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EFFECTS OF BUTYRATE ON THE INSULIN HOMEOSTASIS OF CHICKENS KEPT ON MAIZE- OR WHEAT-BASED DIETS

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The aim of the present study was to investigate the effects of butyrate as a feed supplement on the expression of insulin signalling proteins as potent regulators of metabolism and growth in Ross 308 broiler chickens fed maize- or wheat-based diets. Both diets were supplemented with non-protected butyrate (1.5 and 3.0 g/kg of diet, respectively) or with protected butyrate (0.2 g/kg of diet); the diet of the control groups was prepared without any additives (control). On day 42 of life, systemic blood samples were drawn for analyses of glucose and insulin concentrations, and tissue samples (liver, gastrocnemius muscle and subcutaneous adipose tissue) were taken for Western blotting examinations. The expression of key insulin signalling proteins (IRβ, PKCζ and mTOR) was assessed by semi-quantitative Western blotting from the tissues mentioned. The type of diet had a remarkable influence on the insulin homeostasis of chickens. The wheat-based diet significantly increased IRβ and mTOR expression in the liver as well as mTOR and PKCζ expression in the adipose tissue when compared to animals kept on a maize-based diet. IRβ expression in the liver was stimulated by the lower dose of non-protected butyrate as well, suggesting the potential of butyrate as a feed additive to affect insulin sensitivity. Based on the results obtained, the present study shows new aspects of nutritional factors by comparing the special effects of butyrate as a feed additive and those of the cereal type, presumably in association with dietary non-starch polysaccharide- (NSP-) driven enteric short-chain fatty acid release including butyrate, influencing insulin homeostasis in chickens. As the tissues of chickens have physiologically lower insulin sensitivity compared to mammals, diet-associated induction of the insulin signalling pathway can be of special importance in improving growth and metabolic health.

Key words: Butyrate, insulin signalling, broiler chicken

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The restriction of applying antibiotics and hormones as growth promoters in food-producing livestock gives an expanding space to the use of alternative feed additives, such as the short-chain fatty acid (SCFA) (n-)butyrate in animal production. Butyrate was proved to improve gut health by providing energy for the gastrointestinal epithelium (Roediger, 1982), by regulating cell proliferation and differentiation (Gálfi and Neogrády, 2001), and by enhancing the intestinal absorptive capacity and maintaining the eubiotic gut flora (Hu and Guo, 2007). In addition to free butyrate salts (primarily sodium butyrate), several protected forms (e.g. encapsulated butyrate and esterified derivates) are applied successfully in poultry farming (Antongiovanni et al., 2007; Chamba et al., 2014). Protected forms avoid rapid butyrate absorption from the crop, stomachs or duodenum by providing prolonged release mainly in the distal part of the gastrointestinal tract (Chamba et al., 2014). This SCFA is also the major end-product of microbial fermentation of carbohydrates in the large intestine that can be stimulated by the dietary uptake of resistant starch or non-starch polysaccharides (NSP) with NSP-degrading enzyme (xylanase, glucanase) supplementation to provide oligosaccharide substrates for butyrate-producing bacteria (Jamroz et al., 2002).

Soluble NSP content – mainly constituted by arabinoxylans – is higher in wheat than in maize. These compounds can only be degraded in animals by microbial fermentation (de Lange, 2000). Soluble NSPs – at moderately higher levels – have some adverse effects on digestion by increasing the viscosity of the digesta, decreasing passage rate, thus extending the time for bacteria to thrive (de Lange, 2000). However, they also have prebiotic effects providing substrates for probiotic bacteria. More available bypass substrates promote the microbial fermentation in the caecum, resulting in higher total SCFA and butyrate production (Molnár et al., 2015).

In association with its widespread epigenetic (Kien et al., 2008; Mátis et al., 2013*a,b*) and receptor-mediated (Thangaraju et al., 2009) effects, butyrate could greatly influence insulin homeostasis, primarily insulin signalling. Summarising the main steps of the signalling pathway, if the α subunit of the insulin receptor (IRα) binds insulin, the β subunit (IRβ) is being autophosphorylated due to its tyrosine kinase activity (White and Kahn, 1994). The subsequent phosphorylation of the insulin receptor substrate 1 (IRS-1) activates the MAP-kinase cascade and the phosphatidylinositol 3-kinase (PI3K) enzyme, catalysing the synthesis of phosphatidylinositol triphosphate (PIP_3) (White and Kahn, 1994). The elevating intracellular PIP_3 concentration results in the activation of the downstream elements Akt and – via protein kinase C (PKC) – mTOR (the mammalian target of rapamycin). PKC is mostly involved in the translocation of GLUT-4 containing vesicles, while atypical PKCζ can function either as a mediator or an inhibitor of the insulin signalling pathway depending on the activating stimuli (Turban and Hajduch, 2011). mTOR plays a central role in the stimulation of protein synthesis via p70 ribosomal S6 kinase (White and Kahn, 1994).

Orally administered butyrate was shown to increase systemic insulin sensitivity in mice (Gao et al., 2009). Furthermore, butyrate, applied in a daily oral bolus, could influence insulin signalling of chickens in a tissue-dependent manner, assumedly acting on glucose shifting by selectively increasing the glucose uptake of skeletal muscle via butyrate-associated insulin receptor β subunit (IRβ) up-regulation (Mátis et al., 2015). However, it is not yet elucidated if the type of diets (maize- or wheat-based) and/or butyrate as a feed additive may also affect insulin signalling and thus endocrine metabolic regulation in chicken. Notwithstanding that birds have greatly decreased systemic insulin sensitivity compared to mammals, this hormone is one of the most important regulators of metabolism and growth in chicken as well (Józefiak et al., 2010). Therefore, feed additives modifying insulin homeostasis, such as butyrate, may be potential effectors of growth via endocrine metabolic regulation.

In the present study it was hypothesised that butyrate as a feed additive and/or the application of different dietary cereal types (wheat vs. maize) may alter insulin homeostasis by influencing insulin signalling of various tissues. As a main goal, different forms (non-protected and protected form) of oral butyrate application were aimed to be compared with the effects of diet type (wheat- or maize-based) influencing caecal SCFA production. Monitoring the alterations of insulin homeostasis after dietary butyrate supplementation could highlight the underlying mechanisms beyond the growth-promoting activity of butyrate.

Materials and methods

Chemicals

Chemicals were purchased from Sigma-Aldrich (Munich, Germany) except when specified otherwise.

Animals and treatments

All animal procedures were carried out in strict accordance with the relevant international and national laws as well as with the institutional guidelines. Experimental procedures were approved by the County Food Chain Safety and Animal Health Directorate of Zala, Hungary (ZAI/100/1361-009/2013).

One-day-old male Ross 308 broiler chickens (176 birds, obtained from Gallus Company, Devecser, Hungary) were housed in metal pens (22 birds per pen) on wheat straw litter under controlled environmental conditions according to the recommendations of the breeder company (Aviagen, 2014), over the entire trial at the Georgikon Faculty of Pannonia University, Keszthely, Hungary.

Table 1

Ingredients and calculated nutrient composition of experimental diets in various phases of the study

Ingredient (g/kg diet)	Starter		Grower		Finisher	
	Maize-based	Wheat-based	Maize-based	Wheat-based	Maize-based	Wheat-based
Maize	459	187	526	143	576	111
Wheat	0	300	0	300	0	400
Barley	0	0	0	100	0	100
Soybean meal	285	229	317	236	253	181
Soybean oil	172	200	67	126	82	109
Corn gluten	10	10	0	0	0	0
Sunflower oil	30	30	50	55	50	60
L-Lysine	1	2	1	2	1	2
DL-Methionine	2	2	2	2	2	2
Limestone	17	17	15	15	15	15
MCP	16	15	14	13	13	12
Salt	3	3	3	3	3	3
Vitamin-mineral premix*	5	5	5	5	5	5
Axtra XB 201 enzyme	0	0.15	0	0.15	0	0.15
Total	1000	1000	1000	1000	1000	1000
Nutrient content						
ME_n (MJ/kg)	12.6	12.6	13.0	13.0	13.3	13.3
Crude protein	220.0	220.0	200.0	200.0	180.0	180.0
Crude fibre	33.0	33.0	30.4	33.6	30.0	32.0
Crude fat	84.2	85.3	85.5	96.0	88.8	97.2
Starch	312.7	331.5	349.0	351.0	376.7	390.0
Lysine	13.8	13.8	12.5	12.5	10.9	10.9
Methionine	5.3	5.1	5.2	5.0	5.2	5.1
Methionine + Cystine	9.2	9.2	8.8	8.8	8.6	8.6
Ca	10.0	10.0	9.0	9.0	8.5	8.5
Available P	5.0	5.0	4.5	4.5	4.2	4.2

*Vitamin-mineral premix was supplied by Visonka Kft. (Páhi, Hungary). The active ingredients contained in the vitamin-mineral premix were as follows (per kg of diet): Vitamin-mineral premix in starter and grower feed: Vitamin A: 2.4×10^6 IU, Vitamin D_3: 8×10^5 IU, Vitamin E: 1×10^4 IU, Vitamin K_3: 4×10^2 IU, Monensin Na: 2×10^4 mg, Phyzyme Phytase: 2.5×10^4 mg, Zn: 1.2×10^4 mg, Cu: 3×10^3 mg, Fe: 5×10^3 mg, Mn: 1.8×10^4 mg, Se: 6×10^1 mg; Vitamin-mineral premix in finisher feed: Vitamin A: 9×10^5 IU, Vitamin D_3: 3×10^5 IU, Vitamin E: 3.75×10^3 IU, Vitamin K_3: 1.5×10^2 IU, Phyzyme Phytase: 2.5×10^4 mg, Zn: 1.2×10^4 mg, Cu: 3×10^3 mg, Fe: 5×10^3 mg, Mn: 1.8×10^4 mg, Se: 6×10^1 mg

Animals were randomised into eight experimental groups (n = 22/group): two different basal diets were set in all three dietary phases (starter, grower and finisher) of the experiment: a maize-based control (MBC) and a wheat-based control (WBC) diet and the following treatment groups were formed: MBC +

1.5 g/kg butyrate (MBB1.5), MBC + 3 g/kg butyrate (MBB3), MBC + 0.2 g/kg protected butyrate (MBPB) (Butipearl®, micro-encapsulated form, Kemin Industries, Des Moines, Iowa, USA); WBC + 1.5 g/kg butyrate (WBB1.5), WBC + 3 g/kg butyrate (WBB3) and WBC + 0.2 g/kg protected butyrate (WBPB). All diets were isocaloric, isonitrogenous and were defined according to the requirements of broiler chickens (NRC, 1994). Ingredients and calculated nutrient contents of the diets are shown in Table 1.

The lower concentration of non-protected butyrate complies with the usually applied average dose in poultry nutrition (Mátis et al., 2013*b*), while the application of the higher dose was aimed to test the dose dependence of butyrate effects. The dosage of protected butyrate was determined as indicated by the manufacturer. During the entire 42-day period of the study feed and water were provided *ad libitum*.

Body weights of chickens were recorded on days 1, 10, 24, 35 and 42; feed intake was measured per pen. Birds were in good health condition during the study, no signs of illness were observed in any of the experimental groups. The mean body weight and mean feed intake of chickens are presented in Table 2.

Samplings

Blood samples were drawn from the brachial vein of six randomly selected chickens per group (without previous feed deprivation) into heparinised tubes prior to slaughtering on day 42; blood plasma was separated by immediate centrifugation (2000 *g*, 10 min) and stored at –80 °C until measurement of plasma glucose and insulin concentrations. After slaughtering chickens by decapitation in carbon dioxide anaesthesia, liver, skeletal muscle *(m. gastrocnemius)* and subcutaneous adipose tissue samples were also collected from the same birds as used for the previous blood sampling (n = 6/group). All tissue samples were shock-frozen in liquid nitrogen and kept at –80 °C until further examinations.

Plasma measurements

Plasma glucose concentrations were determined by the colorimetric Fluitest Glucose Assay kit (Analyticon, Lichtenfels, Germany), while plasma insulin levels were measured by a chicken-specific insulin ELISA kit (MyBioSource, San Diego, CA, USA) with intra- and inter-assay variations below 15%, according to the manufacturer's instructions.

Western blot analyses of insulin signalling proteins

The protein expression of key members of the insulin signalling pathway, e.g. that of insulin receptor β (IRβ), atypical protein kinase C zeta (PKCζ) and the mammalian target of rapamycin (mTOR) was assessed from liver, skeletal muscle and subcutaneous adipose tissue samples by semiquantitative Western

blotting in duplicates as described previously (Mátis et al., 2015). Briefly, after tissue homogenisation, lysates were diluted to equal protein concentrations (liver: 1.5 µg/µl, muscle: 2 µg/µl, adipose tissue: 0.5 µg/µl) and processed with (mTOR) or without (IRβ and PKCζ) heat denaturation (Mátis et al., 2015). Proteins were separated by PAGE and blotted to nitrocellulose membranes, which were subjected for blocking in 10% (IRβ and PKCζ) or 5% (mTOR) fat-free milk-containing PBST for 90 min at room temperature. An overnight incubation was performed at 4 °C with the following primary antibodies at concentrations suggested by the manufacturer: IRβ (Santa Cruz Biotechnology, CA, USA), 1:100 in 5% fat-free milk/PBST; PKCζ (Santa Cruz Biotechnology, CA, USA), 1:200 in 5% fat-free milk/PBST; mTOR (Cell Signaling, Frankfurt, Germany), 1:250 in 5% BSA/ PBST. Primary antibodies were detected with the application of an anti-rabbit secondary antibody coupled with horseradish peroxidase (1:2000 in 5% fat-free milk/PBST) for 1 h at room temperature. Finally, chemiluminescence was generated with the SuperSignal West Dura Extended Duration Substrate (Pierce, Rockford, IL) in case of IRβ, whereas with Lumiglo Chemiluminescent Substrate (KPL, Gaithersburg, MA) for PKCζ and mTOR. Band detection and quantification were carried out as described previously, trace quantities were standardised to the Indian ink stained bands.

Statistical analyses

Statistical analyses of data were performed with R 2.14.0 software. Two-way ANOVA was applied to evaluate the main effect (i.e. an effect not conditional on butyrate supplementation) of wheat-based (WB) diet compared to maize-based (MB) diet. Means of the butyrate-supplemented groups were compared to control groups within the two basal diet types (MB and WB) by *post hoc* and Mann-Whitney tests. Significance level was set at $P < 0.05$. The results are expressed as means ± SEM.

Results

Plasma measurements

Insulin concentration of the blood plasma was significantly lower in chickens fed a WB diet than in those given an MB diet (main effect: $P = 0.001$, Fig. 1). However, blood glucose concentration was not significantly affected either by the diet type or by oral butyrate application (Fig. 1).

Insulin receptor β (IRβ) protein expression

Regarding the investigated insulin signalling elements, the protein expression of IRβ was significantly increased by the WB diet compared to the MB diet in the liver (main effect: $P = 0.034$, Fig. 2.1), but no significant alterations could

Table 2

Mean body weight and mean feed intake of broiler chickens fed with different diets

	Maize				Wheat			
	MBC	MBB1.5	MBB3	MBPB	WBC	WBB1.5	WBB3	WBPB
Mean body weight (g)								
Day 1	40.4 ± 0.4	40.0 ± 0.4	39.7 ± 0.4	40.0 ± 0.5	39.1 ± 0.4	40.0 ± 0.4	39.2 ± 0.4	39.3 ± 0.3
Day 10	260 ± 7	246 ± 7	247 ± 11	244 ± 8	252 ± 7	267 ± 6	269 ± 4	250 ± 9
Day 24	1194 ± 28	1207 ± 30	1099 ± 26	1068 ± 34	1247 ± 32	1284 ± 28	1305 ± 20	1211 ± 36
Day 35	2308 ± 54	2291 ± 56	2133 ± 43	2188 ± 55	2449 ± 61	2452 ± 57	2470 ± 33	2366 ± 60
Day 42	2776 ± 58	2711 ± 59	2664 ± 52	2762 ± 68	3077 ± 70	3003 ± 73	3110 ± 47	3079 ± 71
Mean feed intake (g/bird/day)								
Days 1–10	29	31	26	26	29	30	31	31
Days 10–24	101	96	96	92	103	101	103	99
Days 24–35	166	167	161	163	173	174	174	166
Days 35–42	144	140	151	159	172	163	180	186

MBC: maize-based control diet; MBB1.5: maize-based diet + 1.5 g/kg butyrate; MBB3: maize-based diet + 3.0 g/kg butyrate; MBPB: maize-based diet + 0.2 g/kg protected butyrate (Butipearl®, micro-encapsulated form, Kemin Industries, Des Moines, Iowa, USA); WBC: wheat-based control diet; WBB1.5: wheat-based diet + 1.5 g/kg butyrate; WBB3: wheat-based diet + 3.0 g/kg butyrate; WBPB: wheat-based diet + 0.2 g/kg protected butyrate (Butipearl®). Body weight was measured individually on days 1, 10, 24, 35 and 42. Data are expressed as mean ± SEM (standard error of mean). Mean feed intake was calculated as the feed intake in a certain period divided by the number in days between the two measurements and the number of birds concerning the diet group. Total number of chickens: n = 172

be detected in the other tissues (Figs 2.2 and 2.3). Oral butyrate supplementation affected the expression of IRβ in the liver: it was significantly up-regulated by the lower and higher doses of non-protected butyrate within the WB dietary group (P = 0.031 and P = 0.002, respectively; Fig. 2.1).

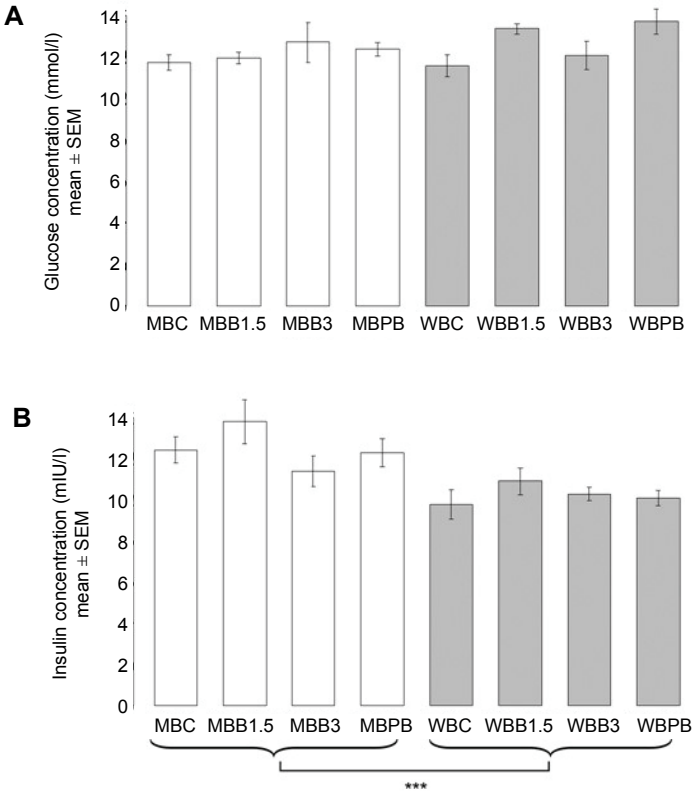

Fig. 1. Effects of diet type and butyrate supplementations on plasma glucose and insulin concentrations in broiler chickens. A. Glucose; B. Insulin. MBC: maize-based control diet, MBB1.5: maize-based diet + 1.5 g/kg butyrate, MBB3: maize-based diet + 3.0 g/kg butyrate, MBPB: maize-based diet + 0.2 g/kg protected butyrate (Butipearl®, micro-encapsulated form, Kemin Industries, Des Moines, Iowa, USA); WBC: wheat-based control diet, WBB1.5: wheat-based diet + 1.5 g/kg butyrate, WBB3: wheat-based diet + 3.0 g/kg butyrate, WBPB: wheat-based diet + 0.2 g/kg protected butyrate (Butipearl®). Asterisks at the columns indicate significant differences gained by *post-hoc* tests, asterisks at the braces indicate significant main effects of the diet. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; $^{\#}P < 0.10$

Mammalian target of rapamycin (mTOR) protein expression

Significant up-regulation of mTOR was found in the liver (main effect: P = 0.003, Fig. 2.1) and in the subcutaneous adipose tissue (main effect: P = 0.006, Fig. 2.2) of animals fed a WB diet compared to those kept on an MB diet.

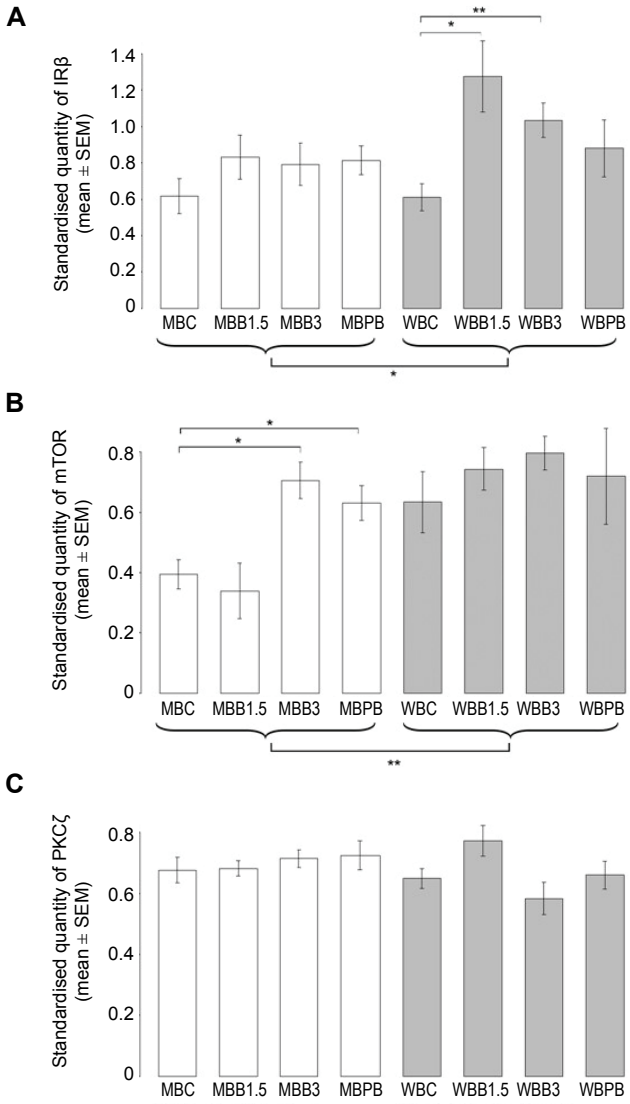

Fig. 2.1. Effects of diet type and butyrate supplementation on the expression of insulin signalling proteins in the liver of broiler chickens. Vertical (y) axis presents the standardised trace quantity values of (A) IRβ, (B) mTOR and (C) PKCζ protein expression. MBC: maize-based control diet, MBB1.5: maize-based diet + 1.5 g/kg butyrate, MBB3: maize-based diet + 3.0 g/kg butyrate, MBPB: maize-based diet + 0.2 g/kg protected butyrate (Butipearl®, micro-encapsulated form, Kemin Industries, Des Moines, Iowa, USA); WBC: wheat-based control diet, WBB1.5: wheat-based diet + 1.5 g/kg butyrate, WBB3: wheat-based diet + 3.0 g/kg butyrate, WBPB: wheat-based diet + 0.2 g/kg protected butyrate (Butipearl®). Asterisks at the columns indicate significant differences gained by *post-hoc* tests, asterisks at the braces indicate significant main effects of the diet. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; $^{\#}P < 0.10$

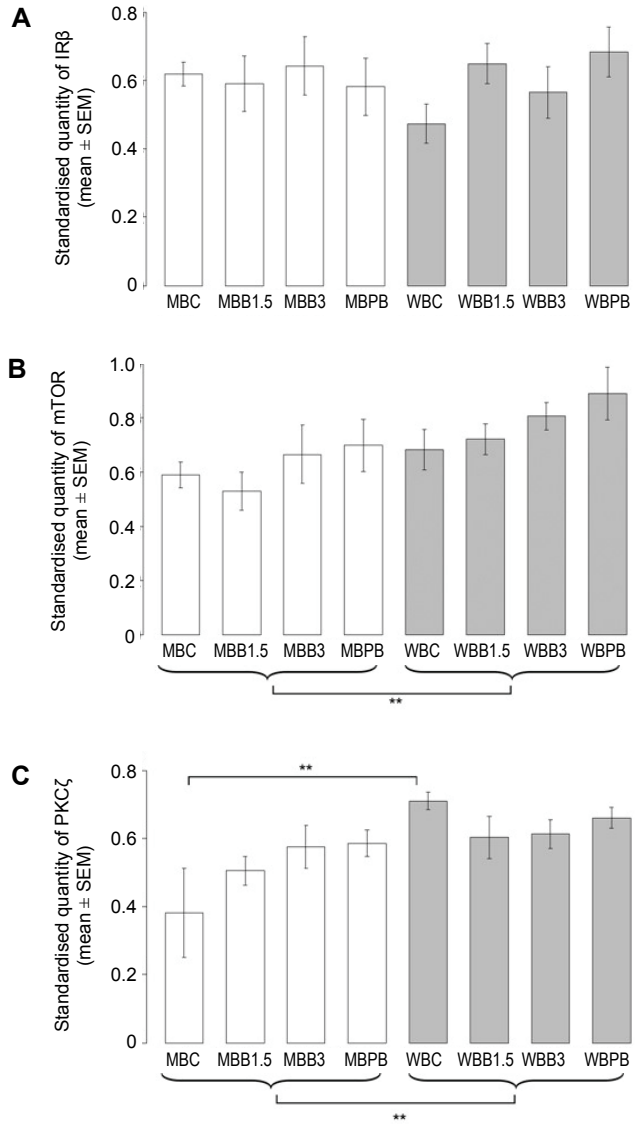

Fig. 2.2. Effects of diet type and butyrate supplementation on the expression of insulin signalling proteins in the adipose tissue of broiler chickens. Vertical (y) axis presents the standardised trace quantity values of (A) IRβ, (B) mTOR and (C) PKCζ protein expression. MBC: maize-based control diet, MBB1.5: maize-based diet + 1.5 g/kg butyrate, MBB3: maize-based diet + 3.0 g/kg butyrate, MBPB: maize-based diet + 0.2 g/kg protected butyrate (Butipearl®, micro-encapsulated form, Kemin Industries, Des Moines, Iowa, USA); WBC: wheat-based control diet, WBB1.5: wheat-based diet + 1.5 g/kg butyrate, WBB3: wheat-based diet + 3.0 g/kg butyrate, WBPB: wheat-based diet + 0.2 g/kg protected butyrate (Butipearl®). Asterisks at the columns indicate significant differences gained by *post-hoc* tests, asterisks at the braces indicate significant main effects of the diet. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; $^{\#}P < 0.10$

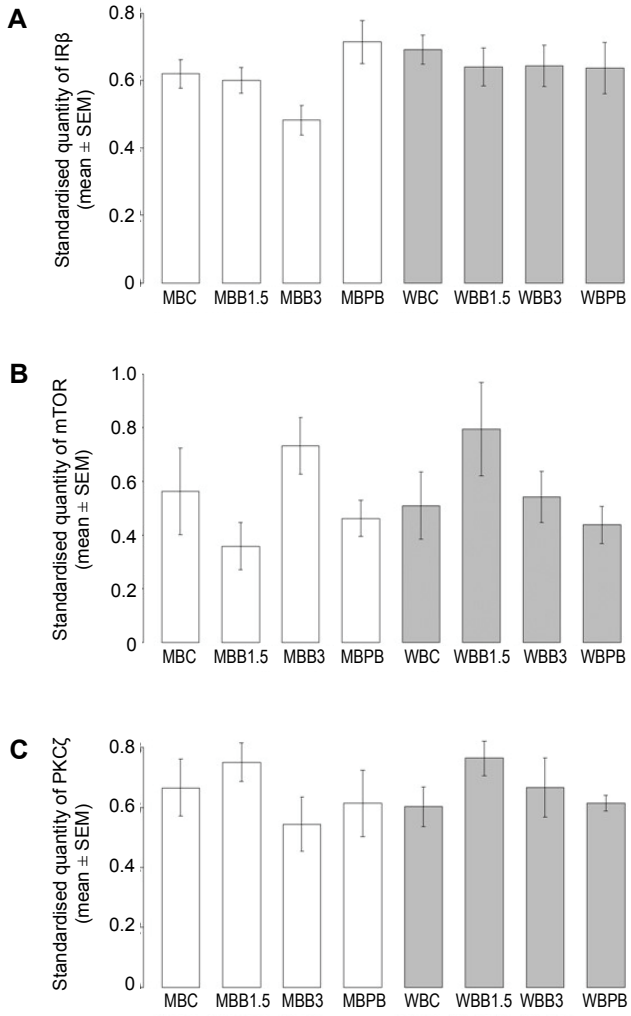

Fig. 2.3. Effects of diet type and butyrate supplementation on the expression of insulin signalling proteins in the skeletal muscle of broiler chickens. Vertical (y) axis presents the standardised trace quantity values of (A) IRβ, (B) mTOR and (C) PKCζ protein expression. MBC: maize-based control diet, MBB1.5: maize-based diet + 1.5 g/kg butyrate, MBB3: maize-based diet + 3.0 g/kg butyrate, MBPB: maize-based diet + 0.2 g/kg protected butyrate (Butipearl®, micro-encapsulated form, Kemin Industries, Des Moines, Iowa, USA); WBC: wheat-based control diet, WBB1.5: wheat-based diet + 1.5 g/kg butyrate, WBB3: wheat-based diet + 3.0 g/kg butyrate, WBPB: wheat-based diet + 0.2 g/kg protected butyrate (Butipearl®). Asterisks at the columns indicate significant differences gained by *post-hoc* tests, asterisks at the braces indicate significant main effects of the diet. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; $^{\#}P < 0.10$

However, no such alterations could be obtained in the skeletal muscle (Fig. 2.3). The hepatic expression of mTOR was significantly increased by the higher dose

of non-protected (P = 0.014) and protected (P = 0.036) butyrate within the MB dietary group.

Protein kinase C zeta (PKCζ) protein expression

PKCζ expression was significantly higher in the subcutaneous adipose tissue of WB-fed chicks compared to birds of the MB dietary groups (P = 0.006, Fig. 2.2), but no significant changes were found in the other tissues (Figs 2.1 and 2.3).

Discussion

Studying insulin homeostasis, a remarkable response to butyrate and to the diet type could be detected in our feeding trial. Concerning the feeding regime applied, it has been already described in our previous study, using the same diet types, that caecal total SCFA and butyrate concentrations are significantly, nearly twofold increased (caecal butyrate concentration from approx. 15 μmol/g to approx. 30 μmol/g) by the WB diet compared to chickens kept on the MB diet (Kulcsár et al., 2015). This finding is mainly associated with the different soluble NSP content of the applied cereals. The soluble arabinoxylan (NSP) content was determined to be 0.88 mg/g of maize and 9.37 mg/g of wheat, measured on the basis of the protocol of Douglas (1981). As indicated by these results, the application of different cereals (maize or wheat) as the main carbohydrate source altered the NSP content of the experimental diets; the MBC had a lower while the WBC a higher soluble NSP level. In the latter case, the elevated soluble NSP content, following its degradation to oligosaccharides by the supplemented xylanase and glucanase enzymes, provided substrates for caecal bacterial fermentation in order to enhance caecal SCFA production including butyrate. It has been thus justified that applying wheat as the main carbohydrate source can stimulate caecal microbial SCFA production (including the production of butyrate) by providing more substrates for the intestinal microbiota (Kulcsár et al., 2015). However, it should be taken into consideration that maize and wheat also greatly differ in some other parameters (such as amino acid and fatty acid profiles), thus the impact of certain further nutrients in the observed diet-associated changes cannot be excluded.

The expression of key insulin signalling proteins was found to be influenced by the diet type and butyrate application in certain tissues. IRβ and mTOR were significantly up-regulated in the liver, while in the subcutaneous adipose tissue of chickens kept on a WB diet compared to the MB groups, mTOR and PKCζ were significantly up-regulated. Dietary butyrate supplementation affected IRβ in the liver, where both doses of non-protected butyrate application increased protein expression in birds fed the WB diet, compared to the WB control birds. Furthermore, mTOR was significantly up-regulated by the higher dose of

non-protected butyrate and by the protected forms of butyrate within the experimental group fed the MB diet. Considering these results, the significant alterations of protein expression levels were mainly associated with the cereal type, suggested to be in connection with the enhanced caecal SCFA production (including butyrate) in the WB groups.

Regarding the investigated blood plasma parameters, no significant differences were found in blood glucose concentration between the experimental groups; however, in our earlier chicken studies, butyrate, applied in a daily oral bolus, increased blood glucose concentration (Mátis et al., 2015). Insulin concentration in the blood plasma was significantly decreased in the WB dietary groups compared to chickens fed an MB diet; however, it was unaffected by butyrate supplementation.

Although birds are known to be less insulin sensitive than mammals (Braun and Sweazea, 2008), our results may suggest that the increased expression levels of insulin signalling proteins could play a role in the maintenance of constant blood glucose level even at a decreased plasma insulin concentration. As all significantly affected insulin signalling proteins were up-regulated by the WB diet or by oral butyrate exposure, the insulin sensitivity of chickens was supposed to be stimulated by the application of wheat as the main carbohydrate source (suggested to be mediated by the NSP-triggered caecal SCFA release including butyrate), and by applying butyrate as a feed additive as well. Since insulin plays a pivotal role in the maintenance of growth, inducing insulin sensitivity in chickens, being physiologically much less insulin responsive than mammals, could be of special importance by improving growth performance and metabolic health.

In our earlier study, butyrate treatment in a daily bolus altered insulin sensitivity in three-week-old broilers in a tissue-specific manner (Mátis et al., 2015). Following a daily oral bolus application of sodium butyrate (0.25 g/kg of body weight) for 5 days, IRβ, mTOR and PI3K were down-regulated in the liver, and decreased IRβ and mTOR protein expressions were also found in the subcutaneous adipose tissue. In contrast, relative protein expression of IRβ was increased in the skeletal muscle. These partly contradictory results of the earlier and the present studies could be associated with the different age of broilers (three weeks vs. six weeks) and the various sources and application forms of butyrate. The arising hypotheses should prompt further studies on the age-, application form- and origin-dependence of butyrate efficacy. Considering the age-dependent differences, the effects of butyrate on the insulin homeostasis of broilers may be less pronounced at the age examined in the present study (6 weeks), because by that time the phase of intensive growth, when insulin as an anabolic hormone is mostly involved in growth regulation, is already completed. It was previously described that the sensitivity of insulin signalling proteins decreased with age in both mammals (Gupte et al., 2008) and chickens (Deng et al., 2014).

Concluding our results, the present study describes new ways of influencing insulin homeostasis of chickens by nutrition, such as by butyrate as a feed

additive and by applying various cereals as dietary carbohydrate sources with different NSP levels. It should be stressed that the application of a wheat-based diet (with higher soluble NSP levels) may have a strong influence on insulin homeostasis by stimulating the intestinal production of SCFA including butyrate. Based on these findings, the role of SCFA as potent effectors of endocrine metabolic regulation, and primarily that of butyrate, was highlighted by comparing different application forms and by describing some underlying molecular mechanisms. Concerning the data obtained, applying higher dietary soluble NSP levels and/or butyrate as a feed additive can be a promising tool in poultry farming to influence insulin homeostasis and thus improve metabolism, growth and animal health.

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References

Antongiovanni, M., Buccioni, A., Petacchi, F., Leeson, S., Minieri, S., Martini, A. and Cecchi, R. (2007): Butyric acid glycerides in the diet of broiler chickens: effects on gut histology and carcass composition. Ital. J. Anim. Sci. **6,** 19–25.

Aviagen (2014): Broiler Management Handbook: Ross 308. Aviagen Ltd., Newbridge, UK.

Braun, E. J. and Sweazea, K. L. (2008): Glucose regulation in birds. Comp. Biochem. Phys. B **151,** 1–9.

Chamba, F., Puyalto, M., Ortiz, A., Torrealba, H., Mallo, J. J. and Riboty, R. (2014): Effect of partially protected sodium butyrate on performance, digestive organs, intestinal villi and *E. coli* development in broilers chickens. Int. J. Poult. Sci. **13,** 390–396.

de Lange, C. F. M. (2000): Characterisation of the non-starch polysaccharides. In: Moughan, P. J., Verstegen, N. W. A. and Visser-Reyneveld, M. I. (eds) Feed Evaluation: Principles and Practice. Wageningen Press, Wageningen, The Netherlands. pp. 77–92.

Deng, H., Zheng, A., Liu, G., Chang, W., Zhang, S. and Cai, H. (2014): Activation of mammalian target of rapamycin signaling in skeletal muscle of neonatal chicks: Effects of dietary leucine and age. Poultry Sci. **93,** 114–121.

Douglas, S. G. (1981): A rapid method for the determination of pentosans in wheat flour. Food Chem. **7,** 139–145.

Gao, Z., Yin, J., Zhang, J., Ward, R. E., Martin, R. J., Lefevre, M., Cefalu, W. T. and Ye, J. (2009): Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes **58,** 1509–1517.

Gálfi, P. and Neogrády, S. (2001): The pH-dependent inhibitory action of n-butyrate on gastrointestinal epithelial cell division. Food Res. Int. **34,** 581–586.

Gupte, A. A., Bomhoff, G. L. and Geiger, P. C. (2008): Age-related differences in skeletal muscle insulin signaling: the role of stress kinases and heat shock proteins. J. Appl. Physiol. **105,** 839–848.

Hu, Z. and Guo, Y. (2007): Effects of dietary sodium butyrate supplementation on the intestinal morphological structure, absorptive function and gut flora in chickens. Anim. Feed Sci. Tech. **132,** 240–249.

Jamroz, D., Wiliczkiewicz, A., Orda, J. and Skorupinska, J. (2002): Performance and intestine fermentation of carbohydrates in chickens fed wheat-barley diets supplemented with microbial carbohydrases. Arch. Geflugelkd. **66,** 59–65.

Józefiak, D., Ptak, A., Kaczmarek, S., Mackowiak, P., Sassek, M. and Slominski, B. A. (2010): Multi-carbohydrase and phytase supplementation improves growth performance and liver insulin receptor sensitivity in broiler chickens fed diets containing full-fat rapeseed. Brit. Poultry Sci. **89,** 1939–1946.

Kien, C. L., Peltier, C. P., Mandal, S., Davie, J. R. and Blauwiekel, R. (2008): Effects of the *in vivo* supply of butyrate on histone acetylation of cecum in piglets. JPEN – Parenter. Enter. **32,** 51–56.

Kulcsár, A., Mátis, G, Molnár, A., Petrilla, J., Wágner, L., Fébel, H., Huber, K. and Neogrády, Zs. (2015): The effect of different application forms of (n-)butyrate on the intestinal activity of cytochrome P450 enzymes in chicken. Proc. Soc. Nutr. Physiol. **59,** 78.

Mátis, G., Kulcsár, A., Turowski, W., Fébel, H., Neogrády, Zs. and Huber, K. (2015): Effects of oral butyrate application on insulin signaling in various tissues of chickens. Domest. Anim. Endocrin. **50,** 26–31.

Mátis, G., Neogrády, Zs., Csikó, Gy., Gálfi, P., Fébel, H., Jemnitz, K., Veres, Zs., Kulcsár, A., Kenéz, Á. and Huber, K. (2013*a*): Epigenetic effects of dietary butyrate on hepatic histone acetylation and enzymes of biotransformation in chicken. Acta Vet. Hung. **61,** 477–499.

Mátis, G., Neogrády, Zs., Csikó, Gy., Kulcsár, A., Kenéz, Á. and Huber, K. (2013*b*): Effects of orally applied butyrate bolus on histone acetylation and cytochrome P450 enzyme activity in the liver of chicken – a randomized controlled trial. Nutr. Metab. **10,** 1–10.

Molnár, A., Hess, C., Pál, L., Wágner, L., Awad, W. A., Husvéth, F., Hess, M. and Dublecz, K. (2015): Composition of diet modifies colonization dynamics of *Campylobacter jejuni* in broiler chickens. J. Appl. Microbiol. **118,** 245–254.

NRC (1994): Nutrient Requirements of Poultry. 9th revised edition. National Academy Press, Washington, DC, USA.

Roediger, W. E. (1982): Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology **83,** 424–429.

Thangaraju, M., Cresci, G. A., Liu, K., Ananth, S., Gnanaprakasam, J. P., Browning, D. D., Mellinger, J. D., Smith, S. B., Digby, G. J., Lambert, N. A., Prasad, P. D. and Ganapathy, V. (2009): GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. Cancer Res. **69,** 2826–2832.

Turban, S. and Hajduch, E. (2011): Protein kinase C isoforms: Mediators of reactive lipid metabolites in the development of insulin resistance. FEBS Lett. **585,** 269–274.

White, M. F. and Kahn, C. R. (1994): The insulin signaling system. J. Biol. Chem. **269,** 1–4.