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EFFECT OF FEEDING DIFFERENT COMBINATIONS OF ORGANIC ACIDS, MONO-GLYCERIDES AND PROBIOTICS ON *CAMPYLOBACTER* COLONIZATION IN BROILERSO. Casabuena¹, M. Chemaly², M. Den Hartog³, P. Vasseur³, and M.I. Gracia¹¹Imasde Agroalimentaria, S.L., Madrid, Spain²Anses, UHQPAP, Ploufragan, France³NEPLUVI, Houten, the Netherlands

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Abstract

An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the effect of a combination of a blend of mono-glycerides and organic acids (MGOA) with a probiotic alone (*B. subtilis* C-3102, P) or with a multispecies probiotic (PS) and a plant extract based on garlic (G) added to the feed on *Campylobacter* counts in broilers. There were three treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1+MGOA at 2.5%+P at 100 g/t and T3: T1+MGOA at 2.5%+PS at 1,000 g/t+G at 1,000 g/t. A total of 126 one-day-old Ross 308 broilers (half male and half female) were divided into the experimental treatments. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1×10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data were analysed with a Kruskal-Wallis test (SPSS v.19.0). At 21 d, the two combinations used consistently decreased the *Campylobacter* counts compared with T1 (by 3-log; $P < 0.05$). At 35 and 42 d, only T2 (MGOA+P) decreased the colonization of *Campylobacter* compared with T1 (3.05 vs 7.15 log₁₀ CFU/g; $P = 0.001$ and 4.41 vs 8.39 log₁₀ CFU/g; $P = 0.003$; for T2 vs T1 at 35 and 42 d, respectively). The results suggest that the supplementation of a blend of mono-glycerides with organic acids together with a probiotic, especially *B. subtilis*, can effectively decrease *Campylobacter* in broilers.

Keywords: mono-glycerides, organic acids, probiotics, *Campylobacter jejuni*, broiler**Introduction**

Campylobacter is the major cause of bacterial gastroenteritis in humans worldwide. The number of confirmed cases of campylobacteriosis in the European Union has followed a significant increasing trend in the last years, along with a clear seasonal trend. In 2013, it was the most commonly reported zoonosis with 214,779 confirmed human cases (EFSA, 2015). Few studies have evidenced a positive role of probiotics in preventing or reducing the shedding of *C. jejuni* in *in vivo* trials (MORISHITA et al., 1997; SANTINI et al., 2010), although *in vitro* studies reported a strong antimicrobial activity of several probiotic strains toward this pathogen (FOOKS and GIBSON, 2002; CHAVEERACH et al., 2004). A wide range of plant extracts and compounds have demonstrated strong bactericidal activity against *Campylobacter* spp. *in vitro* (FRIEDMAN et al., 2002; ROBYN et al., 2013). However, when evaluating *in vivo* results, data are not so conclusive. Also, *in vitro* studies have demonstrated that organic acids, medium chain fatty acids, or their monoglycerides have a strong bactericidal effect on *Campylobacter* spp. (CHAVEERACH et al., 2002; HERMANS et al., 2010). However, inconsistent results have been reported in *in vivo* trials (SOLIS DE LOS SANTOS et al., 2010; HERMANS et al. 2012). Therefore, it is clear that there exist differences in the results *in vitro* and *in vivo*. The objective of this work was to evaluate the effect of a combination of a blend of mono-glycerides and organic acids (MGOA) with a probiotic alone (*B. subtilis* C-3102, P) or with a multispecies probiotic (PS) and a plant extract based on garlic (G) added to the feed on *Campylobacter* counts in broilers.

Material and Methods

A total of 126 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were three treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + MGOA at 2.5% + P at 100 g/t and T3: T1 + MGOA at 2.5% + PS at 1,000 g/t + G at 1,000 g/t. The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. The individual bird was the experimental unit. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. Diets were cereal based diets similar to those used commercially. Prior to the infection of the animals, caecal samples of 9 birds randomly selected were collected to verify that chicks were all *Campylobacter*-free. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing

1×10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca were collected from 12 birds per treatment and *Campylobacter* counts determined (ISO 10272). Detection limit was 2 logs, and animals below limit of detection were assigned 2 logs for statistical analysis. Data were analysed with a Kruskal-Wallis test (SPSS v.19.0). Statistical significance is declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered as a near-significant trend.

Results

At 21 d, the two combinations used consistently decreased the *Campylobacter* counts compared with T1 (by 3.5-log $P = 0.010$ and by 3.0-log $P = 0.032$, for T2 vs T1 and T3 vs T1, respectively). At 35 and 42 d, only T2 (MGOA+P) decreased the colonization of *Campylobacter* compared with T1 (3.05 vs 7.15 \log_{10} CFU/g; $P = 0.001$ and 4.41 vs 8.39 \log_{10} CFU/g; $P = 0.003$; for T2 vs T1 at 35 and 42 d, respectively). This decrease was mainly due to the broilers below detection level, 75 and 8% and 58 and 17% in T2 and T3 at 35 and 42 d, respectively. Birds on T3 showed a non-significant reduction of 1.81-log compared to T1.

Conclusions

Under our experimental conditions, it is concluded that: i) Both the supplementation of a combination of a blend of MGOA with a probiotic alone or with a multispecies probiotic and a garlic extract, significantly decrease *Campylobacter* counts in the ceca of infected birds at 21d, ii) Only the blend of MGOA together with the probiotic based on *B. subtilis* significantly decreased *Campylobacter* counts at 35 and 42d (a decrease by 4-log was reached at the end of the fattening period), and iii) the blend of MGOA with the multispecies probiotic and the garlic extract reached a non-significant decrease of 1.81-log in relation to controls.

The results suggest that the supplementation of a blend of MGOA together with a probiotic, especially *B. subtilis*, can effectively decrease *Campylobacter* in broilers.

Acknowledgements

This work has been done within the project CAMPYBRO, which has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 605835.

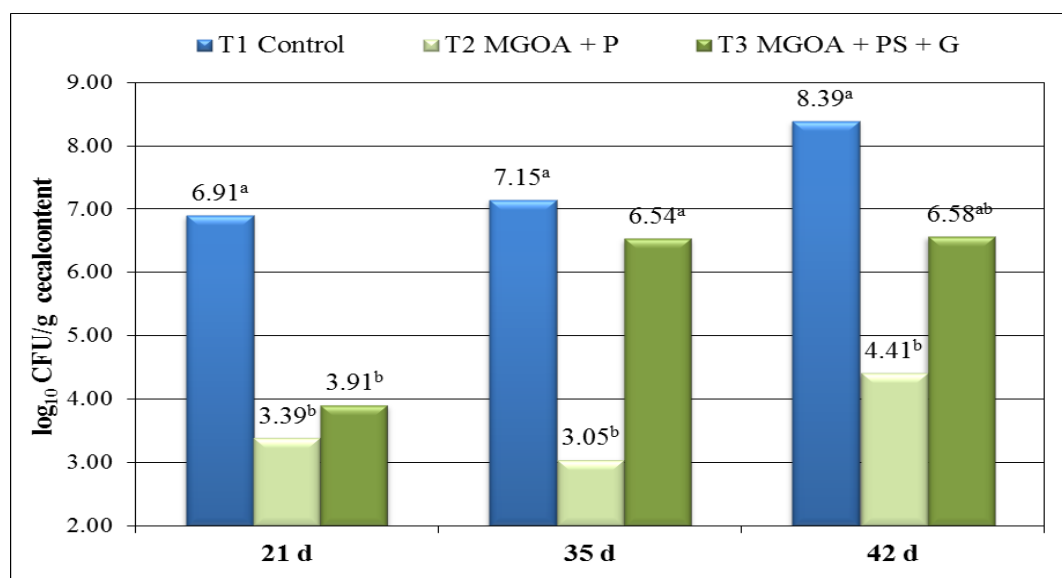


Figure 1. Effect of dietary treatment (T1: Control, T2: T1 + MGOA at 2.5% + P at 100 g/t and T3: T1 + MGOA at 2.5% + PS at 1,000 g/t + G at 1,000 g/t) on *Campylobacter* counts (\log_{10} CFU/g) in the caeca of broilers at 21, 35 and 42 days of age

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P-065 (ID 194)**EFFECT OF SUPPLEMENTING VIA DRINKING WATER A BLEND OF MONO-GLYCERIDES, ALONE OR IN COMBINATION WITH ORGANIC ACIDS, ON *CAMPYLOBACTER* COLONIZATION IN BROILERS****J. Sánchez¹, A. Parra², Y. Carre³, A. Csorbai⁴, and M.I. Gracia¹**¹Imasde Agroalimentaria, S.L., Madrid, Spain²CZ Veterinaria, Porriño, Spain³CIDEF, Rennes, France⁴BTT, Budapest, Hungary**Corresponding author:** mgracia@e-imasde.com**Abstract**

An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the effect of a blend of short and medium chain fatty acids (C3+C4+C6+C10+C12) mono-glycerides (MG) and a mixture (60:40 %) of the same mono-glycerides with organic acids (MGOA) added to the drinking water on *Campylobacter* counts in broilers. There were three treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + MG at 4ml/l from 0-21d and at 2ml/l at 21-42d and T3: T1 + MGOA at 9ml/l from 0-21d and at 4.5ml/l at 21-42d. A total of 126 one-day-old Ross 308 broilers (half male and half female) were divided into the experimental treatments. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10⁵ CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data expressed as log₁₀ CFU/g caecal content were analysed by the nonparametric test of Kruskal-Wallis (SPSS v.19.0). Although treatments, especially T3, decreased the *Campylobacter* counts 0.9 to 2.3-log, no significant differences were detected between the two products tested and the control at any of the times evaluated (21, 35 and 42 d of age). It is concluded that the two mono-glyceride combinations tested did not significantly reduce *Campylobacter jejuni* colonization under the experimental model assayed.

Keywords: mono-glycerides, organic acids, *Campylobacter jejuni*, broiler**Introduction**

Campylobacter is the major cause of bacterial gastroenteritis in humans worldwide. The number of confirmed cases of campylobacteriosis in the European Union has followed a significant increasing trend in the last years, along with a clear seasonal trend. In 2013, it was the most commonly reported zoonosis with 214,779 confirmed human cases (EFSA, 2015). *In vitro* studies have demonstrated that organic acids, medium chain fatty acids, or their monoglycerides have a strong bactericidal effect on *Campylobacter* spp. (CHAVEERACH et al., 2002; HERMANS et al., 2010). However, inconsistent results have been reported in *in vivo* trials (VAN DEUN et al., 2008; SOLIS DE LOS SANTOS et al., 2008, 2010; MOLATOVÁ et al., 2011; HERMANS et al. 2012). Therefore, it is clear that there exist differences in the results *in vitro* and *in vivo*. The objective of this work was to evaluate the effect of a blend of short and medium chain fatty acids (C3+C4+C6+C10+C12) mono-glycerides (MG) and a mixture (60:40 %) of the same mono-glycerides with organic acids (MGOA) added to the drinking water on *Campylobacter* counts in broilers.

Material and Methods

A total of 126 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were three treatments applied to the drinking water from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + MG at 4ml/l from 0-21d and at 2ml/l from 21-42d and T3: T1 + MGOA at 9ml/l from 0-21d and at 4.5ml/l from 21-42d. The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. The individual bird was the experimental unit. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. Diets were cereal based diets similar to those used commercially. Prior to the infection of the animals, caecal samples of 9 birds randomly selected were collected to verify that chicks were all *Campylobacter*-free. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10⁵ CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca were collected from 12 birds per treatment and *Campylobacter* counts determined (ISO 10272). Detection limit was 2 logs, and animals below limit of detection were assigned 2 logs for statistical analysis. Data, expressed as log₁₀

CFU/g caecal content, were analysed by the nonparametric test of Kruskal-Wallis (SPSS v.19.0). Statistical significance is declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered as a near-significant trend.

Results

Both treatments showed numerically lower *Campylobacter* counts than control, with reductions of 0.60, 0.44 and 0.66-log for T2 and 0.90, 2.3 and 1.73-log for T3 at 21, 35 and 42d, respectively (Figure 1). However, the high variability observed mainly due to the broilers below detection level observed in T3 (8 and 42% and 17 and 33% in T2 and T3 at 35 and 42 d, respectively) caused that no significant differences in the *Campylobacter* counts were observed between the two products tested and the control treatment at any of the times evaluated (21, 35 and 42 d of age).

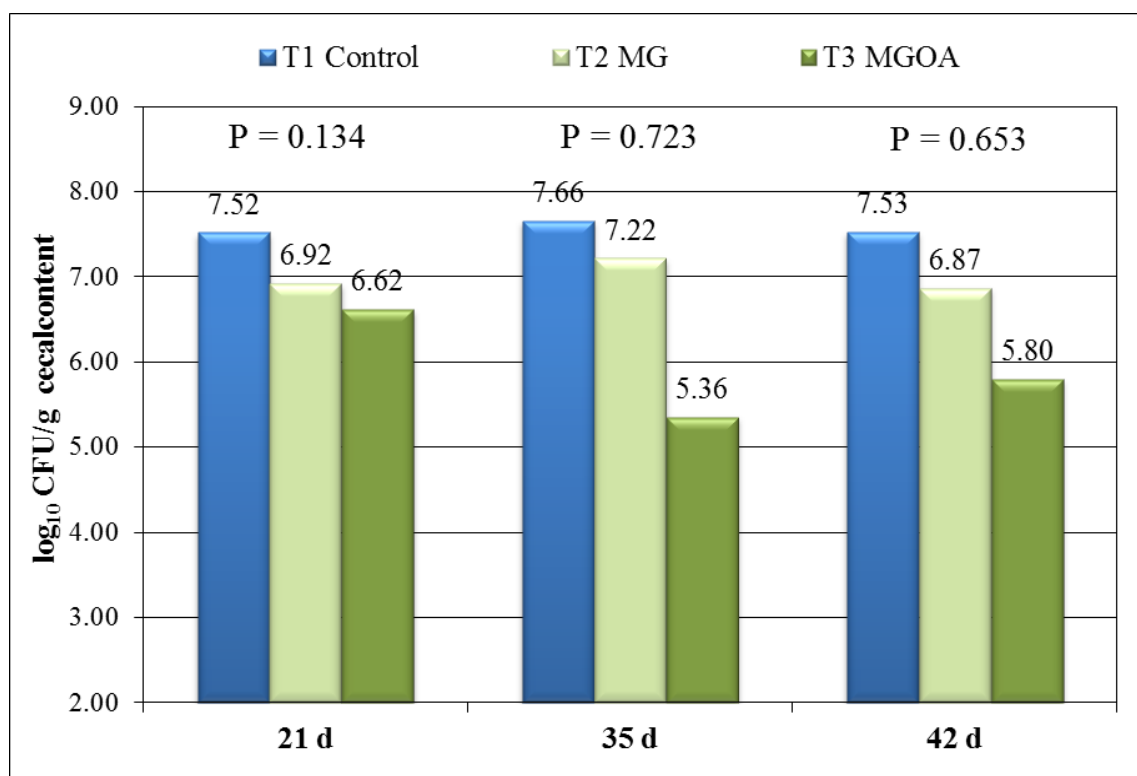


Figure 1. Effect of dietary treatment (T1: Control, T2: MG at 4ml/l from 0-21d and at 2ml/l from 21-42d and T3: MGOA at 9ml/l from 0-21d and at 4.5ml/l from 21-42d) on *Campylobacter* counts (\log_{10} CFU/g) in the caeca of broilers at 21, 35 and 42 days of age.

Conclusions

Under our experimental conditions, it is concluded that the two mono-glyceride combinations tested did not significantly reduce *Campylobacter jejuni* colonization under the experimental model assayed

Acknowledgements

This work has been done within the project CAMPYBRO, which has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 605835.

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P-153 (ID 190)

EFFECT OF TYPE OF CEREAL AND OAT HULLS ADDITION ON GASTROINTESTINAL MORPHOLOGY IN BROILERS ORALLY INFECTED WITH *CAMPYLOBACTER JEJUNI*

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Abstract

An experiment was conducted within the EU-FP7 project CAMPYBRO for evaluating the effect of type of cereal and oat hulls (OH) addition on gastrointestinal (GIT) morphology in broilers. There were five treatments: corn [C], wheat [W] or barley [B], and the inclusion of 5% of OH in C and W diets. A total of 180 one-day-old Ross 308 broilers were divided into cages (3 birds/cage) and experimental treatments (12 cages/treatment). At 14 d, all broilers were orally gavaged with 100 µl of a solution containing 1x10⁵ cfu/ml of ST-45 *C. jejuni*. *Campylobacter* effect on GIT morphology could not be analysed since all birds were infected. On days 35 and 42, the performance parameters and the weight of GIT, empty BW (EBW), proventriculus (PRO), gizzard (GIZ), and the pH at PRO and GIZ were taken. Data were analysed by GLM procedure of SPSS (v.19.0). From 0-42d birds fed B ate more feed than birds fed C-OH. Age of animals significantly affected all the GIT parameters studied decreasing their proportion in relation to BW, GIT or EBW. Birds fed B showed the biggest GIT as %BW, and OH increased the GIT weight in C and W diets and the relative importance of the GIZ, but decreased it in the PRO. Dietary treatment did not affect the pH at GIZ, but OH increased the pH at the PRO, especially in W diet. It is concluded that type of diet modifies the GIT morphology.

Keywords: *Campylobacter jejuni*, cereals, oat hulls, broiler, gastrointestinal tract

Introduction

To date, several research have investigated beneficial effects of adding oat hulls (OH), or other types or fibrous fractions into the diet of broiler chickens (HETLAND et al., 2003). Moderate amounts of some fiber sources dilutes the diet but might improve the motility and function of the gastrointestinal tract (GIT) and, eventually benefit digestive physiology without compromising growth in broiler chickens (MATEOS et al., 2012). The objective of this study was to evaluate the effect of different cereal sourced and oat hulls (OH) addition on gastrointestinal morphology in broilers orally infected with *Campylobacter jejuni*.

Material and Methods

A total of 180 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were five treatments applied from 1 to 42 d of age: corn (C), wheat (W) or barley (B), and the inclusion of 5% of OH in C and W diets. The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10⁵ CFU/ml of ST-45 *C. jejuni* strain. Performance was calculated by cage at 21 and 42 days. On days 35 and 42, twelve broilers per treatment were euthanized and the weight of the GIT, empty BW (EBW), proventriculus (PRO), gizzard (GIZ), and the pH at PRO and GIZ were taken. Data were analysed by GLM procedure (SPSS v.19.0). The model included the dietary treatment, the age and its interaction. A Tukey's test was used for mean comparison.

Results

There was no effect of dietary treatment on growth or feed conversion at any of the studied periods. From 0-21d, broilers fed wheat+OH showed higher feed intake than birds fed corn or wheat-based diets, showing the rest of treatments intermediate results. From 21-42d, broilers fed barley ate more feed than birds fed corn+OH and wheat+OH, showing the rest of treatments intermediate values. For the whole trial, broilers fed barley ate more feed than birds fed corn+OH (Table 1).

Table 1. Effect of dietary treatment on average daily gain (ADG, g), average daily feed intake (ADFI, g) and feed conversion ratio (FCR, g/g) from 0-21, 21-42 and 0-42 d of age

Treatment	0-21 d			21-42 d			0-42 d		
	ADG	ADFI	FCR	ADG	ADFI	FCR	ADG	ADFI	FCR
Barley	31.7	64.1 ^{ab}	2.038	78.1	147.9 ^a	1.921	54.9	106.2 ^a	1.949
Corn	32.4	56.9 ^b	1.815	74.2	137.9 ^{ab}	1.897	53.8	98.3 ^{ab}	1.837
Corn + 5% OH	32.2	64.3 ^{ab}	2.013	69.8	120.6 ^c	1.745	51.1	92.7 ^b	1.817
Wheat	31.5	57.8 ^b	1.849	73.9	135.5 ^{ab}	1.854	53.3	97.7 ^{ab}	1.844
Wheat + 5% OH	28.6	69.5 ^a	2.075	76.0	130.6 ^{bc}	1.731	52.0	100.5 ^{ab}	1.941
SEM (n=12)	1.192	1.445	0.091	1.920	2.062	0.049	0.988	1.447	0.033
P	0.519	0.001	0.509	0.339	0.001	0.244	0.408	0.001	0.193

SEM = Standard error of mean. Different superscripts in same column are significant (a-c: $P \leq 0.05$).

Campylobacter effect on GIT morphology could not be analysed since all birds were infected. Age of animals significantly affected to all the parameters studied showing, in general, bigger size of organs at 42d than at 35d, but lower importance expressed as percentage of BW, GIT, or EBW (Table 2).

Barley-based diets showed the biggest GIT in weight and in %BW, and consequently the lowest EBW, significant compared to corn and wheat diets (Table 3). Unusually, OH inclusion in corn and wheat diets decreased the relative importance of the proventriculus, compared to the cereals alone (4.14^{ab}, 4.65^a, 3.96^b, 4.56^a and 3.91^b %BW for barley, corn, corn+OH, wheat and wheat+OH, respectively). As expected, OH inclusion in corn and wheat diets increased the relative importance of the gizzard compared to the cereals alone (2.87^{ab}, 2.67^{bc}, 3.14^a, 2.47^c and 2.89^{ab} %BW for barley, corn, corn+OH, wheat and wheat+OH, respectively). This was a clear effect of long size particles of fiber, which stimulate the gizzard function and muscle development. Dietary treatment did not affect the pH at gizzard but, unexpectedly, OH increased the pH at the proventriculus, especially in wheat-based diets (3.69^c, 3.72^{bc}, 4.16^{ab}, 3.70^c and 4.26^a, for barley, corn, corn+OH, wheat and wheat+OH, respectively).

Conclusions

Under our experimental conditions, it is concluded that cereal type and quantity of fibre modifies the GIT morphology.

Acknowledgements

This work has been done within the project CAMPYBRO, which has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 605835.

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Table 2. Effect of age, type of cereal and oat hulls (OH) inclusion (mean values at 35 and 42 d) on gastrointestinal tract (GIT) morphology and pH

	BW, g	GIT		Empty BW		Proventriculus			
		g	%BW	g	%BW	g	%GIT	%BW	%EBW
Age, d									
35d	1713	166.1	9.69	1547.3	90.31	6.95	4.23	0.41	0.45
42d	2261	208.3	9.20	2052.4	90.80	8.73	4.26	0.39	0.43
SEM (n=60)	27.3	3.82	0.135	24.87	0.135	0.185	0.094	0.007	0.008
Treatment									
Barley	2060	210.7 ^a	10.26 ^a	1849.0	89.74 ^b	8.63 ^a	4.14 ^{ab}	0.42 ^a	0.47 ^a
Corn	1964	175.3 ^b	8.91 ^b	1788.7	91.09 ^a	8.17 ^{ab}	4.65 ^a	0.41 ^{ab}	0.45 ^{ab}
Corn + 5% OH	1938	182.6 ^b	9.45 ^{ab}	1755.5	90.55 ^{ab}	7.13 ^b	3.96 ^b	0.37 ^b	0.41 ^b
Wheat	2017	180.5 ^b	9.00 ^b	1836.8	91.00 ^a	8.04 ^{ab}	4.56 ^a	0.40 ^{ab}	0.44 ^{ab}
Wheat + 5% OH	1956	186.8 ^b	9.60 ^{ab}	1769.2	90.40 ^{ab}	7.25 ^b	3.91 ^b	0.37 ^b	0.41 ^b
SEM (n=24)	43.2	6.04	0.213	39.33	0.213	0.293	0.149	0.011	0.013
Probability									
Age	<0.001	<0.001	0.012	<0.001	0.012	<0.001	0.867	0.051	0.038
Treatment	0.256	0.001	<0.001	0.359	<0.001	0.001	0.001	0.002	0.002
Age x Treatment	0.817	0.469	0.692	0.876	0.692	0.481	0.833	0.517	0.502

	Gizzard				pH	
	g	%GIT	%BW	%EBW	Proventriculus	Gizzard
Age, d						
35d	50.45	30.44	2.95	3.27	3.90	3.36
42d	59.92	29.11	2.67	2.94	3.91	3.51
SEM (n=60)	1,203	0.458	0.054	0.062	0.072	0.063
Treatment						
Barley	57.92 ^{ab}	27.85 ^b	2.87 ^{ab}	3.20 ^{ab}	3.69 ^c	3.50
Corn	52.29 ^{bc}	29.89 ^b	2.67 ^{bc}	2.93 ^{bc}	3.72 ^{bc}	3.23
Corn + 5% OH	60.54 ^a	33.57 ^a	3.14 ^a	3.47 ^a	4.16 ^{ab}	3.32
Wheat	49.08 ^c	27.39 ^b	2.47 ^c	2.72 ^c	3.70 ^c	3.56
Wheat + 5% OH	56.08 ^{abc}	30.19 ^b	2.89 ^{ab}	3.20 ^{ab}	4.26 ^a	3.55
SEM (n=24)	1.902	0.723	0.085	0.098	0.114	0.100
Probability						
Age	<0.001	0.043	<0.001	<0.001	0.952	0.087
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	0.070
Age x Treatment	0.505	0.066	0.405	0.455	0.373	0.858

SEM = Standard error of mean. Different superscripts in same column are significant (a-c: $P \leq 0.05$).

P-242 (ID 192)

EFFECT OF PARTICLE SIZE AND FEED PRESENTATION (MASH VS PELLETS) ON CECAL MORPHOLOGY AND *CAMPYLOBACTER JEJUNI* COLONIZATION OF BROILERS ORALLY INFECTED

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Abstract

An experiment was conducted within the EU-FP7 project CAMPYBRO for evaluating the effect of particle size (PS) of wheat (W) and feed presentation (FP) in morphology and *Campylobacter jejuni* colonization in the caeca of broilers orally infected. There were four treatments factorially arranged with two W screen sizes (2mm vs 5mm) and two FP (mash vs pellets). A total of 144 one-day-old Ross 308 broilers were divided into cages (3 birds/cage) and experimental treatments (12 cages/treatment). At 14 days of age, all broilers were orally gavaged with 100 µl of a solution containing 1×10^5 cfu/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, caeca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). At 42d, the weight and the pH at caeca were taken. Data were analysed by GLM procedure of SPSS. Pelleting the diet decreased the relative importance of the caeca expressed as percentage of Gastrointestinal tract, body weight or empty body weight ($P < 0.05$). There was an interaction between FP and PS for caeca weight: pelleting of fine size diets decreased the caeca weight and the opposite was observed for 5mm-diets ($P = 0.048$). Neither the PS nor the FP caused a significant variation on *C. jejuni* counts or pH at the caeca of the birds. No interactions were observed between PS and FP. It is concluded that pelleting the diet decreased the relative weight of caeca but did not affect the pH or *C. jejuni* counts.

Keywords: *Campylobacter jejuni*, pelleting, particle size, broiler, gastrointestinal tract

Introduction

Campylobacter is the major cause of bacterial gastroenteritis in humans worldwide. In the EU, it is the most commonly reported gastrointestinal (GI) bacterial pathogen since 2005, with 214,779 human cases reported in 2013 (EFSA, 2015). *Campylobacter* preventive measures aim at reducing the probability of *Campylobacter* colonisation in birds (GHAREEB et al., 2013). For this purpose several approaches, as the use of bacteriophages (WAGENAAR et al., 2005), bacteriocins (LINE et al., 2008), organic acids (CHAVEERACH et al., 2004; SKÅNSEN et al., 2010) and their derivatives (HILMARSSON et al., 2006) or medium chain fatty acids (HERMANS et al., 2010) have been proposed over the past few years. However, few efforts have been done on modifying the upper GI tract in order to combat *C. jejuni* colonization in lower GI tract. HUANG et al. (2006) proposed that the gizzard may be a critical control point for reducing *Salmonella* contamination in growing broilers, and MOEN et al. (2012) have recently shown that a stimulated gizzard delays the horizontal spread of *C. jejuni* in broiler flocks. The aim of this work was to investigate the effect of particle size of wheat and feed presentation on *C. jejuni* colonization in broilers.

Material and Methods

A total of 144 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were four treatments factorially arranged with two W screen sizes (2mm vs 5mm) and two FP (mash vs pellets). The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1×10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca were collected from 12 birds per treatment and *Campylobacter* counts determined (ISO 10272). Detection limit was 2 logs, and animals below limit of detection were assigned 2 logs for statistical analysis. At 42d, the weight of the gastrointestinal tract (GIT), empty BW (EBW), the weight of caeca and its pH were taken. Data were analysed by GLM procedure as a factorial design with PS and FP as main

effects (SPSS v.19.0). Statistical significance is declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered as a near-significant trend.

Results

Neither the PS of the cereal nor the FP caused a significant variation on *C. jejuni* counts in the caeca of broilers at any of the periods studied (Figure 1). Also, no interactions were detected between PS and FP ($P < 0.05$)

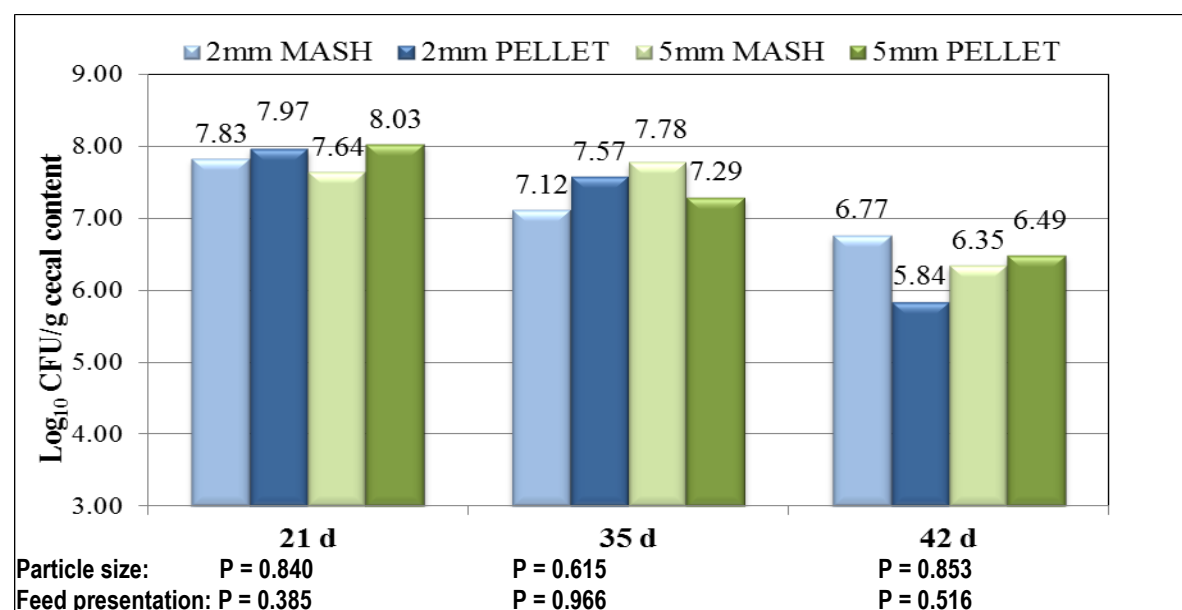


Figure 1. Effect of particle size of cereal (2 vs 5 mm) and feed presentation (mash vs pellet) on *Campylobacter* counts (log₁₀ CFU/g) in the caeca of broilers at 21, 35 and 42 days of age.

Particle size did not affect any of the morphological parameters evaluated (Table 1). Pelleting the diet increased GIT weight (214.3 vs 178.4 g, for pellets vs mash; $P = 0.001$) and empty BW (2557 vs 1986 g, for pellets vs mash; $P = 0.001$). Pelleting also decreased the relative importance of the caeca expressed as percentage of GIT, BW or EBW ($P < 0.05$). There was an interaction between PS and FP for caeca weight: pelleting 2mm diets decreased the caeca weight but the opposite was observed for 5mm-diets (1.17, 1.08, 1.08 and 1.23 g for 2mm mash, 2mm pellet, 5mm mash and 5mm pellet, respectively; $P = 0.048$). Dietary treatment did not affect pH at the caeca of the birds ($P > 0.05$).

Conclusions

It is concluded that pelleting the diet increased BW, GIT weight and empty BW of broilers and decreased the relative weight of caeca without affecting the pH or *C. jejuni* counts.

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Table 1. Effect of particle size of cereal (2 vs 5 mm) and feed presentation (mash vs pellet) on on caeca morphology and pH

and pH

	BW, g	GIT		Empty BW		Caeca				
		g	%BW	g	%BW	g	%GIT	%BW	%EBW	pH
Particle size										
2 mm	2440	191.7	7.91	2249	92.09	1.12	0.60	0.047	0.051	6.67
5 mm	2496	200.9	8.09	2295	91.91	1.15	0.59	0.047	0.051	6.75
Feed presentation										
Mash	2165	178.4	8.27	1986	91.73	1.12	0.64	0.052	0.057	6.78
Pellet	2772	214.3	7.74	2557	92.27	1.15	0.55	0.042	0.045	6.64
SEM (n=24)	59.9	7.13	0.237	56.3	0.237	0.043	0.027	0.002	0.002	0.070
Probability										
Particle size	0.512	0.367	0.607	0.559	0.607	0.591	0.906	0.897	0.884	0.400
Feed presentation	0.001	0.001	0.121	0.001	0.121	0.591	0.017	0.001	0.001	0.188
PS x FP	0.523	0.266	0.329	0.590	0.329	0.048	0.409	0.190	0.174	0.205

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