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Effect of the competitive exclusion culture on the growth, blood parameters, leg health, and innate immunity of broiler chickens*

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The primary aim of poultry production is to obtain a high yield and quality end product. To reduce the risk of disease, many direct-fed microbial products have been developed. That appears to be an excellent tool for disease prevention.
We evaluated the influence of the commercial, competitive exclusion (CE) product, Broilact®, on the growth rate, hematology, serum biochemistry, and innate immunity in male ROSS-308 chickens, randomly divided into two groups (Broilact® treatment and control) raised for 42 days.

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The birds' body weight was determined at 1, 7, 14, 28, 35, and 42 days, and the blood samples were collected at days 22 and 42 of life. We observed lower mortality, better gait score, and higher final body weight in the Broilact® group. At day 22, birds from the treated group presented higher white blood cells counts (WBC) and T cytotoxic lymphocyte (CD8+) counts, higher total protein (TP) (fraction globulin and albumin), and lower triglyceride (TAG) and Ca^{2+} plasma concentrations. No differences were found in acute phase proteins (APPs). At day 42, only the K+ and Na+ concentrations were higher, while the IL-10 was lower in treated birds' blood serum.
Our results indicate that treatment with one dose of the Broilact® product at day one of life has a beneficial influence, which improves the chickens' performance, leg health and some serum enzymes activity, maintains electrolyte homeostasis, and influences leukocyte count with the rise of T CD8+ subpopulations.

KEYWORDS: competitive exclusion / chicken broiler / leg health indicators / hematology / blood biochemistry / innate immunity

List of abbreviations

A:G: albumin/globulin; ALT: alanine transaminase; ANOVA: analysis of variance; AP: alkaline phosphatase; APP: acute phase proteins; AST: aspartate transaminase; Bu-1+: B lymphocyte; Ca: calcium; CD3+: T lymphocyte; CD4+/CD8+: T lymphocyte subpopulations; CE: competitive exclusion; CFU: colony forming units; CP: ceruloplasmin; CRP: C-reactive protein; EDTAK3: ethylenediaminetetraacetic acid tripotassium salt dehydrate; ELISA: Enzyme-Linked Immunosorbent Assay; EPEF: European production efficiency factor; ESBL: extended-spectrum beta-lactamase; GS: gait score; FCR: feed conversion ratio; FGA: fibrinogen Alpha; FITC: fluorescein isothiocyanate; FPD: footpad dermatitis; HP: haptoglobin; IBD: Infectious Bursal Disease; IgA: immunoglobulin A; IgM: immunoglobulin M; IgY: immunoglobulin Y; IL-1β, IL-2,IL-4,IL-6, IL-8, IL-10 : interleukins 1 beta,2,4,6,8 and 10; IFG: interferon Gamma; K: potassium; LZM: lysozyme; MCP-1: monocyte chemotactic protein 1; MD: Marek's Disease; ME: metabolizable energy; Mq+: Monocytes; Na: sodium; ND: Newcastle Disease; NE: necrotic enteritis; ORM1: orosomucoid 1; P: phosphorus; PE: phycoerythrin; RBC: red blood cell; ROSS-308: broiler chicken line; SEM: standard error of mean; sICAM-1/CD54: soluble intercellular adhesion molecule 1; TAG: triglycerides; TP: total protein; WBC: white blood cell.

A mixture of stable microbes derived from the intestinal microbiota of healthy adult animals is the basis for competitive exclusion (CE) products. The application of CE products is based on the so-called Nurmi Concept [Pivnick and Nurmi 1982, Pieczyńska *et al.* 2020, 2022, Akbarimehr *et al*. 2023, Asadollahi *et al*. 2023, Marchewka *et al.* 2023, Nowaczewski *et al.* 2023, Pourreza *et al.* 2023, Wójcik *et al.* 2023]. Combinations of distinct probiotic bacteria have been shown to suppress pathogenic bacteria from the gut of vertebrates through competitive exclusion [Nurmi and Rantala 1973]. The use of CE and the Nurmi Concept in combination with biosecurity measures has been shown to act against food-poisoning by *Salmonella*

spp. and *Campylobacter* spp. in poultry [Hakkinen and Schneitz 1999, Nurmi and Rantala 1973, Palmu and Camelin 1997, Pivnick and Nurmi 1982].

This concept was originally devised to control salmonella infections. In Finland, CE technology was first used in the broiler industry as early as 1976, and by 1981 it was also playing an important role in neighboring Sweden as a part of their national salmonella control strategy for poultry [Wierup *et al.* 1995]. It was also experimentally proved that CE treatment also protects chicks against pathogenic *Escherichia coli* [Hakkinen and Schneitz 1996], *Yersinia enterocolitica* [Soerjadi-Liem *et al.* 1984], and *Campylobacter* spp. [Mead *et al.* 1996, Schneitz and Hakkinen 2016], as well as protecting chickens, turkeys, and pheasants against *Salmonella Infantis* [Schneitz and Renney 2003]. Moreover, CE treatment in broiler chicks decreased the count of *Clostridium perfringens* in the caeca, resulting in reduced mortality from necrotic enteritis (NE) - Elwinger *et al.* [1992].

In 1987, the first commercial CE product: Broilact® was launched in Finland and Sweden. In the beginning, and until 1994, it was sold in liquid form. After that, the original preparation was substituted by the lyophilized product [Schneitz *et al.* 1998]. According to the manufacturer, Broilact® is refined gut microbiota obtained from healthy, adult hens, which colonizes the intestinal surface and establishes a natural, dominant gut microbiota. The more detailed composition of the Broilact® was previously published by Such *et al.* [2021]. Its properties provide the flock with a lifelong natural defense against undesirable bacteria when used in chicks, in a single dose, just after they hatch. Consistent use, together with proper farm hygiene management, reduces the need for antimicrobial treatment. Broilact® can be used with various species of fowl, including chickens, turkeys, and pheasants (https://www.broilact.com/). The Broilact® product has already been tested in other experiments, where its ability to positively modulate gut microbiota, protect against particular gastrointestinal bacteria (*Salmonella* spp., *Clostridium* spp., *Campylobacter* spp.), and reduce extended-spectrum beta-lactamase (ESBL) production, *Escherichia coli* colonization in broilers' intestines as well as improve feed digestibility and production parameters in birds has been proved and appears to benefit chickens, pheasants, and turkeys [Bilal *et al.* 2000, Elwinger *et al*. 1992, Hakkinen and Schneitz 1996, 1999, Kaldhusdal *et al.* 2001, Nuotio *et al.* 2013, Palmu and Camelin 1997, Schneitz 1992, Schneitz and Hakkinen 1998, Schneitz *et al.* 1998]. Broilact® is widely used in Finnish broiler and turkey production as a valuable part of poultry biosecurity procedures [Siekkinen *et al.* 2012]. Due to the favorable epidemiological situation and the judicious use of antibiotics, Finnish broiler production is almost completely antibiotic-free; it is also almost free of *Salmonella* spp. and has a low prevalence of *Campylobacter* spp. [The European Union One Health 2022 Zoonoses Report 2022]. The product is also widely used in other countries (https://www.broilact.com/).

The requirement to diminish the use of antibiotics in animal production imposes a need to apply alternatives. The use of CE products in animal feeding, particularly in poultry, appears to be a promising antibiotic-independent approach "from farm

to fork," which can be used to reduce the prevalence of human infection with by enteropathogens [Ducatelle *et al.* 2015, Heimesaat *et al.* 2021, Mountzouris *et al.* 2009]. We would like to enrich the knowledge about this type of product especially focusing on the general reaction of chicken organisms on contact with the provided external digestive tract microbiota. The aim of our experiment was to evaluate possible influence of this product on hematology, serum biochemical parameters, leg health indicators, and innate immune response, after oral application of the product to one-day-old broilers reared up to 42 days of age. This aim was to be achieved by tracking the quantitative and qualitative changes in selected immune response elements (T cell and B cell subpopulations, monocytes), antibody levels, and changes in blood morphology and biochemical indicators in chickens that received Broilact®. The economic aspects of production were also considered.

Material methods

Birds and experimental design

To simulate in the best possible way the commercial farm conditions a floor pen trial was carried out in the experimental broiler house (RZD Wilanow-Obory, Warsaw University of Life Sciences–SGGW, Warsaw, Poland) we used the largest possible number of experimental birds. In total, 1044 ROSS-308 one day old male chicks, that had been vaccinated against IBD (Infectious Bursal Disease), MD (Marek's Disease), and ND (Newcastle Disease) were purchased from a local commercial hatchery and randomly allocated to 18 pens (58 chicks per pen on litter) in a windowless and thermostatically controlled room. The chickens were divided into two groups: the experimental group treated with Broilact® (B, n=522 chicks) (Orion Pharma, Finland) and the non-treated control group (C, without Broilact®, n=522 chicks). Each group consisted of nine replicated pens, with a predicted stocking density, according to the *EU Council's broiler welfare directive* [2007/43/EC], of 33 kg/m^2 at day 42, post-hatching (EU Broiler Welfare Directive Council Directive 2007/43/EC). Each pen was equipped with two tube feeders and two bell drinkers. The lighting and temperature in the building were adjusted according to the Ross management guide [Ross management guide. Aviagen 2019], with the ambient temperature starting at 34°C at chick placement, which was then gradually reduced to 20°C by day 25, and maintained at this level thereafter. Before administration, the Broilact® was prepared according to the manufacturer's instructions. A dose of 1 mg Broilact® per bird ($\geq 10^7$ CFU/0.3 mL) was diluted in 0.3 ml of regenerant agent solution and administered into the crop of one-day chicks at the hatchery. The birds from the control group received 0.3 ml of regenerant agent solution in the same way. The chickens were fed commercial diets (granulated feed): starter I (0-7 days) and starter II (8-15 days, metabolizable energy (ME)) 3000 kcal/kg, 22.10% protein); grower I (16-27 days, ME 3130 kcal/kg, 20.10% protein); grower II (28-35, ME 3170 kcal/kg, 19.30% protein); and finisher (36-42 day, ME 3190 kcal/kg, 18.70% protein) from Cedrob S.A. Company (Poland) [Official Methods of Analysis of AOAC International, 2005].

Determination of chicken production parameters, food pad lesions, and gait score

The chickens were reared for 42 days. The birds' body weights were determined at 1, 7, 14, 28, 35, and 42 days of life. Final production parameters were also calculated as the total weight of chickens (kg), mortality (%), feed conversion ratio (FCR = feed consumption (kg))/(body weight gain (kg)), and European production efficiency factor (EPEF = (livability (%) × body weight (kg) × 100)/(age (days)× feed conversion ratio (kg)).

The footpad quality was visually defined five days before slaughter according to a modified scale proposed by Rushen *et al.* [2011]. The evaluation consisted of determining the degree to which lesions had advanced on a 0, 1, 2 scale (Tab. 1), based on the Instruction [2017] GIWpr.02010-7/2017, issued by the Polish Chief Veterinary Officer to the veterinarians of the Veterinary Sanitary Inspection for inspecting slaughterhouses, from the Act of Concerning Veterinary Inspection [2004].

The evaluation of the gait score (GS) was performed according to Butterworth [2009] and Kestin *et al.* [1992] - Table 2. The bird's ability to walk was scored on a six- points scale on the thirty-ninth day of life. So that the result was objective, the assessment was carried out by at least two evaluators.

Table 1. Scale of assessment of FPD (footpad dermatitis) lesions in chickens

Score	Description
0	No lesions
1	Superficial lesions, colored lesions with a diameter not exceeding 0.5 cm
2	Deep lesions with a scab and ulceration, colored lesions with a diameter of 0.5 cm or greater

Table 2. Evaluation of the gait score (GS)

GS	Description
0	No detectable abnormality, fluid locomotion, furled foot when raised
1	Slight defect difficult to define
2	Definite and identifiable defect, but it does not hinder the broiler's movement
3	An obvious gait defect that affects the broiler's ability to maneuver, accelerate, and gain speed
4	A severe gait defect, the broiler will walk only a couple of steps if driven, before sitting down
5	Complete lameness, either cannot walk or cannot support weight on the legs

Determination of blood parameters

Blood samples (3 × 1 mL) were collected from the jugular veins of 15 randomly chosen birds from each group at the following time points: 22 and 42 days of life. Blood was collected in two tubes coated with EDTAK3 (ethylenediaminetetraacetic acid tripotassium salt dehydrate), for hematological and flow cytometry analysis, and into one tube without additives for serum intended for biochemical and serological tests. Red blood cell (RBC, 10^6/μL) and white blood cell (WBC, 10^3/μL) counts were determined in a Neubauer hematological chamber, with Natt and Herrick solution used as a solvent.

A chemistry analyzer (Miura One, I.S.E. S.r.l., Albuccione, Italy) was used for determining the following biochemical serum parameters: aspartate transaminase (AST, IU/L), alanine transaminase (ALT, IU/L), alkaline phosphatase (AP, IU/L), glucose (mg/dL), uric acid (mg/dL), total protein (g/L), albumin (g/L), globulin (g/L), bilirubin (mg/mL), cholesterol (mg/dL), triglycerides (TAG, mg/dL); and the ions, total calcium (Ca, mg/dL), phosphorus (P, mg/dL), potassium (K, mmol/L), and sodium (Na, mmol/L).

Commercially available ELISA tests (Fine Test, Wuhan, China) were used according to the manufacturer's instructions to determine HP (haptoglobin), IgY (immunoglobulin Y), IgA (immunoglobulin A), IgM (immunoglobulin M), and LZM (lysozyme); while Elabscience tests (Elabscience, Wuhan, Hubei, China) were used to determine ORM1 (orosomucoid 1), CRP (C-reactive protein), IL-4 (interleukin 4), IFG (interferon Gamma), IL-2 (interleukin-2), IL-6 (interleukin-6), sICAM-1/CD54 (soluble intercellular adhesion molecule 1), IL-1β (interleukin-1-Beta), IL-8 (interleukin-8), MCP-1 (monocyte chemotactic protein 1), FGA (fibrinogen Alpha), IL-10 (interleukin-10), and CP (ceruloplasmin).

Peripheral blood T and B lymphocytes and monocytes were isolated using low-speed centrifugation, and a Histopaque®1077 (Sigma-Aldrich, St. Louis, MO, USA) was used for density gradient separation. Specific monoclonal antibodies, mouse anti-chicken CD3+ and CD4+ (fluorescein isothiocyanate, FITC staining), CD8+, Bu-1+, and monocyte/macrophage+ (phycoerythrin, PE staining) (Southern Biotech, Birmingham, AL, USA), were used. The fluorescence intensities of 100,000 cells were measured using a BD FACSAria™ flow cytometer, and the results were analyzed using BD FlowJo® software (Ashland, OR, USA).

Ethical statement

All animal procedures were approved by the WULS Second Local Ethics Committee for animal experiments to ensure compliance with Polish and European regulations for animal welfare in relation to the use of live vertebrate animals in research and teaching (decision WAW2/174/2019 of 20.11.2019).

Statistical analysis

The results for the determination of chicken production parameters, footpad lesions, and gait score were processed using the PS IMAGO PRO 5.1. The obtained data were developed using T-Student test and significance was determined at $p \leq 0.05$. All data were subjected to the Shapiro-Wilk test to assess normality, and the Levene test was used to verify the equality of variances. If the assumptions for the Student's T-test were not met, its non-parametric equivalent, the Mann-Whitney U test, was used.

The results for the determination of blood parameters were analyzed using the appropriate statistical tests (ANOVA, Tukey's test), and GraphPad Prism 7 software (San Diego, CA, USA), and presented as the mean and standard error of the mean (SEM). Significant differences were accepted at $p \leq 0.05$ with a 95% confidence rate.

Results and discussion

The effect of CE product treatment on production parameters, footpad lesions, and gait score

None of the birds presented any necropsy lesions or clinical symptoms of any diseases. The basic growth performance parameters, such as body weight, average cumulative feed consumption, FCR, and EPEF, are shown in Table 3, while footpad dermatitis and gait score results appear in Table 4.

Table 3. Production parameters of chickens treated with Broilact® (B) and the non-treated control group (C)

Parameter		Group	Mean	SEM
Average body weight (g)	day1	B	44.15	0.16
		C	44.05	0.14
	day7	B	213.20	1.00
		C	213.14	0.94
	day 14	B	553.30	2.41
		C	557.46	2.53
	day 28	B	1909.74	8.17
		C	1923.76	9.67
	day 35	B	2831.02	9.81
		C	2817.85	11.78
	day 42	B	3664.90	16.84
		C	3649.72	19.03
Total weight of chickens (kg)		B	1449.70*	2.11
		C	1382.84*	2.95
Mortality (%)		B	0.96*	0.01
		C	2.30*	0.01
Feed converse ratio (FCR; kg)		B	1.58	0.02
		C	1.60	0.02
European Production Efficiency Factor (EPEF; points)		B	543.58	12.05
		C	539.73	7.17

*Statistically significant differences between groups, $p \leq 0.05$.

Table 4. Footpad dermatitis (FPD) and the gait score (GS) of chickens from the Broilact® and control groups

Indicator	Score	Group			
		B		C	
		%	n	%	n
Footpad dermatitis	0	90.10	319	89.70	312
	1	9.60	34	9.80	34
	2	0.30	1	0.60	2
	p-value	≥0.05			
Gait score	0	93.80	332	88.20	307
	1	5.60	20	9.80	34
	2	0.60	2	1.70	6
	3	0.00	0	0.30	1
	4	0.00	0	0.00	0
	5	0.00	0	0.00	0
	p-value	≥0.05			

There were no statistically significant differences in body weight between the chickens from the Broilact® group (3664.90 kg) and the control group (3649.72 kg) on the forty-second day. The mortality rate percentage was lower in the Broilact® group (0.96 %) in comparison to the non-treated group (2.30 %) - Table 3.

There were statistically significant differences in the gait score results between chickens from the Broilact®-treated group where more birds showed no abnormal gait (Tab. 4).

The effect of CE product treatment on chicken hematology

The influence of Broilact® on the red blood cell (RBC) and white blood cell (WBC) counts in chickens is shown in Table 5. Significant changes were seen in the total WBC count, where the chickens from the Broilact®-treated group showed higher numbers on day 22, which continued until day 42.

Table 5. Average red blood cell (RBC) and white blood cell (WBC) counts in the blood of the experimental chickens treated with Broilact® (B) and the non-treated control group (C)

Parameter	Group	Age of chicken			
		22 days		42 days	
		mean	SEM	mean	SEM
RBC (count x 10^6/µl)	B	1.85	0.14	2.48	0.14
	C	2.13		2.68	
WBC (count x 10^3/µl)	B	30.42*	1.78	30.70	1.78
	C	23.14*		26.84	

*Statistically significant differences between groups, $p \leq 0.05$.

The effect of CE product treatment on chicken lymphocyte and monocyte populations

A flow cytometry analysis was performed to evaluate each WBC population, since the total WBC count was a general parameter (Tab. 6). Among the populations

of leukocytes that were distinguished, on day 22 there was a significantly increased number of T (CD8+) leukocytes in the group treated with Broilact®.

Table 6. Flow cytometry analysis of the percentage of T lymphocytes with CD3+, CD4+, CD8+, CD4+/8+ receptors; B lymphocytes (Bu-1+); and monocytes (Mq+) in the blood of the chickens treated with Broilact® (B) and the control group (C)

Parameter	Group	Age of chicken			
		22 days		42 days	
		mean	SEM	mean	SEM
B lymphocyte (Bu-1+)	B	9.43	0.63	6.91	0.58
	C	8.14		7.12	
T lymphocyte (CD3+)	B	7.41	1.00	6.91	0.93
	C	7.51		7.12	
T lymphocyte (CD4+)	B	6.00	1.21	14.08	1.13
	C	7.25		12.81	
T lymphocyte (CD8+)	B	10.54*	0.79	4.20	0.73
	C	8.70*		4.53	
T lymphocyte (CD4+/8+)	B	3.08	0.50	1.01	0.47
	C	2.51		0.77	
Monocytes (Mq+)	B	1.33	0.18	1.58	0.16
	C	1.15		1.40	

*Statistically significant differences between groups, $p \leq 0.05$.

The effect of CE product on chicken biochemical parameters

The influence of Broilact® on the chickens' biochemical blood parameters during the experiment was evaluated, and some differences among the groups were noticed (Tab. 7). A comparison of biochemical parameters in the serum showed significantly lower concentration of AP on day 22 in the Broilact®-treated group, with an upward trend on day 42. On day 22, this group also tended to have a lower ALT concentration and showed significantly higher concentration of total protein associated with significantly higher concentration of albumin and globulin. On the same day, they demonstrated significantly lower concentration of triglyceride (TAG), and had a tendency to higher concentration of cholesterol in their serum. Additionally, the chickens from the Broilact®-treated group also showed significantly lower serum Ca concentration with a trend towards higher P concentration on day 22 of the study. On day 42, the birds from this group showed significantly higher serum K^+ and Na^+ concentrations.

The effect of CE product on the serum concentrations of lysozyme, acute phase proteins, and immunoglobulins

To evaluate the influence of the Broilact® treatment on immune system function, the levels of lysozyme, acute phase proteins and cytokines (Tab. 8), and immunoglobulins (Tab. 9) were measured in the chicken sera. When comparing the expression of immune-

related proteins and cytokines, statistical analysis revealed no significant differences. Only the IL-10 level, on day 42, was significantly lower in the chickens treated with Broilact® compared to the control group. The levels of IgY, IgM, and IgA did not differ among the groups on day 22 or 42.

Table 7. The concentrations of serum biochemical parameters in chickens treated with Broilact® (B) and the control group (C)

Parameter	Group	Age of chickens			
		22 days		42 days	
		mean	SEM	mean	SEM
AST (IU/L)	B	176.00	21.63	393.00	21.63
	C	217.30		369.80	
ALT (IU/L)	B	5.90	0.96	5.87	0.96
	C	7.89		4.97	
AP (IU/L)	B	1685.00*	387.10	2439.00	387.10
	C	3440.00*		1630.00	
Total Protein (g/L)	B	31.83*	1.39	26.99	1.39
	C	27.52*		27.83	
Albumin (g/L)	B	15.90*	0.57	14.87	0.57
	C	14.48*		13.96	
Globulin (g/L)	B	15.93*	0.96	12.13	0.96
	C	13.04*		13.87	
A:G ratio	B	1.01	0.09	1.29*	0.09
	C	1.13		1.02*	
Glucose (mg/dL)	B	231.00	7.30	225.1.0	7.30
	C	228.50		226.50	
Uric Acid (mg/dL)	B	4.08	0.50	2.21	0.50
	C	3.67		2.67	
Bilirubin (mg/dL)	B	0.51	0.16	0.26	0.16
	C	0.65		0.22	
Cholesterol (mg/dL)	B	145.90	5.00	112.90	5.00
	C	135.30		107.70	
Triglycerides (mg/dL)	B	131.50*	8.98	80.98	8.98
	C	160.20*		79.95	
Ca (mg/dL)	B	8.70*	0.23	8.55	0.23
	C	9.29*		8.85	
P (mg/dL)	B	4.40	0.36	3.18	0.36
	C	3.65		3.27	
Ca:P Ratio	B	2.00*	0.28	2.71	0.28
	C	2.89*		2.84	
K (mmol/L)	B	5.60	0.25	6.27*	0.25
	C	5.30		5.48*	
Na (mmol/L)	B	148.30	2.28	144.80*	2.28
	C	144.80		138.90	

*Statistically significant differences between groups, $p \leq 0.05$.

Table 8. Levels of lysozyme, selected acute phase proteins, and cytokines in the blood of chickens treated with Broilact® (B) and the control group (C)

Parameter	Group	Age of chickens			
		22 days		42 days	
		mean	SEM	mean	SEM
Lysozyme (LZM, pg/ml)	B	8118.00	45.53	8095.00	45.53
	C	8112.00		8118.00	
Ceruloplasmin (CP, ng/ml)	B	1611.00	20.99	1633.00	20.99
	C	1603.00		1596.00	
C-reactive protein (CRP, pg/ml)	B	962.60	27.44	801.50	27.44
	C	994.10		806.8	
Fibrinogen (FGA, ng/ml)	B	21294.00	1003	21713.00	1003.00
	C	21381.00		20232.00	
Interferon gamma (IFG, pg/ml)	B	183.80	11.57	181.00	11.57
	C	205.20		185.10	
Haptoglobin (HP, ng/ml)	B	55.10	36.24	198.90	36.24
	C	62.04		166.70	
Interleukin-1β (IL-1B, pg/ml)	B	80.79	19.59	116.50	19.59
	C	93.82		136.40	
Interleukin-2 (IL-2, pg/ml)	B	575.20	74.51	557.00	74.51
	C	674.20		634.70	
Interleukin-4 (IL-4, pg/ml)	B	353.60	14.04	348.90	14.04
	C	350.60		356.60	
Interleukin-6 (IL-6, pg/ml)	B	498.40	87.97	490.50	87.97
	C	473.70		513.20	
Interleukin-8 (IL-8, pg/ml)	B	134.50	22.36	108.10	22.36
	C	133.50		138.50	
Interleukin-10 (IL-10, pg/ml)	B	244.50	65.69	195.50*	65.69
	C	361.20		375.00*	
Monocyte chemotactic protein 1 (MCP-1, pg/ml)	B	43.22	16.75	88.45	16.75
	C	43.69		63.88	
Soluble intercellular adhesion molecule 1 (sICAM-1/CD54, ng/ml)	B	630.80	301.00	1173.00	301.00
	C	577.40		842.70	
Orosomucoid 1 (ORM1, ng/ml)	B	23.91	27.70	37.28	27.70
	C	11.26		57.23	

*Statistically significant differences between groups, $p \leq 0.05$

Table 9. Serum immunoglobulin (Ig) levels in chickens treated with Broilact® (B) and the control group (C)

Parameter (ng/ml)	Group	Age of chickens			
		22 days		42 days	
		mean	SEM	mean	SEM
IgY	B	39.68	4.79	39.78	4.79
	C	37.05		40.76	
IgA	B	71.42	4.05	52.66	4.05
	C	72.18		54.21	
IgM	B	80.94	6.34	50.19	6.39
	C	78.21		52.65	

The effect of CE product treatment on production parameters, gait score, and footpad lesions

The use of multi-strain products to create positive gut microbiota in chicks seems to be beneficial. This may be the effect of the synergistic action of all the strains present in these products. This synergy can positively improve nutrient utilization, increase the secretion of positive metabolites, synthesize enzymes, and enhance antagonism against pathogens in broilers' guts [Chapman *et al*. 2011].

Production parameters are often used to evaluate the influence of competitive exclusion products with an undefined culture applicated on the day of hatching on the health of chickens. Previous experimental models have shown that these products improve bird performance as higher body weight and feed consumption, and reduced/lower feed conversion and mortality [Abu-Ruwaida et al.1995; Bilal *et al*. 2000; Kaldhusdal *et al*. 2001]. This is in line with our results, where the final total weight of chickens treated with Broilact® was higher and mortality was lower (0.96%) in comparison to the control group (2.3%) (Tab. 3). The higher final total weight of the chickens in the Broilact® group could be explained by lower mortality and, as a result, a higher number of birds on the day of slaughter, which is beneficial for farmers. We additionally observed a tendency for the EPEF value in the Broilact® group to be higher (543.58) compared to the control group (539.73). The EPEF's higher values and lower feed conversion indicated that the body weight gain in the birds was uniform, and the flock was in good health [Bhamare *et a*l. 2016].

The gait score system is an estimation of locomotion deficiency, and is based on a visual judgment of a broiler chicken's ability to walk on a known surface. It was developed by Kestin *et al*. [1992] to assess lameness in broilers, and the methodology consists of empirical, repetitive visual observations of how birds walk on a surface. In our experiment, the gait score results were better in chickens from the Broilact® treated group in comparison to the control group, where more birds had symptoms of lameness. Similar results were described in a work by Bendowski *et al.* [2022], where milk thistle administered to the chickens in drinking water in the amount of 0.36 g/day/animal, significantly reduced the number of feet scoring 1 or 2, i.e., where feet with lesions in the footpad were visually observed. Broilers with lameness problems cannot walk easily, and, as a result, cannot reach the feeder or the drinker, which could also partly explain the final total higher weight of the chickens in the Broilact® group. Additionally, this leg disorder reduces the chickens' quality of life, and, furthermore, movement problems may be painful for the birds, decrease the broiler's activity, and increase various health problems, which, in turn, increase the mortality rate in the flock [Aydin 2018].

The effect of CE product treatment on chicken hematology

The effect of using various symbiotic formulas or probiotics to affect chicken immunity has also been evaluated by other authors [Alimohamadi *et al*. 2014, Alkhalf *et al*. 2010, Beski and Al-Sardary 2015] using various hematological tests. In our experiment, we used a CE product, which is a mixture of different microorganisms

isolated from healthy birds, yet there is still no publication referring to the use of such a formula to affect hematological parameters in chicken broilers. We expected that probiotic bacteria and NGF (normal gut flora) could stimulate the local immune system in the gut, and that their systemic effects could be observable also in the blood. However, different immunomodulatory effects may be induced; thus, identical effects cannot be expected when different probiotic bacterial strains or NGFs are used [Ashraf *et al.* 2014], which complicate the matter of determining reference parameters. The significant differences between the Broilact®-treated group and the control group for the average WBC count present on day 22 may be due to the fact that the bacteria present in Broilact® could have stimulated the intestinal immune system of the chicks and, as a result, led to transient leukocytosis.

There is a lack of specific publications on the hematological changes in blood after the use of CE products. However, such an increment change in the WBC count in birds receiving mono- or multi-strain probiotics was observed by other authors [Deraz 2018; Paryad and Mahmoudi 2008]. Thus, a similar effect was also observed by Hanamanta et al. [2009] after prebiotic, probiotic, and G-probiotic SPL were used in broiler chickens. The supplementation with probiotics positively influenced hematopoiesis, which, among others, increased the WBC counts. As shown in the literature, supplementation with beneficial microorganisms can both enhance immune cell synthesis and stimulate them, which further protects the host against pathogens [Gaggìa *et al*. 2010; Yeşilyurt *et al*. 2021].

The effect of CE product treatment on chicken lymphocyte and monocyte populations

The significant difference observed in the CD8+ cytotoxic lymphocytes T population between the groups may speak in favor of the theory concerning their additional stimulation. The evaluation of the WBC population by flow cytometry showed that the birds that received the Broilact® product, on day 22, presented a significantly higher CD8+ cell count; these are the T cytotoxic lymphocytes that can be activated by the invasion of pathogens and are responsible for generating an effective immune response against them so the permanent and stable amount of CD8+ is necessary [Wong and Pamer 2003]. This mechanism associated with supplemented microbiota was also postulated by others [Deraz 2018, Gaggìa *et al*. 2010]. This may indicate that Broilact® is beneficial for immune system development and leads to faster presence at the proper level of immune defense, however, the mechanism behind this stimulation is, for now, not so clear.

The effect of CE product treatment on lysozyme, acute phase proteins, cytokines, and immunoglobulins

The analysis of lysozyme, acute phase proteins, cytokines (except IL-10), and immunoglobulins did not show any differences between groups. This indicates that the immune system was not negatively affected by the CE supplementation. The only significant difference we noticed was in the anti-inflammatory cytokine, IL-10

concentration which was lower in the supplemented birds on day 42. This interleukin is a multi-action molecule with a range of reported anti-inflammatory and regulatory functions. The effect of action depends on context, including, but not limited to, timing, tissue, and target cell [Rothwell *et al.* 2004]. In cases of infection with viruses, bacteria, fungi, protozoa, or helminths, this cytokine is released and acts as an anti-inflammatory cytokine that controls the nature and extent of the inflammatory responses [Couper *et al*. 2008]. When released before a burst of pro-inflammatory activity at the beginning of inflammation, it diminishes the ability of the animal to develop adaptive immune responses [Sabat *et al*. 2010]. This cytokine plays a particularly central role in intestinal immunity and homeostasis [Manzanillo *et al.* 2015]. The lower levels of IL-10 in the serum of the Broilact®- treated chickens on day 42 of our experiment may be the effect of a lower stimulation of the immune system by gut microflora what could be a sign of positive effect of better protection by the Broilact®. This effect was to be more expected in our experiment, since data presented by Wu *et al*. [2016] indicated that an anti-inflammatory IL-10 response that is too strong may be immunosuppressive in chickens, as it is in mammals, so birds with high concentration of it may also become susceptible to other pathogens as well, including *Campylobacte*r spp. and *Clostridium* spp. that can, potentially, also be transmitted to humans (e.g. *Salmonella* spp.).

Lower concentration of IL-10 may have another positive aspect. In the literature, it was shown that vaccine efficiency in animals was correlated with IL-10 production, and the anti-IL10 treatment increased the nature, magnitude, and efficacy of the vaccine responses in mammals due to vaccination [Darrah et al. 2010; Pitt *et al*. 2012]. As demonstrated by Wu *et al.* [2016], IL-10 has similar functions in birds as it does in mammals. On the basis of our results, we can suggest that CE products not only protect and help to reduce the burden of diseases, but also ensure that chickens are better prepared for vaccination by enhancing the development of a better immune response to vaccine stimuli.

The effect of CE product treatment on chicken biochemical parameters

All the biochemical parameters measured in our experiment appeared within the physiological range for poultry [Café *et al*. 2012], and did not differ significantly between groups, except for the alkaline phosphatase (AP) level. AP is an enzyme derived from various parts of the body, including the bile duct, epithelial cells, intestinal mucosa, bones, red blood cells, and kidneys. AP is found in many isoforms, depending on its origin within the body; but predominantly it is expressed and plays an integral role in metabolism within the liver and digestive tract, and in development within the skeleton. AP activity can be high in young birds during growth but decreases with age, although its activity is also high when birds suffer from osteoporosis [Sanger *et al.* 1966]. We can find many reports in the literature concluding that a biochemical marker such as AP can be used as a general indicator of skeletal development in vertebrates [Gade *et al.* 2011]. We observed significantly lower AP concentration at

day 22 in the Broilact®-treated group. This concurrently correlates with lower total Ca^{2+} concentration in birds from that group. Most of the total body calcium (98 to 99%) is located in the bones. When calcium blood concentration decreases, calcium is mobilized from the bones to maintain Ca^{2+} physiological blood level [Scott et al. 1982]. Moreover, we described less evidence for leg disorders, and in combination with lower levels of Ca^{2+} in the serum, Ca+ had probably already been incorporated into the bones: so, we may suspect that supplementation with Broilact® may help to maintain better bone development.

Low AP concentration also correlated with lower levels of TAG in the Broilact® group. This may be an effect of decreasing lipogenesis in the liver. Similar results were observed by Alimohamadi *et al.* [2014] in chickens fed diets supplemented with black seed (Nigella sativa), cumin seed (Cuminum cyminum), probiotics, or prebiotic.

Interestingly, on day 22, we noticed significantly higher levels of total protein, which can be attributed to higher albumin and globulin values in the birds treated with Broilact®. High values usually reflect a high protein metabolism rate, but it was not correlated with greater body weight in the chickens from that group at that time point. The significantly higher A:G ratio at day 42 in the Broilact® group, combined with lower absolute values for globulins, may indicate weaker immune system stimulation from the digestive tract in these birds, since the microorganisms in Broilact® may probably in some way prevent pathogens from penetrating deeper. As it was shown CE products accelerate the maturation of caecal microbiota [Meijerink *et al.* 2020]. This could be related to the lower mortality in the group treated with Broilact®.

The sodium, potassium, calcium, and phosphorus present in blood and cellular fluids in the form of electrolytes, affect the osmotic pressure and acid-base balance in the body. Further evaluation of ions in the serum showed that the values for chickens varied depending on the bird's age. The possible causes of the fluctuations in Ca^{2+} concentration were discussed above. Aside from $Ca^{2+,}$ significantly higher concentrations of K^+ and Na^+ were also observed in the Broilact® group in comparison to the control group, at day 42. The increase in Na+ concentration is in line with the findings of Aluwong *et al*. [2013] and Silva *et al.* [2007]. In the same experiment, Aluwong *et al*. [2013], observed a decrement in K^+ concentration, which was opposite to our results. This could be due to the fact that they used only yeast supplementation, while we had a mixture of microorganisms that can act in a synergistic way and may improve the absorption of minerals; however, the exact mechanism of interaction needs to be investigated. Concluding: both serum Na^+ and K^+ concentrations were within the normal range, indicating that treatment of broiler chickens with Broilact® did not alter the ratio of electrolytes in the blood.

Conclusions

Our results indicate that Broilact® has a beneficial influence on production parameters. Treatment with one dose of the Broilact® product on day one of a chicks'

life improves their performance, leg health and some serum enzyme activity, and maintains electrolyte homeostasis and affects leukocyte counts by increasing the CD8+ subpopulation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

REFERENCES

1. ABU-RUWAIDA A.S., HUSSEINI M., BANAT I.M., 1995 - Salmonella exclusion in broiler chicks by the competitive action of adult gut microflora. ***Microbios*** 83, 334, 59-69.
2. AKBARIMEHR M., SOLKA M., NOSRATI M., B RASOULI B., VAHABI-ASIL O., SEIDAVI A., HOSSEINTABAR-GHASEMABAD B., MARCHEWKA J., SZTANDARSKI P., JASZCZYK A., HORBAŃCZUK K., PETRYKOWSKI S., SIWIEC D., HORBAŃCZUK J.O., 2023 - Multi-strain probiotic improved feed conversion ratio and selected health indicators in Japanese quail. ***Animal Science Papers and Reports*** 41, 4, 345-358.
3. ALIMOHAMADI K., TAHERPOUR K., GHASEMI H.A., FATAHNIA F., 2014 - Comparative effects of using black seed (*Nigella sativa*), cumin seed (*Cuminum cyminum*), probiotic or prebiotic on growth performance, blood haematology and serum biochemistry of broiler chicks. ***Journal of Animal Physiology and Animal Nutrition*** 98, 3, 538-546.
4. ALKHALF A., ALHAJ M., AL-HOMIDAN I., 2010 - Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. ***Saudi Journal of Biological Sciences*** 17, 3, 219-25.
5. ALUWONG T., HASSAN F.B., RAJI M.S., KAWU M.U., DZENDA T., AYO Y.O., 2013 - Effect of different levels of supplemental yeast on performance indices, serum enzymes and electrolytes of broiler chickens. ***African Journal of Biotechnology*** 12, 35, 5480-5485.
6. ASADOLLAHI H., MAHYARI S.A., TORSHIZI R.V., EMRANI H., EHSANI A., 2023 - Genomic evaluation of body weight traits in a F2 mixture of commercial broiler and native chicken. ***Animal Science Papers and Reports*** 41, 2, 123-137.
7. ASHRAF R., VASILJEVIC T., DAY S.L., SMITH S.C., DONKOR O.N., 2014 - Lactic acid bacteria and probiotic organisms induce different cytokine profile and regulatory T cells mechanisms. ***Journal Functional Foods*** 6, 395-409.
8. AYDIN A., 2018 - Leg Weaknesses and Lameness Assessment Methods in Broiler Chickens. ***Archives of Animal Husbandry & Dairy Sciences*** 1, 1-6.
9. BENDOWSKI W., MICHALCZUK M., JÓŹWIK A., KARWAN Y.K., ŁOZICKI A., KARWACKI J., BIEŃ D., 2022 - Using Milk Thistle (*Silybum marianum*) Extract to Improve the Welfare, Growth Performance and Meat Quality of Broiler Chicken. ***Animals*** 12, 9, 1085.
10. BESKI S.S.M., AL-SARDARY S.Y.T., 2015 - Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. ***International Journal of Poultry Science*** 14, 1, 31–36.
11. BHAMARE K.S., DILDEEP V., SENTHIL-MURUGAN S., CHAVAN S.J., 2016 - Nutritive evaluation of cashew apple waste in broilers. ***International Journal of Science and Nature*** 7, 3, 629–632.
12. BILAL T., OZPINAR H., KUTAY C., ESECELI H., ABAS I., 2000 - The effects of Broilact® on performance and feed digestibility of broilers. ***Archive fur Geflugelkunde*** 64, 3, 134-138.
13. BUTTERWORTH A., 2009 - Animal welfare indicators and their use in society. In: Food Safety Assurance and Veterinary Public Health. Ed. Smulders H, Algers B, Wageningen Academic Publishers, Wageningen, The Netherlands 371-389.

14. CAFÉ M., RINALDI F.P., MORAIS H.R., PRAZEREZ C., 2012 - Biochemical blood parameters of broilers at different ages under thermoneutral environment. Book of abstracts of World Poultry Congress in Salvador. ***World's Poultry Science Journal*** 1,143-146.
15. CHAPMAN C.M., GIBSON G.R., ROWLAND I., 2011 - Health benefits of probiotics: Are mixtures more effective than single strains? ***European Journal of Nutrition*** 50, 1–17.
16. COUPER K.N., BLOUNT D.G., RILEY E.M., 2008 - IL-10: the master regulator of immunity to infection. ***Journal of Immunology*** 180, 9, 5771-5777.
17. DARRAH P.A., HEGDE S.T., PATEL D.T., LINDSAY R.W., CHEN L., ROEDERER M., SEDER R.A., 2010 - IL-10 production differentially influences the magnitude, quality, and protective capacity of Th1 responses depending on the vaccine platform. ***Journal of Experimental Medicine*** 207, 7, 1421-1433.
18. DERAZ S.F., 2018 - Synergetic effects of multispecies probiotic supplementation on certain blood parameters and serum biochemical profile of broiler chickens. ***Journal of Animal Health and Production*** 6, 1, 27–34.
19. DUCATELLE R., EECKHAUT V., HAESEBROUCK F., VAN IMMERSEEL F., 2015 - A review on prebiotics and probiotics for the control of dysbiosis: present status and future perspectives. ***Animal*** 9, 1, 43-8.
20. ELWINGER K., SCHNEITZ C., BERNDTSON E., FOSSUM O., TEGLÖF B., ENGSTÖM B., 1992 - Factors affecting the incidence of necrotic enteritis, caecal carriage of *Clostridium perfringens* and bird performance in broiler chicks. ***Acta Veterinaria Scandinavica*** 33, 369-378.
21. EU BROILER WELFARE DIRECTIVE COUNCIL DIRECTIVE 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:182:0019:0028:EN:PDF Accessed on 8 May 2024.
22. GADE T.P., MOTLEY M.W., BEATTIE B.J., BHAKTA R., BOSKEY A.L., KOUTCHER J.A., MAYER-KUCKUK P., 2011 - Imaging of Alkaline Phosphatase Activity in Bone Tissue. ***PloS One*** 6, e22608.
23. GAGGÌA F., MATTARELLI P., BIAVATI B., 2010 - Probiotics and prebiotics in animal feeding for safe food production. ***International Journal of Food Microbiology*** 141, 15-28.
24. HAKKINEN M., SCHNEITZ C., 1996 - Efficacy of a commercial competitive exclusion product against a chicken pathogenic *Escherichia coli* and *E. coli* O157:H7. ***Veterinary Record*** 139, 6, 139-141.
25. HAKKINEN M., SCHNEITZ C., 1999 - Efficacy of a commercial competitive exclusion product against *Campylobacter jejuni*. ***British Poultry Science*** 40, 619-621.
26. HANAMANTA N., NARAYANA M., VEENA T., NARAYANA H.D., JAYAKUMAR K., 2009 - Effect of prebiotic, probiotic and G-probiotic SPL® on certain haematological parameters in broiler chickens. ***Veterinary World*** 2 9, 344-346.
27. HEIMESAAT M.M., WESCHKA D., MOUSAVI S., BERESWILL S., 2021 - Treatment with the Probiotic Product Aviguard® Alleviates Inflammatory Responses during *Campylobacter jejuni* - Induced Acute Enterocolitis in Mice. ***International Journal of Molecular Sciences*** 22, 13, 6683.
28. Instruction Issued By Chief Veterinary Officer In Poland. 2017 - GIWpr.02010-7/2017: https://www.wetgiw.gov.pl/download/Brojlery-kontrola-lapek-instrukcja-28.06.2017,3766.pdf Accessed on 08 May 2024.
29. KALDHUSDAL M., SCHNEITZ C., HOFSHAGEN M., SKJERVE E., 2001 - Reduced incidence of *Clostridium perfringens*-associated lesions and improved performance in broiler chickens treated with normal intestinal bacteria from adult fowl. ***Avian Diseases*** 45, 1, 149-156.
30. KESTIN S.C., KNOWLES T.G., TINCH A.E., GREGORY N.G., 1992 - Prevalence of leg weakness in broiler chickens and its relationship with genotype. ***Veterinary Record*** 131, 9, 190-194.

31. MANZANILLO P., EIDENSCHENK C., OUYANG W., 2015 - Deciphering the crosstalk among IL-1 and IL-10 family cytokines in intestinal immunity. ***Trends Immunology*** 36, 8, 471-478.
32. MARCHEWKA J., SOLKA M., SZTANDARSKI P., JASZCZYK A., ZDANOWSKA-SĄSIADEK Ż., MASTALERCZUK G., BORAWSKA-JARMUŁOWICZ B., KOMOROWSKA D., HORBAŃCZUK J.O., 2023 - Free range characteristics and use by chickens from two genetic lines. ***Animal Science Papers and Reports*** 41, 2, 139-152.
33. MEAD G.C., SCOTT M.J., HUMPHREY T.J., McALPINE K., 1996 - Observations on the control of *Campylobacter jejuni* infection of poultry by 'competitive exclusion'. ***Avian Pathology*** 25, 69-79.
34. MEIJERINK N., KERS J.G., VELKERS F.C., VAN HAARLEM D.A., LAMOT D.M., DE OLIVEIRA J.E., SMIDT H., STEGEMAN J.A., RUTTEN V.P.M.G., JANSEN C.A., 2020 - Early Life Inoculation with Adult-Derived Microbiota Accelerates Maturation of Intestinal Microbiota and Enhances NK Cell Activation in Broiler Chickens. ***Frontiers in Veterinary Science*** 7, 584561.
35. MOUNTZOURIS K.C., BALASKAS C., XANTHAKOS I., TZIVINIKOU A., FEGEROS K., 2009 - Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with *Salmonella enteritidis*. ***British Poultry Science*** 50, 4, 467-478.
36. NOWACZEWSKI S., JANISZEWSKI S., KACZMAREK S., KACZOR N., RACEWICZ P., JAROSZ Ł., CISZEWSKI A., ŚLÓSARZ P., HEJDYSZ M., 2023 - Evaluation of the effectiveness of alternative methods for controlling coccidiosis in broiler chickens: a field trial. ***Animal Science Papers and Reports*** 41, 2, 97-110.
37. NUOTIO L., SCHNEITZ C., NILSSON O., 2013 - Effect of competitive exclusion in reducing the occurrence of *Escherichia coli* producing extended-spectrum β-lactamases in the ceca of broiler chicks. ***Poultry Science*** 92, 1, 250-254.
38. NURMI E.V., RANTALA M., 1973 - New aspects of *Salmonella* infection in broiler production. ***Nature*** 241, 210-211.
39. Official Methods of Analysis of AOAC International. 2005 - 18th Edition. Ed. W. Horwitz, G. W. Latimer. AOAC International, Gaithersburg, Maryland, USA.
40. PALMU L., CAMELIN I., 1997 - The use of competitive exclusion in broilers to reduce the level of *Salmonella* contamination on the farm and at the processing plant. ***Poultry Science*** 76, 11, 1501-1505.
41. PARYAD A., MAHMOUDI M., 2008 - Effect of different levels of supplemental yeast (*Saccharomyces cerevisiae*) on performance, blood constituents and carcass characteristics of broiler chicks. ***African Journal of Agricultural Research*** 3, 12, 835-842.
42. PIECZYNSKA M.D., SAMPINO S., LAWINSKI M., STACHOWIAK A., ZIETEK M., LIGAS J., SZELAG M., HORBANCZUK O.K., SWIRSKI M., MODLINSKI J.A., HORBANCZUK J.O., 2022 - Dynamics of gut microbiota emergence during fetal development in mice model. ***Animal Science Papers and Reports*** 40, 1, 99-110.
43. PIECZYNSKA M.D., YANG Y., PETRYKOWSKI S., HORBANCZUK O.K., ATANASOV A.G., HORBAŃCZUK J.O., 2020 - Gut microbiota and its metabolites in atherosclerosis development. ***Molecules*** 29;25(3), 594. doi: 10.3390/molecules25030594.
44. PITT J.M., STAVROPOULOS E., REDFORD P.S., BEEBE A.M., BANCROFT G.J., YOUNG D.B., O'GARRA A., 2012 - Blockade of IL-10 signalling during bacillus Calmette-Guerin vaccination enhances and sustains Th1, Th17, and innate lymphoid IFN-gamma and IL-17 responses and increases protection to Mycobacterium tuberculosis infection. ***Journal of Immunology*** 189, 8, 4079-4087.
45. PIVNICK H., NURMI E., 1982 - The Nurmi concepts and its role in the control of Salmonella in poultry." In: Developments in Food Microbiology. Volume 1. Applied Science Publishers. Davis, R., Ed.; Basel, Switzerland 41-70.

46. POURREZA A., JAVANDEL F., SEIDAVI A., FRANCO-ROBLES E., 2023 - The effect of dietary organic acids on performance, carcass characteristics, immunity, blood constitutes and ileal microflora of broiler chickens. ***Animal Science Papers and Reports*** 41, 1, 39-48.
47. ROSS MANAGEMENT GUIDE. AVIAGEN 2019. https://modernhatch.eu/wp-content/uploads/2021/01/Ross-308-broiler-PO-AND-NS-2019-PL.pdf Accessed on 8 May 2024.
48. ROTHWELL L., YOUNG J.R., ZOOROB R., WHITTAKER C.A., HESKETH P., ARCHER A., SMITH A.L., KAISER P., 2004 - Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*. ***Journal of Immunology*** 173, 4, 2675-2682.
49. RUSHEN J., BUTTERWORTH A., SWANSON J.C., 2011 - Animal behaviour and well-being symposium: Farm animal welfare assurance: Science and application. ***Journal of Animal Science*** 89, 4, 1219–122.
50. SABAT R., GRÜT G., WARSZAWSKA K., KIRSCH S., WITTE E., WOLK K., GEGINAT J., 2010 - Biology of interleukin-10. ***Cytokine Growth Factor Reviews*** 21, 5, 331-344.
51. SANGER V.L., BURMESTER B.R., MORRILL C.C., 1966 - Serum Alkaline Phosphatase Levels in Avian Osteopetrosis. ***Avian Diseases*** 10, 3, 364-371.
52. SCHNEITZ C., 1992 - Research note: automated droplet application of a competitive exclusion preparation. ***Poultry Science*** 71, 12, 2125-2128.
53. SCHNEITZ C., HAKKINEN M., 1998 - Comparison of two different types of competitive exclusion products. ***Letters in Applied Microbiology*** 26, 5 338-341.
54. SCHNEITZ C., HAKKINEN M., 2016 - The efficacy of a commercial competitive exclusion product on Campylobacter colonization in broiler chickens in a 5-week pilot-scale study. ***Poultry Science*** 95, 5, 1125-1128.
55. SCHNEITZ C., KIISKINEN T., TOIVONEN V., NASI M., 1998 - Effect of Broilact on the physicochemical conditions and nutrient digestibility in the gastrointestinal tract of broilers. ***Poultry Science*** 77, 3, 426-432.
56. SCHNEITZ C., RENNEY D.J., 2003 - Effect of a commercial competitive exclusion product on the colonization of *Salmonella infantis* in day-old pheasant chicks. ***Avian Diseases*** 47, 4, 1448-1451.
57. SCOTT M.L., NESHEIM M.C., YOUNG R.J., 1982 - Essential inorganic elements. In: ***Nutrition of the chicken***. 297-304.
58. SIEKKINEN K.M., HEIKKILÄ J., TAMMIRANTA N., ROSENGREN H., 2012 - Measuring the costs of biosecurity on poultry farms: a case study in broiler production in Finland. ***Acta Veterinarica Scandinavica*** 54, 12.
59. SILVA P.R.L., FREITAS NETO O.C., LAURENTIZ A.C., JUNQUEIRA O.M., FAGLIARI J.J., 2007 - Blood serum components and serum protein test of Hybro-PG broilers of different ages. ***Brazilian Journal of Poultry Science*** 9, 4, 229-232.
60. SOERJADI-LIEM A.S., SNOEYENBOS G.H., WEINACK O.M., 1984 - Establishment and competitive exclusion of *Yersinia enterocolitica* in the gut of monoxenic and holoxenic chicks. ***Avian Diseases*** 28, 1, 256–260.
61. SUCH N., FARKAS V., CSITÁRI G., PÁL L., MÁRTON A., MENYHÁRT L., DUBLECZ K., 2021 - Relative Effects of Dietary Administration of a Competitive Exclusion Culture and a Synbiotic Product, Age and Sampling Site on Intestinal Microbiota Maturation in Broiler Chickens. ***Veterinary Science*** 8, 9, 187.
62. THE EUROPEAN UNION ONE HEALTH 2022 ZOONOSES REPORT. https://doi.org/10.2903/j.efsa.2023.8442 Accessed 8 May 2024.
63. WIERUP M., ENGSTRÖM B., ENGVALL A., WAHLSTRÖM H., 1995 - Control of *Salmonella enteritidis* in Sweden. ***International Journal of Food Microbiology*** 25, 3, 219-226.

64. WONG P., PAMER E.G., 2003 - CD8 T cell responses to infectious pathogens. ***Annual Review of Immunology*** 21, 29-70.
65. WÓJCIK W., DAMAZIAK K., ŁUKASIEWICZ-MIERZEJEWSKA M., ŚWIDER O., NIEMIEC J., WÓJCICKI M., ROSZKO M., GOZDOWSKI D., 2023 - Dietary supplementation broilers with β-alanine and garlic extract improves production results and muscle oxidative status. ***Animal Science Papers and Reports*** 41, 4, 359-376.
66. WU Z., HU T., ROTHWELL L., VERVELDE L., KAISER P., BOULTON K., NOLAN M.J., TOMLEY F.M., BLAKE D.P., HUMEA D.A., 2016 - Analysis of the function of IL-10 in chickens using specific neutralising antibodies and a sensitive capture ELISA. ***Developmental and Comparative Immunology*** 63, 206–212.
67. YEŞILYURT N., YILMAZ B., AĞAGÜNDÜZ D., CAPASSO R., 2021 - Involvement of Probiotics and Postbiotics in the Immune System Modulation. ***Biologics*** 1, 2, 89-110.