

Effect of inclusion of micronized camelina, sunflower, and flax seeds in the broiler chicken diet on performance productivity, nutrient utilization, and intestinal microbial populations

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ABSTRACT The aim of the study was to evaluate the effect of inclusion of micronized full-fat camelina, flax, or sunflower seeds in the diet for broiler chickens on the performance productivity, nutrient utilization, and composition of intestinal microbial populations and to assess the possibility of modification of the resistance of isolated bacteria to chemotherapeutic agents with different mechanisms of action. The use of micronized oilseeds improved the broiler chicken body weight ($P = 0.035$) and the FCR value ($P = 0.045$) in the final rearing stage by enhancement of the utilization of total protein and organic matter. *Lactobacillus-Enterococcus* spp., *Bifidobacterium* spp., *Escherichia coli*, and *Salmonella* spp. were isolated from small intestinal contents, and *Enterobacteriaceae* taxa were detected in the cecum and cloaca of the broiler chickens. The addition of micronized camelina seeds (**CAM.IR**) contributed to an increase in the *Bifidobacterium* counts in the small intestine, compared with the control treatment ($P < 0.050$). *Escherichia coli* bacteria were not isolated only in the CAM.IR treatment. Nitrofurantoin and chloramphenicol were the most effective agents against the isolates from the cecum

and cloaca in all oilseed treatments, whereas streptomycin exhibited the lowest efficacy. In the CAM.IR and micronized sunflower seed (**SUN.IR**) treatments, there were higher counts of trimethoprim/sulfamethoxazole-resistant *Enterobacteriaceae* strains than in the control and micronized flax seed (**FLA.IR**) treatments ($P < 0.05$). There was a difference between strains isolated from the cecum and cloaca only in the FLA.IR treatment, i.e., increased tetracycline sensitivity was exhibited by strains isolated from the cloaca (13% vs. 50%), also in comparison with the control treatments ($P = 0.054$). In comparison with the CAM.IR and control treatments, reduced numbers of multi-resistant strains were found in the cloaca isolates from the for FLA.IR and SUN.IR variants. Micronized camelina, flax, and sunflower seeds can be used as part of an effective nutritional strategy focused on optimization of the efficiency of rearing broiler chickens, as they positively modify intestinal microbial populations and increase bacterial sensitivity to the analyzed chemotherapeutic agents.

Key words: broiler chicken, micronization, oil seeds, performance, intestinal microbial populations

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INTRODUCTION

Human and animal diseases caused by *Enterobacteriaceae* bacilli are a widespread epidemiological problem (Mon et al., 2015; Silva et al., 2019). Farmed birds play an important role in the spread of *Enterobacteriaceae* due to

the high prevalence of poultry infections caused by these microorganisms (Miranda et al., 2008; Osman et al., 2018). Maintenance of appropriate intestinal integrity is one of the key elements of profitability in poultry production. The avian gastrointestinal tract is colonized by tens of billions of microorganisms that contribute to the normal harmonious functioning of the entire organism. In the chicken microbiome, at least 13 different types of bacteria have been identified with over 90% represented by *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. Approximately 900 species of microorganisms identified are members of over 117 genera. The most numerous are bacteria of the genus

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Clostridium, *Ruminococcus*, *Lactobacillus*, and *Bacteroides* (Binek et al., 2017). They play an important role in the digestion and absorption processes and in maintenance of good health status of the entire organism. A special role in this respect is assigned to bacteria of the genus *Lactobacillus* or *Bifidobacterium*. Their beneficial effects are reflected not only in stimulation of the function of the gastrointestinal tract, but also in inhibition of the growth of pathogenic microorganisms (Sergeant et al., 2014), e.g., *Escherichia coli* or *Clostridium*. They are also a reservoir of antibiotic resistance genes (Zhou et al., 2012). Special epidemiological importance is ascribed to multi-resistant strains and identification of factors inducing the mechanism of bacterial resistance to chemotherapeutic agents (Paterson, 2006; Stecher et al., 2011; Yang et al., 2019).

There is an ongoing search for sources of phytobiotic additives with bacteriostatic, bactericidal, immunomodulatory, and antiinflammatory properties (Kiczorowska et al., 2017; Gheisar and Kim, 2018; Kim and Lillehoj, 2019). This type of research is conducted on oilseed plants, e.g., camelina (Terpinc et al., 2012; Kumar et al., 2017), flax (Gaafar et al., 2013; Palla et al., 2015; Narender et al., 2016), or sunflower (Salgado et al., 2012; Guo et al., 2017). Oilseeds contain large amounts of fat, which is a certain limitation of the size of addition thereof to feed mixtures for poultry (Zajęc et al. 2020). The fat is rich in fatty acids necessary for the proper function of the organism, e.g., n-3 unsaturated fatty acids, including α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Dodin et al., 2005). Additionally, polyunsaturated fatty acids in feed can stimulate the development of a beneficial population of intestinal microorganisms (Cani et al., 2007). Oilseeds also contain phytochemicals, including phytosterols, natural antioxidants, and bioactive carbohydrates. The use of oilseeds in feed mixtures, e.g., sunflower or flax, optimizes blood lipid metabolism by reducing low-density lipoprotein (LDL) cholesterol and increasing high-density lipoprotein (HDL) cholesterol (Meineri et al., 2018). Moreover, the potential ability of plant sterols to regulate the phenomenon of multidrug resistance (MDR) is investigated. It has been hypothesized that phytosterols, which are compounds of natural origin, may be an alternative to the known MDR modulators (Furtula et al., 2013).

Addition of raw materials to feed mixtures does not always yield desired production results in poultry farming (Zajęc et al., 2020); therefore, the feed industry more often applies modern processing of feed raw materials to increase their nutritional value and storage stability (Kiczorowska et al., 2019). Such processes include e.g., micronization (Regulation of the UE Commission, 2013), which contributes to reduction of the diameter of feed material particles, thus increasing the surface area of feed substances. This, in turn, accelerates and improves their solubility, absorption, distribution, and bioavailability (Kiczorowska et al., 2016; Speroni et al., 2019; Kuna-Broniowska et al., 2020). Infrared irradiation applied in the micronization process may also result in multidirectional modifications of

the chemical composition of the raw material, e.g., inactivation of antinutritional substances, increased solubility of poorly bioavailable dietary fiber fractions, gelatinization of starch, and complexation of basic nutrients, such as protein, fat, and sugars (Deepa and Hebbar, 2016; Kiczorowska et al., 2019; Dhiman and Prabhakar, 2020). A prerequisite for achievement of high nutritious value of feed material is individual adjustment of process parameters to the physicochemical properties of the processed raw material (Chen et al., 2018; Kuna-Broniowska et al., 2020).

The aim of the study was to determine the effect of addition of micronized full-fat camelina, flax, or sunflower seeds in the diet of broilers on the performance productivity, nutrient utilization, gastrointestinal tract anatomy, and composition of the intestinal microbiota and to assess the possibility of modification of the resistance of isolated bacteria to chemotherapeutic agents with different mechanisms of action.

MATERIALS AND METHODS

Oil Seeds, Experimental Design, and Management

Raw camelina (*Camelina sativa* L. Crantz) Luna cv., flax (*Linum* L.) Opal cv., and dehulled sunflower (*Helianthus* L.) Lech cv. were purchased in a specialist store (Seed Centre, Lublin, Poland). All oilseeds were micronized twice at a temperature of 180 °C for 60 s. The seeds were heated with infrared rays emitted by an infrared radiation generator with an ESC-1 infrared illuminator panel with a power output of 400W (Elcer, Rzeszotary, Poland). Temperature was measured on the surface of the seeds with a Raynger ST60 infrared thermometer (Raytek, Inc., Santa Cruz, CA).

Raw oilseeds and those micronized immediately after the purchase were subjected to chemical analysis. Nine random samples were collected from each batch of seeds and analyzed in triplicate (250 g seeds/variety). The content of basic chemical composition of raw and infrared-irradiated camelina, flax, and sunflower seeds was determined according to standard AOAC procedures, (2019). The nutrient content in the raw and processed seeds is shown in Table 1.

Birds, Diets, and Experimental Design

The experiment was carried out with the approval from the Second Local Ethics Committee at the University of Life Sciences in Lublin (No. 35/2015).

Two hundred 1-day-old broiler chickens (Ross 308, Aviagen, Cracow, Malopolskie province, Poland) were randomly assigned to 4 dietary treatments (Control, camelina seeds [CAM.IR], flax seeds [FLA.IR], sunflower seeds [SUN.IR]) with 5 cages per treatment and 5 females and 5 males per cage. The body weight of the 1-day-old broiler chickens was 42.6 ± 0.1 g, and the birds were reared in 1-m²cages. During the 6-wk

Table 1. Basic composition of raw and micronized camelina, sunflower (dehulled), and flax seeds added to the feed.

Component	Camelina				Flax				Sunflower			
	Raw	CAM.IR ²	SEM ³	P-value ⁴	Raw	FLA.IR ²	SEM ³	P-value ⁴	Raw	SUN.IR ²	SEM ³	P-value ⁴
Dry matter	862	948	5.18	0.157	917	975	4.34	0.067	849	978	6.26	0.167
Crude ash	43.1	41.5	0.13	0.331	38.3	36.2	0.19	0.206	35.1	34.6	0.24	0.203
Crude protein ¹	246 ^b	295 ^a	2.15	0.027	205	201	2.05	0.098	241 ^b	294 ^a	1.85	0.041
Ether extract	393	402	3.32	0.125	446	418	2.31	0.124	531	534	2.92	0.109
Crude fiber	117 ^a	73.8 ^b	0.75	0.016	81.2 ^a	41.5 ^b	0.57	0.023	22.3 ^a	17.1 ^b	0.43	0.018

Results of 9 samples in 3 replicates.

¹Calculated by Kjeldhal nitrogen N x 6.25.

²IR – infrared irradiation of oil seeds.

³SEM - standard error of the mean.

⁴P < 0.05; ^{a,b}statistical differences.

experiment, the birds had unlimited access to feed and water. Veterinary care was provided throughout the experiment, and the air temperature and humidity were maintained at levels recommended by [Aviagen, \(2014a\)](#). The basal feed diets were made from cereal meal middlings (wheat and corn) and postextraction soybean meal as recommended ([Aviagen, 2014b](#)). The broiler chickens were fed 3 types of diets: starter (0 to 21 d), grower (22 to 35 d), and finisher (36 to 42 d); the detailed composition of the diets in each stage of animal

feeding is presented in [Table 2](#). The same starter diet was administered in all 4 groups. In the period 0–21 d (starter), the birds achieved similar statistically insignificant production parameters, e.g., average body weight (**BW**) of approximately 648 g ($P = 0.145$), average daily feed intake (**ADFI**) - average 58.4 g/d ($P = 0.089$), body weight gain (**BWG**) - average 687.5 g/chicken ($P = 0.209$), and feed conversion ratio (**FCR**) - average 1.64 kg/kg ($P = 0.203$) ([Zajac et al., 2020](#)). The values of these production parameters were calculated as the

Table 2. Dietary ingredients and nutrient content in the experimental diets.

Component	Diets ¹											
	Starter (0 to 21 d)				Grower (21 to 35 d)				Finisher (35 to 42 d)			
	Control	CAM.IR	FLA.IR	SUN.IR	Control	CAM.IR	FLA.IR	SUN.IR	Control	CAM.IR	FLA.IR	SUN.IR
Diet composition, %												
Wheat			20.0		23.0	23.0	23.0	23.0	27.0	27.0	27.0	27.0
Soybean meal, 46% CP ²			39.4		35.7	28.9	30.9	30.4	31.3	25.3	27.3	26.8
Maize			30.04		30.0	29.03	27.03	27.53	29.93	28.95	26.95	27.45
Soybean oil			6.0		7.0				8.0			
Camelina seeds ³						15.0				15.0		
Flax seeds ³							15.0				15.0	
Sunflower seeds ³								15.0				15.0
Dicalcium phosphate		1.83			1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Limestone		1.20			1.00	1.00	1.00	1.00	0.70	0.70	0.70	0.70
NaCl		0.33			0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
DL-Met ⁴		0.36			0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
L-Lys ⁵		0.34			0.34	0.36	0.36	0.36	0.36	0.34	0.34	0.34
Vitamin-mineral premix ⁶		0.50			0.50	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sum, %		100			100	100	100	100	100	100	100	100
Analyzed chemical composition, g/kg												
MEn, MJ/kg ⁷		12.45			13.01	12.98	13.02	13.09	13.35	13.20	13.25	13.28
Gross energy, MJ/kg		14.94			15.61	15.32	15.36	15.45	16.29	16.37	16.43	16.47
CP ²		221.3			209.2	206.2	205.1	207.8	191.5	195.4	195.1	197.8
Lys		14.29			12.75	12.79	12.53	12.82	11.57	11.16	11.75	11.62
Met + Cys		10.51			9.68	9.74	9.31	9.82	8.92	8.79	8.65	9.10
Ca		9.84			8.87	8.56	8.28	8.69	7.86	7.86	7.97	8.02
P		6.61			6.29	6.65	6.53	6.37	6.21	6.57	6.59	6.61
Phonphytate		4.81			4.27	4.41	4.38	4.29	4.03	4.07	4.09	4.02

¹Control – diet without oilseeds. CAM.IR – diet with 15% of infrared-irradiated camelina seeds. SUN.IR – diet with 15% of infrared-irradiated sunflower seeds. FLA.IR - with 15% of infrared-irradiated flax seeds.

²CP – crude protein.

³IR - infrared irradiation.

⁴Evonik Degussa GmbH. Essen. Germany (per kilogram of 990 g methionine).

⁵Ajinomoto Eurolysine S.A.S.. Amiens. France (per kilogram of 780 g lysine).

⁶Added minerals and vitamins per kg of starter diet: Mn. 100 mg; I. 1 mg; Fe. 40 mg; Zn. 100 mg; Se. 0.15 mg; Cu. 10 mg; vitamin A. 15.000 IU; vitamin D3. 5.000 UI; vitamin E. 75 mg; vitamin K3. 4 mg; vitamin B1. 3 mg; vitamin B2. 8 mg; vitamin B6. 5 mg; vitamin B12. 0.016 mg; biotin. 0.2 mg; folic acid. 2 mg; nicotinic acid. 60 mg; pantothenic acid. 18 mg; choline. 1.800 mg. Added minerals and vitamins per kg of grower diet: Mn. 100 mg; I. 1 mg; Fe. 40 mg; Zn. 100 mg; Se. 0.15 mg; Cu. 10 mg; vitamin A. 12.000 IU; vitamin D3. 5.000 UI; vitamin E. 50 mg; vitamin K3. 3 mg; vitamin B1. 2 mg; vitamin B2. 6 mg; vitamin B6. 4 mg; vitamin B12. 0.016 mg; biotin. 0.2 mg; folic acid. 1.75 mg; nicotinic acid. 60 mg; pantothenic acid. 18 mg; choline. 1.600 mg. Added minerals and vitamins per kg of finisher diet: Mn. 100 mg; I. 1 mg; Fe. 40 mg; Zn. 100 mg; Se. 0.15 mg; Cu. 10 mg; vitamin A. 12.000 IU; vitamin D3. 5.000 UI; vitamin E. 50 mg; vitamin K3. 2 mg; vitamin B1. 2 mg; vitamin B2. 5 mg; vitamin B6. 3 mg; vitamin B12. 0.011 mg; biotin. 0.05 mg; folic acid. 1.5 mg; nicotinic acid. 35 mg; pantothenic acid. 18 mg; choline. 1.600 mg.

⁷MEn = metabolizable energy in the mixtures corrected to zero nitrogen balance.

mean of the measurements performed in all 4 groups. After the first rearing period (starter), the birds were left in their groups and entered the experimental grower and starter period in the original division. No random selection of the birds before the beginning of the experimental period was applied so as not to induce additional stress in the birds. Additionally, the birds were not regrouped on rearing d 22 due to their similar number in the cages, which had not changed significantly, as low mortality at the average level of 1.8% was observed in all groups.

From rearing d 22, the broilers were fed according to the methodological design, with 15% of micronized oil-seeds in the diets as an experimental factor: CAM.IR, FLA.IR, and SUN.IR. The experimental mixtures were isoenergetic and isonitrogenous and balanced in accordance with the feeding recommendations for broiler chickens (Aviagen, 2014b).

Productivity Parameters and Apparent Utilization of Nutrients

For each cage, the final body weight of the broiler chickens and the ADFI were recorded at 21, 35, and 42 d of life. The BWG and FCR were calculated in the grower and finisher periods. The mortality rates were recorded daily. The weight of dead broiler chickens was included in the calculations of the average weight gain, feed intake, and FCR.

Feed utilization was evaluated in the finisher mixture by direct balance on 64 birds in 4 replications of 4 birds – 16 birds from the control and each treatment at the final fattening stage (Kussaibat and Leclercq, 1985). For 4 d, the birds were habituated to the feed and droppings were collected for the next 3 d. During the utilization experiment, the amount of consumed feed and the amount of droppings were determined. In the collected droppings, the content of dry matter and organic matter was determined (AOAC, 2019), and the content of nitrogen was determined according to Krogdahl and Dalsgard, (1981). In each mixture, the basic nutrient utilization coefficients and metabolic energy content were calculated based on the difference in the amount of feed intake during the experiment and the dry weight and chemical composition of the droppings. The dry matter and organic matter utilization ratios and the content of nitrogen-corrected metabolizable energy were calculated for each mixture according to formulas given in the European Table of Energy Values for Poultry Feedstuffs (1986).

Twenty birds (2 hens and 2 cocks per pen) were selected from every group for slaughter by decapitation of stunned birds. The body weight of the birds was close to the average body weight in the group (Ziotecki and Doruchowski, 1989).

Sample Collection and Analysis of Microbial Population Counts in the Small Intestine

Three samples of the small intestine from 5 chickens per treatment (one chicken per cage) collected during

the dissection. Fragments of the intestine from the bile duct outlet to Meckel's diverticulum and from Meckel's diverticulum to the outlets of the ceca were excised from the intestine. The samples were weighed (10 g each), diluted with phosphate buffered saline (PBS), and homogenized (Stomacher BagMixer 400; Interscience, Breda, the Netherlands). Next, the supernatant samples were fixed for 16 h at 4 °C in paraformaldehyde (Sigma-Aldrich, Warsaw, Poland). The resultant suspension was homogenized again with glass balls (3 mm) for 5 min (Merck, Darmstadt, Germany). Using centrifugation (5000 × g for 5 min at 4 °C), eukaryotic cells and undigested feed were removed (MPW-350R; MPW Medical Instruments, Warsaw, Poland). The resultant supernatant was placed on white polycarbonate membrane filters (pore size=0.2 μm) (Milipore, Cork, Ireland) by means of sterile filter units (Nalgene, Lima, OH). Samples for further analyses were stored at a temperature of –20 °C. Using the fluorescent in situ hybridization method, microorganisms were determined using group-specific probes (Thermo Scientific, Ulm, Germany) for the *Bacteria* domain, for *Lactobacillus-Enterococcus* spp., and for *Bifidobacterium* spp. as described previously (Kiczorowska et al., 2016). The count of *Escherichia coli* and *Salmonella* spp. in the small intestine was determined by culturing colonies as described previously (Kiczorowska et al., 2016a,b).

Identification of Enterobacteriaceae Taxa From the Cecum and Cloaca

The material for microbiological analysis was collected with sterile swabs once from 5 birds per treatment (one chicken per cage) from the cecum (3 samples per chicken) and from the cloaca (3 samples per chicken). LB nutrient broth (BioMaxima, Lublin, Poland) incubated at 37°C for 24 h was used for laboratory bacterial culture. Next, the material was transferred to Salmonella-Shigella (S-S) agar (BioMaxima, Lublin, Poland) and the culture was grown at 37°C for 24 h. After incubation on a selective medium, bacterial colonies of the experimental isolates were selected for further identification and analysis. All isolated strains were analyzed biochemically. Each strain was tested using the commercial API 20E kit (bioMérieux, Poland).

Tests of Sensitivity to Chemotherapeutics

The sensitivity to chemotherapeutic agents was determined with the disk-diffusion method developed by Bauer, (1966) on Mueller-Hinton agar (BioMaxima, Lublin, Poland) using Oxoid disks (Oxoid, Hampshire, England). This method allowed rapid determination of the efficacy of a chemotherapeutic agent by measuring the diameter of the inhibition zone that resulted from diffusion of the agent into the medium surrounding the disk. The following chemotherapeutics were used for determination of the drug sensitivity of the isolates: chloramphenicol (C 30 μg/mL), tetracycline (TE 30 IU),

trimethoprim/sulfamethoxazole (TMP/SXT 1.25 + 23.75 $\mu\text{g}/\text{mL}$), streptomycin (S 10 IU), nitrofurantoin (FM 300 IU), and ampicillin (AM at a concentration of 10 $\mu\text{g}/\text{mL}$). The plates were incubated at 37°C for 24 h. After incubation, the plates were examined and the diameters of the zone of complete inhibition were observed. The results were interpreted as recommended by the producer of the disks. The diameter of the inhibition zones was measured and expressed as sensitive (S), medium sensitive (MS), and resistant (R) categories.

Statistical Analysis

Each cage served as an experimental unit for assessment of the growth performance (weight gain, feed intake, and FCR) (5 cages per treatment), and the values of nutrient utilization (4 replications of 4 birds), and all microbiological analyses were calculated using a bird sampled from each cage (5 birds per treatment) as an experimental unit. Statistical analysis was performed using Statistica software version 13.1 (Dell Inc., 2016). The normality of data and homogeneity of variances were tested using the Shapiro-Wilk and Brown-Forsythe tests, respectively. The data obtained were analyzed statistically using treatments as an independent variable in the general linear model (GLM) of one-way ANOVA analysis of variance. Detailed comparisons between the means were conducted using Tukey's HSD post hoc test. Variability in the data was expressed as the SEM, and $P < 0.05$ was considered to be statistically significant. The antimicrobial susceptibility data are expressed as percentages (category: sensitive, medium sensitive, and resistant) and differences between the treatments were

compared by the nonparametric χ^2 test with Yates correction. $P < 0.05$ was considered statistically significant.

RESULTS

Chemical Composition of Raw and Infrared Irradiation of Oil Seeds

The micronization process induced changes in the basic chemical composition of the analyzed oilseeds (Table 1). Significantly lower content of total protein, i. e., on average by 20%, was determined in the processed camelina ($P = 0.027$) and sunflower ($P = 0.041$) seeds, in comparison with the raw material. Additionally, the micronization process reduced the crude fiber content by approx. 37% in the camelina seeds ($P = 0.016$), by 49% in the flax seeds ($P = 0.023$), and by 23% in the sunflower seeds ($P = 0.018$) in relation to the unprocessed seeds. No statistically significant differences were found in the content of the other nutrients.

Productivity Parameters and Nutrient Utilization

The addition of 15% of micronized oilseeds to the mixtures for broiler chickens did not have significant effect on the BW, ADFI, BWG, and FCR in the grower period (Table 3). The positive effects of the use of micronized camelina and flax seeds were reflected by an average 9% increase in the body weight of chickens in the CAM.IR and FLA.IR treatments, compared with the control ($P = 0.035$) in the 36 to 42 d period. Concurrently, a ca.

Table 3. Selected productivity parameters of broiler chicken and digestibility of nutrients.¹

Items	Treatments ²				SEM ³	P-value ⁴
	Control	CAM.IR	FLA.IR	SUN.IR		
21–35 d (grower period)						
BW ⁵ . g	1709	1824	1809	1796	55.7	0.131
ADFI ⁶ . g/d	1112	1105	1094	1061	37.5	0.206
BWG ⁶ . g/chicken	1268	1311	1325	1293	4.35	0.144
FCR ⁷ . kg/kg	2.02	1.98	1.94	1.93	0.06	0.107
36–42 d (finisher period)						
BW ⁵ . g	2425 ^b	2651 ^a	2631 ^a	2574 ^{ab}	68.2	0.035
ADFI ⁶ . g/d	1397	1343	1351	1338	87.5	0.129
BWG ⁶ . g/chicken	545	541	543	549	4.03	0.203
FCR ⁷ . kg/kg	1.98 ^a	1.88 ^{ab}	1.78 ^b	1.86 ^{ab}	0.08	0.045
21–42 d (grower and finisher period)						
BW ⁵ . g	2067 ^b	2238 ^a	2220 ^a	2185 ^{ab}	77.1	0.032
ADFI ⁶ . g/d	1255	1224	1223	1200	85.2	0.145
BWG ⁶ . g/chicken	907	926	934	921	5.15	0.484
FCR ⁷ . kg/kg	2.03 ^a	1.93 ^{ab}	1.94 ^b	1.91 ^b	0.23	0.019
Mortality. %	3.08 ^a	2.12 ^b	2.17 ^b	2.07 ^b	0.05	0.026

¹Data represent the mean of 5 cages (10 broiler chickens/cage) per treatment.

²Control – diet without oilseeds. CAM.IR – diet with 15% of infrared-irradiated camelina seeds. SUN.IR – diet with 15% of infrared-irradiated sunflower seeds. FLA.IR – with 15% of infrared-irradiated flax seeds.

³SEM - standard error of the mean.

⁴<0.05; ^{a,b} statistical differences

⁵BW – average final body weight of period.

⁶ADFI – average daily feed intake.

⁷BWG – body weight gain.

⁸FCR – feed conversion ratio.

10% decrease in the FCR value was noted in the CAM.IR treatment ($P = 0.045$).

Throughout the experimental period (21–42 d), it was found that the addition of 15% of the infrared-irradiated seeds had a positive effect on the body weight of broiler chickens in the CAM.IR and FLA.IR treatments ($P = 0.032$) and the FCR value in the FLA.IR and SUN.IR variants ($P = 0.019$). It also substantially reduced the mortality rate by an average of 31% in the experimental treatments (CAM.IR, FLA.IR, SUN.IR; $P = 0.026$), compared with chickens receiving the standard mixture.

The addition of micronized plant seeds in the mixtures for broiler chickens in the 21–35 d rearing period had a positive effect on the utilization of crude protein, especially in the CAM.IR variant ($P = 0.035$), and organic matter ($P = 0.021$) (Table 4). Significantly better utilization of organic matter, i.e., on average by 7% compared with the control treatment, was noted in the group of broiler chickens fed with mixtures supplemented with infrared flax (FLA.IR) and sunflower (SUN.IR) seeds. In the finisher period (36–42 d), there was a beneficial effect of the supplementation of the feed mixtures with the micronized oilseeds on organic matter utilization ($P = 0.017$) in the FLA.IR treatment, in comparison with the control.

The Microbial Populations in the Small Intestine

The bacterial counts determined in the small intestinal contents at 42 d of the experiment are presented in Table 5. The addition of the micronized oilseeds to the diet mixtures had no significant effect ($P > 0.05$) on the *Lactobacillus* and *Enterococcus* counts in the intestinal contents. However, the counts of *Escherichia coli* tended ($P = 0.089$) to decrease in all oilseeds treatments. In turn, the inclusion of micronized camelina seeds (CAM.

IR) increased the counts of *Bifidobacterium*, compared with the control treatment ($P = 0.034$). No *Salmonella* spp. was isolated from the intestinal contents in any of the treatments.

Enterobacteriaceae Taxa in the Caeca and Cloaca of Broiler Chickens

The isolates obtained from the birds in all the treatments belonged to *Enterobacteriaceae* and were represented by 10 and 9 taxa in the cecum and cloaca, respectively (Table 6). *Citrobacter freundii*, *Providencia rettgeri*, *Citrobacter youngae*, and *Escherichia coli* were the most frequent taxa in the cecum, whereas *Citrobacter freundii* and *Escherichia coli* were the predominant taxa in the cloaca. The present study showed the greatest diversity of *Enterobacteriaceae* taxa in the control treatment material, i.e., 7 species were identified in the cecum and 6 species were detected in the cloaca of the control birds. The lowest number of *Enterobacteriaceae* taxa was isolated from the cecum of chickens in the FLA.IR variant, i.e., only 4 species, whereas 6 species were identified in the CAM.IR and SUN.IR treatments. In turn, the analysis of the cloaca contents showed the lowest number of *Enterobacteriaceae* taxa isolated from the birds in the CAM.IR and SUN.IR groups, i.e., 3 species in each variant, whereas 5 species were isolated in the FLA.IR treatment. No *Escherichia coli* bacteria were detected in the cecum and cloaca isolates in the CAM.IR variant.

Sensitivity of Enterobacteriaceae Strains to Chemotherapeutics

The highest activity against the isolates obtained from all the oilseed treatments was exhibited by nitrofurantoin (100% of sensitive *Enterobacteriaceae* strains

Table 4. Apparent nutrient and energy utilization of broiler chicken mixtures %¹.

Components	Treatments ²				SEM ³	P-value ⁴
	Control	CAM.IR	FLA.IR	SUN.IR		
21–35 d (grower) ⁵						
Dry matter	75.6	81.7	83.1	79.7	0.12	0.241
Crude protein ⁶	74.3 ^b	78.3 ^a	77.2 ^{ab}	76.4 ^{ab}	0.24	0.035
Ether extract	74.1	71.4	69.6	70.5	0.13	0.168
Organic matter	81.6 ^b	86.5 ^b	87.3 ^a	86.9 ^a	0.11	0.021
Gross energy	87.6	85.3	86.9	83.3	0.09	0.253
36–42 d (finisher) ⁵						
Dry matter	82.3	85.4	86.1	84.9	0.21	0.231
Crude protein ⁶	75.3	78.3	79.1	76.8	0.09	0.283
Ether extract	73.4	75.6	72.6	78.2	0.31	0.106
Organic matter	82.3 ^b	88.5 ^{ab}	89.8 ^a	85.7 ^b	0.14	0.017
Gross energy	86.3	87.1	90.5	89.6	0.08	0.135

¹Data represent the mean of 5 cages (10 broiler chickens/cage) per treatment.

²Control – diet without oilseeds. CAM.IR – diet with 15% of infrared-irradiated camelina seeds. SUN.IR – diet with 15% of infrared-irradiated sunflower seeds. FLA.IR – with 15% of infrared-irradiated flax seeds.

³SEM - standard error of the mean.

⁴ $P < 0.05$; ^{a,b}statistical differences.

⁵Throughout the rearing cycle, the preparation and analysis of nutrient utilization lasted 7 d and ended on the last d of rearing: 35 and 42d.

⁶Calculated by Kjeldhal nitrogen $N \times 6.25$.

Table 5. Effects of inclusion of micronized oilseeds on the small intestine microbial populations of broiler chickens at d 42.¹

Bacteria	Treatments ²				SEM ³	P-value ⁴
	Control	CAM.IR	FLA.IR	SUN.IR		
Bacterial counts per g of intestinal contents (log ₁₀)						
<i>Lactobacillus</i> and <i>Enterococcus</i> spp.	7.56	8.13	7.92	8.09	0.19	0.183
<i>Bifidobacterium</i> spp.	8.23 ^b	8.99 ^a	8.76 ^{ab}	8.42 ^b	0.21	0.034
<i>Escherichia coli</i>	6.43	6.18	6.22	6.09	0.09	0.089
<i>Salmonella</i> spp.	ND	ND	ND	ND	-	-
Total count	10.1	9.78	9.56	9.83	0.24	0.112

Abbreviation: ND, not detected.

¹Data represent the mean of 5 broiler chickens per treatment.

²Control – diet without micronized oilseeds. CAM.IR – diet with 15% of micronized camelina seeds. FLA.IR - with 15% of micronized flax seeds. SUN.IR – diet with 15% of micronized sunflower seeds.

³SEM - standard error of the mean.

⁴ $P < 0.05$. ^{a,b} statistical differences.

from the cecum and cloaca) and chloramphenicol (70–93% of sensitive *Enterobacteriaceae* strains isolated from the cecum and 70–83% of strains identified in the cloaca isolates). The lowest efficacy was determined for streptomycin (87–100% of resistant strains from the cecum and 90% to 100% of resistant strains from the cloaca) (Table 7). The present study showed slightly higher chloramphenicol sensitivity of the cloaca isolates from the oilseed treatments, compared with the control treatment ($P = 0.079$).

In the CAM.IR and SUN.IR treatments, there were higher counts of trimethoprim/sulfamethoxazole-resistant *Enterobacteriaceae* strains, in comparison with the control and FLA.IR treatments ($P < 0.05$).

Table 6. *Enterobacteriaceae* taxa isolated from the cecum and cloaca of broiler chickens at d 42.¹

Species	Treatments ²			
	Control	CAM.IR	FLA.IR	SUN.IR
Cecum				
<i>Citrobacter braakii</i>	+	-	-	-
<i>Citrobacter freundii</i>	+	+++	+++	+
<i>Citrobacter youngae</i>	-	++	++	+
<i>Escherichia coli</i>	+	-	++	+
<i>Enterobacter cloacae</i>	+	+	-	-
<i>Enterobacter sakazaaki</i>	-	+	-	+
<i>Klebsiella ozaenae</i>	-	-	-	-
<i>Klebsiella pneumoniae</i>	-	+	-	+
<i>Proteus mirabilis</i>	+	-	-	-
<i>Proteus penneri</i>	-	-	-	-
<i>Proteus vulgaris</i>	+	-	-	-
<i>Providencia rettgeri</i>	+	++	++	+
Cloaca				
<i>Citrobacter braakii</i>	+	-	+	-
<i>Citrobacter freundii</i>	+	+++	++	+++
<i>Citrobacter youngae</i>	-	-	+	-
<i>Escherichia coli</i>	+	-	++	+
<i>Enterobacter cloacae</i>	-	-	-	-
<i>Enterobacter sakazaaki</i>	-	+	-	+
<i>Klebsiella ozaenae</i>	-	-	-	-
<i>Klebsiella pneumoniae</i>	-	+	-	-
<i>Proteus mirabilis</i>	++	-	-	-
<i>Proteus penneri</i>	-	-	-	-
<i>Proteus vulgaris</i>	+	-	-	-
<i>Providencia rettgeri</i>	+	-	+	-

¹Data represent the mean of 5 broiler chickens per treatment.

²Control – diet without micronized oilseeds. CAM.IR – diet with 15% of micronized camelina seeds. FLA.IR - with 15% of micronized flax seeds. SUN.IR – diet with 15% of micronized sunflower seeds.

- - Not detected. + - Single. ++ - Numerous. +++ - Very numerous.

Additionally, the highest proportion of ampicillin-resistant *Enterobacteriaceae* isolated from the cecum and cloaca was noted in the FLA.IR variant (80% and 77% resistance, respectively) ($P < 0.1$). There were no significant differences in the sensitivity between the strains isolated from the cecum and cloaca, besides the evident increase in tetracycline sensitivity of strains isolated from the cloaca in the FLA.IR treatment (13% vs. 50%), also compared with the control treatments ($P = 0.054$).

In all the treatments analyzed in the present study, the *Enterobacteriaceae* strains derived from the cecum exhibited the highest frequency of resistance to 2-3 of the tested chemotherapeutic agents (60-77% of the strain pool) (Table 8). In turn, a significant increase in the number of sensitive strains isolated from the cloaca was noted in the FLA.IR and SUN.IR treatments (60% and 63% of the pool of strains resistant to 0-1 chemotherapeutic agents), compared with the CAM.IR and control treatments (83% and 70% of the pool of strains resistant to 2–3 chemotherapeutic agents).

DISCUSSION

Micronization reduces the size of feed particles, which improves their functional and physicochemical properties, e.g., water retention, solubility, and antioxidant properties. It also helps in the preservation of feed by inactivation of microorganisms or inhibition of enzymes. This treatment improves the microbiological safety of processed feed and concurrently extends its storability (Dhiman and Prabhaka, 2020; Kuna-Broniowska et al., 2020). A tendency to increase the dry matter content has been noted in all the irradiated seeds. Micronization has also been found to reduce the level of total protein in irradiated oilseeds. This phenomenon poses a problem in most thermal processes used in feed processing (Kiczorowska et al., 2019). Changes in the protein fraction may be associated with loss of amino acids. Lysine, methionine, and cysteine are extremely sensitive to high temperatures (Khattab et al., 2009). Besides poorly digestible protein-fat complexes, melanoidins, i.e., volatile products of the Maillard reaction responsible for the smell and color of feed, are formed. An undisputable

Table 7. Sensitivity of *Enterobacteriaceae* strains isolated from the cecum and cloaca of broiler chickens at d 42 to chemotherapeutic agents %.¹

Agent	Treatments ²				P-value
	Control	CAM.IR	FLA.IR	SUN.IR	
Cecum					
Chloramphenicol (30 µg/mL) ³					
S	70	90	70	93	0.281
MS	13	0	30	0	
R	17	10	0	7	
Tetracycline (30 IU) ³					
S	27	17	13	17	0.954
MS	50	53	60	53	
R	23	30	27	30	
Trimethoprim/Sulfamethoxazole (1.25 + 23.75 µg/mL) ³					
S	87	0	7	7	0.032
MS	0	30	60	17	
R	13	70	33	76	
Streptomycin (10 IU) ³					
S	30	3	0	0	0.327
MS	0	10	0	0	
R	70	87	100	100	
Nitrofurantoin (300 IU) ³					
S	100	100	100	100	1.000
MS	0	0	0	0	
R	0	0	0	0	
Ampicillin (10 µg/mL) ³					
S	17	27	0	0	0.093
MS	53	50	20	70	
R	30	23	80	30	
Cloaca					
Chloramphenicol (30 µg/mL) ³					
S	70	83	77	70	0.079
MS	0	17	23	27	
R	30	0	0	3	
Tetracycline (30 IU) ³					
S	26	27	50	7	0.054
MS	47	46	40	60	
R	27	27	10	33	
Trimethoprim/Sulfamethoxazole (1.25 + 23.75 µg/mL) ³					
S	90	3	13	3	0.029
MS	0	27	57	27	
R	10	70	30	70	
Streptomycin (10 IU) ³					
S	10	7	0	0	0.514
MS	10	3	0	0	
R	80	90	100	100	
Nitrofurantoin (300 IU) ³					
S	53	100	100	100	0.037
MS	47	0	0	0	
R	0	0	0	0	
Ampicillin (10 µg/mL) ³					
S	13	17	0	0	0.085
MS	77	50	23	60	
R	10	33	77	40	

Abbreviations: S, sensitive; MS, medium sensitive; R, resistant.

¹Data represent the mean of 5 broiler chickens per treatment.

²Control – diet without micronized oilseeds. CAM.IR – diet with 15% of micronized camelina seeds. FLA.IR - with 15% of micronized flax seeds. SUN.IR – diet with 15% of micronized sunflower seeds.

³Concentration of the chemotherapeutic on the disk.

Table 8. Resistance of *Enterobacteriaceae* strains isolated from the cecum and cloaca of broiler chickens at d 42 to chemotherapeutic agents (% of the strain pool).¹

Number of chemotherapeutics to which the isolated strains were resistant	Treatments ²			
	Control	CAM.IR	FLA.IR	SUN.IR
Cecum				
0	7	7		3
1	13	13	20	20
2	60	63	43	33
3	17	10	30	27
4	3	7	7	10
5	0	0	0	0
6	0	0	0	0
Cloaca				
0	20	3	0	7
1	10	7	63	53
2	63	50	30	20
3	7	33	7	17
4	0	7	0	3
5	0	0	0	0
6	0	0	0	0

¹Data represent the mean of 5 broiler chickens per treatment.

²Control – diet without micronized oilseeds. CAM.IR – diet with 15% of micronized camelina seeds. FLA.IR - with 15% of micronized flax seeds. SUN.IR – diet with 15% of micronized sunflower seeds.

advantage of this process is the inactivation of antimetabolic protein compounds, e.g., cyanogenic glycosides, linamarin in flax seeds, or protease inhibitors in camelina seeds, the removal of which improves the nutritional usefulness of micronized seeds (Rehman and Shah, 2005; Arrutia et al., 2020). All the tested oilseeds were also characterized by reduced crude fiber content, which was probably a result of the degradation of the cellulose and hemicellulose polysaccharides to simple sugars. They are more easily digested in the gastrointestinal tract of monogastric animals; concurrently, they increase the energy value of feed, which is desirable in poultry nutrition (Aravind et al., 2012; Pedrosa et al., 2012).

The supplementation of the feed mixtures with micronized oilseeds, especially camelina and flax seeds, exerted a positive influence on the chicken body weight and the FCR value, especially in the final rearing period. The improvement of the rearing efficiency in the CAM.IR and FLA.IR treatments may be associated with the better utilization of organic matter and crude protein. Inactivation of antinutritional substances, especially those that block the availability of protein by infrared irradiation of the feed, can stimulate the growth of birds at the beginning of intensive muscle mass gain (Alonoso et al., 2000; Abdollahi et al., 2011). Significantly lower mortality rates in chickens receiving the mixtures with micronized oilseeds were demonstrated in the present study. A similar phenomenon was observed by Parveen et al., (2016), who supplemented feed mixtures for broiler chickens with high-temperature processed flax seeds and observed an increase in body weight, improved ADFI and FCR values, and a significant reduction in chicken mortality (up to 1%), which was explained by lower contents of antinutritional substances in the feed. Similarly, Feng et al., (2003) have reported that processed flax seeds exhibit reduced levels of cyanide and mucilage, which significantly

improves their nutritional efficiency in poultry in terms of actual utilization of nitrogen and energy (Shen et al., 2005). Reduction of glucosinolates and trypsin inhibitors achieved through micronization of camelina seeds has been reported by Woyengo et al., (2017).

Adverse environmental factors have the greatest impact on intestine development in the first rearing period and may thus exert an unfavorable effect on the overall performance and efficiency (Bar-Shira and Friedman, 2006). More mature broiler chickens also react negatively to nutritional deficiencies or such dietary components as antinutritional substances (Dibner et al., 1996; Tako et al., 2004). The removal of e.g., antinutritional components from the feed allows the intestinal structures to develop optimally, ensuring achievement of desired poultry production results (Loudon et al., 2011; Lilburn and Loeffler, 2015).

The avian gastrointestinal tract is colonized by diverse microbiota exerting a significant impact on health and, consequently, on the production performance through an influence on the physiology of digestion and the immune system (Rubio et al., 2015; Gong et al., 2019). Moreover, the beneficial microbiota is involved in limitation and prevention of colonization by intestinal pathogens through the process of competitive exclusion and production of bacteriostatic and bactericidal substances (Grela and Semeniuk, 1999).

The present study demonstrated that the addition of micronized camelina, flax, and sunflower seeds to the diets did not modify the counts of *Lactobacillus* and *Enterococcus* in the small intestine microbial populations. However, a positive effect of camelina seeds on the increase in the number of *Bifidobacterium* spp. was noted. *Bifidobacterium* bacilli in humans and animals adhere to the intestinal mucosa and produce mucin-degrading enzymes, thus improving the tightness of the intestinal mucosa via competition with other species on the mucus layer (Pan and Yu, 2014). There are few reports in the available literature on the possible modifying effect of camelina on chicken intestinal microbiota. Some investigations conducted on mice indicate that n-3 polyunsaturated fatty acids can modify the composition of the intestinal microbiota by increasing the counts of *Bifidobacteria* spp. (Cani et al., 2007; Kaliannan et al., 2015). A previous study showed an increase in the count of *Bifidobacteria* spp. upon inclusion of flax in the broiler chicken diet; however, the changes were not significant, and the seeds have substantial content of n-3 fatty acids (Kiczorowska et al., 2019). Camelina seeds contain oligosaccharides, i.e., raffinose and stachyose, which are more commonly associated with legume seeds (Berhow et al., 2014) and promote the growth of desired bacterial microbiota, such as *Bifidobacteria* (Yazawa and Tamura, 1982). Birds receiving the diet supplemented with the micronized oilseeds exhibited reduced counts of *Escherichia coli* in the small intestine contents, which may indicate sensitivity of these microorganisms to such active substances with antibacterial properties as camalexin, phenols, phytic acid, glucosinolates present in camelina seeds (Terpinc et al., 2012; Kumar et al.,

2017), phenolic acid, flavonoids, and lignans contained in flax seeds (Gaafar et al., 2013; Palla et al., 2015; Narender et al., 2016), or tannins, saponins, glycosides, alkaloids, and phenolic compounds present in sunflower seeds (Salgado et al., 2012; Guo et al., 2017).

The intestinal microbial populations are present in the entire gastrointestinal tract of broiler chickens. Its taxonomic composition is determined by various modifying factors, i.e., the colonized segment of the gastrointestinal tract, the age of the animal, nutrition, breeding methods and environments, and potential exposure to chemotherapeutic agents (Clavijo and Flórez, 2018). The cecum is an especially important site of functional activity due to the slower flow of intestinal contents than in other intestine segments and the highest water absorption and fermentation of carbohydrates, which significantly increases the taxonomic diversity of microbiota that is important for health and production performance (Pan and Yu, 2014; Rubio et al., 2015). Undesirable microbiota, especially from the *Enterobacteriaceae* family such as *Escherichia* and *Salmonella*, can also be found in the cecum in certain conditions (Mon et al., 2015). In the present study, the isolates from birds in the oilseed treatments were represented by up to 7 taxa of *Enterobacteriaceae* in the cecum and cloaca. However, a greater diversity of bacterial taxa was noted in the material from the control treatments. *Citrobacter freundii*, *Providencia rettgeri*, *Citrobacter youngae*, and *Escherichia coli* were identified in the cecum most frequently, whereas *Citrobacter freundii* and *Escherichia coli* were predominant in the cloaca. The isolated taxa have pathogenic potential and, in certain conditions, may pose a threat to human and animal health and have high epidemic importance (Pepperell et al., 2002; Chuang et al., 2006; Oakley et al., 2014; Osman et al., 2018; Silva et al., 2019). In this study, the supplementation of the diet for broiler chickens with the micronized oilseeds limited the number of isolated *Enterobacteriaceae* taxa both in the cecum and in the cloaca. Importantly, no *Escherichia coli* bacteria were detected in the cecum and cloaca contents in the experimental variant supplemented with the micronized camelina seeds. The limited growth and diversity of *Enterobacteriaceae* strains in the cecum and cloaca of birds receiving micronized seeds may indicate sensitivity of the other taxa to the antimicrobial properties of active substances contained in this additive, which was observed in the small intestine to some extent. Substances with documented antibacterial properties include polyunsaturated fatty acids, whose high contents have been detected in all seeds used in the experiment (McGaw et al., 2002; Kaithwas et al., 2011), antimicrobial peptides (de Souza Candido et al., 2014), or lignans contained in flax seeds (Schmidt et al., 2012; Gaafar et al., 2013; Narender et al., 2016). The higher percentage of *Enterobacteriaceae* strains in the isolates from the CAM.IR and SUN. IR treatments showing resistance to Trimethoprim/Sulfamethoxazole indicates a possible effect/interaction of factors associated with the components and chemical composition of the feed. These results indicate, however,

that it is still necessary to conduct further research in this field.

The increasing prevalence of multi-resistant strains is a clinical and epidemiological problem. The misuse and excessive use of veterinary antibiotics have contributed to the development of bacterial resistance (Yang et al., 2019). In the present study, the strains of all taxa isolated from the cecum and cloaca of all studied birds were characterized by relatively high homogeneity of biochemical features. The analysis of drug resistance of the *Enterobacteriaceae* isolates from all oilseed treatments revealed the highest efficacy of nitrofurantoin and chloramphenicol and the lowest effectiveness of streptomycin. The higher resistance to streptomycin, which is one of the oldest chemotherapeutic agents, may be related to its long-term use before the introduction of fluoroquinolones in human and veterinary medicine (Ojo et al., 2012). Similarly, the increased resistance to trimethoprim/sulfamethoxazole observed in the CAM.IR and SUN.IR treatments may be associated with the fact that trimethoprim, sulfonamides, or trimethoprim-sulfonamide combinations are also a group of long-used chemotherapeutic agents, and microorganisms have evolved various resistance mechanisms associated with mutations in chromosomal and plasmid genes (Then, 1982; Stecher et al., 2011). In the present study, resistance to 2-3 chemotherapeutic agents was most frequently exhibited by the *Enterobacteriaceae* strains isolated from the cecum. The resistance of *Enterobacteriaceae* bacteria to antimicrobial drugs may be an important sign of the presence of resistant strains in the bacterial environment (Ojo et al., 2012). In the case of chickens fed the diet supplemented with micronized flax and sunflower seeds, the isolates from the cloaca were represented by an increased number of strains that were sensitive to all the chemotherapeutic agents. The possible influence of the diet or other environmental factors on the expression or inhibition of chemotherapeutic resistance traits in bacteria isolated from farm animals is a current epidemiological and clinical problem. At present, various multidirectional strategies are employed to reduce bacterial resistance in poultry production. They mainly involve bans on the application of antibiotic growth promoters (AGP), replacement of AGP with new feeds and feed additives, increased biosecurity, or optimization of management in specific areas of poultry production (Gheisar and Kim, 2018; Kim and Lillehoj, 2019; Yadav and Jha, 2019).

CONCLUSIONS

The supplementation of diets for broiler chickens with micronized camelina and flax seeds can improve body weight in the final rearing stage and significantly reduce the FCR value by increasing the utilization of total protein and organic matter in feed. Concurrently, it contributes to reduction of mortality rates throughout the chicken rearing period.

The infrared-irradiated camelina seeds added to the feed mixture also improve the balance of intestinal

microbial populations by increasing the population of *Bifidobacteria* spp. The taxa of the *Enterobacteriaceae* family isolated from the gastrointestinal tract of the analyzed chickens may pose a serious epidemiological problem in suitable conditions. The research results indicate that micronized oilseeds decrease the diversity of potentially pathogenic *Enterobacteriaceae* in the cecum and cloaca in broiler chickens, and flax and sunflower seeds reduce the number of multi-resistant strains.

In summary, the addition of micronized camelina, flax, and sunflower seeds to broiler chicken diets can be considered an effective nutritional strategy improving the effectiveness of rearing by positive modification of the intestinal microbial populations and enhancement of bacterial sensitivity to selected chemotherapeutic agents.

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DISCLOSURES

No potential conflict of interest was reported by the authors.

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