Ann. Anim. Sci., Vol. 17, No. 4 (2017) 1107–1122 DOI: 10.1515/aoas-2017-0021

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EFFECTS OF DIFFERENT DIETARY LEVELS OF WHEY LACTOSE AS A PREBIOTIC DISACCHARIDE ON THE PRODUCTIVE PERFORMANCES AND SELECTED INDICES OF THE CAECAL MICRO-ENVIRONMENT IN BROILER CHICKENS

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Abstract

The primary aim of this study was to investigate the impact of three dietary levels of lactose (LAC) originating from conventional dried whey (DW) and the duration of these treatments (from 8 to 21 or to 42 days of age) on growth performance, basic post-slaughter traits and excreta quality of broiler chickens kept in cages. A secondary purpose was to investigate the effect of LAC level on some parameters of the caecal micro-environment and gross morphology in these birds. A total of 560 Ross 308 chickens (sex ratio 1:1) were assigned to 7 dietary combinations with 10 replicate cages of 8 birds per cage. The control group was fed basal diets consisting of maize, wheat and soybean meal. The other 6 groups received the same basal diets with DW added in amounts equivalent to a LAC dietary levels of 1, 2 or 3%. Only continuous feeding (day 8 to 42) with 1% and 2% levels of LAC was found to yield the overall body weight gain (BWG) during the whole 42-day rearing period, which was significantly higher than that on the control diet, with a larger share of breast meat in carcass at a 2% LAC. However, these effects were associated with greater faecal score values indicating more watery excreta compared with the control. Increasing levels of LAC augmented the relative caecal weight and length. A reduction in the caecal pH was confirmed at day 21 for birds fed 1% and 2% of dietary LAC. The lower pH values were correlated to an increased sum of total volatile fatty acids (VFA), causing large increases in the concentration of undissociated forms of individual VFA. The decline in plate counts of coliform bacteria was observed with 2% and 3% LAC, whereas the counts of lactic acid-producing bacteria (LAB) were higher at these two LAC levels. The present findings lead to the conclusion that the dietary level of 2% LAC originated from DW is the most effective in enhancing the productivity of broilers, with moderate occurrence of undesirable side effects.

Key words: broilers, lactose, dried whey, performance, caecal pH, volatile fatty acids, lactic acid-producing bacteria, coliforms

Biosecurity threats to public and animal health resulting from the increased resistance of pathogens, the accumulation of antibiotic residues in animal products, and in the environment have led to the prohibition of the use of antibiotics as in-feed growth promoters (AGP) for farm animals (Dibner and Richards, 2005). As

a consequence, a number of alternatives to AGP have been investigated in animal production, especially prebiotics and probiotics, to evaluate the usefulness of their incorporation in feed as causative factors for better growth (Alloui et al., 2013).

The use of lactose and high-lactose whey derivatives in animal nutrition, especially newborn ruminants and piglets, is one of the important pathways of their utilisation. However, due to the very low activity of enterocytary β-galactosidase (lactase) birds, unlike growing mammals, are unable to digest lactose to an appreciable extent (Siddons, 1969), and therefore, the bulk of lactose supplied with the diet passes unchanged into their lower gut, including the caeca (Hume et al., 1992). Here, it is fermented by microbiota to short-chain organic acids (volatile fatty acids and lactate, as the major) that can decrease the luminal pH, and to gaseous products (H_2, CH_4 and CO_2). Within the literature some evidence exists demonstrating that, in poultry, purified lactose may promote the growth of lactose-utilising bacteria that compete with enteropathogens and/or lactose protective action against some pathogens can be ascribed to an increased acidity of the intestinal digesta resulting from fermentation of this disaccharide (Rehman et al., 2009).

The research trials looking at the growth performance of broiler chickens fed diets supplemented with dried whey as a source of lactose are relatively scarce and have produced inconclusive results (Kermanshahi and Rostami, 2006; Samli et al., 2007). In particular, there is insufficient knowledge concerning the dose-related efficacy of whey lactose as a feed additive for broilers grown to market age. There are also no clear data regarding dose-dependent side effects of dietary lactose, i.e. distension of the hindgut wall associated with excessive gas formation, and the water-absorbent effect resulting in symptoms of osmotic diarrhoea – a bowel problem well recognised in lactose intolerant (lactase non-persistent) humans (Deng et al., 2015).

With the intent to determine the most efficient dose, our primary objective was to investigate the influence of graded levels of dietary lactose (1, 2, and 3%) resulting from different additions of dried whey on production performances and selected indices of the caecal environment and gross morphology in broilers housed in cages. An additional exploratory aim of this experiment was to examine whether duration of feeding diets containing whey lactose (days 8–21 or 8–42 of life) would affect final production results and basic carcass parameters in 42-day-old broilers.

Material and methods

All experimental procedures used throughout this study were reviewed and approved by the II Local Ethics Committee for Animal Experimentation in Kraków.

Source of dietary lactose

Lactose was provided from commercial dried whey (DW) included in the diets to attain pre-planned lactose levels. Before incorporation into the feed, concentrations of lactose (LAC), total N (CP), and dry matter (DM) content of DW were determined. The results of the relevant analyses were as follows: DM, 86.7% as-fed; LAC, 76.3% of DM; CP (N × 6.38), 12.2% of DM.

Table 1. Composition of the experimental diets (g/kg as-fed)

Item	Prestarter	Starter				Grower			
		basal (control)	1% LAC	2% LAC	3% LAC	basal (control)	1% LAC	2% LAC	3% LAC
Ingredients									
maize	395.0	403.2	383.1	365.0	346.9	378.4	361.3	343.2	326.1
wheat	140.0	170.0	170.0	170.0	170.0	230.0	230.0	230.0	230.0
soybean meal (47.9% CP)	395.0	350.0	350.0	350.0	350.0	300.0	300.0	300.0	300.0
rapeseed oil	25.0	32.0	37.5	40.0	43.0	50.0	52.0	55.0	57.0
$Ca(H_2PO_4)_2$	18.0	18.0	18.0	18.0	18.0	16.0	16.0	16.0	16.0
limestone	16.0	16.0	16.0	16.0	16.0	15.0	15.0	15.0	15.0
NaCl	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
premix[1, 2]	5.0[1]	5.0[1]	5.0[1]	5.0[1]	5.0[1]	5.0[2]	5.0[2]	5.0[2]	5.0[2]
L-lysine (79%)	1.5	1.3	1.3	1.3	1.3	1.2	1.2	1.2	1.2
DL-met (99%)	1.5	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.4
dried whey	-	-	15.1	30.2	45.3	-	15.1	30.2	45.3
Calculated[3]									
crude protein (N × 6.25)	241	222	221	221	221	204	203	203	203
ME (MJ/kg)	12.00	12.32	12.34	12.31	12.29	12.90	12.86	12.83	12.80
lysine (total)	14.2	12.8	12.8	12.7	12.6	11.4	11.4	11.3	11.2
methionine (total)	5.2	4.8	4.8	4.7	4.7	4.6	4.6	4.6	4.5
Ca	11.3	11.2	11.3	11.4	11.5	10.3	10.4	10.5	10.6
P available	5.4	5.3	5.4	5.5	5.6	4.8	4.9	5.0	5.1

[1, 2]vitamin-trace mineral premix provided per kilogram of diet: [1]vit. A – 13500 IU; vit. D – 3600 IU; mg: vit. E – 45; vit. K – 3; vit. B_1 – 3.25; vit. B_2 – 7.5; vit. B_6 – 5; vit. B_{12} – 0.03; biotin – 0.15; folic acid – 1.5; nicotinic acid – 45; calcium pantothenate – 15; choline chloride – 600; Fe – 67.5; Zn – 5; Mn – 100; Cu – 17.5; I – 1; Se – 0.28; Co – 0.4; coccidiostat (diclazuril) 1; [2]vit. A – 12000 IU; vit. D – 3250 IU; mg: vit. E – 40; vit. K – 2.25; vit. B_1 – 2; vit. B_2 – 7.25; vit. B_6 – 4.25; vit. B_{12} – 0.03; biotin – 0.1; folic acid – 1; nicotinic acid – 40; calcium pantothenate – 12; choline chloride – 450; Fe – 65; Zn – 65; Mn – 100; Cu – 15; I – 0.8; Se – 0.25; Co – 0.4; coccidiostat (semduramycin) – 25.

[3]on the basis of chemical analysis of soybean meal, and from tabular data on proximate composition for remaining feed materials (Smulikowska and Rutkowski, 2005).

Animals, housing and feeding

The experiment was carried out on 560 feather-sexed Ross 308 broiler chickens (sex ratio 1:1) obtained from a commercial hatchery. To distinguish a bird's sex in the first 3 weeks of life, male chicks were marked on their head with a water-resistant pen. The marks were refreshed on days 7 and 14 of age. The average body mass of one-day-old chicks, determined by individual weighing of 60 randomly selected birds, was 43.1±0.3 g. The males and females were equally distributed to 70 wire mesh-floor cages (dimensions L × D × H: 120 × 60 × 44 cm) arranged in a three-tier battery in an environmentally controlled broiler house. Each cage was equipped with two nipple drinkers, a trough feeder along the front of the cage (15 cm/bird), and a metal tray placed under the cage floor. The temperature inside the building was maintained at 32°C during days 1–5 and then gradually reduced to 22°C until the end of rearing. The lighting cycle was set to provide continuous fluorescent lighting until day 5 of life and then switched to 18 h light and 6 h dark per day. All the birds were given free access to a mash feeds based on maize, wheat and soybean meal, including: prestarter type diet fed during the pre-experimental phase (day 1 to 7), starter type diets fed from day 8 to 21, and grower type diets fed from day 22 to 42 (Table 1). The diets were formulated to meet the dietary recommendations for commercial broiler chickens (Smulikowska and Rutkowski, 2005) and, apart from anticoccidials, did not contain any specific additives (enzymes, phytobiotics, pH control agents, etc.).

Experimental design and treatments

Seven dietary combinations were tested (Table 2). The control group was fed basal starter and grower diets only. The other 6 treatment groups of chickens received basal diets with DW added in amounts equivalent to a LAC dietary levels of 1, 2 or 3%. Thus, DW was included in the basal diets at 15.1, 30.2 or 45.3 g per kg diet, respectively, at the expense of maize on wt/wt as-fed basis. The metabolisable energy was adjusted by increasing amounts of the vegetable oil in order to keep the diets isoenergetic within phases. Feeding the whey lactose-containing diets was initiated on day 8 of age and was continued for the 14-day starter phase (treatments denoted as 1%LAC8-21, 2%LAC8-21, and 3%LAC8-21), or for the 35-day period of the experiment (treatments 1%LAC8-42, 2%LAC8-42, and 3%LAC8-42). After the termination of the short-term feeding period on starter LAC diets the chickens from treatment groups nos. 2, 3 and 4 were switched to the grower-type basal diet (Table 2).

The experiment was performed in a randomised block arrangement with 10 replicate cages of 8 chickens (4 males and 4 females) assigned to each dietary group after the seven day pre-experimental period. An augmented factorial design, LAC dietary level by period when the chickens were provided LAC-containing diets (3×2) plus one control group, was used (Lentner and Bishop, 1993). The original experimental design comprised the two aforementioned factors, however only the growth performance and post-slaughter data were collected in a manner that made possible the use of full factorial analysis of variance.

Measurements, evaluations and collection of samples

The chickens were weighed by cage on day 8, 21 and 42 after 4 h feed deprivation. The chickens were inspected twice daily for general health status and mortality. Feed intake (FI) was recorded on a cage basis. Body weight gains (BWG) were calculated for the two sub-periods and for the whole 42-day period of rearing. The feed conversion ratio (FCR) values were corrected for the weight of the dead and culled birds. Mean BWG, FI and FCR were used to evaluate growth performance. Over the 7-day pre-experimental period, the average consumption of prestarter diet was determined to be 117 g/bird. The excreta of chickens were visually evaluated on day 14 and 35 on a cage basis by a six-member panel based on the droppings accumulated during 6 h on a clean tray beneath the cage floor. For each dietary treatment, the excreta score was determined according to Douglas et al. (2003) using a 5-point scale, where 1 was faecal material that was dry and well-formed and 5 was material that was very loose or liquid.

Table 2. Scheme of the experimental design, data collection and determination of parameters

Dietary treatment no. abbreviation	Days post hatch (feeding phase)/dietary level of lactose (LAC) originated from dried whey		
	1–7 (prestarter)	8–21 (starter)	22–42 (grower)
1 Control	pretreatment phase[1]	basal [1,2,3,4]	basal [1,2,3,5,6]
2 1%LAC8-21		1% [1,2,3,4]	basal [1,2,6]
3 2%LAC8-21		2% [1,2,3,4]	basal [1,2,6]
4 3%LAC8-21		3% [1,2,3,4]	basal [1,2,6]
5 1%LAC8-42		1% [1,2]	1% [1,2,3,5,6]
6 2%LAC8-42		2% [1,2]	2% [1,2,3,5,6]
7 3%LAC8-42		3% [1,2]	3% [1,2,3,5,6]

[1, 2, 3, 4, 5, 6] parameters monitored or determined: [1]body weight, feed intake, death and culling losses; [2]excreta evaluation; [3]pH of caecal chyme, weight and length of the caeca; [4]volatile fatty acid concentrations in the caecal contents; [5]caecal counts of lactic acid and coliform bacteria; [6] carcass traits and organ weights.

On day 21 of life, 8 birds (sex ratio 1:1) per treatment in groups 1%LAC8-21; 2%LAC8-21 and 3%LAC8-21, and the control group were randomly chosen, labelled, and weighed. All the selected birds were killed by cervical dislocation. The caeca were removed and the pH of their content was determined by direct electrode tip insertion into the gut lumen. All pH measurements were taken in duplicate on a digital pH-meter (CyberScan pH 510, Eutech Instruments, Singapore). Immediately afterwards, caecal chyme samples of four mixed-sex chickens (per eight selected) were taken, placed into sterile plastic containers and deep-frozen (–20°C) to await determination of total volatile fatty acids (tVFA) content. The empty caeca were rinsed with clean water, dried with blotting paper and weighed, and their lengths were recorded. The caecal weight and length were expressed relative to 100 g live body weight (LBW).

After weighing the chickens on day 42, 4 males and 4 females were selected at random from each of the following groups: control, 1%LAC8-42, 2%LAC8-42, and

3%LAC8-42. The birds were sacrificed and their caeca quickly removed and subjected to the above-described measurement procedures (pH determination, weight and length). Samples of caecal chyme were collected, as described above, from 4 mixed-sex chickens per group for enumeration of total lactic acid and coliform bacteria in the fresh material. At the end of the experiment (day 43), eight birds (sex ratio 1:1, with LBW close to the group average) were chosen from each dietary group and used for slaughter analysis. All of the birds were fasted for 12 h, weighed, killed by cervical dislocation, bled out, scalded, and defeathered in a rotary drum-picker. During manual evisceration, the weights of the liver and spleen were recorded. After removal of the feet and heads, the empty carcasses were air-chilled overnight at 4°C, weighed and then dissected to measure the mass of skinless breast muscles and abdominal fat. The carcass percentage yield (carcass recovery) and the relative weights of the liver and spleen were calculated on the basis of LBW. The breast meat yield and abdominal fat deposition were calculated relative to the cold carcass mass.

Chemical analyses and calculations

All compositional analyses were performed in duplicate. The concentrations of proximate nutrients in DW powder and in soybean meal used in diet formulations were analysed according to AOAC International (2000) procedures. The concentration of LAC in DW was determined using the Luff-Schoorl titration method (Egan et al., 1981). For the determination of tVFA, a 2 g sample of thawed caecal chyme was filtered through absorbent wool on a laboratory vacuum station, rinsed, and the filtrate was diluted with deionised water to the final volume of 50 ml. Then, 5 ml of that solution was mixed with 1 ml of 24% metaphosphoric acid, incubated for 30 min, and centrifuged at 13,000 g for 7 min. The supernatant was analysed with a Varian Model 3400 gas chromatograph (Varian Inc., Walnut Creek, USA) equipped with FID and a Zebron ZB-Wax capillary column (30 m long, 0.53 mm i.d., 1 µm film thickness; Phenomenex Inc., Torrance, USA). The concentration of undissociated volatile fatty acids (uVFA) was calculated from the total acid contents with the equation of Henderson-Hasselbalch:

$$pH = pKa + log10\ [A\text{-}] / [HA]$$

where:

A is dissociated acids and *HA* is undissociated acids; with measured caecal *pH*, the *pKa* of each acid, and the total acid concentration as determined by gas chromatography.

The respective *pKa* values used in calculating the concentration of undissociated acid present were: acetic 4.76, propionic 4.88, isobutyric 4.85, and butyric 4.82.

Microbiological analysis

Immediately after collection, digesta samples were placed in a cool box kept at 4°C until further processed (within 2 h) to estimate selected microbial popula-

tions using the routine reference methods of plate counting. The number of lactic acid bacteria was assessed according to the PN-90/A-75052.07:1990 procedures. The number of coliform bacteria was determined in accordance with the PN-ISO 4832:2007 method. All the samples were analysed in two replications and the results were expressed as the average number of colony-forming units (cfu) per g of chyme.

Statistical analysis

The replicate cage served as the experimental unit for growth performance indices, and individual birds of both sexes were the experimental units for statistical analysis of post-slaughter and bio-physiochemical data. Statistical evaluation of all of the results was conducted with the Statistica® ver. 6 package (StatSoft Inc. Tulsa, OK, USA). After transforming data by $x' = \log (x + 2)$ in the case of mortality percentage, the Levene's test provided evidence against equality of variances between the treatment groups. The data on growth performance and carcass characteristics were analysed by factorial analysis of variance with an isolated control group. In this design, for comparing individual treatments with an isolated control group, some pre-planned orthogonal contrasts were tested for significance. The data for mixed-sex chickens resulting from their caeca measurements, caecal microflora and VFA determinations/calculations were subjected to one-way analysis of variance with LAC dietary level (0%, 1%, 2% and 3%) as the explanatory variable. The linear and quadratic polynomial contrasts were performed to test for a trend in the treatment means. If the probability value of F-tests was <0.05, differences among main effect means (2-way ANOVA) and treatment means (1-way ANOVA) were determined with the Tukey's HSD post-test. Differences between means were considered to be significant when $P \leq 0.05$.

Results

During the starter feeding phase (days 8–21), no differences ($P>0.05$) among the 1%LAC and 2%LAC treatments for any of the performance parameters (BWG, FI, FCR) were determined (Table 3). However, significantly reduced BWG in spite of higher FI was observed on the 3%LAC dietary treatment compared with the other two treatments, resulting in deteriorated feed efficiency by 3% LAC level fed up to 21 days of age. Over this period, no significant differences in losses due to death and culling (mortality rate) were observed between inclusion levels of LAC. At day 21, the 2% LAC treatment resulted in higher BWG than the control diet (contrast C2; Table 4). Throughout the starter phase, the broilers from the experimental treatments (all LAC levels) consumed significantly more feed overall than the broilers from the control group (contrasts C1-C3); however, only in the case of 3% LAC level was this found to be negatively associated with feed efficiency (C3 for FCR; Table 4). Mean values of mortality rate over the 8–21-day period were not different between the control diet and all levels of LAC.

Table 3. Effects of dietary level of whey LAC and period of administration in the feed on growth performance, breast meat percentage and excreta scores

Dietary treatment no. abbreviation	Initial BW[1] (g)	BWG (g)			Feed intake (g/bird)		FCR (g feed/g BWG)			Mortality[2] (%)		Breast meat[3] (%)	Excreta scores (points) at day	
		8–21 d	22–42 d	1–42 d	8–21 d	22–42 d	8–21 d	22–42 d	1–42 d	8–21 d	22–42 d		14	35
1 Control	160	564	1739	2421	917	3476	1.69	2.01	1.87	2.50	3.75	25.4	1.53	1.33
2 1%LAC8-21	160	582	1765	2464	963	3565	1.68	2.02	1.89	3.75	1.25	26.4	1.75	1.28
3 2%LAC8-21	161	591	1795	2504	969	3505	1.64	1.95	1.83	0.00	1.25	26.5	2.43	1.35
4 3%LAC8-21	160	546	1767	2431	1003	3589	1.86	2.04	1.94	1.25	5.00	26.6	2.45	1.34
5 1%LAC8-42	159	588	1822	2527	962	3395	1.65	1.86	1.77	1.25	1.11	26.3	1.86	1.67
6 2%LAC8-42	161	602	1816	2536	963	3533	1.63	1.95	1.82	2.50	0.00	26.9	2.45	2.00
7 3%LAC8-42	159	566	1688	2371	1020	3491	1.83	2.08	1.96	8.75	2.50	25.0	2.63	2.45
SEM	1.0	3.9	11.1	12.9	6.2	20.0	0.015	0.017	0.013	0.727	0.617	0.20	0.06	0.06
Main effect dietary whey lactose[4] (LAC)														
1%		585 a	1793 a	2495 a	962 b	3480	1.66 b	1.94 b	1.83 b	2.50	1.18	26.3	1.81 b	1.48 b
2%		596 a	1806 a	2520 a	966 b	3519	1.64 b	1.95 b	1.83 b	1.25	0.63	26.7	2.44 a	1.68 b
3%		556 b	1728 b	2401 b	1011 a	3540	1.85 a	2.06 a	1.95 a	5.00	3.75	25.8	2.54 a	1.89 a
Main effect feeding duration (FD)														
8–21 days of life		-	1776	2466	-	3553	-	2.00	1.89	-	2.50	26.5	-	1.32
8–42 days of life		-	1775	2478	-	3473	-	1.96	1.85	-	1.20	26.1	-	2.04
P-value														
LAC		0.001	0.011	0.001	0.001	0.505	0.001	0.016	0.001	0.116	0.129	0.238	0.001	0.002
FD		-	0.988	0.647	-	0.063	-	0.234	0.145	-	0.331	0.310	-	0.001
LAC × FD		-	0.040	0.125	-	0.166	-	0.064	0.084	-	0.768	0.160	-	0.007

[1]pretreatment body weight at day 8 of age.
[2]includes birds found dead and that were culled due to injuries to wings and leg abnormalities.
[3]treatment means for both sexes.
[4]main effect means with different letters are significantly different at $P \leq 0.05$.

The significant negative responses (across FD) to the 3% LAC compared with 1% and 2% LAC treatments in terms of reduced BWG and decreased feed efficiency (higher FCR) were observed for days 22–42 and for the whole rearing from day 1 to 42. Over the 22–42-day period, increasing amounts of LAC in the diet had no overall effect on FI and mortality, and length of feeding LAC to birds (FD) regarded as an independent variable, did not affect any productive trait throughout the grower phase. Nonetheless, the LAC × FD interaction (P=0.04) showed a clear decrease in the BWG of birds receiving the 3% LAC diet for the longer 8–42-day period (Table 3). Feeding diets with 1 and 2% LAC throughout the 35-day period resulted in higher BWG (contrasts C4 and C5) and better feed efficiency (C4; 1% LAC; P=0.02) than the control diets. However, there were no differences when comparing any individual LAC treatments to the control diet for FI and mortality during this period (Table 4).

Table 4. Significant orthogonal contrasts among the control and specific treatment means for growth performance, carcass characteristics and excreta scores

Parameter[1]	C1	C2	C3	C4	C5	C6
	control vs 1%LAC8-21	control vs 2%LAC8-21	control vs 3%LAC8-21	control vs 1%LAC8-42	control vs 2%LAC8-42	control vs 3%LAC8-42
BWG						
8–21 d		0.006[3]				
22–42 d				0.035	0.050	
1–42 d				0.017	0.010	
Feed intake						
8–21 d	0.009[2]	0.005[3]	0.001[4]			
FCR						
8–21 d			0.001[4]			
22–42 d				0.020		
1–42 d				0.020		
Breast meat yield					0.050	
Excreta scores						
d 14	0.019[2]	0.001[3]	0.001[4]			
d 35				0.028	0.001	0.001

[1]contrasts for all remaining parameters were not significant (P>0.05).
[2]for contrast among treatments no. 1 vs 2 + 5.
[3]for contrast among treatments no. 1 vs 3 + 6.
[4]for contrast among treatments no. 1 vs 4 + 7.

At day 14 of age (after 7 days on LAC-containing diets), excreta scores were higher for chicks provided with 2 and 3% LAC compared to 1% LAC (Table 3). At day 35, when the average data on excreta score values across both periods of feeding LAC were compared, no difference between the 1% LAC and 2% LAC treatments was detected, but the mean value from birds on 3% LAC was higher (P≤0.05). Longer-term feeding period on LAC diets (28 vs 14 days) resulted in higher (P=0.001)

scores obtained during evaluation at day 35, especially with 3% of dietary whey lactose (LAC × FD interaction; P=0.007; Table 3). In comparison with the control group (contrasts C4, C5 and C6; Table 4), distinctly higher excreta scores recorded in older chickens were found on all levels of LAC fed continuously from day 8 to 35 of their life.

No significant differences associated with dietary LAC level, as well as with length of feeding LAC supplements, were noted for post-slaughter indices under study (detailed data not shown, except for breast meat yields included in Table 3). The overall averages (in relative values followed by pooled SEM in parentheses) for carcass recovery, abdominal fat, and the relative weights of liver and spleen were 74.6 (0.19), 2.13 (0.068), 1.80 (0.021) and 0.084 (0.0022), respectively, and the values of the control chickens were similar to those determined in birds fed the LAC-containing diets (P>0.05). The only contrast that was significant showed the superiority of the 2% LAC diet fed during the 8–42-day period relative to the control diets for breast meat yield: 26.9 vs 25.4% carcass mass (Table 3 and 4).

The lumen pH at day 21 decreased in the presence of LAC in the diet, and the significant quadratic responses from the two lactose treatments: 1% LAC = 2% LAC < control were observed (Table 5). At day 42, feeding 2% and 3%LAC diets was found to significantly decrease the pH value compared to that determined for the control group. Overall, for both time points, there were no differences in the caecal pH between the three LAC treatments. Supplementation of 1, 2, and 3% whey LAC to the basal diets resulted in a linear increase in the relative weight and length of the caeca. In younger chickens (day 21) fed 2% and 3% LAC, caecal weights were significantly higher compared with their control group counterparts, whereas caecal lengths at this age were linearly increased on all dietary LAC levels. Only at 3% LAC inclusion level were these two caecal morphometric parameters affected in 42-day-old birds, showing the highest values (3% LAC > control = 1% LAC = 2% LAC; P<0.05).

The contents of the individual total VFA varied among the treatment groups and observed quadratic responses were associated with the presence of whey lactose in the feed. Caecal concentrations of acetic acid (the predominant VFA) in the control birds were lower than in those on the 1% LAC and 2% LAC treatment diets, but statistically similar (P>0.05) to those from the 3% LAC treatment. Propionic acid concentrations in the caeca of chickens that received the control diet were lower than in those on the 1% LAC and 2% LAC treatments, but were not different from the birds given the 3% LAC diet. There was no difference in the content of isobutyric acid between the control and 3% LAC diets but, compared to the 1% and 2% LAC levels the lower concentration of this acid was observed on the non-LAC treatment (P≤0.05). The trend toward increased butyrate production due to whey LAC supplementation at 2% and 3% of diet was small and did not approach statistical significance (linear, P=0.186; quadratic P=0.798). The overall sum of tVFA varied among treatments in the same manner as that observed for acetic acid concentration (quadratic, P=0.019). When the calculated caecal concentrations of undissociated volatile fatty acids were compared (Table 5), significant quadratic responses in the individual uVFA (P<0.001) were observed. It was found that inclusion of whey LAC resulted in substantially higher values than the control diet, and, in most cases the differences were statistically significant.

Table 5. Effect of dietary level of whey lactose on lumen pH, relative weight (g/100 g LBW) and length (cm/100 g LBW) of the caeca, total (tVFA) and undissociated (uVFA) volatile fatty acid concentrations, and selected microbial populations in the caecal chyme of chickens (log_{10} cfu/g)

Item	Days[1]	Dietary treatment				SEM	P-value	
		control	1% LAC	2% LAC	3% LAC		linear	quadratic
pH	21/14	6.81 a	6.15 b	6.21 b	6.41 ab	0.078	0.081	0.006
	42/35	6.85 a	6.63 ab	6.48 b	6.43 b	0.049	0.003	0.478
Relative weight	21/14	0.39 b	0.44 ab	0.47 a	0.51 a	0.009	0.003	0.800
	42/35	0.29 b	0.31 b	0.33 b	0.39 a	0.008	< 0.001	0.127
Relative length	21/14	1.72 c	1.91 b	1.99 ab	2.07 a	0.028	< 0.001	0.142
	42/35	0.76 b	0.79 b	0.80 b	0.89 a	0.017	0.005	0.226
tVFA (mmol/g):	21/14							
acetic		16.86 b	28.52 a	25.52 a	22.72 ab	1.036	0.113	0.003
propionic		0.97 c	1.97 a	1.87 ab	1.19 bc	0.159	0.612	0.005
isobutyric		0.47 b	0.78 a	0.68 a	0.53 b	0.048	0.845	0.009
butyric		10.16	9.78	11.98	12.33	0.678	0.186	0.798
sum of tVFA[2]		28.99 b	42.08 a	40.89 a	37.36 ab	1.907	0.110	0.019
uVFA (mmol/g):	21/14							
acetic		0.149 c	1.116 a	0.874 a	0.498 b	0.0978	0.011	< 0.001
propionic		0.011 c	0.100 a	0.084 a	0.034 b	0.0086	0.221	< 0.001
isobutyric		0.005 b	0.037 a	0.029 a	0.014 b	0.0028	0.141	< 0.001
butyric		0.103 b	0.437 a	0.469 a	0.309 b	0.0389	< 0.001	< 0.001
Lactic acid bacteria	42/35	7.88 b	7.96 b	8.73 a	8.75 a	0.144	0.005	0.892
Coliform bacteria	42/35	8.17 a	7.83 a	7.41 b	6.92 b	0.153	0.001	0.736

[1]age of birds/exposure time to the feed containing whey lactose.
[2] including isovaleric and valeric acids.
Values within a row with different letters are significantly different at P≤0.05.

The highest values for acetic, propionic and butyric acids were obtained with 1% and 2% LAC treatments; approximately 6- to 9-times greater than the corresponding values calculated for the control diet. Caecal populations of lactic acid-producing (LAB) and coliform bacteria in 42-day-old chickens were significantly affected by dietary treatment, showing a linear pattern of changes with an increase in the level of LAC supplementation. In comparison with the control group, the LAB counts were higher and the counts of coliforms lower in whey LAC-treated birds, with significant differences when LAC was present at 2% and 3% of the diet (Table 5).

Discussion

The overall purpose of this study was to evaluate which of the selected dietary levels of whey lactose – 1, 2 or 3% – may be considered the most optimal in terms of broiler productivity, with minimal symptoms pointing to the existence of such side effects as osmotic diarrhoea or flatulence. An attempt to elucidate prebiotic effects of

whey LAC at given dosage levels was based upon shifts in LAB and coliform groups of bacteria and changes in the caecal VFA concentrations and pH.

Feeding broilers with whey LAC (at all applied dietary levels) exclusively during the first 2 weeks of the trial had, in general, no significant effects on major productive performance endpoints, and survivability rate by day 42 of age in comparison with the control birds. Over this period, the use of diet with 2% LAC improved the BWG of broiler starters, whilst their feed efficiency was significantly reduced on the 3% LAC-diet. And yet, no carry-over effects following the short-term feeding diets supplemented with 2 or 3% whey lactose were found on these parameters in the subsequent (grower) phase on the non-LAC diets: neither positive on BGW nor negative on FCR, respectively. Only continuous feeding (from 8 to 42 day) with 1% and 2% levels of dietary LAC was found to yield the overall BWG during the whole 42-day rearing period that was significantly higher than that on the control diet, with a larger share of breast meat in the carcass at a 2% LAC level. However, these effects were associated with greater faecal score values indicating more loose (watery) excreta. On the other hand, significant negative responses to the 3% LAC, reflected in reduced growth and decreased feed efficiency compared with the 1% and 2% LAC treatments but not with the control one, were observed.

Significant increases in total 42-day BWG of chickens provided 1% and 2% dietary LAC compared with the control birds were similar to those reported by Radfar and Farhoomand (2008), Kermanshahi and Rostami (2006) and Gulsen et al. (2002) following 5 to 7 weeks feeding with dried whey providing lactose at 0.75%, 1.4 or 2.7%, and 2.5% of diet, respectively. It can be argued that the above effects could be partially attributed to the action of biologically active proteins contained in dried whey (e.g. 2% LAC addition of dried whey provided over 3 g of these proteins per kg of diet). It was recently shown that feeding small amounts of these proteins can be regarded as a method for elevating production efficiency and meat yield in market-age broilers (Szczurek et al., 2013).

The positive effect of 2% whey LAC supplementation with regard to BWG during days 8 to 21 coincides with the results obtained by Gulsen et al. (2002) after inclusion of 3.85% dried whey (= 2.5% whey LAC) into the starter diets. And taking into account the findings of this research team, it can be speculated that an effect was due to the increase in the length of intestinal villi which could lead to enhanced nutrient absorption. The higher FCR value of broiler starters fed on the 3% LAC treatment diet (Table 3) is consistent with observations by Kermanshahi and Rostami (2006) and Douglas et al. (2003), suggesting that as lactose level in the starter diet exceeds 2%, feed efficiency may be depressed. These latter authors also reported the higher values of a subjective excreta score in chickens treated for 14 days with lactose at 2 and 4% of diet, and the relevant data from the present experiment are in good accordance with their findings. Similarly, in the study of Waldroup et al. (1992) supplementation of 2.5% lactose to the broiler diet has been associated with a two-fold increase in the percentage of caked-over litter, indirectly pointing out the signs of osmotic diarrhoea.

In the above context, it is important to stress here the impact of ingested lactose on gross morphometry of chicken caeca. In the present experiment increasing

amounts of dietary whey LAC caused pronounced rises in caecal relative weight and length in younger chickens (Table 5), and this is in agreement with the studies of Waldroup et al. (1992) and Kermanshahi and Rostami (2006). The observed increases in empty caecal weight could possibly be attributed to the stimulation of mucosal epithelial cell proliferation at low lumen pH or, as suggested by Tellez et al. (1993), to hyperplasia of the caecal enterocytes. It seems very plausible that an increase in caecal lengths, with a striking difference at the level of 3% LAC, was due to the effect of gas distention of the caeca, and the above-mentioned studies support this assertion.

It is evident from the presented data that the pH and VFA concentrations were affected by the presence of whey LAC in the diet. At day 21 of age, decreased caecal pH values were closely correlated to increased overall sum of total VFA determined at this time point, and caused significant increases in the concentration of undissociated form of acetic, propionic and butyric acids. In this experiment, clear differences could also be observed between the LAB and coliform bacterial groups in the caecal material of 42-day-old broilers fed the control and LAC-containing diets. Dietary whey lactose at the 2% and 3% doses significantly reduced the number of coliforms, whereas the viable counts of LAB were found to be significantly higher at these dietary LAC levels. This latter linear effect agrees, to some extent, with the observations of Samli et al. (2007) who evidenced a substantial ($P<0.05$) rise in the population of LAB in the ileum and droppings of broilers supplemented with dried whey provided 2.8% LAC to the diet. Likewise, Pierce et al. (2006) reported that dietary LAC results in increased lactobacilli concentrations in the colon and caecum of weaning pigs. However, in contrast to these results are those obtained by McReynolds et al. (2007) and Józefiak et al. (2008) in purified lactose-treated chickens: no effect with the levels of 2.5 or 4.5%, a significant reduction at 0.2% of diet, respectively.

Taking into account that lactobacilli readily utilize lactose as a substrate for lactic acid production in anaerobic conditions (Fu and Mathews, 1999), it is possible that the drop in caecal pH in 42-day-old birds receiving the 2% and 3% LAC diets was also due to higher lactate concentrations. The majority of such responses have been found for dietary levels of purified lactose, e.g. at 5% (van der Wielen et al., 2002) or at 2.5% (Hinton et al., 1991). But, interestingly, Radfar and Farhoomand (2008) showed that the level of 0.75% of whey LAC in the diet was also able to significantly increase caecal lactate concentration in 28-day-old broilers. Furthermore, the present results on the increased caecal production of total acetate and propionate in chickens provided dietary LAC are in good accordance with observations made by Tellez et al. (1993) and Hinton et al. (1991) in young chickens aged 10 to 19 days.

It is well known that the environmental pH affects the strength of the antibacterial activity of the volatile fatty acids by altering the degree of undissociation of the individual acids according to the principle: the degree of VFA undissociation increases as the pH decreases. When the acid is in the undissociated form it can freely diffuse into the cell cytoplasm and suppress bacterial cell enzymes, and nutrient transport systems (Huyghebaert et al., 2011). Unfortunately, only a few studies related to this topic have been performed to date with broiler chickens offered lactose, and their results seem to be rather inconsistent.

In the present experiment, sharp increases were revealed in the levels of undissociated form of acetic and propionic acids in the caeca of 3-week-old broilers treated with dietary supplemental LAC, especially at 1% and 2%. This is comparable with what was observed in the caeca of chickens when purified lactose was provided in the drinking water at 2.5% wt/vol (Hinton et al., 1991). Although the caecal lactic acid content was not estimated in this study, it can be presumed that the pH-reducing effects of, most probably, elevated lactate concentrations on the 2% and 3% LAC treatments caused an increase in the undissociated form of some caecal VFA in birds aged 6 weeks. Apparently, as a consequence of their activity, the viable counts of members of the coliform bacterial group were reduced in these broilers. In extensive research into the role of VFA in the establishment of the caecal microflora in chickens, van der Wielen et al. (2000) clearly evidenced that undissociated acetate, propionate, and butyrate are responsible for the reduction of counts of *Enterobacteriaceae* in the caeca of broiler chickens during growth. A positive relationship between the presence of lactose (purified or dried whey-sourced) in diets and the abundance of enterococci, *E. coli* or total coliforms in the caeca has been reported by some authors (van der Wielen et al., 2002; Kermanshahi and Rostami, 2006).

The results obtained under the conditions of this experiment lead to the conclusion that the dietary inclusion level of 2% lactose (LAC) originated from dried whey is the most effective in enhancing the productivity of broilers. Though the excreta were less compact and the caeca more distended in chickens fed 2% LAC diets compared with those of the control, this dietary treatment resulted in significant improvements in weight gain by day 21 and throughout the entire grow-out period lasting 42 days. There was also an increase in breast muscle yield of chickens that were fed the 2% LAC diet continuously from week 2 to 6 of their life. Moreover, it was demonstrated that this treatment significantly increased caecal concentrations of total and undissociated VFA, and was efficacious in reducing counts of potentially harmful coliform bacteria, and in promoting caecal LAB species to develop. These latter findings may be regarded as valuable indicators pointing to the prebiotic properties of whey lactose included in broiler diets.

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Received: 23 III 2017
Accepted: 14 VII 2017