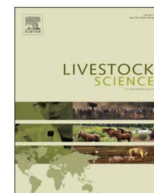
Livestock Science 203 (2017) 54–62

Contents lists available at ScienceDirect

Livestock Science

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



Hepatic proteome changes induced by dietary supplementation with two levels of native chicory inulin in young pigs

Agnieszka Herosimczyk[a,*], Adam Lepczyński[a], Małgorzata Ożgo[a], Marcin Barszcz[b], Edyta Jaszczuk-Kubiak[c], Mariusz Pierzchała[d], Anna Tuśnio[b], Jacek Skomiał[b]

[a] Department of Physiology, Cytobiology and Proteomics, West Pomeranian University of Technology, Doktora Judyma 6 Str., 71-466 Szczecin, Poland
[b] Department of Animal Nutrition, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Instytucka 3 Str., 05-110 Jabłonna, Poland
[c] Department for Large Animal Diseases with Clinic, Warsaw University of Life Sciences, Nowoursynowska 100 Str., 02-797 Warsaw, Poland
[d] Department of Genomics, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Postępu 36a Str., 05-552 Jastrzębiec, Poland

ARTICLE INFO

Keywords:
Pig
Liver
Proteome
Plasma liver enzymes
Inulin-type fructans

ABSTRACT

Inulin-type prebiotics are known as health-promoting dietary components and still remain a promising alternative to replace antibiotics for improving performance of young animals. Recent studies have proven that dietary inulin may positively affect the liver transcriptomic profile. However, its effect on the hepatic proteome in growing pigs is largely unknown. Therefore, the aim of the study was to gain insight into the effect of dietary supplementation with two inulin levels on the accumulation of the hepatic proteins and expression of selected genes. The experiment was carried out on a total of 24 castrated male piglets, which were assigned to three groups, fed from the 10th day of life an unsupplemented cereal-based diet or diets supplemented with 1% or 3% of native chicory inulin. Liver and blood samples were collected after 40 days of feeding and liver proteins were resolved using two-dimensional electrophoresis. To check whether modifications in accumulation of selected proteins were due to higher levels of mRNA and not due to decrease in protein degradation, real-time PCR was employed. Feeding diet with 3% of inulin induced significant down-accumulation of proteins involved in cytoskeleton organization and four isoforms of 14-3-3 protein and its mRNA. Both levels of inulin had also the potential to induce the expression of genes and accumulation of proteins directly or indirectly involved in controlling hepatic triglycerides level by increasing its lipolysis to fatty acid and glycerol. Dietary supplementation with inulin significantly increased liver cholesterol and TG concentration but decreased total plasma cholesterol and HDL cholesterol levels, and additionally reduced fibrinogen concentration, especially at 3% dietary level. Inulin level did not affect plasma liver enzymes, albumin, total protein content and immunoglobulin G levels. In conclusion, 1% of inulin is not sufficient to exert its effects on changes in hepatic protein expression, whereas feeding diet with the 3% inulin addition down-regulates expression of proteins involved in organization of cytoskeleton in hepatocytes and affects lipid metabolism in the liver causing alterations in expression of specific proteins, greater accumulation of cholesterol and TG, and changes in lipid profile of blood plasma.

1. Introduction

Inulin-type prebiotics are known as health-promoting dietary components and still remain a promising alternative to replace antibiotics for improving performance of young animals. Fermentation of inulin by the intestinal microbiota results in an increased formation of short chain fatty acids (SCFAs) of which acetate, propionate and butyrate are the most abundant and may comprise 90–95% of all produced SCFAs. Butyrate is almost completely oxidized by the colonic epithelial cells, but acetate and propionate are absorbed into the bloodstream and taken up by various peripheral tissues and organs, including liver, where they are involved in various physiological processes. For instance, Yasuda et al. (2009) reported that feeding weanling pigs with a corn-soy, iron-deficient diet containing 4% of inulin of different chain length for 49 days caused significant changes in the expression of several genes in the liver including those involved in iron metabolism. Results of the study by Sevane et al. (2014) also indicated that supplementation of broiler diets with 5% of inulin for 34 days affected gene expression in the liver. In particular, inulin supplementation up-regulated genes involved in the synthesis of long chain fatty acids and down-

* Corresponding author.
E-mail address: agnieszka.herosimczyk@zut.edu.pl (A. Herosimczyk).

http://dx.doi.org/10.1016/j.livsci.2017.07.004
Received 12 January 2017; Received in revised form 3 July 2017; Accepted 5 July 2017
1871-1413/ © 2017 Elsevier B.V. All rights reserved.

regulated those participating in the degradation of long branched-chain fatty acids and hydrolysis of phospholipids. However, it still remains unclear whether similar effects can be determined at the protein level. Previously we have demonstrated that the diet supplemented either with 2% of chicory inulin extract (Herosimczyk et al., 2015) or dried chicory root (Lepczyński et al., 2015) caused changes in serum proteins involved in haemostasis and innate immune response. As liver is a major metabolic organ involved in a wide variety of physiological functions including lipid metabolism, innate immune defence and haemostasis, we hypothesized that the implementation of inulin to the diet of growing pigs will induce hepatic proteome changes. Therefore, we decided to assess the influence of feeding a diet supplemented with 1% and 3% of native chicory inulin on the expression of hepatic proteins and selected genes as well as plasma biochemical indices, including liver enzymes, in young pigs.

2. Material and methods

2.1. Animals and sample collection

The experiment was carried out on a total of 24 castrated male piglets (PIC × Penarlan P76), divided into three groups ($n = 8$). At the beginning of the trial piglets were kept with their sows (4 litters and 4 sows per group) in a farrowing pens, on a commercial farm. Sows and their litters were randomly allotted to the different treatments. The sows were in the third and fourth parity. Piglets were kept with sows until weaning at 28 day of life, and during the first 9 days of life received colostrum and milk of their mothers, while from the 10th day were offered an unsupplemented cereal-based diet (control group, C) or diet supplemented with 1% (treatment group 1, T1) or 3% of native chicory inulin (treatment group 2, T2). Inulin (Inulin Orafti®GR, BENEO GmbH, Mannheim, Germany) contained approximately 92% of inulin with degree of polymerization (DP) ≥ 10% and 8% of other carbohydrates (glucose, fructose and sucrose) and was introduced instead of corn starch as it is the most neutral from the point of view of microbiota activity and composition in the large intestine. The remaining components of the diet are presented in the Table 1. The composition of the diets was previously described by Barszcz et al. (2017). At 28 day of life piglets were weaned and weighed, and then 2 males from each litter were chosen, ear-tagged and transported to the experimental facility. Upon arrival, the animals were divided according to dietary treatment and placed in pens of 4 piglets each (2 pens per group) and fed the control and experimental diets for 22 days. Animals were given free access to feed and water. Body weight of animals was measured twice, at weaning and at the end of the experiment. Feed intake and health status was monitored every day during the experimental period. At the age of 50 days, at a final body weight of about 20 kg, animals were stunned by electric shock and then killed by exsanguination. The samples of mixed blood were collected during exsanguination into heparinized tubes and plasma for biochemical analyses was obtained by centrifugation at 3000 rpm for 10 min at 4 °C and stored at –40 °C until used. The samples of liver were taken from the left lobe immediately after sacrifice and washed twice with 0.9% NaCl and thereafter twice with 20 mM Krebs-HEPES buffer (99 mM NaCl, 4.69 mM KCl, 2.50 mM $CaCl_2$, 1.20 mM $MgSO_4$, 1.03 mM K_2HPO_4, 25 mM $NaHCO_3$, 5.60 mM D-(+)-glucose, pH 7.4). Subsequently, tissue samples were snap frozen in liquid nitrogen and then stored at –80 °C until further analysis. The experimental procedures were approved by the Local Commission of Ethics for the Care and Use of Laboratory Animals (No. 13/2012).

2.1.1. Nutrient analyses in the experimental diets

Nutrient contents were analysed by standard procedures of the Association of Official Analytical Chemists (AOAC) (2000) and the metabolizable energy of the diets was calculated based on chemical analyses and diet composition. Fructan content in the diets was determined using Fructan Assay Kit (Megazyme International Ireland, Bray, Ireland) according to the manufacturer's protocol.

Table 1
Composition and nutrient contents of experimental diets.

Ingredients, %	Diet		
	Control	1% of inulin	3% of inulin
Wheat	46.84	46.84	46.84
Barley	20	20	20
Corn starch	3	2	–
Full-fat soybean	5.90	5.90	5.90
Whey	9.70	9.70	9.70
Fish meal	4.00	4.00	4.00
Spray-dried blood plasma	4.00	4.00	4.00
Soybean oil	3.39	3.39	3.39
Calcium formate	0.30	0.30	0.30
Limestone	0.50	0.50	0.50
Dicalcium phosphate	0.60	0.60	0.60
Sodium chloride	0.07	0.07	0.07
L-lysine	0.61	0.61	0.61
DL-methionine	0.23	0.23	0.23
L-threonine	0.26	0.26	0.26
L-tryptophan	0.09	0.09	0.09
Mineral-vitamin premix*	0.40	0.40	0.40
Aroma	0.10	0.10	0.10
Native chicory inulin†	–	1	3
Nutrients (% dry matter)			
Dry matter	90.03	90.15	90.13
Crude ash	4.54	4.54	4.53
Crude protein	20.05	20.04	20.03
Ether extract	6.04	6.04	6.03
Crude fiber	1.52	1.52	1.51
Fructan	1.00	1.52	3.11
EM (MJ/kg)[B]	14.3	14.3	14.3

* Premix composition, per kg: vitamin A 600,000 IU, vitamin D3, 60,000 IU, vitamin E 3000 mg, vitamin K3 120 mg, vitamin B1 120 mg, vitamin B2 240 mg, vitamin B6 240 mg, nicotinic acid 1600 mg, pantothenic acid 800 mg, folic acid 160 mg, biotin 10 mg, vitamin B12 1.6 mg, choline chloride 12 g, Mg 3.2 g, Fe 6 g, Zn 5.6 g, Mn 2.4 g, Cu 6.4 g, I 40 mg, Se 16 mg, Co 16 mg.

† Native chicory inulin with an average degree of polymerization ≥ 10.

2.2. Protein extract preparation

Frozen liver samples were ground with steel beads in 2 ml tubes using a mechanical homogenizer (Tissue Lyser, QIAGEN) at a frequency of 22 000 Hz for 5 min. Next, samples were homogenized in 1500 µl of lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 1% w/v DTT, 2% v/v Biolyte, 1% v/v protease inhibitor cocktail, 0.1% v/v nuclease) at a frequency of 20 000 Hz for 60 min. Insoluble tissue debris were removed by centrifugation (4 °C, 15,000 g, 15 min) and the resulting supernatant was used for two-dimensional electrophoresis.

2.3. Two-dimensional electrophoresis (2-DE)

All gels were performed in duplicate. Total protein concentration was estimated by the modified Bradford assay (Bio-Rad Protein Assay, Bio-Rad). Samples containing 800 µg of proteins, were mixed with lysis buffer to the total volume of 650 µl and applied to 4–7, 24 cm ReadyStrip™ IPG Strips (Bio-Rad). Isoelectrofocusing was run by ramping the voltage to a maximum of 5000 V and finished at 90,000 Vh using a Protean® IEF Cell (Bio-Rad). Next, IPG strips were reduced with DTT in equilibration buffer (6 M urea, 0.5 M Tris/HCl, pH 6.8, 2% w/v SDS, 30% w/v glycerol and 1% w/v DTT) for 15 min and then alkylated with iodoacetamide (2.5% w/v) for 20 min. The second dimension was performed in 12% SDS polyacrylamide gels (20 × 25 cm) at 100 V for 17 h at 15 °C using a Protean Plus™ Dodeca Cell™ electrophoretic chamber (Bio-Rad). After 2-DE separation, the gels were stained with colloidal Coomassie Brilliant Blue G-250.

Download English Version:

https://daneshyari.com/en/article/5543005

Download Persian Version:

https://daneshyari.com/article/5543005

Daneshyari.com