *Lythrum salicaria* extract group, BOE – *Bistorta officinalis* extract group, LSP – *Lythrum salicaria* plant group, BOP – *Bistorta officinalis* plant group; metabolizable energy was calculated according to GfE (2008): ME (MJ/ kg DM) = 0.021503 × crude protein + 0.032497 × ether extract – 0.021071 × crude fibre + 0.016309 × starch + 0.014701 organic residue

HCl at 110 °C for 24 h. For the full amino acid profile (except tryptophan), samples were hydrolysed in the same manner; for tryptophan determination, 6 M NaOH was used at 110 °C for 24 h. Glucosamine and galactosamine in ileum digesta and faeces were detected with the same method (Table S3).

Apparent ileal digestibility and apparent total tract digestibility

Samples of ileal digesta and faeces were lyophilised and subsequently ground to pass through a 0.5 mm sieve. Crude protein, amino acids, celite and minerals were analysed as described for feed samples. Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) were calculated using the following formula:

$$\text{AID resp. ATTD (\%)} = 100 - 100 \times \times \frac{(\text{marker in diet} \times \text{nutrient in digesta})}{(\text{marker in digesta} \times \text{nutrient in diet})}.$$

Histology of duodenum and jejunum tissues

After 24–48 h, formaldehyde was replaced with 70% ethanol. Following dehydration, samples were embedded in paraffin and sectioned using a slide microtome (SM2000R, Leica Microsystems GmbH, Nussloch Germany) at 3 µm thickness. They were then mounted on glass slides, deparaffinised, hydrated and stained with alcian blue-PAS (ABPAS; 1.01646.0001, Merck KGaA, Darmstadt, Germany). Tissue morphometry of the duodenum and mid-jejunum and quantification of goblet cells in the mid-jejunum were performed using a photomicroscope (BX43F, Olympus, Tokyo, Japan) with a digital camera (Olympus DP72, Tokyo, Japan). Image analysis was conducted with cellSens imaging software (v.1.4, Olympus). Ten well-oriented villi were randomly selected and measured at 4× magnification, from the tip of the villus to the crypt mouth, to determine villus height. Crypt depth was measured at 10× magnification from the crypt mouth to the crypt base, along with the crypt area (ten crypt per animal). For the analysis of villus height, crypt depth, and crypt area, mean values per animal were calculated from ten measurements (Figure S1). Using this data, the villus height to crypt depth ratio was calculated (Liu et al., 2014). Goblet cells were counted in ten villi and ten crypts of the mid-jejunum and expressed as the number of cells per 10 000 µm^2. Cells stained in magenta or blue were considered goblet cells according to Liu et al. (2014). Mucin types were not differentiated. Cell counting was performed using QuPath software (version: v0.5.1) (Bankhead et al., 2017).

Jejunal tissue samples from the midsection were stained for eosinophils according to the protocol described by Tanaka et al. (2020). The staining solution was prepared by dissolving 0.8 g of Direct Red 23 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in 200 ml of 50% isopropyl alcohol, followed by the addition of 3.2 g of sodium sulphate. Eosinophil cells in the lamina propria were counted using the same microscope and camera system as described above (Figure S2).

Statistical analyses

Statistical analyses of performance parameters, pH values, AID, ATTD, and histological data were conducted using IBM® SPSS® Statistics (Version 28.0.1.0, Armonk, NY, USA). Outliers in the pH, AID and ATTD, galactosamine and glucosamine datasets, defined as values located more than 2.5 times below the first quartile or above the third quartile, were excluded from the analysis. Normal distribution was assessed using the Shapiro-Wilk test. Differences between feeding groups were determined using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Results are presented as mean values with the standard error of the mean (SEM). Differences were considered significant at $P \leq 0.05$.

Results

Phytochemical standardisation

Quantitative analysis of dry plant extracts. To standardise tannin content in extracts and plant materials, TPC and TTC were expressed per g of dry extract (Table 3). The *L. salicaria* extract contained a higher concentration of total polyphenols (275 µg/mg) compared to the *B. officinalis* extract (228 µg/mg). Tannin content followed the samepattern, with higher values recorded in *L. salicaria* (268 µg/mg) than in *B. officinalis* (211 µg/mg) (Table 3). The extraction yield of *L. salicaria* was approxi-mately 15%, corresponding to 6.70 g of plant material per 1 g of extract.

Table 3. Comparison of polyphenol and tannin content in extracts of *Lythrum salicaria* and *Bistorta officinalis*

Parameter	*Lythrum salicaria*	*Bistorta officinalis*
Total polyphenols, µg/mg extract	275	228
Total tannins, µg/mg extract	268	211
Extract yield, %	15	20
Conversion ratio, g plant/g extract	6.70	5.00
Polyphenols per 100 g plant, g	4.12	4.56
Tannins per 100 g plant, g	4.02	4.22

For *B. officinalis,* the yield was 20%, corresponding to 5.00 g of plant material per 1 g of extract. Based on these ratios, 100 g of *L. salicaria* plant material provided approximately 4.12 g of polyphenols and 4.02 g of tannins, while 100 g of *B. officinalis* rhizomes provided 4.56 g of polyphenols and 4.22 g of tannins. These results indicate that both *L. salicaria* and *B. officinalis* are rich sources of phenolic compounds, including tannins.

Qualitative analysis of dry plant extracts

Qualitative analysis using an ultra-high-performance liquid chromatography system equipped with a diode array detector and ion trap mass spectrometer (UHPLC-DAD-MS) was conducted to characterise the phytochemical profiles of *L. salicaria* and *B. officinalis* extracts. In the *L. salicaria* extract, a total of 14 compounds was identified (Figure S3), including 6 hydrolysable tannins, and *5 flavonoids*, indicating a presence of low-molecular-weight polyphenolic compounds, and 3 unidentified compounds (Table S4). Further characterisation of hydrolysable tannins in *L. salicaria* extract allowed the identification of vescalagin, castalagin, salicarinin A, salicarinin B, 1,6-di-O-galloylglucose, and ellagic acid (Piwowarski and Kiss, 2013). The *B. officinalis* extract contained 26 distinct compounds (Figure S4), including 9 classified as hydrolysable tannins, and 13 determined as condensed tannins, indicating a high proportion of polymeric polyphenols. One compound was classified as phenolic acid, and 3 compounds remained unidentified based on the available spectral data (Table S5).

Animal performance

Performance data of piglets are presented in Table 4. No significant differences in performance parameters were observed between the treatment groups.

Histological parameters

Morphological and histochemical parameters of the duodenum and mid-jejunum are presented in Table 5. Piglets in the BOE group had a higher crypt area in the jejunum compared to the LSP group ($P = 0.022$). No differences were observed in the other parameters.

Apparent ileal nutrient digestibility

The AID of CP, AAs, and minerals is summarised in Table 6. Tannin supplementation had

Table 4. Growth performance of weaned piglets

Performance	CON	LSE	BOE	LSP	BOP	SEM	*P*-value
Body weight day 0, kg*	7.69	7.79	7.72	7.72	7.77	0.11	0.999
Body weight day 13/14, kg*	9.45	9.86	9.22	9.52	9.38	0.15	0.757
Average daily gain, g/day*	131	153	113	133	119	6.82	0.382
Average daily feed intake, g/day$^{\#}$	230	262	226	291	283	10.0	0.125
Feed conversion ratio$^{\#}$	1.86	1.97	2.21	2.29	2.48	0.11	0.426

CON – control group, LSE – *Lythrum salicaria* extract group, BOE – *Bistorta officinalis* extract group, LSP – *Lythrum salicaria* plant group, BOP – *Bistorta officinalis* plant group; * mean calculated per piglet; $^{\#}$ mean value calculated per pen; SEM – standard error of the mean; ^{ab} – means with different superscripts in one row indicate significant differences between groups ($P < 0.05$)

Table 5. Histomorphological parameters in duodenum and jejunum of piglets

Parameter	CON	LSE	BOE	LSP	BOP	SEM	*P*-value
Duodenum							
villus height, μm	458	469	432	450	449	12.5	0.925
crypt depth, μm	569	565	494	565	505	12.6	0.135
villus height/crypt depth	0.82	0.84	0.88	0.81	0.91	0.03	0.799
crypt area, μm^2	29439	28122	22515	28946	22854	1130	0.116
Jejunum							
villus height [μm	473	471	472	474	461	12.7	0.998
crypt depth [μm	391	406	408	350	391	7.38	0.084
villus height/crypt depth	1.23	1.18	1.17	1.37	1.20	0.04	0.626
crypt area [μm^2	22137^{ab}	22026^{ab}	23029^{a}	16536^{b}	21351^{ab}	705	0.022
total goblet cells in villus per 10000 μm^2	1.95	2.49	2.43	1.98	1.84	0.12	0.319
total goblet cells in crypt per 10000 μm^2	15.0	17.0	14.8	14.8	14.0	0.48	0.376
total eosinophil cells per 10000 μm^2	13.2	15.9	15.7	15.6	15.4	0.61	0.655

CON – control group, LSE – *Lythrum salicaria* extract group, BOE – *Bistorta officinalis* extract group, LSP – *Lythrum salicaria* plant group, BOP – *Bistorta officinalis* plant group, SEM – standard error of the mean; ^{ab} – means with different superscripts in one row indicate significant differences between groups ($P < 0.05$)

Table 6. Apparent ileal digestibility of crude protein, amino acids and minerals, %

	CON	LSE	BOE	LSP	BOP	SEM	*P*-value
Proteins							
crude protein[#]	79.3	77.4	74.7	71.9	73.8	0.92	0.089
Indispensable amino acids[##]							
arginine	84.5^{a}	82.9^{a}	81.9^{a}	74.4^{b}	81.1^{ab}	0.92	0.003
histidine	83.0^{a}	81.3^{a}	78.6^{ab}	72.3^{b}	77.7^{ab}	0.99	0.004
isoleucine	75.5	75.5	71.5	69.3	74.1	1.07	0.291
leucine	79.2	79.4	75.3	72.9	76.4	0.97	0.177
lysine	84.3	84.9	83.2	82.6	84.5	0.58	0.735
phenylalanine	79.3	78.6	74.9	72.9	74.6	0.97	0.182
threonine	84.6	84.3	81.9	81.4	83.3	0.64	0.448
valine	78.4	77.5	74.9	73.4	76.5	0.91	0.451
Dispensable amino acids[##]							
alanine	66.9	67.4	63.1	61.9	65.9	1.31	0.639
aspartic acid	76.3	75.0	71.4	72.6	74.4	0.99	0.575
glutamic acid	87.8^{a}	87.0^{ab}	85.2^{abc}	81.0^{c}	81.3^{bc}	0.76	0.003
glycine	70.3	69.2	64.9	62.1	65.9	1.27	0.266
proline	87.1^{a}	87.1^{a}	79.6^{b}	76.5^{bc}	72.0^{c}	1.25	<0.001
serine	81.5	81.3	78.3	74.4	77.6	0.90	0.066
tyrosine	79.7	79.6	73.4	72.9	75.9	0.99	0.064
Minerals[###]							
Ca	59.9	57.6	66.0	74.5	71.7	2.37	0.112
P	51.4	55.9	54.0	60.9	65.1	1.77	0.089
K	73.4	69.7	70.3	73.8	73.3	1.59	0.901
Na	84.6^{a}	78.7^{ab}	82.2^{a}	63.3^{b}	75.7^{ab}	2.06	0.005

CON – control group, LSE – *Lythrum salicaria* extract group, BOE – *Bistorta officinalis* extract group, LSP – *Lythrum salicaria* plant group, BOP – *Bistorta officinalis* plant group; [#] missing data samples: BOE: 2; LSP: 2, BOP: 2; [##] missing data samples: CON: 2; LSE 2 (except cysteine: 3); BOE 3 (except methionine, cysteine, glycine: 4); LSP: 4; BOP: 4; [###] missing data samples: CON: 0 (except Ca: 1); BOE: 2 (except Ca: 3); LSP: 2; BOP; SEM – standard error of the mean; ^{ab} – means with different superscripts in one row indicate significant differences between groups ($P < 0.05$)

Table 7. Apparent total tract digestibility [%] of crude protein, amino acids and the minerals

	CON	LSE	BOE	LSP	BOP	SEM	*P*-value
Proteins							
crude protein[#]	79.3	77.4	74.7	71.9	73.8	0.92	0.089
Indispensable amino acids[##]							
arginine	84.5^{a}	82.9^{a}	81.9^{a}	74.4^{b}	81.1^{ab}	0.92	0.003
histidine	83.0^{a}	81.3^{a}	78.6^{ab}	72.3^{b}	77.7^{ab}	0.99	0.004
isoleucine	75.5	75.5	71.5	69.3	74.1	1.07	0.291
leucine	79.2	79.4	75.3	72.9	76.4	0.97	0.177
lysine	84.3	84.9	83.2	82.6	84.5	0.58	0.735
phenylalanine	79.3	78.6	74.9	72.9	74.6	0.97	0.182
threonine	84.6	84.3	81.9	81.4	83.3	0.64	0.448
valine	78.4	77.5	74.9	73.4	76.5	0.91	0.451
Dispensable amino acids[##]							
alanine	66.9	67.4	63.1	61.9	65.9	1.31	0.639
aspartic acid	76.3	75.0	71.4	72.6	74.4	0.99	0.575
glutamic acid	87.8^{a}	87.0^{ab}	85.2^{abc}	81.0^{c}	81.3^{bc}	0.76	0.003
glycine	70.3	69.2	64.9	62.1	65.9	1.27	0.266
proline	87.1^{a}	87.1^{a}	79.6^{b}	76.5^{bc}	72.0^{c}	1.25	< 0.001
serine	81.5	81.3	78.3	74.4	77.6	0.90	0.066
tyrosine	79.7	79.6	73.4	72.9	75.9	0.99	0.064
Minerals[###]							
Ca	59.9	57.6	66.0	74.5	71.7	2.37	0.112
P	51.4	55.9	54.0	60.9	65.1	1.77	0.089
K	73.4	69.7	70.3	73.8	73.3	1.59	0.901
Na	84.6^{a}	78.7^{ab}	82.2^{a}	63.3^{b}	75.7^{ab}	2.06	0.005

CON – control group, LSE – Lythrum salicaria extract group, BOE – Bistorta officinalis extract group, LSP – Lythrum salicaria plant group, BOP – Bistorta officinalis plant group; [#] missing data samples: CON 1, LSE 1, LSP 1; [##] missing data samples: CON 1, LSE 1, BOE 1, LSP 1, BOP 0; [###] missing data samples: CON 1, LSE 1 (except Na 2), BOE 0 (except Na = 1); LSP 1; SEM – standard error of the mean; ^{ab} – means with different superscripts in one row indicate significant differences between groups ($P < 0.05$) different superscripts in one row indicate significant differences between groups ($P < 0.05$)

no significant effect on most parameters. However, a significant difference was observed for proline (*P* = 0.044), with the CON group showing higher digestibility than the LSP and BOP groups. For most parameters (except for cysteine), all treatment groups (LSE, BOE, LSP, BOP) showed numerically lower digestibility compared to the CON group.

Apparent total tract nutrient digestibility

The ATTD results are presented in Table 7. Significant differences were observed for arginine, with higher ATTD values in the CON, LSE, and BOE groups than in the LSP group (*P* = 0.003). Histidine ATTD was lower in the LSP group compared with the CON and LSE groups (*P* = 0.004). For glutamic acid, the CON group showed higher digestibility than the two groups receiving whole plant material (LSP and BOP) (*P* = 0.003), while the LSE group showed higher values compared to the LSP group. Proline ATTD was highest in the CON and LSE group (*P* < 0.001). Moreover, the BOE group also showed higher proline digestibility than the BOP group. Regarding minerals, sodium ATTD was lower in the LSP group than in the CON and BOE groups (*P* = 0.005).

Discussion

This study evaluated the effects of dietary supplementation with tannin-rich whole plant materials and extracts from *L. salicaria* and *B. officinalis* on animal performance, nutrient digestibility, and gut health in weaned piglets. Despite the commonly reported antinutritive effects of tannin-rich botanical sources, no significant effects on growth performance were observed between treatment groups. This aligns with previous findings, where moderate levels of tannin supplementation (e.g., ≤ 0.5%) were reported to have limited influence on performance outcomes in piglets (Caprarulo et al., 2021; Deng et al., 2024). However, supplementation with 0.45% hydrolysable tannins from chestnut has been shown to improve FCR in weaned piglets after 28 days (Biagi et al., 2010). Moreover, low levels of tannin supplementation are considered to have a positive effect on feed intake and due to that on the performance (Huang et al., 2018; Hassan et al., 2020). Nevertheless, tannins are generally considered as antinutritive factors and are often associated with reduced feed palatability and intake due to their interaction with bitter taste receptors and salivary proteins (Huang et al., 2018; Girard and Bee, 2020; Hassan et al., 2020). Such effects were not apparent in the present study, which might be due to the relatively low inclusion level. In fact, ADFI in the LSP and BOP groups was numerically higher than in the CON and BOE groups, suggesting that the plant matrix or tannin type may have mitigated the astringent properties. Possibly, the extracted condensed tannins (BOE) may exerted a stronger effect on feed intake than tannins present in the plant material (BOP). This interpretation is consistent with literature reports that proline-rich proteins in saliva (Fanali et al., 2008; Cappai et al., 2013) react less with hydrolysable tannins, such as vescalagin and castalagin, present in *L. salicaria*, than for condensed tannins and gallotannins (Tang et al., 2003; Piwowarski et al., 2015), which could explain why ADFI was higher in the LSE and LSP groups. However, hydrolysable tannins derived from acorn have also been shown to bind to proline-rich proteins in a study with growing pigs (Cappai et al., 2013).

The effects of tannins on intestinal morphology appear to be highly variable and likely depend on both their source and dosage. This variability is also reflected in the present study, where the crypt area in the jejunum was lower in piglets from the LSP group than in those from the BOE group. No significant differences were detected for the other morphological parameters. Likewise, tannic acid from gallnut only led to an increase in the villus height at a higher inclusion level of 3 g/kg, while no effect was detected at 1.5 g/kg in the duodenum and jejunum. At the same time, crypt depth in the jejunum and ileum was reduced at 3 g/kg compared to the control (Deng et al., 2024). In another study, tannic acid supplementation (0.05, 0.1, 0.2 or 0.4%) did not affect crypt depth in the duodenum, jejunum, or ileum. However, villus height increased in the duodenum at 0.4% and in the jejunum at 0.05, 0.2 and 0.4% (Song et al., 2021). In contrast, supplementation with 0.2% tannic acid resulted in a decrease of crypt depth in the duodenum of weaned piglets, while 0.1% had no impact. Moreover, the villus height to crypt depth ratio was higher at 0.2%, with no effect observed in the jejunum (Yu et al., 2020). Similarly, the addition of encapsulated tannic acid to a weaner diet (1 g/kg) reduced crypt depth in the duodenum in comparison with the control group, while no difference in jejunum crypt depth was observed. In the latter study, the villus height to crypt depth ratio in the duodenum was elevated (Wang et al., 2020). In another work, dietary supplementation with chestnut tannins at 2.25 g/kg of in weaned piglets led to a reduction in the crypt depth in the ileum, whereas lower (1.13 g/kg) and higher

(4.5 g/kg) inclusion levels did not produce this effect (Biagi et al., 2010). This non-linear response suggests a possible dose-dependent threshold or biphasic effect of tannins on intestinal morphology. In contrast to previous trials, which focused on villus height and crypt depth, the current study additionally evaluated crypt area, an aspect of gut morphology that has received limited attention in the literature. This measure may provide additional information regarding structural changes in the intestinal mucosa in response to dietary tannins (Liu et al., 2014). Furthermore, feeding the treatment diets did not lead to any differences in jejunal tissue regarding total goblet cell counts in villi or crypts per 10 000 μm^2 or total eosinophil counts per 10 000 μm^2. Likewise, no differences were observed in glucosamine or galactosamine concentrations in ileal digesta or faeces. Digesta pH was largely unaffected, although piglets in the LSE group showed a significantly higher pH in the proximal jejunum than the CON animals.

In this study, piglets fed diets supplemented with plant material showed significantly reduced AID of proline, while diets supplemented with dietary plant extracts caused only a numerical reduction compared to the control diet. In growing pigs, short-term supplementation (8 days) with acorn (hydrolysable tannins) increased the secretion of proline-rich proteins in stomach digesta. These proline losses could have originated from saliva or gastric mucins, both characterised by proline content. It is therefore possible that proline-rich proteins may act as an endogenous buffer against tannins in pigs, because among other properties, proteins with a high proline content have a higher affinity for tannins (Cappai et al., 2013). In contrast, no differences in ADFI were observed between the treatment groups in the present study. This suggests that tannins did not elicit a bitter taste response or increased salivary secretion, possibly due to masking effects of the plant material or extract supplements. It is plausible that tannin-proline complexes were more stable in the diets containing plant material than in those with extracts. Under this assumption, the release of tannins from *L. salicaria and B. officinalis* plant material in the stomach may have interfered with digestibility, but not with feed intake. However, this remains speculative, as proline concentrations in stomach digesta and saliva were not determined. Nevertheless, the lower AID of proline observed in this study is consistent with previous reports showing that tannins affect endogenous amino acid secretion.

In a metabolic trial, piglets fed 4% quebracho extract showed a 300% increase in ileal endogenous nitrogen, accompanied by changes in amino acid composition, including elevated proline, arginine, and glycine levels. Proline, for example, increased from 68 to 115 g/160 g N compared to the control group. Apparent nitrogen digestibility was also reduced in the quebracho-supplemented group (Steendam et al., 2004). Similarly, reduced proline AID was observed in growing pigs (60 kg) fed diets containing increasing levels of condensed tannins from different sorghum varieties. In another study conducted by the same research group in growing pigs (53 kg), sorghum varieties with the highest tannin content caused negative or very low proline AID. In addition, these high-tannin varieties were associated with lower arginine and glycine AID compared with low-tannin varieties (Mariscal-Landín et al., 2004).

This effect was not observed in the current study, which may be related to the lower tannin levels of additionally 2 g tannins/kg. In comparison, hightannin sorghum varieties contain substantially higher tannin concentrations (47.2–57.1 g/kg) (Mariscal-Landín et al., 2004). On the other hand, another study reported no effect of tannin content in sorghum on proline AID in growing pigs. However, higher tannin levels reduced the AID of several other amino acids, including lysine, threonine, valine, histidine, arginine, serine, glutamic acid and aspartic acid (Pan et al., 2022).

Moreover, it should be noted that AID of CP and all amino acids (except cysteine in the BOP group) was numerically higher in the CON than in the tannin-supplemented groups. Regarding ATTD, significant differences between the groups were observed for arginine, histidine, glutamic acid and proline. The LSP group had the lowest values for arginine and histidine ATTD digestibility. Overall, the tannin groups (BOE, LSP, BOP) tended to show lower ATTD of CP and most amino acids compared with the CON group, with the exception of alanine. In addition, both plant material groups (LSP and BOP) showed lower ATTD values for glutamic acid and proline.

The antinutritive effect of tannins on CP digestibility in weaned piglets is inconsistent and appears to depend on tannin source and dosage. For instance, dietary supplementation with grape seed extract reduced AID of CP at 100 and 150 mg/kg compared to the control and 50 mg/kg group after 14 days of supplementation in the diet of weaned piglets. In addition, the CP ATTD after 14 days was significantly lower in the 100 mg/kg group in relation to the control and the 150 mg/kg group had the lowest value, whereas the 50 mg/kg group was intermediate between the control and the 100 mg/kg group (Li et al., 2020).

Comparable dose-dependent effects have been reported for tannic acid derived from gallnut. Inclusion levels up to 0.2% exerted no impact on ATTD of CP, while 0.4% caused a significant decrease. A linear relationship between dietary tannic acid content and crude protein digestibility was also observed (Song et al., 2021). In line with these findings, supplementation with 2 g/kg condensed tannins from black wattle resulted in CP ATTD comparable to the control (Souza et al., 2025). Considering individual amino acids, inclusion of 15 g/kg grape tannins in piglet diets resulted in a tendency towards lower AID of arginine and histidine, as well as numerically lower AID of glutamic acid. In contrast, AID of isoleucine and threonine was significantly lower in the tannin group compared to the control, while proline AID was not reported (Myrie et al., 2008). However, positive effects on CP digestibility have also been described. For example, coated chestnut tannins (with hydrogenated palm oil) increased ATTD of CP in weaned piglets (Xu et al., 2022). Likewise, supplementation with 150 mg/kg of grape extract also increased CP ATTD in 23-day-old piglets (Rajković et al., 2021). Taken together, these results suggest that plant material and extracts may differ in their effects on amino acid and CP digestibility. In the present study, the LSE group appeared to exert a less negative influence on both AID and ATTD of amino acids than the other tannin-supplemented groups.

With regard to mineral digestibility, no significant differences were observed between the groups for AID of Ca, P, K, and Na, or for ATTD of Ca, P, and K. In contrast, the ATTD of Na was significant higher in the CON and BOE groups compared to the LSP group. The absence of effects for most minerals is consistent with previous studies reporting that dietary tannin supplementation, particularly at moderate levels, does not affect mineral digestibility in weaned piglets. For example, coated tannins (1.5 g/kg) in a diet for weaned piglets resulted in ATTD values for crude ash, Ca, and P comparable to the control (Xu et al., 2022). Similarly, supplementation with 2 g/kg condensed tannins from black wattle did not alter mineral ATTD in 22-day-old piglets (Souza et al., 2025). Moreover, a chestnut tannin supplementation (4 g/kg tannic acid equivalents) for finishing pigs (153 kg) did not had an impact on crude ash ATTD (Galassi et al., 2019). In contrast, some studies have actually observed improved mineral digestibility. Supplementation with 150 mg/kg grape extract (> 40% total polyphenols, > 30% procyanidins) led to an improved ATTD of crude ash and P during the entire trial period (day 1–53/54), and calcium on days 25–26 (Rajković et al., 2021). These discrepancies may reflect differences in tannin source and inclusion level, with lower doses potentially favouring digestibility.

Conclusions

Overall, the findings suggest that *Lythrum salicaria* and *Bistorta officinalis* are safe for use in piglet diets at the applied inclusion levels, exerting moderate effects on nutrient digestibility under the study conditions. Further trials with longer feeding periods, different dosages, or combined approaches (e.g., encapsulation, synergistic additives) are required to fully validate the potential of these plants as feed components.

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Conflict of interest

The Authors declare that there is no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The Authors declare that AI-assisted tools were used solely to improve the language and clarity of the manuscript. Specifically, DeepL (free translator; https://www.deepl.com/de/translator) and ChatGPT (version: 5.2) were employed for linguistic refinement. No AI tools were used for data analysis, interpretation, or the generation of scientific content.

References

AOCS (American Oli Chemists‘ Society), 2017. Standard procedure Ba 6a-05. Crude fiber in feed by filter bag technique. IL, USA: AOCS Press

Bankhead P., Loughrey M.B., Fernández J.A. et al., 2017. QuPath: Open source, https://doi.org/10.1038/s41598-017-17204-5

Biagi G., Cipollini I., Paulicks B.R., Roth F.X., 2010. Effect of tannins on growth performance and intestinal ecosystem in weaned piglets. Arch. Anim. Nutr. 64, 121−135, https://doi.org/10.1080/17450390903461584

Cappai M. G., Wolf P., Pinna W., Kamphues J., 2013. Pigs use endogenous proline to cope with acorn (*Quercus pubescens* Willd.) combined diets high in hydrolysable tannins. Livest. Sci. 155, 316−322, https://doi.org/10.1016/j.livsci.2013.05.003

Caprarulo V., Giromini C., Rossi L., 2021. Review: Chestnut and quebracho tannins in pig nutrition: The effects on performance and intestinal health. Animal 15, 100064, https://doi.org/10.1016/j.animal.2020.100064

Degano I., Mattonai M., Sabatini F., Colombini M.P., 2019. A mass spectrometric study on tannin degradation within dyed woolen yarns. Molecules 24, 2318, https://doi.org/10.3390/molecules24122318

Deng Z.C., Wang J., Wang J., Yan Y.Q., Huang Y.X., Chen C.Q., Sun L.h., Liu M., 2024. Tannic acid extracted from gallnut improves intestinal health with regulation of redox homeostasis and gut microbiota of weaned piglets. Anim. Res. One Health. 2, 16−27, https://doi.org/10.1002/aro2.51

Directive 2010/63/EU, 2010. European Parlament, Council of the European Union.Directive, EUs, 2010. 63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union 276, 33−79

El-Shazly A., Ateya A. M., Wink M., 2001. Quinolizidine alkaloid profiles of Lupinus varius orientalis, L. albus albus, L. hartwegii, and L. densiflorus. Z. Naturforsch. C. J. Biosci. 56, 21−30, https://doi.org/10.1515/znc-2001-1-204

European Pharmacopoeia, 2019. European Pharmacopoeia 10th Edition., Council of Europe, Strasbourg

Fanali C., Inzitari R., Cabras T., Fiorita A., Scarano E., Patamia M., Petruzzelli R., Bennick A., Messana I., Castagnola M., 2008. Mass spectrometry strategies applied to the characterization of proline-rich peptides from secretory parotid granules of pig (*Sus scrofa*). J. Sep. Sci. 31, 516−522, https://doi.org/10.1002/jssc.200700343

Galassi G., Federico M., Luca R., Crovetto G.M.,Spanghero M., 2019. Digestibility and metabolic utilisation of diets containing chestnut tannins and their effects on growth and slaughter traits of heavy pigs. Ital. J. Anim. Sci. 18, 746−753, https://doi.org/10.1080/1828051X.2019.1570361

Gericke S., Kurmies B., 1952. Colorimetric determination of phosphoric acid with vanadate-molybdate (VM-Method) (in German). Z. Anal. Chem. 137, 15−22, https://doi.org/10.1007/BF00452421

GfE (Society for Nutritional Physiology, in German), 2006. Recommendations for energy and nutrient supply for pigs (in German). Frankfurt am Main, Germany: DLG-Verlags-GmbH

GfE (Society for Nutritional Physiology, in German), 2008. Prediction of metabolisable energy of compound feeds for pigs. Proceedings of the society of nutrition physiology 17

Girard M., Bee G., 2020. Invited review: Tannins as a potential alternative to antibiotics to prevent coliform diarrhea in weaned pigs. Animal 14, 95–107, https://doi.org/10.1017/S1751731119002143

Gresse R., Chaucheyras-Durand F., Fleury M.A., Van de Wiele T., Forano E., Blanquet-Diot S., 2017. Gut microbiota dysbiosis in postweaning piglets: Understanding the keys to health. Trends Microbiol. 25, 851–873, https://doi.org/10.1016/j.tim.2017.05.004

Hagermann A.E., 2002. Tannin chemistry. Miami University, Oxford, OH

Hassan Z.M., Manyelo T.G., Selaledi L., Mabelebele M., 2020. The effects of tannins in monogastric animals with special reference to alternative feed ingredients. Molecules 25, 4680, https://doi.org/10.3390/molecules25204680

Huang Q., Liu X., Zhao G., Hu T., Wang Y., 2018. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Anim. Nutr. 4, 137−150, https://doi.org/10.1016/j.aninu.2017.09.004

Huang Q., Yang W., Li X., Xie L., Tang D., Qian Z., 2024. Screening of α-glucosidase inhibitors in extracts of Polygonum bistorta based on spectrum-effect relationship. J. Exp. Clin. Appl. Chin. Med. 5, 69−81, https://doi.org/10.62767/jecacm504.6382

Ivanov S.A., Nomura K., Malfanov I.L., Sklyar I.V., Ptitsyn L.R., 2011. Isolation of a novel catechin from Bergenia rhizomes that has pronounced lipase-inhibiting and antioxidative properties. Fitoterapia 82, 212−218, https://doi.org/10.1016/j.fitote.2010.09.013

Lallès J.P., Bosi P., Smidt H., Stokes C.R., 2007. Nutritional management of gut health in pigs around weaning. P. Nutr. Soc. 66, 260−268, https://doi.org/10.1017/S0029665107005484

Li Q.H., Yan H.S., Li H.Q., Gao J.J., Hao R.R., 2020. Effects of dietary supplementation with grape seed procyanidins on nutrient utilisation and gut function in weaned piglets. Animal 14, 491−498, https://doi.org/10.1017/S1751731119002234

Liu P., Pieper R., Rieger J., Vahjen W., Davin R., Plendl J., Meyer W., Zentek J., 2014. Effect of dietary zinc oxide on morphological characteristics, mucin composition and gene expression in the colon of weaned piglets. PLOS One 9, e91091, https://doi.org/10.1371/journal.pone.0091091

Ma M., Chambers J.K., Uchida K., Ikeda M., Watanabe M., Goda Y., Yamanaka D., Takahashi S. I., Kuwahara M., Li J.Y., 2021. Effects of supplementation with a quebracho tannin product as an alternative to antibiotics on growth performance, diarrhea, and overall health in early-weaned piglets. Animals 11, 3316, https://doi.org/10.3390/ani11113316

Ma M., Enomoto Y., Takahashi T., Uchida K., Chambers J.K., Goda Y., Yamanaka D., Takahashi S.I., Kuwahara M., Li J., 2024. Study of the effects of condensed tannin additives on the health and growth performance of early-weaned piglets. Animals 14, 2337, https://doi.org/10.3390/ani14162337

Makkar H. P. S., Blümmel M., Borowy N. K., Becker K., 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric. 61, 161−165, https://doi.org/10.1002/jsfa.2740610205

Mariscal-Landín G., Avellaneda J.H., Reis de Souza T.C., Aguilera A., Borbolla G.A., Mar B., 2004. Effect of tannins in sorghum on amino acid ileal digestibility and on trypsin (E.C.2.4.21.4) and chymotrypsin (E.C.2.4.21.1) activity of growing pigs. Anim. Feed Sci. Tech. 117, 245−264, https://doi.org/10.1016/j.anifeedsci.2004.09.001

McDonald S., Prenzler P.D., Antolovich M., Robards K., 2001. Phenolic content and antioxidant activity of olive extracts. Food Chem. 73, 73−84, https://doi.org/10.1016/S0308-8146(00)00288-0

Myrie S. B., Bertolo R. F., Sauer W. C., Ball R. O., 2008. Effect of common antinutritive factors and fibrous feedstuffs in pig diets on amino acid digestibilities with special emphasis on threonine. J. Anim. Sci. 86, 609−619, https://doi.org/10.2527/jas.2006-793

Pan L., Feng S., Li W., Zhu W., 2022. Comparative digestion and fermentation characteristics of low-tannin or high-tannin sorghum grain in the porcine gastrointestinal tract. J. Anim. Sci. 100, skac300, https://doi.org/10.1093/jas/skac300

Pawłowska K. A., Hałasa R., Dudek M. K., Majdan M., Jankowska K., Granica S., 2020. Antibacterial and anti-inflammatory activity of bistort (*Bistorta officinalis*) aqueous extract and its major components. Justification of the usage of the medicinal plant material as a traditional topical agent. J. Ethnopharmacol. 260, 113077, https://doi.org/10.1016/j.jep.2020.113077

Piwowarski J.P., Granica S., Kiss A.K., 2015. Lythrum salicaria L.-Underestimated medicinal plant from european traditional medicine. A review. J. Ethnopharmacol. 170, 226−250, https://doi.org/10.1016/j.jep.2015.05.017

Piwowarski J.P., Kiss A.K., 2013. C-glucosidic ellagitannins from Lythri herba (European pharmacopoeia): Chromatographic profile and structure determination. Phytochem. Anal. 24, 336-348, https://doi.org/10.1002/pca.2415

Rajković E., Schwarz C., Tischler D. et al., 2021. Potential of grape extract in comparison with therapeutic dosage of antibiotics in weaning piglets: Effects on performance, digestibility and microbial metabolites of the ileum and colon. Animals 11, 2771, https://doi.org/10.3390/ani11102771

Rauha J.P., Wolfender J.L., Salminen J.P., Pihlaja K., Hostettmann K., Vuorela H., 2001. Characterization of the polyphenolic composition of purple loosestrife (Lythrum salicaria). Z. Naturforsch. C. J. Biosci. 56, 13−20, https://doi.org/10.1515/znc-2001-1-203

Regulation 152/2009. European Parliament, Council of the European Union, 2009. Regulation (EC) No 152/2009 of the European Parliament and of the Council of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union L54, 42−46

Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition, chapter II article 11. L268, 18/10/2003, https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0029:0043:EN:PDF (accessed at 21.07.2025)

Regulation 152/2009. European Parliament, Council of the European Union, 2009. Regulation (EC) No 152/2009 of the European Parliament and of the Council of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union L54, 42−46

Scalbert A., 1991. Antimicrobial properties of tannins. Phytochemistry 30, 3875−3883, https://doi.org/10.1016/0031-9422(91)83426-L

Song Y., Luo Y., Yu B. et al., 2021. Tannic acid extracted from gallnut prevents post-weaning diarrhea and improves intestinal health of weaned piglets. Anim. Nutr. 7, 1078−1086, https://doi.org/10.1016/j.aninu.2021.04.005

Souza K.L.D, Dias C.P., Callegari M.A., Friderichs A., Paes A.O.S., de Carvalho R.H., da Silva C.A., 2025. Performance and intestinal health of piglets in the nursery phase subjected to diets with condensed black wattle (*Acacia mearnsii*) tannin. Anim. Biosci. 38, 117−130, https://doi.org/10.5713/ab.24.0112

Steendam C.A., Tamminga S., Boer H., de Jong E.-J., Visser G.H., Verstegen M.W.A., 2004. Ileal endogenous nitrogen recovery is increased and its amino acid pattern is altered in pigs fed quebracho extract. J. Nutr. 134, 3076−3082, https://doi.org/10.1093/jn/134.11.3076

Tanaka T., Watanabe N., Kato T., Aoki R., Ogiso T., Sugiyama A., Tomita E., 2020. Utility of direct fast scarlet staining in the histopathological diagnosis of eosinophilic esophagitis: A short report. Gastrointest. Disord. 2, 448−455, https://doi.org/10.3390/gidisord2040040

Tang H.R., Covington A.D., Hancock R.A., 2003. Structure-activity relationships in the hydrophobic interactions of polyphenols with cellulose and collagen. Biopolymers 70, 403−413, https://doi.org/10.1002/bip.10499

VDLUFA, 1976. Methodology book Volume III. The chemical analysis of animal feed (in German). Darmstadt, Germany, VDLUFA-Verlag

Wang M., Huang H., Hu Y., Huang J., Yang H., Wang L., Chen S., Chen C., He S., 2020. Effects of dietary microencapsulated tannic acid supplementation on the growth performance, intestinal morphology, and intestinal microbiota in weaning piglets. J. Anim. Sci. 98, skaa112, https://doi.org/10.1093/jas/skaa112

Wang S.-T., Yang H., Gao W., Li H.-J., Li P., 2016. Trace enrichment and characterization of polyphenols in bistort rhizoma using weak anion-exchange solid phase extraction and high performance liquid chromatography-quadrupole time-of-flight mass spectrometry. J. Pharm. Biomed. Anal. 119, 91−98, https://doi.org/10.1016/j.jpba.2015.11.033

Xu T.T., Ma X., Zhou X.C., Qian M.Q., Yang Z.R., Cao P.W., Han X.Y., 2022. Coated tannin supplementation improves growth performance, nutrients digestibility, and intestinal function in weaned piglets. J. Anim. Sci. 100, skac088, https://doi.org/10.1093/jas/skac088

Yang W.-Q., Qian Z.-M., Wu M.-Q., Gao J.-L., Huang Q., Zou Y.-S., Tang D., 2023. Online microextraction coupled with HPLC-ABTS for rapid analysis of antioxidants from the root of *Polygonum bistorta*. Evid. Based. Compl. Alt. Med. 7496848, https://doi.org/10.1155/2023/7496848

Yang Y.-n., Li F.-s., Liu F., Feng Z.-m., Jiang J.-s., Zhang P.-c., 2016. A novel adduct of ECG fused to piceid and four new dimeric stilbene glycosides from *Polygonum cuspidatum*. Rsc. Adv. 6, 60741−60748, https://doi.org/10.1039/C6RA11135A

Yu J., Song Y., Yu B. et al., 2020. Tannic acid prevents post-weaning diarrhea by improving intestinal barrier integrity and function in weaned piglets. J. Anim. Sci. Biotechnol. 11, 87, https://doi.org/10.1186/s40104-020-00496-5

Yu S.J., Kong X.B., Jin X. et al., 2025. Systematic elucidation of the effective constituents and potential mechanisms of *Scrophulariae radix* against neoplasm based on lc-ms, network pharmacology, and molecular docking approaches. Front. Plant Sci. 16, 1615076, https://doi.org/10.3389/fpls.2025.1615076

Supplementary data

Table S1. pH-values in gastrointestinal tract compartments of piglets

pH$^{\#}$	CON	LSE	BO	LSP	BOP	SEM	*P*-value
Stomach proximal	4.50	4.53	4.61	4.36	4.44	0.10	0.963
Stomach distal	3.13	3.20	3.20	3.68	3.15	0.17	0.836
Stomach mixed	2.39	2.98	2.33	3.18	2.43	0.19	0.504
Duodenum	6.10	6.22	6.09	6.00	6.07	0.09	0.970
Jejunum proximal	5.91^{b}	6.70^{a}	6.13^{ab}	6.46^{ab}	6.42^{ab}	0.09	0.034
Jejunum distal	6.47	6.70	6.53	6.65	6.70	0.05	0.575
Ileum	6.60	6.87	6.54	6.69	6.76	0.06	0.536
Caecum	5.79	5.80	6.15	5.99	5.93	0.06	0.379
Colon	6.22	6.13	6.17	6.27	6.27	0.06	0.923
Faeces	6.96	6.63	6.66	6.45	6.73	0.07	0.173

CON – control group, LSE – *Lythrum salicaria* extract group, BOE – *Bistorta officinalis* extract group, LSP – *Lythrum salicaria* plant group, BOP – *Bistorta officinalis* plant group; # missing data samples: duodenum: CON: 1, LSE: 3, BOE: 1, BOP: 3, jejunum proximal: CON: 3; LSE: 2; BOE: 3, LSP: 1, BOP: 2; jejunum distal: LSE: 1, BOE: 2, LSP: 1, caecum: LSE: 2, faeces: CON: 1, LSE: 2; SEM – standard error of the mean; ^{ab} – means with different superscripts in one row indicate significant differences between groups ($P < 0.05$)

Table S2. Analysed crude nutrient content of extracts and plant materials

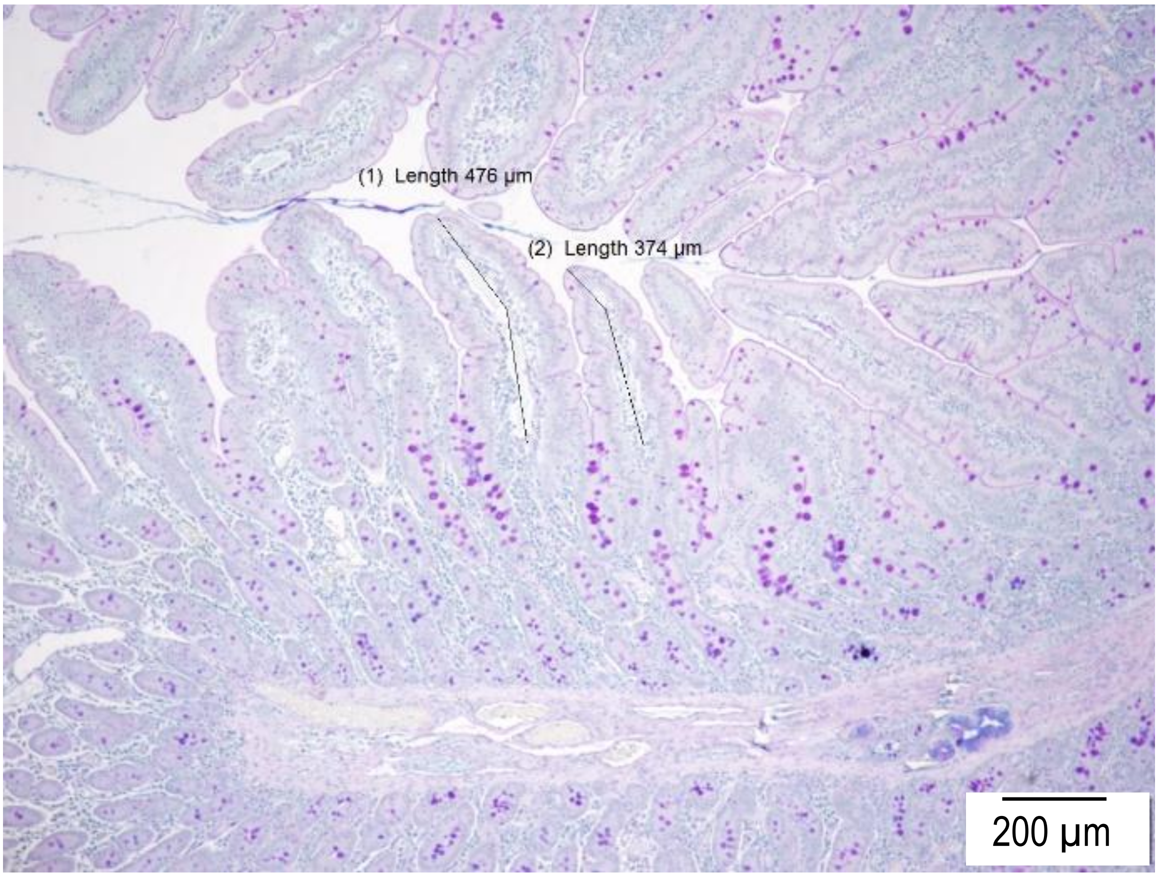
	Lythrum salicaria extract	*Bistorta officinalis* extract	*Lythrum salicaria* plant material	*Bistorta officinalis* plant material
Dry matter (DM), g/kg	932	930	932	923
Nutrient content, g/kg DM				
crude ash	164	58.8	59.3	39.6
crude protein	n.a.	n.a.	110	116
ether extract	n.a.	n.a.	20.8	5.83
crude fibre	n.a.	n.a.	286	70.0
sugar	239	535	50.4	176
glucose	73.0	99.9	n.a	n.a.
fructose	207	187	n.a.	n.a.
saccharose	7.22	156	n.a.	n.a.
starch	n.a.	n.a.	69.9	127
neutral detergent fibre	n.a.	n.a.	498	294
acid detergent fibre	n.a.	n.a.	324	173
acid detergent lignin	n.a.	n.a.	77.3	224
total dietary fibre	n.a.	n.a.	570	314
insoluble dietary fibre	n.a.	n.a.	532	271
soluble dietary fibre	n.a.	n.a.	44.1	42.4
calcium	10.2	4.06	12.8	12.9
total phosphorus	7.16	6.10	2.44	2.13
potassium	48.5	14.6	11.4	4.27
magnesium	6.63	4.25	2.68	1.84

n.a. – not analyzed

Table S3. Galactosamine and galactosamine concentration in the ileum digesta and faeces

	CON	LSE	BOE	LSP	BOP	SEM	*P*-value
Ileum digesta$^{\#}$							
galactosamine, g/kg DM	1.66	1.55	1.56	1.41	1.87	0.11	0.801
glucosamine, g/kg DM	2.09	2.21	2.22	1.95	2.25	0.13	0.963
Faeces$^{\#\#}$							
galactosamine, g/kg DM	0.82	0.70	0.81	0.65	0.82	0.03	0.283
glucosamine, g/kg DM	1.68	1.41	1.60	1.67	1.92	0.06	0.117

CON – control group, LSE – *Lythrum salicaria* extract group, BOE – *Bistorta officinalis* extract group, LSP – *Lythrum salicaria* plant group, BOP – *Bistorta officinalis* plant group; $^{\#}$ missing data samples: duodenum: CON 2; LSE 2; BOE 2; LSP 3; BOP 3; $^{\#\#}$ missing data samples: CON: 1; LSE 1 (except glucosamine: 2); BOE 1; SEM – standard error of the mean; ^{ab} – means with different superscripts in one row indicate significant differences between groups ($P < 0.05$)



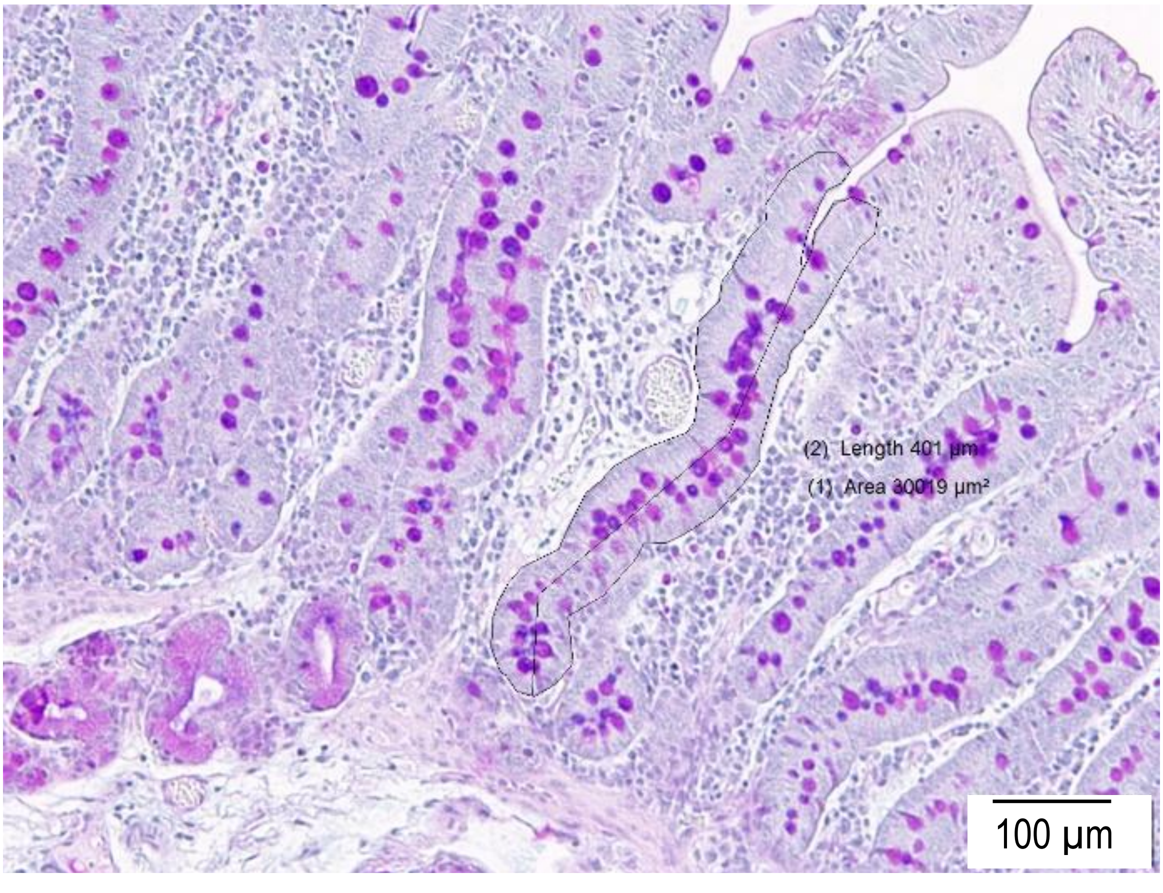


Figure S1. Example of a histological section of the duodenum after the alcian blue-PAS staining

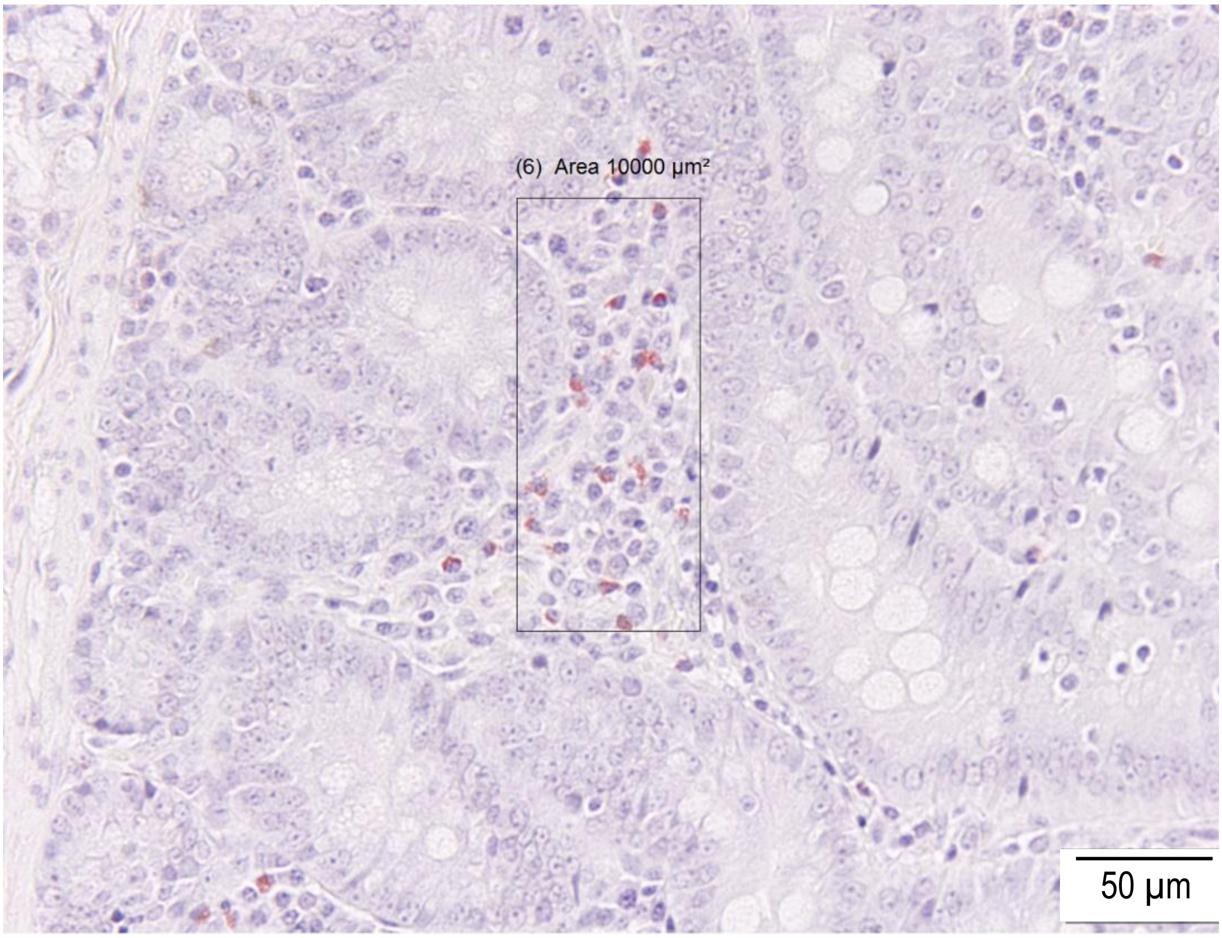


Figure S2. Example of a histological section of the duodenum after the staining for the eosinophilic cell counting

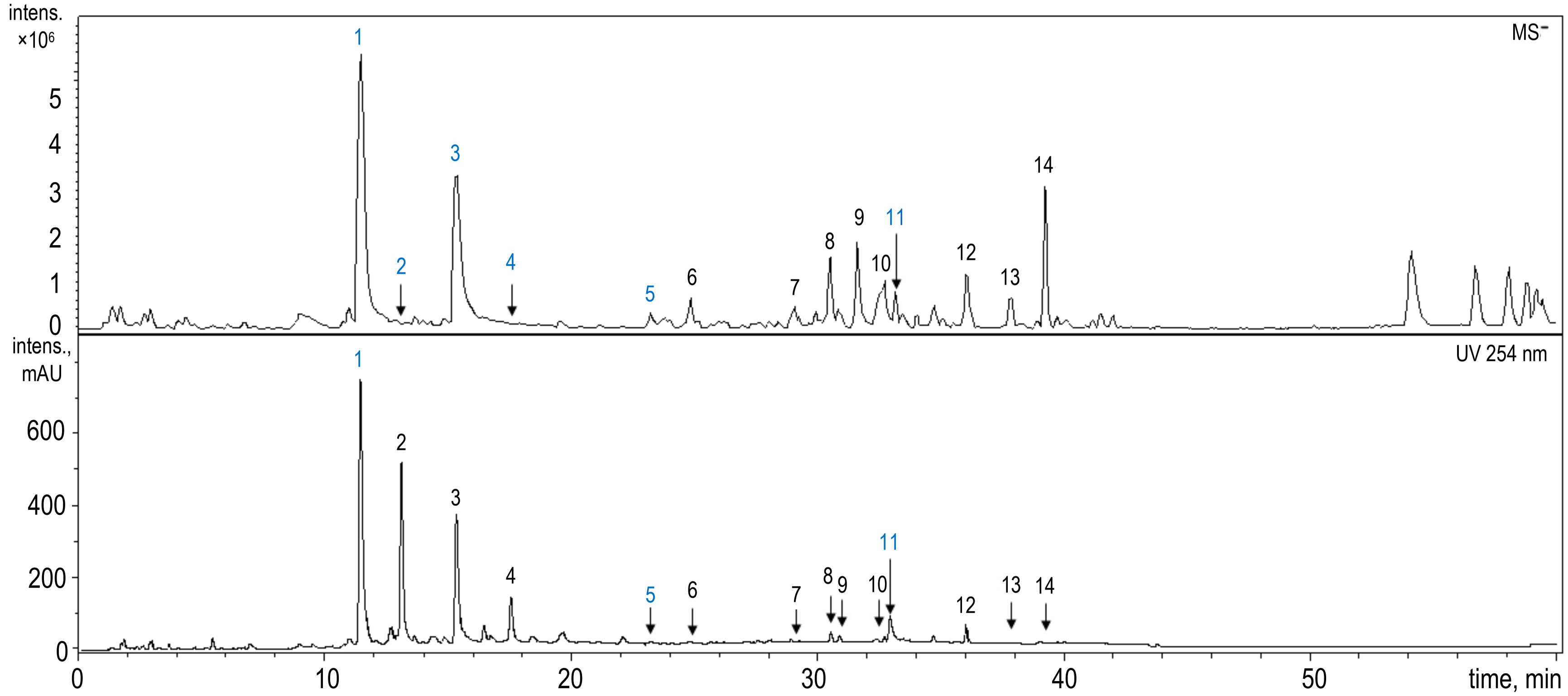


Figure S3. Chromatogram (254 nm) and MS- spectra of L. salicaria extract. Compounds are classified according to Table S4. Hydrolysable tannins are marked in dark blue.

Table S4. Retention times, MSn spectra and UV maxima of compounds found in *L. salicaria* extract. Bold stands for main peak.

Pn	Class	Retention time, min	UV-Vis, nm	[M-H] (-), m/z	MS2 ions	[M+H] (+), m/z	MS2 ions	Citation	Identification
1	HT	11.5	225	933, **466**	916, 872, 737, 614, 570, 491, 445, 301, 275	935	918, 615, 597, 535, 517, 505, 455, 437, 277	(Piwowarski and Kiss, 2013)	Vescalagin
2	HT	13.3	225	933, **466**	1490, 1391, 1219, 1087, 1083, 944, 916, 895, 569, 489, 343, 302			(Piwowarski and Kiss, 2013)	Salicarinin A
3	HT	15.3	204, 221	933, **466**	915, 898, 631, 570, 443, 346	935	918, 874, 704, 634, 616, 597, 554, 535, 495, 469, 455, 437, 303, 277	(Piwowarski and Kiss, 2013)	Castalagin
4	HT	17.6		933, **466**	1481, 915, 897, 632, 569, 441, 302			(Piwowarski and Kiss, 2013)	Salicarinin B
5	HT	23.2	217	483	647, 629, 465, 423, 397, 301			(Rauha et al., 2001; Piwowarski et al., 2015)	1,6-di-O-galloylglucose
6	F	24.9	217	431	385, 223, 205			(Rauha et al., 2001)	-
7	F	29.1	217	463	417, 381, 327, 301			(Piwowarski et al., 2015)	-
8	F	30.6	213, 349	447	429, 357, 327	449	431, 395, 383, 353, 329, 299	(El-Shazly et al., 2001)	-
9	-	31.7	218	497 (451)	451	453	435, 336, 226, 139		-
10	F	32.8	216, 265, 330	431	413, 341, 311	433	415, 379, 367, 337, 313, 283	(Rauha et al., 2001)	-
11	HT	33	219	**301**, 463	255, 463			(Piwowarski and Kiss, 2013)	Ellagic acid
12	F	36.1	216	607	299, 284	609	463, 301	(Piwowarski et al., 2015)	-
13	-	37.9	219	455	425, 375, 345, 260	457	439, 409, 359, 287, 189		-
14	-	39.3	216	439	421, 409	411	313, 287, 249, 189		-

Pn – peak number; Class: HT – hydrolysable tannin, F – flavonoid

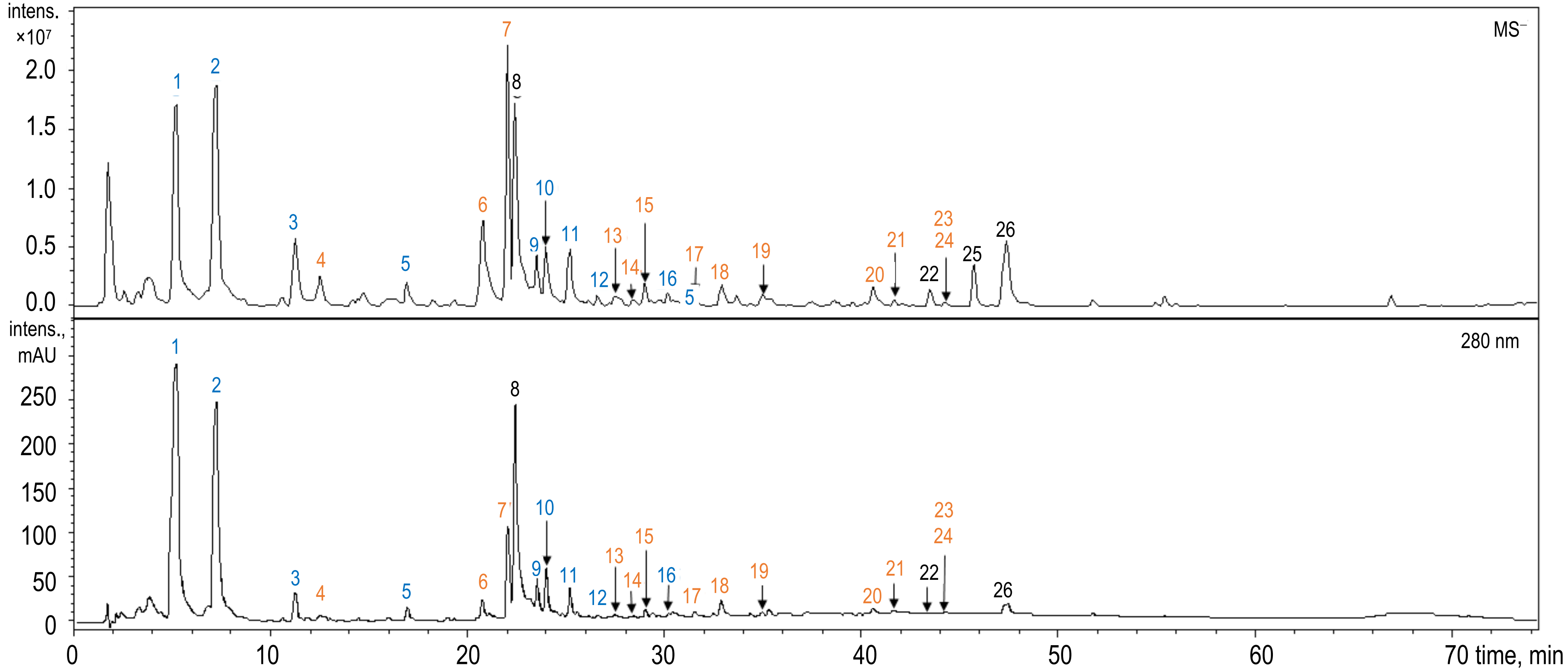


Figure S4. Chromatogram (280 nm) and MS- spectra of B. officinalis extract. Compounds are classified according to Table S5. Hydrolysable tannins are marked in dark blue, condensed tannins in orange.

Table S5. Retention times, MSn spectra and UV maxima and classification of compounds found in B. officinalis extract. Bold stands for main peak

Pn	Class	Retention time, min	UV-Vis, nm	[M-H] (-), m/z	MS2 ions	[M+H] (+), m/z	MS2 ions	Citation
1	HT	5.3	214, 272	331	271	-	-	(Wang et al., 2016)
2	HT	7.3	214, 272	331	271, 241	-	-	(Wang et al., 2016)
3	HT	11.3	213, 273	493	331, 271, 211, 193, 169	-	-	(Pawłowska et al., 2020)
4	CT	12.6	204, 273	593	467, 425, 407, 315, 289, 273	595	443, 425, 317, 305, 299, 291, 287	(Ivanov et al., 2011)
5	HT	17.0	212, 273	483	295, 235, 271, 211, 193	-	-	(Huang et al., 2024)
6	CT	20.9	200, 278	577	559, 451, 407, 425, 299, 289	579	417, 409, 355, 303, 289, 247	(Pawłowska et al., 2020)
7	CT	22.1	225, 278	289	-	291	-	(Yang et al., 2023)
8	PA	22.4	230, 290, 325	353	191	355	-	(Huang et al., 2024)
9	HT	23.5	211, 281	483	465, 313, 301, 271, 235, 211,193, 168	-	-	(Pawłowska et al., 2020)
10	HT	24.0	214, 276	483	373, 313, 301, 271, 211, 169	-	-	(Pawłowska et al., 2020)
11	HT	25.3	212, 274	483	439, 313, 287, 271, 169	-	-	(Degano et al., 2019)
12	HT	26.2	211, 275	453	437, 373, 327, 313, 298, 275, 179, 169	-	-	(Wang et al., 2016)
13	CT	27.5	209, 275	577	451, 425, 407, 381, 289, 245	579	561, 453, 427, 409, 301, 275, 247	(Yang et al., 2023)
14	CT	28.7	211, 277	745	727, 619, 593, 575, 557, 467, 457, 423, 405, 305	-	-	(Pawłowska et al., 2020)
15	CT	29.1	202, 225, 275	289	-	291	-	(Pawłowska et al., 2020)
16	HT	30.2	211, 275	453	416, 313, 299, 289, 271, 258, 221, 183, 169, 125	455	437, 401, 315, 273, 267, 249, 231, 213, 195, 183, 165, 141	(Wang et al., 2016)
17	CT	31.6	211, 275	603	585, 451, 361, 331, 313, 271, 241, 211	-	-	(Yang et al., 2016)
18	CT	33.0	208, 277	729	577, 559, 451, 437, 425, 407, 289	731	579, 441, 411, 301, 291, 271	(Pawłowska et al., 2020)
19	CT	35.1	209, 275	577	531, 451, 425, 407, 287	579	453, 409, 289, 163	(Pawłowska et al., 2020)
20	CT	40.7	209, 275	441	331, 313, 289, 273, 259, 245	443	273	(Yu et al., 2025)
21	CT	41.8	212, 275	881	729, 711, 603, 577, 559, 541, 441, 407, 289	-	-	(Pawłowska et al., 2020)
22	U	43.5	215, 275	487	441, 307, 205, 163, 145	-	-	-
23	CT	44.4	214, 277	613	451, 341	-	-	(Huang et al., 2024)
24	CT	44.4	214, 277	729	653, 603, 577, 559, 541, 451, 425, 407, 269	-	-	(Pawłowska et al., 2020)
25	U	45.8	214, 275	517	471, 307, 163, 145	-	-	-
26	U	47.4	209, 279	451	408, 341, 323, 299, 297, 231, 217, 189	453	343, 313, 301	-

Pn – peak number; Class: HT – hydrolysable tannin, CT – condensed tannin, PA – phenolic acids, U – unkown