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THE EFFECT OF DIFFERENT MEDIUM-CHAIN FATTY ACIDS, CALCIUM BUTYRATE, AND SALINOMYCIN ON PERFORMANCE, NUTRIENT UTILIZATION AND GASTROINTESTINAL TRACT OF CHICKEN OF POLISH GREEN-LEGGED PARTRIDGE HEN

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Abstract

The present study aimed to determine the effect of diets supplemented by feed additives, on the growth performance and digestive tract functioning in chicken of Green-Legged Partridge hen (GLPH). Two types of diets were used. Both the "low-digestible" (LD) diet characterized by high level of crude fiber and lard, and the "high-digestible" (HD) diet characterized by low content of crude fiber and soybean oil were supplemented by different additives such as 0.34% DM (dry matter) salinomycin, 0.30% DM medium-chain triglycerides (MCT), 1.00% DM calcium butyrate (CB), or 0.85% DM medium-chain fatty acids (MCFA). The experiment was conducted on 960 one-day-old male chickens of GLPH, randomly divided into 10 treatment groups. One group consisted of 12 replications (each with 8 birds). Chickens fed HD diet presented significantly higher body weight gain (BWG) in 15–56 days and also 0–56 days of experiment and lower feed conversion ratio (FCR) during whole time (0–56 days) of experiment (P<0.05). Addition of CB and MCFA did not affect the differences in BWG (15–56; 0–56 days) and FCR (15–56 days) in comparison with the control diet. Significant interaction between the type of diet and additives (P<0.05) was observed for feed intake (FI) throughout the experiment. The highest FI was found on LD diet with CB. On LD diet, all additives resulted in significantly worse nitrogen retention, however, on HD diet, it was observed only for salinomycin. The additives had no impact on fat digestibility when chickens were fed HD diet. There was significant (P<0.05) lower-fat digestibility when LD diet with MCT, CB, and MCFA was used. The greatest value of nitrogen-corrected apparent metabolizable energy (AME_N) was found on HD diet (P<0.05). The addition of MCT, CB, and MCFA increased the percentage share of ileum, and MCT also significantly increased the percentage share of the liver. The lactic acid bacteria and *C. perfringens* counts were lower on HD diet (P<0.05). The addition of MCT and CB increased the count of lactic acid bacteria in caeca (P<0.05). Many interactions between experimental factors in this experiment showed that the efficacy of using different feed additives depends on the type of diet. The results suggest that MCFA may be the most beneficial feed additive for GLPH chicken as an alternative for antibiotic growth promoters (AGP).

Key words: chicken, Green-Legged Partridge hen, medium-chain fatty acids, calcium butyrate

Many types of pathogens present in the poultry production may affect its profit and are the source of human diseases. Campylobacteriosis is one of the most common zoonoses, caused by contact with sick birds or by the consumption of contaminated poultry products (Hovorková et al., 2015). Another dangerous disease affecting humans is salmonellosis. Although in recent years there has been a decrease in the number of sick people, the situation still has to be controlled (Koutsoumanis et al., 2019). Many countries have their own monitoring systems for protecting poultry production against different serotypes of *Salmonella* (Kuczkowski et al., 2015). Until 2006, antibiotic growth promoters were popularly used for protection against many pathogens in poultry nutrition. However, the use of AGP is currently forbidden in Europe. The growth parameters when AGP were used, were improved by limiting the development of pathogenic microbiota in the gastrointestinal tract of birds and reduced the use of nutrients by pathogens. Using AGP limited the development of pathogenic microflora which led to improved effectiveness of gastrointestinal tract processes, which used the positive bacteria (Brennan et al., 2003; Knarreborg et al., 2004).

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Salinomycin is an ionospheric antibiotic, which exhibits anticoccidial properties. After its addition to the feed, salinomycin enters the protozoa cells, leading to high ionophore concentration inside the cells and death of the cells (Danforth et al., 1977; Bolder et al., 1999). In general, coccidiostats are used popularly in poultry nutrition for protecting birds against *Eimeria* species, which cause gastrointestinal diseases. Through the control of coccidia with salinomycin and others, the poultry production is made safer, which may prevent the risk related to *Eimeria* and improve the economical aspect (Engberg et al., 2000; Johansen et al., 2007). Either way, the use of coccidiostats creates some controversy among consumers, and their residues in products can affect animal or human health. However, the using of salinomycin is allowed in chicken nutrition (Commission Implementing Regulation (EU) 2017/1914).

Butyric acid and medium-chain fatty acids (MCFA) may have an effect on growth performance, body weight, and feed intake (FI) and can restrict the development of deleterious bacteria in the gastrointestinal tract by reducing the pH value (Evans and Sayers, 2000; Namkung et al., 2011; Lipiński et al., 2016; Van der Aar et al., 2017). Alternative additives generally exhibit a similar mechanism of action like that of AGP with effects that could improve the growth performance and promote proper functioning of the gastrointestinal tract (Huyghebaert et al., 2011). In addition, inclusion of MCFA into the drinking water or feed yields positive results in limiting the development of *Campylobacter jejuni* (Solis de Los Santos et al., 2008; Hermans et al., 2010). On the other hand, other data suggest that both MCFA and butyric acid did not significantly improve chicken production (Hejdysz et al., 2012; Ocejo et al., 2017). The use of feed additives such as AGP, coccidiostats, or others creates much controversy among consumers. Most often, many consumers believe that the products from organic farms are of much higher quality. Eco-trend has gained significant attention in the past years (Grashorn and Serini, 2006). Our previous experiments were focused on fast-growing chickens that were maintained and fed conventionally (Hejdysz et al., 2018). However, the Polish breed Green-Legged Partridge hen (GLPH) can be successfully used in organic farms and be a source of meat and eggs free from many food additives, which have a negative effect from the consumer point of view (Grashorn and Serini, 2006). Organic acids are not forbidden in organic poultry production and could make it much safer from a microbiological aspect with regard to human health. Previously presented papers did not show sufficiently clear results as to whether MCFA or butyrate may improve the growth parameters and can be treated as good alternatives for AGP or coccidiostats (Hejdysz et al., 2018). Therefore, as a continuation, the present study aimed to determine the effect of diets with different crude fiber contents and fat sources (low digestible (LD) and high digestible (HD)), which were supplemented by salinomycin, MCFA, medium-chain triglycerides (MCT), or calcium butyrate (CB), on the growth performance and digestive tract functioning in chicken of GLPH.

Material and methods

All the research methods and procedures were conducted according to the relevant European Union (EU) directives, and the study protocol was approved by the Local Ethics and Animal Experimentation Committee at Poznań University of Life Sciences. All the requirements concerning animal welfare were followed.

Table 1. Composition of experimental diets – starter (0–14 day) and grower (15–56 day) (LD)

Item, % of diet	Starter (1–14 day)					Grower (15–56 day)				
	K	S	MCT	CB	MCFA	K	S	MCT	CB	MCFA
1	2	3	4	5	6	7	8	9	10	11
Wheat	30.31	29.96	30.40	29.31	29.46	33.16	33.15	33.20	32.50	32.65
Rye	5.00	5.00	5.00	5.00	5.00	4.90	4.90	4.90	4.90	4.90
Barley	25.00	25.00	25.00	25.00	25.00	30.00	30.00	30.00	30.00	30.00
Soybean meal	23.50	23.50	23.50	23.50	23.50	16.30	16.30	16.30	16.30	16.30
Rapeseed meal	6.00	6.00	6.00	6.00	6.00	5.00	5.00	5.00	5.00	5.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Lard	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Monocalcium phosphate	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Limestone	0.42	0.42	0.42	0.42	0.42	0.40	0.40	0.40	0.40	0.40
$NaHCO_3$	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
NaCl	0.26	0.26	0.26	0.26	0.26	0.20	0.20	0.20	0.20	0.20
DL-methionine	0.21	0.21	0.21	0.21	0.21	0.15	0.15	0.15	0.15	0.15
L-lysine HCl 98	0.26	0.26	0.26	0.26	0.26	0.20	0.20	0.20	0.20	0.20
L-threonine	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Premix	1.00[1]	1.00[1]	1.00[1]	1.00[1]	1.00[1]	1.00[2]	1.00[2]	1.00[2]	1.00[2]	1.00[2]

Table 1 – contd.

1	2	3	4	5	6	7	8	9	10	11
TiO_2	–	–	–	–	–	0.30	0.30	0.30	0.30	0.30
Addition	–	0.34	0.30	1.00	0.85	–	0.34	0.30	1.00	0.85
Calculated composition (%)										
ME (MJ/kg)		12.00					11.90			
crude protein		21.00					19.00			
non phytate P		0.45					0.42			
calcium		1.00					1.00			
lysine		1.10					1.00			
methionine		0.47					0.41			
Determined value (%)										
crude protein		20.87					18.90			
crude fat		5.92					5.97			
crude fiber		4.42					4.07			

[1]Composition: vit. A 12000 IU. vit. D_3 3000 IU; vit. E 35 mg; vit. K 2.5 mg; vit. B_1 3 mg; vit. B_2 6 mg; vit. B_6 8 mg; vit. B_{12} 0.03 mg; niacin 30 mg; d-pantothenic acid 15 mg; folic acid 2 mg; d-biotin 1 mg; choline 200 mg; betaine 125 mg.

[2]Composition: vit. A 10000 IU. vit. D_3 2400 IU; vit. E 30 mg; vit. K 2 mg; vit. B_1 2 mg; vit. B_2 5 mg; vit. B_6 5 mg; vit. B_{12} 0.03 mg; niacin 24 mg; d-pantothenic acid 17.4 mg; folic acid 0.8 mg; d-biotin 0.8 mg; choline 200 mg; betaine 100 mg.

K – control, S – salinomycin, MCT – triglyceride form of capric acid and caprylic acid (1.38:1), CB – calcium butyrate, MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1).

Table 2. Composition of experimental diets – starter (0–14 day) and grower (15–56 day) (HD)

Item	Starter (1–14 day)					Grower (15–56 day)				
	K	S	MCT	CB	MCFA	K	S	MCT	CB	MCFA
Ingredient (%) of diet										
corn	46.63	46.28	46.63	45.63	45.86	50.77	50.76	50.81	50.11	51.01
soybean meal 44%	35.40	35.40	35.40	35.40	35.60	35.30	35.30	35.30	35.30	35.30
wheat	10.00	10.00	10.00	10.00	10.00	5.00	5.00	5.00	5.00	5.00
soybean oil	4.13	4.13	4.13	4.13	4.13	4.74	4.74	4.74	4.74	4.74
monocalcium phosphate	1.11	1.11	1.11	1.11	1.16	1.02	1.02	1.02	1.02	1.02
limestone	0.57	0.57	0.57	0.57	0.16	0.48	0.48	0.48	0.48	0.06
$NaHCO_3$	0.10	0.10	0.10	0.10	0.10	0.43	0.43	0.43	0.43	0.05
NaCl	0.29	0.29	0.29	0.29	0.33	0.33	0.33	0.33	0.33	0.33
DL-methionine	0.37	0.37	0.37	0.37	0.37	0.26	0.26	0.26	0.26	0.26
L-lysine HCl 98	0.28	0.28	0.28	0.28	0.29	0.01	0.01	0.01	0.01	0.06
L-threonine	0.11	0.11	0.11	0.11	0.14	–	–	–	–	–
premix	1.00[1]	1.00[1]	1.00[1]	1.00[1]	1.00[1]	1.00[2]	1.00[2]	1.00[2]	1.00[2]	1.00[2]
TiO_2	–	–	–	–	–	0.30	0.30	0.30	0.30	0.30
addition	–	0.34	0.30	1.00	0.85	–	0.34	0.30	1.00	0.85
Calculated composition (%)										
ME (MJ/kg)			12.00					11.90		
crude protein			21.00					19.00		
nonphytate P			0.45					0.42		
calcium			1.00					1.00		
lysine			1.10					1.00		
methionine			0.47					0.41		
Determined value (%)										
crude protein			21.03					18.98		
crude fat			6.02					6.31		
crude fiber			3.27					3.01		

[1]Composition: vit. A 12000 IU. vit. D_3 3000 IU; vit. E 35 mg; vit. K 2.5 mg; vit. B_1 3 mg; vit. B_2 6 mg; vit. B_6 8 mg; vit. B_{12} 0.03 mg; niacin 30 mg; d-pantothenic acid 15 mg; folic acid 2 mg; d-biotin 1 mg; choline 200 mg; betaine 125 mg.

[2]Composition: vit. A 10000 IU. vit. D_3 2400 IU; vit. E 30 mg; vit. K 2 mg; vit. B_1 2 mg; vit. B_2 5 mg; vit. B_6 5 mg; vit. B_{12} 0.03 mg; niacin 24 mg; d-pantothenic acid 17.4 mg; folic acid 0.8 mg; d-biotin 0.8 mg; choline 200 mg; betaine 100 mg.

K – control, S – salinomycin, MCT – triglyceride form of capric acid and caprylic acid (1.38:1), CB – calcium butyrate, MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1).

Animals and feeding program

The experiment was conducted on 960 one-day-old male chickens of GLPH, randomly divided into 10 treatments (average weight 41±2 g). Each group consisted of 12 replications (each with 8 birds). The experiment was performed in totally controlled environmental conditions: the birds were reared on wood shavings litter in pens of dimensions 1.2 × 0.8 m; the room temperature was gradually reduced from 30°C to 21–22°C; the lighting program comprised 24 h of light until 10 days, followed by 19 h of light and 5 h of darkness. Both LD and HD diets were supplemented with salinomycin – 0.34%; triglyceride form of capric and caprylic acid (MCT; 1.38:1) – 0.3%; CB – 1.0%; and mixture of caproic, caprylic, and capric acids (MCFA; 1:1:1) – 0.85%. Salinomycin was purchased from Huvepharma NV, while the other additives from Brenntag Polska Sp. z o.o. The chickens were fed isoprotein and isoenergetic experimental diets: in the period of 1–14 days with starter; in days 15–56 – with grower mixtures based on wheat, rye, barley, rapeseed meal, and fish meal – LD (Table 1) or diet based on corn, wheat, and soybean meal – HD (Table 2). Each kind of diet was composed of five treatments. The linear optimization based on own previous dates was used to calculate the composition of feed mixtures. Chickens were fed mixtures in mashed form, *ad libitum* throughout the experiment, and coccidiostats were not added because all the additives used exhibit some anticoccidial properties. Nutrients met or exceeded the breeder recommendations for slow-growing chickens (Smulikowska and Rutkowski, 2018). Nonabsorbable marker such as titanium dioxide was added to all mixtures (3 g/kg) and used to calculate nitrogen retention (NR), crude fat digestibility coefficient, and nitrogen-corrected apparent metabolizable energy (AME_N) value in the excreta.

Data collection

Excreta was collected in four days at the end of the experiment, twice a day in each cage on floor covered with plastic foil (Rutkowski et al., 2016). Subsequently, excreta (pooled per cage and n = 10 for each group) were homogenized and frozen to be prepared for chemical analysis. In total, 36 birds were selected randomly (3 birds per replication) at the end of the experiment (56 days) and slaughtered by cervical dislocation. Then, the organs and digestive tract were removed at once. A part of fresh contents of the crop, ileum, and caeca from 21 chickens per treatment was used for microbiological assays.

Analytical methods

Before chemical analysis, excreta samples were subjected to lyophilization (Christ 1825 Medizinischer Apparatebau 326; Martin Christ GmbH, Osterode, Germany) and grinding. In the starter and grower diets as well as in the excreta samples, the parameters were determined in accordance with the AOAC standards (2007): level of crude fat (method 920.39) using a Soxtec System HT 1043 Extraction Unit (Foss Tecator, Denmark) and level of crude protein (method 976.05) using a Kjell-Foss Automatic instrument, model 16210 (A/S N. Foss Electric, Denmark). To determine the amino acid content in the mixtures, Amino Acid Analyzer AAA-400 (INGOS s.r.o., Praha, Czech Republic) was used, and ninhydrin was used for 10 postcolumn derivatizations. All the samples were hydrolyzed with 6 N HCl for 24 h at 110°C in advance (procedure 994.12; AOAC, 2005). Methionine was determined as methionine sulfone, and cystine was determined as cysteic acid with procedure 994.12, alternative 3; 15 (AOAC, 2005) after cold performic acid oxidation before hydrolysis. Calcium was analyzed according to the procedure of the AOAC (2007), and phytate was analyzed in the mixtures according to the method of Haug and Lantzsch (1983). Nonphytate-P was calculated as total P minus phytate. The level of crude fiber in the mixtures was analyzed using PN-EN ISO 6865. In both the mixtures and the excreta, the level of titanium dioxide was determined using the method of Short et al. (1996), and the samples were prepared according to the method presented by Myers et al. (2004). A calorimeter bomb standardized with benzoic acid (KL 12Mn, Precyzja-Bit PPHU, Poland) was used to evaluate the gross energy in the mixtures and samples.

Microbial status of gastrointestinal tract

For bacterial enumeration, subsamples obtained from the ileum (lactic acid bacteria and *Clostridium perfringens*) and caeca (lactic acid bacteria) of 2.5 g were diluted in 30 mL of sterile prereduced salt medium, and the suspensions were homogenized for 2 min in CO_2-flushed plastic bags using a stomacher homogenizer (Interscience, France). Subsequently, the samples were serially diluted in 10-fold steps using prereduced salt medium (Miller and Wolin, 1974). Lactic acid bacteria were enumerated by spread plating on de Man, Rogosa, and Sharpe agar (CM0361, Oxoid Limited, United Kingdom). *C. perfringens* were enumerated on Iron Sulfite Agar (42603, Biomerieux, Marcy l'Etoile, France) with d-cycloserine supplement (42619, Biomerieux) and incubated for 24 h at 38°C in anaerobic jars (Anaerocult A, Merck 1.13829).

Calculation and statistical analysis

Total tract digestibility of crude fat (CF), nitrogen retention and AME_N values were determined using the following formula:

$$TTD = \left\{1 - \left(\frac{TiO2\left[\frac{g}{kg}diet\right]}{TiO2\left[\frac{g}{kg}EX\right]}\right)\left(\frac{CF\left[\frac{g}{kg}EX\right]}{CF\left[\frac{g}{kg}diet\right]}\right)\right\} \times 100\,\%$$

where: *TTD* = total tract digestibility, *EX* = excreta, and *CF* = crude fat.

Table 3. The effect of organic acids and diets on performance results of Green-Legged partridge hen[1]

Group	Additive	Diet	BWG			FI			FCR		
			0–14 day	15-56 day	0–56 day	0–14 day	15–56 day	0–56 day	0–14 day	15–56 day	0–56 day
1	-	LD	72 D	638	710	217 b	2155 A	2372 AB	2.92	3.39	3.35
2	Salinomycin	LD	75 CD	609	684	220 b	2127 A	2344 ABC	2.96	3.48	3.42
3	MCT	LD	74 D	600	674	219 b	2047 A	2262 BCD	2.92	3.42	3.36
4	CB	LD	70 D	642	712	244 a	2179 A	2440 A	3.20	3.34	3.34
5	MCFA	LD	71 D	638	708	216 b	2034 A	2210 CD	3.02	3.19	3.18
6	-	HD	95 AB	678	773	224 b	1811 BC	2011 EF	2.36	2.56	2.57
7	Salinomycin	HD	97 A	656	751	223 b	1892 B	2135 DE	2.32	2.89	2.82
8	MCT	HD	89 B	650	731	215 b	1751B CD	1942 F	2.47	2.65	2.63
9	CB	HD	81 C	657	740	207 b	1658 D	1937 F	2.49	2.58	2.57
10	MCFA	HD	94 AB	657	750	216 b	1739 CD	1979 F	2.41	2.69	2.65
SEM			1.21	4.04	4.54	2.19	22.54	22.69	0.04	0.04	0.04
Main effect											
		LD	73	625 B	697 B	223	2109	2327	3.00 a	3.36 A	3.33[A]
		HD	91	660 A	749 A	217	1772	1999	2.41 b	2.68 B	2.65[B]
	-		84	658 a	741 a	220	1999	2199	2.63	2.99 b	2.96
	Salinomycin		85	633 c	717 ab	221	2004	2240	2.63	3.18 a	3.10
	MCT		81	623 c	701 b	217	1899	2095	2.70	3.02 ab	2.98
	CB		76	650 ab	726 a	225	1930	2188	2.82	2.94 b	2.94
	MCFA		83	648 ab	729 a	216	1893	2090	2.71	2.94 b	2.91
Diet			<.0001	<.0001	<.0001	0.134	<.0001	<.0001	<.0001	<.0001	<.0001
Addition			0.001	0.021	0.018	0.693	0.048	0.008	0.230	0.029	0.106
Interaction											
Addition x Diet			0.029	0.437	0.444	0.012	0.038	0.031	0.708	0.211	0.343

SEM – standard error of the mean; MCT – triglyceride form of capric acid and caprylic acid (1.38:1); CB – calcium butyrate; MCFA – mixture of caproic acid; caprylic acid and capric acid (1:1:1); LD – low digestible diet; HD – high digestible diet; a–b vs. A–B Means with a column with no common letters differ significantly (P≤0.05 vs. P≤0.01). [1]Means represent 12 pens of 8 chicks each.

$$AMEN = \left[GE\left[\frac{kcal}{kg} EX\right] \left(\frac{TiO2\left[\frac{g}{kg} diet\right]}{TiO2\left[\frac{g}{kg} EX\right]} \right) \right] - 33.4$$

$$\left[N\left[\frac{g}{kg} EX\right] - \frac{TiOw\left[\frac{g}{kg} diet\right]}{TiO2\left[\frac{g}{kg} EX\right]} \right]$$

where: *GE* = gross energy, *N* = nitrogen, and *34.4* = the energy equivalent of uric acid nitrogen (Hill and Anderson, 1958).

The results were statistically analyzed using the general linear model of Statsoft (2011), according to the following general model:

$$Yij = \mu + \alpha i + \beta j + (\alpha\beta)ij + \delta ij$$

where: *Yij* was the observed variable; μ was the overall mean; αi was the effect of diet; βj was the effect of supplement; $(\alpha\beta)ij$ was the interaction between diet and supplements; and δij was the random error.

In cases where the overall effect was significant ($P \leq 0.05$, $P \leq 0.01$), means were compared pairwise (pdiff). Results are expressed as the least squares means with pooled standard error of the mean (SEM). Two-way analysis of variance (ANOVA) and Duncan's test were used to identify significant statistical differences and compare means.

Results

Growth performance

The mortality was low (<1%) throughout the experiment (groups 1, 2, 5, 8, 9 – 1.04% in each, group 6 – 2.08%). Table 3 shows the effects of using different diets and different additives on performance results in chicken of GLPH. The interaction between diet and additives was observed for body weight gain (BWG) in the first period of the experiment (0–14 days). In the first period, additives in LD diets had no significant impact on BWG, but CB added to HD diet significantly reduced the BWG compared with control diet and other additives. MCT also significantly reduced BWG when compared to salinomycin in HD diets. However, no interaction for BWG was observed in the next period of the experiment (15–56 days) and in general (0–56 days). The kind of diet (HD or LD) used had a significant effect on BWG ($P<0.05$); HD diet led to greater BWG in 15–56 days and 0–56 days of the experiment. The interaction was observed for FI for each part of the experiment (0–14 days; 15–56 days; 0–56 days). FI in the first period (0–14 days) of the experiment was higher only on LD diet with the addition of CB ($P<0.05$). In the second part of the experiment (15–56 days), the impact of using additives was not observed on LD diet. However, addition of CB and MCFA to HD diet significantly reduced FI compared to salinomycin included diet during 15–56 days. In this period, CB addition to HD diet resulted in lower FI when compared to control diet as well ($P<0.05$). There was no interaction for FCR in the experiment. FCR was significantly higher on LD diet in every period of the experiment ($P<0.05$). Addition of salinomycin led to a significantly higher ($P<0.05$) FCR than the other groups (except MCT) in the second period of the experiment (15–56 days). In comparison with the control diet, the other additives had no impact on FCR in the second part (15–56 days) of the experiment. The effect of used additives on FCR was not observed in periods 14 days and 0–56 days.

NR, crude fat digestibility, and AME_N

The effect of using feed additives and different diets on NR, crude fat digestibility, and AME_N is shown in Table 4. The significant interaction ($P<0.05$) between the kind of diets and additives used was observed for NR and crude fat digestibility. The additives used, except salinomycin, did not have any significant effect on NR in HD diet as they reduced nitrogen retention (%) in LD diets when compared to control group ($P<0.05$). There was no significant difference ($P>0.05$) in crude fat digestibility on HD diet. The additives reduced crude fat digestibility on LD diet ($P<0.05$); however, salinomycin diet was not significantly different from control diet. Interaction was not observed ($P = 0.084$) for AME_N; however, significant difference ($P<0.05$) was found only for the kind of diets. The AME_N value was significantly higher ($P<0.05$) on an HD diet, and the additives used had no impact.

Gastrointestinal tract measurements

Table 5 shows the effect of treatments on the relative weights of the liver, pancreas, ileum, and caeca in terms of body weight of GLPH. The interaction between the type of diet and additives was observed for the percentage share of caeca only ($P<0.05$). Salinomycin used on LD diet resulted in lower percentage share of caeca than MCFA ($P<0.05$). LD diet led to a decrease in the percentage share of the liver but an increase in the percentage share of the ileum ($P<0.05$). The additives used had a significant effect on the percentage share of the liver and ileum ($P<0.05$). Addition of MCT resulted in an increase in the percentage share of the liver. Furthermore, enhancement in the percentage share of the ileum on CB, MCT, and MCFA diets was observed and the difference was significant ($P<0.05$).

Table 4. The effect of organic acids and diets on crude fat digestibility, nitrogen retention and apparent metabolizable energy value (kcal/kg) of Green-Legged partridge hen[1]

Group	Additive	Diet	Nitrogen retention (%)	Crude fat digestibility (%)	AME_N (kcal/kg)
1	-	LD	39.5 AB	73.3 B	2769
2	Salinomycin	LD	24.0 CD	72.7 B	2623
3	MCT	LD	25.6 CD	65.0 C	2700
4	CB	LD	27.5 C	63.0 C	2689
5	MCFA	LD	20.7 D	67.5 C	2669
6	-	HD	40.7 A	83.0 A	2718
7	Salinomycin	HD	33.8 B	86.5 A	2705
8	MCT	HD	40.5 A	84.6 A	2803
9	CB	HD	40.2 A	81.3 A	2798
10	MCFA	HD	42.5 A	86.9 A	2873
SEM			1.0	1.0	15
Main effect					
		LD	27.9	68.3	2691 B
		HD	39.6	84.4	2781 A
	-		40.1	78.1	2743
	Salinomycin		29.1	79.6	2664
	MCT		34.1	75.2	2752
	CB		34.1	72.1	2747
	MCFA		33.1	77.6	2776
Diet			<.0001	<.0001	0.003
Addition			<.0001	0.001	0.148
Interaction					
Addition × Diet			<.0001	0.035	0.084

SEM – standard error of the mean; MCT – triglyceride form of capric acid and caprylic acid (1.38:1); CB – calcium butyrate; MCFA – mixture of caproic acid; caprylic acid and capric acid (1:1:1); LD – low digestible diet; HD – high digestible diet; a–b vs. A–B Means with a column with no common letters differ significantly ($P \leq 0.05$ vs. $P \leq 0.01$). [1]Means represent 12 pens of 8 chicks each.

Table 5. The effect of organic acids and diets on percentage share of organs in body weight of Green-Legged partridge hen[1]

Group	Additive	Diet	Liver	Pancreas	Ileum	Caeca
1	–	LD	2.57	0.30	3.42	0.48 abc
2	Salinomycin	LD	2.46	0.27	3.18	0.39 c
3	MCT	LD	2.70	0.31	4.17	0.48 abc
4	CB	LD	2.44	0.26	4.01	0.45 bc
5	MCFA	LD	2.40	0.28	4.23	0.50 ab
6	–	HD	2.63	0.27	3.22	0.48 abc
7	Salinomycin	HD	2.60	0.26	3.20	0.56 ab
8	MCT	HD	2.86	0.28	3.83	0.49 ab
9	CB	HD	2.67	0.26	3.95	0.43 bc
10	MCFA	HD	2.63	0.27	3.73	0.52 ab
SEM			0.03	0.003	0.06	0.01
Main effect						
		LD	2.52 B	0.28	3.81 a	0.46
		HD	2.68 A	0.27	3.59 b	0.50
	-		2.61 B	0.28	3.32 B	0.48
	Salinomycin		2.54 B	0.26	3.20 B	0.47
	MCT		2.78 A	0.29	4.00 A	0.49
	CB		2.56 B	0.26	3.98 A	0.45
	MCFA		2.52 B	0.28	3.98 A	0.51
Diet			0.001	0.079	0.021	0.045
Addition			0.004	0.066	<.0001	0.307
Interaction						
Addition × Diet			0.763	0.657	0.389	0.023

SEM – standard error of the mean; MCT – triglyceride form of capric acid and caprylic acid (1.38:1); CB – calcium butyrate; MCFA – mixture of caproic acid; caprylic acid and capric acid (1:1:1); LD – low digestible diet; HD – high digestible diet; a–b vs. A–B Means with a column with no common letters differ significantly (P≤0.05 vs. P≤0.01); [1]Means represent 15 chickens per treatment.

Table 6. The effect of organic acids and diets on bacterial counts in digesta (log cfu/g of digesta) of Green-Legged Partridge hen[1]

Group	Additive	Diet	Lactic acid bacteria		*C. perfringens*
			ileum	caeca	ileum
1	-	LD	7.69	8.48	3.80
2	Salinomycin	LD	7.79	8.52	3.61
3	MCT	LD	8.19	8.63	4.66
4	CB	LD	7.86	8.72	4.62
5	MCFA	LD	8.03	8.46	4.35
6	-	HD	7.54	7.91	2.78
7	Salinomycin	HD	7.55	7.90	2.94
8	MCT	HD	7.80	8.39	3.18
9	CB	HD	7.30	8.28	3.05
10	MCFA	HD	6.72	8.17	3.49
SEM			0.09	0.05	0.13
Main effect					
		LD	7.91 A	8.57 A	4.21 A
		HD	7.38 B	8.13 B	3.09 B
	-		7.62	8.20 b	3.28
	Salinomycin		7.67	8.21 b	3.24
	MCT		7.99	8.51 a	3.92
	CB		7.58	8.50 a	3.75
	MCFA		7.37	8.30 ab	3.92
Diet			0.002	<.0001	<.0001
Addition			0.220	0.019	0.060
Interaction					
Addition × Diet			0.192	0.440	0.530

SEM – standard error of the mean; MCT – triglyceride form of capric acid and caprylic acid (1.38:1); CB – calcium butyrate; MCFA – mixture of caproic acid, caprylic acid and capric acid (1:1:1); LD – low digestible diet; HD – high digestible diet; a–b vs. A–B Means within a column with no common letters differ significantly (P≤0.05 vs. P≤0.01); [1]Means represent 36 chickens in 12 pooled replicates per treatment.

The number of bacteria in the ileum (lactic acid bacteria and *C. perfringens*) and caeca (only lactic acid bacteria) is shown in Table 6. No interaction between the type of diet and kind of additives was observed for bacterial counts in the digesta. However, the additives used had an effect ($P<0.05$) on the counts of lactic acid bacteria in the caeca only, while MCT and CB led to an increase of the bacterial counts in comparison with the control diet. Significant differences between the kind of diets were observed for the concentration of both kinds of bacteria, and LD diet led to significantly ($P<0.05$) higher counts of lactic acid bacteria in both the ileum and caeca. In addition, LD diet enhanced the counts of *C. perfringens* in the ileum. No effects of the additives used were observed for the counts of lactic acid bacteria and *C. perfringens* in the ileum.

Discussion

In presented experiment conducted on Green-Legged Partridge hen chickens fed LD diet (barley, rye, wheat, rapeseed meal) or HD diet (with corn and wheat as grain components, soybean oil), also characterized by diversified crude fiber and NSP – the fact that these contents can potentially modify the gastrointestinal functions and microbial population and the effect of bioactive feed supplements were not clearly confirmed. Many authors had reported the beneficial effects of different organic acids on performance parameters (Panda et al., 2009; Hassan et al., 2010; Fascina et al., 2012). The interaction ($P<0.05$) between the kind of diets and additives used was observed in the first period (0–14 days) for BWG. The better results were observed when the birds were fed HD diet with salinomycin and MCFA and it was not significantly different from HD control diet. The addition of CB lowered BWG in the first period (0–14 days) among treatments on HD diet, however, FI and also FCR were not significantly different in this period. Many authors have reported positive impact of using butyrate on weight gain of broiler chickens (Panda et al., 2009; Mansoub, 2011; Hernandez et al., 2013). Similarly to this study, Zhang et al. (2011) did not find the effect of using CB on weight gain. The starter period could be too early to see how it influenced the development of villi of gastrointestinal tract as effect of butyrate addition. Chotikatum et al. (2009) and Sabour et al. (2019) have shown significantly improved final body weight on a diet that was supplemented by MCFA. MCFA works by directly entering the pathogen cells and releasing H^+ cations, significantly reducing the intracellular pH, and thus disrupting their functioning. In addition, it limits the development of pathogens in the intestine by lowering the pH (Khan et al., 2016). In general, young chickens are exposed to many pathogens from hatcheries that may cause deaths in the first period. No impact of the use of butyric acid on BWG was observed by Czerwiński et al. (2012). In contrast, another study (Kaczmarek et al., 2016) showed an increase in BWG in the first period (0–14 days) due to the use of encapsulated butyric acid, which had an effect on the more accurate use of CB. BWG was worse during the whole period (0–56 days) on the MCT diet. Lai et al. (2014) suggest that main factor affecting improvement in body gain is the dose of MCT. Furuse et al. (1992) showed that reduced growth of chickens could be due to the use of energy from MCT to increase the efficiency of NR, which, however, has not been confirmed in this study. In study conducted on pigs, authors have shown that protein digestibility was higher when the dose of MCT increased, however the differences in BWG were not observed (Lai et al., 2014). Importantly, diets with MCT, CB, and MCFA were associated with significantly better FCR in contrast to diet with salinomycin. It could be partially explained by the function of organic acids as a promoter for development of the gastrointestinal tract. The surface of ileum was enhanced on MCT, CB and MCFA treatments. The HD diet with salinomycin exhibited significantly worse NR ($P<0.05$), which could lead the birds to consume more feed to obtain the necessary nutrients. In addition, the salinomycin diet exhibited a slightly lower AME_N value, which forced higher FI to meet the energy needs of the birds. The growth-depressive effect after the use of salinomycin was also shown by Harms et al. (1989). On the other hand, no effect on the production parameters of broiler chickens after the use of salinomycin was reported by Engberg et al. (2000) and Kierończyk et al. (2016). The different effects of salinomycin can partly be attributed to the conditions under which the study was conducted and whether a coccidiostats was required depending on the environmental conditions. Significantly lower FI was noted on the HD diet with MCT, CB, and MCFA when compared to salinomycin diet throughout the experiment. A possible explanation is the effect of MCFA on cholecystokinin secretion, which plays a great role in feeding behavior as shown by a study conducted on rats (Douglas et al., 1990). Contrasting result was presented by Engberg et al. (2006), who showed insignificant differences in FI. In general, an interaction was observed in every part of an experiment; however, the direct impact on FI by the use of MCFA, CB, or MTC was not observed, which was consistent with the results obtained by Khatibjoo et al. (2017). Additionally, other data present the interaction ($P<0.05$) between using organic acids and crude fiber level, which had no effect on FI (Sabour et al., 2018). In the second part (15–56) of the experiment and in the entire experiment (0–56), the use of LD diet resulted in significantly worse BWG ($P<0.05$). In addition, LD diet resulted in higher FCR during every part of the experiment, which was related to lower AME_N value ($P<0.05$); the same result was reported by Hejdysz et al. (2018) and Hetland and Svihus. (2001). Birds first meet their energy needs and then protein, which results in increased consumption if the diet is less energetic. The LD diet was composed of ingredients that contain many fractions of NSP, which change the viscosity of the nutrient content,

increase the time of passage of the content through the intestines, and as a result, worsen the digestibility, which is consistent with the reports of Shakouri et al. (2006). On the contrary, no effect on BWG was observed after using a standard poultry diet as control and three diets with a different source of crude fiber (Amerah et al., 2009). The diet with a high level of crude fiber stimulates the development of the gastrointestinal tract. In the present study, the percentage share of the ileum was significantly greater ($P<0.05$) on LD diet, which is in accordance with other results (Hetland and Svihus, 2001; Kalmendal et al., 2011). It suggests that the development of ileum surface could increase the time when the ileal digesta are being digested and absorbed, although the components of an LD diet can be classified as difficult to digest. Kalmendal et al. (2011) have reported that the use of diets with a high level of crude fiber (insoluble) can positively affect the gut development and does not have any effect on the digestibility of nutrients. LD diet had an effect on the percentage share of the liver and ileum ($P<0.05$). The LD diet led to a greater percentage share only in ileum, while the liver exhibited a smaller percentage share. Soybean oil used in HD diet is a source of easier-to-digest fatty acids. The greater percentage share of the liver in terms of body weight can be attributed to the accumulation of these fatty acids. The only additive that contributed to the greater weight of the liver was the MCT stored in it. The addition of MCFA did not have any impact on the liver weight, and the same result was presented by Shokrollahi et al. (2014). The addition of salinomycin in LD diet led to the lowest weight of the caeca, which is really interesting because the same additive in HD diet led to the highest weight of the caeca, thereby showing a significant interaction ($P<0.05$). As a result of the use of salinomycin, proliferation of bacteria in the caeca should be limited, as reported by Knarreborg et al. (2004). Previously, it has been confirmed that butyric acid has a beneficial effect on gut development, and the working mechanism of MCFA is determined to be similar. The beneficial effects of butyrate on gut development have been reported earlier (Choct, 2009; Wu et al., 2016). Enterocytes can absorb butyric acid quickly and simply, which increases the concentration of goblet cells, enhances the bowel efficiency, and changes the ratio of crypts to villi (Adil et al., 2011). Feed components and dietary constituents commonly used in feed mixtures for sensitive growing chicks to a substantial degree have an effect on the development, histological structure of ileal walls, and digestive functions of the alimentary tract (Jørgensen et al., 1966; Noy and Sklan, 1995; Uni et al., 1998; Jamroz et al., 2006). The expected reduction of the number of potential pathogens (anaerobes, *C. perfringens*) by the bacterial action of MCFA was not observed. In many publications, the beneficial effect of these fatty acids and butyrate was confirmed (Biagi et al., 2007; Guilloteau et al., 2009; Dahiya et al., 2017), especially the use of encapsulated MCFA. The accumulation of fatty acids into the bacteria cell, dissociation and change of hydrogen ions, and pH value can reduce the bacterial enzymes activity and their vitality (Guilloteau et al., 2009). MCT and CB significantly increased the number of lactic acid bacteria in the caeca ($P<0.05$). It is well known that addition of butyric acid can reduce the number of pathogens and increase the number of positive bacteria such as lactic acid bacteria (Abdelquader et Al-Fataftah, 2016; Makled et al., 2019). Morover, Kulkarni et al. (2007) have shown that butyric acid can limit harmful bacteria gene expression. In general, organic acids limit the development of bacteria, a phenomenon which it is difficult to explain. Despite this, lactic acid bacteria are beneficial for poultry and necessary for the proper functioning of the gastrointestinal tract; furthermore, this effect is not treated like a defect (Taheri et al., 2009). All LD diets exhibit a higher content of both beneficial lactic acid bacteria and harmful bacteria such as *C. perfringens*. It was suspected that the addition of organic acid would limit the development of *Clostridium* and enhance the development of lactic acid bacteria. Previously, similar result was observed with other additives (rice husk) on poultry gut microbiota (Abazari et al., 2016). No effect of salinomycin on the counts of lactic acid bacteria was observed in this study and by Johansen et al. (2007). Engberg et al. (2000) observed decreasing of lactic acid bacteria in caeca in broiler fed diet with salinomycin. In general, salinomycin is a coccidiostat which is dedicated for protecting against *Eimeria* spp. and antibacterial properties against other pathogens are mostly not found or much poorer (Johansen et al., 2007).

In conclusion, the alternative feed additives such as MCT, CB, and MCFA could be used as alternative for salinomycin in both "low-digestible" diet and "high-digestible" diet for light strain chickens without any negative effect on the performance. However, there was no reduction in the number of pathogens. A diet with easier-to-digest nutrients can lead to better performance. MCFA and butyrate can be good alternatives for AGP and coccidiostats in poultry nutrition, especially in an ecological system of production, when native breeds are used. The results of this study suggest that MCFA may be the most beneficial additive for GLPH nutrition.

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