Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2022 / v.24 / n.1 / 001-004

http://dx.doi.org/10.1590/1806-9061-2020-1441

Original Article

Effects of Medium Chain Fatty Acid Supplementation in Broiler Diet on Microbiological Quality of Litter

■Author(s)

Zimborán A[I] https://orcid.org/0000-0003-2666-4035
Erdélyi M[II] https://orcid.org/0000-0003-2928-3545
Szabó RT[I] https://orcid.org/0000-0001-8879-7197
Weber M[I] https://orcid.org/0000-0002-8973-2472

[I] Institute of Animal Breeding, Hungarian University of Agriculture and Life Sciences, Hungary.
[II] Institute of Physiology and Nutrition, Department of Feed Safety, Hungarian University of Agriculture and Life Sciences, Hungary.

■Mail Address

Corresponding author e-mail address
Ágnes Zimborán
H-2103, Gödöllő, Páter Károly utca 1, Hungary.
Phone: 0036 (28) 522000
Email: zimboranagnes@gmail.com

■Keywords

Coconut oil, litter, medium chain fatty acids, microbiology, palm oil.

Submitted: 28/February/2021
Approved: 03/September/2021

ABSTRACT

In our experiment, coconut and palm oil supplementation was added to the diet of broiler chickens to prove the hypothesis that medium chain fatty acids (MCFAs) can reduce the occurrence of potential pathogens (*Clostridium perfingens, Coliform*) in gut microflore and therefore improve bird welfare. Cobb 500 cockerels were divided in five groups. Control birds were fed commercial broiler diet, while birds in the four treatment group diets were supplemented with coconut, palm oil, or a combination of the two, respectively. As a positive control, sunflower oil supplementation was included in the diet of the fifth group. During the 28 days of the study (from 14 to 42 days of life), 5 samplings were scheduled, when excreta samples were taken from the litter to analyse total microbial count and the number of *Clostridia*, *Coliforms,* and *Salmonella*. According to the results of microbiological analysis, coconut oil supplementation led to the continuous decline of *Clostridium perfringens* numbers until they vanished by the end of the experimental period. A similar but faster decline was found as a result of palm oil and the combined (palm oil+coconut oil) treatment. However, the number of *Coliforms* and total microbial count changed only slightly by the end of the study. No *Salmonella spp*. was present in the samples throughout the experiment. Altogether, coconut and palm oil supplementation has a beneficial effect on the microbiological composition of poultry litter.

INTRODUCTION

Medium chain fatty acids (MCFA) are saturated fatty acids with 6 to 12 carbon atoms in their aliphatic chain. Natural MCFAs normally have a paired number of carbon atoms in their molecules, like caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0). These acids are present in high proportion (60%) in coconut oil, which provides 10 per cent of the world's kitchen oil production. MCFAs are also present in palm oil (8%) and cuphea oil, but in much lower proportions (Bhatnagar *et al.*, 2009; Dayrit, 2015; Wang *et al.*, 2015).

Chemical properties of MCFAs are determined by their amphyphil character. They have antibacterial effect, as they deteriorate cell membrane and cause cell leakage, consequently resulting in the death of the bacteria. However, the mechanism behind this process is still unknown (Kim & Rhee, 2013). Due to their generous antibacterial effect and natural origin, the use of MCFAs in human medicine, agricultural production, and food industry is more and more popular (Desbois & Smith, 2010).

Organic acids such as propionic acid and MCFAs that have growth promoting and antimicrobial effects might be good alternatives to antibiotics (Paul *et al.*, 2007). According to Jang *et al*. (2007), an unnamed blend of essential oils has reduced the mortality in bacterial infections

cases in chicken flocks, as the number of *Escherichia coli* in small intestines declined. In the study, the colony forming units (CFU) of *Escherichia coli* in the ileo-cecum digesta of the antibiotic-treated group (10 mg/kg) was significantly lower as compared to the control group. However, there was no difference in the CFU of *E. coli* between the antibiotic-treated group and essential oil-treated (25 mg/kg and 50 mg/kg) groups.

Using combined treatment with capric and lauric acids results in an efficient eduction of *Clostridium perfringens* counts in chickens' *jejuna* and *ilea* (Jansman *et al.*, 2006). There are several studies in the literature proving beneficial effects of these supplements on the feed efficiency of broiler chickens (Dibner & Richards, 2005; Jansman *et al.*, 2006), but there are controversial results, as well (van Gerwe *et al.,* 2010).

According to literature data, MCFAs might be good alternatives to antibiotic growth promoters. In some scientific reports, the use of 1.2 per cent caprylic and capric acid in combination with other acids was beneficial for layers, as laying intesity and eggshell strength were increased, and the presence of *Lactobacilli* and *E. coli* in the excreta was also improved (Lee *et al.*, 2015).

In another research, the effect of MCFA supplementation used in two different concentrations (1.5 and 2 g/kg feed, respectively (Aromabiotic® Vitamex, Drongen, Belgium)) on the performance (live weight, feed efficiency) of broiler chicken was studied (Khosravinia, 2015). A high dose (2 g/kg) of MCFAs resulted in reduced mortality. Furthermore, MCFAs were also beneficial even in case of high flock density (16 and 18 birds/m^2), as they reduced the frequency of foot diseases.

Based on the aforementioned literature data, our research studied further effects of different oil supplements on microbial composition of excrets in a broiler feeding experiment.

MATERIAL AND METHODS

Cobb 500 cockerels (n=245) were used in our experiment at the reference farm of the Hungarian University of Agriculture and Life Sciences (predecessor: Szent István University, Gödöllő, Hungary). Birds were distributed among five groups with two replicates each, as follows: C (control group fed with commercial broiler diet), CO (control diet supplemented with 5% coconut oil), PO (control diet supplemented with 5% palm oil), COPO (control diet supplemented with 2.5% coconut oil and 2.5% palm oil) and SO (control diet was supplemented with 5% sunflower oil).

The fatty acid composition of coconut oil, palm oil, and sunflower oil used in the study was determined by an free-lance laboratory (EUROFINS - FoodAnalytica) in accordance with the MSZ EN ISO 12966-4: 2015 test method.

Litter was sampled in each group and replicate on a weekly basis. Samples were taken from the same place (mixed samples from several sampling points) from fresh excreta (not older than 3 hours after excretion). Samples were stored at -20°C until analysis.

Laboratory test was done by the accredited laboratory EUROFINS - FoodAnalytica (NAH-1-1582/2016), following the relevant standards. *Salmonella spp*. (MSZ EN ISO 6579:2002/A1/2007), *Clostridium perfringens* (MSZ EN ISO 7937:2005), *Coliform* (ISO 4832:2006) and total bacteria count (MSZ EN ISO 4833-1:2014) were tested as part of the analysis.

Results were recorded in Microsoft Excel 2010 (Microsoft Corp.) and data were converted into logarithmic values. The database was analysed statistically with the R 3.6.1. software (Bell Laboratories (formerly AT&T, now Lucent Technologies) by John Chambers and colleagues) and one-way ANOVA analysis was conducted at significance level of $p<0.05$. Shapiro-Wilk test and Q-Q test were run to evaluate normal distribution of the results. When significant difference was found between two data, a Tukey test was also conducted.

RESULTS AND DISCUSSION

Based on the results of fatty acid analyses, the composition of the three selected oils was different. Measurements have shown that coconut oil is high in lauric acid, palm oil is rich in palmitic acid, and sunflower oil is rich in linoleic acid (Table 1).

Table 1 – Fatty acid composition of coconut oil, palm oil, and sunflower oil used in the study

Fatty acids			CO	PO	SO
MCFA	Caprylic acid	C8:0	8.86	0	0
	Capric acid	C10:0	6.24	0	0
	Lauric acid	C12:0	43.26	0.23	0
LCFA	Myristic acid	C14:0	18.17	0.94	0
	Palmitic acid	C16:0	10.04	43.16	6.63
	Stearic acid	C18:0	3.05	4.70	3.48
	Elaidic acid	C18:1n9t	6.87	39.87	28.86
	Linoleic acid	C18:2n6c	1.66	9.66	59.83
	α- and γ-Linoleic acid	C18:3n6/3	0	0.15	0
Others			1.85	1.29	1.20

CO – coconut oil, PO – palm oil, SO – sunflower oil, MCFA – medium chain fatty acids, LCFA – long chain fatty acids.

All the samples were free of *Salmonella spp*. according to the results of the microbiological tests. Results of other bacterial counts are presented in Table 2. No significant difference was found between the groups.

Considering the *Clostridium perfringens* count, not all the samples were contaminated with the pathogen. Occurrence varied not only among groups, but also in time. For instance, at the first sampling, all samples in Group CO were contaminated with a concentration of 3.03 log10 CFU/g, while at the second sampling it reduced to 2.15 log10 CFU/g, and at the fourth sampling to 1.89 log10 CFU/g (Table 3). Also, only 50 per cent of the samples were contaminated at this stage, and by the end of the experiment no samples were contaminated with *Clostridia*. In Group PO and COPO, the concentration of the bacteria was lower, than in Group CO. At the first sampling, concentrations were as low as 0.74 and 0.50 log10 CFU/g, respectively, with contamination frequency at 50 per cent. These values increased to 0.95 and 1.00 log10 CFU/g by the time of the second sampling, and contamination vanished completely later on. *Clostridia* were present in high numbers throughout the experiment in Group SO, but the level of occurrence was variable in time. No significant difference was found between the groups.

Table 2 – Occurrence and concentration of Clostridium perfringens, Coliforms, and total bacteria in the litter (five sampling averages) samples collected during the experiment.

Group	*Clostridium perfringens*		*Coliforms*		Total bacteria	
	Average concentration log10 CFU/g	Occurrence	Average concentration log10 CFU/g	Occurrence	Average concentration log10 CFU/g	Occurrence
C	0.51	2/10	5.46	10/10	9.63	10/10
CO	1.41	5/10	5.84	10/10	9.58	10/10
PO	0.34	2/10	5.57	10/10	9.51	10/10
COPO	0.30	2/10	5.91	10/10	9.48	10/10
SO	4.97	5/10	4.94	10/10	9.62	10/10

C – control group, CO – coconut oil group, PO – palm oil group, COPO – coconut and palm oil group, SO – sunflower oil group.

Table 3 – Concentration of *Clostridium perfringens* in the litter (average concentration log10 CFU/g / sampling) samples collected during the experiment.

Group	Sampling number				
	1	2	3	4	5
C	0.80	ND	1.75	ND	ND
CO	3.03	2.15	ND	1.89	ND
PO	0.74	0.95	ND	ND	ND
COPO	0.50	1.00	ND	ND	ND
SO	2.27	0.80	0.74	ND	1.16

C – control group, CO – coconut oil group, PO – palm oil group, COPO – coconut and palm oil group, SO – sunflower oil group. ND means not detectable.

Coliform count was the lowest at the first sampling in the different groups. It was increased in time in all the groups except for Group SO. In the latter group, relatively low levels of *Coliform* counts were found at the second and fourth samplings (Table 4). No significant difference was found between the groups.

Table 4 – Concentration of *Coliforms* in the litter (average concentration log10 CFU/g / sampling) samples collected during the experiment.

Group	Sampling number				
	1	2	3	4	5
C	4.60	5.48	5.61	6.38	5.23
CO	4.91	5.58	6.01	5.93	6.78
PO	4.65	5.68	5.95	5.38	6.18
COPO	4.93	6.19	5.56	5.45	7.46
SO	5.01	4.60	6.08	2.15	6.88

C – control group, CO – coconut oil group, PO – palm oil group, COPO – coconut and palm oil group, SO – sunflower oil group.

Minimal changes have occurred in total microbial count among the groups and among the different samplings, but the tendencies were similar to the one found for *Coliform* counts (Table 5). However, significant difference occurred among the groups at the last sampling (SO-CO p=0.00334, PO-CO p=0.02649, SO-C p=0.02997, SO-COPO p=0.00758).

The MCFA content according to Bhatnagar *et al.* (2009) in coconut oil is 59.7%, while in palm oil and in sunflower oil it is 0% (no data). In our own measurement, proportions were found to be similar: 58.4% in coconut oil, 0.23% in palm oil and 0% in sunflower oil. In the experiment by Wang *et al.* (2015), the MCFA content of feed containing 1.5% coconut oil was 17.89%. In our study, this value

was 38.13% in relation to feed for the CO group. So the feed containing 5% coconut oil contained lower level of MCFA (calculated value) for 1 per cent supplementation than in the referred experiment. Jang *et al.* (2007) showed that essential oils reduced the presence of *Coliforms* in chickens, as the control group CFU (log10 / g) decreased from 3.8 to 2.8. We could not prove this in our study. Jansman *et al.* (2006) described that capric acid and lauric acid reduce the concentration of *Clostridium perfringens*. We came to the same conclusion for the CO group over time. The attenuating effect of MCFAs on the presence of *Coliforms* conflicts with the results of Lee *et al.,* (2015) as we could not confirm this effect.

Table 5 – Concentration of Total bacteria in the litter (average concentration log10 CFU/g / sampling) samples collected during the experiment.

Group	Sampling number				
	1	2	3	4	5
C	9.53^{a}	9.18^{a}	9.68^{a}	9.88^{a}	9.86^{ab}
CO	9.56^{a}	9.89^{a}	9.28^{a}	9.76^{a}	9.45^{a}
PO	8.91^{a}	9.61^{a}	9.42^{a}	9.54^{a}	10.00^{bc}
COPO	9.37^{a}	9.33^{a}	9.59^{a}	9.49^{a}	9.62^{ab}
SO	9.59^{a}	9.31^{a}	9.57^{a}	9.15^{a}	10.40^{c}

C – control group, CO – coconut oil group, PO – palm oil group, COPO – coconut and palm oil group, SO – sunflower oil group. a, b, c: different superscript letters show significant differences ($p \leq 0.05$) between groups (C, CO, PO, COPO and SO) in the same column by Tukey test.

In summary, coconut and palm oil supplementation containing MCFAs has changed the occurrence of *Clostridium perfringens*. Palm oil supplementation resulted in reduced *Coliform* count, while total microbial count was reduced efficiently by the combined supplementation of coconut and palm oil in the diet. Based on these results, we recommend performing additional studies with a larger number of samples to support more reliable statistical proof. Furthermore, it is recommended that tests are performed from the same broiler chicken individually each time.

ACKNOWLEDGEMENT

This work was supported by the EFOP-3.6.3-Vekop-16-2017-00008 and co-financed by the European Union and the European Social Fund.

REFERENCES

Bhatnagar AS, Prasanth Kumar PK, Hemavathy J, Gopala Krishna AG. Fatty acid composition, oxidative stability, and radical scavenging activity of vegetable oil blends with coconut oil. Journal of the American Oil Chemists Society 2009;86:991–999.

Dayrit FM. The properties of lauric acid and their significance in coconut oil. Journal of the American Oil Chemists Society 2015;92:1–15.

Desbois AP, Smith VJ. Antibacterial free fatty acids: sctivities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology 2010;85:1629–1642.

Dibner JJ, Richards JD. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Science 2005;84:634–643.

Jang IS, Ko YH, Kang SY, Lee CY. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Animal Feed Science and Technology 2007;134(3-4):304–315.

Jansman AJM, Wagenaars CMF, Schonewille A, Snel H, Van der Klis JD. Bestrijding van *Clostridium* en *Campylobacter* infecties in pluimvee via natuurlijke antimicrobie¨ le voedingscomponenten. Wageningen: Animal Sciences Group; 2006. p.50.

Khosravinia H. Effect of dietary supplementation of medium-chain fatty acids on growth performance and prevalence of carcass defects in broiler chickens raised in different stocking densities. Journal of Applied Poultry Research 2015;24:1–9.

Kim SA, Rhee MS. Marked synergistic bactericidal effects and mode of action of medium-chain fatty acids in combination with organic acids against *Escherichia coli* O157:H7. Applied and Environmental Microbiology 2013;79:6552–6560.

Lee SI, Kim HS, Kim I. Microencapsulated organic acid blend with MCFAs can be used as an alternative to antibiotics for laying hens. Turkish Journal of Veterinary Animal Sciences 2015;39:520–527.

Paul SK, Halder G, Mondal MK, Samanta G. Effect of organic acid salt on the performance and gut health of broiler chicken. The Journal of Poultry Science 2007;44(4):389–395.

Van Gerwe T, Bouma A, Klinkenberg D, Wagenaar JA, Jacobs-Reitsma WF, Stegeman, A. Medium chain fatty acid feed supplementation reduces the probability of *Campylobacter jejuni* colonization in broilers. Veterinary Microbiology 2010;143:314-318.

Wang J, Wang X, Li J, Chen Y, Yang W, Zhang L. Effects of dietary coconut oil as a medium-chain fatty acid source on performance, carcass composition and serum lipids in male broilers. Asian-Australasian Journal of Animal Sciences 2015;28:223–230.